Book of Abstracts
GPZ Meeting of AG Cytogenetics
30–31 March 2017

Chromosome biology and genome editing in the context of plant breeding
In light of the ongoing progress in the field of cytogenetics and genome editing, the focus of the conference is “Chromosome Biology and Genome engineering in the context of plant breeding”.

The program will address a broad spectrum of fundamental and applied aspects of these topics.

The meeting provides excellent opportunities to stimulate scientific discussion and interact with international colleagues involved in genome editing, chromosome engineering, advanced cytogenetics and plant breeding.

**Support**

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Thursday, 30 March 2017
13:00 Welcome
Chair: Ingo Schubert

13:10 Jaroslav Doležel Institute of Experimental Biology, AS CR, Olomouc, Czech Republic
Chromosome genomics supports alien introgression in breeding and gene cloning in wheat

13:35 Gabriella Linc Centre for Agricultural Research, Hungarian Academy of Sciences, Mátészalka, Hungary
Molecular cytogenetic tools in characterization of pre-breeding materials produced with Agropyron species

14:00 Petr Capal Institute of Experimental Biology, AS CR, Olomouc, Czech Republic
Single chromosome genomics

14:15 Veit Schubert IPK, Gatersleben SIM and PALM - two super-resolution methods feasible with the Zeiss Elyra microscope
14:30 coffee break

Chair: Veit Schubert

15:30 Thomas Schmitt Technische Universität Dresden
Comparative Cirrus FISHing

15:55 Jiri Macas Biology Centre AS CR, České Budějovice, Czech Republic
Centromere evolution in Fabaceae

16:10 Eva Hřibová Institute of Experimental Biology, Olomouc, Czech Republic
Comparative analysis of repetitive DNA in eight representatives of species and ryegrasses

16:25 Phuong Hoang IPK, Gatersleben
Cytogenetics of duckweeds, an emerging crop

16:40 Katrijn Van Laere Institute for Agricultural, Fisheries and Food Research, Melle, Belgium
FISH-guided genome assembly in Rosa wichuraana

16:55 Lars-Gernot Otto IPK, Gatersleben
Ploidy variation within cultivated Matricaria recutita L. - Towards breeding of sterile triploid chamomile

17:10 Michal Kveták Institute of Plant Genetics of the Polish Academy of Sciences, Poznań, Poland
Constitution and transmission of chromosomes of distant hybrids obtained by intergeneric hybridizations between selected species of grasses (Aegilops spp.) and Triticeae (×Phleum sect. Wittmack)

17:25 Svetlana Khrustaleva Russian State Agrarian University-Entseregy Agricultural Academy, Russia
Cytogenetic mapping in Allium and its application for onion breeding

17:40 Joanna Łusinska University of Slavica, Kalwaria, Poland
Analysis of Brachypodium karyotypic structure and evolution using cross-species chromosome barcoding

17:55 End
19:00 Brewery Quedlinburg

Friday, 31 March 2017
9:00 Robert Hasterok University of Slavica, Kalwaria, Poland
Dissecting grass genome organization at the cytomolecular level using the model genus Brachypodium

9:25 Thorben Sprink JKI, Quedlinburg, Germany
Different aspects of Genome Editing in plants using CRISPR/Cas9

9:50 Stefan Hieske IPK, Gatersleben
Haploid induction after targeted mutagenesis of CenH3 in barley

10:15 Katharina Unkotel JKI, Quedlinburg, Germany
Targeted modifications of centromeric haxone H3 (CENH3) by using CRISPR/Cas9 in carrots (Daucus carota L.)

10:30 Takayoshi Isbii IPK, Gatersleben
Dynamics of cpeops CENH3 towards haploid induction

10:45 coffee break

Chair: Jörg Fuchs

11:30 Stefan Heckmann IPK, Gatersleben
Can we harness meiosis for crop plant breeding?

11:55 Nico de Stormi Ghent University, Belgium
PROTEIN PHOSHATASE 2A protects centromeric sister chromatid cohesion in Arabidopsis male meiosis by maintaining REC8 at the chromosomes

12:10 Steven Dreisig IPK, Gatersleben
Single pollen genotyping

12:35 Arifa Kus University of Slawisk in Katowice, Poland
Establishing Brachypodium distachyon as a model in analysis of plant genomes stability after mutagenic treatment

12:50 Mahmoud Said Institute of Experimental Botany, Olomouc, Czech Republic
The karyotypes of Agropyron cristatum and its comparison with that of bread wheat using FISH with single gene probes

13:05 Mirko Vanetti European Application Manager Functional Genomics
Improved CRISPR genome editing using chemically-modified crRNA:tracrRNA complexes and Cas9 protein

13:20 Lunch break, IPK Casino
14:00 End of meeting

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Abstract

Chromosome genomics supports alien introgression breeding and gene cloning in wheat
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The analysis of nuclear genomes remains challenging in many plant species due to genome complexity and large size. To overcome the difficulties, our team has been developing chromosome-centric approaches, which rely on the ability to dissect nuclear genomes to chromosomes. This is achieved by preparing suspensions of intact mitotic chromosomes from root tip meristems and isolating chromosomes of interest by flow cytometry. The lossless complexity reduction facilitates de novo genome assembly as well as validation of already available whole genome assemblies. For species where reference genome assemblies are not yet available, chromosome genomics provides a cost-effective way to identify a majority of genic sequences and order them along chromosomes. Chromosome-derived sequences facilitate development of DNA markers to support alien introgression breeding. With the growing number of finished reference genome sequences for important crops, the future of chromosome genomics lies in the ability to target particular genome regions. This results in a significant reduction of costs and, if needed, it allows analyzing a chromosome of interest isolated from different genotypes (mutants). The applications include identification of chromosomes with integrated transgenes, characterization of alien chromatin in introgression lines and development of molecular markers. Gene cloning is becoming one of the most important applications of chromosome genomics. The targeted approach greatly streamlines gene cloning and reduces project costs. Two chromosome-based gene cloning approaches, namely MutChromSeq and TACCA (targeted chromosome-based cloning via long-range assembly) have been developed and validated recently. Chromosome genomics can be applied in any species from which a liquid suspension of intact mitotic chromosomes can be prepared and the number of uses of flow-sorted chromosomes in plant genomics keeps growing. This work has been supported by the National Program of Sustainability (grant award LO 2014).
The analysis of complex polyploid and highly repetitive plant genomes can be significantly simplified by dissecting them into their natural subunits – chromosomes – by flow cytometry. The sorting of chromosomes in plants has its limitations due to their similar size and DNA content. To overcome this limitation a method for obtaining DNA from single copies of chromosome was developed. Each individual copy of a chromosome is 106 amplified to obtain microgram quantities of chromosome-specific DNA that is suitable for various downstream applications including next-generation sequencing. Utilizing this approach it is possible to identify genic sequences on particular chromosomes, to develop chromosome-specific DNA markers, to verify assignment of DNA sequence contigs to individual pseudomolecules, and to validate whole-genome assemblies. The protocol expands the potential of chromosome genomics, which may now be applied to any plant species from which chromosome samples suitable for flow cytometry can be prepared, and opens new avenues for studies on chromosome structural heterozygosity and haplotype phasing in plants.
Wild relatives of cultivated wheat represent a rich potential source of genetic variation for many agriculturally significant characteristics. Perennial 
Triticeae species - genotypes of the Thinopyrum genus - are important as tertiary gene pools for wheat improvement. Understanding the organization of the genomes in the Thinopyrum genus and their phylogenetic relationships with other related species will greatly facilitate the utilization of these species for transferring agronomically useful genes into bread wheat.

Detailed FISH-based karyotype of three diploid wheatgrass species, Agropyron cristatum [(L.) Beauv.] v. Agropyron cristatum (L.) Gaertn., Thinopyrum bessarabicum [(Savul. & Rayss) A. Löve], Pseudoroegneria spicata [(Pursh) A. Löve], the supposed ancestors of hexaploid Thinopyrum intermedium [(Host) Barkworth & D.R. Dewey] compiled using DNA repeats and microsatellite markers. Fluorescence in situ hybridization (FISH) with repetitive DNA probes was suitable for the identification of individual chromosomes of the diploid JJ, SS and PP genomes. Among seven tested microsatellite markers only (GAA)n trinucleotide sequence is appropriate to use as single chromosome marker for the Ps. spicata 1S chromosome. Based on COS marker analysis, phylogenetic relationship between diploid wheatgrasses and the hexaploid bread wheat genome was established. One of these findings supports that J and E genomes are in the neighbouring clusters.

A Thinopyrum intermedium × Thinopyrum ponticum synthetic hybrid wheatgrass is an excellent source of leaf and stem rust resistance. Pre-breeding materials have been developed in Martonvásár and wheat line Mv9kr1 was crossed with this hybrid (Agropyron glael) in order to transfer its advantageous agronomic traits into wheat. Progenies were screened by in situ hybridization and disomic translocations were selected.

This work was supported by National Science Foundation Grants OTKA K10855, K104 382; and the MTA KEP 5/2016 (Hungarian Academy of Sciences).
Comparative FISHing of saffron (Crocus sativus L.) and related Crocus species

Thomas Schmidt, Gerhard Menzel
Department of Biology, Technische Universität Dresden, 01062 Dresden

The flowers of saffron (Crocus sativus) contain the most expensive spice of the world, and the species is grown as crop in rural regions of Spain, Greece, Iran and Kashmir. Saffron is a triploid hybrid (3n = 24, x = 8) with a genome of approximately 10.5 Gp. Due to its infertility it is only propagated vegetatively, and hence the species shows only very low genetic variability. The parental species of C. sativus are yet not known. Crocus cartwrightianus is considered as a donor, however, also autopolyplody is discussed.

Several karyotyping experiments have been performed including mostly chromosome staining and banding. However, due to the lack of discriminating probes a clear and unequivocal karyotype has not been established yet. We have performed genome sequencing of saffron to isolate probes for FISH. Using RepeatExplorer, we have analysed the major classes of repetitive sequences including many satellite DNA families.

Multi-colour FISH with satellite DNA probes and rDNA genes generated up to four cytogenetic landmarks per chromosome resulting in an unequivocal chromosome identification and establishment of a FISH karyotype. In six of the nine triplets we found heteromorphic chromosomes strongly indicating the allopolyploid nature of saffron. By integrating FISH signals with reported staining karyotypes we identified the sequence composition of large C-banding sites.

Expanding the multi-colour FISH enabled the chromosome identification in seven related Crocus species. Comparative FISH of the karyotype of C. cartwrightianus (2n = 16) with saffron chromosomes showed many inconsistencies. Although C. cartwrightianus has most likely contributed to the chromosome complement of saffron, not all saffron triplets contain two homologues of C. cartwrightianus suggesting that C. cartwrightianus itself has heteromorphic chromosomes. This is in line with the variability in the number of satellite sites found among C. cartwrightianus plants tested.
The legume tribe *Fabeae* includes four main genera, *Vicia, Lathyrus, Pisum* and *Lens*, that exhibit extraordinary diversity in the structure and sequence composition of their centromeres. While *Vicia* and *Lens* have monocentric chromosomes, the primary constrictions of metaphase chromosomes in *Lathyrus* and *Pisum* are extended up to a third of chromosome length and carry multiple domains of CenH3-containing chromatin. Since these constrictions carry histone phosphorylation patterns similar to holocentric chromosomes, it has been speculated that they represent a transition between monocentrics and holocentrics. In this talk, I will review our new data on sequence composition of centromeres across various *Fabeae* species differing in centromere organization. I will also present results of detailed comparative analysis of homeologous centromeres between two *Pisum* species, *P. sativum* and *P. fulvum*, revealing various mechanisms of their diversification.
The genus *Rosa* has important economic value in the ornamental sector and many breeding activities are going on supported by molecular studies. To extend the genomic toolbox for rose breeding we initiated genome sequencing of *Rosa wichurana*, a diploid species involved in the origin of many modern rose cultivars and a valuable source of resistance genes. Using Illumina (2x250 reads) sequencing, Hi-C sequencing and Lachesis, a genome contact-probability map with 16771 scaffolds >10kb ordered into 7 pseudochromosomes (≈0.7 genome equivalent) was built. To validate this draft genome assembly, high sensitive Tyramide-FISH with 14 single-copy probes along the 7 *Rosa* chromosomes have been performed, revealing good co-linearity between the cytogenetic map and the Hi-C based map. In addition tyramide-FISH with 18 single-copy probes located on chromosome 7 was done and the order of the genes was determined. This shows that the long arm of chromosome 7 is mostly made up of euchromatin, while the short arm consists of heterochromatin, which is very difficult to order properly by Lachesis. More FISH markers are needed to come to a good anchoring in this region. FISH is very valuable to map repetitive regions and to integrate genome assembly with chromosomal landmarks, such as heterochromatin and (peri) centromeric regions, which enables to understand their evolution and function. To determine the centromere structure and position on the *R. wichurana* pseudochromosomes, we identified rose tandem centromeric repeat sequences in the repeatome, and visualized those by FISH on mitotic and pachytene chromosomes.

Future work involves the creation of a GBS-based genetic map and further integration of complementary cytogenetic, physical and genetic maps. The resulting high-quality genome assembly, together with ongoing RNAseq data analysis can be further applied in rose breeding.
Ploidy variation within cultivated *Matricaria recutita* L. – Towards breeding of sterile triploid chamomile

**Lars-Gernot Otto**¹, Wolfram R. Junghanns², Andreas Plescher³, Marlies Sonnenschein³, Bartolome Plocharski³ and Timothy F. Sharbel¹

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*Matricaria recutita* L. (German chamomile) has a long history of medicinal use, being already mentioned by Hippocrates (5th century BC). Chamomile is one of the most important medicinal plants in Germany. New fields for cultivation are difficult to gain, since the seeds can lay dormant for 10 to 15 years in the soil. Thus, also crop rotation is inhibited, leading to the accumulation of chamomile specific diseases. Cultivated varieties are diploid or artificially generated tetraploid. A sterile triploid chamomile variety could be a solution, like in many fruit and ornamental crop plants for which seeds are dispensable.

The ploidy variation in various chamomile varieties and populations was determined by flow-cytometry. Several tetraploid varieties contained to some extent diploid, triploid and aneuploid plants. As a proof of concept, triploid chamomile plants could be generated by interploid crosses between di- and tetraploid parents. The triploid plants were highly sterile, and of comparable agricultural performance as di- or tetraploid plants.
Constitution and transmission of chromosomes of distant hybrids obtained by intergeneric hybridizations between selected species of goatgrasses (Aegilops spp.) and triticale (×Triticosecale Wittmack)

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Spontaneous hybrids arisen from interspecific and intergeneric crosses are one of the main parts of evolution. Chromosome rearrangements are utilized in order to transfer desirable traits into cultivated plants. The aim of our study was to determine the dynamics of changes in chromosome constitution of intergeneric hybrids obtained by crossing hexaploid triticale (×Triticosecale Wittmack) with wild Aegilops species. The main assumptions of this study was (1) to precisely identify and compare the chromosomes of Aegilops species and triticale (including relatives from Triticum genus); (2) to evaluate the localization of chromosome breakpoints and (3) to select and study hybrid forms bearing valuable traits.

We used five Aegilops spp. × Secale cereale amphiploid forms for reciprocal distant hybridizations with triticale varieties. We assumed, that using such forms will have a significant impact on F1 hybrid stability because of R-genome chromosomes, which will be able to pair during prophase I of meiosis and will ensure the functional daughter cells formation and sufficient level of vital pollen grains, as a consequence.

Firstly, we established and compared the FISH patterns on chromosomes of several Aegilops and Triticum species and triticale using repetitive sequences from BAC library of wheat ‘Chinese Spring’. The differences between localization of cytogenetic markers in homoeologous chromosomes were detected in several species of Aegilops and Triticum genus. The most informative probes were used for karyotyping of Aegilops-triticale hybrids. Chromosome dynamics was observed in subsequent generations of hybrids during mitotic metaphase of root meristems and first metaphase of meiosis of pollen mother cells. Fluorescence in situ hybridization (FISH) and immunolocalization and was applied in order to detect DNA sequences and synaptonemal complex which are involved in chromosome pairing.

We developed several monosomic/disomic alien addition/substitution triticale forms which are crucial for transfer of genes from wild relatives into cultivated varieties. Our cytogenetic study, supported by the marker assisted selection using Pm13 marker and visual evaluation of infection by Blumeria graminis, allowed to select triticale hybrids carrying chromosome 3S (derived from Ae. variabilis) which were tolerant to the powdery mildew. We allocated chromosome 2D of Ae. tauschii in triticale background, which resulted in changes of its organization, what was related to varied expression of agronomically important traits. Moreover, we investigated the least known gametocidal action of 4Mg chromosome (derived from Ae. geniculata) during the meiosis of pollen mother cells of monosomic 4Mg addition triticale plants. We adapted the gametocidal system in purpose to induce the chromosome aberrations between Triticum and Secale chromosomes of triticale. We applied this mechanism in a combination with DH lines production, which provided a sufficiently large population of homozygous doubled haploid individuals of triticale with two identical copies of translocation chromosomes.

The study was supported by a grant from the National Sciences Centre (NCN SONATA 6; 2013/11/D/NZ9/02719).
Cytogenetic mapping in Allium and its application for onion breeding.

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Fluorescence in situ hybridization (FISH) has not been readily exploited for physical mapping of molecular markers in plants due to the technical challenge of visualizing small single-copy probes. Signal amplification using tyramide FISH can increase sensitivity up to 100 fold. We have applied tyramide-FISH for visualization of gene/marker on the Allium chromosomes.

Tyramide FISH was used to locate relatively small genomic amplicons (846 to 2251 bp) and a cDNA clone (666 bp) from molecular markers linked to Ms locus onto onion chromosome 2 near the centromere, a region of relatively low recombination. This result explains why several labs have identified molecular markers tightly linked to the Ms locus after screening relatively few DNA clones or primers and segregating progenies. Although these markers are still useful for marker-aided selection, our results indicate that map-based cloning of Ms will likely be difficult due to reduced recombination near this gene.

Onion chromosome 5 carries major quantitative trait loci (QTL) of interest to breeders that control dry-matter content, pungency and storability of bulbs etc. We used EST clones and sequences of onion from the NCBI database to develop DNA probes for in situ hybridization. We produced DNA probes that carried introns to increase the hybridization specificity of the probes. Through the integration of genetic and chromosomal maps we were able to estimate the distribution of recombination events along onion chromosome 5.

We cloned, sequenced and located the alliinase (probe 1100bp) and lacrymatory factor synthase (LFS, probe 550bp) genes that encode enzymes operating in a biochemical pathway that produces the compounds responsible for the onion’s characteristic flavour. A disruption of collinearity between homeologous chromosomes was revealed by mapping the alliinase genes in a number of Allium species closely related to onion. This information can be useful for effective interspecific breeding because genome collinearity is a strong prerequisite for homologous recombination and transferring desirable traits from donor species.

This study was financially partly supported by a research grant 16-16-10031 from the Russian Science Foundation.
The enormous diversity of angiosperm plants is a reflection of great variation in their genomes. Recent data indicate that polyploidisation and dysploidy are the major evolutionary forces driving the success of flowering plants and also the most important mechanisms, which determine a numerical alteration of chromosomes. Paleogenomic studies based on the bioinformatics analyses of DNA sequences have revealed that nested chromosome fusions played an important role in the divergence of modern grasses.

The genus *Brachypodium* represents a particularly suitable model system for the analysis of grass karyotype evolution. It comprises 15–20 species with different basic chromosome numbers, size, morphology and ploidy levels. Present study elucidate the mechanisms of the chromosome rearrangements that have shaped the structure of *Brachypodium* karyotypes, using comparative chromosome barcoding by BAC-FISH (fluorescence *in situ* hybridisation with bacterial artificial chromosomes as probes). The karyotypes of selected *Brachypodium* species were compared with reference to the model grass *B. distachyon*. Single-locus BAC clones derived from the *B. distachyon* genomic libraries were selected from the assemblies of FPCs (FingerPrinted Contigs) that had previously been assigned to the chromosomes of *B. distachyon*. This comparative chromosome barcoding approach can be used to study the organisation of karyotypes and reconstruct mechanisms of the chromosome rearrangements that have shaped the structure of the extant grasses karyotypes.

This work is supported by the National Science Centre Poland (grant no. 2012/04/A/NZ3/00572).
Dissecting grass genome organisation at the cytomolecular level using the model

*Brachypodium*


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Modern molecular cytogenetics combines various methodological approaches of cytology, molecular genetics and advanced digital image analysis. It focuses on the study of nuclear genomes at the microscopic level. The cytomolecular organisation of plant genomes is still rather poorly investigated, compared to that of animals. Most plant genomes, including those of economically and ecologically crucial cereals and forage grasses, are usually large and saturated with repetitive DNA, which hampers detailed molecular cytogenetic analyses.

Model organisms possess a combination of features, which makes them more amenable to scientific investigation than others. One of the most recent and rapidly developing model systems are representatives of the *Brachypodium* genus, particularly *B. distachyon*. They possess small, and in some cases, already sequenced genomes with a low repeat content, diverse basic chromosome numbers and ploidy levels. They also have an interesting phylogeny, short life cycles and simple growth requirements, complemented by a rapidly and continuously growing repertoire of various experimental tools.

This presentation outlines and discusses our current projects and their future prospects, using *Brachypodium* species for research on various aspects of grass genome organisation, e.g. (i) karyotype structure and evolution, (ii) distribution of chromosome territories within the nucleus, (iii) dynamics of epigenetic modifications of chromatin during embryo development and cell differentiation, (iv) true nature of selective silencing of rRNA genes in some *Brachypodium* allopolyploids and (v) instability of a small grass genome induced via mutagenic treatments.

This work is supported by the National Science Centre Poland (grants no. 2012/04/A/NZ3/00572, 2014/14/M/NZ2/00519 and 2015/18/M/NZ2/00394).
Many plant breeding programs rely on the generation of homozygous lines after cross-combination of parental plants with different desired traits. Genetically stable lines can be produced either by time-consuming and laborious selfing over numerous generations or in just one step by employing haploid technology. Likewise, methods of doubled haploid production can also greatly facilitate the generation of homozygous experimental recombinants, induced mutants as well as transgenic and genome engineered plants. Due to various constraints of current haploid technology (e.g. genotype-dependency, challenging cell culture procedures, recombination bias in DH-populations) there is a strong demand for alternative or even universally useful methods applicable in many crop species. Therefore, we aim to establish a novel method in the model cereal crop barley based upon uni-parental genome elimination as a result of a functional modification in the centromere-specific histone 3 (CENH3) - a principle recently discovered in *Arabidopsis*. CENH3 replaces canonical HISTONE 3 in centromeric nucleosomes in many eukaryotic species. The CENH3 protein recruits key components of the kinetochore complex and is therefore essential for proper chromosome segregation during meiotic and mitotic cell divisions. In *Arabidopsis*, maize and more recently in *Brassica juncea*, the replacement of native CENH3 by an altered derivative (GFP-tailswap-CENH3) was demonstrated to result in plants having a certain capacity of producing haploid progeny. Crossed with any CENH3 wild type plant of the same species, these lines trigger the elimination of their own chromosomes during early embryo development (Kelliher et al., 2016; Ravi and Chan, 2010; Watts et al., 2017).

To produce such inducer-lines for barley, we stably expressed a GFP-tailswap-HvCENH3α transgene and confirmed the localization of its product to all barley centromeres. In addition, we created functional knock out (KO) alleles of HvCENH3 via targeted mutagenesis using RNA-guided endonucleases (RGENs). Plants carrying a cenh3 KO allele in homozygous condition exhibit a normal growth phenotype but do not produce any progeny upon self-pollination. However, crossing of these mutants with wild type barley results in the elimination of the cenh3 KO allele-carrying genome, which, via embryo rescue, can entail the formation of haploid plants. Surprisingly, the GFP-tailswap-HvCENH3 is neither essential for the induction of haploidy nor does it rescue the infertility of HvCENH3 loss-of-function mutants.

Literature:


Targeted modifications of centromeric histone H3 (CENH3) by using CRISPR/Cas9 in carrots (*Daucus carota* L.)

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Carrot (*Daucus carota* L.) is the most widely grown species of the genus *Daucus*, with F1 hybrid breeding as the main breeding technique. However, as a cross-pollinated biannual species, the production of genetically homogeneous parental lines through several subsequent steps of inbreeding is long lasting and might lead to inbreeding depression. Haploid production by tissue culture techniques is inefficient in Apiaceae species, and genome elimination by interspecific hybridization has not yet been reported for the *Daucus* genus. Modification of the kinetochore specific centromeric histone H3 (CENH3) - which plays a major part in proper segregation of chromosomes during cell division - might result in uniparental genome elimination during early embryogenesis and has been proposed as a new accelerated method for haploid induction. In eudicots CENH3 consists of a highly conserved C-terminal Histone Fold Domain (HFD) and an N-terminal tail showing variations in length and sequence between species. We used the RNA guided endonuclease (RGEN) technique CRISPR/Cas9 to induce mutations at different sites of the HFD region of *DcCENH3* with the objective to generate sequence variants which impair the function of CENH3 in the light of creating potential haploid inducer lines. To complement the possible loss of function of carrot CENH3, we additionally co-transformed carrot varieties with the *PgCENH3* gene cloned from *Panax ginseng*, a member of the Araliaceae plant family belonging to the order Apiales. Due to the high regeneration rate of hairy roots, an early screening method to identify the most promising hairy root lines is essential prior to plant regeneration via somatic embryogenesis. Among other mutation detection techniques, we used high-resolution fragment (HRF) analysis via an automatic LICOR sequencing apparatus as a pre-selection tool to identify multiple CRISPR/Cas9 induced mutations inside the coding sequence of *DcCENH3*. We show that we were able to induce mutations in the CENH3 gene of carrot by RGEN which led to visible changes in the CENH3 phenotype in some hairy root lines.
During meiosis, the cohesin complex that maintains sister chromatid cohesion is lost in a stepwise manner. In yeast and vertebrates, the meiosis-specific cohesin subunit Rec8 is cleaved only along the chromosome arms at meiosis I; up till Metaphase II it is protected at the centromeres by the action of Shugoshin (Sgo) and Protein Phosphatase 2A (PP2A). In plants, centromeric sister chromatid cohesion from Metaphase I to II is protected by two Sgo orthologs and by a plant-specific protein PATRONUS (PANS), however the detailed mechanism by which sister chromatid cohesion is maintained at centromeres is still poorly understood. The Arabidopsis genome contains nine PP2A B’ subunit homologs. Using genetic studies we here demonstrate that *pp2a b’* double mutants display premature separation of sister chromatids in meiosis starting from anaphase I, whereas single mutants do not show any alteration in cohesion release, indicating that AtPP2A B’ and AtPP2A B’ are redundantly required for the maintenance of centromeric sister chromatid cohesion during meiosis I. Furthermore, we demonstrate that the AtREC8 cohesin subunit is prematurely depleted from the centromeric regions in male meiocytes of the *pp2a b’ pp2a b’* double mutant, suggesting that PP2A maintains centromeric sister chromatid cohesion from Metaphase I to II by protecting REC8 from cleavage. Finally, we also found that AtPP2A B’ and α are dispensable for mitotic cell progression in Arabidopsis.
Establishing *Brachypodium distachyon* as a model in analyses of plant genome stability after mutagenic treatment

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Due to their high sensitivity, higher plants are widely used for screening and monitoring environmental genotoxicity. *Brachypodium distachyon*, an internationally accepted model grass species, would be a convenient system in mutagenesis to analyse ‘hot spots’ (and ‘cold spots’) of DNA damage in nuclear genome and consequently could find practical application in the environmental monitoring. The chromosome rearrangements are commonly identified using classical cytogenetic techniques. Physical mapping technology together with the availability of BAC libraries of *B. distachyon* nuclear DNA, allow comprehensive analyses of mutagenic effects at the chromosomal level and extend our understanding of the mechanisms of chromosomal aberrations. The visualisation of mutagen-induced genome changes, including micronuclei formation and alterations of chromosome territories in interphase nuclei using fluorescence *in situ* hybridisation (FISH) with selected chromosome-specific BAC clones, as well as ribosomal DNA and chromosome region-specific, i.e. centromeric and telomeric probes here is presented.

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The karyotype of *Agropyron cristatum* and its comparison with that of bread wheat using FISH with single gene probes

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*Agropyron cristatum* L. (2n=2x=14, PP) commonly known as crested wheatgrass is a wild relative of wheat and an attractive source of novel genes for its improvement. As alien gene transfer by interspecific hybridization is affected by chromosome colinearity, it is important to establish the syntenic relationships between the chromosomes of the donor alien species and wheat. To date, identification of all chromosomes of *A. cristatum* is not possible and its molecular karyotype has not been developed. With the aim to identify chromosomes of *A. cristatum* by FISH, its genomic DNA was sequenced and several tandem repeats were discovered. Their location on mitotic chromosomes by FISH revealed specific distribution pattern for six of them. The use of one tandem repeat together with 45S rDNA as probes for FISH enabled identification of all seven chromosomes of *A. cristatum*. In order to analyze the structure and homoeology of *A. cristatum* chromosomes, 45 FLcDNA from the seven chromosome groups of wheat were localized by FISH on chromosomes of crested wheatgrass cv. Parkway. Important structural rearrangements were observed for chromosomes 2P, 4P, 6P and 7P, while, no major rearrangements were detected for the remaining three chromosomes. The results of this work provide new insights into the genome evolution within the tribe Triticeae and will facilitate the use of crested wheatgrass in alien introgression breeding of bread wheat.
Increased CRISPR efficiency using chemically-modified and length-optimized crRNA:tracrRNA complexes.

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The natural CRISPR-Cas9 system in S. pyogenes employs two RNA molecules, a 42-nt target-specific CRISPR RNA (crRNA) and an 89-nt universal trans-activating RNA (tracrRNA). Through systematic testing of truncations in the RNAs, we developed length-optimized versions that exhibit improved editing performance in mammalian cells. Although unmodified RNA oligonucleotides can be used to direct Cas9 cleavage, they are rapidly degraded by serum or cellular nucleases, limiting their functional activity. Further, unmodified RNAs can trigger an innate immune response in mammalian cells. Extensive studies of chemical modification strategies for both the crRNA and the tracrRNA were performed. Over 400 RNA oligos were compared for functional performance in various settings, systematically evaluating the tolerance of each base for modification. Highly functional modified variants were developed where as high as 78% of the crRNA and 84% of the tracrRNA residues were substituted with 2’OMe RNA. Use of phosphorothioate modified internucleotide linkages or other end-blocking strategies were also helpful in preventing 5’- or 3’-exonuclease attack. The new chemically-modified crRNA:tracrRNA synthetic oligonucleotides can be annealed, complexed with recombinant Cas9 protein and introduced into mammalian cells using lipofection or electroporation to achieve high editing efficiency with minimal side effects. Functional validation has been obtained in a variety of systems including: mice, zebrafish, nematodes, mammalian tissue culture cells, iPSCs, and primary T-cells isolated from human donors.
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