Comparative chromosomal localization of 45S and 5S rDNAs in 76 purple-fleshed sweet potato cultivars

Dan Su (1353139269@qq.com)
Jiangsu Normal University https://orcid.org/0000-0003-1671-5644
Lei Chen
Jiangsu Normal University
Jianying Sun
Jiangsu Normal University
Luyue Zhang
Jiangsu Normal University
Runfei Gao
Jiangsu Xuhuai Regional Xuzhou Institute of Agricultural Sciences
Qiang Li
Jiangsu Xuhuai Regional Xuzhou Institute of agricultural Sciences
Yonghua Han
Jiangsu Normal University
Zongyun Li
Jiangsu Normal University

Research

Keywords: purple-fleshed sweet potato, fluorescence in situ hybridization, rDNA

Posted Date: May 12th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27377/v1

Abstract

Background: In recent years, purple-fleshed sweet potato has been paid more and more attention because of its high nutritional value. However, the current studies on purple-fleshed sweet potato were still focused on the research and production of the related products. The research on its cytogenetics is relatively lagging behind, which cannot satisfy the study of genetic diversity of purple-fleshed sweet potato. Therefore, we carried out cytogenetic analysis on 76 purple-fleshed sweet potato cultivars, aim to analyze the chromosome structure and distribution of 45S rDNA and 5S rDNA in 76 purple-fleshed sweet potato cultivars.

Results: We have found that only 62 purple-fleshed sweet potato cultivars with 90 chromosomes, and the others were aneuploid with 88, 89, 91, 92 chromosomes. The number of 45S rDNA in 76 purple-fleshed sweet potato cultivars varies from 16 to 21, with different signal sizes and intendities, and localized at the terminal or satellite of chromosomes. The number of 5S rDNA were relatively stable, 74 of the varieties investigated contained 6 sites, located at the terminal of chromosomes and near centromere. Only the Quanzishu 96 has 7 5S rDNA sites, and Yuzixiang 10 has 5 5S rDNA sites. In addition, rDNA analysis was also performed on two parents of Quanzishu 96. Both the two parents had 18 45S rDNA sites and 6 5S rDNA sites, which were different from the results of Quanzishu 96.

Conclusions: For hexaploid sweet potato cultivars, there is genetic instability between purple-fleshed sweet potato cultivars. The 45S rDNA sites showed numerical variation, whereas conserved number of 5S rDNA sites were observed.

Background

Sweet potato [*Ipomoea batatas* (L.) Lam., 2n=6x=90], belonging to the genus of *Ipomoea* in Convolvulaceae, is the seventh most important food crop in the world. It has been widely distributed from the tropical, subtropical, and warm temperate regions. China is the largest producer with an annual output of about 72 million tons, accounting for 63.84% of the total production in the world [1].

As a special type of sweet potato, purple-fleshed sweet potato has gradually developed into cash crops and medicinal crops besides being a food crop [2]. It is rich in vitamins, dietary fiber, anthocyanins, and carotenoids [3-5]. The identification of the free radical-scavenging activity of these functional components has attracted the interest of health-conscious consumers, which is related to slowing the aging process and preventing chronic degenerative diseases such as cancer [6-8].

At present, the research on purple-fleshed sweet potato is mainly focused on the extraction and purification of anthocyanin pigment from purple potato, the physiological health function and the production of related products, and has made some progress [9-11]. However, due to its large number of extremely small chromosomes and the thick cytoplasm that difficult to remove, the research on cytogenetics is still weak, there are only a few related reports, which cannot satisfy the development of high-quality purple-fleshed sweet potato. Therefore, the cytogenetic analysis of purple potato is particularly urgent [12].

Fluorescence *in situ* hybridization (FISH) is a relatively mature molecular cytogenetic technique that focuses on the study of phylogeny at the chromosomal level. It could analyze the position, qualitative, and relative quantitative analysis of target sequences by combining labeled nucleic acid probes with chromosomes, interphase nucleus or DNA fibers. It has been widely used for the identification of specific chromosome regions, analyzing the composition, spatial location and dynamic changes of chromatin in the cell cycles [13-15]. In addition, it has been widely used in the research of the physical map of genome, the clarification of the structure and evolution of the genome, and analyzing the relationship between species [16-21].

Ribosomal RNA genes (rDNA) are multi-copy sequences of tandem repeats that are highly repetitive and conserved in species and are often used in comparative studies to elucidate homology between species [22]. The rDNA of higher plants containing 45S rDNA and 5S rDNA, which are independent transcription units and mostly distributed on different chromosomes [23-24]. The 45S rDNA is located in the nucleolar organizer region and consists of tandem repetitive units of the 18S-5.8S-28S rRNA genes and non-transcribed spacer regions [25-26]. 5S rDNA contains a highly conserved coding region of 120 bp and a non-transcribed spacer (NTS region that varies between 100 and 900 bp). As relatively stable and reliable molecular cytogenetic markers, the characteristics of 45S and 5S rDNA on chromosomes provide useful information for chromosome research [27-29]. The FISH positions of rDNAs on chromosomes directly provide an effective method for chromosome identification and the research of chromosome organization and evolution. And it is an effective means to distinguish and identify plant chromosomes, especially tiny chromosomes [30-32]. In

the past few decades, the distribution and organization of 5S and 45S rDNA of hexaploid *Ipomoea batatas* by using FISH only found in a few studies, and they detected different 45S rDNA sites and 6 5S rDNA sites [33-36].

In previous studies, genetic diversity of 76 purple-fleshed sweet potato cultivars has been analyzed from morphological, quality and molecular level, so as to clarify the differences among different purple-fleshed sweet potato varieties and provide guidance for the improvement, development and utilization of parents selection and breeding varieties of purple sweet potato varieties [37]. In our study, we conducted FISH experiments with 45S and 5S rDNA probes on metaphase spreads of 76 purple-fleshed sweet potato cultivars to investigate their numbers and physical positions. The analysis of its chromosome structure and distribution of 45S rDNA and 5S rDNA are of great significance for further understanding of chromosomal relationships, and which could also provide a basic cytological basis for the study of related germplasm resources of sweet potato.

Results

Seventy-six purple-fleshed sweet potato cultivars were analyzed in this study. Double-color FISH was used to investigate the distributions and site numbers of 45S rDNA and 5S rDNA. The patterns with well-spread chromosomes and distinguishable FISH signals were used for the analysis. At least 20 metaphase or prephase spreads were studied to generate the FISH results for each variety. The number and intensity of rDNA sites of 76 varieties were summarized in Table 1. Chromosomal positions, the number of 45S and 5S rDNA sites of some varieties were presented in Figure 1-4.

Chromosome number of 76 purple-fleshed sweet potato cultivars

In the 76 purple-fleshed sweet potato cultivars, the chromosome number of 62 varieties was 90. In the other 14 varieties, the chromosome number of 4 varieties was 88, 5 varieties were 89, 3 varieties were 91 and 2 varieties were 92 (Table 1).

Distribution and number of 45S rDNA sites

In the 76 purple-fleshed sweet potato cultivars, the number of 45S rDNA showed differences between different varieties. 43 varieties showed 18 45S rDNA sites (The results of 30 varieties are shown in Figure 1), 18 varieties showed 20 45S rDNA sites (12 are shown in Figure 2), 7 varieties showed 16 45S rDNA hybridization signals (6 are shown in Figure 3, c1-c6), 2 varieties showed 17 45S rDNA sites (Figure 3, d1-d2), 3 varieties showed 19 45S rDNA sites (Figure 3, e1-e3), and 21 45S rDNA sites was detected in 3 varieties (Figure 3, f1-f3). The results of the other 20 varieties were shown in Figure 4. The signal was mainly located at the terminal and satellite of chromosomes. The signal intensity of different varieties showed significant differences, and the signal intensity of different chromosomes of the same variety also has great differences (Table 1, Figure 1-4).

Table 1 Summary of rDNA results of 76 purple-fleshed sweet potato varieties

No.	Varieties	Chromosome number (2n)	No. of 45S rDNA	No. of 5S rDNA	No.	Varieties	Chromosome number (2n)	No. of 45S rDNA	No. of 5S rDNA
1	Quzishu 57	90	18(1S,12M,5W)	6	39	Chuanzishu 4	90	18(1S,11M,6W)	6
2	Neiyuzi 2	92	18 (6S, 8M, 4W)	6	40	Fushu 24	90	20(4S,8M,8W)	6
3	Qinzishu 2	90	18(3S,11M,4W)	6	41	Guijingshu 5	90	18(3S,11M,4W)	6
4	Longzishu 4	90	18(6S,10M,2W)	6	42	Chuanzishu 2	88	18(2S,12M,4W)	6
5	Longzishu 6	90	18(3S,13M,2W)	6	43	Guijingshu 9	90	18(4S,14M)	6
6	Zhezishu 1	90	18(4S,12M,2W)	6	44	Fangzishu 9	90	20(2S,16M,2W)	6
7	Qinzishu 3	90	18(3S,13M,2W)	6	45	Shangxuzi 1	90	20(3S,11M,6W)	6
8	Ningzishu 3	90	18(2S,6M,10W)	6	46	Jixushu 2	90	16(4S,9M,3W)	6
9	Jihei 2	90	18(1S,13M,4W)	6	47	Yuzixiang 10	90	18(4S,6M,8W)	5
10	Longjinshu 1	90	18(2S,9M,7W)	6	48	Xuzishu 3	90	18(2S,10M,6W)	6
11	Fushu 1	90	18(2S,13M,3W)	6	49	Yusuzi 43	90	16(1S,11M,4W)	6
12	Taizhong 11	90	18(1S,12M,5W)	6	50	Yuzishu 7	90	20(4S,14M,4W)	6
13	Yanzishu 3	89	18(1S,13M,4W)	6	51	Pengzishu 3	90	20(1S,11M,7W)	6
14	Jizishu 1	90	18(3S,12M,3W)	6	52	Yuzishu 3	90	18(2S,8M,8W)	6
15	Wan W-36-1	90	20(16M,4W)	6	53	Yuzi 263	88	18(2S,12M,4W)	6
16	Longjinshu 3	90	18(2S,14M,2W)	6	54	Xuzishu 5	90	18(2S,14M,2W)	6
17	Zhanzishu 2	90	20(3S,14M,3W)	6	55	Guijingshu 6	90	16(1S,14M,1W)	6
18	Jizishu 2	90	18(2S,14M,2W)	6	56	Guijingshu 7	90	18(1S,10M,7W)	6
19	Yanzishu 2	90	19(2S,12M,5W)	6	57	Guijingshu 3	90	18(3S,7M,8W)	6
20	Ningzishu 4	90	21(4S,12M,5W)	6	58	Nanzishu 008	91	18(2S,10M,6W)	6
21	Ningzishu 1	88	18(4S,10M,4W)	6	59	Nanzishu 014	90	16(1S,13M,2W)	6
22	Xuzishu 2	88	18(1S,13M,4W)	6	60	Nanzishu 015	90	16(1S,14M,1W)	6
23	Ningzishu 2	90	16(2S,10M,4W)	6	61	Qianzishu 1	91	17(1S,13M,3W)	6
24	Ningzishu	89	18(2S,11M,5W)	6	62	Funingzi 3	90	21(1S,18M,2W)	6

	5								
25	Luozishu 1	90	18(1S,11M,6W)	6	63	Funingzi 4	90	20(4S,11M,5W)	6
26	Xuzishu 8	90	18 (6S, 8M, 4W)	6	64	Quanzishu 96	90	18(2S,12M,4W)	7
27	Xuzishu 6	90	20(1S,12M,7W)	6	65	Puzishu 3	90	19(4S,8M,7W)	6
28	Yanzishu 4	90	18(4S,12M,2W)	6	66	Puzishu 18	89	18(2S,13M,3W)	6
29	Jizishu 1	90	18(3S,9M,6W)	6	67	Xushu33	91	18(1S,13M,4W)	6
30	Guizishu 1	90	16(3S,9M,4W)	6	68	Jizishu 3	90	18(2S,12M,4W)	6
31	Mianzishu 9	90	20(1S,16M,3W)	6	69	Guangzishu 9	90	20(2S,12M,6W)	6
32	Mianyuzi 11	90	20(4S,12M,4W)	6	70	Guangzishu 10	90	18(1S,14M,3W)	6
33	Wanzi 56	90	18(2S,13M,4W)	6	71	Guangzishu 11	89	19(2S,11M,5W)	6
34	Ezishu 13	90	20(6S,11M,3W)	6	72	Guangzishu 1	90	20(2S,15M,3W)	6
35	Ezishu 12	89	17(2S,12M,3W)	6	73	Guangzishu 2	90	20(2S,16M,2W)	6
36	Guizishu 3	90	20(2S,15M,3W)	6	74	Guangzishu 8	90	20(2S,13M,5W)	6
37	Guiziwei 1	90	18(5S,12M,1W)	6	75	Jizishu 18	90	21(1S,17M,3W)	6
38	Fuzishu 404	92	20(2S,12M,6W)	6	76	Aya	90	18(2S,14M,2W)	6

Note: S: Strong signal; M: Medium signal; W: Weak signal

Distribution and number of 5S rDNA sites

Compared with 45S rDNA, the number of 5S rDNA showed conversation with 2 exceptions. 74 varieties in our study showed 6 5S rDNA sites (Figure 1-4, Table 1), 7 sites of 5S rDNA was detected in Quanzishu 96 (Figure 1, a26) and 5 in Yuzixiang 10 (Figure 1, a19). 5S rDNA is mainly located at the sub-terminal of chromosomes and near centromere. The 5S rDNA site size and intensity were different between varieties and within individual varieties.

Colocalization of 45S and 5S rDNA sites

Colocalization of 45S and 5S rDNA sites was investigated based on the FISH signals in 20 varieties. There were only two varieties showed colocalization of 45S and 5S rDNA sites in the same chromosome (Figure 3, d1, f1, arrows).

Distribution and number of 45S rDNA and 5S rDNA sites in two parents of Quanzishu 96

Based on the presence of 7 5S rDNA sites in Quanzishu 96, a double-color FISH mapping of 45S rDNA and 5S rDNA was also carried out for its two parent materials (Longshu 9 and Quanshu 10). However, Longshu 9 and Quanshu 10 showed 18 45S rDNA and 6 5S rDNA hybridization signals, hadn't found the same phenomenon as Quanzishu 96 (unshown).

Discussion

rDNA is highly repetitive and conserved in species. For plant species with a large number of chromosomes and small size, the appearance of rDNA probe provides a cytological approach for studying their genetic relationship. The distribution pattern of rDNA in

different species is generally different. Observing the difference in the number and distribution position of rDNA sites can further analyze the chromosomal behavior of inter-genus species, which is a relatively reliable and stable molecular cytogenetic marker.

In this study, we carried out a comprehensive statistical analysis of the chromosome numbers and distribution patterns of 45S rDNA and 5S rDNA in all purple-fleshed sweet potato cultivars. We have found that only 62 varieties with 90 chromosomes, and the others were aneuploid with 88, 89, 91, 92 chromosomes. Wu et al. [38] implemented a statistical test to detect 6× +1 and 6× -2 aneuploidy in sweet potato cultivars based on read depth, indicating that the discovery of aneuploidy may present an extreme form of structural variation that clearly would affect transcript dosage and consequently, phenotypic variation. Analyses in potato also revealed extensive structural variation, including presence/absence variation of sequences up to 575 kb in length that impacts transcript dosage [39-41].

For hexaploid sweet potato cultivars, the number of 45S rDNA was variable. In the previous studies, researchers found the number of 45S rDNA varied from 12 to 22 and the number of 5S rDNA is 6. Over the past few decades, researchers have found that the intrarespecific variation in the number and intensity of 45S rDNA signals are common, and the distribution patterns of rDNA sites often vary between closely related species, the characteristics of highly variable and unstable have been found and confirmed in many species [42-45]. Compared with previous studies, our results confirmed the high variability of 45S rDNA in varieties, ranging from 16 to 21. Excluding the influence of objective factors, the intraspecific variation in the number and location of rDNA sites may be attributed to three mechanisms: the first is the unequal crossing over and transposition event. Due to the transposon activity, the intra-genome migration of rRNA genes has been widely reported in seed plants, and it is speculated that this is one of the main factors driving the evolution of rDNA sites. Schubert et al. [46] found that the entire 45S rDNA repeat sequence in the chromosomes of Allium and its subgenus can be freely transferred from one site to another, indicating that 45S rDNA may move as a transposable element. The second is the occurrence of chromosomal structure fracture and rearrangement. In recent years, the view that the 45S rDNA region is a fragile site (brittle site) prone to chromosomal damage has been confirmed in several species [47]. The increase in the number of 45S rDNA sites above the critical threshold also increases the possibility of chromosomal breakage in the ribosome DNA region [48]. Thomas et al. [49] found that 45S rDNA cleavage in rye grass led to the rearrangement of chromosome structure, and the position and number of 45S rDNA sites were different. The third is the change of different degree in the process of polyploidy [50-52]. Srisuwan et al. considered that the variation in the number of 45S rDNA in hexaploid *I. batatas* might be due to the fact that the hexaploid genome of *batatas* is always in the process of diploidization, and the genome is unstable, which leads to the interspecific and intraspecific variation in the number of 45S rDNA. In addition, 45S rDNA is mainly located at the terminal and satellite of chromosomes, which may also contribute to the variable number of 45S rDNA. As 45S rDNA often falls from the terminal to the satellite, which may be more prone to unequal crossing over or ectopic recombination. Mantovani et al. [53] also considered that a large number of hybridization events during cultivation may also be the cause of rDNA site changes.

In previous studies, the number of 5S rDNA sites in cultivated sweet potato was relatively stable, and 6 5S rDNA sites were detected. In this study, among the 76 purple-fleshed sweet potato varieties, the number of 5S rDNA was 6 in 74 varieties, and the other two were different, 7 sites of 5S rDNA was detected in Quanzishu 96 (Figure 1, a26) and 5 in Yuzixiang 10 (Figure 1, a19). However, Longshu 9 and Quanshu 10 showed 18 45S rDNA and 6 5S rDNA hybridization signals, which was the parent materials of Quanzishu 96, hadn't found the same phenomenon. Some studies have suggested that the increase of the number of 5S rDNA may be caused by the amplification of the covert rDNA copy number during the crossing and transposable events or due to the translocation of rDNA gene to chromosomes without rDNA sites, and the loss may be caused by other DNA sequence fusion [54-55].

The signal size and intensity of 45S rDNA and 5S rDNA are different in different varieties or the same variety, the intensity of the signal is positively related to the copy number, and the weak signal may mean that the copy number is relatively low. Events such as amplification, deletion and unequal crossing over can also affect the copy number and result in signal differences.

In general, the evolution of 45S rDNA and 5S rDNA is independent and they tend to be distributed on different chromosomes due to physical distance [56]. Roa and Guerra [57-58] believe that 5S-45S colocalization exists in at least one species of each genus, although the probability of colocalization is relatively low, which has been reported in the *Hordeum*, *Cucumis* and *Brassica* [59-60]. Among the varieties we studied, there was an adjacent situation of 5s-45s in two varieties, and in the study of Sun, there was a colocalization phenomenon in three cultivars of sweet potato, which may be the result of the interaction between the characteristics of rDNA instability and some other unknown factors.

Conclusion

In the present study, rDNA-FISH was used to study the molecular cytogenetics of 76 purple-fleshed sweet potato cultivars. For hexaploid sweet potato cultivars, there is genetic instability between purple-fleshed sweet potato cultivars. The 45S rDNA sites showed numerical variation, whereas conserved number of 5S rDNA sites were observed. It provides direct cytological information for the identification of sweet potato chromosomes, and also provides a cytological basis for the development of high quality purple-fleshed sweet potato germplasm resources.

Methods

Plant materials

76 purple-fleshed sweet potato cultivars analyzed in this study were collected from the Institute of Sweet potato, Chinese Academy of Agricultural Sciences. The test varieties Longshu 9 and Quanshu 10 (the parent varieties of Quanzishu 96) were provided by the Institute of Agricultural Sciences of Quanzhou.

Preparation of chromosome spreads

The procedure of mitotic chromosome preparation was basically the same as published protocols [61] with minor modifications. The root tips were induced by cutting away the tip of the main root. Root tips of about 1-2 cm were pretreated in 0.002M 8-hydroxyquinoline at darkroom temperature for 2-4 h to collect metaphase dividing cells, then fixed in Carnoy's fixative solution (ethanol/acetic acid, 3:1, v/v) for at least 1 day. Before enzymatic hydrolysis, deionized water was used to thoroughly clean the root tip, then root tips were digested in enzyme mixtures (2% cellulase and 1% pectinase) for 2.5 h at 37°C in a water bath. The enzyme solution was replaced by deionized water for 30 min. The slides were obtained using a "flame-dried" method, root tips were transferred on to a slide, mashed, and flame dried.

Probe preparation

The 5S rDNA oligonucleotide probes 5S-1 and 5S-2 were the 1-59 and 60-118 base sequences of the 5S rRNA coding region in *Arabidopsis thaliana* (L) Heynhold, respectively (Table 2). The 45S rDNA oligonucleotide probes 45S-1, 45S-2 and 45S-3 were derived from partial sequences of the 5.8S, 18S and 25S rRNA coding regions of *Arabidopsis thaliana*, respectively. 5S-1 and 5S-2 were labeled with 6-carboxyl-tetramethyl rhodamine (TAMRA) at the 5'-terminal and then mixed together as a 5S rDNA probe. 45S-1, 45S-2 and 45S-3 were labeled with 6-carboxyl fluorescein (6-FAM) at the 5'-terminal and mixed together as a 45S rDNA probe. These oligonucleotide probes were synthesized by Sangon bioengineering (Shanghai) co., LTD.

Table 2 Name, sequence, and sources of oligonucleotide probes for fluorescence in situ hybridization (FISH) analysis

Probe Name	Sequence and Fluorochrome Label	Sequences Used to Develop Probes (GenBank Accession Number)
5S-1	TAMRA-5´ggatgcgatcataccagcact aatgcaccggatcccatcagaactccgc agttaagcgt3´	1–59 bases in coding region of 5S rRNA from *Arabidopsis thaliana* (GenBank AJ307346.2)
5S-2	TAMRA-5´gcttgggcgagagtagtacta ggatgggtgacctctcgggaaatcctcg tgttgcatc3´	60–118 bases in coding region of 5S rRNA from *Arabidopsis thaliana* (GenBank AJ307346.2)
45S-1	6-FAM -5´AAAACGACTCTCGGCAACGGA TATCTCGGCTCTCGCATCGATGAAGAACG TAGCGAAAT3´	coding region of 5.8S rRNA from *Arabidopsis thaliana* (GenBank NR141643.1)
45S-2	6-FAM -5´TACCTGGTTGATCCTGCCAGTA GTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTG3	coding region of 18S rRNA from *Arabidopsis thaliana* (GenBank NR141642.1)
45S-3	6-FAM-5´CCCGCTGAGTTTAAGCATATCAA TAAGCGGAGGAAAAGAAACTAACAAGGATTCCCTTA3´	coding region of 25S rRNA from *Arabidopsis thaliana* (GenBank X52320.1)

Fluorescence *in situ* hybridization and signal detection

Fluorescence *in situ* hybridization was performed according to the method of Jiang et al. [62] and slightly modified. The hybridization solution is as follows: deionized formamide 10 μL, 50% dextran sulphate 4 μL, 20×SSC 2 μL, salmon sperm DNA 2 μL(40 ng), each probe DNA 1 μL(40 ng), respectively. The slides were baked at 65℃ for 45 min, cooled and denatured in 70% deionized formamide at 85℃ for 2.5 min, then dehydrated in 70%, 90% and 100% alcohol for 5 min at -20℃, respectively, and dried in air. The hybridization solution was applied to the pretreated chromosome slide at 20 μL per tablet, and incubated at 37℃ overnight.

The hybridized slides were eluted with 2×SSC and 1×TNT and carried out on a shaker. The 45S and 5S signals were detected using anti-biotin AlexaFluor488 and rhodamine-conjugated anti-digoxigenin antibody (Roche), respectively. The chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in VectaShield antifade solution. The photographs were taken by Leica dmra 2 fluorescence microscope or Olympus BX63 microscope, and Adobe Photoshop 5.0 was used for image contrast.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the data generated in the present research is contained in this manuscript.

Competing interests

The authors declare that they have no competing interests.

Fundings

This study was supported by the National Natural Science Foundation of China (31771367), China Agriculture Research System (Grant No. CARS-10-B03), and the Colleges and Universities in Jiangsu Province plans to graduate research and innovation (KYCX18-2137), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Author's contributions

Conception and design of the research: YH, ZL; performe experiments: DS, LC, JS, LZ, RG; statistical analysis: DS, JS; drafting the manuscript: DS; revision of manuscript for important intellectual content: JS, QL, YH, ZL. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Food and Agriculture Organization of the United Nations. 2017; FAOSTAT Statistics Database. Available from http://www.fao.org/faostat/.
2. Xie FL, Burklew CE, Yang YF, Liu M, Xiao P, Zhang BH, et al. De novo sequencing and a comprehensive analysis of purple sweet potato (*Impomoea batatas*) transcriptome. Planta. 2012;236(1):101-113.
3. Katayama K, Kitahara K, Sakai T, Kai Y, Yoshinaga M. Resistant and digestible starch contents in sweet potato cultivars and lines. Journal of Applied Glycoscience. 2011;58(2):53-59.
4. He W, Zeng MM, Chen J, Jiao YZ, Niu FX, Tao GJ, et al. Identification and quantitation of anthocyanins in purple-fleshed sweet potatoes cultivated in China by UPLC-PDA and UPLC-QTOF-MS/MS. Journal of agricultural and food chemistry. 2016;64(1):171-177.
5. Zhao HY, Zhang SS, Wang FB, Zhao N, He SZ, Liu QC, et al. Comparative transcriptome analysis of purple-fleshed sweet potato provides insights into the molecular mechanism of anthocyanin biosynthesis. Frontiers of Agricultural Science and Engineering. 2018;5(2):214-225.
6. Yoshimoto M, Okuno S, Yamaguchi M, Yamakawa O. Antimutagenicity of deacylated anthocyanins in purple-fleshed sweetpotato. Bioscience, biotechnology, and biochemistry. 2001;65(7):1652-1655.
7. Chen PN, Chu SC, Chiou HL, Chiang CL, Yang SF, Hsieh YS. Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis in vitro and suppress tumor growth in vivo. Nutrition and cancer. 2005;53(2):232-243.
8. Luceri C, Giovannelli L, Pitozzi V, Toti S, Castagnini C, Routaboul JM, et al. Liver and colon DNA oxidative damage and gene expression profiles of rats fed Arabidopsis thaliana mutant seeds containing contrasted flavonoids. Food & chemical toxicology. 2008;46(4):1213-1220.
9. Terahara N, Shimizu T, Kato Y, Nakamura M, Maitani T, Yamaguchi M, et al. Six diacylated anthocyanins from the storage roots of purple sweet potato, *Ipomoea batatas*. Bioscience, biotechnology, and biochemistry. 1999;63(8):1420-1424.
10. Terahara N, Konczak-Islam I, Nakatani M, Yamakawa O, Goda Y, Honda T. Anthocyanins in callus induced from purple storage root of *Ipomoea batatas*. Phytochemistry. 2000;54(8):919-922.
11. Tian QG, Konczak I, Schwartz SJ. Probing anthocyanin profiles in purple sweet potato cell line (*Ipomoea batatas* Cv. Ayamurasaki) by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. Journal of Agricultural and Food Chemistry. 2005;53(16):6503-6509.
12. Magoon ML, Krishnan R, Bai KV. Cytological Evidence on the Origin of Sweet Potato. Theoretical and Applied Genetics. 1970;40(8):360-366.
13. Zhang ZT, Yang SQ, Li ZA, Zhang YX, Wang YZ, Cheng CY, et al. Comparative chromosomal localization of 45S and 5S rDNAs and implications for genome evolution in *Cucumis*. Genome. 2016;59(7):449-457.
14. Li JH, Webster MA, Wright J, Cocker JM, Smith MC, Badakshi F, et al. Integration of genetic and physical maps of the Primula vulgaris S locus and localization by chromosome in situ hybridization. New Phytologist. 2015;208(1):137-148.

15. Sun JY, Zhang ZH, Zong X, Huang SW, Li ZY, Han YH. A high-resolution cucumber cytogenetic map integrated with the genome assembly. BMC Genomics. 2013;14(1):461.
16. Han YH, Zhang ZH, Liu CX, Liu JH, Huang SW, Jiang JM, et al. Centromere repositioning in cucurbit species: implication of the genomic impact from centromere activation and inactivation. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(35):14937-14941.
17. Heslop-Harrison JS, Schwarzacher T. Organization of the plant genome in chromosomes. Plant Journal. 2011;66(1):18-33.
18. Winterfeld G, Wolk A, Roser M. Genome evolution in alpine oat-like grasses through homoploid hybridization and polyploidy. AoB Plants. 2016;8:plw039.
19. Yang SQ, Qin XD, Cheng CY, Li Z, Lou QF, Li J, et al. Organization and evolution of four differentially amplified tandem repeats in the *Cucumis hystrix* genome. Planta. 2017;246(4):749-761.
20. He L, Braz GT, Torres GA, Jiang JM. Chromosome painting in meiosis reveals pairing of specific chromosomes in polyploid *Solanum* species. Chromosoma. 2018;127(4):505-513.
21. Xin HY, Zhang T, Wu YF, Zhang WL, Zhang PD, Xi ML, et al. An extraordinarily stable karyotype of the woody Populus species revealed by chromosome painting. The Plant Journal. 2020;101(2):253-264.
22. Han YH, Zhang ZH, Liu JH, Lu JY, Huang SW, Jin WW. Distribution of the tandem repeat sequences and karyotyping in cucumber (*Cucumis sativus*) by fluorescence in situ hybridization. Cytogenet Genome Research. 2008;122:80-88.
23. Snowdon RJ, Friedt W, Ko¨hler A, Ko¨hler W. Molecular cytogenetic localisation and characterisation of 5S and 25S rDNA loci for chromosome identification in oilseed rape (*Brassica napus*). Annals of Botany. 2000;86:201-204.
24. Galián, JA, Rosato M, Rosselló JA. Early evolutionary colocalization of the nuclear ribosomal 5S and 45S gene families in seed plants: evidence from the living fossil gymnosperm Ginkgo biloba. Heredity. 2012;108(6):640-646.
25. Lapitan NLV. Organization and evolution of higher plant nuclear genome Genome. 1992;35(2):171-181.
26. Heslop-Harrison JS. Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. Plant Cell. 2000;12(5):617-636.
27. Garcia S, Garnatje T, Kovařík A. Plant rDNA database: ribosomal DNA loci information goes online. Chromosoma. 2012;121(4):389-394.
28. Garcia S, Gálvez F, Gras A, Kovařík A, Garnatje T. Plant rDNA database: update and new features. Database. 2014;bau063-bau063.
29. Vitales D, Ambrosio UD, Gálvez F, Kovařík A, Garcia S. Third release of the plant rDNA database with updated content and information on telomere composition and sequenced plant genomes. Plant Systematics and Evolution. 2017;303(8):1115-1121.
30. Martina O, Frantisek M, Mikulas O. A chromosome study and localization of 18S rDNA in *Khawia saurogobii* (Cestoda: Caryophyllidea). Parasitol Research. 2010;106(3):587-593.
31. Zhao X, Lu JY, Zhang ZH, Hu JJ, Huang SW, Jin WW. Comparison of the distribution of the repetitive DNA sequences in three variants of *Cucumis sativus* reveals their phylogenetic relationships. Journal of Genetics and Genomics. 2011;38(1):39-45.
32. Tran TD, Cao HX, Jovtchev G, Novák P, Vu GTH, Macas J, et al. Chromatin organization and cytological features of carnivorous Genlisea species with large genome size differences. Frontiers in Plant Science. 2015;6:613.
33. Srisuwan S, Sihachakr D, Siljak-Yakovlev S. The origin and evolution of sweet potato (*Ipomoea batatas*) and its wild relatives through the cytogenetic approaches. Plant Science. 2006;171(3):424-433.
34. Choi EY, Seo JH, Seo BB. Sequence polymorphism and chromosomal localization of 5S rDNA of three cultivated varieties of sweet potato (*Ipomoea batatas* (L.) Lam.). Genes and Genomics. 2009;31(4):325-332.
35. Tang JL, Qi DS, Zhang Y, Liu HJ, Sun JY, Cao QH, et al. FISH analysis of chromosomes of sweet potato(*Ipomoea batatas*Xushu No.18). Hereditas. 2010;32(2):177-182.
36. Sun JY, Yu LX, Cai ZX, Zhang A., Jin WW, Han YH, et al. Comparative karyotype analysis among six *Ipomoea*species based on two newly identified repetitive sequences. Genome. 2019;62(4):243-252.
37. Gao RF. Genetic Diversity Analysis of Purple-fleshed Sweetpotato Bred in China Cultivars Revealed by Morphology and SSR Markers. Chinese Academy of Agricultural Sciences. 2019 (in Chinese).
38. Wu S, Lau KH, Cao QH, Hamilton JP, Sun HH, Zhou CX, et al. Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. Nature Communications. 2018;9(1):4580.

39. Iovene M, Zhang T, Lou QF, Buell CR, Jiang JM. Copy number variation in potato-an asexually propagated autotetraploid species. The Plant Journal. 2013;75(1):80-89.
40. Hardigan MA, Crisovan E, Hamiltion JP, Kim J, Laimbeer P, Leisner CP, et al. Genome reduction uncovers a large dispensable genome and adaptive role for copy number variation in asexually propagated *Solanum tuberosum*. The Plant Cell. 2016;28,388-405.
41. Pham GM, Newton L, Wiegert-Rininger K, Vaillancourt B, Douches DS, Buell CR. Extensive genome heterogeneity leads to preferential allele expression and copy number-dependent expression in cultivated potato. Plant Journal. 2017;92:624-637.
42. Pedrosa-Harand A, de Almeida CCS, Mosiolek M, Blair MW, Schweizer D, Guerra M. Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris*) evolution. Theoretical and Applied Genetics. 2006;112(5):924-933.
43. Singh M, Kumar R, Nagpure NS, Kushwaha B, Mani I, Chauhan UK, et al. Population distribution of 45S and 5S rDNA in golden mahseer, Tor putitora: population-specific FISH marker. Journal of Genetics. 2009;88(3):315-320.
44. Liu B, Davis TM. Conservation and loss of ribosomal RNA gene sites in diploid and polyploid *Fragaria* (Rosaceae). BMC Plant Biology. 2011;11(1):157.
45. Sochorová J, Coriton O, Kuderová A, Lunerová J, Chèvre A, Kovařík A. Gene conversion events and variable degree of homogenization of rDNA loci in cultivars of Brassica napus. Annals of Botany. 2017;119(1):13-26.
46. Schubert I, Wobus U. In situ hybridization confirms jumping nucleolus organizing regions in *Allium*. Chromosoma. 1985;92(2):143-148.
47. Huang M, Li H, Zhang L, Gao F, Wang P, Hu Y, et al. Plant 45S rDNA clusters are fragile sites and their instability is associated with epigenetic alterations. PLoS One. 2012;7(4):e35139.
48. Dutrillaux AM, Carton B, Cacheux L, Dutrillaux B. Interstitial NORs, fragile sites, and chromosome evolution: a not so simple relationship-the example of Melolontha melolontha and genus *Protaetia* (Coleoptera: Scarabaeidae). Cytogenetic & Genome Research. 2016;149(4):304-311.
49. Thomas HM, Harper JA, Meredith MR, Morgan WG, Thomas ID, Timms E, et al. Comparison of ribosomal DNA sites in *Lolium*species by fluorescence in situ hybridization. Chromosome Research. 1996;4(7):486-490.
50. Badaeva ED, Dedkova OS, Gay G, Pukhalskyi VA, Zelenin AV, Bernard S, et al. Chromosomal rearrangements in wheat: their types and distribution. Genome. 2007;50(10):907-926.
51. Mondin M, Aguiar-Perecin MLR. Heterochromatin patterns and ribosomal DNA loci distribution in diploid and polyploid *Crotalaria* species (Leguminosae, Papilionoideae), and inferences on karyotype evolution. Genome. 2011;54(9):718-726.
52. Rosato M, Kovařík A, Garilleti R, Rosselló JA. Conserved Organisation of 45S rDNA Sites and rDNA Gene Copy Number among Major Clades of Early Land Plants. PLoS One. 2016;11(9):e0162544.
53. Mantovani M, Douglas dos Santos Abel L, Moreira-Filho O. Conserved 5S and variable 45S rDNA chromosomal localisation revealed by FISH in *Astyanax scabripinnis* (Pisces, Characidae). Genetica. 2005;123(3):211-216.
54. Leitch IJ, Heslop-Harrison JS. Physical mapping of the 18S-5.8-26S rRNA genes in barley by in situ hybridization. Genome. 1992;35:1013-1018.
55. Hanson RE, Islam-Faridi MN, Percival EA, Crane CF, Ji YF, McKnight TD, et al. Distribution of 5S and 18S-28S rDNA loci in a tetraploid cotton (*Gossypium hirsutum*) and its putative diploid ancestors. Chromosoma. 1996;105(1):55-61.
56. Martins C, Jr G. Conservative distribution of 5S rDNA loci in *Schizodon* (Pisces, Anostomidae) chromosomes. Chromosome Research. 2000;8(4):353-355.
57. Roa F, Guerra M. Distribution of 45S rDNA sites in chromosomes of plants: structural and evolutionary implications. BMC Evolutionary Biology. 2012;12(1):225.
58. Roa F, Guerra M. Non-random distribution of 5S rDNA sites and its association with 45S rDNA in plant chromosomes. Cytogenetic and genome research. 2015;146(3):243-249.
59. Taketa S, Harrison GE, Heslop-Harrison JS. Comparative physical mapping of the 5S and 18S-25S rDNA in nine wild *Hordeum* species and cytotypes. Theoretical and Applied Genetics. 1999;98(1):1-9.
60. Kamisugi Y, Nakayama S, O'Neil CM, Mathias RJ, Trick M, Fukui K. Visualization of the Brassica self-incompatibility S-locus on identified oilseed rape chromosomes. Plant Molecular Biology. 1998;38(6):1081-1087.

61. Han YH, Zhang ZH, Huang SW, Jin WW. An integrated molecular cytogenetic map of *Cucumis sativus* chromosome 2. BMC Genetics. 2011;12(1):18.
62. Jiang JM, Gill B S, Wang GL, Ronald PC, Ward DC. Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. 1995;92:4487-4491.

Figures

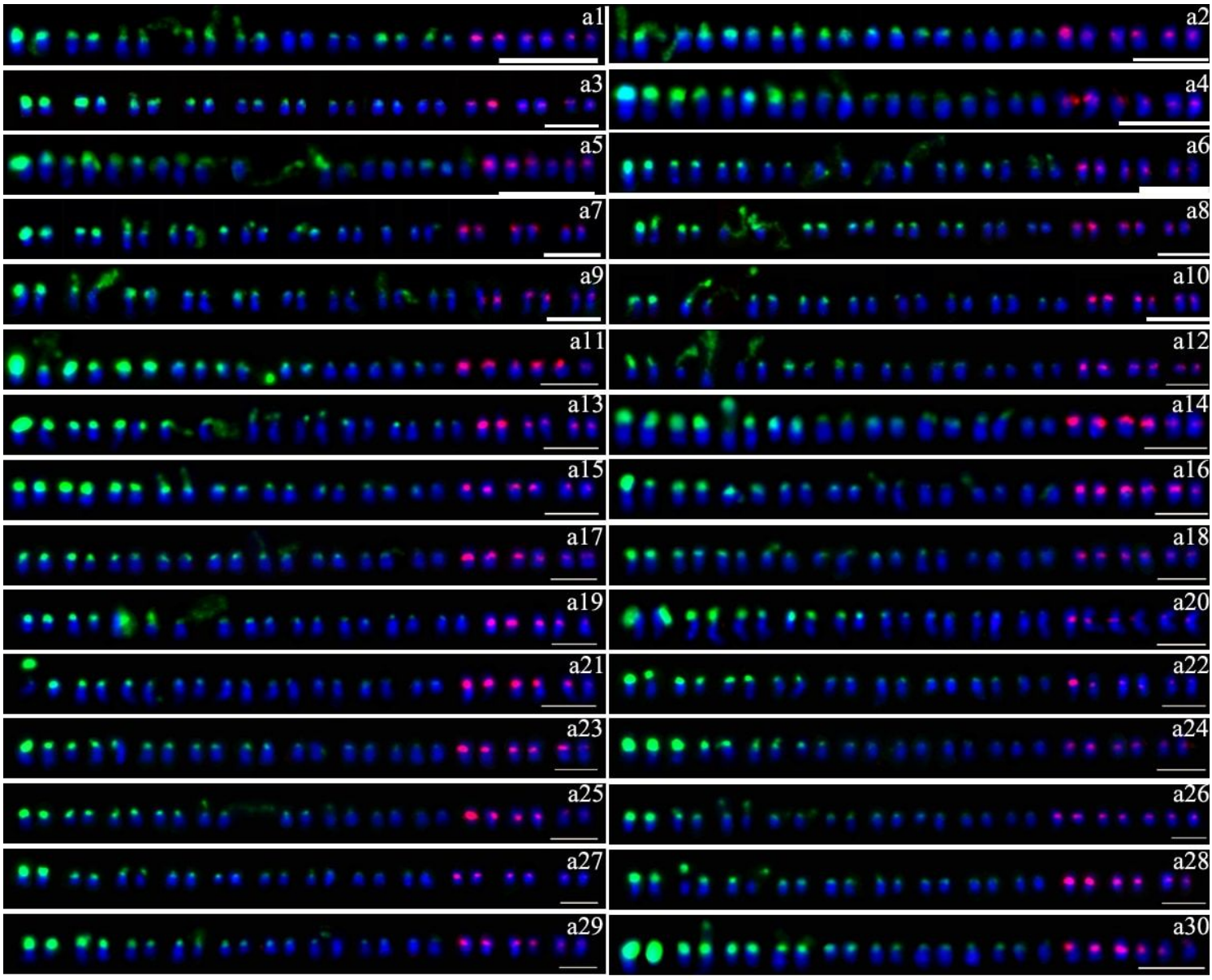

Figure 1

Distribution of 45S (green) and 5S (red) rDNA sites detected by FISH with the number of 45S rDNA is 18. a1: Neiyuzi 2; a2: Qinzishu 2; a3: Longzishu 6; a4: Zhezishu 1; a5: Jihei 2; a6: Fushu 1; a7: Taizhong 11; a8: Yanzishu 3; a9: Jizishu 1; a10: Jizishu2; a11: Ningzishu 1; a12:Luozishu 1; a13: Xuzishu 8; a14: Yanzishu 4; a15: Guiziwei 1; a16: Chuanzishu 4; a17: Guijingshu 5; a18: Chuanzishu 2; a19: Yuzixiang 10□a20: Xuzishu 3; a21: Yuzishu 3; a22: Xuzishu 5; a23: Guijingshu 7; a24: Guijingshu 3□a25: Nanzishu 008; a26: Quanzishu 96; a27: Puzishu 18; a28: Xushu 33; a29: Jizishu 3; a30: Aya. Scale bars, 5 μm.

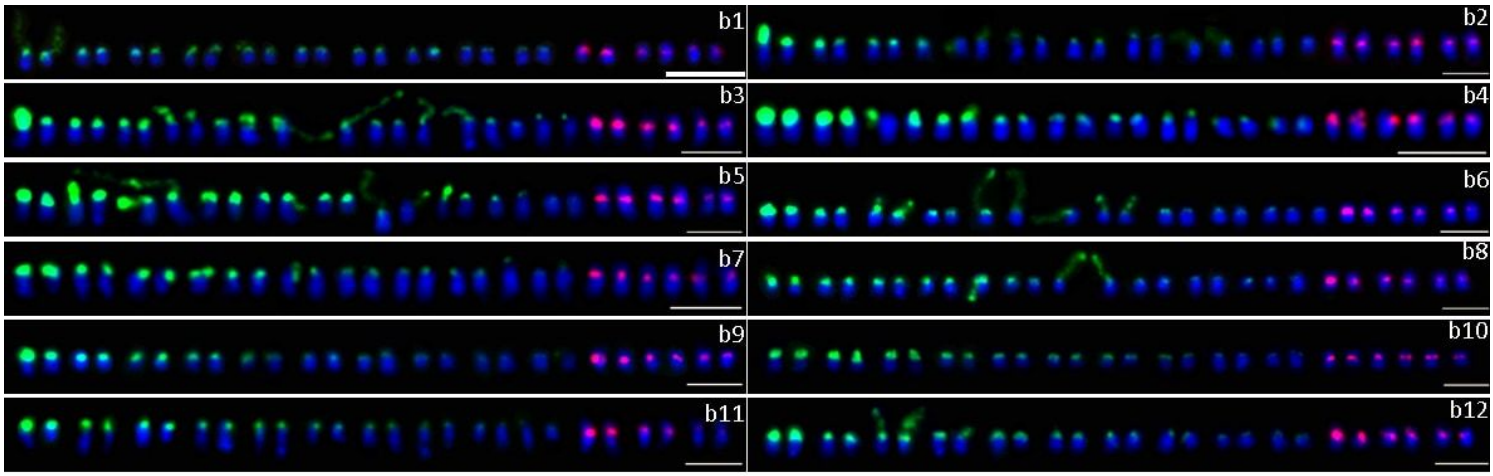

Figure 2

Distribution of 45S (green) and 5S (red) rDNA sites detected by FISH with the number of 45S rDNA is 20. b1: Wan W-36-1; b2: Zhanzishu 2; b3: Mianzishu 9; b4: Mianyuzi 11; b5: Ezishu 13; b6: Fuzishu 404; b7: Fangzishu 9; b8: Yuzishu 7; b9: Pengzishu 3; b10: Funingzi 4; b11: Guangzishu 9; b12: Guangzishu 8. Scale bars, 5 μm.

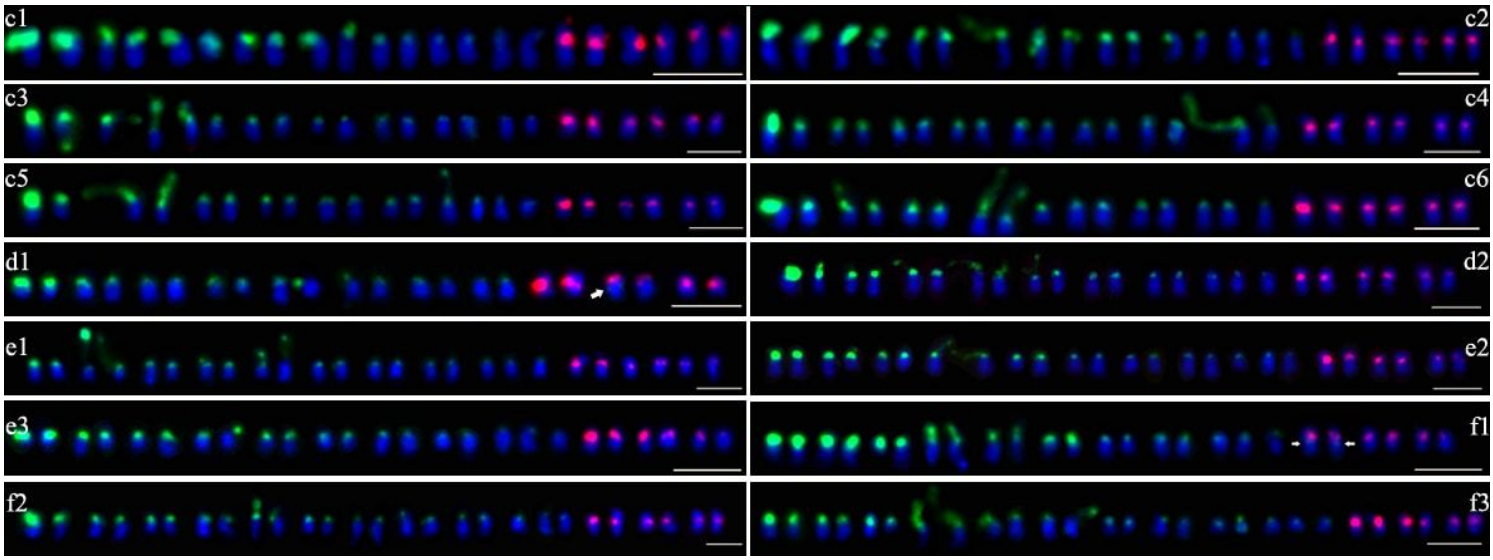

Figure 3

Distribution of 45S (green) and 5S (red) rDNA sites detected by FISH with the number of 45S rDNA is 16,17,19,21. c1-c6: the number of 45S rDNA is 16, c1: Ningzishu 2; c2: Guizishi 1; c3: Yusuzi 43; c4: Guijingshu 6; c5: Nanzishu 014; c6: Nanzishu 015. d1-d2: the number of 45S rDNA is 17, d1: Ezishu 12, the colocalization site was shown by arrowheads; d2: Qianzishu 1. e1-e3: the number of 45S rDNA is 19, e1: Yanzishu 2; e2: Puzishu 3; e3: Guangzishu 11. f1-f3: the number of 45S rDNA is 21, f1: Ningzishu 4, the colocalization sites are shown by arrowheads; f2: Funingzi 3; f3: Jizishu 18. Scale bars, 5 μm.

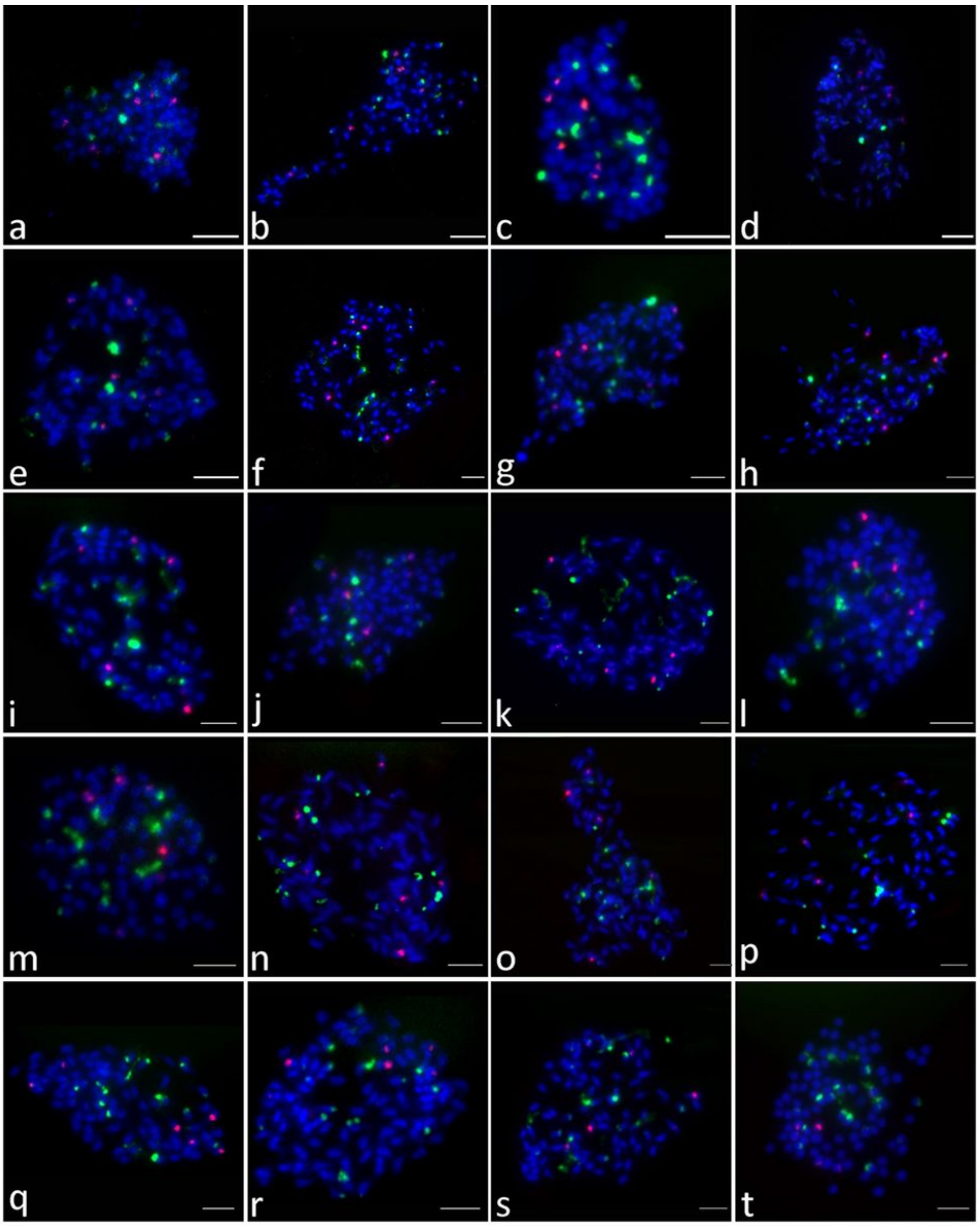

Figure 4

Distribution of 45S (green) and 5S (red) rDNA sites detected by FISH in the remain 20 purple sweet potato varieties. a: Quzishu 57; b: Longzishu 4; c: Qinzishu 3; d: Ningzishu 3; e: Longjinshu 1; f: Longjinshu 3; g: Xuzishu 2; h: Ningzishu 5; i: Xuzishu 6; j: Jizishu 1; k: Wanzi 56; l: Guizishu 3; m: Fushu 24; n: Guijingshu 9; o: Shangxuzi 1; p: Jixuzi 2; q: Yuzi 263; r: Guangzishu 10; s: Guangzishu 1; t: Guangzishu 2. Scale bars, 5 μm.