Chromosomes associate premeiotically and in xylem vessel cells via their telomeres and centromeres in diploid rice (*Oryza sativa*)

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With 3 figures and 2 tables

SUMMARY

Studies of the meiosis of diploid plants such as *Arabidopsis*, maize and diploid progenitors of wheat have revealed no premeiotic association of chromosomes. Premeiotic and somatic association of chromosomes has only been previously observed in the anther tissues and xylem vessel cells of developing roots in polyploid plants such as hexaploid and tetraploid wheat, polyploid relatives of wheat and artificial polyploids made from the progenitor diploids of wheat. This suggested that this association was confined specifically to polyploids or was induced by polyploidy. However we developed procedures for *in situ* hybridization on structurally well-preserved tissue sections of rice, and analyzed two diploid rice species (*Oryza sativa* and *Oryza punctata*). Contrary to expectation, this has revealed that centromeres and telomeres also associate both in the xylem vessel cells of developing root and in undifferentiated anther cells in these diploids. However in contrast to wheat and related polyploids, where the initial association in undifferentiated anthers is between either non-homologous or related chromosomes and not homologous chromosomes, the initial association of rice chromosomes seems to be between homologues. Thus in contrast to the diploid dicot model *Arabidopsis*, meiotic studies on the diploid model cereal, rice, will now need to take into account the effects of premeiotic chromosome association.

Key words: centromeres, telomeres, polyploidy, meiosis, cereals, chromosome pairing, confocal microscopy

INTRODUCTION

The studies of meiosis in the diploid plants, *Arabidopsis* and maize as well as the diploid (A and D genome) progenitors of wheat have revealed that chromosomes only associate during meiosis (Armstrong et al. 2002; Comai et al. 2003; Bass et al. 1997; Martinez-Perez et al. 2000). Premeiotic association of chromosomes has not been reported in these species. In contrast, studies of chromosome pairing in hexaploid (*T. aestivum)* and tetraploid (*T. durum)* wheat, both allopolyploids, and their polyploid relatives have shown that chromosomes associate in pairs via their centromeres during anther development prior to meiosis (Martinez-Perez et al. 1999; Martinez-Perez et al. 2000; Maestra et al. 2002). The initial association of the chromosomes during early anther development is not homologous being either non-homologous or homoeologous (between related chromosomes from the three wheat genomes). At the onset of meiosis and just prior to the formation of the telomere bouquet, the chromosomes cluster via the centromeres into 7 groups (Martinez-Perez et al. 2003). At this stage, the level of association of the homologous chromosome increases to over 90% suggesting that the homologous chromosomes locate to the same group. Centromere association also occurs in the xylem vessel cells of developing wheat roots (Martinez-Perez et al. 2001). The *Ph1* locus which affects both premeiotic and meiotic chromosome pairing also affects this somatic pairing in the roots. An artificial polyploid made between the A and D progenitors of wheat does exhibit premeiotic centromere pairing (Martinez-Perez et al. 2000). These studies of diploid and polyploid plants suggested that premeiotic association was restricted to polyploids or could be induced following polyploidisation.

Rice (*Oryza sativa*) has a relatively small genome (430Mbp) compared with many cereals and grasses. A draft of its genome sequence is available. It is generally

now considered as a model for the cereals. Its overall gene order is quite similar to that of other cereals including wheat and maize. The genes on the rice map can be grouped into sets and these same sets of genes (rice linkage segments) also describe the genetic maps of all the chromosomes of barley, foxtail millet, maize, sorghum, sugar cane and wheat (Moore et al. 1995). Thus the structure of the rice genome provides the solution to representing cereal synteny. Rice is not only a model for the cereals, but is one of the major cereal crops. Understanding the factors that affect chromosome pairing and recombination in this species would have important consequences for breeding strategies.

Based on our previous studies of wheat and its diploid and polyploid relatives, and on the studies of maize, we predicted that rice would not exhibit premeiotic association. We therefore undertook a study to test this hypothesis. Studies on wheat and maize have all shown the importance of examining well-preserved material, both to be confident of the 3-D preservation of delicate nuclear structure, and to be confident of the cell types that are being analysed (Bennett et al. 1979; Dawe et al. 1994; Aragon-Alcaide et al. 1998). For example, tapetal cells and meiocytes in anthers or xylem vessel cells and other cells in the root can be misclassified, and polarised centromere or telomere clusters can easily be displaced or brought together artefactually in highly squashed preparations. Procedures have not previously been determined for undertaking 3D in situ approaches on either of the monocot or dicot model species, rice and *Arabidopsis*. We therefore initially determined conditions for labelling chromosome sequences in thick rice root and anther sections by *in situ* hybridization and visualised the resulting specimens by three-dimensional confocal microscopy. Using this approach with probes for centromeres and telomeres, as well as BAC probes for other chromosomal regions, we found, unexpectedly, that in two diploid and one polyploid

rice species, the chromosomes associate as pairs early during floral development in all the cells in the anthers, and in the xylem vessel precursor cells of developing roots. This has led us to reassess the origin and extent of this premeiotic association, which we had previously ascribed to polyploidy in the close wheat relatives.

RESULTS

Centromere and telomere organisation in roots

Initially using root tissue, we developed a whole mount sectioning method for rice using a vibratome, on which *in situ* hybridization could be reliably performed (see Materials and Methods). Each root section made is approximately 20 µm thick and contains about 2-3 cell layers. Using longitudinal root sections, we examined the morphology of the developing root tissue of diploid cultivated rice (*Oryza sativa*) at early stages (Figure 1). Within such sections, it is possible to identify clearly the developing xylem vessel cells, as the central column of large cells (Figure 1). There is a substantial increase in size of the nuclei of the cells of the developing xylem vessel column compared with the surrounding tissues. This suggests that endoreduplication may be occurring, as has been described previously for the xylem vessel cells in hexaploid wheat (Martinez-Perez et al. 2001). It is apparent that in most root nuclei there is no preferential organization of the centromeres and telomeres and they are both dispersed around the periphery of the nuclei. However, in the xylem vessel cells, the centromeres and telomeres do localise to opposite poles of the nuclei, suggesting that there is a Rabl configuration in these cells (Figs 1c and 1d).

Somatic pairing of centromeres and telomeres

In situ hybridization with the RCS2 sequence and (TTTAGGG)n telomere sequences as probes was used to detect the centromeres and telomeres in the interphase nuclei of the intact root sections in *O. sativa* (Figure 1). The nuclei within the tissue section are all clearly and specifically labelled with the two probes. We next examined 3D confocal stacks such as those shown in Figure 1 to determine whether centromere association occurs in diploid cultivated rice (*O. sativa*, A genome). We counted the number of

centromeric sites in the developing xylem vessel cells. This showed, surprisingly, approximately half the expected number of centromere sites (about 12 sites seen for the 24 centromeres present – Figures 1 and 2; Table I.) and suggested that centromeres are associated in pairs in the xylem vessel cells. In contrast, the number of centromere sites in the surrounding root cells equated to the number of chromosomes (an average of 22 sites visible) (Figures 1 and 2; Table 1). Therefore the centromeres remain largely unassociated in these cells. The association of centromeres in the xylem vessel cells and not in the surrounding cells of the root is similar to that observed previously in the root tissues of hexaploid wheat (Martinez-Perez et al. 2001). The 48 telomeres of *O. sativa* also reduce to less than half the number of sites (15 sites) in the xylem vessel cells, but not in the surrounding cells (32 sites) (Table 1; Figure 2). The association of centromeres and telomeres is unexpected in this diploid species as association of chromosomes had previously only been reported in a polyploid situation. The nucleolus is bigger in the xylem cells and could displace some volume of the nucleus causing a redistribution of centromeres and telomeres and resulting in their apparent "passive" association. Both the *in situ* hybridization and resulting statistical analysis clearly reveal close to half the number of centromere sites with little deviation from this number between the cells analysed. This is consistent with an active process of association rather than a passive one where both the numbers of centromeres and the size of the associated centromere groups would vary markedly between cells analysed.

In order to determine whether these centromere and telomere associations are specific to *O. sativa*, we also examined the developing root of another diploid rice species, *O. punctata* (B genome). This revealed that the 24 centromeres in this species also reduce to approximately half the number (12 sites) in the xylem vessel cells (Table 1; Figure 2). Again, the 48 telomeres also reduce to less than half the number of sites

(15) (Table 1; Figure 2). Thus, the chromosomes are associating by their centromeres and their telomeres in the developing column of xylem vessel cells of this diploid rice species. Therefore, the expression of centromere association is already present in at least two diploid rice species and does not depend on development of polyploidy.

These observations in diploid rice species *O. punctata* and *O. sativa* show similarities between the diploid rice species and hexaploid wheat, but contrast with those of the wheat diploid relative *T. monococcum*, which did not exhibit centromere association in either the xylem vessel cells or the surrounding tissue in the roots (Martinez-Perez et al. 2001). Telomere behaviour in the xylem vessel cells of hexaploid wheat was not assessed so a comparison of telomere behaviour cannot be made with the diploid rice species

Since centromeres and telomeres unexpectedly associate in the diploid rice species, this raises the question of what happens in polyploid rice species. We therefore examined the rice polyploid *Oryza minuta* (BBCC), which possesses 48 centromeres, to determine centromere behaviour. Unfortunately the centromere probe used in this study only detected the 24 B genome centromeres and not the C genome centromeres. Moreover no centromeric signals were detected when *in situ* hybridization was carried out using our centromere RCS2 probe in the rice diploid *O. officinalis* (C genome). We were therefore unable to examine the nature of pairing in the C genome by *in situ* hybridization using this probe.

Despite being only able to label half the centromeres, analysis of the xylem vessel cells of a developing root in this polyploid showed that the 24 centromeres of the B genome were visualised as approximately half the expected number of sites (12) (Table 1), again suggesting association of at least the B genome centromeres.

Furthermore the 96 telomeres also associated reducing to approximately half (47) the number of expected sites (Table 1). The centromeres and telomeres in the surrounding tissue cells did not associate (Table 1). Thus this polyploid rice species showed very similar behaviour to the two diploid rice species described above.

Premeiotic association of centromeres and telomeres

In hexaploid wheat, the centromeres associate premeiotically in the developing anther tissues reducing to approximately half the number of sites (Martinez-Perez et al. 1999). Labelling of early undifferentiated anther tissue isolated from *O. sativa* (AA) also showed that the 24 centromeres also reduce to an average of 12 sites during pre-meiotic floral development (Table 1; Figures 1 and 2). As in the case of the xylem vessel cells in rice, the 48 telomeres also associate at the same time reducing to less than half the number (16). Thus in common with hexaploid wheat, diploid rice associates its centromeres in both the xylem vessel cells and the anthers. Telomere behaviour has not been assessed in early undifferentiated anthers in hexaploid wheat. Thus a direct comparison of telomere behaviour in hexaploid wheat and rice during anthesis cannot be made.

The chromosome association is likely to be homologous

The chromosomes are associated both via their centromeres and telomeres. This observation raises two questions. Are these associations mostly homologous or non-homologous and are the chromosomes associated along their complete length? There is no centromere or telomere specific probe available to ask whether specific homologous

centromeres or telomeres are associating. We therefore tested 30 mapped BACs from chromosome 1, making a probe for each one and testing each individually for hybridization at a single, unique chromosome position. Of the 15 that have a clear, single site, we chose the closest mapped BACs to one of the telomeres and the centromere of the chromosome, and used these as chromosome-unique probes for these chromosomal regions.

In situ hybridization with both these BACs in both the xylem vessel cells and the anther tissue of *O. sativa* clearly shows two sites of hybridization for each BAC (Figure 3). Thus the homologous chromosomes are separated at these sites, and the chromosomes are not associated along their complete length but only at the centromeres and telomeres. However the homologous pairs of sites appeared closer together in the xylem vessel and anther nuclei than in most of the other somatic cells. To quantify whether homologous sites are closer together in those tissues in which centromeres and telomeres are associating, the 3-dimensional distance between the pairs of hybridization sites was compared in the xylem vessel cells and anther cells with cells in which no centromere/telomere association occurred. The statistical analysis confirms that the hybridising sites are in much closer proximity to each other for both BACs located in centromere and telomere regions than in those tissues in which the telomeres and centromeres are not associating (Table 2). The simplest interpretation of this observation is that the centromere and telomere associations are likely to be between homologous chromosomes, and that this brings the intervening parts of the chromosomes closer together, but that there is not close association along the length of the chromosome arms.

DISCUSSION

In hexaploid wheat and its polyploid relatives, centromeres associate premeiotically. On the other hand, there seems to be no premeiotic association of the chromosomes in rye, *Arabidopsis*, the ancient allotetraploid maize and the A and D diploid progenitors of wheat (Bass et al. 1997; Martinez-Perez et al. 2000; Mikhailova et al. 2001; Comai et al. 2003). Moreover there is no general evidence of premeiotic associations of chromosomes reported in the dicots. An artificial polyploid derived from the A and D genome progenitors does exhibit premeiotic centromeres association (Martinez-Perez et al. 2000). We therefore previously ascribed the centromere association in the wheat relatives to the ploidy of the genome, with polyploid species undergoing centromere association and diploid species not doing so.

The present study reveals that rice associates both its centromeres and telomeres premeiotically in anther cells and non-meiotically in xylem vessel precursor cells. This is similar to the situation occurring in wheat and related polyploids. The difference is that in hexaploid wheat, the initial chromosome associations in undifferentiated anthers are mostly not homologous (being either non-homologous or homoeologous) but in the undifferentiated rice anthers are homologous. This difference is likely to reflect the presence of related chromosomes derived from the different genomes in the polyploid situation. The observation means that we need to reconsider premeiotic chromosome association as being polyploid specific. It suggests that the potential to associate chromosomes either via centromeres (and telomeres) both premeiotically and in certain other cell types may be widespread in the cereals.

The cereals (the family Gramineae) are part of the subclass Commelinidae, which includes the rushes and sedges. The evolution of the cereals within the

Gramineae through breakage and fusion events resembles chromosome evolution within the rushes and sedges (Moore et al. 1997). These species have extensively modified their meiosis so that association between homologous chromosomes is retained through meiosis I (Nordenskiold 1962). In fact a recent study suggests that the rushes also show premeiotic association of their chromosomes, which may explain their unusual meiotic behaviour (Haizel et al. 2003). The potential to associate the chromosomes during anther development may therefore have arisen in the ancestor of the cereals and rushes and has been retained in some but not all cereals. This then raises the issue of what is the consequence of its retention in rice and not for example maize? RFLP mapping of the maize genome revealed an ancient tetraploid structure (Weber et al. 1989; Whitkus et al. 1992; Ahn and Tanksley 1993; Moore et al. 1995). However recent sequencing of regions of the maize genome indicates that it has extensively diploidised itself deleting duplicated copies (Ilic et al. 2003). Thus the maize genome is relatively unstable.

In contrast, when the first synteny analyses were undertaken, it was suggested that the genome structure of rice is closer to the genome structure of the ancestor of the cereals than that of the other cereals. Over the last 5 years, more detailed synteny comparisons at the sequence level have been undertaken. They reveal that most rearrangements detected in comparisons between other cereals and rice are found in the other cereal genome rather than in rice (Bennetzen and Ma 2003). This supports the theory that the present-day rice genome is more similar to the ancestral cereal genome than those of the other modern cereals. It also raises the question of why the chromosomes of rice are relatively more stable than those of other cereals. The only other cereal genome which has been reported to be relatively stable is hexaploid wheat, which also has not undergone major rearrangements of its genome following polyploidisation (Moore 2000). When the rice genome was first organised into linkage

segments in the syntenic map with the other cereals, it was known that telomeres defined the ends of some segments (Moore et al. 1995). However it was not known what genomic structures defined the remaining ends (Moore et al. 1997). The mapping of rice centromeres revealed that the linkage segments were in fact defined both by centromere and telomere locations. This suggests that telomeres and centromeres are important in defining the chromosome rearrangements that have taken place during cereal evolution. The stability of the rice genome may therefore be connected with the constraints placed on its chromosomes of pairing premeiotically both at centromeres and telomeres. The loss of these restraints as occurred in maize may relate to its apparent genome “instability”.

Finally the elucidation of the chromosome pairing process in wheat both premeiotically and meiotically has demanded extensive sectioning and 3-D analysis. It is clear that the chromosome pairing in the cereal model rice will not resemble that reported for *Arabidopsis.* Moreover the presence of premeiotic chromosome pairing will complicate the exploitation of the knowledge on meiotic processes derived from other plant systems to underpin rice breeding strategies. However the procedures described in the paper will provide tools for developing a framework on which future rice meiotic studies can be based.

EXPERIMENTAL PROCEDURES

Plant material

Rice lines used in the present study were a cultivated rice (*Oryza sativa*, AA, 2n=24, cv. Bengal) and three wild *Oryza* species: *O. punctata* acc. 105980 (BB), *O. minuta* acc. 101141 (BBCC) and *O. officinalis* acc. 100896 (CC). *O. punctata, O. minuta* and *O. officinalis* lines were kindly provided by Dr. Darshan S. Brar from the Plant Breeding, Genetics and Biochemistry Division in the International Rice Research Institute (IRRI), Metro Manila, Philippines.

Seeds were pre-treated at 37°C for 24 h and then germinated for 3 days at 24°C on moist filter paper. The root-tips were excised and fixed in 4% (w/v) formaldehyde freshly prepared from paraformaldehyde in PEM buffer (50 mM PIPES/KOH pH6.9; 5 mM EGTA; 5 mM MgSO4) for 1 h at R.T. and then washed in TBS (10 mM Tris-HCl, pH7.4; 140 mM NaCl) for 10 min. Sections (20-50μm) were cut from the root tips using a Vibratome Series 1000 (TAAB Laboratories Equipment Ltd., Aldermarston, UK).

Rice plants were grown to flowering in a controlled environment room to. Rice spikes at different developmental stages were fixed for 1 hour at room temperature in 4% (w/v) paraformaldehyde in PEM buffer. To facilitate penetration of the fixative, vacuum infiltration was used. Single spikelets were detached from the spike and sectioned (50-100 μm thick sections) under water using a Vibratome series 1000.

Sectioning and fluorescence in situ hybridization

Vibratome sections from roots or spikes were allowed to dry on multi-well slides (ICN Biomedicals Inc.). The slides were pre-treated by washing in 3% Decon for at least 1 hour, rinsing thoroughly with distilled water and then coated with a freshly prepared solution of 2% (v/v) 3-aminopropyl triethoxy silane (APTES, Sigma) in acetone for 10

seconds and activated with 2,5% (v/v) glutaraldehyde in phosphate buffer for 30 min., rinsed in distilled water and air dried.

The RCS2 centromeric probe (GenBank Accession No. AF058902) was obtained by PCR amplification of total genomic *O. sativa* DNA with a pair of primers (5´-TACTGGAATCAAAAAGTTCAAAAAGAGCCA-3´ and 5´-TCGTGTATGCACTTGGCATTAAATGAC-3´) based on sequences that flank the conserved centromeric region of rice (Dong et al. 1998). Two bacterial artificial chromosomes (BACs) for rice chromosome 1 were also used as probes (Chen et al. 2002). The a0002C21 clone is located in contig 1 of chromosome 1 and mapped approximately between 1 and 7 cM. The a0040G11 clone is located in contig 16 in rice chromosome 1 and mapped approximately at 100 cM (Chen et al. 2002). The centromere of chromosome 1 is mapped to single genetic position at 73.4 cM).

Fluorescence in situ hybridization (FISH) on rice root sections

To improve the permeability step, the tissue sections were first dehydrated in a methanol series of 30%, 50%, 70% and 100%. Immediately after, the tissue sections were treated with the enzyme mix, 1% (w/v) cellulase (Onozuka R-10), 0.5% pectolyase (Kikkoman, Japan) in TBS for 30 min. at R.T., washed in TBS for 10 min., and dehydrated again in a methanol series 30%, 50%, 70% and 100%. The hybridization mixture contained 50% deionised formamide, 20% dextran sulphate, 0.1% sodium dodecyl sulphate, 10% 20×SSC, 200ng of centromeric, telomeric or BAC probe, 1 µg of sonicated salmon sperm as blocking DNA. Probes were labelled with digoxigenin-11-dUTP (Boehringer Mannheim Corp. Indianopolis, IN) or biotin-16-dUTP (Boehringer Mannheim) by nick translation. Denaturation of the hybridization mixture was carried out at 95ºC for 5 min., cooled in ice for another 5 min. and immediately applied to the

sections. Target DNA denaturation was carried out in a modified thermocycler (Omnislide, Hybaid LTD., Long Island, NY) at 78ºC for subsequent hybridization at 37ºC overnight. Post-hybridization washes were carried out using 20% formamide in 0.1SSC at 42ºC. Probes labelled with digoxigenin were detected by an anti-digoxigenin antibody conjugated to FITC (Boehringer Mannheim Corp., Indianopolis, IN) and biotin-labelled probes were detected with extravidin-cy3 (Sigma, Chemical Co.).

Confocal fluorescence microscopy, image acquisition and data analysis.

Confocal optical section stacks were collected using a Leica TCS SP confocal microscope (Leica Microsystems, Heidelberg GMbH, Germany) equipped with a Krypton and an Argon laser. The microscopy data were recorded and then transferred to NIH image (a public domain program for the Macintosh by W. Rasband available via ftp from ftp://Zippy. nimh.nih.gov) and composite using Adobe Photoshop 5.0 (Adobe systems Inc., Mountain View, CA). Final images were printed on a Pictography P3000 printer.

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TABLES

Table 1. Statistics of the numbers of centromere and telomere sites in root and anthers of diploid and polyploid rice. All centromere and telomere sites were counted on the original three-dimensional confocal stacks. The standard deviation (SD) is given in parentheses. The p value indicates the probability of the null hypothesis, that the two means in the somatic cells and the rest of cell types are the same.

Species	Tissue	Cells analysed	Centromeres Average (SD)	p value	Telomeres Average (SD)	p value
O. sativa (AA) 2n = 2x = 24	Non-xylem root cells	80	22.1 (1.7)		32.2(9.0)	
	Xylem vessel	43	12.5(0.8)	p<0.001	14.7(2.9)	p<0.001
	Non-meiotic anther cells	16	12.6(1.3)	p<0.001	16.5(2.4)	p<0.001
O. punctata (BB) 2n = 2x = 24	Non-xylem root cells	47	22.9(1.1)		33.2(8.2)	
	Xylem vessel	24	12.1(1.5)	p<0.001	15.1(2.9)	p<0.001
O. minuta (BBCC) 2n = 4x = 48	Non-xylem root cells	74	23.3(0.8)		47.2(2.2)	
	Xylem vessel	24	12.9(0.8)	p<0.001	23.9(2.2)	p<0.001

Table 2. Distance between two BAC signals (μm) in root and anther tissues of *O. sativa*. The standard deviation (SD) is given in parentheses. The p value indicates the probability of the null hypothesis, that the distances between two BACs signals in the somatic cells and in the rest of the cell types are the same.

	Cells analysed	40G11 average (SD)	p value	2C21 average (SD)	p value
Non-xylem root cells	47	5.1 (0.3)		5.1 (1.1)	
Xylem vessel	22	2.1(0.5)	p<0.001	2.1 (0.3)	p=0.01
Non-meiotic anther cells	62	2.0 (0.5)	p<0.001	2.3 (0.4)	p<0.001

FIGURE LEGENDS

Figure 1. DAPI and fluorescence *in situ* confocal images of root sections of *Oryza sativa*.

(a) Projection of three adjacent optical sections of the root showing a whole central column of vessel cells. The focal step size between sections was 4 μm. Chromatin was counterstained with DAPI. (b) Fluorescence *in situ* hybridization in the cells inset with centromere (green) probe. The image is a single confocal optical section to show the different distribution of centromeres between xylem vessel and non-xylem cells in the root. A vessel cell (1) and a non-xylem cell (2) are arrowed. (c) 3-D rotation of the vessel cell (arrow 1 in b) exhibiting around 12 centromeres grouped in one side of the cell nucleus. (d) 3-D rotation of the same vessel cell in c, showing telomeres (red) distributed in the opposite side of the centromeres. (e) 3-D rotation of a non-xylem root cell (arrow 2 in b) exhibiting around 24 centromeres dispersed in the entire cell nucleus. (f) 3-D rotation of the same non-xylem root cell in e, showing that telomeres are distributed around the entire cell. The 3-D rotations (0,45,135 and 180 degrees) are generated from the projections of 8-10 adjacent optical sections with a 0.6-0.8 μm focal step size between sections.Bar scale represented 20 μm in a, 10 μm in b and 5 μm in the rest of the panels.

Figure 2. Centromere and telomere behaviour on diploid rice.

All images are projections from confocal sections spaced 0.5 μm. The projections have been produced to show the centromeres (green) and the telomeres (red) in root cells and in floral tissue at early developmental stages. The number of centromeres is haploid in vessel cells and in somatic cells in anthers. Not all the centromeres or telomeres are

visible in these projections and some of them can be very close or superposed because of the superposition of the images.

Bar scale = 5μm.

Figure 3 Labelling of BACs in *Oryza Sativa.*

(a) Fluorescence *in situ* confocal images of two bacterial artificial chromosomes (BACs) for rice chromosome 1 in root sections and in floral tissue at early developmental stages of diploid rice. Bar scale = 5μm.

(b) Location of the a0040G11 clone and the a0002C21 clone at the chromosome 1 of rice. The centromere of chromosome 1 is shown in yellow (adapted from Chen *et al*., 2002).

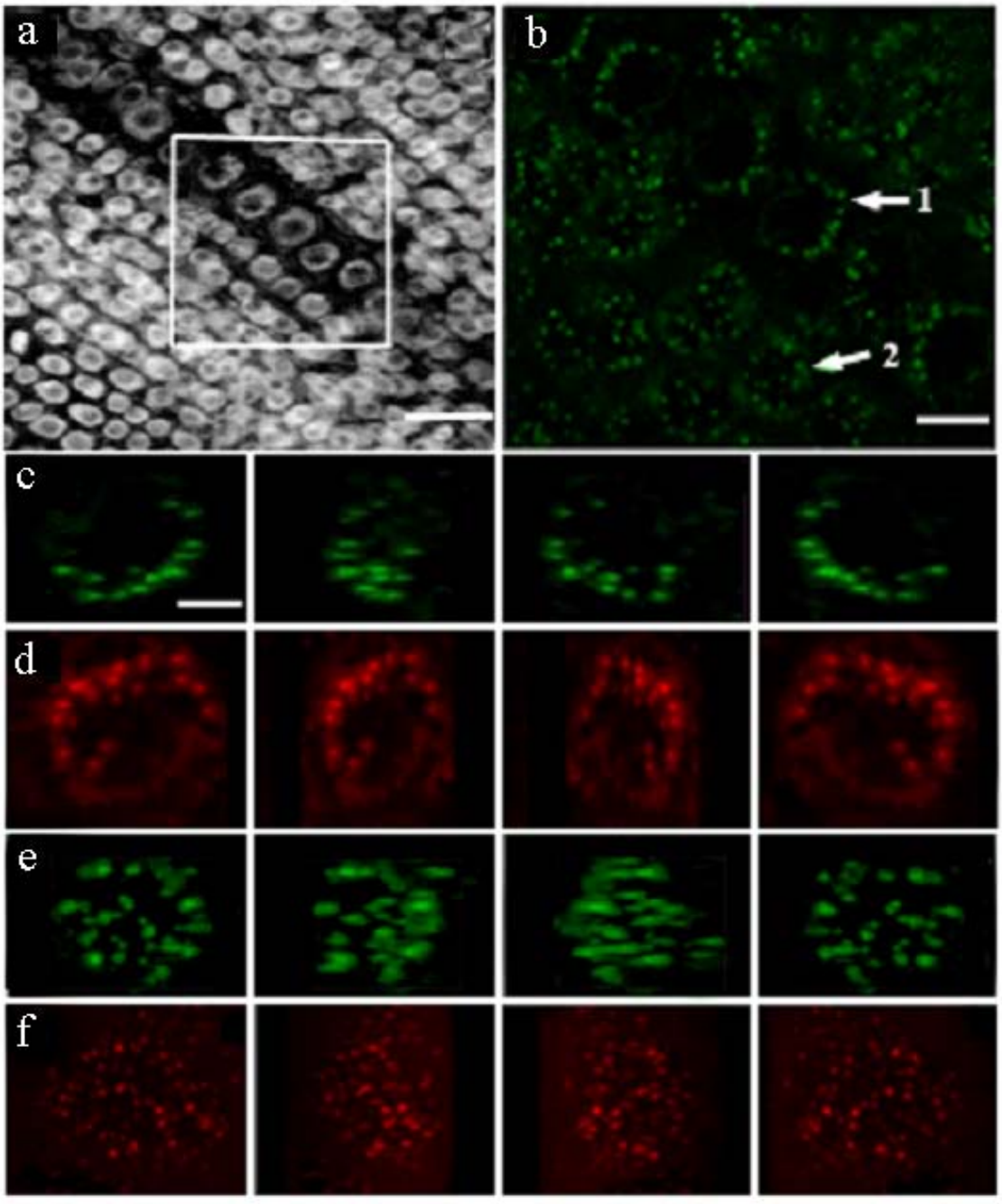

Figure 1

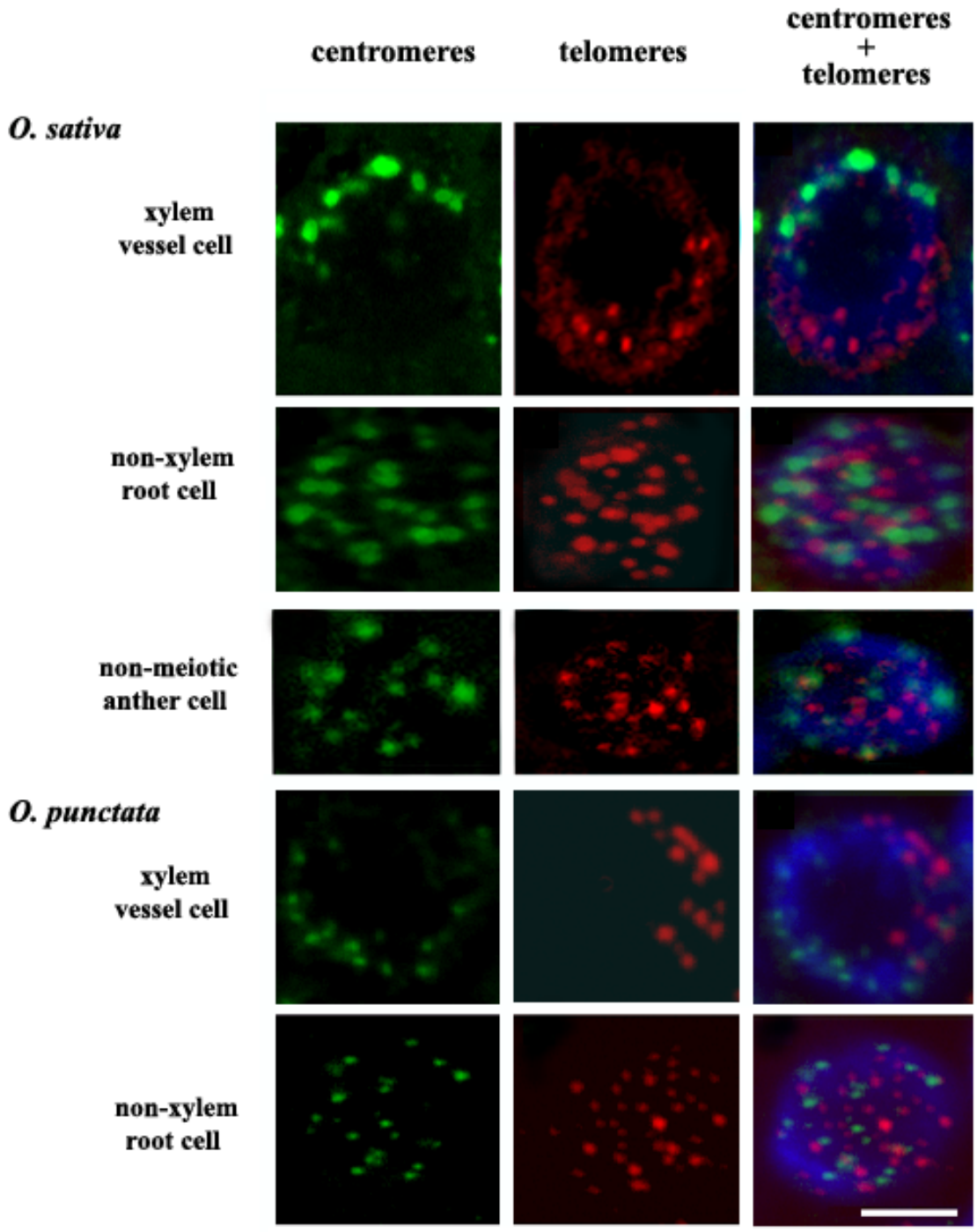
centromeres
telomeres
centromeres + telomeres
O. sativa
xylem vessel cell
non-xylem root cell
non-meiotic anther cell
O. punctata
xylem vessel cell
non-xylem root cell

Figure 2

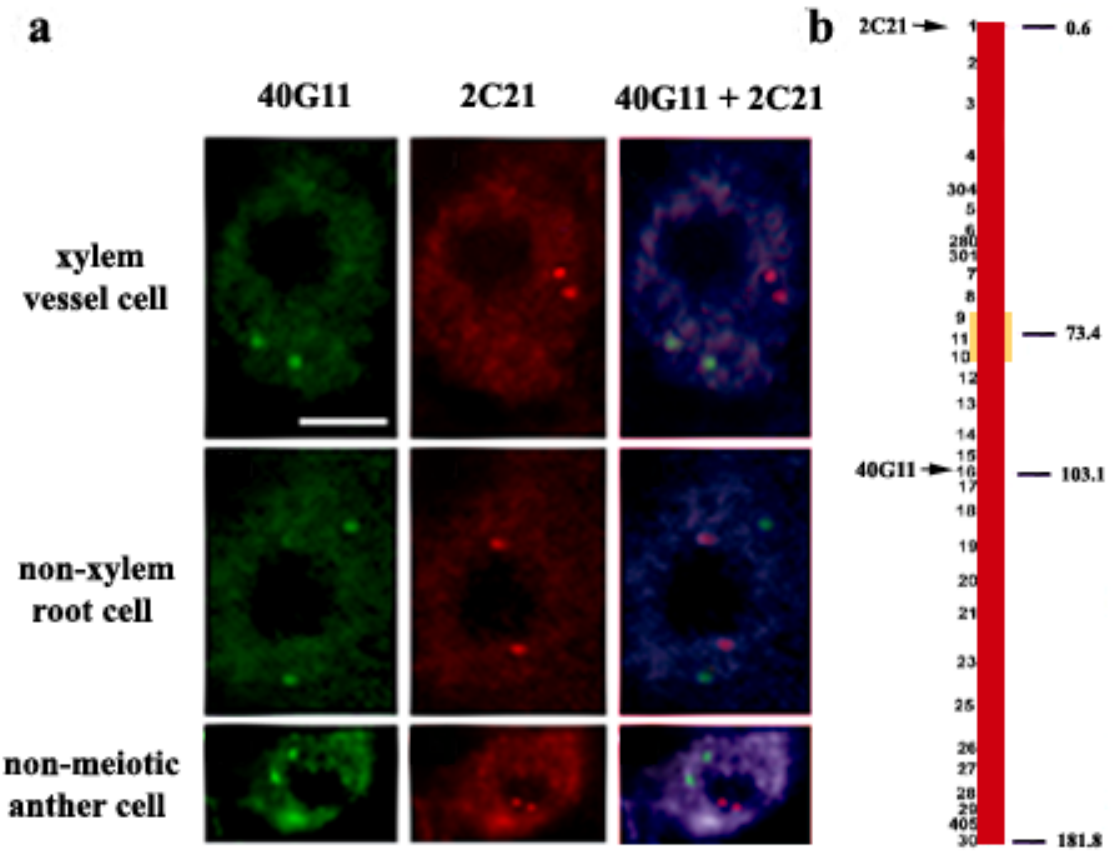
a
40G11
2C21
40G11 + 2C21
xylem vessel cell
non-xylem root cell
non-meiotic anther cell
b
2C21
40G11
0.6
73.4
103.1
181.8

Figure 3