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Fluorescent *in situ* hybridisation in plants: new ways to link DNA sequence resources and chromosome loci – a review

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Abstract

Fluorescent *in situ* hybridisation (FISH) that allows chromosome DNA labelling has revolutionised plant molecular cytogenetics. With the development of FISH techniques, diverse opportunities to explore and understand the structure, origin, and evolution of plant karyotype have emerged. This review aims to summarize and discuss the latest advances in the application of the FISH technique in crop plants and some wild species. Variable patterns of the genome rearrangements in the newly made distant hybrids are covered as well as contribution of genomic *in situ* hybridisation (GISH) in revealing allopolyploid species phylogeny at the evolutionary scale. Currently, oligo-FISH increased chromosome loci detection and visualisation at a precision never recorded before. This approach requires skills in computational analysis of DNA resources and oligo-probe design. The advantages of synthetic bulked oligo-FISH probes for genome mapping and gene localisation are emphasised in this review. This technique significantly compliments DNA sequencing data, highlights new findings in chromosome collinearity, and shows some unusual instances of plant genome plasticity.

Keywords: fluorescent *in situ* hybridisation (FISH), genomic *in situ* hybridisation (GISH), oligo-FISH, chromosome painting, crops, allopolyploids.

Introduction

Until the 1970s, classical cytogenetics was based on chromosome number counts and their morphometric features, centromere position, chromosome arms' length, and the presence or absence of secondary constriction. The 1980s mark the beginning of the era of molecular cytogenetics, when the *in situ* hybridisation technique emerged, firstly by using radioactive probe labelling (Gerlach, Bedbrook 1979; Schubert, Wobus, 1985), but very soon non-radioactive fluorescent compounds were applied instead (Rayburn, Gill, 1985). FISH technology has proven to be superior to previous *in situ* methods providing a better spatial resolution combined with the ability of simultaneous multi-probe labelling by using different fluorochromes in the same cytological specimen (Anamthawat-Jónsson et al., 1990; Mukai et al., 1993; Sánchez-Morán et al., 1999). In mitotic and meiotic chromosome spreads, FISH started by the detection of continuous blocks of repetitive sequences. These are the probes for the ribosomal DNA gene clusters, 45S and 5S rDNA (Jiang, Gill, 1994; Thomas et al., 1996), the satellite repeats in telomeres (Cox et al., 1993) and centromeres (Ananiev et al., 1998). Further, physical chromosome loci mapping based on DNA inserts in bacterial artificial chromosomes (BAC) clones was introduced (Jiang et al., 1995). Currently, oligo-FISH, a new principle linking the synthesis of short computationally selected DNA oligomers and their fluorescent labelling, was developed which highly increased the precision and range of the targets in plant chromosome painting (Waminal et al., 2018; Liu, Zhang, 2021).

The application of FISH ranges from karyotype analysis to gene localisation. In a chromosome spread, fluorescent DNA probes bind differentially to the chromosomes and/or their segments by targeting complementary sequences. Thus, in cytogenetics, FISH is used to detect and localise the presence or absence of specific DNA sequences in the chromosomes, and *in situ* refers to the chromosome as the "original site" for the nuclear DNA to be present. In plants, the cytological preparations of the metaphase chromosome spreads are made from the meristematic root tip cells. For meiotic chromosomes, the preparations of squashed anthers at the early meiosis, prophase or metaphase I, are used. Routinely, the cytological preparations are screened for a sufficient number of cells at a certain stage of division, and the selected spreads are physically adhered to the objective slide by hard freezing.

The use of FISH is very broad. Depending on the experimental approach, FISH allows differential colour-labelling of the chromosomes of the parental species in the hybrids or discriminates individual chromosomes in the karyotype, or highlights loci of the specific sequences in a particular chromosome(s) (Raina, Rani, 2001; Jiang, Gill, 2006; Huber et al., 2018; Jiang, 2019). Many groups

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of researchers employed FISH to study genome structure in hybrid plants building up physical genome maps and as a tool to study phylogenetic relationships, and there are a few reviews covering these aspects (Markova et al., 2007; Zhang et al., 2007; Zhang, Friebe, 2009; Younis et al., 2015; Jiang, 2019). The advantages of FISH have been demonstrated in genetic studies of cultivated plants, many of which are hybrids or introgression lines; hereby, wheat has become a model for cytogenetics of allopolyploids (reviewed in Zhang et al., 2007). Over the years, FISH/GISH has been a key approach for the discovery hybrid origin of many other allopolyploids. The first reports about revealing genome origin were made in *Avena* (Jellen et al., 1994), *Festuca* (Humphreys et al., 1994), *Coffea* (Raina et al., 1998), *Arachis* (Raina, Mukai, 1999), and *Musa* (D'Hont et al., 2000) species. Meanwhile, the extended list of the allopolyploid genomes dissected by GISH is covered in a few FISH cytogenetics reviews (Raina, Rani, 2001; Jiang, Gill, 2006; Silva, Souza, 2013; Younis et al., 2015).

This article aims to review the latest advances in plant cytogenetics achieved by FISH with a specific focus on economically important crops. Some examples of natural plant species are included to demonstrate the marked potential of chromosome site mapping by oligo-FISH. This very recent FISH technique allows a high resolution at the chromosome level and significantly compliments DNA sequencing in plant genome studies. Literature references search was performed in the Web of Science and PubMed databases using appropriate keywords, "FISH plants", "oligo-FISH", and others. The priority was given to the references covering the last 10 years; also, earlier classical studies are referred to as well.

FISH methods

Specific DNA probes. In plant cytogenetics, the rDNA clusters, 5S and 45S rDNA, telomeric and subtelomeric repeats, and the repeats found in centromeres are commonly targeted (Devi et al., 2005; Younis et al., 2015; Iwata-Otsubo et al., 2016; Zwyrtková et al., 2020). Such satellite repeats array probes, which target distinct region(s) on one or multiple chromosome(s), are used to study chromosome structure, label individual chromosomes, and follow phylogenetic relationships among species by comparative FISH mapping (Jiang et al., 1995; Jiang, Gill, 2006; Markova et al., 2007). Simple sequence repeats (SSRs), which are dispersed through the plant genome, in some cases also provide chromosome banding patterns; this has been achieved in barley and wheat chromosomes by GAA-repeat (Pedersen et al., 1996; Danilova et al., 2012). Similarly, the physical location of genes or positions of non-coding DNAs in chromosomes can be determined. With the advancement of FISH, plasmid insert based specific probes for loci detection have been developed in a number of plants such as wheat and other Triticeae (Danilova et al., 2012; 2014), *Solanum* (Lou et al., 2010; 2014; Torres et al., 2011), *Cucumis* (Lou et al., 2014), *Allium* (Kirov et al., 2017), and *Trifolium* (Dluhošová et al., 2018) species. Although some probes with less than 5 kb of target sequences can be localised using FISH, usually, DNA fragments less than 10 kb in length are not reliably detected; therefore, larger inserts are required. This was achieved by bacterial artificial chromosomes (BAC)-FISH, the next significant step in FISH development.

BAC-FISH. The first plant BAC libraries were created and used in FISH for the species with relatively small genomes: rice (Jiang et al., 1995), barley (Lapitan et al., 2002), *Arabidopsis*, melon, tomato, soybean, and sorghum bicolor (review on BAC-FISH in Jiang and Gill, 2006). The BAC-FISH is based on fluorescent labelling of genomic DNA clones transformed into artificial bacterial chromosomes. BAC vectors hold the fragments up to 300 kb. BAC-FISH includes the construction of BAC libraries, screening, fluorescent labelling of the BAC clones, and hybridisation onto the chromosome spreads. BAC-FISH has been an efficient method for identifying individual chromosomes and for the assembly of physical maps in coffee (Noir et al., 2004), cotton (Wang et al., 2006), common been (Fonsêca et al., 2010) and potato (Gaiero et al., 2017). However, BACs often generate strong background signals, especially in large complex allopolyploids such as wheat genomes (Jiang, Gill, 2006; Suzuki et al., 2012).

Oligo-FISH. The current advent of FISH, synthetic oligo-FISH, started around the 2000s with the introduction of unique short oligonucleotide probes for labelling and mapping them to chromosomal regions. Currently, oligo-FISH is rapidly replacing the traditional plasmid-cloned probes, pTA71, pTa535, pAs1, and many others as well as some BAC-FISH probes (Tang et al., 2014; Liu et al., 2020b). An ample set of short oligonucleotide sequences of up to 25,000–40,000 can be used in FISH run. In plant cytogenetics, chromosome labelling with the bulk oligo-based probes has become a great tool to study genome structure, chromosome rearrangements, meiotic pairing, and recombination at the precision never recorded before. Oligos, 40–60 nt in length, selected throughout chromosome regions at a density of every 2–10 kb can be employed to generate a barcode signal allowing the identification of the entire set of individual chromosomes in a single FISH experiment. The oligo-FISH barcode system has now been developed for several species including potatoes, corn, rice, sugarcane, maize and other crops (Lou et al., 2014; Braz et al., 2018; Albert et al., 2019; de Oliveira Bustamante et al., 2021). Hereby, the oligo-FISH method became the common way to line up the gene loci and non-coding DNAs for any plant species with a genome sequence available (Waminal et al., 2018; Liu, Zhang, 2021).

Genomic DNA probes. A specific technique of FISH is genomic *in situ* hybridisation (GISH), which is based on the fluorescent probes of total genomic DNAs of species (Schwarzacher et al., 1989). In brief, the total genomic DNA of a species is labelled with a fluorochrome and directly hybridized to chromosome spreads on a glass slide. In this way, parental genomes can be discriminated and hybrid genome composition revealed in allopolyploid plants, and the degree of recombination events can be estimated visually as well. Thus, over last three decades, GISH became one of the most important and versatile tools for the disclosure of hybrid genome structure in natural species and bringing the light on genomic rearrangements in the experimental hybrids (Silva, Souza, 2013; Younis et al., 2015). Often, to improve the performance of species differentiation, a targeted genomic DNA of a species is used in the hybridisation mixture together with an unlabelled DNA from another genome (blocking DNA) to avoid excessive "cross-hybridisation" with the non-target chromosomes. Blocking DNA is added at a high concentration, 20–50-fold in excess of probe DNA, and it is required for hybrids derived from closely related species due to the high degree of homology (Lee et al., 2011; Silva, Souza, 2013). Notably, there are many hybrids where GISH targeting can be applied without blocking DNAs (Silva, Souza, 2013). One of the major advantages of this technique is its use for chromosome behaviour analysis in the meiosis of natural and artificial hybrids. Using GISH, it is possible to analyse the patterns of chromosome pairing and assess the frequency of chiasma showing recombination events between homologous and homoeologous chromosomes (Ji, Chetelat, 2007; Zwierzykowski et al., 2008; He et al., 2018). The ratio of bivalent, multivalent, and univalent formation can be determined by linking them to the progenitors in natural allopolyploids or to the parental genomes in experimentally produced hybrids. Specifically, this is useful in the absence of differences in chromosome morphology of the species involved. A great advantage is that this technique can visualise the factors leading to irregular meiosis and relate this data to hybrid fertility (Silva, Souza, 2013).

FISH in agricultural crops

Many of the world's crops are of polyploid origin (Salse, 2016; Zhang et al., 2019). Hybridisation and polyploidization are widely used in breeding to produce new cultivars of *Triticale*, cotton, tomato,

banana, *Festulolium* grasses and other plants. Therefore, cytogenetic studies in allopolyploid plants and an understanding of the genome changes are important not only to illustrate the evolution of plants but also to facilitate the improvement of crops (Zwierzykowski et al., 2006; Kopecký et al., 2008; Lideikytė, Pašakinskienė, 2007; Danilova et al., 2012; Gaiero et al., 2017; He et al., 2018; Šimoníková et al., 2019).

Wheat and its relatives have greatly benefited from FISH cytogenetics. The chromosome structure was defined by targeting rRNA gene clusters, centromeres, subtelomeres, and telomeres; cytogenetically based physical maps for all homoeologous groups have been constructed; wheat genetic stocks featuring specific translocations, introgression, and deletions were developed; some detailed physical gene maps produced (Zhang et al., 2007; Danilova et al., 2012; 2014). Recently, Tang and co-authors (2014) developed oligonucleotide probes that have replaced plasmid-cloned ones traditionally used in the FISH of wheat, rye and their hybrids. FISH-oligo probes from different wheat sub-genomes were used for karyotyping of common wheat (*Triticum aestivum* L.) cultivar 'Chinese Spring' (2n = 6x = 42), which successfully labelled all 21 wheat chromosome pairs. These probes were also tested on metaphase chromosomes of octoploid *Triticale* (2n = 8x = 56) adding a rye genomic DNA probe. Such multiple FISH allowed the discrimination of 14 rye chromosomes and also uniquely labelled individual wheat chromosomes. In this study, a synthetic oligo-FISH probe (oligo-pTa71) for the 45S rDNA cluster generated strong signals on 1B, 6B wheat, and 1R rye chromosomes.

Subsequently, by applying such oligo-FISH probes (Oligo-pSc119.2 and Oligo-pTa535), Wang et al. (2021) followed stem rust resistance in new synthetic hexaploid wheat developed through the introgressions from the crosses with the accessions of *T. turgidum* subsp. *dicoccum* (2n = 4x = 28, AABB) and *Aegilops tauschii* (2n = 2x = 14, DD). This study revealed an interesting link between stripe rust resistance, and the changes in chromosome collinearity discovered by FISH. The plants with a chromosome pattern identical to the parents were severely susceptible to stripe rust. However, certain plants that showed variation in chromosome structure compared with that of the parents were superior for resistance to stripe rust.

Modern sugarcane (*Saccharum* spp.) (2n = 100–120) cultivars are complex polyploids that emerged around a century ago from interspecific hybridisation between *S. officinarum* and *S. spontaneum*. GISH was used to determine the contribution of the species' components. *S. officinarum* and *S. spontaneum* genomic and 45S rDNA probes were used. By the assessment of the recent cultivars, it was revealed that *S. officinarum* chromosomes account for 70–80% of the genome, 15–27.5% comes from *S. spontaneum*, and 8–13% of chromosomes are recombinant. Interspecific hybrids were confirmed to correspond to 2n + n parental chromosome composition pattern: 2n = 80 from *S. officinarum* and n = 32 or n = 40 from *S. spontaneum* (Piperidis et al., 2010). The cytogenetic analysis in modern sugarcane cultivars has been recently extended by oligo-FISH probes replacing the species' total genomic DNA labelling. This study provided a deep insight into the chromosome set rearrangements revealing highly variable patterns of genome composition in ten modern sugarcane cultivars (Wang et al., 2021).

The genus *Gossypium* consists of 45 diploid species (2n = 2x = 26) that fall into eight different genome groups (from A to G, and K), and 5 tetraploid species (2n = 4x = 52) that belong to the AD genome group (Wendel et al., 2009). The most widely grown cotton is upland or Mexican cotton, allotetraploid *G. hirsutum* (2n = 4x = 52). In the group of allotetraploid cotton, Wu and co-authors (2013) studied *G. mustelinum* (2n = 4x = 52) using species GISH and a 45S rDNA probe and showed that the 45S rDNA cluster size and location in *G. mustelinum* are different from that of other allopolyploid species. Recently, FISH in cotton species was extended by the bulked oligo-FISH approach. The bulked oligos for two arms of chromosome 7 were developed from the genome sequence of *G. raimondii* (DD, 2n = 2x = 26) with 12,544 oligos synthesized for each arm. In this study, chromosome 7 was easily identified in both D and AD genome cotton species, including common allotetraploid cotton, *G. hirsutum* (Liu et al., 2020a).

FISH is widely used in the common bean (*Phaseolus vulgaris* L.) (2n = 2x = 22) cytogenetics (David et al., 2009; Fonsêca et al., 2010; Bonifácio et al., 2012; Iwata-Otsubo et al., 2016). The oligo-FISH signals and their intensities were sufficient to discriminate all 11 chromosome pairs of common beans by a set of oligo-FISH probes targeting variable centromeric satellite repeats, a 25 bp *khipu* subtelomeric repeat, 5S rDNA, and a BAC clone (Iwata-Otsubo et al., 2016). The same probe set was applied to common beans from the Andean gene pool, Mesoamerican gene pool, and *P. vulgaris* wild relatives and landraces. Considering that similar FISH patterns were generated, these results suggest that the chromosomal distributions of the repeats were fixed before the divergence of Andean and Mesoamerican gene pools occurred, about 100,000 years ago. In this oligo-FISH study, satellite centromeric and subtelomeric *khipu* repeats were found to evolve actively in the karyotype of *Phaseolus* spp. creating unique footprints in common bean accessions (Iwata-Otsubo et al., 2016).

Lou and co-authors (2014) used an original approach of pooled single-copy genes' FISH probes for cucumber (*Cucumis sativus* L.) (2n = 2x = 14) FISH mapping in the mitotic metaphase and meiotic pachytene chromosomes. A set of probes of the five neighbouring genes on chromosome 5 was used employing repeat-free genes every 50–300 kb. FISH signal positions on chromosome 5 in the metaphase coincided with those at the pachytene. Therefore, fluorescent probes of the five neighbouring genes were visualised for their physical positions on the chromosome. Also, these contiguous gene probes provided reliable labelling of a specific chromosome region. Moreover, FISH was extended to other chromosome pairs, and distinct FISH painting patterns were observed in *C. sativus* and its relatives, *C. melo* and *C. metuliferus*, localizing the region of sequential 133 pooled genes covering 8 Mb in chromosome 4 (Lou et al., 2014). By this approach, a comparative chromosome map of this region was constructed between cucumber and melon.

The application of GISH has been highly effective for disclosing relationships within the *Lolium-Festuca* species complex, and for assessing the ways and the degree of genome recombination that goes on in the hybrids (Pašakinskienė, Jones, 2005; Kopecký et al., 2006; 2008; Zwierzykowski et al., 2006; Majka et al., 2018; 2019). Earlier, GISH studies revealed *F. arundinacea* (2n = 6x = 42) as a natural allohexaploid originating as a hybrid between *F. pratensis* and *F. glaucescens* structurally defined as FpFpFgFgFgFg (Humphreys et al., 1994). Further, *L. multiflorum* was found to be the third species involved in the development of *F. arundinacea* by its introgression within the *F. pratensis* subgenome (Pašakinskienė et al., 1998). The crosses between two ryegrasses, *L. multiflorum* and *L. perenne*, and diploid *F. pratensis* or hexaploid *F. arundinacea* demonstrate particularly high compatibility providing the germplasm for the development of commercial grass cultivars (Kopecký et al., 2006; 2008; Zwierzykowski et al., 2006; Lideikytė et al., 2006; Lideikytė, Pašakinskienė, 2007; Humphreys, Zwierzykowski, 2020). In nearly three decades, FISH/GISH studies highlighted a wide spectrum of intergenomic adjustments in *Festulolium* hybrids. In this way, certain regularities were revealed: *Lolium* chromosomes dominate over *Festuca*; a high portion of chromosomes are species-recombinant; 45S rRNA sites show high fragility; atypical interstitial positions of telomeric repeats appear in the chromosomes of these hybrids (Canter et al., 1999; Kopecký et al., 2006; 2019; Zwierzykowski et al., 2006; Lideikytė, Pašakinskienė, 2007; Majka et al., 2018; 2019; Glombik et al., 2021). Also, rare and more unusual cases of instant re-diploidization of the *F. pratensis* sub-genome were described in F1C0 and F2C1 of *L. multiflorum* × *F. arundinacea* (2n = 8x = 56/2n

= 6x = 42) octoploid/hexaploid group (Pašakinskienė et al., 1997; Pašakinskienė, Jones, 2005). This particular striking phenomenon of *F. pratensis* "escaping" and rebuilding itself is considered to be a rare event of the burst of chromosomal fragmentation and reassembly. Thus, GISH studies bring new knowledge by highlighting the broad plasticity of *Festulolium* hybrid genomes and enable us to better understand the evolutionary pathways and processes that have led to the present status of *Lolium* and *Festuca* species.

Preferential genome removal in the hybrids

Hybridisation affects the integrity of genomes of the species involved and may cause a chain of genomic rearrangements in new hybrids altering genome size and the composition of a chromosome set as observed within the broad range of taxa (Liu, Wendel, 2002; Jones, Pašakinskienė, 2005; Gaeta, Pires, 2010; Wendel, 2015; Alix et al., 2017; Glombik et al., 2020; Yu et al., 2021). In addition, chromatin rearrangements, changes in DNA methylation, alterations in gene expression, and activation of transposable elements are recorded in distant hybrids. Moreover, several instances are documented, when the chromosome set of one of the parents has been removed, and GISH has enabled scientists to follow these exceptional cases of genome deletion visually. In earlier studies (Laurie, Bennett, 1989), rapid preferential loss of single-parent chromosomes in the cells of wheat × maize hybrid embryos via lagging and displacement of maize component from the metaphase plate was described. Later, the loss of single-parent chromosomes through lagging was confirmed in many distant crosses (reviewed in Gernand et al., 2005). Meanwhile, GISH experiments visualized another way of the selective deletion of parental chromosomes during early embryo development. In the *Triticum aestivum* × *Pennisetum glaucum* hybrid, it was discovered that sorghum chromatin is actively deleted by "budding" (Gernand et al., 2005). By this mechanism, sorghum nuclear DNA content is extruded and physically displaced at interphase from the hybrid nucleus of embryo cells. The same was observed in the nucleus of *Hordeum vulgare* and *H. bulbosum* hybrid where *H. bulbosum* chromatin was discarded by budding (Gernand et al., 2006).

Allopolyploid species and FISH

Natural hybridisation and polyploidization play a key role in the evolution of plant taxa. Interspecific hybridisation increases genetic diversity at the population level and leads to the emergence of new species. Many plant species have formed in nature through distant hybridisation with an estimated 50% of flowering plants being allopolyploid hybrids (Tang et al., 2008; Soltis, Soltis, 2009; Cenci et al., 2010; Soltis et al., 2010; Zhang et al., 2019). In particular, FISH and GISH have been remarkably helpful in revealing the hybrid origin of plants, and new information is rapidly growing in this field.

There is a well-documented classical example of the formation of allopolyploid species in North America. Around the 1900s, three diploid species of *Tragopogon*: *T. dubius*, *T. porrifolius*, and *T. pratensis* (2n = 2x = 12), were introduced to North America from Europe. Around the 1950s, scientists observed intermediate *Tragopogon* forms in the wild and proposed that they were two different hybrids formed between the alien species. These naturally occurring neo-allopolyploids were discovered specifically in the western part of North America and have not been documented in Europe. Even before the use of molecular markers, the origin of *Tragopogon* hybrids was predicted by the hybridisation test, when Ownbey and McCollum (1954) discovered that *T. dubius* and *T. pratensis* could be the parents of the *T. miscellus*. Confirmation of this hypothesis occurred later using molecular methods. In the first FISH experiment, Pires and co-authors (2004) studied the distribution of a set of fluorescent probes for several satellite repeats, 45S rDNA and 5S rDNA, in diploids and allopolyploids. Such FISH labelling allowed the identification of the parental chromosomes in the hybrids. It was confirmed that *T. miscellus* (2n = 4x = 24) originated from the intercrosses *T. dubius* × *T. pratensis* and *T. mirus* (2n = 4x = 24) from *T. dubius* × *T. porrifolius* (Pires et al., 2004). Further, by applying multicolour FISH with 45S rDNA, subtelomeric, and a centromeric repeat probes, individual chromosomes were traced, and extensive parental chromosome substitutions and patterns of chromosome set variation were revealed in the populations after *ca*. 40 generations of these neo-allopolyploids (Soltis, Soltis, 2009; Chester et al., 2012).

Another distinct example of the allopolyploid karyotype assessment comes from the study of two diploid species from the *Aster* genus, *A. ageratoides* (2n = 2x = 18) and *A. iinumae* (2n = 2x = 18), and their tetraploid hybrid *A. microcephalus* var. *areus* (2n = 4x = 36). Previously, cytological studies have shown that the chromosomes of *A. agaratoides* are significantly larger than those of *A. iinumae* and that *A. microcephalus* var. *areus* chromosomes are likely to be inherited from both species. Subsequently, GISH with the genomic DNA probes from *A. iinumae* and *A. ageratoides*, confirmed that *A. microcephalus* var. *areus* (2n = 4x = 36) is an allotetraploid formed by the intercross of these two diploids. The *A. iinumae* probe labelled small chromosomes, and the *A. agaratoides* probe labelled large chromosomes, accordingly. It is notable that the *A. agaretoides* simultaneously highlighted the centromeres of *A. iinumae*. This demonstrates that cross-hybridisation has occurred in the centromere region of the opposite species, and *A. agaretoides* genomic probe has served as a centromere-specific FISH probe altogether (Matoba et al., 2007).

Waminal and co-authors (2021) used eight oligo-FISH probes targeting tandem repeats covering >12% of the *Senna tora* genome and determined their distribution in related *Senna* species, *S. tora* and *S. occidentalis*. This study provided cytological evidence of extensive chromosomal rearrangements and highlighted a significant role of tandem repeats in (re)building the chromosome structure during *S. tora* speciation. Also, in this very complex study, two oligo-probes for labelling telomere and subtelomere sites were used, namely, the standard plant telomeric repeat TTTAGGG and the 180 bp subtelomeric repeat from the *S. tora* genome. In the *S. occidentalis* karyotype, both repeats were localised at the chromosome ends as was expected, and only rarely appeared at the interstitial positions. Meanwhile, in the *S. tora* karyotype, the canonic plant telomeric repeat TTTAGGG was detected at many pericentromeric and interstitial loci with an intense signal.

Thus, the unusual distribution of tandem repeats detected by oligo-FISH in *S. tora* chromosomes not only allowed for easy identification of individual chromosomes but also revealed massive chromosomal rearrangements that are likely to play an important role in the recomposing of this genome through species evolution.

Chromosome barcoding by oligo-FISH

Theoretically, bulked oligo-FISH probes can be designed for any plant species with a genome sequence available. Jiang (2019) described multiple oligo-FISH probes marking a single chromosome in autopolyploid switchgrass (*Panicum virgatum* L.) (2n = 4x = 36). First, a specific probe for chromosome 8a comprising 27,000 FISH oligomers was developed. All oligomers with greater than 75% homology to chromosome 8b were removed to reduce cross-hybridisation of probe 8a to chromosome 8b. This probe generated strong signals on chromosome 8a and was only very weak on chromosome 8b. In the next step, an oligo-FISH probe was developed for both short arms of chromosomes 4a and 4b. Only oligomers with greater than 90% sequence similarity between 4a and 4b were selected. This resulted in FISH signals of equal intensity on the short arms of chromosomes 4a

and 4b. Another example comes from the cytogenetic analysis of banana (*Musa balbisiana* Colla) cultivar 'Tani' (Šimoníková et al., 2019). In this study, oligo-FISH probes marking chromosomes 1 and 3 were developed and applied to metaphase and pachytene chromosomes. As an outcome, chromosome rearrangements were detected in the 'Tani' karyotype with the translocation of the long arm of chromosome 3 into chromosome 1. Multiple chromosome-marking by oligo-FISH probes also has been developed for cucumber and potato and served in comparative karyotype analysis within the genera of *Cucumis* (Han et al., 2015) and *Solanum* (Braz et al., 2018). Also, chromosome barcoding has been achieved in maize (Braz et al., 2020; 2021), beans (de Oliveira Bustamante et al., 2021), oats (Jiang et al., 2021), wheat (Li et al., 2021), rice (Liu et al., 2020a), and sugarcane (Piperidis, D'Hon, 2020; Wang et al., 2022).

In the future, it is expected that the oligo-FISH karyotyping could be extended to any other plant species using the sequence data from the reference genomes available.

EPSPS gene copy number expansion detected by FISH in weeds

Duplication of gene sequences in crop species, including wheat, cotton, and soybean, has contributed to the improvement of important agronomic traits such as grain quality, fruit shape, and disease and stress resistance (Panchy et al., 2016). Therefore, the studies of the mechanism and impact of gene duplication are important in understanding how duplicate genes may contribute to new traits. However, there is a "dark side" of this phenomenon related to the expansion of gene copies in weed species, which represents their strategy for survival. One such example is an increase in gene copy number for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) resulting in weed resistance to the herbicide glyphosate. FISH has shown to be a powerful tool in detecting these acute gene multiplication events by using a fluorescent probe targeting EPSPS gene clusters. In *Amaranthus palmeri* weed resistant to glyphosate, FISH highlighted EPSPS loci *de novo* dispersed along all chromosomes, indicating massive amplification of this gene (Gaines et al., 2010). Further FISH studies revealed that the amplification of the EPSPS gene in *A. palmeri* was based on a 297-kb extrachromosomal circular DNA (eccDNA) molecule, which is transmitted through mitosis and meiosis by a mysterious mechanism of tethering to chromosomes (Koo et al., 2018). The elevation of herbicide resistance in weed populations by EPSPS gene expansion and *de novo* development of a gene carrier by extrachromosomal circular DNA demonstrates remarkable genome plasticity and raises the question of sustainable use of glyphosate-resistant GM crops.

Conclusion

Fluorescent *in situ* hybridisation (FISH) in plants went through many improvements since it started nearly 30 years ago. Modern cytogenetics requires new skills in computational analysis of DNA resources and oligo-probe design. With the advent of oligo-FISH in plant cytogenetics, true chromosome painting has become feasible. Oligo-FISH increased chromosome loci detection and visualisation at a precision never recorded before. This technique significantly compliments DNA sequencing, highlights new findings in chromosome collinearity and shows new striking instances of plant genome plasticity. As many crops are hybrids, oligo-FISH enables us to follow chromosomes and chromosomal fragments visually down the generations and backcrosses from intergeneric and interspecific hybrids as well as through the trait introgression schemes. Therefore, in many ways, it opens new prospects in non-model plant cytogenetics and facilitates physical mapping in large genomes of many crops.

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Fluorescencinės *in situ* hibridizacijos augaluose apžvalga: nauji būdai susieti DNR sekų išteklius ir chromosomų lokusus

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Santrauka

Fluorescencinė *in situ* hibridizacija (FISH), leidžianti ženklinti chromosomų DNR, sukėlė augalų molekulinės citogenetikos revoliuciją. Tobulėjant FISH technikoms atsirado įvairių galimybių tyrinėti ir suprasti augalų kariotipo struktūrą, kilmę ir evoliuciją. Apžvalgos tikslas – apibendrinti naujausių FISH metodų taikymą žemės ūkio augalams ir kai kurioms laukinėms rūšims. Atskleidžiamas genominės *in situ* hibridizacijos (GISH) indėlis išaiškinant gamtinių alopoliploidinių rūšių kilmę evoliucijos mastu, taip pat aptariami įvairūs genomų persitvarkymai naujai sukurtuose tolimuosiuose hibriduose. Pastaraisiais metais oligo-FISH pagerino chromosomų lokusų aptikimą ir vizualizavimą tokiu dideliu tikslumu, kuris iki šiol nebuvo pasiektas. Šis metodas reikalauja naujų įgūdžių analizuojant DNR išteklius ir projektuojant oligomerinius zondus. Apžvalgoje išryškinami sintetinių masinių oligo-FISH zondų pranašumai genomo kartografavimui ir genų lokalizacijai. Šis metodas reikšmingai papildo DNR sekų duomenis išryškindamas netikėtus chromosomų linijinės struktūros pokyčius ir demonstruodamas ryškius augalų genomo plastiškumo pavyzdžius.

Reikšminiai žodžiai: fluorescencinė *in situ* hibridizacija (FISH), genominė *in situ* hibridizacija (GISH), oligo-FISH, chromosomų spalvinimas, žemės ūkio augalai, alopoliploidai.