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Abstracts

S1 Preharvest abiotic stress

S1.1 Plant metabolomics: a useful tool for the analysis of phytochemicals

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Abstract:
Plant Metabolomics, both un-targeted and targeted, have emerged as exciting analytical tools in plant sciences. These analytical methods provide a high throughput screening of plant metabolites, with high sensitivity and the possibility of quantification in complex matrices, as far as authentic standards are available. These are based in the combination of UPLC with Q-TOF and Triple Quadrupole detectors, together with powerful biostatistics and bioinformatics applications. This has become a powerful technique for different aspects of research on leafy vegetables that is now available to most scientists.

Some examples of the application of metabolomics to lettuce quality research will be presented. Metabolomics has been successfully used for identification of biomarkers of susceptibility to wound-induced browning in iceberg lettuce. In addition, this technique has also been used for the identification of the metabolites responsible for the anti-listeria effects of browned lettuce. From a human health perspective metabolomics can also be fruitfully used to evaluate the interaction of leafy vegetable phytochemicals with gut microbiota and their potential physiological effects.

S1.2 Development of a dedicated breeding strategy for nitrogen-use efficiency in spinach

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Keywords: Spinach; NUE; Hydroponics; QTL mapping

Abstract:
Spinach is recognized as an important food crop with an increasing consumption; the Netherlands is the leader in seed production worldwide. It is a crop with a high demand for nitrogen in order to rapidly produce a good yield of harvestable leaves with preferably a dark green colour as required by the market; yet it has low nitrogen use efficiency. The current production system consequently requires a high input of nitrogen, but puts a lot of strain on the environment. For this reason there is a need for cultivars adapted to low-input growing conditions to enable sustainable conventional as well as organic spinach cultivation, but the current cultivars do not meet these requirements. This problem has not yet been addressed by the breeding sector. Therefore, the aim of this presentation is to give an overview of the current status of knowledge and tools available to set up a breeding program for the development of varieties that perform well (good yield stability and quality) with low input of nitrogen. The following aspects are addressed: i) genetic diversity for nitrogen use efficiency found in spinach cultivars using the Ingestad model on hydroponics, ii) analysis of Genotype x Environment (GxE) interaction under different N treatments and iii) construction of a molecular map and quantitative trait locus analysis of nitrogen use efficiency in spinach. The results presented in this study provide a first step towards molecular breeding for complex traits in spinach. The identified QTLs may be targets for breeding programs. However, the implication of the QTLs found under hydroponic conditions using the Ingestad model for spinach cultivation under field conditions still have to be validated.

S1.3 Effects of water stress and salinity on the quality of different genotypes of basil

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Keywords: Water stress; Salinity; Basil; quality

Abstract:
Fresh basil (*Ocimum basilicum* L.) has become increasingly popular in recent years because of its superior flavour. Control of abiotic stresses during cultivation can improve quality and extend shelf life of fresh basil. The aim of this work was to study the influence of water stress and salinity on the quality after harvest and during storage of three fresh basil genotypes, Green Iranian, Purple Iranian and Genovese. To evaluate the influence of water stress, three treatments were compared: control that was established according to field capacity, 25% and 50% deficit irrigation. To evaluate the effect of salinity, two levels of salinity included 40 and 80 mM NaCl were compared with 0 mM NaCl as control. After harvest, plants were packed in plastic trays and stored in air at 12 Â° C for 7 days. In general, reducing irrigation dose did not affect the visual quality of any of the genotypes studied. However, salt stress positively affected the visual quality of Green Iranian basil. Moreover, increasing water stress increased phenolic compounds and essential oils content of all genotypes although the content of total phenolic acids and anthocyanins did not show differences between irrigation treatments. During storage, salinity stressed Green Iranian increased the content of individual and total phenolic acids compared to control leaves. In conclusion, salinity reduced the visual performance while improved product quality whereas water deficit did not affect either the performance or visual quality. The increase in the content of phenolic compounds and essential oils was observed under both water stress and salinity growing conditions.

PS1.1 Developing methods to assess and quantify abiotic stress response in Brassica oleracea and Lactuca sativa

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Keywords: Brassica oleracea; Lactuca sativa; lettuce; abiotic stress; screening

Abstract :
Brassica oleracea includes many cultivars familiar as food crops such as cabbage, cauliflower, broccoli and kale. Lettuce (Lactuca sativa) is a popular leafy salad crop. Climatic and population changes are increasing the need for the development of commercial lines with improved tolerance of environmental stresses such as heat and drought. This research forms part of the Vegetable Genetic Improvement Network (VeGIN) project, a collaboration between Harper Adams and Warwick Universities. We are exploring whole plant abiotic stress physiology in brassicas and lettuce, developing and using multiple stress protocols in order to analyse the response of Diversity Fixed Foundation Sets (DFFSs) to environmental fluctuations and to assess tip burn in lettuce. This will allow the identification of stress-tolerant brassica and tip-burn tolerant lettuce lines. Such lines will be examined in further detail, such as the identification of stress tolerance-associated QTLs, and could provide useful genetic material for crop breeding programmes.

PS1.2 Effect of UV-B light applied on pigmented lettuce cultivar to increase functional compounds

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Keywords: UV-B light; abiotic stress; preharvest

Abstract:
With the aim to increase functional properties of vegetables, application of abiotic factors, such as UV-B radiation over crops, is presented as an innovative agronomic practice. The responses in vegetables exposed to high doses of UV-B are defined by the variety, morphology and absorbing compounds, among other characteristics. This study reports physical and chemical modified responses in two commercial cultivars of lettuce (*Lactuca sativa* L.) with different pigmentation. The cultivars Kristine RZ and Versa RZ present red and green oak leaves, respectively and were grown in hydroponic systems under a regimen of UV-B applied in different doses (0 to 58 mW/cm2) daily for 30 min for a period of 10 days.

Doses of 58 mW/cm2 caused reductions of 25% of height in Kristine RZ cultivar compared to its control. The foliar area as well as fresh and dry weight of lettuce was affected by the exposition of doses over 16 mW/cm2 which were enough intensities to reduce in 6% production weight. The concentration of absorbing pigments was different in each cultivar, Kristine RZ cultivar presented 10 times more chlorophyll a, b and carotenoids than Versa RZ. In Kristine RZ as the doses of the UV-B were incremented less chlorophyll a content was quantified reaching 22% less content compared to control. In cultivar Versa RZ doses of 33 mW/cm2 diminished in about 50% antioxidant capacity and total phenol content compared to control and other doses applied monitored by DPPH and FRAP methods respectively.

PS1.3 A role for oxidative stress in mediating tipburn of lettuce?

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Keywords: lettuce; tipburn; oxidative stress; vertical farming

Abstract:
Vertical Farming Systems (VFS) can increase crop yield per unit area of land and improve crop resource efficiency, especially in urban settings where space is limited. We have identified tipburn of lettuce as a key limitation to crop quality in a proprietary commercial VFS. This physiological disorder causes collapse and necrosis of leaf margins and the apex of developing leaves. Symptoms were only observed in the top layers of the VFS, which were exposed to supra-optimal environmental conditions (high light intensity, temperature and nutrient supply). Tipburn has previously been linked to localised calcium deficiency. In addition, supra-optimal environmental conditions are known to induce oxidative stress in plants. However, the mechanisms underlying the symptoms of tipburn under these conditions are not fully understood. We have used a deep flow hydroponic system, eliminating gradients in the growing environment observed in VFS, to investigate the links between oxidative stress and tipburn induction. Two light intensities (high light: 450 μmol m^{-2} s^{-1} and low light: 170 μmol m^{-2} s^{-1}) were applied, simulating the conditions within the top and bottom layers of the VFS and changes in antioxidant enzyme activity and lipid peroxidation were evaluated pre-symptomatically (first visual tipburn symptoms appeared on day 16). When treatments statistically differed, values were always higher in the high light treatment. This provides evidence of a role for oxidative stress in mediating tipburn of lettuce, under supra-optimal environmental conditions.

PS1.4 Characterization of Pseudomonas species associated with tomato wilt disease

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Keywords: Characterization; bacteria; tomato; disease diagnosis

Abstract:
Bacterial wilt is an extremely serious plant disease of tomato and other crops. Infected plants wilt rapidly and often die. The disease is caused by a soil bacterium *Pseudomonas* and infects plants through the roots. The causal agent of tomato wilt (*Pseudomonas* species) was identified from the collected eleven varieties of tomato plant. Eleven local varieties of tomato were sown in separate earthen pots to be grown up to nursery and then transplanted in separate rows. Simple random sampling technique was adopted for collection of diseased tomato samples. The growth of pathogen was carried out on different specified culture media viz. triphenyl tetrazolium chloride (TTC) and yeast peptone glucose agar. The bacterial isolate was characterized by morphological, biochemical tests and its phytopathogenicity was verified by simple Rapid Streaming test.

PS1.5 Microbiological Quality Of Rocket Salad In Supply Chain Context

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Keywords: Rocket salad; ready to eat food; storage temperature; microbiological quality; biofilm

Abstract:
Rocket is a crop that is widely consumed in ready-to-eat (RTE) salads. RTE salad is an ideal medium for microbial growth; there are numerous examples of food-borne illness outbreaks from salad consumption. In this study, we would like to determine the effects of storage conditions on bacterial growth and discover the distribution of bacteria on RTE rocket, so the same techniques could be used to monitor the efficacy of future sanitation treatments. Bagged RTE wild rocket was supplied by Alresford Salads and analysed at The University of Reading. Samples taken on the day of arrival at Reading were the control. Samples were stored in typical temperatures throughout the supply chain: 13°C at the retailer, 4 °C in a domestic fridge post-purchase. Adverse treatments were 25°C for 4 hours, indicates a failure to purchase until the 'display until' date. Bags were opened and the leaves were mixed by hand to simulate consumers who do not consume the whole bag in one day. Micrographs of rocket leaves were taken to observe the distribution of bacteria using scanning electron microscopy (SEM). Results showed a short disruption of the cool chain did not cause increased microbial growth, provided that the produce was subsequently maintained in the cool chain after the incidence. High bacterial count was linked with samples that underwent prolonged exposure to 13°C. The SEM showed that opening the bag introduces a new bacterial inoculum, but counts indicate that storing at 4°C restricts growth. SEM images revealed that bacteria were distributed on and inside the leaves. Formation of biofilms was also observed. Temperature plays an important role in determining the growth of bacteria in RTE salads. Improper storage conditions can result in significantly higher bacterial growth and production of biofilms.

PS1.6 Is the light exposure the only factor promoting an abiotic stress during postharvest storage of baby spinach?

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Keywords: baby leaves; quality; fresh produce; physiology

Abstract:
Minimally processed products are generally exposed to low temperature and uncontrolled lighting conditions during the supply chain. The positive effect of low temperature on baby spinach is widely reported, but there is no information available about the light effect on the sensorial quality. The objective of this study was to evaluate the effect of different lighting conditions on the visual quality of baby spinach during storage. This study was carried out in 2 consecutive trials. In the first one, minimally processed baby spinach was packaged in passive Modified Atmosphere Packaging (MAP) and stored for 10 days at 7ºC under 3 light conditions: continuous light, 12 h photoperiod and continuous darkness. During storage, the 3 different light conditions led to 3 different headspace gas compositions within the bags due to photosynthesis and respiration reactions. Quality characteristics of baby spinach were strongly affected. However, it was not possible to elucidate if there was an effect by the lighting condition or by the different O_2 and CO_2 levels generated in the bags. Thus, in the second trial, baby spinach was minimally processed and stored under 2 Controlled Atmosphere (CA) conditions (0.5% O_2 + 10% CO_2 and air) combined with 2 lighting conditions (continuous light and darkness). The results showed that there were no differences by the different light conditions. High CO_2 level was responsible of the reduction in chlorophyll fluorescence (Fv/Fm) and the increase in 'off' odours development while controlled the growth of Pseudomonas sp. and the histological damage. Moreover, baby spinach stored under air showed higher lipid peroxidation without differences between light conditions. Our results showed that quality of baby spinach could be affected by the light condition during storage because of the modified atmosphere generated; high levels of CO_2 under darkness and high levels of O_2 under light conditions.

PS1.7 Metabolomics to evaluate the browning potential of Iceberg lettuce

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Key words: Metabolomics; lettuce; principal component analysis; phenolics; wounding; biomarkers

Abstract:
Metabolomics is a powerful tool to measure metabolic profiles with high accuracy of two subjects of similar nature. This is the case of two varieties of iceberg lettuce that showed different browning susceptibility after cutting. The aim of this study was the analysis of the metabolomic profile of these two varieties after cutting and storage for 6 days.

Analysis of UPLC was carried out with a C 18 column (1.8 mm particle size) of high resolution for the separation of isomers and a mass analyzer ESI-QTOF-MS for detection. Data was processed using principal component analysis (PCA) to detect those metabolites responsible for differences between varieties. The identification was carried out by comparison with different databases (NIST 11 Mass Bank; Metlin, MMCD), authentic standards matching, if available, structural analysis by MS-MS (HPLC-ESI Ion Trap), and when necessary by HPLC-coupled Q-TOF NMR (BML-NMR) using an solid phase extraction available at the CEBAS-CSIC metabolomics service. The analyses provided a large amount of data, and around five thousand metabolites were detected for each variety. These results were submitted to further analyses with statistical tools and filters to identify the most significant metabolites.

The most susceptible variety to cut-induced browning showed a rich metabolic profile of phenolic compounds of the phenylpropanoid pathway such as caffeoyl quinic acids while the least susceptible one presented a metabolic profile related to benzoic acid derivatives, lignans and lignin routes.

The variety with the highest browning susceptibility also showed a number of plasma membrane lipid metabolites such as monoacyl glycerol-phospho inositols and lysophosfatidyl ethanolamine, which were already present just after cutting and were not observed in the least susceptible cultivar. These metabolites have been related to the production of jasmonic acid and therefore are part of the wound response signals that as a response induces browning. It seems that the most susceptible variety releases these membrane constituents immediately after cutting, while the membrane composition or its integrity after wounding is higher in the least browning susceptible cultivar. This study suggests that these metabolic biomarkers could probably be used for the selection of lettuce cultivars for fresh-cut. The analysis of these biomarkers in a high number of lettuce cultivars is actually going on in our laboratory to validate these metabolites as general biomarkers for browning susceptibility.

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S2 Biotic stress: from plants to people

S2.1 Comparative genomics of lettuce and *Bremia lactucae* to inform strategies for more durable disease resistance.

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Abstract:
We have been characterizing variation in both lettuce and its pathogens in order to enable informed deployment of resistance genes to provide more durable control. Over 50 phenotypic resistance genes are known in lettuce, many for resistance to *Bremia lactucae* the causal agent of downy mildew. Over 25 phenotypic resistance loci co-localize to three of five major resistance clusters (MRC) on chromosomes 1, 2, and 4. Additional resistance genes are being rapidly mapped using next-gen sequencing of near-isogenic lines. We are currently refining the reference genome of lettuce cv. Salinas as well as sequencing additional genotypes. The majority of candidate resistance genes that encode nucleotide binding-leucine rich repeat (NLR) proteins genetically co-localize with phenotypic resistance loci. Each MRC spans several tens of Mb containing ~25 to 100+ NLR encoding genes as well as hundreds of genes which are not related to NLRs. Forward and reverse genetic approaches have been applied to dissect the MRCs to identify the causal genes. In parallel, we have monitored variation in *B. lactucae* in California since 1982 at the phenotypic and sequence levels. We have sequenced ~50 isolates of *B. lactucae* that differ in geographical and temporal origin as well as virulence phenotype, including the BL type isolates from Europe. These data are providing the basis for deploying pyramids of resistance genes. Different resistance genes are being deployed in different lettuce types in order to fragment the selection pressure on the pathogen population.

S2.2 Screening for host-plant resistance against Nr:0 and Nr:1 biotypes of Nasonovia ribisnigri

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Keywords: Nasonovia ribisnigri; Nr gene; Lactuca; Nr:0; Nr:1; host-plant resistance; aphid

Abstract:
Lettuce cultivars resistant to the currant-lettuce aphid, Nasonovia ribisnigri, were first released in 1999 and have since been widely cultivated throughout Europe. The resistance was sourced originally from the Institute for Horticultural Plant Breeding (IVT) Lactuca gene bank where some accessions of Lactuca virosa provided nearly complete resistance to N. ribisnigri (Nr:0) via the Nr gene.

During 2007, a new biotype of N. ribisnigri (Nr:1) was identified in Germany and France which was able to develop and reproduce on resistant lettuce cultivars with the Nr gene. It was confirmed during 2009 that this new resistance-breaking biotype had reached the United Kingdom and samples of aphids were collected from a crop of aphid-resistant lettuce in Kent and sent to Warwick Crop Centre.

With the emergence of this new resistance-breaking biotype, the future effectiveness of the current Nr gene cultivars for the control of this pest was uncertain, and the development of cultivars with new mechanisms of resistance became necessary.

A lettuce diversity set consisting of 96 lines obtained from the Vegetable Genetic Improvement Network (VeGIN) at Warwick Crop Centre was screened for novel resistance to wild type (Nr:0) and resistance-breaking biotypes (Nr:1) of N. ribisnigri. The diversity set represented a range of morphological and genetic variation in domesticated L. sativa accessions and wild Lactuca species. The screening showed a range of susceptibility to both N. ribisnigri biotypes, confirming genetic variation for the resistance trait between lines. Novel resistance genes with different types of resistance mechanisms to the Nr gene were identified. Additional screens investigated the phenotype for resistance in several mapping parent lines.

New mapping populations are currently being developed using individuals from the extremes of the resistance distribution for both biotypes. These will be genotyped using the same panel of KASP SNP markers used to screen the diversity set.

S2.3 Genetics and breeding of bacterial leaf spot resistance

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Keywords: disease; disease resistance; distributive breeding; *Xanthomonas hortorum; Lactuca sativa*

Abstract :
Bacterial leaf spot (BLS) caused by the pathogen *Xanthomonas* campestris pv. vitians (Xcv) is a globally important disease of whole head and baby leaf lettuce that reduces crop yield and quality. Host resistance is the most feasible method to reduce disease losses. Screening *Lactuca* accessions has identified resistance, though most cultivars used for commercial production are susceptible. Batavia Reine des Glaces, Iceberg, Salad Crisp and other cultivars express incomplete resistance to Xcv. High-level race-specific resistance is known in a few lettuce cultivars and wild species. The Batavian cultivar La Brillante along with the Latin cultivars Pavane and Little Gem express a hypersensitive response (HR) to California strains of Xcv resulting in high-level resistance. The HR in La Brillante is due to a single dominant gene, named Xanthomonas resistance 1 (Xar1), located on linkage group 2. The genes conferring the HR in Pavane and Little Gem are either allelic or closely linked with Xar1. Breeding resistant iceberg cultivars has resulted in eight publicly released inbred lines using either the Xar1 gene or resistance derived from Iceberg and Salad Crisp. Breeding resistant cultivars for use in baby leaf production is challenged by the need to breed resistance into the diverse types grown as baby leaf. We developed a breeding scheme that uses early generation testing to select populations that are uniform for BLS resistance but variable for leaf morphology and color. To demonstrate the breeding scheme, we conducted selection for BLS resistance and red-colored leaves among progeny from Batavia Reine des Glaces Ã— Eruption (red colored leaves and Xcv susceptible). Two populations were developed with uniform levels of resistance equivalent to Batavia Reine des Glaces and variable leaf morphology and color. These populations can be used to select for diverse types of lettuce suitable for baby leaf production and with BLS resistance.

S2.4 Breeding and Genetics of Lettuce against Verticillium Wilt Race 2

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Keywords: Breeding; genetics; lettuce; Verticillium wilt, Verticillium dahlia

Abstract:
Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae*, is a destructive disease of lettuce in California. Verticillium resistance 1 (Vr1) confers resistance to race 1 isolates but is defeated by race 2 isolates, which are predicted to increase in prevalence. Three Plant Introductions (PI) with partial resistance to race 2 were intercrossed with each other and a susceptible cultivar to determine the inheritance of resistance to race 2. Ninety-nine F3 families from PI 171674 Ã— PI 204707, their parents, and the susceptible check Salinas™ were inoculated with race 2 and evaluated for disease incidence (DI), disease severity (DS), foliar symptoms (FS) and days to flowering (DF). The experiment was conducted twice. F3 family means were quantitatively distributed, indicative of segregation for all traits. F3 family means ranged from 7 to 67% for DI, 0.2 to 3 for DS, 6 to 65% for FS, and 91 to 155 for DF. PI 171674 and PI 204707 significantly differed from each other and Salinas™ with 17, 52 and 70% for DI, 0.8, 2.3 and 3.4 for DS, 13, 30 and 51% for FS, and 158, 108 and 178 for DFF, respectively. The segregation and high resistance found for this population is useful for identifying QTLs and breeding resistant cultivars. A population of 99 F3 families from Salinas Ã— PI 169511 was evaluated in one greenhouse experiment and no significant differences were found for DI, DS, or FS. To identify better sources of race 2 resistance, 98 and 34 cultivars were inoculated in two replicated greenhouse experiments. PI 358038 had a median DI of 0% in both experiments with a maximum of 20%, while susceptible Salinas™ had median DI of 20% and 65% with maximums of 25 and 100%, respectively. Complete resistance to race 2 has not been observed to date.

S2.5 Is gut microbiota a missing link for understanding bioactivity of dietary polyphenols?

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Abstract:
Dietary polyphenols protect against metabolic syndrome, despite limited absorption and digestion, raising questions about their mechanism of action. We hypothesized that one mechanism may involve the gut microbiota. To test this hypothesis, C57BL/6J mice were fed a high fat diet (HFD) containing 1% Concord grape polyphenols (GP). Relative to vehicle controls, GP attenuated several effects of HFD feeding, including weight gain, adiposity, serum inflammatory markers (TNFα, IL-6, LPS), and glucose intolerance. GP lowered intestinal expression of inflammatory markers and increased intestinal expression of proglucagon and genes involved in barrier function and triglyceride metabolism. 16S rRNA gene sequencing and quantitative PCR of cecal and fecal samples demonstrated that GP dramatically increased the growth of *Akkermansia muciniphila* and decreased the proportion of Firmicutes to Bacteroidetes, consistent with prior reports that similar changes in microbial community structure can protect from diet-induced obesity and metabolic disease. These data suggest that GP act in the intestine to modify gut microbial community structure, resulting in lower intestinal and systemic inflammation and improved metabolic outcomes. The gut microbiota may thus provide the missing link in the mechanism of action of poorly absorbed dietary polyphenols. To deliver beneficial health effects of polyphenols, we have developed polyphenol-rich Rutgers Scarlet Lettuce (RSL) (*Lactuca sativa* L.) using somaclonal variation and selection in tissue culture. RSL may have some of the highest reported contents of polyphenols and antioxidants amongst all common fruits and vegetables (95.6 mg/g dry weight and 8.7 mg/g fresh weight gallic acid equivalents and 2721 µmol/g dry weight and 223 µmol/g fresh weight Trolox equivalents). Three main compounds accumulate at particularly high levels in RSL: chlorogenic acid (5-caffeoyl quinic acid) up to 27.6 mg/g DW, cyanidin-malonyl-glucoside, up to 20.5 mg/g DW, and quercetin-malonyl-glucoside up to 35.7 mg/g DW. Major polyphenolic constituents of RSL have been associated with health promotion as well as anti-diabetic and/or anti-inflammatory activities.

S3 Improving structural and functional crop quality

S3.1 Tracking quality changes through the supply chain for leafy vegetables using multitrait analyses

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Keywords: rocket salad; volatile organic compounds; sensorial quality; stress responses; post-harvest storage

Abstract:
As leafy vegetables transit through the food supply chain they are subjected to numerous stresses that can affect quality. Key post-harvest stresses include poor handling at harvest, breaches to the cold supply chain and processing damage. These stresses induce changes in the physiology and biochemistry of the leaves that can be detected through changes in gene expression and alterations in the levels of key metabolites. Some of these metabolites affect nutritional quality, others such as changes in the composition of volatile organic compounds (VOCs) affect also sensorial perception. To identify key changes in gene expression, rocket salad leaves (*Diplotaxis tenuifolia*) were subjected to short term post-harvest stresses in darkness: cold storage and ambient temperature with or without dehydration stress or wounding. RNAseq was used to identify unique and shared changes in gene expression compared to control leaves at harvest. These include 336 genes whose expression was altered by all stresses, and uniquely activated or repressed genes associated with wounding (865), dehydration stress (810) ambient temperature alone (348) and cold (534). Real-time PCR confirmed the validity of the RNA-seq experiments. VOC profiles were also altered by the stress treatments and statistical analyses discriminated profiles associated with cold, ambient temperature alone and wounding treatments compared to freshly harvested material. Changes in VOCs were combined with analyses of nutritionally relevant compounds and used in multifactorial analyses of post-harvest storage at commercially relevant temperatures to derive modules of compounds that indicate changes in quality and sensorial perception. Finally at the retail end of the food supply chain VOCs can be used to chart changes in pre-bagged ready to eat rocket salad over time as they reach their use-by date providing a useful marker for overall quality.

S3.2 Application of thermal desorption gas chromatography time-of-flight mass spectrometry (TD-GC-TOF-MS) as a tool for analysis of fresh cut produce quality

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Keywords: thermal desorption gas chromatography time-of-flight mass spectrometry; rocket salad; *Listeria monocytogenes*

Abstract:
Volatile organic compounds (VOCs) are closely associated with food flavour and the VOC profile of fresh cut produce is affected by processing and storage. Several methods are available for capture and analysis of VOCs; here we collected VOCs from headspace on thermal desorption (TD) tubes and analysed them using TD-GC-TOF-MS. This set up offers several advantages over other methods such as solid phase microextraction (SPME) or solvent desorption: a) collection of VOCs from headspace of vegetables is easy, b) TD tubes can be stored and transported enabling remote sampling, c) use of TD and TOF is reputed to offer ~ 100 times higher sensitivity. We have developed a technique for the sampling of VOCs from the headspace over rocket leaves with TD tubes, and statistical analyses to identify whole-profile changes. We used the methods to investigate whether VOC profiles discriminated a) species and degree of wounding, b) duration and temperature of storage and c) contamination with Listeria monocytogenes. For species and degree of wounding we used leaves from two commercial varieties of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) with different degrees of wounding. A total of 55 compounds were putatively identified from the samples by their mass spectra and retention indices based on NIST spectral library data comparisons. Here, abundance of compounds correlated to the degree of wounding, storage time and temperature were discriminated by significant changes in the entire VOC profile. Contamination with *L. monocytogenes* was also detectable and VOC profiles discriminated for storage time and inoculation levels. Further statistical analysis identified a subset of VOCs indicative of *Listeria* contamination. VOC profiles thus may be useful markers to monitor species and damage, effects of time and temperature on quality and a subset of the profile has the potential to be developed into a safety marker system.

S3.3 Application of multivariate accelerated test for the estimation of shelf life of fresh-cut lettuce

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Keywords: Multivariate modeling; principal components; variance; lettuce leaves; shelf-life.

Abstract:
For fresh-cut produce the individuation of the quality attribute to consider as benchmark for shelf-life definition is very critical since several degradation reactions occur simultaneously. The aim of this study was to obtain more realistic shelf life estimation by considering contemporaneously several sensorial, physical and chemical attributes of fresh-cut melons by using the Multivariate Accelerated Shelf life Testing (MASLT). Iceberg lettuce heads were washed, cut and packaged in PP bags with an initial gas composition of 5 KPa O2 and 20 KPa CO2, simulating commercial conditions. Samples were stored over 11 days at 0, 5 and 15°C, periodically analyzing sensorial attributes, phenols, and antioxidant activity. On the data a Principal Component Analysis (PCA) was performed. By using this method the PC scores were used to build a multivariate kinetic chart which resumes the information of the degradation of all the quality attributes studied. Then, establishing a shelf-life limit for each of the attribute included in the model it was possible to estimate the shelf life of the product taking into account the changes of all quality attributes. A total variance of 79.6% was explained by two PC components. The multivariate degradation kinetic was better described by a zero-order kinetic showing r2 greater than 0.97 while, the temperature dependence of the multivariate rate constant, km, was well fitted by the log-logistic model. A cut-off criterion of - 1.52 was calculated giving shelf- life of ~12.4, ~10.4 and ~3.7 days for fresh-cut lettuce stored at 0, 5 and 15°C. This method certainly gives an overall and accurate description of the degradation phenomena occurring contemporaneously during storage.

S3.4 Using the *L. sativa* x *L. serriola* lettuce mapping population to direct breeding for flavour and nutrition

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Keywords: *Lactuca*, quantitative trait loci, nutrition, flavour, metabolomics, molecular markers

Abstract:
The publication of crop genome sequences has paved the way for the development of a range of further genomic resources and investigation of many more commercially useful and fundamental traits. We have combined a traditional QTL mapping approach with an innovative computational pipeline to define genomic markers and putative regulatory genes for traits related to nutrition and flavour quality in lettuce. The study made use of the genetic diversity present in the *Lactuca sativa* cv. Salinas x *Lactuca serriola* mapping population; a fully annotated genome sequence exists for both parent lines and transcriptome sequence has been obtained from the progeny. We combined data from three experimental growing environments for a wide range of metabolite traits that were characterized using NMR and verified using complementary techniques. We subsequently used a trained human sensory panel to establish that our biochemical measurements were paralleled by the human olfactory system and to understand how the interaction of different metabolites associated with bitterness and sweetness were perceived by humans upon consumption. Our combined trait-to-gene and gene-to-trait approach has enabled the identification of putative regulatory genes for sesquiterpenoid lactones which are key metabolites in lettuce linked to antimicrobial, antiherbivory, human health benefits and bitterness perception. This refined approach has enabled us to differentiate between sesquiterpenoid lactones linked to either health benefits or taste, to the extent that pre-breeding markers have ben identified for use in molecular breeding programmes which aim to improve one trait without compromising the other.

Poster session #2

PS2.1 Freshness of fresh-cut lettuce: from the field to the table

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Key words: Lettuce; heat shock; browning; packaging

Abstract:
The study is included in the project entitle "Freshness of fresh cut products" that was started in 2012 and will finish at the end of 2015. This project is supported by the Spanish Ministry of Economy and Competitiveness under the 2012 National Program of Public-Private Cooperation –subprogram INNPACTO (project IPT-2012-0169-060000). The main objective is the study of different strategies that are able to preserve "freshness" of fresh-cut lettuce, prolonging the primary and secondary shelf-life. The term freshness is related to some quality characteristics of lettuce such as fresh appearance, absent of discoloration and decay, crispy texture and good flavour. Moreover, the terms primary and secondary shelf-life are related to the maximum period of time in which the product has an acceptable quality just immediately after opening the bag and after 24 h in air, respectively. The main objective will be reach by means of 4 secondary objectives which are related to cultivar selection, heat shock treatment, washing disinfection technologies and packaging design. Regarding lettuce cultivars, different commercial iceberg and romaine cultivars have been studied during two consecutive years to select those ones with the lowest browning potential and off-flavour development. Additionally, the industrial application of heat shock treatment has been evaluate to prolong the shelf life by improving the visual quality of fresh-cut iceberg and romaine lettuce. Regarding the washing disinfection technologies, the main objective was to identify and validate effective disinfection agents to eliminate the risks of cross-contamination and disinfection by products by the use of static and dynamic washing systems. In addition, different factors that influence the packaging design have been evaluated including the study of the film permeability to O_2, CO_2 and H_2O, the film microperforation, the respiration rate at low O_2 concentrations and the optimal O_2 and CO_2 levels.

PS2.2 Chemical, physical and sensorial characterization of fresh quinoa sprouts (Chenopodium quinoa Willd.) and effects of modified atmosphere packaging

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Keywords: Chenopodium quinoa; sprouts; ready-to-eat; modified atmosphere packaging (MAP)

Abstract:
The aim of study was the characterization of the quality attributes and the evaluation of postharvest performance of Real and Regalona Baer quinoa (*Chenopodium quinoa* Willd.) sprouts. Beside germination ratio, weight, water content, texture, color, phenolic content, and antioxidant activity were evaluated on sprouts. Regalona Baer gave a better performance showing higher germination and weight increase (44% and 49%, respectively) than Real. Moreover, Regalona Baer showed a higher phenolic content with value of 9.1 µmol gallic acid g^{-1} DWB, while Real only had 4.9 µmol gallic acid g^{-1}. A separate experiment was performed to study the effects of different modified atmosphere packaging conditions in order to extend the shelf life of ready-to-eat fresh quinoa sprouts. The following MAP treatments were tested: 1) Passive MAP - air in microperforated PP bags; 2) Active MAP - 5 % O_2 + 20 % CO_2 in microperforated PP bags. Chemical (ethanol and acetaldehyde content), physical (texture and color) and sensorial attributes of quinoa sprouts were evaluated during storage at 5°C. Among sensorial properties appearance, color, green odor, grassy odor and off-odor were evaluated using anchored subjective scales. Results show that the Real quinoa sprouts in Passive MAP showed the shortest shelf life characterized by a fast decrease of texture, browning on the surface, as well as a production of off-odor after 1 day of storage. In the same storage conditions Regalona Baer sprouts were still marketable, showing a higher appearance and color scores. Regalona Baer in Active MAP showed a greater turgidity and crispiness compared to samples stored in Passive MAP. Therefore, Regalona Baer quinoa sprouts, which showed the better quality attributes, packaged in PP microperforated with a gas composition of 5% O_2 + 20% CO_2 could be a potential solution for extending the shelf life as a ready-to-eat product.

PS2.3 Comparison of overall sensory profile of raw, fresh-cut and sous-vide borage (*Borago officinalis*) by means of a sorting task strategy

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Keywords: *Borago officinalis*; sous-vide; conventional cooking; sensory profile; sorting task

Abstract:
Borage (*Borago officinalis*) is an annual vegetable of high nutritional value cultivated mainly in Aragon (North-east Spain). Its preparation is quite laborious as it requires separating the leaves from the stalks, cleaning them in order to eliminate the fibre threads, and cooking them for at least 15 minutes. Sous-vide borage is a fast and valuable alternative to raw and fresh-cut products as it avoids the traditional cooking process that produces a loss of colour, flavour and nutritional compounds.

The main objective of this work is to evaluate if a borage obtained by this culinary technique (cooked sous-vide at 90 ÂºC for 16 min and then regenerated in a microwave oven at 700 W for 1.5 minutes), improved the sensory profile in comparison to raw and fresh-cut borage (cooked for 15 min in boiling water) by means of a sorting task strategy.

Ready-to-consume samples (sous vide, raw and fresh-cut) were presented in duplicate to a group of 18 borage consumers. They were asked to sort the 6 samples on the basis of similarity attending to the global sensations perceived in the mouth (flavour, texture, persistence). After sorting, they were also asked to provide a few descriptors that characterised each of the groups they had formed. Data was analysed by multidimensional scaling (MDS) and Hierarchical Cluster Analysis (HCA).

In the MDS chart, both replicates of sous-vide borage were displayed close together, and far apart from the other samples, which indicates that this product has marked organoleptic properties easily recognizable by consumers. Sous-vide borage was defined as crunchier, more intense in flavour and with desirable textural properties. Results highlight therefore that sous-vide treatment is better preferred than traditional cooking of either raw or fresh-cut borage.

PS2.4 Optimization of processing of fresh-cut endive

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Keywords: Endive; fresh-cut; browning; modified atmosphere; maturity indicators; flower stalk

Abstract:
Successful processing of endive to render fresh-cut vegetable requires a thorough knowledge of their sensitivity to browning reactions and microbial growth, their respiratory metabolism, and their behavior under modified atmosphere packaging. The decisive factors in obtaining fresh-cut products are the quality of the raw material, methods applied in the process and finally, the type of packaging used for the marketing of the product. Development and execution of this study has been raised according to key factors when it comes time to optimize of minimal processing of fresh-cut endives as varieties selection, optimization of cutting and packaging. First of all, two different varieties of endive (Baccara and Crenoline) in their commercial ripening stage were evaluated for suitability for minimal processing. We observed that var. Baccara is better than var. Crenoline, because the respiration rate and the ethylene production was lower in Baccara, and consequently the rate of deterioration was lower too. The maturity stage or harvest time (month) of whole endive influenced the response to fresh-cut processing thus emphasizing the fact that shelf life was reduced with advanced maturity stage and harvest time. Better appearance and lower browning susceptibility were observed for the endives with smaller flower stalk (at start of season), besides, if the cutting was did in cold tap water (absence of O_2) gave rise to less susceptibility to enzymatic browning, both in Baccara than Crenoline endives. Finally, a MAP of minimally processed vegetables (8%O_2, 12%CO_2 and the remainder N2) combined with storage at refrigeration temperatures (7^{o}C) was considered the best way of maintaining the sensory and microbiological quality of fresh-cut products. In our case, we have demonstrated that this fresh-cut processing is the best option to get a fresh-cut endive safe and with good appearance during the shelf life period (3 days at 4^{o}C + 7 days at 6^{o}C)

PS2.5 Creation of new forms of dill by means of intervarietal hybridization

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Keywords: Dill; hybridization; variety; diversity

Abstract:
Dill is a popular leaf crop for fresh production as well as for production of raw materials for drying, freezing and essential oil extraction. The existent dill varieties are suitable for these purposes. When breeding new varieties with other combinations of characteristics it is necessary to have a diverse parent material. Hybridization between varieties is one of the ways to create a large diversity of dill plants with regard to the set of features.

Our researches were conducted in this direction. 4 dill varieties with the following specific for the variety distinguishing features were used in the crossings as parental material:

- variety A - long harvesting period in long day conditions (15-45 days), many leaves in a rosette (15-25 pieces);
- variety B - shortened stem internode, thick and broad leaf segment;
- variety C - no wax bloom, attractive green glossy leaves;
- variety D - thin and long leaf segment.

As a result of pairwise crosses of the above mentioned varieties the following dill plants with the new combinations of characteristics were selected in F2 hybrid generation:

- plants with many leaves, with no wax bloom and with a long harvesting period;
- dill plants with shorter stem internodes, thick and broad leaf segment without wax bloom and with a bright green color;
- plants with a long harvesting period, with shortened stem internodes, with thick and broad leaf segments;
- plants with thin and very long leaf segments and with a long harvesting period.

Obtained new forms of dill will be used to create new varieties and in further breeding work to obtain plants with other combinations of characteristics.

PS2.6 Screening of wild Lactuca materials against infection of a Bremia lactucae race from Murcia (Spain)

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Keywords: breeding; Bremia; germplasm; lettuce; wild species

Abstract:

Introduction of resistance to Bremia lactucae is a priority in commercial breeding programmes in lettuce. Several wild relatives in genus Lactuca have been used as sources of resistance against an important number of B. lactucae races. We have screened a collection of 78 accessions of wild lettuce, including three accessions of L. perennis, 9 of L. saligna, 63 of L. serriola and 3 of L. virosa against isolate BLV-1 of B. lactucae from Murcia (Spain). As a susceptible control the L. sativa varieties Romana, Olaf and Green Towers were used. Plantets of each accessions were cultivated in mini greenhouses with a capacity for 60 plantlets and were kept in a climatic chamber with a 12h/12h light/dark photoperiod, a temperature of 16ºC and a relative humidity around 80%. Plantlets were inoculated when they had two leaves developed. The BLV-1 has been biologically characterized and does not correspond to any of the B. lactucae catalogued by the International Bremia Evaluation Board. Inoculum was maintained in Romana leaves. Concentration of spores for inoculation was adjusted to $5x10^4$ spores ml^{-1}. We evaluated the number of plants presenting sporulation at 7, 10 and 15 days after inoculation. Susceptible controls presented a high degree of sporulation (97.2% for Olaf and 100% for Romana and Green Towers). Wild Lactuca species displayed a variable performance. Two accessions of L. perennis, two of L. saligna, 11 of L. serriola and one of L. virosa did not present sporulation in any of the plantlets tested. These materials may be of interest for the development of lettuce cultivars resistant to this new race of B. lactucae.

PS2.7 European harmonization of evaluation of resistance of lettuce to *Bremia lactucae*

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Keywords: Lettuce, Bremia lactucae, Downy mildew, resistance, notation scale

Abstract:
The aim of the Harmores 2 project (co-founded by CPVO) is to harmonize, at the European level, resistance tests to seven vegetable diseases including *Bremia lactucae* on lettuce. Previous ringtest results on comparison of different substrates and different notation scales showed comparable results between laboratories. Different assessors did not record the same results for some varieties. Moreover, we observed that different official notation scales (CPVO, UPOV and IBEB) were described.

In the framework of this project, GEVES organized in 2014 a workshop to draw the line between resistance and susceptibility around light or sparse sporulation, and to define a common notation scale and interpretation of observed symptoms. National offices of seven European countries and ESA representatives participated.

PS2.8 IBEB and ABEB propose a streamlined lettuce differential set for Bremia lactucae

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Abstract:
Bremia lactucae, the causal organism of lettuce downy mildew, continues to evolve its resistance-breaking capacities to match the changing resistance genetics in the field. The International Bremia Evaluation Board (IBEB) has responded to this situation by denominating three new isolates, Bl: 29, 30 and 31, and by adding new differentials for differentiation of the new isolates.

Resistance tests with specific isolates of *Bremia lactucae* are needed for a defining description of a lettuce variety. Without an official description it is not possible to bring a variety to the market or obtain intellectual property rights, i.c. Plant Breeders Rights. UPOV provides the legal framework for official variety descriptions with a worldwide validity. CPVO uses this framework to regulate the European market. The current UPOV guideline "TG/13/10 rev. 2" includes 17 *Bremia* isolates: Bl: 2, 5, 7, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26 and 27. The addition of the new isolates Bl: 29, 30 and 31 will be proposed to UPOV during the revision if this guideline this summer.

IBEB, together with her newly formed sister, the American Bremia Evaluation Board (ABEB), has also recognized the fact that Bl: 1 to 15 are no longer relevant for the market. Seed companies have agreed to mention only isolates Bl: 16 and higher in their seed catalogues from 2014 onwards. This means that Bl: 1, 3, 4, 6, 8, 9, 10, 11, and 13 are no longer needed for practical purposes. This development creates an opportunity for reducing the number of differentials.

IBEB and ABEB have worked out a new proposal for an updated and streamlined differential set. Information about this set is given on this poster and will be available through the ISF website: http://www.worldseed.org/isf/ibeb.html.

Thursday 16 April

S4 Postharvest quality

S4.1 Agronomy - the route from genotype to desirable phenotype

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Abstract:

The leafy vegetable sector is under multiple pressures to keep on innovating whether in response to environmental concerns, price reductions, healthy eating campaigns or food safety worries. Plant scientists (and funders!) often focus on genetic approaches to meet these challenges. However, manipulating the growing environment through agronomic inputs and treatments has significant impact on the expression of both desirable and undesirable traits. In addition, for many traits a grower is concerned about crop phenotype at a field-scale rather than an individual plant. This presentation will discuss desired phenotypes and highlight the combination of improved agronomy and genetics in a 'phenomics' approach to improve the phenotype of leafy vegetable crops.

S4.2 Biomarkers of quality for leafy vegetable crops

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Abstract:
In the postharvest chain of fresh leafy vegetables, the main objective for growers, processors and consumers is to guarantee quality. The fresh-cut industry has a long list of factors that impact quality, but there is little or no scientific evidence for adequate tools or biomarkers to predict quality. Therefore, there is a need to develop novel quality monitoring tools. Quality losses concerns in leafy vegetables are mainly due to 1) browning of cut edges due to tissue disruption and oxidative processes, 2) increased respiration and metabolic reactions with development of off-odours and 3) tissue softening. The aim of the QualityLeaf project (AGL2013-48529-R) is to study different biomarkers as predicted tools to determine the impact of intrinsic and/or extrinsic factors related with the quality characteristics. The main expected result from the development of quality biomarkers will be the selection of genotypes, environmental conditions, agricultural practices, handling conditions, processing and storage practices that guarantee the quality maintenance of leafy greens. The QUALITY BIOMARKERS included in this study are:

1) Plant metabolites. Phenolic compounds and other primary and secondary metabolites by metabolomics analysis as biomarkers of enzymatic browning under different extreme environmental conditions during pre and postharvest.
2) Volatile compounds. Changes in gaseous and volatile compounds from the respiratory and non-respiratory metabolisms as biomarkers for off-odours development by the changes in respiration rate, respiratory quotient, fermentation induction point and the type and concentration of volatiles developed under different extrinsic and/or intrinsic factors.
3) Biomechanical properties. Leaf turgor, elasticity, plasticity, cell size, cell number and cell turgor, photosynthetic and transpiration rates as potential biomarkers for the loss of texture under different agronomical conditions.

S4.3 Salad research

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Abstract:

S4.4 Elucidating the genetic basis of antioxidant status in lettuce (*Lactuca sativa*)

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Keywords: lettuce breeding; phytonutrients; quantitative trait loci (QTL); antioxidant; phenolics; recombinant inbred lines (RILs); quercetin

Abstract:
A diet rich in phytonutrients from fruit and vegetables has been acknowledged to afford protection against a range of human diseases, but many of the most popular vegetables are low in phytonutrients. Wild relatives of crops, however, may contain allelic variation for genes determining these beneficial nutrients and therefore understanding the genetic basis of this variation is important for breeding efforts to enhance nutritional quality. In this study, lettuce recombinant inbred lines (RILs), generated from a cross between wild and cultivated lettuce (*Lactuca serriola* and *L. sativa*, respectively), were analysed for antioxidant potential, carotenoid, chlorophyll, and total phenolic content, and concentrations of individual phenolic compounds, many of which are important phytonutrients. When grown in two environments, 96 quantitative trait loci (QTL) were identified for these nutritional traits: four for antioxidant potential, two for carotenoid content, three for total chlorophyll content, and 87 for individual phenolic compounds (average 2 per compound). Usually, the *L. serriola* alleles conferred an increase in total antioxidants and individual metabolites. Candidate genes that mapped to these QTL were identified by BLAST searches; in several cases these had functions that suggested involvement in phytonutrient biosynthetic pathways. Analysis of a QTL on linkage group 3, which accounted for >30% of the variation in antioxidant potential, revealed a candidate gene encoding flavanone 3-hydroxylase, an enzyme involved in the biosynthesis of the flavonoids quercetin and kaempferol, which are known to have powerful antioxidant activity. An Arabidopsis line with a T-DNA insertion in the orthologue of this gene showed a 33% reduction in overall antioxidant potential. These results offer exciting opportunities to improve the nutritional content and health benefits of lettuce through marker-assisted breeding.

S4.5 Improving postharvest quality of leafy vegetable crops – Ammonia as postharvest freshness indicator for leafy greens

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Abstract:
Most leafy vegetables require assiduous temperature management to maintain their postharvest quality and their perceived freshness. Freshness is a very desirable but somewhat elusive characteristic for all fresh produce, but especially leafy greens. The weakest points in the cold chain are typically the period after harvest before cooling and then again during final distribution. Modified atmospheres are a supplement to good temperature management but are generally much less important in postharvest handling. Examples of quality loss in relation to temperature and modified atmospheres will be discussed for several intact and fresh-cut leafy green including lettuces, spinach, arugula, kale, and grape leaves. This discussion will focus specifically on ammonia and its relationship with other postharvest quality indicators for leafy greens. In harvested aging tissues (dependent on length of storage and storage temperature) and in stressed tissues (inappropriate modified atmospheres; temperature abuse) there is a decline in the activity of glutamate synthetase and an accumulation of ammonia, which is toxic to plant cells. Although there is probably no single measurement of freshness, there is evidence that for fresh and fresh-cut leafy vegetables, sensing of ammonia could be a useful freshness indicator.

S4.6 Pre and postharvest strategies to reduce microbial risks of leafy vegetable crops

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Key words: food safety; foodborne pathogens; phyllosphere; epiphytic microbiota; leafy vegetables

Abstract:
Leafy greens present challenges for maintaining food safety as they have been identified as one of the five top groups of risk food/pathogen associated commodities. Contamination of leafy greens with foodborne pathogens may occur at any step in the farm to consumer chain (growing, harvest, processing, wholesale storage, transportation, retailing and handling at home) from environmental, animal or human sources. Recent publications highlighted several pre-harvest sources as the most probable origins of potential contamination including: contaminated water, soil amendments and fecal contamination from wildlife. The process and processing environment are also significant factors for the microbiological safety of fresh-cut products. Water disinfection is one of the most recommended intervention strategies for irrigation and process water if the microbial quality is out of the recommended range. The selection of environmentally friendly technology to reduce, control or eliminate efficiently the risk of microbial pathogen contamination for irrigation and process water will be presented. Additionally, the phyllosphere of plant surfaces is colonized by millions of bacteria. Enteric human pathogens that pretend to colonize plant surface must co-exist in an ecological niche already populated by bacterial epiphytes. Thus, the fate of enteric pathogens is determined by their ability to compete with the epiphytic microbiota. Their survival will be largely influenced by the niche overlap between them and the epiphytic microbiota. Fluctuations of the epiphytic microbiota may also influence survival of enteric human pathogens in leafy greens and this could be part of an integrated strategy to control the growth of pathogens. Understanding the variations of epiphytic microbiota due to intrinsic and extrinsic factors could help to understand the different susceptibilities of leafy vegetables to be affected by pathogenic enteric bacteria.

S4.7 Different glucosinolates kinetic in italian and dutch kales as a result of cooking systems: boiling, steaming, and stir-frying

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Keywords: kales; glucobrassicin; indole-3-carbinol; cooking; kinetic

Abstract:
Kales (Brassica oleracea ssp. acephala) represent a traditional leafy vegetable of several European countries, generally used in the preparation of traditional dishes such as soups or filling of pastries. Kales contain high amount of indolic glucosinolates, with glucobrassicin representing the most abundant compound. Indole-3-carbinol, identified as the major breakdown product from glucobrassicin, is particularly important for its putative anticancer properties. Glucosinolates are subject to different forms of degradation during cooking. The aim of this study was to compare the effect of boiling, steaming and stir-frying on glucobrassicin and indole-3-carbinol, through kinetic parameters describing the reaction rate constants.

Three local kale populations, the Dutch Boerenkool, and the Italians Broccolo Lavagnino and Nero di Toscana, were obtained from an experimental trial established in Wageningen (The Netherlands). For boiling and steaming four cooking times (5, 10, 30, and 60 min) were selected, three for stir-frying (5, 10, and 20 min). Samples of kales and cooking water were analyzed for their content in glucobrassicin and indole-3-carbinol.

Boiling and stir-frying produced higher losses in glucobrassicin. However in the case of boiling most losses were due, at least at the beginning, to leaching in the cooking water. In most cases glucobrassicin thermal degradation fitted with a second order kinetic model, meaning that more than one process was involved in its degradation.

Indole-3-carbinol showed higher contents in raw leaves. After boiling it was only detectable in the cooking water. In all cases it was observed a slight increase of indole-3-carbinol on the cooking time, especially if considered Nero di Toscana.

The three kale population were well differentiated showing different degradation rate constants during thermal treatment.

S4.8 The influence of pre-harvest and postharvest factors on cut-end browning in celery

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Keywords: Celery; ethylene; browning; crop maturity

Abstract:
Celery (Apium graveolens L.) is a foliage crop which is commonly consumed for its long and thick petioles. Colour is a key quality parameter used by consumers to determine stalk freshness. Postharvest browning, which manifests as black/brown stains on cut ends, is a physiological disorder that occurs in celery during storage. It is believed to be due to the activity of polyphenol-oxidases (PPOs), which oxidase phenolic compounds to produce brown pigments. It represents an economic issue for growers and the fresh produce industry as customers tend to reject affected products. For this reason, the effect of crop maturity and ethylene treatment on postharvest browning of cut ends was investigated in this study. The experiment was conducted in Spain (Murcia) and replicated across two growing seasons. In each trial, celery cv. Monterey plants were harvested every week in order to obtain immature, mature and over-mature plants. Subjective and objective colour analyses were performed at regular time intervals during storage at cut petiole and butt ends of the samples. Cut-browning increased with storage time and crop maturity, with the blackening/darkening developing sharply in the first 6 days of storage. On the other hand, within each stage of crop maturity, continuous ethylene application did not influence browning. In summary, these results indicate that crop maturity impacts on postharvest discolouration of celery, whilst no significant effect was observed with exogenous ethylene.

Friday 17 April

S5 Exploring genetic diversity – ways to improve elite cultivars

S5.1 Linking gene banking and plant breeding: opportunities and challenges

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Keywords: genebank services; germplasm utilization; strategic alliances

Abstract:
Due to technological advances, increasing numbers of sequencing data can nowadays be generated against decreasing costs. As a result, large-scale resequencing projects have been initiated in various crops to identify variation at genes. These developments are expected to boost genomics research and crop improvement. As genebanks generally harbour large numbers of samples of a crop's gene pool, their collections are considered important targets for resequencing initiatives. To better facilitate the user community, genebanks may have to strengthen their core business, including improvement of the composition of their collections and improvement of the accessibility thereof to users. In addition, genebanks should anticipate the new demands from the research community and the plant breeding industry, including the introduction of novel collection types and the development of novel information services. How the functioning of genebanks may change is the subject of this presentation. It is argued that to improve their user-oriented services, genebanks may need to increase the communication and strengthen the collaboration with the user community. Examples from leafy vegetables are used for illustration.

S5.2 Downy mildew effector screening in lettuce (*Lactuca*) germplasm and its responses in *L. saligna*

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Keywords: effectors; non host resistance; lettuce; downy mildew

Abstract:
Lettuce downy mildew, caused by the oomycete *Bremia lactucae*, leads to high losses in cultivated lettuce (*Lactuca sativa*) worldwide. Plant pathogenic oomycetes are known to secrete effector proteins inside plant cells to promote infection. These effectors can manipulate plant processes either through their own biochemical activity, or by interacting with plant target proteins (to suppress PAMP Triggered Immunity).

To identify new resistances, *Bremia* candidate effector genes have been identified and transiently expressed in lettuce germplasm using *Agrobacterium*. Effector recognition by the plant leads to a cell death response at the site of infiltration that can be visually scored. By expressing single effectors, new plant R-genes or plant target genes could be identified, as the effects of one effector cannot be masked by the presence of others effectors like in a classical disease test. Segregating populations of lines that respond differently to specific effectors can be used to map the loci responsible for effector recognition.

Previously, a set of 26 candidate effectors have been tested on a *Lactuca* (L.) germplasm set of 129 accessions. One effector, BLG01, was recognized by many accessions of the wild lettuce species *L. saligna* and the response has been mapped to the bottom of chromosome 9. *L. saligna* is considered as a nonhost for Bremia, which can be crossed with L. sativa and may therefore provide a valuable source of durable resistance. Recently, 10 new candidate effectors have been screened. One of these effectors is recognized by 3 *L. saligna* accessions. The effector response was found to be associated with resistance in segregating populations. Mapping this effector response is currently in progress and could lead towards new, potentially durable, resistance loci.

S5.3 Exploring the phytochemical diversity of rocket salad

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Keywords: rucola; *Eruca; Diplotaxis*; phytochemicals; glucosinolates; isothiocyanates; Flavonols; plant breeding; germplasm; gene bank

Abstract:
The species of rocket salad, *Eruca sativa*, is a crop with great potential for improvement. In collaboration with two industrial partners, the phytochemical diversity of 19 *E. sativa* gene bank accessions and 16 commercially available varieties (including *Diplotaxis tenuifolia*) was investigated. Plants were grown in triplicate groups of four under controlled environment conditions and harvested after 30 days. Leaf samples were analysed using LC-MS and concentrations of 13 glucosinolate (GSL) and 11 flavonol compounds were determined. Both commercial and germplasm material showed a high degree of variability, however a general trend was established that germplasm showed higher accumulation of GSLs and commercial had higher flavonols. Six gene bank accessions were selected on their GSL and flavonol profile (high, medium, low) along with one commercial E. sativa variety, for further study. TD-GC-TOF-MS was used to determine the diversity of volatile compounds including isothiocyanates (ITCs). In addition, amino acid content was determined by GC-MS, and CE determined sugar and organic acid content. Accessions all showed a high degree of variability and numerous significant differences were observed. Results under controlled environment conditions indicate that germplasm and commercial genotypes are highly variable in terms of phytochemistry, however under field conditions, this may not be the case. To test this hypothesis and determine changes in phytochemical profile a field trial was conducted in the summer of 2014 using five germplasm accessions and one commercial D. tenuifolia variety as a comparator. Future analyses will determine GSL, flavonol, sugar, amino acid and ITC content at the point of harvest, and at nine other points in the course of processing and shelf life. The information gathered in these studies will help to inform future plant breeding programs, and future work aims to utilise uniform *E. sativa* breeding lines to develop mapping populations, and ultimately QTL for phytochemical traits.

S5.4 Resistance of wild Lactuca genetic resources to diseases and pests, and their exploitation in lettuce breeding

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Keywords: wild lettuce; germplasm; diseases; pests; resistance sources; breeding

Abstract:
The taxonomy, bio-geography and gene pools, biodiversity and resistance variation of wild *Lactuca* species are described. The reported variation in reaction to pathogens and pests of wild *Lactuca* spp. is summarized, including viral pathogens (e.g. Lettuce mosaic virus - LMV, Mirafiori lettuce virus /Lettuce big vein virus LBV, Beet western yellows virus - BWYV, Tomato spotted wilt virus TSWV, Lettuce necrotic stunt virus LNSV), bacterial pathogens (corky root - *Rhizomonas suberifaciens*, bacterial leaf spot - *Xanthomonas campestris* pv. vitians), oomycete and fungal pathogens (downy mildew - *Bremia lactucae*, powdery mildew - *Golovinomyces cichoracearum*, anthracnose - *Microdochium panattoniana*, stemphylium leaf spot - *Stemphylium* spp., sclerotinia drop - *Sclerotinia* spp., verticillium wilt - *Verticillium dahliae*, fusarium wilt - *Fusarium* spp., pythium wilt - *Pythium tracheiphylum*), nematodes (potato cyst nematode - *Globodera rostochiensis*, root-knot nematode - *Meloidogyne* spp., incognita, hapla, javanica, enterolobii), insects and mites (the green lettuce aphid - *Nasonovia ribisnigri*, the green peach aphid - *Myzus persicae*, the potato aphid - *Macrosiphum euphorbiae*, leafminer - *Liriomyza* spp.). The approaches used to exploit wild *Lactuca* spp. in lettuce breeding are discussed and known examples of lettuce cultivars with traits derived from wild *Lactuca* spp. are described.

S5.5 Genetic diversity in natural populations of rare wild Lactuca spp.; The Wild Lettuce Gene Bank (WLGB) project

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Keywords: genetic diversity; wild *Lactuca* spp.

Abstract:
The objectives of my research are related to collecting and studying genetic diversity in natural populations of wild *Lactuca* species. Specifically, the study is devoted to: surveying, collecting, and eco-geographical characterization of rare wild *Lactuca* spp., that are taxonomically close to the cultivated lettuce (*L. sativa*); species definition; taxonomic validation; morphological traits; identification of natural putative hybrids; genetic diversity; out-crossing rate; diseases resistance (*Bremia lactucae*); chemical composition; and bolting control (*L. georgica*).

L. sativa is part of a reproductively isolated group that includes the wild species *L. serriola, L. saligna* and *L. virosa* (in order of decreasing sexual compatibility with *L. sativa*). Of these three wild species, only *L. serriola* represents the primary gene pool. Six other wild species are crop wild relatives to the cultivated lettuce: *L. aculeata, L. georgica, L. scarioloides, L. altaica, L. azerbaijanica,* and *L. dregeana*. However, these are less widespread and the status of some of them as distinct species is unclear. Studies are ongoing to clarify their taxonomic status and crossing potential.

Our studies based mainly on new collections of *L. serriola, L. aculeata, L. saligna,* and *L. georgica* from Israel and Armenia, as well as some accessions collected in past from Jordan, Turkey and addition countries. Described and ongoing results from the WLGB project were obtained by various levels of national and international collaborations. Variation obtained in our studies suggest that these species are largely untapped sources for breeding resistances against biotic and a-biotic stresses, as well as other traits, into the cultivated lettuce. This increases also the rationale to identify and collect additional accessions representing these four species, as well as new and unique germplasm of additional wild *Lactuca* spp. that mentioned above from multiple locations throughout their geographic distributions. These collections may serve as potentially novel sources for breeding programs.

PS5.6 Survey of reproductive barriers in an interspecific lettuce hybrid, *L. saligna* x *L. sativa*

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Keywords: reproductive barrier; transmission ratio distortion; introgression breeding; wild Lactuca species; non host

Abstract:
Hybrids between species are sometimes inviable, sterile or show other, less extreme, negative phenotypes. These so-called 'hybrid incompatibilities'(HI) are of evolutionary interest for their role in speciation as reproductive isolating barriers. The HI genetic architecture is predominantly explained by interactions between at least two genes (known as the Bateson Dobzhansky "Muller interaction model). Because HI can severely arrest exchange of genetic variants between species, it results in transmission ratio distortion (TRD). So TRD can be used as an indicator of HI /reproductive barriers.

In our quest for alternative downy mildew resistance in lettuce (*Lactuca sativa*), we study introgression of nonhost resistance from the secondary gene pool species, *Lactuca saligna*. In research activities on interspecific F2 and BC1 progenies between cultivated (*L. sativa* cv. Olof) and wild lettuce (*L. saligna* CGN05271), we have observed at least twelve loci for TRD (TRDL) over six of the nine lettuce chromosomes. In the F2 progeny, the average chi2 value for these TRDL was 32, but three TRDL showed extreme distortions with chi2 values of almost 100. Patterns in genotype frequency curves plotted along the genetic map, allowed for classifications of barrier type: three gametophytic and nine zygotic. For at least four TRDL a digenic interaction was proven. Further discussion involves potential biological explanations for the observed TRD and consequences for lettuce introgression breeding with *L. saligna* as resource germplasm.

S5.7 Effect of the lettuce genotype on the protective efficacy of plant defence stimulators against *Bremia*

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Keywords: lettuce; *Lactuca sativa*; downy mildew; *Bremia lactucae*; stimulator of plant defences; genetic diversity

Abstract:
Different stimulators of plant defences (SPD) were tested on large number of lettuce accessions (*Lactuca sativa*) to evaluate in laboratory the protective efficacy against downy mildew due to *Bremia lactucae*. Three products, BION 50WG, CalFlux and a formulation with yeast extract (ABE IT 56), were used on 402 lettuce accessions. Five days after SPD treatment, the young plants at the 3-4 leaves stage were sprayed with *Bremia* spore suspension, strains Bl: 25 or Bl: 26. The three tested SPD could induce resistance in lettuce against *Bremia* but a majority of the tested accessions were not protected by any tested SPD. Among the 3 SDP, BION was the most effective: close to half accessions of the 302 susceptible to Bl: 25 and a quarter of the 110 accessions tested with Bl: 26 were well protected against the tested strain by BION and some of these were also protected by CalFlux (13% and 14% respectively) and few by ABE IT 56 (10% and 4% respectively). The reactive accessions were identified as well among the old cultivars as among the modern cultivars. Protected cultivars belong to each group, butterhead, crisphead, leaf lettuce or cos lettuce. For 15 accessions susceptible to Bl: 25 and to Bl: 26, the protection by the 3 SDP was tested against the both isolates. The induced resistance seems to be strain-specific as some accessions were protected against one strain and not protected against another strain; nevertheless some promising accessions were protected against both strains in our test conditions. This genetic variability could be interesting to use in breeding program to increase the level of the protection by SPD in culture. In the future, it could be a genetic character to cumulate with resistance gene to try to increase the level and the durability of the resistance.