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Poster Presentation Abstracts

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Poster Title	Application of molecular data to construct common bean core collection (Central and South Eastern European germplasm example)
Abstract	<p>Genetic diversity of different genetic resources, including common bean has been evaluated in numerous studies using different DNA markers. Information on genetic diversity of common bean from Central and Eastern Europe is scarce; therefore our study was a basis for construction common bean core collection using Central and East European germplasm. Regarding to basic multi-crop passport descriptors and seed characteristics including geographic origin, biological status, ancestral data, phenotypic seed characteristics and different phaseolin type (corresponding Andean/Mesoamerican origin), 782 accessions from 9 gene banks and 12 geographic origins were selected. Genotyping procedure was performed to assess their genetic background. Based on results of our recent studies and literature survey, we decided for the application of SSR markers as being informative, reliable, codominant and cost-effective. For that purpose, we selected 33 genome-specific and highly polymorphic nSSR and EST-SSR markers with different repeat motives covering genetic diversity of <i>P. vulgaris</i> germplasm with equal distribution among all linkage groups among <i>P. vulgaris</i> genome (24 markers), gene pool determination (3 indel-spanning markers of <i>Pv SHP1</i> gene) and some trait related loci (6 markers). Fragment analysis was performed using Genetic Analyser ABI3130XL. In total, we genotyped 25.806 electropherograms and codominant matrix of allelic profiles was obtained. Bioinformatics was performed applying different programs and software packages including GenAIEx, Structure, Structure Harvester, GenePop, Arlequin, Identity, Populations, Genetix, TreeView, MSToolkit. Specific parameters of genetic variability showed extremely high level of genetic diversity ($H_e=0.822$); cluster analysis with Bayesian approach determined real $K=10$ ($H_e > 0.65$). It was also proven that selected set of nSSR and EST-SSR markers was highly informative and polymorphic ($PIC>0.8$). Obtained molecular data provided reliable information about the genetic structure, origin and genetic relations among 782 geographically and phenotypically diverse genotypes. With obtained molecular data we proposed core collection encompassing 63 highly diverse accessions covering diverse environments representing rich source of agronomically important traits and (multiple) alleles related to nutritional value and resistance potential to biotic and abiotic stress.</p>

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Poster Title	The effects of <i>Ascophyllum nodosum</i> extract and its possible oligosaccharides on the soybean gene expression.
Abstract	<p>The application of brown seaweed <i>Ascophyllum nodosum</i> and its corresponding extract (ANE) on plants has been widely reported to be beneficial for both the promotion of growth and the alleviation of plant stress. However, the bioactive components within ANE and associated modes-of-action are still poorly understood. The fungus, <i>Mycophycias ascophylli</i>, is a naturally occurring mutualistic endosymbiont of <i>A. nodosum</i>. Under the hydrolyzing processes of making ANE, polysaccharides from the cell walls of both <i>A. nodosum</i> and its microsymbiont, <i>M. ascophylli</i>, create corresponding oligosaccharides which make up the major components within ANE. To investigate the bioactive oligosaccharides within ANE and its associated actions at the molecular level, whole genome wide transcriptome profile of soybeans treated with ANE, oligo-alginate, oligo-chitin, and oligo-chitosan were compared and analyzed. Our results demonstrate similarity in plant gene expression patterns among ANE, oligo-chitin, and oligo-chitosan in treated soybeans. Treatment with ANE, oligo-alginate, oligo-chitin and oligo-chitosan up-regulated the expression of genes related to plant stress responses, especially with respect to the synthesis of plant secondary metabolites. Interestingly, three of the treatments (ANE, oligo-chitin and oligo-chitosan) inhibited the expression of a large number of genes involving in ribosomal protein biosynthesis and other energy consuming processes. It appears that the enhancement of stress responses following the treatments with ANE, oligo-chitin and oligo-chitosan were at the cost of the plant's general energy consuming process. Results from this study suggest that ANE and compositional oligosaccharides of <i>M. ascophylli</i> are promising agents that can potentially prime the plants to deal with the impending adversities and, at the same time, save the plant energy. As ANE is a mix of a myriad of components, additional studies are needed to explore the full spectrum of bioactive components within it, the result of which could increase the effective use of ANE in agricultural practices.</p>

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Poster Title	Combining Protein Sequence Similarities and GO-term Semantic Similarities Improves Protein Function Prediction in Arabidopsis
Abstract	<p>We use machine learning to automatically assign Gene Ontology (GO) terms to Arabidopsis Thaliana proteins. Our method creates a similarity profile of each protein based on its sequence similarity to a set of annotated proteins. GO terms are then assigned to unannotated proteins based on the annotations of proteins with the most similar profiles. We exploit the inherent redundancy of GO terms imposed by their hierarchical structure and transform them into a more compact function representation that is easier for machine learning methods. We propose two new such transformations one based on the GO hierarchy and one based on semantic similarity of terms.</p>

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Poster Title	RNA-Seq analysis of Orobanche resistance in tobacco: development of molecular markers for breeding recessive resistance from Wika tobacco variety.
Abstract	<p>Orobanche spp. (broomrapes) is an obligate root parasite that can attack a wide spectrum of plants, including tobacco. It is responsible for economic losses in Europe since 2002 and its incidence in many tobacco growing countries is increasing. Preventive and curative methods exist, including the use of agrochemicals, however dissemination is important due to a high rate of multiplication of the parasite and very small seeds.</p> <p>The tobacco variety Wika shows lower or later germination of Orobanche seeds. This seems to be conditioned by a single recessive gene (Cailleateau, Coresta 2006). An artificial testing in Petri dishes was developed to evaluate the ability of tobacco plantlets to stimulate seeds germination. Different lines derived from Wika, with susceptible control lines, were tested and studied by RNA-Seq. Candidate markers including SNPs or genes differentially expressed between susceptible and resistant lines were identified. A F2 population segregating for Wika recessive tolerance was then used for validation and mapping. All the candidates mapped on chromosome 14 of the tobacco genetic map. The Nicotiana collection of varieties from Imperial Brands was also tested with these markers, highlighting or confirming others potential donor.</p> <p>KASP™ genotyping or markers for conventional gel electrophoresis are now available to pilot the transfer of Wika recessive tolerance into elite lines.</p> <p>RNA-Seq technology combined with good experimental testing has proven again its high efficiency to identify useful markers for tobacco breeding.</p>

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Poster Title	Simple and robust genotyping by next generation sequencing of plant DNA.
Abstract	<p>Genotyping by next generation sequencing has significant advantages over traditional genotyping methods, including the ability to interrogate thousands of markers at high resolution while discovering newly introduced SNPs that may contribute to phenotypic changes. Plant genotyping presents additional challenges in target enrichment design, library preparation, and data analysis, arising from incomplete or absent reference genomes, repetitive sequences, and the presence of enzyme-inhibiting contaminants in DNA samples.</p> <p>In an attempt to provide a simple and inexpensive method for NGS-based plant genotyping, we modified the NEBNext Direct target enrichment method to optimize the method for GBS and tested it with a variety of plant species, both with and without reference genomes. In the NEBNext Direct protocol, DNA is enzymatically nicked, denatured, and then enriched through hybridization-based capture. After capture, the desired fragments are bound to beads, washed, and converted into Illumina-compatible libraries using an enzyme-based strategy to reduce the conversion of non-target sequences into library. The results indicate highly specific target enrichment with 90-99% of the inserts mapping to the targeted regions. Of note, blocking repetitive DNA regions during hybridization was not required to achieve this level of specificity. No difference was observed in the specificity of capture between species that had a reference genome available for bait design versus species with only amplicon sequences available. In addition, libraries prepared from CTAB-purified DNA demonstrated only a small decrease in conversion compared to libraries prepared with higher quality, column-purified DNA, suggesting that contaminants present in CTAB-purified DNA are compatible with the NEBNext Direct method. Thus, our modified NEBNext Direct target enrichment protocol provides cost-effective and robust enrichment of plant targets without the need for costly and time-consuming DNA purification and repetitive-sequence blocking DNA, and this approach can be applied to species with little available genomic information.</p>

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Poster Title	In situ mapping in the whole-genome sequencing era
Abstract	<p>The rapid progress in genome sequencing and handling very large datasets have opened boundless prospects in genome study, comparative genomics and gene manipulations. It seems now we are able to get information about structure and genome position of any target gene. Is still room for molecular cytogenetics? While modern tools can produce very long reads the chromosome level assembly is still major bottleneck in genome sequencing projects. The whole-genome sequencing has encountered assembly problems. Previously developed genetic maps greatly assist the assembly of genomes. However, the regions of recombination suppression remind uncovered in genetic maps. Using <i>in situ</i> mapping it is possible to build a reliable backbone for the chromosome level genome assembly. In the last decade, the sensitivity and resolution of <i>in situ</i> mapping have been significantly improved. A robust and routine method, SteamDrop, for high quality plant chromosome preparations suitable for repetitive and unique DNA visualization was developed (Kirov et al. 2014). To accelerate karyotype studies, we developed DRAWID (DRAWing IDiogram), java-based cross-platforming program for physical mapping (Kirov et al. 2017). An ultra-sensitive Tyramide-FISH method allowed to visualize a small unique DNA sequence (Khrustaleva & Kik 2001). To increase in situ specificity of large multigene family mapping we used transcriptome data to produce genomic amplicons from expressed regions that carried both exons and introns for probe preparation (Romanov et al. 2015). With these approaches we were able to map highly expressed alliinase multigene families on physical chromosomes of <i>Alliums</i> unraveling unique genome reorganization events. In the future, the construction of high density cytogenetic maps will accelerate of whole-genome assembly. An integrated approach is needed including different sequencing strategies as well as independent instruments such as molecular cytogenetic mapping.</p> <p>The work was supported by Russian Scientific Foundation (grant №16-16-10031).</p>

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Poster Title	Plant repeatome: bridging the gap between linear DNA sequence and genome structure
Abstract	<p>Genome sequences are becoming more useful and attractive in a light of knowledge about the genome organization and structure. However, we still have limited number of techniques allowing making a bridge between linear DNA sequence and genome structure. Molecular cytogenetics provides a panel of methods for DNA sequence visualization on nucleus or chromosomes bridging the gap between in silico genome research and in vivo genome organization. Probably, one of the most useful current applications of cytogenetics is to draft the physical map of repeatome (the complement of repeated sequences in a genome). We are studying repetitive DNA sequences of huge <i>Allium</i> genome to shed light on plant repeatome biology and to develop chromosome markers that are useful for plant breeding. Several multicopy tandem repeat sequences have been isolated from <i>Allium cepa</i> and <i>A.fistulosum</i> genomes. These sequences were located in heterochromatin and centromeric regions using cytogenetic techniques. Based on the results we found that <i>Allium</i> may possess the longest functional centromere satellite sequence known to date. In addition, this sequence in combination with other <i>Allium</i> satellites is applicable as chromosome markers and can be further used for integration of genomic data with heterochromatin structure. In addition, using RNAseq NGS data we found the transcriptomic context of the repeat location and showed that putative lncRNAs carrying repeats present in <i>Allium</i> cells. The future perspectives of repeatome biology research using genome editing will be discussed.</p> <p>The work was supported by Russian Scientific Foundation (grant № 17-46-07005)</p>

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Poster Title	DRAWID: user-friendly java software for chromosome measurements and idiogram drawing
Abstract	<p>Chromosome morphology is known to be important source of knowledge for genome organization and evolution studies. The data integration from classical chromosome analysis and idiogram construction and molecular cytogenetics (FISH, GISH, banding etc.) is important for better understanding of karyotype features. An idiogram construction following chromosome measurements is a versatile tool for cytological, cytogenetic and phylogenetic studies. However, none of these programs is able to simultaneously measure chromosome parameters and chromosomal landmark positions (e.g. band, FISH and GISH signals), allowing idiogram construction. We developed the DRAWID (DRAWing IDiogram) – program for chromosome analysis and idiogram construction. DRAWID is a user-friendly and freely available (under GNU General Public License) java-based software program that facilitates basic as well as ISH-based karyotype analysis. DRAWID is equipped with an intuitive graphical user interface. DRAWID has number of advantages including a user-friendly interactive interface, possibility for simultaneous chromosome and FISH/GISH/banding signal measurement and idiogram drawing as well as number of useful functions facilitating the procedure of chromosome analysis. The output of the program are Microsoft Excel table and publish-ready idiogram picture. DRAWID and the manual for its use are freely available on the DRAWID website at http://www.drawid.xyz.</p> <p>The work was supported by Russian Scientific Foundation (grant №16-16-10031).</p>

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Poster Title	Candidate genes for resistance to <i>Fusarium oxysporum</i> f.sp. <i>cubense</i> Race 1 in the wild diploid banana species <i>Musa acuminata</i> var. <i>malaccensis</i>
Abstract	<p>Banana is one of the most important fruit crops in the world and a staple food in many tropical countries. The current annual production is over 100 million tons, but this is threatened by one of the most devastating fungal diseases: Fusarium wilt. The causal agent is <i>Fusarium oxysporum</i> f.sp. <i>cubense</i> (<i>Foc</i>). During the 1950s the “Gros Michel” banana based industry was devastated by <i>Foc</i> Race 1. However, the epidemic was quenched and the banana industry was saved by replacing “Gros Michel” with resistant “Cavendish” bananas. Surprisingly, the responsible gene(s) for resistance are still unknown. We used the self-compatible wild banana accession <i>Musa acuminata</i> var. <i>malaccensis</i> (<i>Mam</i>, AA, 2n=22) from the Sumatra <i>Musa</i> population to generate a mapping population and to investigate the inheritance of resistance to <i>Foc</i> Race 1. Initial greenhouse bio-assays confirmed that <i>Mam</i> is resistant to Race 1. The F1 population was generated from 272 pollinated flowers and resulted in 3,458 seeds of which 718 were embryo rescued, and eventually 255 off-spring survived and were maintained in tissue culture. The mapping population was genotyped (N=244) using DArTseq markers and subsequent phenotyping (N=225) revealed segregation for resistance. After strict filtering, 4,171 SNP markers were used for genetic mapping. Analyses of the genotyping and phenotyping data showed the inheritance of a single dominant resistance gene that mapped near the distal part of chromosome 10, based on the reference genome of doubled haploid ‘Pahang’, which is also a <i>Mam</i> accession. The recombination between the markers among the selected recombinants, together with the position of the putative resistance gene, were further analyzed using graphical genotyping and resulted in markers that flank a 360 kb genetic region containing at least 14 NBS-LRR like resistance gene candidates, including the identified candidate gene for resistance to <i>Foc</i> Race 1.</p>

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Poster Title	Identification of novel priming agents by monitoring the expression of defence genes in rice cell suspension cultures
Abstract	<p>Priming agents are compounds that put the plant defence system in an increased state of alertness. Upon subsequent stress exposure, primed plants activate their defence response in a faster and more robust way. Due to the current ban on a large number of traditional pesticides in the EU, discovery of novel priming agents is urgently needed.</p> <p>We are constructing a platform for high-throughput screening of potential priming agents to screen libraries of natural compounds, among which potentially novel priming agents. This platform is based on a set of rice defence associated genes (DAGs), which we have identified using a 'weighted gene co-expression network analysis' (WGCNA). In this meta-analysis, more than 350 micro-array datasets of rice under a variety of biotic stresses were analysed. A list of 36 potential DAGs, activated by biotic stress, was selected for further investigation.</p> <p>The upregulation of the DAGs was validated using an <i>in planta</i> experiment. Expression levels of the 36 potential DAGs were monitored by RT-qPCR, after treatment with different pathogen/damage-associated molecular patterns (PAMPs/DAMPs): lipopolysaccharides, oligogalacturonides and "NemaWater". NemaWater, a nematodal PAMP, was found to be most potent in terms of induction of the selected DAGs. A final set of 10 consistently induced DAGs was selected based on these results.</p> <p>By monitoring the expression of the 10 DAGs in rice cell suspension cultures, the intensity of the plant defence response can now be investigated. As a positive control, the priming activity of β-Aminobutyric acid (BABA) is currently being validated in rice cells. To mimic pathogen attack, we add PAMPs to the cell culture, and we screen for enhanced defence gene expression upon previous exposure to BABA.</p>

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Poster Title	Study of the physiological importance of rice EUL lectins in root development and drought stress response using CRISPR technology.
Abstract	<p>Rice is a highly important agricultural crop, providing more than one fifth of the calories consumed worldwide by humans. In addition, rice is also a very attractive model plant for monocots. Unfortunately drought is an important threat for rice, and can reduce the crop yield by more than 50%.</p> <p>Due to climate change and the increasing world population, the severity and the frequency of drought periods are increasing. The Euonymus lectin (EUL) family represents a group of stress inducible lectins that is ubiquitous in all terrestrial plants. In the rice genome, the EUL family consists of five genes. The five members can be further divided in the D-type EUL genes and S-type EUL genes and carry respectively two and one EUL domain. We have obtained evidence that the expression of the D-type EUL genes in rice is altered as a response to dehydration and treatment with the drought hormone abscisic acid. GUS promoter fusion constructs have revealed the activity of EUL promoter sequences in the vascular system as well as in the root tips of the main and lateral roots.</p> <p>To further confirm the role of D-type EULs in root development and their adaptation against stresses, transgenic lines will be used including overexpression and knock out (KO) lines. The CRISPR technology is used to create single KO lines for three individual EUL genes, named OsEULD1a, OsEULD1b and OsEULD2. Because of the high degree of sequence similarity between the EUL domains (77-88% sequence identity/ 85-95% sequence similarity) it was possible to envisage one triple KO targeting all the D-type genes.</p> <p>The transgenic lines are being evaluated for the growth and performance under normal and stress conditions. The perception of stress by the plant can result in alteration of metabolic processes, repression of cell growth, and reduction of photosynthesis. Therefore the plant experiments will be complemented with biochemical and physiological analyses.</p>

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Poster Title	SES: Synthetic expression system for plants
Abstract	<p>We have developed a novel orthogonal expression system (SES) that functions in a wide spectrum of eukaryotic organisms, including fungi and plants. The expression system is based on a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by a universal core promoter, which was obtained by a specifically designed screening assay. The universal core promoter provides highly constitutive expression level of the sTF, which is employed in the SES system as a potent transcription activator for the target gene. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter, making the whole expression system independent of the host's native regulation and therefore functional in diverse species. The varying number of the sTF-binding sites in combination with a choice of core promoters enable adjustment of the target gene expression levels over a wide range, from very low to very high, which is particularly difficult in plant hosts with current genetic tools. This expression system provides robust and stable expression levels of target genes in a broad spectrum of host organisms with numerous applications in metabolic engineering and protein/enzyme production.</p> <p>The method for selecting the universal core promoters, construction of the expression system, and demonstrating its performance in comparison with the established CMV promoter system will be presented. In addition, the utility of the expression system will be demonstrated for the production of diverse recombinant proteins in tobacco leaves.</p>

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Poster Title	Comparative <i>de novo</i> transcriptomic profiling of salinity stress responsiveness in contrasting pearl millet lines
Abstract	<p>Pearl millet (<i>Pennisetum glaucum</i> (L) R. Br.) is a staple crop of more than 90 million poor farmers. It is known for its drought, salinity and high temperature tolerance. To understand the molecular mechanism underlying its salinity tolerance, a comparative transcriptome analysis between salinity tolerant (ICMB 01222) and susceptible (ICMB 081) lines were conducted under control and salinity conditions. Sequencing using Illumina HiSeq 250 generated total 977 million reads, and these reads were <i>de novo</i> assembled into contigs corresponding to gene products. A total of 11,627 differentially expressed genes (DEGs) were identified in both lines. In ICMB 01222, 2965 genes were up-regulated by salinity stress, and 2946 genes were down-regulated. In ICMB 081, 2243 were up regulated by salinity stress, and 3473 were down-regulated. Of these DEGs, 1287 up-regulated genes and 1451 down-regulated genes were common across both lines. These DEGs are involved in various metabolic pathways such as plant hormone signal transduction, mitogen activated protein kinase signalling pathways, etc. Genes involved in ubiquitin-mediated proteolysis and phenylpropanoid biosynthesis pathways were up-regulated in the tolerant line. In contrast, genes involved in glycolysis/gluconeogenesis and genes for ribosome were down-regulated in the susceptible line. Genes encoding SBPs (squamosa promoter binding proteins), which are plant-specific transcription factors, were differentially expressed only in the tolerant line. Ten randomly selected DEGs expressions were confirmed by quantitative real time PCR. Identified functional genes and pathways can provide useful clues for improving salinity stress tolerance in pearl millet</p>

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Poster Title	Towards the pBC'4 in accelerated introgression breeding for fire blight resistance using a "Fast Track" approach in apple
Abstract	<p>The bacterial disease fire blight, caused by the <i>Erwinia amylovora</i>, is a major threat of the apple fruit production all over the world. Breeding for fire blight resistance is main topic in the apple breeding program at Agroscope since the devastating disease outbreak in Switzerland 2007. The aim is to combine strong resistances from wild apple genotypes with high fruit quality, good storage abilities, regular yield security and high tolerance/resistance to other diseases. The use of fire blight resistant cultivars in combination with an integrated control management is a promising control strategy (Baumgartner et al. 2014). However, currently only a few partially resistant cultivars are ready for the market (e.g. 'Ladina', Kellerhals et al., 2011). Strong fire blight resistances are found in the natural genetic diversity of wild <i>Malus</i> species, e.g. <i>M. x robusta</i> 5 (MR5), <i>M. baccata</i>, <i>M. fusca</i> (Peil et al. 2009; Emeriewen et al. 2014). However, fruit size and quality of wild resistance genitors are insufficient and not comparable with modern apple cultivars available on the market. In order to achieve commercial quality combined with fire blight resistance from a wild species, several pseudo-backcrosses (pBC') with high quality parents are required. To accelerate the long generation cycle of <i>Malus</i> (4 to 5 years till first blooming), a low-input "Fast Track" breeding approach under greenhouse conditions was tested and continuously improved. This method combined with marker based selection allows us to speed up the generation cycle (approximately 2 years till first blooming) using a non GMO approach. The established method used today and results of the latest generations carrying <i>FB_MR5</i> and <i>Fb_E</i> will be presented, including results of artificial shoot inoculation, population studies, breeding achievements and fruit quality development.</p> <p>Baumgartner I.O., Patocchi A., Lussi L., Peil A. und Kellerhals M.: Accelerated introgression of fire blight resistance from <i>Malus x robusta</i> 5 and other wild germplasm into elite apples. Acta Hort. 1056, 281–287, 2014.</p> <p>Kellerhals, M., Franck, L., Baumgartner, I.O., Patocchi, A., and Frey, J.E. 2011. Breeding for Fire Blight Resistance in Apple. Acta Hort. (ISHS) 896: 385-389.</p> <p>Peil, A., Bus, V.G.M., Geider, K., Richter, K., Flachowsky, H., and Hanke, M.-V. 2009. Improvement of Fire Blight Resistance in Apple and Pear. Intl. J. Plant Breed. 3: 1-27.</p> <p>Emeriewen, O., Richter, K., Kilian, A., Zini, E., Hanke, M.-V., Malnoy, M., Peil, A., 2014: Identification of a major quantitative trait locus for resistance to fire blight in the wild apple species <i>Malus fusca</i>. Molecular Breeding 34, 407-419.</p>

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Poster Title	GFP-based transient gene editing marker system to support selection of agronomic traits: A case study of targeting elevated tryptophan content in maize
Abstract	<p>The development of efficient and reliable tools for generation of precise, targeted changes in the genome of plant cells is a long-standing goal for researchers. Although gene technology and conventional transgenesis became integrated components in basic plant science and crop improvement, there are great expectations to develop efficient and widely applicable genome editing methods for plants. Developments in strategies of the oligonucleotide targeted nucleotide exchange (OTNE) and CRISPR/Cas9 system for effective genome editing are in focus of ongoing research and can provide breakthroughs in breeding applications. In the present study, as a novel approach, we test the combination of a non-functional green fluorescent protein gene marker (mGFP) and a selectable agronomic trait as induction of feedback insensitive anthranilate synthase (AS) mutants. The AS enzyme plays a key role in the biosynthesis of tryptophan (Trp), and the alpha subunit of AS is susceptible to feedback inhibition by Trp or its analogues (e.g.: 5-methyl tryptophan, 5-MT). Well characterized AS mutations confer Trp insensitivity (Saika et al, 2011). Experiments presented here are based on using stable transgenic maize cell lines carrying the non-functioning mutant GFP gene (Tiricz et al. 2017) integrated into the genome, as well as a transient transformation-based system on rice, cell suspensions. In the latter case, the mutant pEGAD-mGFP marker plasmid was co-delivered with SDO^{GFP} and SDO^{AS} molecules. The CRISPR based genome editing was also tested in both experimental setups. We conclude that both approaches are suitable for mGFP correction which helps locate and enrich potential “genomic target” mutants (in our case AS gene) confirmed by their 5-MT resistance. Cells expressing successfully edited GFP gene are identified by using confocal and stereo fluorescence microscopy and picked for further growth in isolation. We propose this approach as a general methodology for producing novel genotypes of useful mutations in agronomic traits and selection of mutants even in the case when the target character has no phenotype at the cell level <i>in vitro</i>.</p> <p>Saika H. <i>et al</i> (2011) Application of gene targeting to designed mutation breeding of high-tryptophan rice. <i>Plant physiol.</i>, doi.org/10.1104/pp.111.175778 Tiricz et al (2017) Relaxed chromatin induced by histone deacetylase inhibitors improves the oligonucleotide-directed gene editing in plant cells. <i>J Plant Res</i> doi: 10.1007/s10265-017-0975-8.</p>

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Poster Title	Comparison of two different genome-editing technologies (OTNE and CRISPR/Cas9) in maize (<i>Zea mays</i> L.) and rice (<i>Oryza sativa</i> L.) cells
Abstract	<p>Beside the traditional plant breeding methods, the modern technologies, such as “directed mutagenesis” can play pivotal role in the generation of the desired cultivars. These novel methods allow us to modify target gene sequences with high precision, hence significantly shorten the duration to develop new genotypes. In this study, we compared the efficiency of two different genome-editing methods namely OTNE (oligonucleotide-targeted nucleotide exchange) and CRISPR/Cas9 (Clusters of Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) in correcting the gene sequence of a mutant, non-functional green fluorescent protein (mGFP) in cultured maize and rice cells. We tested the efficiency of DNA correction both using a transient transformation based system on rice (<i>Oryza sativa</i>) cell suspensions, as well as by using stable transgenic maize cell lines carrying the non-functioning mutant GFP gene integrated into the genome (Tiricz et al. 2017). In the transient delivery experiments, plasmid with mGFP gene was co-delivered with correcting oligo or CRISPR components and correction of GFP expression was verified by fluorescence microscopy of maize and rice cells. To increase the resistance of oligonucleotides against cellular exonucleases, the oligonucleotides were synthesized with chemical modification by incorporation of phosphorothioate (PTO) linkages near both the 5' and 3' ends . Unmodified, native oligonucleotides were also incorporated in experiments as comparison. We also tested the editing efficiency of different lengths of template oligonucleotides. According to the preliminary data, both OTNE and CRISPR-based approaches can equally be used for biolistic delivery-based gene editing in plant cells. We discussed the advantages and disadvantages of both approaches including the importance of parameters such as chemical modification and length of template oligonucleotides used in editing experiments.</p>

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Poster Title	Chemically modified template ssDNAs and gRNAs for increasing the efficiency of plant gene editing
Abstract	<p>Improving the efficiency of the directed gene mutations is a prerequisite for their wide application in plant science and breeding. Homologous recombination (HR) is the key mechanism that allows their incorporation. In order to increase the efficiency and gain a wide range of applicability, the frequency of the HR and the specificity of the gene editing have to be increased. These can be achieved by using chemically modified template oligonucleotides and gRNAs (Kelley et al., 2016; Kunwoo et al., 2017). Therefore, we aimed to design and synthesize chemically modified template ssDNA and gRNA molecules to clarify the influence of the chemical structural factors on the mutation efficacies. On one hand, lipid molecules were coupled to template oligonucleotides to enhance their uptake into plant protoplasts and increase their nuclease stability and on the other hand, chemical modifications were incorporated into crRNAs to enhance their specificity and stability against endogenous RNases.</p> <p>These chemically modified ssDNAs and gRNAs were tested in our phenotypic assay, based on transgenic maize cell lines expressing the non-functional Green Fluorescent Protein (mGFP) gene carrying a premature TAG stop codon (Tiricz et al, 2017). These transgenic cells were bombarded with template oligonucleotides and ribonucleoprotein complexes of chemically modified gRNAs and Cas9 protein, to recover GFP expression. In parallel, protoplasts of mGFP expressing transgenic maize cell lines were treated with lipid modified template oligonucleotides. In both experiments, the repair of green fluorescent protein function was monitored by confocal fluorescence microscopy.</p> <p>Kelley ML et al (2016) Versatility of chemically synthesized guide RNAs for CRISPR-Cas9 genome editing. <i>J Biotechnol.</i> 233:74-83.</p> <p>Kunwoo Lee et al. (2017) Synthetically modified guide RNA and donor DNA are a versatile platform for CRISPR-Cas9 engineering, <i>eLife</i> 6:e25312</p> <p>Tiricz H et al. (2017) Relaxed chromatin induced by histone deacetylase inhibitors improves the oligonucleotide-directed gene editing in plant cells. <i>J Plant Res.</i> 2018 Jan;131(1):179-189. doi: 10.1007/s10265-017-0975-8</p>

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Poster Title	Identification of genes differentially expressed in resistance reaction to <i>Didymella pinodes</i> in <i>Pisum sativum</i> L. field experiment
Abstract	<p>Ascochyta blight, caused by <i>Didymella pinodes</i>, is the most destructive foliar pathogen of dry peas in the temperate zones of Europe. This disease causes 10% yield losses as an average and can reach 50% under some conditions. Current control practices are uneconomic and inefficient. Very little is known about the mechanisms or genes that control host plant resistance against these fungus. Flavonoids are compounds with antioxidant and antimicrobial properties. Isoflavone synthase is the key enzyme catalyzing the biosynthesis of isoflavonoids, which have demonstrated efficient antibacterial and antifungal activities. Herein, we investigated the expression change of isoflavone synthase in 8 pea accessions: resistant (Wt 2266, Cud Kelvedonu, Messire, Venus) and susceptible (PI 41369, Wt401, P665, Radley) before and several time points after inoculation. Trials with controlled inoculation were performed at Radzików, Poland. Disease severity assessment was done, according to Xue et al. (1996) (0 – resistant, 9 – susceptible). The expression profiles of chosen gene were analyzed by qRT-PCR in control and <i>D. pinodes</i>-infected plants of chosen accessions. The results showed that a gene level expression increased 3.2 times in resistant accessions and 2.8 times in susceptible accessions 4 hours after inoculation with <i>D. pinodes</i>. Twenty four hours after inoculation the difference between a gene expression in resistant and susceptible accessions was the strongest (1.6). Further analyses could confirm the role of the secondary metabolism genes in defense and finally provide more resistant breeding materials.</p> <p>The study was supported by National Multi-Year Program in Poland.</p>

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Poster Title	Impact of Colorado potato beetle gut microbiome in the adaptation of plants to drought and heat stresses associated to climate change
Abstract	<p>The detrimental effects of intensification practices in modern agriculture have created the need of new environmentally-friendly approaches to maintain sustainable agricultural production and to overcome threats that lead to loss of crop yield, including plant stresses associated with adverse environmental conditions, such as drought, high temperature or soil salinity, as well as biotic stress induced by plant pathogens and pests. In this context, exploiting natural microbial communities for improved plant performance in integrated plant disease management systems appears as a promising effective alternative since microorganisms have been proved beneficial for plants directly by enhancing crop nutrition or indirectly by reducing damage caused by pathogens or environmental stress. Herbivores possess diverse microbes in their digestive systems, and recent research has demonstrated that these gut microbes can manipulate plant-insect interactions and in turn, modify the plant response to other biotic or abiotic stresses. In the case of the Colorado potato beetle (CPB, <i>Leptinotarsa decemlineata</i>), an exceptionally devastating pest of Solanaceae plants, it has been reported that when larvae were reared on different hosts, variation in the composition and structure of CPB bacterial communities was observed, which correlated with differential plant defense response to insect attack.</p> <p>The aim of our present work is to identify gut bacterial communities in CPB larvae fed on tomato varieties resistant to drought and high temperature by means of metagenomic analysis using 16 S rRNA amplicon sequencing and to investigate their potential to induce resistance to abiotic stresses in a tolerant tomato variety.</p>

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Poster Title	Reliably detecting copy number variation in plant genomes
Abstract	<p>Copy number variants (CNVs) are a type of genetic variation comprising deletions and duplications of 50 bp or larger. They are the largest source of genetic variation between individuals of the same species, estimated to affect about 5 times as many base pairs as single nucleotide polymorphisms (SNPs). In plants, they are suspected to play an important role in mediating adaptation and evolution. One clear example of such adaptation was found in several individuals of the weed species <i>A. palmeri</i> that managed to obtain resistance to a widely used herbicide through duplication of the EPSPS gene.</p> <p>Despite the clear biological impact of CNVs, characterizing them in plant species is challenging. The main approach to discover CNVs is to utilize computational pipelines combining multiple algorithms that detect CNVs based on alignments of Illumina next-generation sequencing reads of a sample of interest to a reference genome. However, such pipelines were optimized using human data and it is unknown whether they perform as well when applied to plant species. Moreover, their practical design is strongly tailored towards human research, which makes it difficult to run them on plant datasets.</p> <p>To tackle these problems, we present a computational approach that merges the output of multiple CNV callers, using a strategy optimized using simulated plant datasets. Our approach detects significantly more CNVs than any of the individual tools it employs, while remaining highly accurate. We expect that our approach will enable the plant science community to obtain a more comprehensive overview of CNVs in plant species, resulting in new insights into how genetic variation can drive phenotypic diversity.</p>

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Poster Title	A fast and reliable method for detecting single base editing
Abstract	<p>One of the most powerful applications of genome editing is the introduction of base changes in specific genomic sites that mimic single nucleotide polymorphisms (SNPs) related to diseases or the introduction of stop codons to generate gene knockouts. However, screening a large number of clones to identify those containing the engineered base of interest is still a bottleneck, especially in the absence of a phenotypic readout. To address this need, we developed a SNP-detection method that allows quick screening of clones from a 96-well plate. The method is based on PCR amplification of the genomic target site, followed by an enzymatic assay with fluorescence readout. The overall workflow takes approximately four hours and any positive fluorescent signal is highly correlated with the successful introduction of the desired SNP. SNP detection using this method is independent of the engineered nucleotide substitution and the surrounding targeted genomic loci.</p>

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Poster Title	Enhancing Soil Fertility in Northern Mountainous Areas of Vietnam by Developing Rhizobial Inoculants for Cowpea <i>Vigna Unguiculata</i> (var. <i>Cylindrica</i>) in Intercropping Systems
Abstract	<p>Cassava and maize are widely grown in the Northern mountainous regions of Vietnam. By applying huge amount of mineral fertilizers and promoting monocropping, the farmers have hugely contributed to soil degradation with as direct consequence to the decline of crop yield by unsustainable management practices. Legume-based intercropping system, one of the most widespread agroecology practices, have been widely promoted by scientists and the local authorities in Northern Vietnam because of their numerous advantages: better use of land, economic benefit, diminishing soil erosion, and also gradually increasing soil N content through biological nitrogen fixation as well as mulching legume residues. Nevertheless, the upland farmers have no idea about the use of commercial rhizobial inoculants and their potentials. This study aimed at assessing the needs to promote such rhizobial inoculants in the 2 districts in Yen Bai province of Vietnam, where cowpea is intercropped with cassava and maize. Our results showed that the natural nodulation of cowpea was really low at different soil characteristics and slope categories. There are indigenous rhizobial strains in the soils but it is not sufficient for them to form effective symbiosis with cowpea. Unfortunately, there is no valid commercial inoculant product on the markets in Vietnam until now, it is imperative to develop effective rhizobial inoculants for grain legumes for Vietnam, as well as in the whole South East Asia. There are markets for private companies willing to open new business on such activities. The presence of commercial inoculants on the markets would help in sense of agroecology to improving soil health and building sustaining agricultural systems in this zone.</p>

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Poster Title	The EU official control system for GM food and feed
Abstract	<p>The EU operates a multi-layer monitoring and control system for ensuring that food and feed sold in the EU is safe and in compliance with EU legislation, including the one on the presence of GMOs. Any GMO on the market needs the prior authorisation from the European Commission following a risk assessment done by the European Food Safety Authority (EFSA). Any food or feed product containing (authorised) GMOs at a mass fraction of > 0.9 % per mass of ingredient needs to be labelled to inform the consumer on the presence of GMOs in the product. The EU Reference Laboratory for GM Food and Feed (EURL GMFF) is responsible for the validation of the analytical methods for the detection and quantification of GMOs and maintains a database of all methods. It also organises proficiency tests and provides guidance and support to National Reference Laboratories (NRLs) and Official Control Laboratories involved in the routine testing of food and feed products on the market in their Member State. This ensures a harmonised approach for GMO testing, based on the same validated methods, the same reference materials, international quality control systems, networking, workshops and training. The European Commission is investigating if gene edited organisms would fall under the GMO Regulations. If so, the GMO control system would monitor their presence in food and feed in the EU.</p>

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Poster Title	CRISPR/Cas9 GoldenBraid toolkit application to generate tomato chlorophyll-retaining <i>gf1</i> mutants
Abstract	<p>GoldenBraid is a modular cloning standard and DNA assembly framework, recently adapted for the assembly of CRISPR constructs.</p> <p>The tomato (<i>Solanum lycopersicum</i> L.) <i>green-flesh (gf1)/ stay-green (sgr) locus</i> encodes the Mg-dechelatase, which is involved in the early steps of chlorophyll degradation. Naturally occurring <i>gf</i> mutants of tomato show a distinct stay-green phenotype marked by chlorophyll retention both in senescent green tissues and in ripening fruits. Chlorophyll retention has been found associated to an increase in carotenoids content resulting in a greater antioxidant power, feature which enhances shelf life and tolerance to saprophytic microorganisms.</p> <p>We chose the tomato <i>gf1/sgr locus</i> as target to test the efficiency of the GoldenBraid-based CRISPR system. Targeting <i>gf1</i> for producing knock out mutants in tomato combines the advantage of providing a visual system with a prospective positive effect on fruit storability.</p> <p>We performed <i>Agrobacterium</i>-mediated transformation of tomato cotyledons of the cv 'MoneyMaker' with a CRISPR/Cas9 construct targeting <i>gf1</i>. Overall, 63% of the regenerated plantlets carried mutations in the target gene indicating a good mutation/edit rate; all of which showed a staygreen phenotype in both leaves and fruits. Genotyping of these plants, through Sanger sequencing of the <i>gf1 locus</i>, revealed that the most frequent mutation was a single nucleotide insertion (a T insertion in the 67% of cases). We also investigated potential off-target effects by Illumina deep sequencing of amplicons of putative off-target sites identified <i>in silico</i>. The absence of off-target mutations confirmed the high specificity of the system. Moreover, a T₁ progeny from regenerated plants was obtained, allowing the selection of segregating lines that stably inherited the <i>gf1</i> mutation, while no longer carried the transgene construct.</p> <p>Evaluation of the content of chlorophyll and other pigments in fruit from eight independent T₁ plants confirmed the retention of chlorophyll during ripening in these <i>gf1</i> edited plant.</p>

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Poster Title	Knock-out of Polyphenol Oxidase Genes in Eggplant Fruits using the CRISPR/Cas9 GoldenBraid toolkit
Abstract	<p>Eggplant (<i>Solanum melongena</i>) berries are rich in phenolic compounds. The latter, after cutting, are oxidized by polyphenol oxidase enzymes (PPOs) causing browning of the fruit flesh with a negative impact on fruit quality for both fresh consumption and industrial transformation. Ten PPO genes (named PPO1-10) were isolated also thanks to the recent availability of a high quality and annotated eggplant genome sequence. Their qPCR expression profile was assessed in the fruit flesh and peel of three eggplant varieties, immediately and 30 min after cutting. The <i>ppo1-3-4</i> and <i>5</i> presented strong increases in gene expression in the flesh.</p> <p>A CRISPR/Cas9 toolkit was developed within the GoldenBraid cloning standard, which allows simple and practical assembly of constructs for plant gene editing. Two eggplant varieties, namely Black Beauty and the double haploid Ecavi, were selected for <i>Agrobacterium</i>-mediated transformation. Seed-derived cotyledons were transformed with a CRISPR/Cas9 construct targeting a conserved region of <i>ppo4-5</i> as well as <i>ppo6</i> (due to the high homology between these gene family members).</p> <p>Mutations at the target sites were assessed by sequencing genomic DNA extracted from <i>calli</i> and <i>in vitro</i> regenerated shoots. Mutations found in edited plantlets were predominantly small deletions (1 to 4 bp, being the 1 bp deletion the most frequent). One Black Beauty edited plantlet showed the highest editing efficiency for all three <i>loci</i>: <i>i.e.</i> 66% for <i>ppo4</i>, 75.3% for <i>ppo5</i> and 55% for <i>ppo6</i>.</p> <p>We also investigated potential off-target effects through Illumina deep sequencing of amplicons for putative off-target sites identified <i>in silico</i>. The lack of off-target mutations confirmed the high specificity of the system.</p>

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Poster Title	CRISPR-Cas9 Takes Several Bites in the Potato Genome - Efficient Targeted Multiallelic Mutagenesis in Tetraploid <i>Solanum tuberosum</i>
Abstract	<p>Potato is a tetraploid crop with tetrasomic inheritance and high heterozygosity, making traditional cross-breeding a long term effort. Therefore, breeding technologies where only one or few traits can be introduced into an elite background are of major interest. The recent development of genome editing technologies has opened new doors for plant breeders. The methods can be used for inducing targeted mutations in the genome without stable DNA integration, resulting in a plant that might not be regulated as genetically modified.</p> <p>We have implemented CRISPR-Cas9 as a new breeding technology for potato, demonstrated by the development of amylopectin-starch potato by mutating the gene coding for granule bound starch synthase (GBSS) ¹. This results in a potato producing a desirable starch quality in-planta, extractable and ready-to-use without any further modifications. The method used then was DNA transfection and transient expression in protoplasts, which has been shown to give integration of unintended plasmid DNA into the genome, which from a regulatory perspective is undesired.</p> <p>With the aim to obtain transgene free mutated lines ², we here demonstrate a development of the method by delivery of preassembled ribonucleoproteins (RNP), with either synthetically produced or invitro-translated gRNA to the protoplasts.</p> <p>Funding The work was supported by Lyckeby Research Foundation (Stiftelsen Stärkelsen Forskning Utveckling) and Einar och Inga Nilssons stiftelse.</p> <p>[1] Andersson, M., Turesson, H., Nicolia, A., Fält, A.-S., Samuelsson, M., and Hofvander, P. (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (<i>Solanum tuberosum</i>) by transient CRISPR-Cas9 expression in protoplasts, <i>Plant Cell Reports</i> 36, 117-128.</p> <p>[2] Andersson, M., Turesson, H., Olsson, N., Falt, A. S., Olsson, P., Gonzalez, M. N., Samuelsson, M., and Hofvander, P. (2018) Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery, <i>Physiol Plant</i>.</p>

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Poster Title	Genetic diversity among Turkish <i>Corylus avellana</i> cultivars
Abstract	<p>European hazel (<i>Corylus avellana</i>) is a diploid (2n=22), monoecious and wind-pollinated species. It is grown throughout Europe and the West Iberian Peninsula and mostly cultivated for its nuts. Turkey is the world-leading producer of hazelnut, maintaining 70%-80% of the world's export capacity. Its great economic impact makes this species one of the most valuable agricultural products in Turkey. In order to develop open-public data for hazel genomic researches and support sustainable hazelnut production in Turkey, we conducted the first genome-wide survey of genetic diversity of Turkish hazel trees. Wild hazels, from the East Black Sea, and cultivars, from the West and East Black Sea Regions, were sampled, with regard to spacial and climate parameters, to organize subpopulations. Every individual was subjected to double-digest restriction enzyme associated DNA sequencing (ddRADseq) and a RADtag library was created. These RADtags were aligned to the draft genome assembly (Tombul variety) using samtools. The Stacks reference genome pipeline was utilized to identify unique haplotypes and polymorphisms of the subpopulations; RaxML and FigTree software were used to create phylogenetic trees to show evolutionary relationship between subpopulations. Pairwise fixation index (F_{ST}) tests were used to indicate genetic distance between subpopulations. FineRADStructure was used to draw co-ancestry matrix and determine population structure for the cultivars. Observed heterozygosity and homozygosity within cultivars ranged between 0.1-0.21 and 0.78-0.89 respectively; whereas expected heterozygosity and homozygosity ranged between 0.06-0.21 and 0.78-0.93. Presence of heterozygosity within cultivated subpopulations could be explained by the obligate outcrossing nature of hazel trees. This indicates that hazel cultivation might have increased the homozygosity level by continuously selecting and fixing same genes of interest. Pairwise F_{ST} showed that one wild hazel was found very close to all cultivars ($F_{ST} < 0.1$). We can conclude that the origin of gene flow might have started from the East Black Sea region. In conclusion, based on the given results, we could characterize genomic information of cultivated and wild hazels for the purpose of using them in sustainable hazel production.</p>

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Poster Title	Reducing the acrylamide-forming potential of wheat, rye and potato: Variety selection, genetic improvement and crop management
Abstract	<p>Acrylamide (C₃H₅NO) is a processing contaminant produced predominantly in the Maillard reaction during frying, baking, roasting or high-temperature (> 120 °C) processing. It is a Group 2A carcinogen, and EFSA's Expert Panel on Contaminants in the Food Chain (CONTAM) has stated that the margins of exposure for dietary acrylamide indicate a concern for tumour-inducing effects. Potato, coffee and cereal products are the major contributors to dietary acrylamide intake. The European Commission has just introduced (April 2018) strengthened risk management measures for acrylamide in food, including compulsory Codes of Practice and the setting of Benchmark Levels.</p> <p>Acrylamide forms from free (non-protein) asparagine and reducing sugars that are naturally present in the tubers, edible roots, grains and beans of crop plants. In potato tubers, the main determinant of acrylamide-forming potential is the concentration of reducing sugars, but free asparagine concentration may be the better target for genetic interventions because the colours, flavours and aromas that are also produced by the Maillard reaction are less likely to be affected. In cereal grain, in which there is a lower ratio of free asparagine to reducing sugar concentration, free asparagine concentration correlates closely with acrylamide formation. Varieties of wheat have been identified with consistently low free asparagine concentration in the grain under good growing conditions, but the free asparagine concentration of wheat grain is very sensitive to crop management, and sulphur deficiency and poor disease control, in particular, must be avoided. Genetically modified (GM) potato varieties with very low concentrations of free asparagine and reducing sugars are already on the market in the USA. The amount of acrylamide that forms in products made from these potatoes is so low that GM varieties have the potential to solve the acrylamide problem altogether, at least in the context of current Benchmark Levels. We are using mutagenesis and genome editing (CRISPR-Cas) to achieve similar outcomes in wheat. We have characterised the asparagine synthetase (ASN) gene family, and are targeting the <i>ASN2</i> gene, which is highly expressed specifically in the grain. This work is supported by the BBSRC, the Agriculture and Horticulture Development Board, and a consortium of wheat breeders: KWS UK, Syngenta Seeds, Limagrain UK, RAGT Seeds, and Saaten-Union UK.</p>

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Poster Title	Determination of chalcone synthase isoforms and their specific gene expression in flax
Abstract	<p>Nowadays among the society so called „functional food” gained the major popularity, especially food rich in the antioxidants, such as flavonoids. Identification of factors that control their biosynthesis in crops enables the improvement of commercially available variants. Analysis of genes involved in the flavonoids synthesis showed to be crucial in many cultivars. Since flax has been acknowledged by American National Cancer Institute as one out of six the most important sources of nutraceuticals, the research concerning its flavonoid synthesis pathway deserves the special attention as well. The aim of the research was the identification of genes encoding all chalcone synthase gene isoforms (CHS) in flax <i>Linum usitatissimum</i> L. Chalcone synthase is a key enzyme in the biosynthesis of flavonoids. The activity of the CHS gene is essential for regulation of many living functions in plant and it depends on the organ-specificity, developmental stage and environmental stress factors. So far the CHS genes family was not identified thoroughly in flax. The recognition and characteristic of all CHS gene isoforms will significantly enlarge the existing knowledge about flavonoid compounds synthesis. Moreover the defining of the specificity of particular isoforms will provide information about their function and activity regulation, which will support the characteristic of flax genome and physiology. Additionally the obtained results will serve in the future for generation of new usable types of flax with improved properties.</p>

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Poster Title	Oligodeoxynucleotides can lead to the directed and hereditary epigenetic modifications in CHS gene
Abstract	<p>Chalcone synthase (CHS) has been recognized as a crucial enzyme in the phenylpropanoid biosynthesis pathway. Apart from the leading role in the production of phenolic compounds beneficial to biomedicine, CHS is well appreciated in science. The CHS gene is one of the most intensively studied genes in flax. In our study, we investigated engineering of the CHS gene through genetic and epigenetic approaches. Considering the numerous restrictions concerning the application of genetically modified (GM) crops, the main purpose of this research was optimization of the plant's modulation via epigenetics. In our study, plants modified through two methods were compared: a widely popular agrotransformation and a relatively novel oligodeoxynucleotide (OLIGO) strategy. It was recently highlighted that the OLIGOs technique can be used in quick analysis of the gene function. In order to evaluate the use of ODNs as a tool for predictable and stable gene engineering, we concentrated on the integration of gene expression and gene-body methylation. The treatment of flax with series of short oligonucleotides homologous to a different part of CHS gene isoforms revealed that those directed to regulatory gene regions activated gene expression, directed to non-coding region caused gene activity reduction, while those homologous to a coding region may have a variable influence on its activity. Gene expression changes were accompanied by changes in its methylation status. However, only certain (CCGG) motifs along the gene sequence were affected. The analyzed DNA motifs of the CHS flax gene are more accessible for methylation when located within a CpG island. The methylation motifs also led to rearrangement of the nucleosome location. The obtained results suggest that OLIGO action is highly specific and lead to the induction of heritable genome diversification, thus oligodeoxynucleotides technology might be a valuable alternative for improvement of crops.</p>

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Poster Title	Network modelling unravels mechanisms of crosstalk between ethylene and salicylate signalling in potato
Abstract	To provide means for novel crop breeding strategies, it is crucial to understand the mechanisms underlying the interaction between plants and their pathogens. Network modelling represents a powerful tool that can unravel properties of complex biological systems. Here, we build on a reliable previously manually built <i>Arabidopsis thaliana</i> L.) immune signalling model, extending it with the information from diverse publically available resources. The resulting prior knowledge network (20,012 nodes, 70,091 connections) was then translated to potato (<i>Solanum tuberosum</i> L.) and superimposed with an ensemble network inferred from potato time-resolved transcriptomics data. We used different network modelling approaches to generate specific hypotheses of potato immune signalling mechanisms. An interesting finding was the identification of a string of molecular events, illuminating the ethylene pathway modulation of the salicylic acid pathway through NPR1 gene expression. Functional validations confirmed this modulation, thus supporting the potential of our integrative network modelling approach for unravelling molecular mechanisms in complex systems.

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Poster Title	Delivery of plasmids and gene editing oligonucleotides into plant protoplasts by using a modified cationic polymer
Abstract	<p>Gene editing methods such as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) and OTNE (oligonucleotide-targeted nucleotide exchange) are becoming increasingly prevalent in plant biology, biotechnology and plant breeding. However, delivery of the necessary components for editing (plasmids, oligonucleotides, RNA, protein) into plant cells pose a challenge due to tightly woven cellulosic cell walls and the fragile plasma membranes under turgor pressure. There are several methods used to deliver macromolecules into plant protoplasts. Among these polyethyleneglycol (PEG), liposome, electroporation, cationic polymer and Ca²⁺ mediated delivery methods can be listed. Each of these methods has its own strengths and weaknesses. Nevertheless, the PEG-based delivery is the most commonly used method for plant protoplasts due to its high transfection efficiency; however, this method also results in increased protoplast fusions as well as reduced cell viability. We have synthesized a new, modified cationic polymer to be used in gene editing experiments to deliver plasmid and oligonucleotides into plant protoplasts. We have optimized several parameters such as DNA-polymer ratios, different cell pre-treatments and culture media components to yield a highly efficient method with versatile applicability in plants. This new and efficient cationic polymer delivery method is applicable to various plant species of monocot and dicot origin such as maize (<i>Zea mays</i>, H1233) rice (<i>Oryza sativa</i>, Unggi9) and Arabidopsis. We have shown highly efficient delivery of plasmids expressing green and red fluorescent proteins and fluorescent oligonucleotides. Using optimized conditions of polymer-DNA ratios and culture media, we have also achieved successful gene editing by converting a mutant GFP gene sequence in stable transgenic maize cells back to its original, coding for the functional fluorescent form of this protein.</p>

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Poster Title	Copy number variation and expansion contributes to plant adaptation and evolution
Abstract	<p>Copy number variation (CNV) is a common and important type of genetic variation in plants. CNVs are DNA sequences larger than 50 base pairs that are present in different copy numbers between individuals of the same species. We study the impact of CNV and copy number expansion in the genomes of two Brassicaceae species, <i>Noccaea caerulea</i> and <i>Hirschfeldia incana</i>. These species have adapted in their own way to different challenging environments, which are highly stressful for most other plant species. <i>Noccaea caerulea</i> is a heavy metal tolerant and hyperaccumulating species. However, among different <i>Noccaea</i> accessions there is considerable variation in the level of metal tolerance. We created the <i>Noccaea</i> reference genome and compared it to the close, non-hyperaccumulator relatives <i>Eutrema salsugineum</i> and <i>Arabidopsis thaliana</i> (Severing <i>et al</i>, unpublished). This revealed an overrepresentation of metal homeostasis genes among the genes that have been duplicated in <i>Noccaea</i>. Moreover, we resequenced six other <i>Noccaea</i> accessions with variable levels of metal tolerance, and found CNV for several of these duplicated metal homeostasis genes.</p> <p><i>Hirschfeldia incana</i> achieves extremely high rates of photosynthesis under high light intensities. Compared to other C3 plants, <i>H. incana</i> is much more efficient under such high light conditions, and achieves comparable or even slightly higher efficiencies relative to C4 plants. A homozygous inbred line was created and sequenced with the 10X Genomics and Illumina NGS techniques. The genome was compared to close relatives such as <i>Arabidopsis thaliana</i> and <i>Brassica rapa</i>, and again we find an important contribution of copy number expansion during evolution. Genes involved in photosynthesis and the response to high light are enriched among the duplicated genes in <i>Hirschfeldia</i>. Taken together, these results indicate that CNV and copy number expansion has played an important role in adaptation and evolution of these species.</p>

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Poster Title	Improving the aphid resistance in barley breeding through genome editing
Abstract	<p>Aphids are important pests to control in agricultural crops, as they withdraw nutrients from the hosts and transfer viruses that cause negative impact on the hosts. Barley is an economically important crop and aphid attack is often a serious problem. There are examples of successful breeding in gene-for-gene resistance to green bug and Russian wheat aphid in barley. Gene-for-gene resistance is typically when a major resistance gene in the plant recognizes some specific proteins from the intruder and starts plant defense reactions. However, the major problem with such resistance is that it is generally not durable. Recently, breeding against susceptibility by knocking out susceptibility genes in the host has attracted more attention for achieving better resistance durability.</p> <p>Analyses of aphid-susceptible and -resistant barley lines from a traditional breeding program showed that β-1,3-glucanase gene expression is often higher in susceptible compared to resistant lines. In order to improve the resistance to bird cherry - oat aphid in barley, we have used the genome editing technique, CRISPR/Cas9 to knock out two β-1,3-glucanase candidate gene for susceptibility to this aphid. The CRISPR-constructs was delivered into the cv. Golden Promise via Agrobacterium, performed at John Innes Centre, U.K. Mutation lines have been generated and homozygous mutation lines will be tested for aphid resistance in a near future.</p>

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Poster Title	<i>Pseudomonas aeruginosa</i> rhamnolipids elicitation of Brassicaceae – Perception, molecular responses and protection triggered
Abstract	<p>Microbe-associated molecular patterns (MAMPs) from pathogenic (or non-pathogenic) microorganisms elicit plant defense mechanisms. Some are considered as a promising alternative or a complementary tool to deal with pests in crops. Their perception is generally mediated by plant transmembrane recognition receptors. However a direct interaction with the membrane has been considered for some structurally different elicitors. In this study, we focused on <i>Pseudomonas aeruginosa</i> rhamnolipids. These amphiphilic glycolipids are known to induce grapevine and <i>Arabidopsis thaliana</i> defense responses but their mode of perception by plants and their efficiency toward crops are still unknown. Biophysical studies combined with an in planta approach on Brassicaceae have been carried out.</p> <p>As amphiphilic compounds, rhamnolipids have been proposed to interact directly with plasma membrane lipids. We explored the possibility of an elicitation triggered by a direct interaction with membrane lipids by solid-state NMR and molecular dynamic simulation studies on different models of plant plasma membrane. In order to characterize the early gene expression modifications triggered by rhamnolipids, a micro-array study was realized on <i>A. thaliana</i> seedlings, revealing the large transcriptional signature affecting defense associated genes. The potential use of rhamnolipids to protect the agronomic plant <i>Brassica napus</i> against pest diseases was studied. The efficiency of rhamnolipids was shown in reducing foliar lesions due to the opportunistic pathogenic fungus <i>Botrytis cinerea</i>. <i>B. napus</i> defense responses to rhamnolipids were characterized and the physiological effects were investigated.</p>

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Poster Title	Transcription factor StZPR1 participates in the reproductive development of <i>Solanum tuberosum</i>
	<p>Zinc finger proteins belong to large family of regulators among Eukaryotes. Zinc finger domains are present in proteins involved in a wide range of cellular processes like: replication, transcription and translation to cellular metabolism, signal transduction, cell division and cellular apoptosis. Owing to the presence of zinc binding domains in their structure, these proteins can interact with nucleic acids and cellular proteins. Among plants, zinc finger proteins are important growth and development regulators and also participate in stress response. <i>StZPR1</i> gene, which encodes StZPR1 protein belonging to the C₄ type zinc finger proteins, was isolated from <i>S. tuberosum</i>, cv. Ursus by Gold Yeast One-Hybrid System. Its protein product was bound to the „CIRC” (<i>Circadian Regulated</i>) promoter region of the <i>StBBX24</i> gene, which encodes a protein containing two tandemly arranged B-box zinc finger motifs. Analysis shown that <i>StBBX24</i> gene expression exhibits circadian cycling. To determine the biological function of the StZPR1 protein, <i>S. tuberosum</i> transgenic plants with silenced expression of the <i>StZPR1</i> gene were prepared. Analysis of the four alleles of the <i>StZPR1</i> gene was made to select regions without polymorphism. <i>StZPR1</i> mRNA fragments showing no allelic polymorphism were selected using WMD3 program (Web MicroRNA Designer). Molecular analysis of <i>S. tuberosum</i> transgenic lines was then performed to evaluate the level of exclusion of <i>StZPR1</i> gene expression by real-time PCR and Western blot using a specific antibody directed to the StZPR1 protein. Two lines exhibiting strong silencing of <i>StZPR1</i> expression were chosen for further analysis. The phenotypic analysis of <i>S. tuberosum</i> <i>StZPR1</i>-silenced lines showed that they exhibited early flowering compared to wild-type. Meanwhile they do not display visible changes with respect to growth rate, leaves and tubers. To demonstrate whether StZPR1 protein has an effect on the expression of other circadian-regulated genes, a large-scale screen of transgenic and wild-type plants was performed using a RNA-seq approach performed on leaves of 3-week-old phytotron-grown plants. Comparison of transcriptome of wild-type plants and amiRNA mutants allowed to identify 337 genes functioning in various biological processes with different expression between non-transformed and transformed plants. Interestingly, the lack of StZPR1 protein affects the expression pattern of critical genes associated with circadian clock and with the floral transition process, unveiling a function of StZPR1 in flowering.</p>

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Poster Title	StBBX20 protein regulates salt stress tolerance and the timing of floral development in <i>Solanum tuberosum</i> .
Abstract	<p>The appropriate setting of the transition time from vegetative growth to reproductive development and an ability to tolerate abiotic stresses are essential for successful achievement of plant life cycle and has also commercial significance for crop plants. B-box proteins named BBX comprise one of the most important family in plants. They play essential roles in the regulation of plant growth and development including seedling photomorphogenesis, photoperiodic regulation of flowering, shade avoidance and responses to biotic and abiotic stresses. We identified 30 genes encoding B-box proteins in the potato genome (<i>Solanum tuberosum</i>, ssp. <i>tuberosum</i>, cv. Desiree), and classified them into five structural groups based on the presence of the B-box and CCT domains. Among them StBBX20 protein that belong to structural group IV contains two tandem repeat B-box motifs in the N terminus but lack the CCT domain. By physiological and genetic approaches, we dissected the physiological function of StBBX20 in potato using silenced lines. We showed that these lines displayed susceptibility to high salinity and strongly decreased expression of genes encoding Na⁺ transporters that mediate salt tolerance. Moreover, we showed that these lines exhibit much earlier flowering than wild-type plants. Interestingly, transcriptome and RT-qPCR analyses of <i>StBBX20</i>-silenced lines revealed substantial modifications in the expression of critical genes associated with the floral transition process. In addition the genes promoting and repressing reproductive development exhibited up- and down-regulation at the transcript level, respectively. We suggest that StBBX20, a component in the transduction of high salt signal, is also involved in repression of floral development in potato.</p>

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Poster Title	<i>Lupinus albus</i> and <i>Lupinus luteus</i> transcriptome sequencing, towards identification of genes involved in quinolizidine alkaloid biosynthesis
Abstract	<p>This study has been devoted to understanding thus far obscure biosynthetic pathway of quinolizidine alkaloids (QA) in white lupin (<i>Lupinus albus</i> L.) and yellow lupin (<i>Lupinus luteus</i> L.). The knowledge on the biosynthesis of these anti-nutritional compounds is fundamental to facilitate breeding efforts in providing valuable lupin sources for food and feed. The aim of our research was the transcriptome sequencing of white and yellow lupin genotypes with contrasting alkaloid content in seed, in order to identify differentially expressed genes potentially involved in the alkaloid biosynthesis pathway.</p> <p>The experiment was carried out on the Illumina platform (100 PE, HiSeq4000). A total of 16 white lupin samples have been incorporated into RNA-seq, including two tissue types: leaves and pods. A set of 24 yellow lupin samples have been sequenced, including two tissue types: leaves and flowers. High-confidence short RNA-Seq reads for selected high-alkaloid (bitter) accession were chosen to assemble <i>de novo</i> the reference transcriptome for both species, based on the assumption that genes involved in QA biosynthesis are upregulated in bitter accessions. For white lupin the reference transcriptome was assembled from the short reads of a bitter accession BGR 6305. A total of 109607 transcripts have been received with the average contig length of 761,5 bp (N50 = 1249 bp), and GC content of 38,8%. The transcriptomic data confirm deep transcriptome coverage with Illumina paired-end reads with aid of assembly method (total assembled bases = 59528660 bp; >60x coverage). Afterwards, differentially expressed genes between bitter and sweet accessions were detected which will serve as a basis for future selection of candidate genes based on annotations and gene ontology terms. Similar strategy will be employed for yellow lupin, where a bitter accession: Tremoco Flor Amarela will be used as a reference transcriptome.</p>

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Poster Title	Advancing plant synthetic biology with Plant X-tender, a system for the assembly, delivery and expression of multigene constructs in plants
Abstract	<p>Cloning multiple DNA fragments for delivery of several genes of interest into the plant genome is one of the main technological challenges in plant synthetic biology. Despite several modular assembly methods developed in recent years, the plant biotechnology community has not widely adopted them yet, probably due to the lack of appropriate vectors and software tools. Here we present Plant X-tender, an extension of the highly efficient, scar-free and sequence-independent multigene assembly strategy AssemblX, based on overlap-dependent cloning methods and rare-cutting restriction enzymes. Plant X-tender consists of a set of plant expression vectors and the protocols for most efficient cloning into the novel vector set needed for plant expression and thus introduces advantages of AssemblX into plant synthetic biology. The novel vector set covers different backbones and selection markers to allow full design flexibility. We have included ccdB counterselection, thereby allowing the transfer of multigene constructs into the novel vector set in a straightforward and highly efficient way. Vectors are available as empty backbones and are fully flexible regarding the orientation of expression cassettes and addition of linkers between them, if required. We optimised the assembly and subcloning protocol by testing different scar-less assembly approaches: the noncommercial SLiCE and TAR methods and the commercial Gibson assembly and NEBuilder HiFi DNA assembly kits. Plant X-tender was applicable even in combination with low efficient homemade chemically competent or electrocompetent <i>Escherichia coli</i>. We have further validated the developed procedure for plant protein expression by cloning two cassettes into the newly developed vectors and subsequently transferred them to <i>Nicotiana benthamiana</i> in a transient expression setup. Thereby we show that multigene constructs can be delivered into plant cells in a streamlined and highly efficient way. Our results will support faster introduction of synthetic biology into plant science.</p>

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Poster Title	Plant growth-promoting bacteria from an Olive grove for the production of tailor-made biofertilizers
Abstract	<p>Until the new EU Fertilizer Regulation will be approved (expected by 2020), European fertilizer products based on microorganisms are currently covered by national legislation. The access to the EU market of novel fertilizing products depend on mutual recognition between Member States. In the specific case of Spain, the Royal Decree (RD 999/2017) defining the production, registration and marketing of these products was recently approved. To be included in the National Fertilizer Registry, a biofertilizing product must meet several criteria: purity, identification by means of rRNA genes (16S in prokaryotes and ITS18S in eukaryotes) and absence of pathogens. In addition, it is mandatory to demonstrate its plant growth-promoting (PGP) effect in real agroecosystems. However, although this new legislation enhances the expansion of biofertilizers, their introduction in intensive agriculture is still poor due to barriers such as their unpredictability in terms of productivity. The same microorganism in different soils and/or different crops will hardly produce the same beneficial effect on plants. To overcome this limitation, isolation of native strains adapted to particular soils and agricultural crops will allow the production of tailor-made biofertilizers. With this aim, we performed an isolation process from a rhizospheric soil of olive tree. 109 bacterial strains were obtained. All of them were screened for PGPR activities, obtaining the following results (percentage of positive strains): potassium solubilizers (41.2%), production of auxins (30%), phosphate solubilizers (26%), siderophores (22%), nitrogen fixers (15.5%) and enzyme ACC deaminase (1.8%). Only 1 strain produced measurable amounts of at least 5 of the 6 assessed activities, thus suggesting that products based on bacterial consortiums might be more effective than individual strains. Further research will be focused on assessing the effect of selected bacterial consortiums (combination of strains with high PGP activity from our culture collection) in improving productivity and sustainability of Mediterranean olive groves.</p>

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Poster Title	Parentage assignment in tetraploid plant species using NGS-derived markers
Abstract	<p>Next generation sequencing (NGS) technologies have revolutionized many areas of biological research taking the discovery and application of molecular markers to high-throughput levels, thus broadening and enhancing plant breeding prospects.</p> <p>In plant breeding programmes it is important to establish and track the exact parentage of families and individuals, and early assignment of genetic relationships between parents and offspring can be of undoubtable utility. DNA-based molecular markers have been widely and successfully used in this regard, but they are particularly challenging in polyploid species analyses because of the presence of a large number of homologues which difficult reliable polymorphisms detection.</p> <p>In this work we present the consistent results obtained for the molecular characterization of a tetraploid plant species using NGS-derived markers. The amount and quality of the newly discovered molecular markers allow, at a rationale cost, the accurate parentage assignment of individuals belonging to the same cultivar. Duplicate analysis of 13 samples corresponding to 3 different families (two fathers and three descendants each) have been performed in order to establish the optimal analytical conditions, both capable, at the wet and dry lab, of generating reliable genetic-based results with a high degree of confidence. Real filiation relationships according to genetic data revealed 2 misclassified individuals formerly thought to have a particular parentage, but later on confirmed to be in concordance with the results reported in this study.</p> <p>The analytical strategy here presented is a simplified version of RADseq technique. Using an <i>in silico</i> predicted suitable combination of restriction enzymes to reduce genome complexity, the resulting fragments are selected according to their size (200-500 bp) and finally sequenced using the Illumina 2500 ultrasequencing platform. NGS data is analysed by means of our in-house bioinformatic pipeline to achieve the final parentage assignment mainly based on the genetic distances detected among individuals.</p>

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Poster Title	Exploring the genome and R gene complement of Turkish hazelnut cultivar 'Tombul'
Abstract	<p>European hazelnut (<i>Corylus avellana</i> L.) is an economically important tree crop worldwide, with particular significance for Turkish farmers in the Black Sea region, where about 70% of the world's hazelnuts are produced. However, in spite of the challenges faced by hazelnut producers - including pests, disease outbreaks and climate change - genomic studies on this species are limited. To date, only a draft genome sequence for the American cultivar 'Jefferson' has been released.</p> <p>Therefore, we carried out whole genome shotgun sequencing (Illumina) for two individuals from the Turkish variety 'Tombul', which is considered to give the best nut quality. A variety of assembly programs and parameters were compared including ABySS, SOAPdenovo and SGA, of which the best results were obtained using ABySS, giving an N50 of 8.7 kb and assembly length of 523 Mb. This is c. 25% longer than the predicted genome size, which is believed to reflect a high level of heterozygosity in the genome.</p> <p>Genome assemblies from both individuals were annotated for the presence of repetitive elements, tRNAs, and protein-coding genes. About 13% of the genome was found to consist of transposable elements and 32,460 high-confidence gene models were identified. In order to assess the similarity and differences between individuals and identify putative disease resistance (R) genes, the NBS-LRR gene family was annotated in detail. The two Tombul individuals contained 264 and 273 putative NBS-LRR genes, of which less than 50% were shared between the two. These were further compared with 183 NBS-LRR genes from the draft 'Jefferson' genome, from which only 41 were shared with Tombul. These findings suggest that there is significant diversity in the R gene complement of cultivated hazelnuts, including within single varieties.</p>

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Poster Title	Determination of species of the newly emerging powdery mildew pathogen on hazelnut
Abstract	<p>European hazel (<i>Corylus avellana</i> L.) is a crop tree of well-known health benefits and great economic importance. Hazelnuts, Turkey's most valuable agricultural export, are a fundamental source of income for many families in the Black Sea Region. Even though it has a significant place in agriculture, a limited number of studies exists about <i>C. avellana</i> at the molecular level.</p> <p>Powdery mildew is a plant disease caused by parasitic fungi of the Erysiphaceae family, which infected leaves and other organs, resulting in impairment of photosynthesis and increased senescence of host tissue. On hazelnut, powdery mildew was previously observed to be a mild infection caused by <i>Phyllactinia guttata</i>, for which intervention was generally unnecessary. However, in the last 4 years a new form of powdery mildew with much more severe disease symptoms has been reported in cultivated hazelnuts in Turkey. This disease is caused by a fungus with different morphological features from <i>P. guttata</i>, and tentatively assigned to the genus <i>Erysiphe</i>.</p> <p>The aim of this study was to confirm the species of the newly emerging powdery mildew pathogen, and the degree of genetic diversity within the growing epidemic. DNA barcode analysis of the ITS and D1/D2 regions of its 28S ribosomal RNA determined that the causative agent is <i>Erysiphe corylacearum</i>, a parasite of several <i>Corylus</i> species that has not previously been reported in Turkey. Sanger sequencing of the barcode regions revealed that samples collected from three different Turkish states in the Black Sea region, and also from Georgia, belonged to <i>E. corylacearum</i>; furthermore, no genetic variation was observed within the barcode region of these samples. For further research, the sequences, along with comparative genomics data from 2 other <i>Erysiphe</i> species that have been sequenced previously, <i>Erysiphe pisi</i> and <i>Erysiphe necator</i>, will be used to develop molecular markers that are able to distinguish between powdery mildew species and carry out a genetic survey of the spread of this species in the Black Sea Region. Determining the genetic characteristics of the new hazel pathogen will help to inform control measures, and may provide clues as to its origin and possible natural reservoirs. This will be important in understanding the epidemiology of the disease, and plant-host interactions and give farmers and scientists the information needed to respond effectively to this emerging pathogen.</p>

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Poster Title	TALEN construct evaluation for PHS-specific loci in Triticale
Abstract	<p>Although Triticale is an agriculturally important crop, only a few mutants with altered seed dormancy have been developed so far. Locus-specific mutagenesis gives opportunity to directly modify traits of polyploid plants. Pre-harvest sprouting (PHS) in cereals is a complex phenomenon regulated i.a. by the level of abscisic acid (ABA). Homologs encoding ABA 8'-hydroxylase (ABA8'OH) have been reported in wheat (Chono et al. 2013). New loss of function mutant <i>pdf1</i> that exhibits enhanced seed dormancy phenotype was detected in Arabidopsis (Miatton, 2012), suggesting PDF1 as another negative regulator of seed dormancy. We intend to produce TALEN constructs as to modify PHS-related loci in Triticale. Transcription activator-like effector nucleases (TALENs) has emerged as one of genome editing tools suitable to alter gene function. TALENs mode of action relay on TALE domains which specifically interact with a target sequence. TALEN construct activity evaluation prior to stable transformation is desirable for successful mutagenesis and minimizing time-consuming work with constructs with poor activity or high off-target activity.</p> <p>To determine whether designed TALENs are active in plant cells, target sequences were amplified on Triticale genomic DNA template and cloned into YFP containing construct (pNB1) causing a frameshift (Budhagatapalli et al. 2016). Plasmids carrying TALENs, target-YFP construct and mCHERRY (pNB2) as internal control marker were co-delivered into barley and tobacco leaves via gold particle bombardment. YFP- and mCherry-emitting cells were counted, allowing to estimate activity of designed TALENs based on yellow to red cells ratio. All tested TALEN pairs were able to induce mutations which were measured by the repair of YFP frameshift in the transient expression system.</p>

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Poster Title	Towards the breeding of a new generation of rice cultivars adapted to the use of biostimulants
Abstract	<p>Many experimental works, performed in laboratory/greenhouse, suggest that the inoculation of beneficial microorganisms could have multiple impacts on rice growing such as improvement of plant growth or resistance to biotic/abiotic stresses. Unfortunately, these results are often very difficult to reproduce in the field. This is a major obstacle to the development of bacterial products for biostimulation or biocontrol. An explication of the lack of reliability of PGPR could be that the cultivars which are used nowadays in agriculture are not adapted to beneficial microorganisms. To test the feasibility of such breeding program we screened in greenhouse condition, the effect of Rhizocell®, a biostimulant containing <i>Bacillus amyloliquefaciens</i> IT45 commercialized by the company Lallemand Plant Care on several cultivars, representing the available diversity in rice. This screen allow to draw the conclusion that there is a strong effect of the genotype on the rice response to Rhizocell®. In order to advance in the discovery of the Rhizocell® modes of action on rice growth, a QTL search was carried out on a population of 188 RILS resulting from a cross between IR64 and Azucena. In our experiment, the biofertilizer proved to be very effective since it induced gains in size, tillering and fresh weight of respectively 9, 35 and 69%. We have shown that the growth gains obtained by Rhizocell® treatment are heritable, with heritabilities varying between 0.21 and 0.5 depending on whether we are interested in dry weight or chlorophyll content. This study leads to identify 12 QTL of responses to Rhizocell® for different growth-related parameters (height, tillering, fresh weight, dry weight, chlorophyll content). These QTLs are for the most part minor because they account for less than 10% of the variations observed, and are distributed on all chromosomes except chromosomes 3, 4 and 11. This work opens a way for the identification of genes involved in the response of rice to Rhizocell® and is an important step for the creation of a new generation of cultivars able to derive all the benefits of an interaction with beneficial microorganisms.</p>

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Poster Title	Expression profiles of genes involved in quinolizidine alkaloid biosynthesis in different plants organs of narrowed-leafed lupin (<i>Lupinus angustifolius</i> L.)
Abstract	<p>The seeds of narrow-leafed lupin (<i>Lupinus angustifolius</i> L.) are rich in proteins and fiber, what make them a valuable component of animals forages and also an interesting alternative of a protein source in human diet. The main obstacle to use lupin seeds are quinolizidine alkaloids (QAs) they contain. QA are toxic derivates of lysine amino acid found within the genus <i>Lupinus</i>, the biosynthesis pathway of which is still poorly understood.</p> <p>In order to better understand the molecular basics of the QAs biosynthesis in <i>L. angustifolius</i>, we selected seven QA candidate genes related to plant secondary metabolism based on comparative transcriptomic analysis of high and low alkaloid accessions as well as on literature data. Genes expression analyses were conducted for five plant organs: young leaves, flowers, young pods in the earliest and mid-stages of their development and also for green seeds. Two narrow-leafed lupin cultivars: Oskar and Regent, with contrasting seeds alkaloids content were incorporated into the qPCR analyses with three biological replications for each plant organs. PCR amplification efficiencies ranged from 1,95 to 2,05. The expression levels of the analyzed genes were normalized using of three reference genes with most stable expression for particular plant organs. Alkaloid content in the analyzed tissues was assessed by gas chromatography.</p> <p>Five out of seven analyzed genes showed the highest relative changes of expression level in green parts of the plant – especially in young leaves, but also in pods in early- and mid-stages of their development. At the same time we have not detected major changes in genes expression in seeds and flowers in the group of sweet and bitter accessions. We observed that in each of the plants organ incorporated into the analyses different gene showed the highest relative change of the expression level. Our results are in concurrence with previous studies that large parts of QAs biosynthesis takes place in plants leaves from where they are further transported and eventually stored in seeds. We also show that the involvement in QA biosynthesis of pods and seeds are significantly lower than leaves and role of flowers are marginal in this process.</p> <p>The results of our study shed new light on QA biosynthesis in different organs of narrow-leafed lupin.</p> <p>This research was supported by National Science Center, Poland, project no. 2014/13/N/NZ9/03943.</p>

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Poster Title	MOLECULAR CHARACTERIZATION OF WILD VINE POPULATIONS BY SSR MARKERS, FOR THE DETECTION OF HEALTHY COMPOUNDS IN THE TABLE GRAPES GENOME.
Abstract	<p>The genetic diversity of the vine is an important tool to face the challenges of viticulture in the 21st century, such as climate change, sustainable production and competition in a global market. This has generated a renewed interest in knowing the genetic resources available in this species (both those preserved in collections and those cultivated in isolation) to be used in genetic breeding programs.</p> <p>Nowadays, there is a growing interest in so-called "functional foods", which contributing to improve human health. In grape (<i>Vitis vinifera</i> L.), these components (resveratrol, polyphenols ...) are present in wine and table grapes. Many of these bioactive compounds are phytoalexins, and are produced in plant as a defense against an external attack, so, the study of the environmental effect is particularly relevant.</p> <p>The study of the wild vine presents some challenges, as to find populations with genetic variability. Another handicap is the increase of the regression of these populations due to the deterioration of their habitats.</p> <p>Using localization maps, 7 populations of <i>V. vinifera</i> subsp. <i>Sylvestris</i>, have been prospected in the North of Spain, and kept in the experimental farm "El Encín" (IMIDRA-Madrid). All prospected accessions have been genotyped using 26 pairs of SSR markers. So far, of the 7 populations analyzed, 36 different putative genotypes have been identified. Mature grapes have been collected in the populations since 2016, and sent to IMIDRA for compound analysis.</p> <p>The present work is being developed in NEIKER in collaboration with CIFA and IMIDRA, within the project funded by INIA-RTA2014-00083-C03, and the European Regional Development Fund "FEDER".</p>

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Poster Title	Strains of <i>Erwinia amylovora</i> bacteriophages Hena1 and Hena2 isolated in Belarusian orchard
Abstract	<p>In Belarus, as in other countries of the world, the species <i>Erwinia amylovora</i> is of quarantine concern, and a number of phytosanitary measures are being taken for control. <i>E. amylovora</i> is the causative agent of fire blight disease of fruit crops, affecting plants of the family <i>Rosaceae</i>. The phytopathogenic bacteria control methods using specific bacteriophages are currently being developed. On the territory of Belarus Vil-like <i>E. amylovora</i> bacteriophage phiEa2809 earlier was isolated (doi: 10.1093/femsle/fnv031). In the present study 2 new strains of <i>E. amylovora</i> bacteriophages, Hena1 and Hena2, were isolated from the soil samples in the apple and pear orchard in the Grodno region of Belarus. When growing on Luria–Bertani (LB) agar, Hena1 formed point plaques, whereas Hena2 plaques had uneven edges and 1-1.5 mm size. Liquid lysates (10^9-10^{10} PFU/ml) of bacteriophage strains were obtained when incubated in LB broth. Differences between the genomes of Hena1 and Hena2 were stated based on the RAPD-PCR results using OPL5 (50-ACGCAGGCAC-30) and P2 (50-AACGGGCAGA-30) (doi: 10.1111 / j.1574-6968.2011.02342.x.) primers. In the RFLP analysis of bacteriophages DNA samples EcoRI and DraI sites in genomes of Hena1 and Hena2 were detected. Bacteriophages possessed lytic activity against a number of bacteria strains of <i>E. amylovora</i> isolated in Belarus, as well as strain 1 / 79Sm (Germany, Spontaneous Sm-resistant mutant of 1/79, <i>Cotoneaster</i> sp., 1979). Using spot tests, the ability of Hena2 to cause lysis of individual cultures of <i>Erwinia herbicola</i> was observed. Determination of physiological properties of viruses is of great importance for developing of new antimicrobials based on bacteriophages. Supply of 0.1% sodium citrate into the culture medium reduced ability of bacteriophages to form plaques on bacterial lawn that indicated the need for divalent metal ions as adsorption cofactors. A primary evaluation of the survival of bacteriophages after UV-irradiation was carried out (G15W lamp, 40 cm above the samples, 2 m of exposure, medium: LB broth). In this study a decrease in the titer within one order of magnitude after UV-irradiation was recorded for both bacteriophages. Representatives of <i>E. amylovora</i> bacteriophages as an insufficiently characterized group of viruses, as well as potential agents of bacterial control, are of interest for further research.</p>

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Poster Title	Retikulin oxidase in flax - gene recognition, expression and its modification studies.
Abstract	<p>Flax (<i>Linum usitatissimum</i>) plants show wide range of valuable health-promoting components, like most commonly mentioned omega-3 fatty acids, lignans or phenylpropanoids, but also lately identified terpenophenols - cannabinoids. Beside the identification and preliminary biological activity measurements of these compounds from flax we propose the protein gene that can be responsible for the biosynthesis of cannabinoids. Using comparisons of flax genomic sequences with cannabinoid synthesis related genes from <i>Cannabis</i>, we identified coding region for reticulín oxidase - like protein showing 55% nucleotide and 45% aminoacid identity to the <i>Cannabis sativa</i> CBDA synthase gene.</p> <p>Reticulin oxidases have been identified in several plants as proteins engaged in the synthesis of alkaloids, for example in poppy, tobacco or hemp. They contain berberin bridge enzyme domain and covalently bound FAD cofactor. The reaction catalyzed by this type of protein, as it is in <i>Cannabis</i> plants, is most commonly the oxidative cyclization of prenylated alkilrezorcinols. We have analyzed the promoter of the gene that showed many interesting regulatory elements like light response elements, ABA regulated domains or pathogen infection related sequences. The expression studies showed actual dependence of the transcript production on the mentioned factors, as observed for <i>Cannabis</i> plant.</p> <p>Plants with reduced or elevated expression of the investigated gene were analyzed, where oligonucleotides treatments were used for gene expression silencing and enhancing. The results showed the strong dependence of the planned hybridization site localization with increased expression of the investigated gene when using promoter region sequence and silencing effects with oligonucleotides hybridizing in the coding region of the transcript. The plants with elevated levels of potential reticulín oxidase gene expression showed decreased transcript levels of genes involved in early stages of terpenoid synthesis, while down-regulation of investigated gene caused reverse reaction. These observations are similar to the relations observed in <i>Cannabis</i> what can indicate the functional similarity of the gene, but also confirms expression regulation conservation of the biosynthesis pathways across species.</p>

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Poster Title	Effect of a bioprotectant on Resistance Gene Expression in Wheat
Abstract	<p>Wheat is a staple dietary component and the most extensively grown crop in the world. However, high yield losses are recorded globally each year due to fungal pathogens. As a block, Europe is the highest wheat producer in the world and due to high intensive cultivation, also accounts for about 80% of the worldwide cereal fungicide market. Since many fungal pathogens build up resistance to fungicides, novel and alternative methods of pathogen control need to be developed.</p> <p>An innovative approach to improving productivity in crops challenged by biotic stress, is to stimulate the plant's own defence mechanisms. In a primed state, plants may better respond to biotic and abiotic stresses. The aim of the present project is to determine if a fermentation-based bioprotectant can prime plant defences by inducing the expression of endogenous defence-related genes.</p> <p>To date, results have suggested that such a bioprotectant may have the capacity to affect the expression of genes associated with the salicylic acid dependent signalling pathway. The functions of these genes have been associated with elicited defence responses to specific biotrophic pathogens, such as powdery mildew. Future work will seek to better elucidate the genes and pathways that are induced by the bioprotectant to mediate a plant defence response in wheat.</p>

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Poster Title	Gene Editing Approaches to Address Mycotoxin Contamination of Maize
Abstract	<p>Fungi produce a remarkable number of toxic secondary metabolites, collectively referred to as mycotoxins. Of considerable concern to human health are carcinogenic mycotoxins such as aflatoxin B₁ (produced by <i>Aspergillus flavus</i>), which is often described as the most carcinogenic naturally occurring compound known to mankind. Maize (<i>Zea mays</i>) is particularly susceptible to aflatoxin contamination when environmental conditions induce physiological stress during reproductive development and grain filling. Although genetic resistance to aflatoxin accumulation has been identified, it mostly originates from tropical corn germplasm, which is not suitable for modern agricultural production systems. As a result, decades of conventional breeding in public- and private-sector research programs have failed to produce agronomically viable maize hybrids with acceptable resistance to aflatoxin. Thus, the goal of this project is to identify genes underlying genetic resistance and susceptibility to aflatoxin accumulation in maize, which will then be modified via gene editing approaches. The overarching strategy is to blend descriptive approaches (such as gene expression profiling) with conventional and molecular genetics to identify genes underlying maize responses to infection and aflatoxin production by <i>A. flavus</i>. Progress to date includes the in-house optimization of maize transformation and transfection techniques, as well as the initial identification of candidate genes for further functional analysis.</p>

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Poster Title	High throughput NGS based mutagenesis screen to identify UV-B sensitive mutants in Arabidopsis
Abstract	<p>Solar ultraviolet (UV) light represents approximately 4% of total sunlight reaching the earth surface. Of three UV light, UV-B is the most damaging of all. Cellular targets of UV-B radiation include DNA, cell membrane, organelles such as mitochondrias and, in plant, chloroplasts. Plant being a sessile organism, has evolved a complex mechanism to cope with potentially damaging consequence of UV-B radiation. How various cellular processes coordinate to response to UV-B-induced damage in plant is poorly understood. We previously showed that UV-B causes increased abnormal chromosome behaviour and altered cytoskeleton distribution during mitosis [1]. To further investigate the molecular mechanisms mediating UV-induced abnormal cell division, we set out to screen for UV-B sensitive or resistant mutants in Arabidopsis using next generation sequencing (NSG) based approaches. As the first step of the project we generated multiple Arabidopsis transgenic lines carrying gene fusions between the GFP reporter and various cytoskeleton genes. These transgenic lines have been extensively characterised in terms of the patterns of transgene expression and the subcellular locations of cytoskeleton proteins, with or without UV-B radiation. The establishment of such transgenic reporter bank provides an important asset for subsequent mutant phenotypic characterisations. Work is in progress to identify genes that are responsible for UV-B-induced abnormal cell division using NGS approaches.</p> <p>References</p> <ol style="list-style-type: none">1. Chen, H. and R. Han, <i>Characterization of Actin Filament Dynamics during Mitosis in Wheat Protoplasts under UV-B Radiation</i>. Sci Rep, 2016. 6: p. 20115.

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Poster Title	Unraveling the regulatory pathways in plants using the Universal Plant ChIP-seq kit
Abstract	<p>Chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) is a powerful tool to study protein-DNA interactions for histones or other proteins like transcription factors. This technique contributes to the understanding of the regulatory pathways <i>in vivo</i> through the identification of target DNA sequences at the genome level. However, ChIP-seq experiments are usually time-consuming with several critical steps that need to be optimized for each specific tissue and/or species especially in plants. To overcome these difficulties, Diagenode, utilizing its expertise in epigenetics, has developed a specific solution for plant samples by optimizing each step of the protocol: the Universal Plant ChIP-seq kit. This user-friendly kit generates more chromatin and more immunoprecipitated DNA with higher specific enrichment than standard protocols. It was extensively validated by qPCR and by next generation sequencing on a number of model plant species and tissues using ChIP-seq grade Diagenode antibodies. It was also adapted for automation on the Diagenode IP-Star Compact Automated System.</p> <p>The Universal Plant ChIP-seq kit was developed by Diagenode in conjunction with the EpiTRAITS consortium.</p>

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Poster Title	Converting fruit and vegetable byproducts to compost with biopesticide/biofertilizer effect on pepper seedling
Abstract	<p>Compost from organic waste and by-products of fruit and vegetable processing industry can be used as organic suppressive substrates. In this study, we have evaluated five compost from different mixtures of organic waste and by-products from fruit and vegetable processing to use as partial substitute of peat and their suppressiveness against <i>Phytophthora parasitica</i> in pepper crop. Monitoring of piles composting process and characteristics of compost obtained were evaluated. Compost showed a suitable macro and micro-nutrients similar to other agro industrial compost and physical properties values accepted within values to be considered as an "ideal" organic substrate.</p> <p>The seedbed assay showed that different compost showed a similar even higher seedling growth than peat with suitable characteristics for transplantation into field. Also, some of them proved to be suppressive against <i>P. parasitica</i>. Compost C2 and C7, both containing pepper and vine pruning, showed the lower incidence percentage (30%). Between abiotic properties studied from different compost no significant differences between compost were observed. Both compost reached temperatures of 60 °C and it could be an important point to recolonize compost with antagonist capacity microorganisms.</p> <p>These results open a hopeful way of replacing peat by other organic materials which may come from waste and by-products of fruit and vegetable processing industry. This would by one hand the rational disposal of these wastes and by-product, and by other hand to get alternative organic substrates for peat. Also, these new organic substrates can incorporate an added value in their biofertilizer, bioestimate and/or biopesticide properties, which opens a potential market consumption of these composts in a demanding as is the seedling market.</p>

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Poster Title	Exploring the yield potential of novel spike architecture in hexaploid wheat by targeted mutagenesis using RNA-guided Cas9 endonuclease and genome wide association mapping
Abstract	<p>Grain number, measurable as per unit area, per plant, or per spike is an important yield component of wheat. To enhance grain number per plant in wheat, we aim for modifying its inflorescence architecture. Wheat and barley mutants that display non-canonical spike-branching have been characterized in our laboratory of which one tetraploid wheat mutant, 'Miracle Wheat' (i.e. <i>branched head, bh</i>), produced significantly more grains per spike that consequently led to higher spike yield. Moreover, we positionally cloned and identified mutant alleles of two other spike architecture genes, <i>compositum 1 (com1)</i> and <i>Six-rowed spike 4 (vrs4)</i> in barley, that resulted in branch formation. Therefore, the three above-mentioned genes—<i>Com1</i>, <i>bh</i>, <i>Vrs4</i>—represent exciting targets for engineering novel spike architecture in hexaploid wheat to potentially produce more spikelets and subsequently more grains per spike. To this end, we aim to employ genome engineering technology, namely CRISPR/Cas9, to knock-out these three negative regulators of spike-branching in hexaploid wheat to generate mutant plants with improved seed number or sink capacity.</p> <p>So far, we have targeted at least two positions per gene. The corresponding gRNA constructs designed for all three genes were checked for mutation efficiency by two different approaches prior to final transformation into wheat embryos. The efficiency prediction tests included an <i>in silico</i> analysis followed by a wet-lab experiment both of which revealed a high efficiency for the creation of mutations. Thus, these tested gRNAs were used to knock-out the aforementioned genes using Cas9 restriction endonuclease for which the corresponding transformed plants are being screened for mutation.</p> <p>In a complementary approach to modify the inflorescence architecture, a Genome Wide Association Scan (GWAS) and mapping approach is being implemented in a diverse bread wheat germplasm pool to discover genotypes carrying resource-efficient and/or large-grained related QTLs. We aim to introduce such QTLs into our grain number-elevated, branched-spike genotypes (obtained through CRISPR/Cas9) to overcome the known trade-off effect between grain number and grain size/weight.</p>

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Poster Title	SNP Genotyping for Barley Variety Identification and Purity Assessment
Abstract	<p>In this work we identified a small number of SNPs that can be used to distinguish and identify barley varieties. 38 SNPs have been used to genotype the barley varieties certified since 2012 in Scotland to create reference genotypes for each variety. The 38 SNP set is able to distinguish these varieties. Development and maintenance of this reference genotype database will be necessary and the reference genotype database will continue to expand as more varieties are genotyped with the 38 SNPs.</p> <p>Genotyping new varieties with the 38 SNPs is ongoing at SASA, and the SNP set will be continually assessed for its ability to distinguish new varieties. Additional SNPs will be added if necessary to the 38 SNP set.</p> <p>The work presented here is a tool which can be used to identify and confirm barley varieties. A future aim is to develop a barley variety identification tool to determine varietal purity. This will involve either performing high throughput barley identification on single seeds or by genotyping pooled seeds using a more sensitive SNP detection system which can detect differences of genotypes at very low levels.</p> <p>Another future aim is to assess the usefulness of SNP genotyping (and this SNP set in particular) for supporting plant breeders' rights. In order to register a new variety and protect it, the variety must be tested to be distinct, uniform and stable (this is called DUS testing).</p>

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Poster Title	Systematic analysis of epistatic interactions in photosynthesis efficiency
Abstract	<p>Photosynthesis is the primary mechanism that constitutes plant growth. Plants with high photosynthesis efficiency assimilate more biomass while using less amounts of water, which makes genetically improving this trait very potential considering future challenges of human population growth. Unfortunately, photosynthesis efficiency is a trait that is difficult to reliably phenotype for and that has a genetic architecture is very complex with thousands of genes involved. Therefore, not much effort has been put into studying photosynthesis efficiency using forward genetics approaches.</p> <p>Chromosome substitution libraries (CSLs) are new mapping population types that segregate on the complete chromosome level – i.e. without recombination. As such, CLSs present a highly simplified genomic layout compared to DH, RIL or F2 populations. The number of unique lines in a CSL is finite, rather than (nearly) infinite compared to segregating populations – for <i>Arabidopsis</i>, only 2⁵ unique lines are possible. As a result, mapping power is much higher because all loci are equally balanced. In addition, this high power characteristic allows the study of interchromosomal epistatic interactions. By making specific crosses, the exact genomic regions of interest can then be finemapped in populations that segregate for single chromosomes only by which genetic variation in the background is reduced.</p> <p>Phenovator® is a high throughput phenotyping facility that can reliably evaluate the photosynthetic performance of 1440 plants within 50 minutes. Due to the finite numbers of lines, CSLs can be grown in high numbers of replicates which improves the precision of individual line performance. Together, Phenovator® and CSLs are potent to reveal genetic variation in genes that are involved in photosynthesis and have the potential to highlight genetic interactions that otherwise might be lost in otherwise randomly segregating genetic background populations.</p>

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Poster Title	Regulatory variation CO ₂ Concentration Mechanisms in <i>Moricandia</i>
Abstract	<p>Plants with C₃-C₄ photosynthesis display lower CO₂ compensation points than related C₃ species and Kranz-like anatomy. C₃-C₄ intermediates do not have the C₄ photosynthetic mechanism, but possess an efficient system for recycling of photorespiratory released CO₂ by transporting glycine into bundle sheath cells, where CO₂ released from mitochondria can be efficiently recaptured by numerous, adjacent chloroplasts. Within the project, we would like to investigate the importance of different biochemical and anatomical features for the function of this specific photorespiratory cycle as well as their manifestation in plant genomes. In <i>Moricandia</i>, species with C₃ and C₃-C₄ intermediate photosynthesis exist in close phylogenetic proximity and provide suitable materials to explore regulatory variation associated with this specific CO₂ concentration mechanism.</p> <p>Allele specific gene expression was assessed at a total of 120,200 SNP sites in six interspecific <i>M. arvensis</i> (C₃-C₄) x <i>M. moricandioides</i> (C₃) hybrids. On average, 30 % and 7 % of the SNP sites were regulated by only cis- and only trans- regulatory variation, respectively. In all hybrid lines, more ASE-SNPs had bias toward the C₃-C₄ intermediate, <i>M. arvensis</i>, allele than toward <i>M. moricandioides</i> allele. In addition to photorespiratory genes, many transcripts associated with photosynthesis related pathways were controlled by regulatory variances in the F₁ hybrid. The results of this study will provide valuable insights into the specific CO₂ concentration mechanism in C₃-C₄ intermediates and early evolutionary steps of C₄ photosynthesis.</p>

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Poster Title	Crops as model species: case studies on sorghum and wheat
Abstract	<p>The advent of high quality genome sequence data in crops together with the recent advances in mutagenesis and genome editing technologies opens great opportunities to transfer the knowledge acquired from model species directly into crops. From a broader point of view, crops could be directly exploited as model species to investigate plant physiology and molecular biology in an innovative and often overlooked context.</p> <p>Sorghum (<i>Sorghum bicolor</i> [L.] Moench) is the world's fifth most produced cereal crop. It is a highly resilient crop, it can survive high temperature and it is successfully grown in dry areas. Furthermore, sorghum is an excellent model for C4 grasses, a group that comprises maize and sugar cane.</p> <p>In our research group at CRAG (ES), we are using sorghum as a model species to study brassinosteroid pathways. Brassinosteroids (BR) are a class of plant hormones that control many aspects of plant growth and development, including key agronomic traits. Despite being a highly promising breeding target, BR pathways are still largely unstudied in C4 grasses. Our investigation could generate critical knowledge in BR research that might directly benefit plant breeders and agriculture.</p> <p>Wheat (<i>Triticum aestivum</i> [L.]) is the main cereal crop in Europe, and one of the most important food grain source for humans worldwide.</p> <p>In hexaploid bread wheat, the identification of loss-of-function mutants through conventional forward genetics-based approaches is highly impractical due to the gene redundancy afforded by the homoeologous copies present in the three sub-genomes, often hiding monoallelic mutations. Genome editing technologies offer unprecedented opportunities to simultaneously mutagenize homoeoalleles and to produce lines that are difficult to obtain using traditional mutagenesis. In our project at Rothamsted Research (UK), we obtained a domain-specific simultaneous triple mutant of the homoeoalleles of the gibberellin receptor Rht1. These mutant lines will be a valuable tool to gibberellin research in wheat.</p>