Global Engage's

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Poster Presentation Abstracts

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Poster Title	Unexpected editing and off-target issues: What have we learnt from editing the *TaASN2* gene in wheat?
Abstract	Acrylamide is a carcinogenic processing contaminant that was discovered in cooked food products in 2002. It forms from free (non-protein) asparagine and reducing sugars during baking, frying, toasting and roasting. In cereal products, the free asparagine concentration determines the amount of acrylamide that forms. A gene responsible for asparagine biosynthesis in the grain, *TaASN2*, was identified as a good target for genetic interventions to reduce the acrylamide-forming potential of wheat. Wheat plants with successful CRISPR/Cas9 editing of the *TaASN2* gene are being trialled in Europe's first genome edited wheat field trial. The plants were generated through the biolistic bombardment of three constructs into immature wheat embryos, comprising a codon-optimised *Cas9* gene, a *bar* marker gene, and a polycistronic gRNA gene. The polycistronic gene contained 4 gRNAs, tandemly arrayed with tRNAs to allow intracellular cleavage and the release of 4 mature gRNAs. The edits were characterised in the T1 and T2 generations using NGS, which provided information on the types of edits that had been induced and highlighted some issues in using this technology in wheat. For example, there was evidence for transgenerational editing, with edits appearing in the T2 generation that were not identifiable in the T1 generation. In some lines, multiple alleles were present in the T2 generation, each with new edits appearing in addition to the edits seen in the T1 parents. Most importantly, off-target editing was detected in one of the lines during preparation for the field trial, in the form of a 37bp deletion in the related *TaASN1* gene. The edit was present only in the B genome *TaASN1* genes and at only one of the four potential editing sites. This was the site that was most similar to the corresponding target site in *TaASN2*, but it still showed two mismatches with the gRNA.

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Poster Title	Identifying and characterising a multi-environment grain asparagine content QTL
Abstract	Quantitative trait loci (QTL) controlling grain asparagine content have been sought since the discovery of acrylamide in food in the early 2000s, but stable QTL across environments have been lacking. The recent availability of multiple wheat genomes has greatly facilitated and enabled research into the genetic control of important quality traits. Such resources enabled the identification and characterisation of a QTL controlling grain asparagine content across two field trials undertaken in the UK in 2012 and 2013. Available wheat genomes and commercial UK cultivars from these trials were screened for the presence of the asparagine synthetase 2 B homeolog (TaASN-B2), which is absent from the Chinese Spring reference genome. The TaASN-B2 deletion was shown to associate with lower grain asparagine content under conditions of sulphur sufficiency, but not under conditions of sulphur deficiency; indicating an interaction between the two. The deletion occurs adjacent to an Inga retrotransposon and was also found in some tetraploid species; indicating the probable historic origins of the deletion in progenitors of hexaploid wheats. Expression profiling of the TaASN2 genes was further undertaken to confirm its expression patterns in grain tissue. Our results indicate that the TaASN-B2 deletion may be a stable QTL that can lower grain asparagine content in sulphur-sufficient environments.

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Poster Title	BLSSpeller: case study to discover novel regulatory motifs in maize
Abstract	With the decreasing cost of sequencing and availability of larger numbers of sequenced genomes, comparative genomics methods are becoming increasingly attractive to complement experimental techniques for the task of transcription factor binding site identification. We redesigned BLSSpeller, a motif discovery algorithm, to cope with larger sequence datasets. BLSSpeller was used to identify novel motifs in *Zea mays* in a comparative genomics setting with 16 monocot species. We discovered 61 motifs of which 20 matched previously described motif models in Arabidopsis. In addition, novel, yet uncharacterized motifs were detected, several of which are supported by available sequence-based and/or functional data. Instances of the predicted motifs were enriched around transcription start sites and contained signatures of positive selection. Moreover, the enrichment of the predicted motif instances in open chromatin and transcription factor binding sites indicates their functionality, supported by the fact that genes carrying instances of these motifs were often found to be coexpressed and/or enriched in similar GO functions. Overall, we showed that BLSSpeller, a high-performance motif discovery tool, can successfully be used to predict novel motifs in a comparative setting. Combining such predictions with available genomics and functional data allowed further elucidating transcriptional regulation in *Zea mays*. Although their impact requires further characterization, the provided motifs offer a large and valuable source for further investigation. The candidate motifs that might help our understanding of the genotype to phenotype association in crops.

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Poster Title	Discovery of Novel Genetic Features and Molecular Pathways for Cassava Mosaic Disease (CMD) and Dry Matter Content (DMC) Using Genome-Wide Combinatorial Network Analyses
Abstract	Cassava (Manihot esculenta) is one of the most important edible crops across the tropics and is fundamental to food security in the developing world. However, the genetics of cassava is less studied than other major crops, such as rice or maize, in terms of disease resistance, yield or content. Our aim was to discover novel genetic targets and molecular pathways for cassava mosaic disease (CMD) and dry matter content (DMC), using our proprietary Synomics discovery platform. The Synomics discovery platform is based on constructing pairwise to higher orders (6-way +) interactions of SNPs and uses constraint-based models and machine learning methods. We analysed 101,521 SNPs generated from 3,465 healthy and diseased Nigerian cassava plants sampled after 1-month and 3-months. Our novel technology captures GWAS top SNPs, alongside novel combinations of SNPs that GWAS does not, including 44 and 4 quantitative trait nucleotides (QTNs) related to CMD and DMC, respectively. Individual SNPs taken from significant combinations were mapped to genes, resulting in 435 and 237 candidate genes for CMD and DMC, respectively. These genes were biologically validated by previous studies. GO term enrichment analysis was performed on the candidate genes, showing significance ($p<0.0001$) in important immune response GO terms for CMD and DNA maintenance pathways for DMC, thereby validating our results. We show that the Synomics discovery platform finds new biologically relevant targets for disease and yield traits, that cannot be found by current best practices alone. Therefore, this disruptive technology has potential in plant breeding programs and pesticide target discovery.

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Poster Title	Genetic Analysis of Hexaploid Wheat (*Triticum aestivum* L.) Using Complete Chloroplast DNA Sequences
Abstract	The aim of the presented study is genetic characterization of the hexaploid wheat *Triticum aestivum* L. The complete sequencing of chloroplast DNA was carried on the NovaSeq 6000 and assembled by SOAPdenovo2. The complete chloroplast DNA sequences of 13 hexaploid wheat samples were determined. Free threshing - *T. aestivum* subsp. *aestivum*, 1 sample; *T. aestivum* subsp. *compactum*, 2 samples; *T. aestivum* subsp. *sphaerococcum*, 1 sample; *T. aestivum* subsp. *carthlicoides,* 4 samples. Hulled - *T. aestivum* subsp. *spelta*, 3 samples; *T. aestivum* subsp. *vavilovii* jakubz.*,* 2 samples. The comparative analysis of complete cpDNA sequences of 20 hexaploid wheat samples (13 samples in this article) + 7 samples in 2018 (Gogniashvili et al., 2018) sequenced in this laboratory was carried out. 20 samples can be divided into two groups - *T. aestivum* subsp. *spelta* 3 samples + *T. aestivum* subsp. *vavilovii* collected in Armenia and the remaining 16 samples, including *T. aestivum* subsp. *vavilovii* collected in Europe (Sweden). If we take the cpDNA of Chinese Spring as a reference, 25 SNPs can be identified, 13-14 SNPs in *T. aestivum* subsp. *spelta* and subsp. *vavilovii* (Vav1) (collected in Armenia). In the other samples up to 11 SNPs were detected. 22 SNPs are found in the intergenic regions, 2 found in introns; 10 SNPs were found in the genes, seven of these are synonymous, which does not alter the amino acid. One 35bp insertion and three inversions (56bp, 58bp and 25bp in lengths) have been identified in *Triticum aestivum* subsp. *spelta* and *T. aestivum* subsp. *vavilovii* (Vav2) samples. One 38bp inversion with 4bp loop has been identified in *T. aestivum* subsp. *vavilovii* (Vav1).

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Poster Title	PCR Analysis of Three Homoeologous *Wknox1* Gene of Hexaploid Wheats (*Triticum aestivum* L.)
Abstract	South Caucasus (notably Georgia) and their earlier residents played an important role in wheat formation. 17 domesticated species and subspecies of *Triticum* are known. Georgian endemic wheat species include one *Triticum* species and four subspecies (Menabde, 1948; Menabde, 1961; Hammer et al. 2011): 1. *Triticum turgidum* subsp. *palaeocolchicum* (Menabde) A. Love 2. *Triticum turgidum* subsp. *carthlicum* (Nevski) A. Love 3. *Triticum timopheevii* subsp. *zhukovskyi* (Menabde & Ericzjan) L. B. Cai 4. *Triticum zhukovskyi* Menabde & Ericzjan 5. *Triticum aestivum* subsp. *macha* (Dekapr. & Menabde) McKey PCR-based haplotype analysis of the 4th intron of *Wknox1d* and of the 5th-to-6th exon region of *Wknox1b* of 20 hexaploid wheat samples was carried out. The PCR products were excised from the agarose gel and sequenced on an Applied Biosystems 3700 genetic analyzer. PCR-based haplotype analysis of the 4th intron of *Wknox1d* and the 5th-to-6th exon region of *Wknox1b* provide an opportunity to make an assumption that hexaploid wheats *T. aestivum* subsp. *macha* var. *palaeocolchicum* and var. *letshckumicum* actually differ from other macha samples by the absence of 42 bp insertion (AGTTTGCACACCTGAACATTTTGCATTATGTTCGGGAGCCTA) in the 4th intron of *Wknox1d*. It can be assumed that two *Ae. tauschii* (A) and (B) participated in the formation of hexaploids through the D genome: *Ae. tauschii* (A) - macha (1-5, 7,8,10-12) - and *Ae. tauschii* (B) - macha M6, M9, *T. aestivum* subsp. *aestivum* cv. ‘Chinese Spring’ and cv. ‘Red Doly’. Another possible explanation would be that the insertion might have arisen during the diversification process of common wheat after polyploidization.

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Poster Title	The correctness, completeness and contiguity of a *de novo* genome assembly depend on the assembler utilized.
Abstract	Plant genomes are challenging to assemble due to their large size and extensive repetitive sequences. The reference genomes of model or economically important plant species have been built using a combination of sequencing technologies and are considered as the most accurate genome models available. We carried out an independent benchmarking exercise on assembly algorithms using Pacific Bioscience sequencing data, publicly available for download from NCBI. The most used assemblers such as (Hi)Canu, Flye, Raven, Wtdbg2, Smartdenovo and Hifiasm, were used. We assembled the genomes of *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor*, *Solanum tuberosum* with the different assemblers and assessed the generated assemblies for their correctness, completeness and contiguity using BUSCO, BANDAGE, QUAST and D-GENIES. We also compared the resulting assemblies to each other and the reference genome of each species for misassemblies. The assemblies differ greatly in contiguity as measured by N50. For example, in the case of Arabidopsis, Flye gave an assembly with an N50 of 9.4 Mbp while the assembly generated by Canu for the same dataset has an N50 of 1.8 Mbp. In this case, the assembly of Flye is also the most complete as assessed by BUSCO, with a score of 97.8 %. All assemblies, regardless of assembler used are fragmented especially near the centromeric and telomeric regions. The Arabidopsis assembly using Wtdbg2 is made up of 346 contigs while that generated by Raven has 41 contigs. The number of misassembles as calculated by QUAST also different with the lowest being for Wtdbg2. This leads us to conclude that a genome assembly has to be assessed using several matrices. This also implies that, reference genomes, already published for a large number of plant species may include assembly artifacts which need to be considered in the conclusions drawn. Thus, to date, *de novo* genome assembly must rely on other technologies such as optical maps and Hi-C to generate a chromosome level assembly.

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Poster Title	A Collection of 755 Diverse Bread Wheat Landraces Reveals Broadened Genetic Diversity and Novel Loci Associated With Powdery Mildew Resistance
Abstract	With climate change and an ever-growing human population to feed, crop adaptability and a broad genetic basis for breeding is needed. However, during wheat domestication and breeding, a man-made genetic bottleneck was created, resulting in reduced genetic diversity in modern cultivars. Bread wheat landraces, a crop status between wild relative and elite cultivar, display a higher genetic diversity than modern breeding material, while guaranteeing sexual compatibility in breeding due to their hexaploid nature. To exploit this untapped gene potential, we assembled a diverse collection of 755 bread wheat landraces (*Triticum aestivum* L.) of worldwide origin, with a focus on the region around the Fertile Crescent. This collection was genotyped with the Axiom TaBW35K SNP array, resulting in 29556 high-quality, non-redundant and polymorphic SNPs that were anchored to the Chinese Spring reference genome RefSeq v1.0. Genotypic analysis revealed a broadened genetic diversity compared to earlier studies on landrace collections and affirmed geographic origin as an appropriate proxy for relatedness. An exemplary usage of this collection was conducted focusing on the yield reducing fungal disease powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*. Here, we scored phenotypic resistance against ten different powdery mildew isolates at seedling stage. Genome-wide association studies (GWAS) detected multiple novel regions associated with powdery mildew resistance alongside the known locus of the cloned *Pm2* resistance gene on chromosome 5DS. The latter was formerly unknown to be present in wheat landraces and introduced into hexaploid wheat from its diploid wild relative *Aegilops tauschii*. This study illustrates the utility of hexaploid wheat landrace collections for the detection of novel genetic loci that were believed to be preserved solely in wild relatives.

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Poster Title	Increasing Iron and Zinc in Milled Rice through promoter editing of a Nicotianamine synthase gene in mega variety IR64
Abstract	Iron and Zinc malnutrition or hidden hunger is affecting 2 billion people globally especially pregnant women and children in developing countries like the Philippines, Bangladesh, and Indonesia. Hidden hunger could lead to impaired cognitive development, maligned immune system, and stunting. In a field transgenic study lead by the International Rice Research Institute (IRRI), it has been shown that increasing iron and zinc is possible by constitutively overexpression of rice Nicotianamine Synthase 2 (OsNAS2). OsNAS2 is involved in the mugenic acid family phytosiderophore production in rice roots. In this study, we are exploring the possibility of utilizing CRISPR-Cas9 technology to edit the promoter region of OsNAS2 to achieve the target of increased iron and zinc levels in polished rice grains. As proof of concept, this could potentially achieve the primary target while circumventing the arduous deregulation process required in products developed through transgenic approach. Currently, transgene-free and/or homozygous lines are being assessed through sequencing, off-target study, and mineral content analysis are being performed. Agronomic data will also be collected after and identifying primary candidates from these promising entries and seed increase.

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Poster Title	Vicine-free faba beans - a case for gene editing
Abstract	Faba bean (*Vicia faba*) is a legume crop cultivated for its high-protein seeds and exceptional nitrogen fixing capabilities. It is adapted to a wide variety of climates, thus offering an opportunity to reduce the import of soy from overseas, and boost local protein production for food and feed in Europe. However, the accumulation of the antinutrients vicine and convicine (V&C) in faba bean seeds poses food safety and feed efficiency issues. Upon ingestion, V&C can cause favism, a haemolytic anaemia, in people with glucose-6-phosphate dehydrogenase deficiency - a genetic condition affecting 400 million people worldwide. In addition, V&C is linked to production loses in chickens, laying hens and pigs. Thus, the elimination of V&C is fundamental to broadening the use of faba beans for more sustainable and diverse protein production. We created a gene expression and metabolite atlas spanning 8 different faba bean tissues to help identify V&C biosynthesis genes. Using gene-to-metabolite correlations and fine mapping approaches, we identified *VC1* – a gene encoding for a GTP cyclohydrolase II. We then demonstrated that overexpression of *VC1* complements a low vicine phenotype *in vivo.* Moreover, VC1 protein converts GTP *in vitro*, while feeding labelled GTP precursor to faba bean seedlings results in its incorporation in V&C. These results support the new hypothesis of V&C biosynthesis, and help to build a targeted approach for V&C elimination from faba bean. Given these biochemical insights into V&C synthesis, gene editing might be essential to address the antinutritional properties of faba bean. This requires reliable, stable transformation methods that would facilitate robust gene testing. So far, we are able to transiently express genes of interest using leaf infiltration, as well as create composite plants with transgenic roots. Tissue culture or *de novo* meristem induction methods prove difficult and require further investigation to produce stable transgenic lines of faba bean.

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Poster Title	A chromosome-level, haplotype-phased genome assembly for *Vanilla planifolia* highlights that partial endoreplication challenges accurate whole genome assembly
Abstract	The genome of *Vanilla planifolia* is prone to partial endoreplication, also called strict partial endoreplication (SPE), which manifests by the several rounds of replication of only one fraction of the genome without cell division, thus leading to unbalanced DNA content in cells. We report here first molecular evidence of SPE at chromosome scale by the assembly and annotation of an accurate haplotype-phased genome of *Vanilla planifolia*. Using PacBio HiFi and optical mapping, we assembled and phased a diploid genome of 3.4 Gb with a scaffold N50 of 1.2 Mb, which is therefore close with the size estimated by cytogenetic data, that demonstrated a diploid genome size of 4.09 Gb, with 16 chromosome pairs although aneuploid cells are frequently observed. This assembly represented 82% of the estimated *V. planifolia* genome size and provides a significant step towards its elucidation. Atypical k-mers frequencies and uneven sequencing depth observed along the chromosomes agreed with the expectation of an unbalanced genome representation caused by SPE. Furthermore, from the 59,128 annotated protein-coding genes, sixty-seven percent were scattered over a little less than one third of the genome, putatively linking the endoreplication phenomenon with gene-rich regions. On the contrary, the low coverage regions (which we suppose are non-endoreplicated) were rich in repeated elements but also contained 33% of the annotated genes. Additionally, this assembly showed distinct haplotype-specific sequencing depth variation patterns suggesting a complex molecular regulation of the SPE along the chromosomes. Further efforts will be needed in order to understand this phenomenon and the mechanisms involved in its regulation.

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Poster Title	Genomic selection for resistant genes in F2 hybrids of common bean (*Phaseolus vulgaris* L.)
Abstract	Marker-assisted selection (MAS) for resistant genes is of great importance for the development of new varieties with improved traits with respect to tolerance to biotic stress. A set of 13 trait-associated DNA markers was used to screen the entire phenotypically promising F2 hybrid generation of common bean, which originated from a 18 combinations of Slovenian and worldwide germplasm (targeted hand pollination) and exhibited dwarf and indeterminate growth types. From these, we identified F2 plants with resistance genes for the most common bean pathogens (viruses, bacteria, fungi) and for one of the most important pests (bruchid). For the analysis of association with bean mosaic virus (BCMV) and bean mosaic and necrosis virus (BCMNV), we used 4 SCAR (Sequence Characterised Amplified Region) markers and one CAPS (Cleaved Amplified Polymorphic Sequences) marker. To detect the presence of bean rust resistance genes, a SCAR marker was included in the genetic analysis. In addition, 4 SCAR markers were used to analyse the association of hybrids with bean rust tolerance. To test for the presence of angular leaf spot disease resistance genes, 2 SCAR markers were used. There was also 1 SCAR marker used that was strongly associated with susceptibility to bruchid resistance. In addition, for the entire F2 generation of a breeding material, we used the combination of Phs markers with indel-spanning markers of the Pv SHP1 locus to determine the genetic origin of our breeding material. Of the 247 F2 hybrids of common bean, we selected only 4 (3 of indeterminate growth type and one of dwarf growth type) that exhibited the resistant traits at all loci examined and for different types of biotic stress caused by economically important pathogens in common bean. The combination of phenotypic and genomic selection allows us to selectively and efficiently identify the most suitable hybrids for the development of new varieties to achieve breeding goals faster and with less effort.

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Poster Title	Developing a multigene CRISPR/Cas9 targeting assay in potato
Abstract	*Phytophthora infestans* (*Pi*) causes 16% of losses in global potato production. Efforts to make resistant cultivars through breeding via the insertion of *Resistance* genes is slow and field resistance is quickly overcome by the rise of new *Pi* strains. An alternative method is knocking out *Susceptibility* genes (*S*-genes): innate plant genes that, when removed, can lead to lower disease susceptibility. Here, 33 genes were targeted in potato using PEG mediated protoplast transfection and CRISPR/Cas9. Highly multiplex amplicon sequencing (HiPlex) primers flanking CRISPR cut sites were designed and tested on wild type ´Bintje´ and ´Spunta´ tetraploid DNA. 44 gRNAs targeting 33 genes were selected and cloned into separate expression vectors. gRNA vectors were divided into four pools containing 11 gRNAs each: single or multiple pools were used in protoplast transfections to generate various mutation profiles. Edited alleles were found in both ´Bintje´ and ´Spunta´ pooled and single callus samples. The most efficient gRNA (EXLA2_2) had an average editing efficiency of 20%. Of the 44 gRNAs tested, 24 led to indels (~55%). Interestingly, results show that single calli were edited in multiple genes (up to 11 genes in Spunta). ´Spunta´ calli were regenerated into shoots (~15-20% regeneration efficiency), and will be used for detached leaf assays to test for susceptibility to *Pi*. These results show that multiple genes in different combinations can be edited using a multiplex CRISPR/Cas9 approach, and provides a starting point to engineer *Pi* resistance in potato.

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Poster Title	Increasing durum wheat resistance to *Fusarium graminearum* by using cell wall-derived oligogalacturonides
Abstract	Plant diseases cause substantial crop losses worldwide and compromise food safety because of the presence of toxins associated to fungal contamination. In addition to microbial diseases, climate changes negatively impact crop production as well as water and land availability for agriculture, while favoring pest distribution. Thus, sustainable yield increase, diminishing usage of chemicals and toxic compounds, enhancing crop resilience to biotic stress represent the main, concomitant, targets to be pursued in agriculture in the shortest period. Among the current approaches to crop protection, the use of elicitors, able to activate the natural defense mechanisms of the plant, as alternatives/complementary to pesticides and genetic resistances, is a strategy gaining increasing attention. Numerous studies indicate that local application of plant cell wall (CW)-derived elicitors, such as oligogalacturonides (OGs) derived from partial degradation of homogalacturonan, a major pectin component of the plant CW, induce broad-spectrum, long-lasting, and systemic resistance against pathogens in different plant species. The aim of this study was to establish the efficacy of OGs in protecting durum wheat, characterized by an extreme susceptibility to fusariosis caused by *Fusarium graminearum*. Our results demonstrate that OGs are indeed active elicitors of plant defenses in wheat triggering both a priming effect and the induction of typical immune marker genes, accompanied by the downregulation of genes involved in the fungus mycotoxins biosynthesis. No differences associated with growth and development of wheat seedlings were detected after elicitation with OGs. Furthermore, the study of durum wheat plants with potentially altered endogenous OG levels, i.e. OG-machine lines, is facilitating the elucidation of molecular mechanisms regulating plant defense activation upon sensing danger signals in cereals.

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Poster Title	CRISPR editing of durum wheat to reduce a-amylase/trypsin inhibitors content in the grain
Abstract	The incidence of wheat-related disorders is continuously increasing in the world population. The protein fraction of the wheat kernel is the main cause triggering adverse reactions to wheat. Structural and metabolic proteins, like a-amylase/trypsin inhibitors (ATI), are mainly involved in the onset of bakers' asthma, wheat allergies and Non-Celiac Wheat Sensitivity (NCWS). The ATIs are a protein family including different subunits that can be classified into monomeric (WMAI), homodimeric (WDAI), and heterotetrameric forms (WTAI) based on their aggregation degree. ATI subunits are the most IgE-reactive proteins of the wheat kernel, particularly the subunits WDAI-0.19, WTAI-CM2, WTAI-CM3, and WTAI-CM16. In the present work, the multiplex editing of two genes encoding WTAI-CM3 and WTAI-CM16 subunits were obtained through CRISPR-Cas9 in the Italian durum wheat cultivar Svevo. A marker free approach led to produce GM-free plants from the first generation. The use of multiple gRNAs targeting each gene allowed to enhance the editing efficiency producing homozygous mutant phenotypes with large deletions in the T_0 generation. Enzyme-linked immunosorbent assay (ELISA) test and western blot analysis showed no response to the monoclonal antibody against WTAI-CM3 and WTAI-CM16 proving the knockouts of the target genes. Twelve different ATI, extracted from T_3 plants, were quantitated using heavy isotopic labeled peptides and a LC-MS/MS-SRM method on a triple quadrupole system. Absolute quantitation verified silencing of the two ATIs in the mutant durum wheat lines and no off-target effects were detected on non-target ATIs. In conclusion, we obtained edited durum wheat lines containing stable and heritable mutations with reduced amounts of ATI proteins that might allow both the development of novel wheat cultivars with a lower triggering potential and enable a better understanding of the role of these components in wheat related pathologies.

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Poster Title	A major QTL for stem rust resistance in Italian ryegrasses explains 53 % of the phenotypic variation
Abstract	Stem rust caused by the ascomycete *Puccinia graminis* ssp. *graminicola* is one of the most important fungal diseases in Italian ryegrass (*Lolium multiflorum* Lam.). Increasing average temperatures due to climate change will lead to an even higher stem rust pressure on ryegrasses within the next years. The main disease symptoms usually occur late in the season, right before ripening of the seeds and have a negative impact on forage quality and seed yield. Therefore, breeding for stem rust resistance is imperative, and a detailed understanding of the inheritance of resistance is needed to enable efficient breeding strategies for this important trait. In this study, we developed a biparental F_1 mapping population derived from a reciprocal cross between one genotype of the cultivar Rabiosa and one genotype of the cultivar Sikem. The population consists of 124 single plants which were phenotyped for stem rust resistance in replicates in three environments. Stem rust phenotypes from the field were scored on a 1-9 scale, where 1 is the absence of symptoms and 9 is highly infected. Both parental plants showed an intermediate stem rust resistance. A genetic linkage map, spanning a total of 785 cM over seven linkage groups, was constructed with 1,528 single nucleotide polymorphism markers produced by genotyping-by-sequencing. First quantitative trait locus (QTL) analysis revealed a major QTL on linkage group 7, which explained 53% of the phenotypic variation for stem rust resistance. These results indicate the presence of a major resistance gene for stem rust. Candidate genes for resistance will now be identified and validated using the recently established high quality genome assembly for the parental cultivar ‘Rabiosa’. Moreover, a nested associated mapping population was established and will be used additionally for fine mapping. Already now, the markers closely linked to the QTL provide a valuable resource for marker-assisted breeding of Italian ryegrass.

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Poster Title	Interrogating introgressed chromatin associated with powdery mildew resistance in wheat
Abstract	Allohexaploid bread wheat (*Triticum aestivum*), a staple crop for worldwide food security, is threatened by diseases and pests. *Blumeria graminis* f. sp. *tritici* (Bgt), the causal agent of wheat powdery mildew, is responsible for 5% of these global yield losses. Host resistance mediated by race-specific resistance genes is of vital importance to reduce pesticide use while controlling for the disease. Striving to discover novel race-specific powdery mildew resistance genes, we employed a global collection of 886 wheat accessions that were each subjected to a seedling stage infection test with 24 different Bgt Isolates. A SNP-based genome-wide association analysis was conducted to identify genetic markers associated with the resistance phenotype. Seven regions in the wheat genome showed significant association with race-specific mildew resistance. Among these, four lie in proximity or within coding sequences of already cloned powdery mildew resistance genes (*Pm2*, *Pm4*, *Pm5*, *Pm8*). In contrast, the three other identified regions were not previously characterized to harbour cloned powdery mildew resistance genes. Haplotype analyses suggest that the significantly associated SNP variants for all three candidate regions are contained in alien introgressions compared to the IWGSC reference genome. By comparing the resistance associated haplotypes to published sequence information we identified putative chromatin donors for the three candidate regions. Two of these introgressions possibly originate from *Triticum timopheevi*, whereas the remaining one seems to stem from an *Aegilops tauschii* accession of the just recently identified L3 lineage. Owing to the low recombination rate in the introgressed regions, a precise localization of the resistance conferring locus remains challenging. Although these three introgressed chromosome segments are associated with powdery mildew resistance, not all putative introgression carrying accessions are resistant to powdery mildew. Presumably indicating that there are different introgression variants which are not discernible by the initial genotyping.

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Poster Title	Application of a multiplex genome editing strategy in durum wheat to improve yield and grain nutritional quality
Abstract	Among staple crops, wheat represents one of three major cereals cultivated worldwide, following rice and maize. Its adaptability to cultivation, good nutritional profile and versatility are the main reasons for its success. Wheat and derived foods play an important role in human nutrition as they represent a good source of carbohydrates, proteins, dietary fiber and micronutrients. The impact of climate change and the need to satisfy dietary requirements due to rapid population growth make the challenges facing agriculture and scientific research more difficult. In this scenario it is essential to improve crop yield and food quality in a sustainable manner and CRISPR/Cas technology represents a precise and powerful genome editing tool to achieve these goals. The research here discussed concerns the development of two multiplex genome editing strategies based on the CRISPR/Cas9 system in durum wheat to improve yield and nutritional quality, respectively. In principle, the multiplex strategy relies on exploiting the endogenous tRNA processing system of plants. The first strategy was developed with the aim to improve yield through the editing of two genes involved in floret fertility, determining the number of grains per spikelet (*GNI1A*), and in leaf angle increasing the capacity to intercept light (*SPL8*). Agrobacterium-mediated transformation was used and the selection of transformed plants is in progress. The second strategy was developed with the aim to biofortify genetically durum wheat in β-carotene, precursor of vitamin A. Three genes coding for three key enzymes were targeted: *lycopene epsilon cyclase* (*LCYE*) and β-*carotene hydroxylase* (*BCH-1*), involved in the biosynthesis of carotenoids and *lipoxygenase* (*Lpx-1*), responsible for the oxidative degradation of carotenoids during the post-harvest. Wheat transformation by biolistic method was carried out using two vectors: one containing Cas9 and one containing two gRNAs for each target gene. Homozygous mutants of *Lpx-1* gene were identified showing a reduced lipoxygenases activity.

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Poster Title	A streamlined platform for optimizing base editor activity in wheat and maize
Abstract	The development and optimization of genome editing tools requires testing large panels of variants and configurations. Among the recently developed tools, base editors are built by the fusion of a nickase with a cytidine deaminase to specifically introduce Cytidine (C) to Thymidine (T) mutations at desired genomic sites. Although large numbers Cytidine Base Editors (CBE) have been used in plants, current methods only allow for a few comparisons between vector variants, limiting the testing and optimization of reagents. To facilitate the systematic testing of base editors, we have set up a transient, quantitative and versatile base editing optimization platform for wheat and maize protoplasts. Using a combination of modular Golden Gate cloning, arrayed protoplast transfections, high content imaging and automated analysis, we are able to process up to 96 samples per day. In parallel, we have increased the throughput of genome editing detection at endogenous target sites by improving Fluorescence-Activated Cell Sorting protocols that allow genotyping from two thousand protoplasts. We validate this platform using APOBEC3A-nCas9 CBE and propose that it could be broadly used for step-wise optimization of Base Editors (BE) or Prime Editors (PE).

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Poster Title	Elucidation of the *Arabidopsis NPK1-related Protein kinases* (ANPs) role in danger signalling
Abstract	Plants deploy an enormous number of cell-surface and intracellular immune receptors to perceive danger signals. Danger signal is transmitted inside the plant cells by activated key immune signaling modules and involves several subcellular organelles and interactions between them, coordinated by the exchange of metabolites and signaling molecules, including calcium and reactive oxygen species. Membrane dynamics, cytoskeletal rearrangements and protein transport are essential for the proper activation of plant immune mechanisms and, therefore, critical plant trafficking regulators represent favorite targets of pathogen effectors. Arabidopsis NPK1-related Protein kinases ANP1, ANP2 and ANP3 are MAP kinase kinase kinases that have been shown to be key regulators of essential physiological processes, such as cytokinesis, resistance to pathogens, ROS homeostasis. Moreover, multiple *anp* double KO and silenced triple mutants display reduced growth, spontaneous cell death and constitutive resistance to Botrytis as well as higher susceptibility to Pst DC3000 hrcC-. Most of the above listed processes are also regulated by JA, which in fact was found to be considerably higher in *anp* double and triple mutants, suggesting a possible role for ANPs as negative regulators of JA biosynthesis/accumulation. We show here that, lack of ANPs also prevents the expression of downstream jasmonate target genes *VSP1* and *VSP2*, accompanied by an overexpression of *ORA59*, *PDF1.2* and *THI2.1*, hinting a role of these MAP3Ks in regulating the MYC2-dependent branch in response to JA. Moreover, we show here a novel function of these kinases in the actin cytoskeleton organization and Golgi bodies motility. Elucidation of how danger signalling is generated at a cellular level and transmitted sub-cellularly and to the hole plant will allow the combination of basic research derived knowledge with fast and precise genetic engineering techniques to finely tune immune responses for the benefit of both crop defence and yield.

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Poster Title	Re-Waste: promoting agriculture sustainability by recycling wet-organic waste
Abstract	In the EU, an estimated 20% of the total food produced is lost or wasted. Food waste is a rich in highly valuable biomolecules: phytochemicals such as pigments, phenolic bioactive compounds, oligosaccharins and minerals able to cause changes in crop important physiological processes. The purpose of the current study is to exploit waste by-product to develop a sustainable strategy for crop plant growth, development and resilience to (a)biotic stresses. We, thus, conducted agronomic trials on two model species for agriculture (wheat and tomato) by using agro-food waste, i.e. orange peel and crustacean shells, that was compacted and dehydrated, and the solid residue (SR) obtained at the end of the compaction cycle was directly added to growth substrate. Different morpho-physiological parameters (e.g. SPAD Index, chlorophyll fluorescence, stomatal conductance and plants biomass) were positively influenced by the addition of low doses of both SRs hinting that they may be effective in regulating plant physiology. These SRs represent a potential reservoir of bioactive products, including oligosaccharins, such as chitooligosaccharides - derived from partial degradation of chitin/chitosan - and oligogalacturonides - derived from partial degradation of homogalacturonan, a major pectin component of the plant cell wall - both potential alternative to traditional agrochemicals. Moreover, SRs could correct the hydrogeological and breathable characteristics of soils as well as determine soil remediation effects by positively affecting plant-microbiome relationships, optimizing interactions with beneficial microorganisms. We propose here a novel, safe and sustainable strategy based on a model that promotes circular economy by recycling wet-organic waste, replacing chemical fertilizers, reducing pollution and high management costs by cutting off waste collection, transport, and delivery.

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Poster Title	Gene App: Direct method for gene editing in plants
Abstract	Although gene editing is a powerful tool for improving crop traits, there is a limitation in application to elite cultivars because of the lack of a practical delivery system. In many cases, the callus formation and shoot regeneration process are highly genotype-dependent, inhibiting the wide use of gene editing in crops. Here, we have developed a high-efficient gene-editing method that does not require Agrobacterium-mediated transformation, callus formation, and shoot regeneration, hence applicable to various plants. First, we designed a highly efficient expression vector to enable gene editing in the transient expression of editing tools. We combined this vector with the mechanical introduction directly into the plant meristem to obtain gene-edited seeds simply by growing the plant in normal conditions. We will present the successful results in several tomato commercial cultivars and other plants, including soybean and Perilla.

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Poster Title	Identification and Annotation of Resistance Gene Analogs in *Vanilla planifolia*
Abstract	Vanilla cultivation is limited by numerous diseases, including the Root and Stem Rot Disease caused by the soil fungus *Fusarium oxysporum* f. sp. *radicis-vanillae* (Forv). Obtaining crops with high levels of disease resistance is a major challenge for breeders. The genome of the main cultivated *Vanilla planifolia* is now available and provides the sequences of genes involved in resistance mechanisms. Well-documented bioinformatics tools were used for identification and annotation of Resistance Gene Analogs (RGA) in *V. planifolia* genome CR0040. Plant resistance genes are mainly encoded by three large Leucin-Rich Repeat (LRR)-containing receptor subfamilies: the LRR-receptor-Like Kinase (RLK), LRR-Receptor-Like Protein (RLP) and Nucleotide-binding LRR Receptor (NLR) subfamilies. The genome-wide analysis of the NLR genes of *V. planifolia* revealed extremely low numbers of disease resistance genes, and these results were consistent with the low number of such genes known in the Orchidaceae family. Genome-wide RGA analysis is a crucial step towards identification of genes involved in Forv resistance and provides new information for breeding programs.

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Poster Title	Analysis of Homology-Directed Repair Patterns in CRISPR-MAD7 Transfected *N. benthamiana* Protoplasts Using Deep Sequencing
Abstract	The clustered regularly interspaced palindromic repeats (CRISPR) system has become the prominent system used for genetic engineering in a range of organisms, including plants. However, to date, the utilization of the CRISPR system for commercial crop improvement is still limited by several factors. One of these factors is the still limited efficiency with which precise gene editing can be performed. CRISPR gene knockouts can be efficiently made, as the predominant repair pathway of double-strand breaks (DBS) in somatic cells is the error-prone non-homologous end joining (NHEJ) pathway which often creates loss-of-function mutations. Although a gene knockout can be very valuable, allowing the determination of gene function, their application in crop improvement is limited, as many desirable plant traits are connected to specific alterations of, not disabling, specific genes. We explore the alternative DSB repair pathway, termed homology-directed repair (HDR), which when leveraged, allows precise genome editing. Using a protoplast transfection procedure, we introduce MAD7-based ribonuclease proteins (RNPs) together with HDR templates in N. benthamiana protoplasts and evaluate all types of gene alterations using nanopore sequencing after 48h incubation. We demonstrate a significant impact of HDR template design on overall editing efficiency and associated HDR-mediated repair types. In one example we found overall gene editing frequencies at the target locus of 32% of which 10.62% are associated with HDR-mediated repair and targeted DNA insertion. This model system will allow us to study the HDR repair mechanism and develop efficient strategies to facilitate efficient precise gene editing in plants.

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