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Poster Presentation Abstracts

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Poster Abstracts:

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Poster Title	Generation of Gynoecious Melon Lines using CRISPR/Cas9
Abstract	Gynoecious lines carry only female flowers and have been extensively exploited in *Cucurbitacea* family breeding. The use of a gynoecious lines allow the production of hybrids earlier, with a higher yield, with more concentrated fruits and eliminates the need for hand emasculation, reducing labour costs of crossing. The phenotypic flowering in melon is determined according two genes, CmWIP1 and CmACS-7, with two alleles each, G/g and A/a, respectively. The combination of those alleles determines the phenotypic flowering of plants, which can be as follows: Andromonoecious (*aaGG*), Monoecious (*AAGG*), Hermaphrodite (*aagg*) or Gynoecious (*AAgg*). CmWIP1 gene acts as an inhibitor of carpel development in the bisexual floral meristem and CmACS-7inhibits the development of stamen in the bisexual floral meristem. CRISPR/Cas9 genome-editing system allows a precise editing of DNA target sequences by double strand breaks. CRISPR/Cas9 system will be applied to target CmWIP1 gene of melon in protoplasts and in plants, to validate RNA guides and to obtain new dysfunctional alleles of the gene CmWIP1, respectively. Melon species is very recalcitrant to transformation and is highly genotypic dependent. Efficient CRISPR/Cas9 editing system in melon has been recently reported in our group. Therefore, we will optimise the different aspects of the process to efficiently regenerate genome edited plants via *Agrobacterium*-transformation. We will produce Gynoecious lines by knock-out of CmWIP1 gene of melon and to use them for breeding purposes in hybrid seed production.

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Poster Title	Genome-editing for Low Acrylamide Wheat
Abstract	Acrylamide (C_3H_5NO) is a Group 2a human carcinogen which was unexpectedly found in many cooked foods in 2002. It is a food processing contaminant, meaning that it is not present in the raw foodstuff. Acrylamide forms from the reaction of free (non-protein) asparagine with reducing sugars, such as glucose, fructose and maltose, during the Maillard reaction, the name given to a complex series of non-enzymatic reactions between free amino acids and reducing sugars that also give fried, roasted and baked products their signature colours and flavours. Wheat represents one of the major sources of dietary acrylamide intake with products such as biscuits, breakfast cereals and toast all showing relatively high acrylamide concentrations. The European Food Standards Agency has declared that the margins of exposure to dietary acrylamide indicate a concern for tumour-inducing effects, and the European Commission has recently tightened its acrylamide regulations. Asparagine concentration is the determining factor for acrylamide formation in cereal products. Asparagine synthesis is catalysed by a family of enzymes called asparagine synthetases (ASNs), and five ASN genes (*TaASN1, TaASN2, TaASN3.1, TaASN3.2* and *TaASN4*) have been identified in wheat, with *TaASN2* showing grain-specific expression. The CRISPR/Cas9 system was successfully applied for the targeted knockout of *TaASN2,* generating wheat material with low grain asparagine concentration and, therefore, reduced acrylamide-forming potential. A multiplexed guide-RNA construct, containing four gRNAs interspaced with tRNAs, was designed to target the first exon of *TaASN2*. The construct was co-bombarded into wheat (*T. aestivum*) cv. Cadenza isolated embryos via particle bombardment, alongside a *Cas9*-containing vector. A range of editing events were identified through Next Generation Sequencing. Single seed amino acid testing was performed to indicate the successful production of very low-asparagine wheat. Acknowledgement: Sarah Raffan is supported by the Biotechnology and Biological Sciences Research Council through a South West Biosciences Doctoral Training Partnership CASE studentship, with the following partners: Agriculture and Horticulture Development Board, KWS UK Ltd., Saaten Union UK Ltd, RAGT Seeds Ltd; Syngenta UK Ltd, and Limagrain UK Ltd.

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Poster Title	CRISPR/Cas genome editing toolbox for engineering bacterial leaf blight resistance in *indica* rice
Abstract	Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a destructive rice disease in Asia and Africa. The development of CRISPR/Cas genome editing toolbox to control this disease in *indica* rice is in great demand. Upon infection, X*oo* transcription activator-like (TAL) effectors induce three rice sucrose transporter-encoding genes including *OsSWEET11, OsSWEET13* and *OsSWEET14*. The activation of these SWEET sugar efflux transporters likely provides nutrients to the pathogen, allowing it to amplify and cause the disease. In previous studies, six TAL effectors binding elements (EBEs) in SWEET promoters were found: PthXo1 in the *SWEET11* promoter, PthXo2 in the *SWEET13* promoter, TalC, TalF, PthXo3 and AvrXa7 in the *SWEET14* promoter. Editing these EBEs prevents binding of *Xoo* TAL effectors and the activation of *SWEET* genes, leading to broad-spectrum resistance against *Xoo*. In this study, we provide a genome editing toolbox including CRISPR/Cpf1 constructs and transformation pipeline to edit these EBEs and develop broad-spectrum resistance of *indica* rice against *Xoo*.

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Poster Title	Nanoscale Automation of a Full-Length RNA Sequencing Library Preparation Method on a Microfluidic Circuit
Abstract	RNA sequencing (RNA-seq) has become the gold standard of expression profiling methods. We have developed an elegant microfluidics-based chemistry and workflow leveraging the Juno™ instrument called the Advanta™ RNA-Seq NGS Library Prep Kit (PN 101-9187). Our RNA-Seq Kit supports simultaneous processing of up to 48 samples with a one-click script on our instrument. The Juno NGS system automates the RNA-seq workflow along with a new, nanoscale integrated fluidic circuit (IFC) called 48.Atlas™, which is the size of a standard microtiter plate. The Advanta RNA-Seq NGS Library Prep Kit also includes reagents necessary to generate full-length, stranded RNA-seq libraries from the polyadenylated RNA fraction present in as little as 10 ng of total RNA from eukaryotic organisms. Herein, we show performance characteristics from an internal analytical study and compare our kit to results obtained from the Illumina® TruSeq® Stranded mRNA Kit (PN 20020594).

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Poster Title	Naturally diversity of functional disease resistance genes in wild potato species
Abstract	Wild germplasm collections contain a wealth of functional disease resistance genes that, so far, remain largely poorly characterised. Using targeted genome enrichment sequencing technologies, RenSeq and dRenSeq, we have developed a pipeline to rapidly identify novel sources of resistance for prioritisation in genetic studies, as well as to identify natural variants of functional disease resistance genes. A study of 12 accessions of *Solanum bulbocastanum* from the Commonwealth Potato Collection has identified three novel resistances and functional variants of *R3a*, *Rpi-blb1* and *Rpi-blb2*. Intriguingly, a susceptible accession, *S. bulbocastanum* 7648, contained a variant of *Rpi-blb1* with only four non-synonymous sequence polymorphisms. A structure-functional analysis has identified the two SNPs that are critical for function and which both reside in the coiled-coiled domain. With the ability to identify such critical polymorphisms in cultivars, gene editing can be deployed to render non-functional genes into functional variants and/or improve the existing recognition specificity.

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Poster Title	QTL analysis complemented by the transcriptome sequencing to identify candidate genes associated with wheat heading time
Abstract	Variability of heading time may affect wheat adaptation to different environments. Thus, the detection of new heading time determinants is important for improving cereals. In this work, we used common wheat cultivar Chinese Spring (CS) and the substitution line of CS with 5B chromosome from *T. dicoccoides* (CS-5Bdic), different in their flowering time by two weeks, to detect determinants of heading time on 5B chromosome. Using the RICL population from a cross of CS x CS-5Bdic, we detected QTL in pericentromeric region of chromosome 5B, that was significantly associated with heading time. To determine candidate genes, that affect the trait of our interest, we analyzed transcriptomes of CS and CS-5Bdic using the RNA-seq. Three replicate samples from each genotype were harvested at four time points over 24 hours since the beginning of the light period (0, 3, 9 and 16 hours). The genes showed differential expression between the substituted line and CS were identified in each time point. GO analysis revealed that the DEGs were mainly involved in nitrogen assimilation and metabolism, photosynthesis, regulation of transcription, ATP metabolism. Genes demonstrating the most expression difference were associated with nitrogen metabolism (*glutamate dehydrogenase*, *aspartate kinase*, *ferredoxin-like nitrite reductase*, *nitrate reductase*), with carbohydrate status (*phosphoenolpyruvate carboxykinase*, *6-phosphogluconate dehydrogenase*) and some DEGs encode *APETALA2/ERF*, *NAC* and *WRKY* transcription factors. qPCR confirmed the revealed differences in the expression level. Summing up the obtained results, we detected the general tendencies, which consist in the change of the core metabolic processes (nitrogen compound metabolism and carbohydrate processes). Thus, we may assume that the introgression of alien chromosome may disturb connections existing between genes, negatively influencing their expression rate. This can explain and later flowering of the substitution line CS-5Bdic as compare to CS. This study was supported by the Kurchatov Genomics Center of IC&G (075-15-2019-1662)

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Poster Title	Small RNA regulatory networks in tolerance and resistance responses of potato to potato virus Y infection
Abstract	Plant small RNAs (sRNAs) have been shown to be essential for the regulation of numerous biological processes. Recent studies showed that certain plant sRNAs play a central role in gene expression reprogramming and fine-tuning of defence responses against various pathogens, including viruses. The aim of our study was to identify key players in sRNA-regulatory networks controlling the establishment of tolerant and resistance responses of potato to potato virus Y (PVY), one of the most important viruses affecting potato, responsible for huge economic losses in worldwide potato production. Employing high-throughput sequencing technology, we have identified and quantified sRNAs in the two tolerant potato cultivars (cv. Désirée, Pentland-Squire), as well as a resistant one (cv. Rywal) and compared it to two sensitive potato genotypes, impaired in salicylic acid accumulation of salicylic acid (NahG-Désirée, NahG-Rywal). This information was linked to expression profiles of their target transcripts to link sRNA level responses to physiological processes. Besides the already described regulation of transcripts encoding immune receptors, we have discovered an interesting novel sRNAs gibberellin regulatory circuit that is activated only in the tolerant response of cv. Désirée. By comparing responses between the two tolerant potato genotypes we identified six commonly responding sRNAs, one of which presents an additional regulatory path directed towards the suppression of GA signalling that promotes tolerance against PVY. Additionally, several sRNAs have been found to be regulated in resistant but not in tolerant plants, showing that different sRNA-regulatory networks are activated for the establishment of tolerance and resistance. Taken together, we identified several sRNAs associated with the tolerant or resistant response of potato to PVY, and those candidates that are currently being functionally analyzed to better understand tolerance and resistance responses or as their potential to be used as breeding markers.

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Poster Title	Resilience to salinity in tomato
Abstract	Agriculture will have to feed an increasing world population, using a decreasing arable land surface. Salinity is an increasing problem, in particular in coastal or irrigated areas. Due to climate change, these traditionally fertile areas are suffering from increased soil salinity, which has reached concentrations higher than tolerated by current cultivation practices. In the near future these areas will no longer be suitable for cultivating food unless we adopt novel production practices, including the use of novel resilient plant varieties. For plants to be resilient to abiotic stresses such as salinity and drought, the root system is of vital importance. Roots are the primary organs that adapt their architecture and physiology to drought and salt stress. Their performance is key to the ability of the whole plant to take up nutrients and water. However, we have limited knowledge of how the root functions and this translates into a limited capability to control plant resilience to abiotic stress. Novel developments in biostimulants show that it is possible to affect root functioning and resilience towards abiotic stress such as high-salinity. However, despite their potential for agriculture, there is very limited knowledge on the mechanisms through which biostimulants act. In this study we use genome-wide association mapping and CRISPR/Cas9-mediated forward genetics to understand the mechanism by which biostimulants contribute to tomato resilience under salinity stress conditions. The link between changes in root architecture and salt stress resilience will be discussed together with its importance in understanding salt tolerance in tomato.

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Poster Title	Can we use tobacco for alternative metabolite production? An integrated approach exploration
Abstract	Tobacco leaves have been characterized as bio-factories as they can be used to produce important substances. They are a valuable source of aromatic and bioactive compounds such as phenolics and alkaloids. Greek cultivars have been long known for their distinct aromatic properties, but their full potential has not been explored yet, as we have minimal information on their genetic makeup. In this study presented here we report the analysis of the leaf transcriptome of three oriental tobacco varieties that were developed with traditional breeding techniques at the Institute of Plant Breeding and Genetic Resources and are cultivated extensively by Greek farmers. Un-biased detailed transcriptomics and metabolomics approaches were employed to identify and quantify changes in transcript and metabolite profiles in leaves of the three oriental tobacco varieties. Using modern tools such as next generation sequencing (NGS) and gas chromatography mass spectrometry (GC-MS) technologies, we detected a wide variety of transcripts and metabolites and we explored the metabolic diversity among varieties. We also identified genes with largest expression differences in the leaves; among them three were commonly over-expressed in two varieties in comparison with the third variety. We also emphasized on exploring the expression of genes that participate in the phenylpropanoid pathway. Notably, significant expression differences were recorded in phenylalanine ammonia lyase (PAL) genes that are key genes of phenylpropanoid biosynthesis. Furthermore, metabolomics analysis has shown that polyphenolic compounds varied widely among the three varieties with statistically significant differences in soluble sugars, alcohols, organic acids, amino acids and other metabolites also recorded. The use of combined *-omics* approaches in determining diversity of tobacco varieties offers important insights into the genetic background of important metabolites and constitutes a useful resource for future studies. As tobacco cultivation is set to decline, the exploration of alternative uses of this crop would be of the utmost importance.

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Poster Title	Using GT-seq as a cost effective next-generation SNP genotyping method for plant variety identification
Abstract	Single-nucleotide polymorphisms (SNPs) are used for many applications in agricultural genetics, including variety identification. With these applications, combined with the reduced costs for next-generation sequencing, it is now possible to efficiently identify thousands of SNPs directly from sequencing data. Genotyping-in-Thousands by sequencing (GT-seq) is a cost-effective next-generation genotyping method based on multiplexed targeted amplicon sequencing of relatively small SNP panels (50 – 500) for thousands of plant individuals at once (Campbell et al., 2015). With GT-seq we were able to generate SNP genotypes for more than a thousand raspberry individuals in one Illumina HiSeq run, using a panel of 214 SNPs. GT-seq follows a simple protocol and offers many benefits compared to SNP allele detection assays, including higher resolution, robustness, scalability, and compatibility for polyploid plants. Genotypes generated with this method can be stored in genotype databases, which can subsequently be used for variety identification and trueness-to-type control.

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Poster Title	Combining Various Genomic Strategies With New Technologies to Decipher the Complex Structure of Plant Genomes
Abstract	Among living organisms, plants display a high level of genomes complexity due to their large size, variations in ploidy levels and high percentage and variability of repetitive elements. Despite the Next Generation Sequencing revolution including the recent long read technologies, it remains challenging to obtain high quality assemblies at the genome scale. In order to be efficient, when addressing a scientific question, it is important to choose the relevant strategy according to with the raised topic: exhaustive information on whole genome is not always required while reliable and quality information of the region of interest is crucial and necessary. A reliable sequence information linked to a trait of interest in specific genotypes is essential to understand the role of a genomic region in a phenotype. The French Plant Genomic Resources Center (CNRGV) provides various innovative and efficient genomic tools to better characterize plant biodiversity and understand how plants adapt to their environment through the analysis of their genomes and the intra/inter-species variability. We develop several strategies combining large fragment genomic DNA libraries, CRISPR-CATCH targeting strategy and optical mapping technology combined with long reads sequencing technologies to obtain very high quality sequence. The complementarity of these strategies allows the production of reliable sequence information, which is essential to link a genotype to a phenotype.

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Poster Title	Development of a CRISPR/Cas9 Large DNA Fragment Targeting Technique for Plant Genomes
Abstract	To accelerate plant breeding, high quality assemblies are essential. They help to understand genome structure and to identify genes involved in agronomic traits including resistance to various environmental stresses, resistance to pathogens and high yield. However, the exploration of plant genomes remains challenging due to the complexity of plant genomes in terms of size, repetitive elements content and various levels of ploidy. Moreover, because of a high intra-species variability, a quality reference sequence is not enough to obtain a precise and reliable information of a genomic region linked to a trait of interest in a specific genotype. New strategies for efficiently targeting large regions of interest in complex genomes are really needed to be able to link a phenotype to a genotype. Here, we investigate the potential of the CRISPR/Cas9 system to target a 120 kbp genomic region of interest from a complex genome, the sunflower Helianthus annuus. We improved and adapted the first steps of the CATCH method (Cas9-Assisted Targeting of CHromosomal segments as described by Jiang et al., 2015). Then, we sequenced the targeted region with long reads sequencing approach coupled to the PacBio low input protocol. This strategy allowed the enrichment of the genomic region with high quality assembly. Thus, we propose a CRISPR/Cas9 based method amplification-free, with a simplified bioinformatical pipeline and a potential for multiplexing to sequence large genomic region of interest from plant genomes.

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Poster Title	Carrot genome editing using Cas9:gRNA complexes by PEG-mediated protoplast transformation
Abstract	Genome editing using the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) is a new and fast developing technology. Most currently used CRISPR systems consist of two components, i.e. a Cas protein responsible for the cleavage of DNA and a guideRNA (gRNA), which directs the Cas protein to the target region in genome, where both DNA strands are cut. The subsequent repair of a double-strand break is not perfect and often results in InDel mutations. As a result, the reading frame usually shifts, leading to the synthesis of a truncated and non-functional protein. Genome editing can be initiated by the delivery of the Cas9 gene and gRNA inserted to DNA vectors, but it causes the introduction of foreign DNA to the target cell. An alternative approach relies on the delivery of ribonucleoprotein (RNP) complexes composed of Cas9 protein and gRNA . The benefits are multifaceted, there is no need to optimize gene codons nor select the right promoter, and in particular, the edited plants contain no traces of foreign DNA . In the present research, we edited carrot genome using Cas9 RNP complexes. We designed gRNAs homologous to various sequences of the carrot flavanone 3-hydroxylase (F3H) gene, which is critical for anthocyanin biosynthesis. After in vitro transcription, gRNAs were incubated with the Cas9 protein. The resulting complexes were introduced into carrot protoplasts through PEG-mediated transformation. After a two-day incubation, the protoplasts were collected and DNA was isolated. Molecular analyzes including PCR, restriction digestion and Sanger sequencing were performed. PCR amplified F3H fragments were digested with endonucleases recognizing sequences of gRNA targets. Fractions of non-digested products were identified that indicated that DNA sequences of these target sites were modified. Genome editing was confirmed by Sanger sequencing, which revealed mutations. Hence, we show, for the first time, a successful carrot genome editing by the delivery of RNP complexes to protoplasts. **Acknowledgments** The financial support of the National Science Centre, Poland is acknowledged (decision Nos. 2018/02/X/NZ9/02629 and 2016/21/B/NZ9/01054).

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Poster Title	Unravelling genes in association with the multi-carpellary gynoecium in *Vuralia turcica*
Abstract	*Vuralia turcica* is an endangered, perennial, herbaceous, and endemic species found near the Eber and Akşehir lakes in Central Anatolia (Turkey) and kept under protection at Nezahat Gökyiğit Botanical Garden, Istanbul (NGBB). It is a member of the Fabaceae family and Papilionoidae sub-family. *Vuralia turcica* is special for its multi-carpellary gynoecium, a feature that has not been observed in other Papilinoidae species. This feature makes *Vuralia turcica* an attractive genetic resource for understanding the genetic basis of multiple carpel formation; once the responsible genes are identified, these could then be engineered by gene editing techniques into other nutritionally important Fabaceae species, such as faba bean and common bean. Fresh plant material was collected from NGBB between March and May 2019. Samples were collected as 18 different developmental stages including seven generative stages from flower buds to embryo formation. RNA isolation was carried out using the Qiagen RNeasy Plant Mini Kit with the Qiagen DNase Kit used to eliminate DNA molecules. RNA samples were sequenced using paired-end Illumina RNAseq techniques. After receiving the raw reads, adapters were trimmed off and quality control was performed using *fastqc.* Transcripts were assembled using Trinity *de novo* assembly pipeline. 96-98% of reads were assembled for every sample. Blastx, Transdecoder, and Trinotate were used to investigate identified genes found in gene ontology databases. RSEM was conducted to count transcript abundance and edgeR to calculate differential expression between different developmental stages. As a result, 100 transcripts showed increasing fold change (≥2, *p*-value≤0.001) at the stage when first flower buds emerged. Hypothetically, that should be the moment when floral meristem cells activate cadastral genes and initiate generation of carpels, however, this still requires confirmation with electron microscopy. Out of these 100, 10 transcripts were previously identified in the literature to be highly expressed in floral organs (carpels and stamens) and shoot apex inflorescence. There were yet many other candidates that might regulate multi-carpel generation in *Vuralia turcica* and some of these might be specific to this endemic species. Mapping of the differentially expressed genes on to known plant signalling and metabolic pathways *in silico* will help to identify the strongest candidates for regulation of carpel development. The next step will be the confirmation of these candidate genes through knock-out mutants using CRISPR/Cas9 methods in *Vuralia turcica*. In future, they might contribute increasing seed production in other Fabaceae species.

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Poster Title	Molecular Combing, new perspectives for plant genomics
Abstract	Many plant genomes are large, full of repeats and often polypoid, making them challenging to sequence and assemble using traditional sequencing technologies alone. Here we present how Molecular Combing, a proprietary technology to directly visualize single DNA molecules, can address some of these challenges and resolve complex regions of plant genomes. Associated with a unique detection strategy, the Genomic Morse Code, Molecular Combing allows to analyse mid-size to large structural variants (SV) or rearrangements (>2kb to several Mb) in a single experiment. We will present and discuss different examples that illustrate how Molecular Combing allows (i) the digital quantification of rare and complex rearrangements or SV (ii) the analysis of transgene copy number and pattern of integration; (iii) the analysis of repeats (rDNA, telomeric repeats…).

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Poster Title	The use of grafting to study the influence of canopy growth rate on earliness of tuber initiation and metabolite composition of potato tubers
Abstract	Potato (*Solanum tuberosum* L.) is an important staple food, which represents an excellent source of nutrients including carbohydrates, proteins, vitamins and minerals. However, potato is very sensitive to environmental conditions and adversely affected by different stresses. Early bulking potato cultivars are more profitable for the growers and are candidates for stress escapers, as they can complete their life cycle before stress becomes a serious constraint. Thus, breeding early tuberization potatoes is a major goal of plant breeders. Our study was aimed to find out whether tuber metabolites are influenced by the earliness of tuber initiation. To accomplish this goal grafting experiments with two Hungarian potato cultivars, 'White Lady' (WL) and 'Hópehely' (HP), different in tuber metabolite composition, were carried out under greenhouse conditions with 16/8 light/dark cycle in pots. Measuring the leaf area and counting the tuber number at an early stage of plant development a positive correlation between canopy growth rate and earliness of tuber initiation was found. At the end of the vegetative period the tubers were harvested and a non-targeted metabolite analysis was performed using gas chromatography-mass spectrometry (GC-MS). Based on the amounts of 30 polar metabolites a significant difference in metabolite composition of WL and HP tubers was detected. Although, this difference was less than that detected earlier under field conditions. Principal component analysis (PCA) of the data did not show a significant influence of homo- and heterografting on the metabolite composition of tubers. The metabolite composition of source leaves collected at the early stage of plant development was also analysed by GC-MS. A significant difference in galactinol concentration of HP and WL leaves was found, which was slightly influenced by grafting. In sum, our results supported the previous finding that the earliness of tuber initiation is determined by mobile signals originating from leaves, while, in contrast, the concentrations of major polar metabolites of tubers are not influenced by scion in the case of cultivars HP and WL.

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Poster Title	Comparative transcriptome analysis of spikes of rye (*Secale cereale*) hybrids with differential ergot response
Abstract	***Background*** Rye is used as food, feed and for bioenergy production and remain an essential grain crop for cool temperate zones in marginal soils. Ergot is known to cause severe problems in cross-pollinated rye. The genetics of underlying mechanisms of this disease is still poorly understood due to complex infection pattern and large genome size of rye. RNA sequencing (RNAseq) can provide astonishing details about transcriptional landscape of specific disease pathway. ***Results*** In this study we generated two *de novo* assemblies of rye hybrids with contrasting responses to ergot infection. The final transcriptome assemblies of ergot susceptible and ergot resistant hybrids contain 208690 and 192116 contigs, respectively. By applying the BUSCO pipeline, we confirmed that these transcriptome assemblies contain more than 90% of gene representation of the available ortholog groups at *Virdiplantae odb10*. We employed different strategies using two different datasets to count the differentially expressed genes (DEGs). The gene expression comparisons revealed that 389 genes linked to ergot and GO enrichment analysis of DEGs associate them to metabolic processes, hydrolase activity, pectinesterase activity, cell wall modification, pollen development and pollen wall assembly. Similarly, gene set enrichment analysis (GSEA) of DEGs linked them to cell wall modification and pectinesterase activity. These results emphasized that combination of different pathways, particularly cell wall modification and pectinesterase activity could contribute to underlying mechanism leading to resistance against ergot in rye. ***Conclusion*** We generated two de novo assemblies from the spikes of two rye hybrids and demonstrated how ergot influence transcript expression that has allowed us to identify and annotate transcripts associated with ergot resistance. Our results may pave the way to rye breeders to develop methods and to select genetic material to improve resistance against ergot. Furthermore, this sequence data and de novo assemblies are valuable scientific resources for future studies in rye.

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Poster Title	Xdrop™ Strategies for Targeted Enrichment to Close Sequence Gaps in Poorly Assembled Genomes
Abstract	Large genome size and polyploidy of many eukaryotes has prevented whole genome sequencing and a thorough characterization of these genomes to the extent known from human and some primates. Apart from the bare size of some eukaryote genomes, regions with repeats and high GC content remain a challenge for the next generation sequencing technologies. The consequence is that many draft genomes include mis-assemblies and gaps, making it difficult to understand genetics and utilize genomic information e.g. in precision breeding even for some of the eukaryotes with moderate genome sizes. Long read sequencing technologies like Oxford Nanopore and PacBio are able to sequence through repeats and GC-rich regions but costs of whole genome sequencing can be prohibitive. Here we present Xdrop™, a new microfluidic droplet-based technology for targeted enrichment of genomic regions up to 100-150kb, compatible with downstream analysis by both long and short read sequencing technologies. The Xdrop™ enrichment is unaffected by repeats or high GC content and enables gaps-closing in unknown genomic regions. The only requirement for the enrichment is that a primer set can be designed to generate a short 150-180bp amplicon from a region neighboring the unknown region. The limited requirement for the known sequence also makes the approach ideal for resolving genomics landscapes with only partial sequence information, such as the one deriving from mRNA transcripts. Here we describe the potential of the Xdrop™ enrichment technology to resolve sequence gaps, characterize CG-rich or repetitive regions, distinguish genes from pseudogenes, analyze paralogues, and phase alleles to significantly improve poorly assembled genomes.