Palacký University in Olomouc
Eucarpia
(European Association for Research on Plant Breeding)
Czech Society for Plant Pathology

9th International Conference on Genetics and Breeding of Leafy Vegetables

Eucarpia Leafy Vegetables
2019

Programme and Proceedings of Abstracts

Editors

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on Genetics and Breeding of Leafy Vegetables

Eucarpia Leafy Vegetables 2019

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Introduction and Welcome

Ladies and Gentlemen, colleagues and friends,

On behalf of the Organizing Committee of **Eucarpia Leafy Vegetables 2019**, the 9th International Conference on Genetics and Breeding of Leafy Vegetables, I would like to welcome all of you to the Czech Republic and the historic city of Olomouc. This meeting is addressing a broad swath of theoretical, methodological and applied topics related to genetics and breeding of leafy vegetables – general aspects (cultivation geography and systems, economic importance, new crops etc.); genetic resources (genebanking, taxonomy, botany, biogeography, crop wild relatives, biodiversity etc.); abiotic and biotic stress (diseases and pests); molecular genetics, genomics and genetic improvement (complex of omics activities and genetics in breeding); postharvest quality (nutrition/flavour/appearance/safety); new trends and visions in leafy vegetables research, breeding, growing and marketing; challenges for future research, developments and plant breeding applications. All these aspects represent very broad, vibrant and fast developing areas of plant biology, plant breeding, seed production and growing.

Personally, I am delighted that I have, together with my colleagues on the organizing committee, the opportunity to organize this conference in the city of Olomouc, within the historic Czech Lands, which became from the former Czechoslovakia the Czech Republic in January 1993. The history of this conference has two main periods, i.e. before and after 1999. The 4th Eucarpia Leafy Vegetables conference organized by us in Olomouc in June 1999 could be considered as a recovery action after a long period of fifteen years (the last one was held in Versailles, France 1984). Currently we can conclude that it was very successful recovery, because starting June 1999 the conference has been organized regularly every four years (2003 - the Netherlands; 2007 - UK; 2011 - France; 2015 - Spain). After twenty years we are back in the historical city of Olomouc and on the floor of the second oldest university in the Czech Republic, Palacký University.

There are many interesting historical aspects of this conference. In the second half of the 1970s (1976/1977) I established strong contacts with Dr. Ian R. Crute (NVRS (later HRI), Wellesbourne, UK) in the area of research of interactions between lettuce and wild *Lactuca* spp. and *Bremia lactucae*. These contacts yielded our first joint paper in 1981 (published in Euphytica). This coincides also with the period that I have been involved in this field of research, starting in 1975 not so far from the city of Olomouc, at the Plant Breeding Station at Smržice, which at that time was part of the Research and Breeding Institute of

Vegetable Crops in Olomouc. Hence this region and Olomouc can be considered as the place where the first Czech experimental research on lettuce germplasm and lettuce breeding was initiated. From my viewpoint the most crucial time for development of international contacts and cooperation was the period February 1988 till January 1989 when I had the opportunity to stay for 12 months in the Netherlands as a research fellow of Institute of Horticultural Plant Breeding (IVT, Wageningen) and Institute of Plant Protection (IPO, Wageningen). At that time I established very strong linkage with researchers and breeders in the Netherlands, in some other European countries, as well as in the USA (Dr. E.J. Ryder (Salinas), prof. R.W. Robinson (Geneva), prof. R.W. Michelmore (Davis)) where I stayed in September /October 1988. All these contacts from the "early days" substantially contributed to the building of an exciting network with many people around the globe which was more efficiently exploited after the Velvet Revolution of November 1989 when our country received again its freedom and returned again to the group of democratic Euro-Atlantic countries. Year 2019 is very special for our country because we are celebrating the 30th anniversary of the Velvet Revolution and 15 years of membership in European Union. All these events significantly contributed to us being here for this conference.

In 1994 I moved from the former Plant Breeding Station in Smržice to the Department of Botany, Faculty of Science, Palacký University in Olomouc. Soon, a research group focusing mostly on downy and powdery mildews was established, and research now covers the ecology, plant pathology, physiology and genetics of host-pathogen interactions, population biology and epidemiology, and resistance breeding (mostly vegetable crops and their wild relatives, especially lettuce and cucurbits).

The historic city of Olomouc is situated in the Central Moravia region, the main part of which is formed by the fertile and hospitable Haná lowlands. The name Haná was first used by Jan Blahoslav and Jan Amos Comenius on a map of Moravia in 1627. Historically the city of Olomouc is considered as the capital of Central Moravia, and a prominent centre of education and culture. It is well known that advances in culture and science in the Czech Lands have been, and are still, closely related to the developments of higher education and the University in Olomouc. In 1573 the city was granted university rights; the University in Olomouc is the second oldest in the Czech Lands (after Charles University in Prague, established in 1348). The era of the old University is associated with the names of numerous outstanding scientists, including the founder of genetics Gregor Johann Mendel, who studied philosophy and theology here in 1840-1843. The recent name „Palacký University“ was given (in 1946) in recognition of the historian František Palacký, known as „the Father of the Nation“. At present, the University has eight faculties, with over 1500 teaching staff and ca 25,000 students (www.upol.cz).

The main venue of The 9th Conference Eucarpia Leafy Vegetables 2019, the monumental complex of baroque buildings named „Konvikt“, is very closely linked to the rich history of the city, the Jesuit Boarding School (Jesuit Seminary, Jesuit College) and the University in Olomouc. The early history of this building dates from medieval times. The present building was finished in 1708 and used for a long period by Olomouc University. Unfortunately, towards the end of 18th century the building of the former Jesuit College was handed over to the military for use as a barracks. However, the building was returned to the University after the events of 1989. Extensive reconstruction and modernization was completed in 2002, and it is once again used for its original academic purpose. Thus the building has extraordinary historic and artistic value. It is now used as the Art Centre of Palacký University, with the conference halls being used as a venue for international events such as the present conference.

Olomouc is well known both as a university city, as well as a scientific centre. Research related to natural sciences, plant biology, breeding and growing of vegetable crops has a long tradition in this area. The Research Institute of Vegetable Crops (RIVC) was established here in January 1951, and in the end of 1950s the Institute of Experimental Botany of the Czechoslovak Academy of Sciences. However, the dramatic political and economic changes occurring after November 1989 resulted in closure of RIVC in 1994. The buildings and facilities were given to the Faculty of Science at Palacký University, which established a Biocentre here, focusing on biology and especially the plant sciences. Recently new facilities were built to house the Centre of the Region Haná for Biotechnological and Agricultural Research. This Centre combines activities of the University, the Institute of Experimental Botany of the Czech Academy of Science and the Gene Bank of the Research Institute of Crop Production in Prague.

From the contents of the book of proceedings it is evident that substantial progress has been made in various branches of leafy vegetables research, its application in plant breeding and disease control. The scientific and professional part of this meeting is based on 28 oral contributions and 25 posters, abstracts of most of them are published in this book. From the programme and the contents of the proceedings it is evident that there is a good balance between fundamental science, theoretical and methodological approaches, as well as practical applications of the results. Increasing international contacts and cooperation in this area provides an excellent foundation for future developments.

We are very happy that Palacký University in Olomouc, and the Czech Republic, are hosting this gathering of scientists, breeders and professionals from 20 countries and five continents. We will do our best to make **Eucarpia Leafy Vegetables 2019** a most informative and enjoyable experience for all participants. We believe that the conference will take place in the spirit of cooperation and friendship, and that everybody will enjoy the science, the historic city of Olomouc, Moravia and the Czech Republic.

Aleš Lebeda

Olomouc, May, 2019

Programme

Eucarpia Leafy Vegetables 2019,
9th International Conference on Genetics and Breeding of Leafy Vegetables
(24-28 June 2019, Olomouc, Czech Republic)

24 June, Monday

Hotel Flora

Accommodation

15.00-20.00 ***Registration***

25 June, Tuesday

Palacký University, Konvikt

8.00-9.30 ***Registration, setting up the posters***

Palacký University, Konvikt „Auditorium“

9.30-10.00 ***Opening ceremony, Introduction to the programme***

10.00-11.15 ***General aspects*** *(history, cultivation geography and systems, economic importance, new crops etc.)*
Chairperson: J. Monaghan

10.00-10.45 **I. Simko,** B. Mou, K.R. Richardson, J.D. McCreight (USA)
Trends in lettuce and spinach production in the United States

10.45-11.15 **L. Halinski,** A. Topolewska, P. Stepnowski (Poland)
Chemical aspects of plant selection mechanisms in domestication of new indigenous African leafy vegetables (*Solanum* spp.)

11.15-12.30 ***Genetic resources*** *(genebanking, taxonomy, botany, biogeography, crop wild relatives, biodiversity etc.)*
Chairpersons: Ch. Kik, J. Šuštar-Vozlič

11.15-11.30 **J. Šuštar-Vozlič** (Slovenia), R. van Treuren (the Netherlands), U. Lohwasser (Germany), P. Coquin (France)
ECPGR Working Group on Leafy Vegetables:
Prospects and Challenges for Phase X

11.30-12.00 **Ch. Kik** (the Netherlands)
The first blow is half the battle: collecting and regenerating leafy vegetables

12.00-12.30 **L. Majeský,** M. Kitner, E. Křístková, A. Lebeda (Czech Republic)
Current view on phylogeny and taxonomy of the Lactucinae subtribe

12.30-14.00 ***Lunch***

14.00-15.30 ***Genetic resources*** *(genebanking, taxonomy, botany, biogeography, crop wild relatives, biodiversity etc.)*
Chairpersons: J. Šuštar-Vozlič, Ch. Kik

14.00-14.30 **E. Křístková,** A. Lebeda (Czech Republic)
Ecogeography and field observations of wild *Lactuca* species

14.30-15.00 **A. Lebeda,** E. Křístková, I. Doležalová, M. Kitner (Czech Republic), M.P. Widrlechner (USA)
Genetic resources of wild *Lactuca* spp. in North America, complex view on underestimated topic

15.00-15.15 **M. Kitner,** L. Majeský, E. Křístková, A. Lebeda (Czech Republic)
Genetic variability of wild *Lactuca* species germplasm

15.15-15.30 **J. Šuštar-Vozlič et al.** (Slovenia, Czech Republic)
Slovene autochthonous lettuce variety 'Ljubljanska ledenka': genetic diverstiy and reintroduction to the market

15.30-16.00 ***Coffee break***

16.00-18.30 ***Abiotic and biotic stress*** (*diseases and pests*)
Chairpersons: B. Maisonneuve, J. Correll

16.00-16.30 **J. Correl** et al. (USA)
Economically important spinach diseases: an evolving problem

16.30-17.00 **G. Gilardi,** M.L. Gullino, A. Garibaldi (Italy)
Emergence of a new pathogens on leafy vegetables

17.00-17.30 **F. Lecompte,** P.C. Nicot (France)
Integrated disease and pest management in lettuce

17.30-17.50 **A. Lebeda,** B. Mieslerová, M. Kitner, E. Křístková (Czech Republic)
Lettuce powdery mildew – exciting but unknown disease of lettuce

17.50-18.10 **B. Maisonneuve,** C. Jouan, S. Gimenez (France)
Resistance to root-knot nematodes in the genus *Lactuca*: hope for breeding

18.10-18.30 **A. Lebeda,** I. Petrželová, E. Křístková, (Czech Republic), F. Runge (Germany)
Variation in wild plant pathosystem *Lactuca* spp. – *Bremia lactucae* and its importance for lettuce resistance breeding

19.00-21.00 ***Welcome Party at Historical Rector Building of Palacký University***

26 June, Wednesday

Palacký University, Konvikt "Auditorium"

8.30-10.30 ***Molecular genetics, genomics and genetic improvement***
(*complex of omics activities and genetics in breeding*)
Chairpersons: I. Simko, R.W. Michelmore

8.30-9.30 **R.W. Michelmore** (USA)
Next-generation genomics of lettuce

9.30-10.30 **H. Kuang** (China)
Genetic analysis of complex traits in lettuce

10.30-11.00 ***Coffee break***

11.00-12.30 ***Molecular genetics, genomics and genetic improvement***
(*complex of omics activities and genetics in breeding*)
Chairpersons: R.W. Michelmore, I. Simko

11.00-11.45 **I. Simko** (USA)
Marker-assisted selection in lettuce breeding

11.45-12.30 C. Meisrimler, A. Pelgrom, J. Klein, M. Neilen and **G. Van den Ackerveken** (the Netherlands)
Revealing functions of downy mildew effectors in spinach and lettuce

12.30-14.00 ***Lunch***

14.00-15.30 ***Molecular genetics, genomics and genetic improvement***
(*complex of omics activities and genetics in breeding*)
Chairpersons: G. Van den Ackerveken, M. Kitner

14.00-14.45 **M. El-Esawi** (Egypt)
Overexpression of *Arabidopsis thaliana* WRKY transcription factor improves salt and drought tolerance in lettuce (*Lactuca sativa* L.)

14.45-15.30 **A. Stojakowska** (Poland)
Wild *Lactuca* species and their specialized metabolites

15.30-16.00 ***Coffee break***

16.00-17.30 ***Postharvest quality*** (*nutrition/flavour/appearance/safety*)
Chairpersons: C. Wagstaff

16.00-16.30 **R. van Treuren** (the Netherlands)
Metabolite variation in *Lactuca*

16.30-17.00 **M. Chadwick** et al. (UK)
Screening of metabolites linked to pink and brown discolouration in lettuce (*Lactuca sativa*) using the Sal × Ice mapping population

17.00-17.30 **B. Mou** (USA)
Genetic variation and improvement of nutritional traits in lettuce and spinach

17.30-18.30 ***Poster Session***

27 June, Thursday

7.30-21.00 ***South Moravia - Excursion***

7.30 ***Departure from Hotel Flora***

9.00-12.00 ***Johann G. Mendel Museum of Genetics and Augustinian Abbey in Old Brno***
Lecture of P. Smýkal: J. G. Mendel – from Moravian peasant´s son to world genius
Guided tour in J. G. Mendel Museum

13.00-15.30	***The Castle of Austerlitz (Slavkov), Lunch***
16.30-19.30	***Visit of company Vinselekt Michlovsky (Rakvice, South Moravia), Moravian wine tasting***
19.30	***Departure from Rakvice to Olomouc***
21.00	***Arrival to Olomouc***

28 June, Friday

Palacký University, Konvikt "Auditorium"

9.00-12.00	***New trends and visions in leafy vegetables research, breeding, growing and marketing*** (*general and specific aspects related to these topics*) **Chairperson: K. Reinink**
9.00-9.45	**C. Wagstaff** et al. (UK) Rocket Science - a journey from field to genotype
9.45-10.30	**J. Monaghan** et al. (UK) Discovering traits of commercial interest in diversity collections
10.30-11.00	***Coffee break***
11.00-12.00	***Closing and Challenging lecture*** **Chairperson: K. Reinink**
11.00-12.00	**D.A. Jacobson** (USA) Integrative and computational systems biology and their exploitation in plant breeding
12.15-12.45	***Closing session and closing ceremony***
13.00-14.00	***Lunch***
14.00-16.00	**IBEB-G open meeting** (chaired by J. Schut)
16.00-18.00	**ILGC3 Meeting** (consortium participants only)

Farewell Evening, Palacký University, Konvikt "Atrium" and surrounding area

19.00-19.30 Baroque music concert in Konvikt Chapel

19.30-23.00 Farewell dinner

**** some minor changes may appear in the programe structure/content***

Posters

Eucarpia Leafy Vegetables 2019, Olomouc, Czech Republic
(in alphabetical order of first authors)

1. **Acharya Ch.B., Najarro M., Fletcher K., Michelmore R.W. (USA)**
   Development of molecular markers to explore population dynamics of *Bremia lactucae* (lettuce downy mildew)

2. **Balashova I.T., Bespal’ko L.V., Molchanova A.V., Sirota S.M., Soldatenko A.V. (Russia)**
   Cultivation of leafy plants *Lamiaceae* family at the multi circle hydroponic construction: study on the biochemical composition and some innovations

3. **Beacham A.M., Hand P., Barker G.C., Teakle G.R., Monaghan J.M. (UK)**
   Using vegetable crop genetic resources to identify tolerance to abiotic stress and other key performance traits

4. **Beharav A. (Israel)**
   New insights into the characterization and taxonomy of wild *Lactuca* relatives

5. **Bragalini C., Poluzzi G., Malatrasi M., Donati F. (Italy)**
   Application of Marker Assisted Selection in lettuce (*Lactuca sativa* L.) breeding programs to rapidly asses selfing and crossing rates in experimental F1 progenies

6. **Contente A., Oruna-Concha M.J., Wagstaff C. (UK)**
   Culinary Herbs: determining the basis of variation in flavour

7. **Drozdková N., Křístková E., Lebeda A. (Czech Republic)**
   Biodiversity and ecogeography of wild *Lactuca* species in Hungary

8. **Ebrahimzadeh R., Ellialtıoğlu S.S., Sönmez K., Smilde D., Dolar F.S. (Turkey, the Netherlands)**
   Evaluation of the existence of a new race of *Bremia lactucae* in Turkey

9. **Gerrano A.S., Jansen van Rensburg W.S., Bairu M., Amoo S., Venter S., Mavengahama S. (South Africa)**
   Nutritional characterization of mineral elements and total protein content in the leaf tissues of *Amaranthus*

10. **Gerrano A.S., Jansen van Rensburg W.S., Bairu M., Venter S. (South Africa)**
    Selection of cowpea genotypes [*Vigna unguiculata* (L.) Walp.] for nutritional compositon in the fresh leaves for nutritional quality breeding

11. **Hubrová H., Křístková E., Lebeda A. (Czech Republic)**
    Prickly lettuce in Czech herbals from the first half of 20th century

12. **Jasper J., Elmore S., Wagstaff C. (UK)**
    Defining, predicting and delivering 'fresh' in the context of leafy salads

13. **Kovár M., Brestič M., Mlynáriková-Vysoká D., Živčák M., Botyanszá L., Chovanček E., Barboričová M., Lebeda A. (Slovak Republic, Czech Republic)**
    High-throughput phenotyping of drought stress responses of lettuce genotypes using the optical imaging techniques

14. **Křístková E., Kitner M., Lebeda A., El-Esawi M.A. (Czech Republic, Egypt)**
    Morphological and genetic variation of lettuce from Egypt

15. **Maisonneuve B., Bellec Y., Martin E., Lot H., Gognalons P., Moury B. (France)**
    Lettuce lines with potyvirus resistance: differential set for study of strains and new genitors of resistance

16. **Perrot S. (France) et al.**
    Validation of differentials for new *Fusarium oxysporum* f. sp. *lactucae* race 4 (Fol: 4) - Lettuce

17. **Puranik M., Bell L., Wagstaff C. (UK)**
    Measuring sugars in a mapping population of salad rocket (*Eruca sativa*) at two different locations

18. **Razgallah N., Chikh-Rouhou H., Hassen A., M’hamdi M. (Tunisia)**
    Effects of some nitrogen fertilizers on growth parameters and nitrate accumulatio in lettuce (*Lactuca sativa* L.)

19. **Richardson K.L., Simko I., Wintermantel W. (USA)**
    Breeding lettuce for resistance to impatiens necrotic spot virus, Verticillium wilt, and tipburn in California

20. **Sandoya-Miranda G., Kreutz G., Bhadha J. (USA)**
    Identifying lettuce for phosphorus use efficiency

21. **Smilde D., Correll J.C. (the Netherlands, USA)**
    A future-proof differential set for *Peronospora effusa* in spinach

22. **Thomas V., Himmel P., Brown P., Ruthner S., Grimault V. (France, USA, Switzerland)**
    ISF guidelines on the nomination of novel plant pest races

23. **Turner L., Lignou S., Gawthrop F., Wagstaff C. (UK)**
    Identifying changes in the flavour profile *Apium graveolens* harvested at different maturities

24. **Zorrig W., Cornu J.-Y., Maisonneuve B., Rouached A., Sarrobert C., Shahzad Z., Abdelly C., Davidian J.-C., Berthomieu P. (France, Tunisia)**
    Genetic analysis of cadmium tolerance and accumulation in lettuce (*Lactuca sativa L.*)

25. **Živčák M., Brestič M., Kovár M., Mlynáriková-Vysoká D., Botyanszá L., Chovanček E., Barboričová M., Lebeda A. (Slovak Republic, Czech Republic)**
    Chlorophyll fluorescence imaging in assessment of the drought stress tolerance in lettuce parental lines

Abstracts

Lectures

(in alphabetical order according the first author)

Screening of metabolites linked to pink and brown discolouration in lettuce (*Lactuca sativa*) using the Sal × Ice mapping population

M. Chadwick[a], **S. Lignou**[a], **M.-J. Oruna-Concha**[a], **B. Radha**[a], **P. Hunter**[b], **D. Pink**[b], **P. Hand**[b], **J. Heath**[b], **J. Monaghan**[b], **G. Barker**[c], **A. Hambidge**[c], **C. Wagstaff**[a]

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[b] *Crop and Environment Sciences Department, Harper-Adams University, UK*
[c] *School of Life Sciences, University of Warwick, UK*
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Many leafy vegetables are susceptible to postharvest discolouration that is classified as either 'pinking' or 'browning', the symptoms of which cause fresh produce to fail shelf life targets. Postharvest wastage of lettuce accounts for approximately 1.36m tonnes annually in the UK alone, with fresh cut product being the most vulnerable commodity. The main metabolites involved in browning are phenolics, oxidised to quinones by polyphenol oxidase (PPO), then polymerised to brown melanins. The pinking process is not fully characterised, but is widely regarded as involving quinone conjugates, and consequently involves compounds from the same phenylpropanoid metabolic pathway.

Differences in the development of pink and brown discolouration in cut lettuce products were quantified as part of a genetic mapping study. A population of 100 recombinant inbred lines, from the Sal x Ice mapping population was harvested over two growing seasons in in early and late summer 2016, metabolites related to discolouration were quantified. The population was screened for total phenolics, total quinones, 11 individual selected phenolics, and 15 as yet unidentified phenolic-like compounds, in addition to the activity of PPO and phenylalanine ammonia lyase (PAL), which catalyses the first reaction in the phenylpropanoid biosynthesis. Individual phenolics were assessed by HPLC and other traits by plate spectrometry methods. To determine changes related to shelflife, samples were taken at harvest, as well as one and three days postharvest under commercially relevant conditions.

Differences within the population were large, as expected from the Sal x Ice cross, but there were also statistically significant differences explained by the environment over both trials, as well as arising from the time postharvest. There were significant differences arising from postharvest time for; caftaric acid ($p=0.004$), chlorogenic acid ($p=0.002$), caffeoylmalic acid ($p=0.019$), chicoric acid ($p=0.039$), dicaffeoyltartaric acid ($p= 0.038$), di-O-caffeoylquinic acid ($p=0.018$), quercetin-3-glucuronide ($p=0.029$), quercetin-3-malonylglucoside ($p=0.017$). All identified compounds except chicoric acid showed a strong significant difference in concentration between field trials ($p<0.0001$), as did total phenolics, total quinones, PPO activity and PAL activity. PPO ($p=0.037$) and PAL ($p<0.0001$) also showed statistical differences between shelflife days.

This information will be used to inform QTL mapping with the aim of identifying markers suitable for breeders to produce varieties less prone to postharvest discolouration. Changes in metabolite concentration over shelflife varied between lines, suggesting potentially informative breeding targets, however this may be complicated by the strong environmental influence.

Acknowledgements

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Economically important spinach diseases: an evolving problem

J.C. Correll, Ch. Feng, B. Deep, S. Dhillon, A. Shi, B. Liu, G. Bhattarai, M. Villarroel-Zeballos

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(e-mail: jcorrell@uark.edu)

Spinach continues to increase in popularity as a healthy nutritious leafy green vegetable in the human diet. However, a number of economically important diseases represent major production constraints, as they are difficult to manage, particularly in organic production systems. Major economically important diseases include downy mildew, or blue mold (*Peronospora effusa* [=*P. farinosa* f. sp. *spinaciae* (Pfs)]), Fusarium wilt disease (*Fusarium oxysporum* f. sp. *spinaciae*), white rust (*Albugo occidentalis*), leaf spots (*Colletotrichum dematium, Stemphylium* spp., *Cercospora* spp.), and damping off pathogens (*Pythium aphanidermatum, P. ultimum, F. oxysporum, Rhizoctonia solani* AG4). Due to the popularity of pre-washed bagged spinach and the value of the crop, production practices have intensified. Production practice changes including increased acreage, limited crop rotation, high density plant populations, and an increase in demand for seed have all been factors increasing overall disease pressure. For example, there has been a rapid increase in the number of races of the spinach down mildew pathogen with 17 races currently described, many in recent years. White rust disease, a disease that has been endemic and restricted to certain areas of the U.S. for many years, has now been found in Mexico, Greece, Turkey, and other parts of the E.U. In addition, both Fusarium wilt and leaf spot diseases are increasing in incidence and severity in many production areas. The most effective disease management strategy for reducing the impact of these economically important diseases of spinach is to improve disease resistance. We have developed a number of genetic and molecular resources to improve and expedite breeding for disease resistance in spinach. The resources developed include near isogenic lines, host and pathogen sequence resources, molecular markers linked to major genes for resistance or QTL. In addition, efforts are underway that focus on the functionality of RPF genes using VIGs, CRISPR/Cas editing, plant transformation with RPF resistance genes, gene expression profiles with NILs containing or lacking RPF genes using RNAseq, and sequencing of resistant and susceptible lines including target regions on chromosome

3 with known RPF genes. Molecular markers linked to disease resistance to the white rust and leaf spot pathogens are also being developed for breeding programs.

Overexpression of *Arabidopsis thaliana* WRKY transcription factor improves salt and drought tolerance in lettuce (*Lactuca sativa* L.)

M.A. El-Esawi

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Lettuce (*Lactuca sativa* L.) is a high-value leafy vegetable grown worldwide. High salinity and drought stresses could adversely influence lettuce growth and productivity. Improving lettuce growth and tolerance to such abiotic factors is urgently required to maintain the yield of this economically important crop. WRKY transcription factors could enhance plants tolerance to abiotic factors. Therefore, this study investigated whether *Arabidopsis thaliana* WRKY30 transcription factor could enhance lettuce growth and tolerance to salt and drought stress. *Arabidopsis thaliana* WRKY30 transcription factor was transformed and expressed into lettuce plants. Growth, gas-exchange attributes, photosynthetic pigments content, relative water content, proline content, soluble protein and sugar content, and antioxidant enzymes activities of the WRKY-overexpressing lettuce plants were higher than that of the wild-type lettuce plants. In contrast, the levels of malondialdehyde, electrolyte leakage, and hydrogen peroxide of the transgenic lettuce plants were lower than that of the wild-type lettuce plants. Additionally, the expression of antioxidant enzymes-encoding genes as well as stress-related genes were up-regulated in the transgenic lettuce plants compared to wild type. In conclusion, the results demonstrated that *Arabidopsis thaliana* WRKY30 overexpression improves salt and drought tolerance in lettuce by up-regulating antioxidant machinery, gas-exchange attributes, osmolytes synthesis, and stress-responsive gene expression.

Emergence of a new pathogens on leafy vegetables

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The recent evolutions of the diseases that affect lettuce, wild and cultivated rocket, lamb's lettuce, and spinach are reported. *Plecosphaerella cucumerina,* on wild rocket, endive and lamb's lettuce, *Fusarium equiseti* on wild and cultivated rocket and lettuce, Myrothecium leaf spot on spinach, wild rocket and lamb's lettuce, *Allophoma tropica* on lettuce and different species of *Alternaria* on rocket are among the emerging foliar pathogens and are described. Among soil-borne pathogens, *Pythium aphanidermatum*, *P. irregulare*, *Pythium* Cluster B2a, *P. sylvaticum* have frequently been isolated on spinach and swiss chard, lamb's lettuce and lettuce, while *Fusarium oxysporum* f. sp. *lactucae,* which causes lettuce wilt, is gradually spreading to new countries.

Some of the new pathogens are seed transmitted and typical of tropical areas, and are thus favoured by the rises in temperature that currently characterize the climate change scenario. The possible role of globalisation, climate change and intensive cultivation on the appearance and spread of new pathogens of leafy vegetables is critically discussed.

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Chemical aspects of plant selection mechanisms in domestication of new indigenous African leafy vegetables (*Solanum* spp.)

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Several species of Solanaceous plants, including the gboma eggplant (*Solanum macrocarpon* L.) and scarlet eggplant (*S. aethiopicum* L.), are widely grown in Africa as green leafy vegetables. Their wild relatives (*S. dasyphyllum* Schum. and Thonn. and *S. anguivi* Lam., respectively), while described as separate taxa, are often considered as wild forms of cultivated species. In the present study, the comparison of chemical traits in wild and cultivated plants was performed in order to estimate the phase of domestication of both eggplant species and to shed some light on mechanisms of plant selection at early stages of domestication process.

The amount of selected nutrient (fatty acids, sterols, phenolic compounds and their antioxidant activity) and anti-nutrient (steroidal glycoalkaloids, saponins) compounds was determined in leaves of cultivated and wild lines of *S. macrocarpon* – *S. dasyphyllum* and *S. aethiopicum* – *S. anguivi* complexes. Additionally, two accessions of *S. scabrum* Mill., which is considered as being at the very initial stage of domestication, were also analysed. The results obtained suggest that the only difference between wild and cultivated lines is in their morphology, i.e. in the number of spines present on above-ground parts of the plant. No increase in nutrients or decrease in anti-nutrient compounds was found in cultivated plants when compared to their wild relatives. Similarly, no difference was found between plant lines grown for edible leaves and fruits. Potentially harmful amounts of toxic substances were found in leaves of selected plant accessions; they can be, however, reduced by short-term thermal leaf processing. The highest amount of potentially toxic substances and the lowest of beneficial compounds was found in *S. scabrum* leaves, which somehow supported its cultivation status. In addition, the chemical composition of leaf cuticular waxes was studied in above-mentioned plant species and in related species grown only for edible fruits (the brinjal eggplant, *S. melongena* L. and wild allied taxa). Reduction in the total cuticular wax load was determined as characteristic for plant lines grown for edible leaves. Taking all the results into account, unconscious mechanisms of plant selection based on the reduction of mechanical means of protection are suggested as dominating at early stages of domestication of solanaceous plants grown for edible leaves.

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Integrative and computational systems biology and their exploitation in plant breeding

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The cost of generating biological data is dropping exponentially, resulting in increased data that has far outstripped the predictive growth in computational power from Moore’s Law. This flood of data has opened a new era of systems biology in which there are unprecedented opportunities to gain insights into complex biological systems. Integrated biological models need to capture the higher order complexity of the interactions among cellular components. Solving such complex combinatorial problems will give us extraordinary levels of understanding of biological systems. Paradoxically, understanding higher order sets of relationships among biological objects leads to a combinatorial explosion in the search space of biological data. These exponentially increasing volumes of data, combined with the desire to model more and more sophisticated sets of relationships within a cell, across an organism and up to ecosystems and, in fact, climatological scales, have led to a need for computational resources and sophisticated algorithms that can make use of such datasets. The traits or phenotypes of an organism, including a its adaptation to its surrounding environment and the interactions with its microbiome, are the result of orchestrated, hierarchical, heterogeneous collections of expressed genomic variants regulated by and related to biotic and abiotic signals. However, the effects of these variants can be viewed as the result of historic selective pressure and current environmental as well as epigenetic interactions, and, as such, their co-occurrence can be seen as genome-wide associations in a number of different manners. We have developed supercomputing and explainable-AI approaches to find complex epistatic architectures responsible for all measurable phenotypes as well as an organism’s ability to detect and modulate its microbiome. The result is a comprehensive systems biology model of an organism and how it has adapted to and responds to its abiotic and biotic environment which has applications in bioenergy, precision agriculture, and ecosystem studies.

The first blow is half the battle: collecting and regenerating leafy vegetables

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Collecting and regenerating seed samples are the first two important steps in a process which ultimately should lead to the distribution of these samples to users like research organisations and breeding companies, which use these samples for innovative research and breeding of new cultivars respectively. However how to collect and regenerate in the most effective way has been the subject of many research, but a clear answer to this question is not present yet.

First of all the question how genetic variation is distributed within and among populations is unclear, as most studies focussed on single populations only. Furthermore it has been argued that the focus during collecting should be to sample so-called local common alleles, alleles which are thought to be of adaptive value which have a frequency higher than 5%. However one may question this concept in the face of for example collecting disease resistance genes which is of considerable importance in current leafy vegetable breeding.

Branching process theory has been applied to analyse the efficiency of regenerating seed samples of self- and cross-fertilizing crops. It was shown that bulk regeneration often results in a substantial decrease of genetic variation after a few generations, depending upon allele frequency of traits to be conserved in the seed sample for both breeding systems and additionally for cross-fertilizing species also on the ratio population size and effective population size (N/Ne). It has been shown that the appropriate way to regenerate seed samples and maintaining the genetic variation present in the sample is to regenerate on an individual plant basis for both breeding systems, which results in case of a self-fertilizing crop, like lettuce, in selfing plants from seeds of separately kept seed progenies per generation and in case of a cross-fertilizing crop, like spinach, of carrying out bi-parental crossings.

However the genebank practise is often different and bulk collecting and regeneration mostly takes place. The question how detrimental this practise is for the maintenance of genetic variation in genebank collections has not been answered yet but would be of considerable importance. Therefore it is suggested to initiate research into this area which might be crucial in the face of the currently occurring loss of biodiversity.

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Genetic variability of wild *Lactuca* species germplasm

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In order to access the reservoir of favorable alleles within wild germplasm, knowledge about the genetic variation and the population structure of wild species is needed. We applied different molecular markers (Isozymes, SSR, AFLP, DArT-seq) to study genetic variability of collection of wild lettuce species brought together by authors during the last 25 years, and linked to the knowledge of eco-geographic conditions of original collecting sites, morphological and phenological traits of plants, as well as to their ability to withstand the infection of important races of lettuce downy mildew and lettuce powdery mildew.

Although a low level of genetic diversity and high genetic homogeneity is expected in populations of wild *Lactuca* species, due to predominant selfing, morphological and genotypical differences among individual plants within a single population can be traced. As the most prominent example are natural populations of *L. serriola*, *L. saligna*, and *L. aculeata* in the Near East, the center of diversity of the genus *Lactuca* s. str. where natural populations of these three species are not homogeneous. Elevated diversity (both morphological and genetic) is caused by frequent gene flow among populations and immigration of seeds from neighboring populations. Gene flow occurs even at an interspecific level, as evident from presence of interspecific hybrids. With increasing geographical distance, from the diversity center, diversity of populations (morphological as well as genetic) decrease and populations are becoming less differentiated at the larger geographical area. In contrast, species populations at the edge of its distribution area are highly uniform at the intrapopulation as well as the interpopulation level. Thus, in the autochtonous area, wild lettuce species occurring near the center of their diversity, with suitable ecological conditions, display more genotypic and phenotypic variability. Conversely, at the edge of the distribution area, where less favorable conditions exist, the selection prioritizes stable and well-adapted genotypes.

The three wild lettuce species differ also in their colonizing ability and some species (e.g. *L. aculeata*) have a relatively narrow area of distribution and limited ability for spreading, while others are considered as noxious weeds (e.g. *L. serriola*) distributed over great parts of the world. The investigation of a large sample set of *L. serriola* samples collected across the USA and Canada (area colonized only recently at the end of the 19th century) suggests the recent colonization of the North American sub-continent took place from two regions – northern part of the USA (Oregon, Wyoming, Wisconsin) and Canada (Quebec, Ontario); and western part of the USA (California, Nevada, Arizona), divided by natural barriers of Rocky Mts. and Great Plains. Structure of *L. serriola* populations in Northern America, its low genotypic variation and nearly absent differentiation, is affected by founder events, fast-spreading facilitated by human activities, ecological conditions, and geographical barriers.

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Eco-geography and distribution of wild *Lactuca* species

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Research into the eco-geopraphy, distribution and diversity of wild *Lactuca* species in their natural habitats has been conducted by staff members of the Department of Botany (Palacký University in Olomouc, Czech Republic) and colleagues for almost 25 years. From 1995 – 2018 the distribution of wild *Lactuca* species and the characteristics of plant populations and their natural habitats were studied in approximately 5000 locations in 24 countries in Europe (AUT, BEL, BIH, CHE, CZE, CRO, GBR, GER, GRC, ESP, FRA, HUN, ITA, LICHT, LIT, LUX, NLD, NOR, POL, PRT, SLO, SRB, SVK, SWE), 9 countries in Asia (CHN, GEO, ISR, JOR, KAZ, SKOR, THAI, TUR, USSR), 3 countries in North and South America (CAN, USA, CHL), 2 countries in Africa (EGY, ZAF), and in New Zealand. Species of focus (N = 21) included *L. aculeata, L. biennis, L. canadensis, L. capensis, L. floridana, L. garetti, L. georgica, L. graminifolia, L. indica, L. livida, L. ludoviciana, L. perennis, L. quercina, L. saligna*, cultivated *L. sativa, L. serriola, L. sibirica, L. tatarica, L. tuberosa, L. viminea, L. virosa*), and several undetermined *Lactuca* species as well as members of related genera (e.g. *Mycelis*, *Chondrilla*). In total 7037 seed samples were collected.

Lactuca serriola (5684 samples) was the most frequently observed species, recorded in all five continents. *Lactuca saligna* (876 samples) was recorded in Europe, North America and Asia. *Lactuca virosa* (129 samples) was monitored in Europe and North and South America. These data demonstrate the global spread of these originally Euro-Asian species. *Lactuca viminea* (100 samples) was recorded in Europe and Asia. The distribution of remaining species was restricted to one continent only. North American autochthonous species were recorded only in Canada and the USA, *L. indica* and *L. aculeata* in Asia, and *L. capensis* in South Africa exclusively.

The presence of several species in one location was recorded in Europe (*L. serriola* + *L. saligna*), America (*L. serriola* + *L. virosa*), or in the Near East (*L. serriola* + *L. saligna* + *L. aculeata*). The hybrid origin (natural intraspecific and interspecific hybridization) was proved in some plants from Near East and also from the Czech Republic. Intraspecific variability of morphological traits and phenological characteristics of plants were influenced by specific ecological/climatic conditions, e.g. quality and quantity of indumentation as a response to amount of precipitations and water availability, beginning of bolting, flowering and fruit set in relation to the length of growing season. Such traits are genetically fixed. The majority of plant populations were located within areas of their distribution described by local floras, however in America some allochthonous species were observed in unusual habitats and in high elevations.

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Genetic analysis of complex traits in lettuce

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Lettuce can be divided into several horticultural types based on their architecture. The leaf shapes vary dramatically among horticultural types or even within a horticultural type (such as loose leaf). The prominent leaf morphologies in lettuce include curvature at leaf edges and cup-shape leaves leading to heading. To dissect the heading trait in lettuce, we constructed three segregating populations derived from crosses between a crisphead cultivar and a cultivar from other horticultural types. Heading was shown to be a quantitative trait, controlled by multiple loci in each population. A total of eight loci contributing to the heading trait have been identified. We are constructing sub-families that segregate each individual locus, and use those sub-families to clone the gene through map-based cloning approach. We have cloned the first gene controlling heading in lettuce. It encodes a STM homolog. The gene is activated through an insertion of a transposon in its exon1. The insertion did not abolish its function but upregulated its expression. This gene is required but not sufficient for the heading trait in lettuce. Interestingly, the upregulated STM gene is also responsible for leaf curvature in some loose-leaf cultivars. We also identified candidate genes for the second heading locus and other important traits in lettuce. The genetic analysis of leaf traits in lettuce not only sheds light on leaf development, but also facilitates future breeding for ideal leaf or plant shapes of lettuce. Other traits, such as leaf color, curvature and flowering time are also controlled by several loci. The genetic analysis on these quantitative traits will be discussed. Our genetic analysis will provide useful information for future lettuce breeding programs.

Integrated disease and pest management in lettuce

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Lettuce crops are threatened by a large range of pests (including insects and nematodes) and plant pathogens (including fungi, oomycetes and bacteria). The pressure exerted by these biotic agents depends on the mode of production, the season, and the geographical area. Integrated pest management (IPM) in its modern definition can be seen as a toolbox of technical measures aimed at limiting crop damages by pest and pathogens, while limiting the use of pesticides. A recent societal and regulatory push towards a significant reduction in the use of pesticides is observed worldwide, exemplified by the UE directive 2009/128. Despite this, the preventive use of pesticides is still frequent in many regions of lettuce production to secure the visual quality of the produce and farmers' income.

Some level of genetic control has been achieved for several pests and pathogens in modern lettuce varieties. However, the management of other pests and pathogens cannot rely on genetic control only, either because limited quantitative resistance can be obtained, or because quantitative or qualitative resistance identified in genetic resources has not been introduced in modern varieties.

Based on a recent review of the literature, we address here an overview of techniques available for the management of the main diseases and pests of lettuce. Biocontrol agents, defence inducers, biopesticides, as well as the control of GxE interactions, cultural methods, soil management techniques, greenhouse climate control and prophylaxis can be mobilized to limit pest and pathogen pressure. Several biocontrol agents, which have proved effective in controlling lettuce pathogens in laboratory tests, have not yet been deployed optimally in the field, probably because of insufficient research efforts. In countries where the deployment of GMOs in the field is feasible, host-induced gene silencing relying on RNA interference can be considered as a new opportunity to control pathogens and pests.

We illustrate the description of some of these techniques with a few examples of IPM programs in France and the USA, and show how the implementation of IPM can help to reduce pesticide application. Risk assessment and cost-benefit studies are crucial to persuade farmers to adopt integrated protection methods. Joint work involving scientists and agricultural development agents is needed to produce practical tools and user-friendly decision support systems which will guarantee sustainable lettuce production systems.

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Genetic resources of wild *Lactuca* spp. in North America, complex view on underestimated topic

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In North America, eleven wild and weedy species, eight autochthonous (*L. biennis*, *L. canadensis*, *L. graminifolia*, *L. floridana, L. hirsuta*, *L. ludoviciana*, *L. terrae-novae*, *L. tatarica* subsp. *pulchella*) and three allochthonous (*L. saligna, L. serriola, L. virosa*) represent the genus *Lactuca* (Asteraceae). In addition, the genus comprises cultivated lettuce (*L. sativa*) a highly important vegetable crop. We present pertinent literature on the history of the use of cultivated lettuce and its wild North American relatives, the diversity of wild *Lactuca* taxa and their importance in lettuce breeding, and an overview of the taxonomy of this genus. The characterization, biogeography, distribution, ecology, genepools, and specific examples of phenotypic variation, genetic diversity and disease resistance of these species are summarized from past literature and key results from our own observations and experiments. The exploitation of wild *Lactuca* relatives in lettuce breeding is examined and presented in the broader context of crop improvement. The current status of *in situ* and *ex situ* conservation of wild and weedy North American *Lactuca* is reviewed; gaps and perspectives are illustrated and analyzed. The effective protection, conservation and exploitation of autochthonous North American *Lactuca* species requires comprehensive analyses of their diversity, phylogeny and taxonomy, knowledge of their floristics, biogeography and ecology, a broad sampling of original germplasm of wild *Lactuca* species, including material arising from collecting activities in North America, and evaluations of their environmental adaptation, phenotypic and genetic variation, and disease, pest, and abiotic-stress resistance, to determine their true potential for lettuce improvement. The most recent knowledge of these topics was presented by Lebeda et al. (2019).

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Lettuce powdery mildew - exciting but unknown disease of lettuce

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Lactuca, commonly known as lettuce, is a genus of flowering plants in family Asteraceae. The genus includes at least 100 species, distributed worldwide, but mainly in temperate Eurasia. Cultivated (garden) lettuce (*Lactuca sativa*) with its many varieties is the best-known representative, on the other hand wild-growing relatives (e.g. *L. serriola, L. saligna*, *L. aculeata* etc.) belong among primary and secondary gene-pool of lettuce and are considered as a valuable sources of resistance.

At least representatives of 3 powdery mildew genera can infect *Lactuca* spp.; namely *Golovinomyces, Podosphaera* and *Leveillula*. The most common on *Lactuca* is *Golovinomyces orontii* (long time named as *Golovinomyces cichoracearum* (prev. *Erysiphe cichoracearum*)) (Takamatsu et al., 2013); however recently this species of powdery mildew is described as *Golovinomyces bolayi* with host range including *Cichorium* and *Lactuca* (Braun et al., 2019). Concerning other powdery mildew genera, *Leveillula lactucae-serriolae* and *Leveillula lactucarum* were confirmed on *Lactuca* in Asia and Caucasus (Khodaparast et al., 2010; Lebeda et al., 2019). Rarely there are reports of infections caused by *Podosphaera xanthii* (on *Lactuca sativa* in Korea) and on wild *Lactuca* in Eurasia and North America (Shin et al., 2006).

The more than twenty years of intensive study of pathosystem *Lactuca* spp. - lettuce powdery mildew (*Golovinomyces* cf. *bolayi*) was realized in Department of Botany, Palacký University in Olomouc. The research was focused on collection of samples of *Lactuca* spp. infected by powdery mildew worldwide (Europe, Asia, America) and their taxonomic determination; study of distribution and degree of infection of powdery mildew in populations of wild *Lactuca serriola* in the Czech Republic, and study of pathogenic variability of lettuce powdery mildew isolates from the Czech Republic. During the study there was confirmed the occurrence of *Golovinomyces* cf. *bolayi* on samples of *Lactuca* spp. worldwide, however also *Leveillula lactucae-serriolae* was recorded in Jordan. Expanded was the knowledge about disease distribution and incidence in wild populations of *L. serriola* and last but not least there was confirmed the large virulence variation of *G. bolayi* isolates and was supposed the existence of different races, including their differentiation. The obtained results can help to understand the complexity of relationships in studied pathosystem.

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Variation in wild plant pathosystem *Lactuca* spp. – *Bremia lactucae* and its importance for lettuce resistance breeding

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Extensive research on the wild-plant pathosystem, *Lactuca* spp. (lettuce) - *Bremia lactucae* (lettuce downy mildew), has been conducted in the Czech Republic since the end of the 1970s. Studies were focused on pathogen incidence and distribution, host range, variation in symptom expression and disease severity, and sexual reproduction of *B. lactucae*, as well as on interactions of *B. lactucae* with different wild *Lactuca* spp., the structure and dynamics of virulence within populations of *B. lactucae,* comparison of virulence structure and changes in *B. lactucae* populations occurring in wild (*L. serriola*) and crop (*L. sativa*) pathosystems, variation in resistance within and between natural populations of *Lactuca* spp., and location of sources of resistance for lettuce breeding because *B. lactucae* from wild pathosystems could be dangerous for *L. sativa*.

Populations of *L. serriola* from the Czech Republic, Germany, the Netherlands and the United Kingdom were generally highly susceptible to *B. lactucae* under *in-vitro* tests. However, large variation in *L. serriola* resistance was found both among and within individual countries. There was a clear gradient of increasing uniformity of race-specificity moving from central to western Europe, as well as a slight decrease in the diversity of R-phenotypes. Populations in the United Kingdom were the most different in terms of resistance structure from other geographic regions, and also were the most homogeneous, most likely as a consequence of the relatively greater degree of spatial isolation from other regions.

Temporal changes in virulence frequencies over the period were recorded in *B. lactucae* populations on *L. serriola*. In many v-factors (v11, v14, v16, and v25–28), fluctuations were observed, some (v14 and v17) shifting to higher frequencies, and others (v5/8 and v23) decreasing. The occurrence of mating B1 and B2 was recorded in *B. lactucae* populations in the Czech Republic bearing a higher potential of adaptation to changing resistance structures. Many of the *Dm* resistance genes in cultivated lettuce originate from *L. serriola*, but often a fast overcoming of the resistance in the crop by new downy mildew races was observed.

Recent phylogenetic studies suggested that *B. lactucae* might be restricted only to cultivated *L. sativa* and wild growing *L. serriola*. Moreover, population structure of two *B. lactucae* populations from *L. serriola* and *L. sativa*, respectively, have been investigated. While the pathogen population from *L. serriola* was characterised by fast dispersal with a balanced selection pattern, the pathogen population from *L. sativa* was characterised by fast dispersal as well, but also by a directional selection pattern indicating founder effects most likely caused by host jumps and resulting in a fast radiation and colonization of susceptible lettuce cultivars. Thus, it seems likely that since cultivation of lettuce not a parallel co-evolution of both pathosystems occurred, but rather multiple host jumps cause the periodical occurrence of downy mildew epidemics in lettuce production based on the overcoming of race-specific resistance genes introduced from *L. serriola*.

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Lettuce lines with potyvirus resistance: differential set for study of strains and new genitors of resistance

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Lettuce mosaic virus (LMV; genus *Potyvirus*) is distributed worldwide. This potyvirus could be destructive for lettuce crops and some strains are seed-transmitted. Two resistance alleles of the *mo1* gene were identifed: *mo1*^{1} from Gallega de Invierno was used in European breeding and *mo1*^{2} from PI 251245, a wild Egyptian lettuce, was used in breeding in the USA. Among different LMV isolates collected in Europe, both alleles conferred a partial resistance and untransmissibility by seeds to the common isolate LMV-0, and were overcome by isolates LMV-E and LMV-13. However, these genes showed a differential reaction to isolates LMV-1 and LMV-9, since only *mo1*^{2} was resistant to isolates LMV-1 and LMV-9. To study new LMV isolates or new potyviruses, the set of differential hosts includes butterhead genotypes for susceptibility and for *mo1*^{1} and iceberg genotypes for *mo1*^{2}. The growth of these lettuce types could be different; moreover iceberg lettuce is more difficult for inoculation and symptom observation due to the leaf structure and head shape. Besides that material, a new resistance gene (*Mo3*), efficient against all LMV isolates, has been identified in *L. virosa* PIVT1398 and introgressed into butterhead-type lettuce. Projects were developed to create a set of near-isogenic butterhead lettuce lines useful to characterize LMV and potyvirus pathotypes.

To created near-isogenic lines with *mo1*, a backcrossing programme was used for introducing the *mo1*^{1} gene from the butterhead variety Mantilia or the *mo1*^{2} gene from the iceberg variety Salinas88 into LMV-susceptible Girelle and Mariska butterhead cultivars. Between backcrosses (BC), resistance tests were made on F_2 plants with LMV-0 for *mo1*^{1} screening and LMV-9 for *mo1*^{2}. With this method, four lines with butterhead phenotypes were obtained after four or five BC for *mo1*^{1} and eight BC for *mo1*^{2}; these lines are named Girellemo1^1, Girelle-mo1^2, Mariska-mo1^1 and Mariska-mo1^2. That material could be useful for research on new LMV isolates or new potyviruses.

The *Mo3* was introgressed from *L. virosa* into two butterhead lettuce by backcrosses after interspecific F_1 hybrids (Maisonneuve et al, 2018. Plant Pathol). The screenings for *Mo3* were made by tests of resistance to LMV-13, LMV-0 or LMV-9. The two near-isogenic lines created, named Girelle-Mo3 and Mantilia-Mo3, were very useful to demonstrate the efficiency of *Mo3* against a new potyvirus present in South-East France: lettuce Italian necrotic virus (LINV). Some physiological disorders (necrotic streaks on stem or corky stem basis), resulting probably from interspecific crosses, were still observed in bolting and flowering despite seven or nine BC by lettuce cultivars. Nevertheless these lines could be interesting for research and as genitors for breeding.

All $mo1^{+}$, $mo1^{1}$, $mo1^{2}$ and *Mo3* near-isogenic lines are released for research laboratories.

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Current view on phylogeny and taxonomy of the subtribe Lactucinae

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The Asteraceae family – one of the largest plant families comprises about 10-12% of flowering plants (25.000-33.000 species). The family evolved ca. 41-50 mya (Funk et al. 2009), but many of the recognized tribes are younger and experienced rapid radiation and speciation only recently (e.g. Tremetsberger et al. 2013; Kilian et al. 2017). This radiation was facilitated by frequent hybridization and backcrossing and accompanied by subsequent isolation. Contrasting to the family species richness only around 40 species were domesticated for various purposes. Omitting the taxa used as ornamentals (*Ageratum* spp., *Aster* spp., Daisies, Dahlias, Knapweeds, etc.), or important for the production of various chemicals (guayule, safflower, dandelion) only a handful species are important for the food industry (sunflower, Jerusalem artichoke, artichoke). The three economically highly important species are however from the tribe Cichorieae: lettuce (*Lactuca sativa*), endive (*Cichorium endivia*) and chicory (*Cichorium intybus*). Although superficially similar these two genera represent only distantly related lineages classified as subtribes Lactucinae and Cichoriinae. The subtribe Lactucinae with around 200 accommodated species has mainly Northern Hemisphere-Northern African distribution. The estimated time of origin of the tribe Cichorieae is 26 mya (Oligocene) and the North African–Mediterranean region was inferred as the most plausible place of origin of the tribe (Tremetsberger et al. 2013). The two largest subtribes, accommodating the most species diversity Lactucinae and Crepidinae, are not clearly delimited what is caused by early and rapid diversification and area expansion during the Middle and Late Miocene leading to parallel character evolution creating a number of homoplasious characters. The delimitation of diversified lineages into genera is further complicated by reticulation (hybridization) of the early diversifying lineages (e.g. Wang et al. 2013; Kilian et al. 2017). Thus the recent, rapid and reticulated history of the subtribe Lactucinae (Lactuca alliance) hampers an unambiguous generic classification. In past different taxonomic approaches were used to deal with the richness of the subtribe, which ranged from a broad definition of genera to narrow concepts across intermediate. However, no one was widely adopted by the academic and wide public (e.g. in local Floras). In recent decade number of molecular studies provides new data calling for the reevaluation of the relationships within the Lactucinae subtribe (e.g. Wei et al. 2016; Wang et al. 2013; Kilian et al. 2017; etc). These show that the various classifications of the alliance, which is used presently, is not natural and frequently treated morphologically similar but evolutionary distantly related taxa are hampered together into heterogeneous groups which are rather paraphyletic. The remaining question is how to accommodate and translate the new findings into practical and sustainable taxonomy of the *Lactuca* alliance.

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Revealing functions of downy mildew effectors in spinach and lettuce

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Downy mildews are the most prominent diseases of the leafy greens lettuce and spinach. These crops are at severe risk because of constantly evolving races of downy mildew that break newly introduced resistances at a high pace. Monogenic dominant resistance is generally mediated by specific recognition of pathogen effector proteins. We have compared the genomes of denominated races of the spinach downy mildew *Peronospora effuse* and identified candidate effector genes that are mutated in resistance-breaking isolates and are currently being validated experimentally. From the lettuce downy mildew *Bremia lactucae*, we have identified six effectors that are specifically in selected wild or cultivated *Lactuca* lines. Recognition of effector BLR38 by *L. serriola* LS102 is particularly interesting, as it is mediated by two unlinked loci.

Pathogens have not evolved effectors to get recognized by host plants. Instead, their primary function is in pathogen virulence, e.g. as suppressors of plant immune responses. We used 43 *B. lactucae* effectors to search for possible plant targets by using the yeast two-hybrid system. A total of 61unique protein-protein interactions were identified involving 21 *B. lactucae* effectors and 46 lettuce proteins. Strikingly, four *B. lactucae* effectors target a lettuce tail-anchored NAC transcription factor, LsNAC069. We propose that the effectors have evolved to keep the NAC protein in the ER membrane to avoid it from moving to the nucleus where it could activate plant immune responses.

Effector research can reveal mechanisms of resistance gene function and have the potential to uncover new genetic sources of resistance. On the other hand, their use to find effector targets greatly aids in identifying important plant genes in immune regulation and disease susceptibility. These targets are, therefore, important leads for quantitative resistance that are hopefully more durable.

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Next-generation genomics of lettuce

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We have developed and are applying genomics approaches to the analysis of lettuce and *Bremia lactucae* that causes downy mildew. We have deployed a variety of genomic tools to analyze multiple lines of lettuce and isolates of *B. lactucae* in order to generate annotated chromosome-scale genome assemblies for multiple individuals of both host and pathogen. The advent of genome editing provides a powerful addition to the toolbox for crop improvement. It is now possible to knock-out many genes and technologies for allele replacements and gene additions are advancing rapidly; however, a looming constraint will be having causal genes to edit. We have been characterizing resistance genes in lettuce and effector genes in *B. lactucae* with the goal of knowledge driven deployment of resistance genes in order to provide durable disease resistance. To implement this we are developing and using genome editing technologies to identify disease resistance genes and ultimately to generate stacks of resistance genes that will represent a large evolutionary hurdle for the pathogen to overcome in order to become

Discovering traits of commercial interest in diversity collections

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The leafy vegetable category has developed over the last 30 years from one of a limited range of wholehead products sold loose with seasonal availability to a dynamic sector driven by innovation with all year round availability. Customers have moved up the value chain from loose wholehead products to prepacked multi-ingredient mixes. Breeding of new and novel lines has been a key factor in enabling this progress. However, growers are also under pressure to maintain or improve crop production whilst using fewer plant protection products and coping with more and pest and disease (biotic stress) resistance traits are a key focus. In addition to biotic stress, abiotic stress resilience is also a trait of interest enabling commercial production to be maintained in a more variable growing environment.

This presentation will discuss the use of diversity collections at the UK Vegetable Genebank based at University of Warwick and will give examples from the Defra funded Vegetable Genetic Improvement Network (VeGIN) covering both biotic and abiotic stress resistance traits and consumer traits funded by the BBSRC Horticulture and Potato Initiative (HAPI).

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Genetic variation and improvement of nutritional traits in lettuce and spinach

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Lettuce (*Lactuca sativa*) is the most important vegetable crop produced for fresh market in the United States in terms of acreage, production, and market value. Crisphead lettuce has much lower contents of vitamins and minerals than leaf and romaine lettuces. Our research suggests that the lower nutritional value of crisphead lettuce is due to the enclosure of its leaves in the head structure. Spinach (*Spinacia oleracea*) is called a “superfood” due to its high contents of beta-carotene (pro vitamin A), lutein, folate, vitamin C, calcium, iron, phosphorous, and potassium. Spinach is also known to have a large amount of oxalic acid, which may combine with minerals to form insoluble oxalate crystals, thus reducing the bioavailability and absorption of minerals, and depositing in the kidneys of certain people as a common form of kidney stone. Efforts by public health organizations and the produce industry to increase the consumption of fruits and vegetables have met limited success due to dietary habits and economic or cultural reasons. Elevating the nutritional levels of vegetables would, potentially, increase nutrient intake without requiring an increase in consumption. A breeding program to enhance the nutritional quality of lettuce and spinach must start with an assessment of the existing genetic variation. We found significant variation among and within different types of lettuce and spinach in concentrations of vitamins, minerals, and oxalic acid, which is also influenced by the environment and the moisture content of the plant. Beta-carotene, lutein, and chlorophyll concentrations are highly correlated in lettuce, suggesting that carotenoid content could be selected indirectly through chlorophyll or color evaluation. We are conducting research on molecular marker assisted selection and breeding to improve the nutritional value of lettuce and spinach.

Marker-assisted selection in lettuce breeding

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Cultivated lettuce is a self-fertilizing diploid ($2n = 2x = 18$ chromosomes) species in the family of Compositae (Asteraceae). Development of elite lettuce cultivars is a lengthy process that involves manual cross-pollination, several rounds of selection, development of homozygous lines, and performance testing of advanced lines. Molecular markers linked to genes of interest enable more rapid and, frequently also for more accurate, selection of desirable genotypes than phenotype-based selection. This marker-assisted selection (MAS) is used by lettuce-breeding programs for identification of genotypes with the favorable combination of genes. Assays have been developed to detect genes and alleles for race specific resistances to downy mildew, Verticillium wilt race 1, Fusarium wilt race 2, corky root, lettuce mosaic, lettuce dieback, and the rate of deterioration of fresh cut lettuce. Genes associated with resistances to turnip mosaic, root downy mildew, powdery mildew, big-vein, Fusarium wilt, anthracnose, and tipburn, bolting, and other economically important traits have been mapped on the molecular linkage map of lettuce and can be used for development of MAS assays. Sequencing of lettuce genome paved the road to more rapid mapping, cloning, and functional validation of genes and for more efficient development of molecular marker-assays used in MAS of lettuce.

Trends in lettuce and spinach production in the United States

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Lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) production in the United States is concentrated mostly in California and Arizona, where both leafy vegetables are grown year-round. Lettuce cultivars are divided into eight horticultural types on the basis of the shape and size of the head; the shape, size, and texture of leaves; stem length; and seed size. U.S. lettuce products are categorized into three market uses: whole heads, bulk harvest (for salad processing, food service, or value-added products), and baby leaf or spring mix. The three main lettuce types produced in the United States are iceberg, romaine, and leaf. Yields of all lettuce types continue to increase. For example, in California from 1992 through 2017, summer production yield of iceberg increased 32% in Salinas Valley, and winter production yield of iceberg in Imperial Valley increased 71%. During this same period, yield of romaine and leaf types increased 28% in Salinas Valley but remained essentially unchanged in Imperial Valley. Area planted to iceberg decreased 40% and romaine increased 500% from 1992 to 2017 in Salinas Valley, and similar trends have occurred in the winter production areas of California and Arizona.

The U.S. is the 2nd largest spinach producer in the world, with more than 26,000 ha planted and 330,000 metric tons harvested annually, with a worth of $423M. About 85% of the crop is produced in California and Arizona. Spinach yield has steadily increased over the years, largely due to the use of disease-resistant hybrid cultivars and high seeding rates on wide beds. A new downy mildew race appears every 20 months, on average. It is especially challenging for organic spinach growers without effective organic fungicides. At least 40% of the area planted to spinach in California is organic. Spinach is mostly consumed as bagged salad in the United States. Fresh spinach consumption in the U.S. has not fully recovered from the *Escherichia coli* outbreak in 2006, now at 0.7 kg per person annually. Per capita spinach use in the U.S. is highest among Asians and women 40 years and older.

J. G. Mendel – from Moravian peasant´s son to world genius

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Gregor Johann Mendel has left an indelible mark on the history of science. His work together with Darwin's evolutionary theory established the basis of today's biology. Mendel was born on 20th of July 1822 at the small village Heinzendorf bei Odrau (then in the Silesian part of Moravia within the Austrian empire, now Hynčice u Vražného in northern Moravia in the Czech Republic) and was baptized Johann. He was involved in tending the farm and field work from early childhood. He went to the primary school at his native village and later to the Piaristic school at Lipník. He then attended the Grammar School (today's Mendel's gymnasium) at Opava, followed by studies (1840-1843) at the Philosophical Institute (today's faculty of Philosophy of the Palacký University) at Olomouc where the Department of Natural History and Agriculture was headed by Johann Karl Nestler who conducted research of hereditary traits of plants and animals. In 1844 he joined the St. Augustine order, famous for its support of education and science, at the Moravian capital Brno. He studied at the University of Vienna (1851 to 1853), attending the courses of the famous physicist Christian Doppler, mathematician Andreas von Ettingshausen and botanist Franz Unger which worked on transmission of variable traits from mother to daughter plants through seeds. Apart from acquiring knowledge in mathematics and physics, Mendel also received training in methodology of conducting experiments, which he used in the careful planning and design of his work. There are reasons to view that his initial interests concerned crop improvement but over time became more interested in fundamental questions about inheritance, fertilization, and natural hybridization. Around 1854 he started experiments to answer a long standing question of hybridization which culminated in famous "Versuche über Plfanzen-Hybriden" ("Experiments in Plant Hybrids") presented in 1865 and published year later. To describe biological phenomena by a mathematical model, such as the famous ($A + 2Aa + a$), was absolutely new in science, but became the basic principle of modern scientific research in any natural science. After the *Pisum* studies, Mendel worked mainly on *Hieracium* where he found constant hybrids. Recently, it was shown that it is incorrect to view Mendel's *Hieracium* experiments as intended to verify his findings on pea. He clearly recognized two contrasting types of hybrid (constant and variable) and he chose to study both. Mendel’s interest in hybrids (both inter- and intraspecific) was broadly based and encompassed the mechanism of their formation, inheritance in general, as well as the consequences of hybridization for evolution.

Wild *Lactuca* species and their specialized metabolites

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Lettuce (*Lactuca sativa* L.), one of the most popular leafy vegetables, is present on the market in a wide variety of cultivars, which differ in their taste, color, texture, pathogen resistance and value as a functional food. This diversity is strictly connected with an array of specialized metabolites produced by the plants. In a search for new varieties of lettuce, meeting consumer's demands and expectations, knowledge on wild relatives of the garden lettuce, which can provide a pool of genetic resources for the cultivated species, is of importance.

The most extensively studied specialized metabolites of the garden lettuce are phenolics, especially flavonoids and hydroxycinnamates, which are believed to carry some health benefits. Another group of widely investigated compounds produced by the plant are terpenoids, including carotenoids, pentacyclic triterpenes and sesquiterpene lactones. The latter ones are responsible for the bitter taste of lettuce and chicory as well as, to some extent, for inhibition of insect feeding. Pharmacological studies proved, that some sesquiterpene lactones isolated from lettuce and chicory plants, demonstrated anti-inflammatory and antinociceptive activity. Recently, some reports concerning lignan presence in cultivated lettuce plants and their possible health-beneficial effects were also published.

About 20% of known wild *Lactuca* species has been phytochemically investigated, so far, including 18 species which have been analyzed in our Department. Plants from almost all of the studied taxons produced sesquiterpene lactones, biologically active compounds which are chemosystematic markers in the tribe Cichorieae of the Asteraceae. Only two species – *L. tuberosa* Jacq. and *L. undulata* Ledeb. (section Micranthae) – did not accumulate the sesquiterpenoids in their tissues. Caffeic acid derivatives, which demonstrated antioxidative, chemopreventive and antiviral activity, remained less investigated constituents of the wild lettuces. Interestingly, the phytochemical research on *Lactuca* sp. brought about some new findings concerning lignans biosynthesized by *Lactuca* sp. The group of specialized plant metabolites seems to be of importance as an estrogenic component of human diet.

Majority of wild lettuces, which constitute genetic resource for *Lactuca sativa* L and could possibly enrich the plant with some disease resistance related or health-enhancing features, remain unexplored. This gives us a great opportunity to discover new compounds and explain their function either in plant or in humans. On the other hand, the research done in the area of specialized metabolites of wild lettuces has already revealed evidences of their biological activity and open the way for the improvement of the important vegetable crop.

ECPGR Working Group on Leafy Vegetables: Prospects and Challenges for Phase X

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The European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative programme among most of the European countries that aim at contributing to rationally and effectively conserve *ex situ* and *in situ* Plant Genetic Resources for Food and Agriculture (PGRFA), provide access to them and increase their utilization (http://www.ecpgr.cgiar.org). The Programme operates through 23 Working Groups dealing with groups of crops or with general themes related to plant genetic resources. One of them is the Leafy Vegetables Working Group (WG).

The Leafy vegetables WG, as all others WGs, is composed of experts nominated by the National Coordinators. WG members carry out activities based on specific ECPGR objectives that are defined for a four-years-period. In 2019 ECPGR entered into Phase X (2019-2023). The objectives were revised and address the following issues: i) to efficiently conserve and provide access to unique germplasm in Europe through AEGIS and the European Collection; ii) to provide passport and phenotypic information of actively conserved European PGRFA diversity *ex situ* and *in situ* through the EURISCO catalogue; iii) to improve *in situ* conservation and use of crop wild relatives; iv) to promote on-farm conservation and management of European PGRFA diversity and v) to promote use of PGRFA.

The main achievements of the Leafy Vegetables WG in Phase IX will be presented and the planned implementation of the ECPGR objectives for Phase X will be outlined.

Slovene autochthonous lettuce variety ‘Ljubljanska ledenka’: genetic diverstiy and reintroduction to the market

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‘Ljubljanska ledenka’ is an old Slovenian lettuce (*Lactuca sativa* L.) variety that has been grown and consumed in Slovenia for centuries. It originates from the region of Ljubljana and was later spread to other countries in Europe, Austria and Czech Republic. It is belongs to the group of Crisphead lettuce. The original population is of batavia type with leaf edge that has more or less expressed red hew. ‘Ljubljanska ledenka’ is considered as an ‘umbrella variety’ and some of the derived varieties are included in the Common Catalogue of Vegetable Varieties of the EU (CCVV).

The aim of the study was to elucidate genetic diversity of the traditional lettuce landrace. A set of 51 accessions, including a number of accessions with the name ‘Ljubljanska ledenka’ and synonyms obtained from the Slovene Plant Genebank (SPGB) and from the genebanks abroad (Germany, the Netherlands, Czech Republic, USA) were characterized by morphological, phenological and DNA markers and screened for resistance to 12 races of *Bremia lactucae*.

High phenotypic variation was observed among the accessions for 26 morphological traits from the UPOV descriptor list. While all tested accessions showed close genetic relatedness as revealed by AFLP analysis it was possible to differentiate recent accessions from Slovenian collection against the old accessions from foreign gene banks on phenotypic level. The most striking difference was observed for anthocyanin coloration of leaf edge. ‘Ljubljanska ledenka’ that is cultivated nowadays in Slovenia lost its characteristic reddish leaf edge, but the rest of the traits have very been much preserved over the decades. Most of the accessions were highly susceptible to all tested races of *B. lactucae*; 12 accessions expressed full susceptibility, but in some interactions clear race-specific reaction patterns appeared. Expression of race-specificity was not uniform across the set of accessions and at least 11 different reaction patterns were recorded, meaning that different race-specific resistance factors or genes could be expected.

The results of the study were applied for reintroduction of ‘Ljubljanska ledenka’; in 2015 the variety was inscribed in the CCVV.

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Metabolite variation in *Lactuca*

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Many key crop traits are known to be directly or indirectly related to plant metabolite composition. To identify promising genetic resources for the improvement of the phytochemical composition of lettuce varieties, metabolite variation in the genus *Lactuca* was studied. Phytochemical variation was investigated by untargeted metabolic profiling using 150 *Lactuca* gene bank accessions, including both cultivated and wild germplasm of the lettuce gene pool. Hierarchical clustering of the variation in 2026 phytochemicals was largely in line with the known lettuce gene pool structure, indicating the genetic basis of the observed variation. Data on phytochemical variation were related to traits associated with plant health or nutritional value. In addition to the untargeted approach, the study materials were also investigated for variation in vitamin C levels, which were found to be relatively high in the more primitive lettuces and the closely related wild relatives. It was concluded that the genus *Lactuca* harbours ample phytochemical variation to improve nutritional quality and sustainable production in lettuce.

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Rocket Science - a journey from field to genotype

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Salad rocket (*Eruca sativa*) is an important leafy vegetable crop globally, but is under-domesticated in terms of developing consistent traits for taste and flavour. Chemical compounds within leaves called isothiocyanates (ITCs) are responsible for creating the distinct hot and peppery flavour of leaves. They are also known to have a range of positive impacts on promoting healthy human physiology and preventing non-communicable diseases. Their formation is very unpredictable, and differs according to environmental conditions and underlying genetic variability of plants. Equally, human perception of some of the key aspects of taste and flavour is genetically controlled, leading to a complex array of factors that contribute to the final eating experience.

In this paper, we show that rocket biochemistry is impacted by its journey through a commercial supply chain, but sometimes in a positive way. We demonstrated that taste and flavour are highly dependent upon ITC abundance and relative amounts of free sugars and that consumers of rocket can be characterised in three distinct groups of preferences. We have developed a range of genetic resources for rocket and, through these, obtained transcriptomic insights into the expression of genes associated with relevant biochemical pathways. This strategy will speed up the production of sensorially enhanced varieties, and at the same time provide health benefits for the end consumer.

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Abstracts

Posters

(in alphabetical order according the first author)

Development of molecular markers to explore population dynamics of *Bremia lactucae* (lettuce downy mildew)

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Bremia lactucae (Regel), the obligate biotrophic oomycete that causes downy mildew in lettuce, causes significant yield losses worldwide. Most lettuce programs are actively breeding for resistance to *B. lactucae*, but the variability of *B. lactucae* makes creating cultivars with durable resistance to *B. lactucae* challenging. We continually collect and phenotype isolates of *B. lactucae* to monitor changes in virulence in the Western US.; however, this method of characterization is too labor intensive and slow to enable interventions in real time. We are currently developing molecular markers to genotype individual lesions from the field prior to culturing and phenotyping. Polymorphic simple sequence repeats (SSRs) have been identified using the largest scaffolds of the reference genome of SF5 and whole genome sequences from diverse isolates. Dye labeled, multiplex PCR is being used to quickly genotype isolates. We have so far characterized over 40 unique genotypes and are currently implementing a workflow to genotype numerous lesions within a week and then to selectively phenotype representative isolates. This will enable the characterization of large numbers of isolates and understanding of the diversity of populations within and between fields. Oligonucleotide primers for PCR amplification of these markers will be made available as a standard set for analysis of isolates worldwide.

Cultivation of leafy plants *Lamiaceae* family at the multi circle hydroponic construction: study on the biochemical composition and some innovations

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The progress of the Russian Federation in the twenty first century is provided with innovations in many fields and in vegetable production too. Regular supplying people with fresh vegetables can be due to new technologies, such as the multi circle hydroponic technology (MHT) on narrow benches. MHT is a new modular technology of vertical farming, which gives an opportunity to use vertical area of the greenhouse and to save energy, water and nutrient resources. It is very important for Northern areas, where many Russian people are living now. We obtained new tomato varieties for MHT, using sporophyte selection and target hybridization. And we would like to extend the number of plants for the cultivation on the multi circle hydroponic construction. Leafy vegetables are available for that purpose very well.

Plants of *Lamiaceae* family are well known as aromatic and medicinal plants with high content of essential oils. Monoterpenes and its derivatives present the main group of healthy substances in essential oils with antioxidant activity. Researches of Kaliningrad Botanical Garden show that the above part of *Monarda fistulosa* plant contain 5.52 mg/g of antioxidants phenolic nature. It allows use this plant as a base for the functional nutrition. Leafy parts the plants of *Lamiaceae* family may be used as aromatic and healthy additions to the traditional foods, such as salads, soups and sauces. So, we can regard the plants of *Lamiaceae* family as leafy vegetables too.

Investigation the biochemical composition of *Monarda fistulosa* L. have been cultivated with multi circle hydroponic technology show, that it wasn't change significantly depends on the circle of construction. But the time of leaves cutting is important for the content of biologically active compounds. The total antioxidant content in the leaves of the first cutting was significantly higher, than in leaves of the second and next cuttings. So, we can to recommend the leaves of *Monarda fistulosa* L. plants of the first cutting for the healthy additions in the scheme of the functional nutrition. At the second part of investigation we study the influence plant secondary metabolites steroid glycosides (SG) on the mass of leafy part the plants of *Lamiaceae* family. Seed treatment with water solutions of SG changed significantly the weight of leaves: it was increased in *Monarda citriodora* Cerv. ex Lag. and in *Melissa officinalis* L., but it was decreased in *Monarda fistulosa* L..

Using vegetable crop genetic resources to identify tolerance to abiotic stress and other key performance traits

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Changing climatic conditions are placing constraints on agricultural production and so breeding to provide new environmentally-durable varieties with increased tolerance of abiotic stresses and low nutrient availability is required. The development of pre-breeding genetic resources such as the Vegetable Genetic Improvement Network (VeGIN) Diversity Fixed Foundation Sets (DFFSs) and mapping populations provides the opportunity to identify new sources of genetic material for downstream breeding programmes. We have phenotyped the response of lines of the VeGIN vegetable brassica (*Brassica oleracea)* populations to a range of single and combined abiotic stresses (Brassica). Significant ($P \leq 0.05$) response variation was found across the populations for drought, waterlogging and salinity stress and tolerant lines highlighted. Abiotic stress assays are now being used to phenotype VeGIN lettuce (*Lactuca sativa*) populations and are being adapted for the screening of carrot (*Daucus sativa*) and onion (*Allium* spp.) populations. Future studies will expand the investigation of responses to multiple simultaneous stresses. Such populations have also been used to investigate a range of traits including establishment, post-harvest discolouration, tipburn resilience and pathogen resistance. The results are promising for the future development of more robust vegetable varieties.

New insights into the characterization and taxonomy of wild *Lactuca* relatives

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In recent years we initiated extensive studies on the characterization of the population structure of wild *Lactuca* relatives (WLRs) originating from their center of origin and diversity in Southwest Asia. Our studies have been based mainly on new collections of *Lactuca serriola*, *Lactuca aculeat*a, *Lactuca georgica*, and *Lactuca altaica* (representing the primary Lettuce Gene Pool, LGP-1) and *Lactuca saligna* (LGP-2) from Israel and Armenia. A comprehensive studies are ongoing to determine the most suitable taxonomic position and crossing potential of the critical mass of collected samples with the crop, based on: (i) selected morphological and phenological characteristics, (2) molecular data, and (3) quality and quantity of sesquiterpene lactones content. This presentation will highlight the major advances in knowledge on these themes.

Recently, we completed a collection of *L. aculeata* samples from 46 locations, representing its ecogeographic distribution in northeastern Israel. Our collection is likely the largest and most diverse collection of this important WLR. From regeneration of 185 original collected plants, only 7 (0.27 %) out of 2630 progenies were identified as hybrids *L. aculeata* × *L. serriola*. The meaningful of this observation will be discussed.

During regeneration of unique germplasm that were collected in Armenia, we have meantime identified 19 samples, which we believe could be genuine *L. altaica*. In some morphological traits they were more similar to *L. saligna* plants, but in some other traits more similar to *L.* serriola. A comparative phytochemical study of nine sesquiterpene lactones was recently performed, based on 7 out of the 19 seed samples mentioned.

The higher genetic distance values obtained between our *L. georgica* samples and cultivated Lettuce (*Lactuca sativa*) samples, based on molecular data compared with certain samples of wild *Lactuca* spp., and initial hybridization experiments (personal communication) question the placement of *L. georgica* in the LGP-1. The importance of looking deeply on the raw molecular data as well as the computed genetic distance values will be discussed.

Our observations question also the commonly-accepted "fact" regarding the definition of *L. georgica* as an absolutely biennial or perennial plant. The chance to collect in nature seed samples of *Lactuca scarioloides*, *Lactuca azerbaijanica*, and *Lactuca dregeana*, in order to complete a germplasm representing all wild lettuces that constitute the LGP-1 will be discussed.

Acknowledgements
Described results have obtained by various levels of national and international collaborations. I'll acknowledge them during the presentation.

Application of Marker Assisted Selection in lettuce (*Lactuca sativa L.*) breeding programs to rapidly asses selfing and crossing rates in experimental F1 progenies

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Cultivated lettuce (*Lactuca sativa* L.) is a diploid autogamous species with 2n= 2x= 18 chromosomes and is one of the major crops of the group of leafy vegetables.

Conventional breeding is commonly carried out with crossing, selfing and pedigree selection. Hybridization between different parental pure lines to produce new lettuce varieties, is a process frequently used and it is achieved using hand-made emasculation and pollination protocols.

Traditional emasculation technique consists of washing anthers with water in the early hours of the morning, then manual pollination is performed by rubbing the anthers of the male parent on the stigma of the female parent. This breeding approach is time-consuming and technically demanding, moreover the rate of hybridization success is empirically estimated by counting the seeds obtained from each manual-pollinated flower. Therefore, morphological evaluation is a necessary step to detected F1 and self-pollinated derived plants and it can be extremely difficult when the two parents are phenotypically very similar.

The aim of this work was to develop a panel of molecular markers to rapidly identify F1 plants and self -pollinated plants derived from hand-made hybridization. The molecular markers were developed for High Resolution Melting (HRM) analysis system, a simple, fast and high- throughput technique. The panel of markers was composed by neutral markers, developed from the analyses of a high number of SNPs generated by a genotyping by sequencing (GBS) approach of 141 cultivars belonging to different typologies of L. sativa, as well by markers derived from bibliography associated to disease resistances.

DNAs from about 70 parental lines and from more than 1000 plants derived from manual crosses were extracted and analysed by means of HRM with our molecular markers panel. The analysis allowed the detection of true- crosses and self -pollinated plants rapidly, so it can be adopted to increase efficiency in lettuce breeding programs, moreover it provides, in a short time, a number of information not always retrievable just by morphological analysis.

Culinary Herbs: determining the basis of variation in flavour

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The consumption of culinary herbs has increased due to the campaign of salt reduction, resulting in food manufacturers and home cooks seeking alternative ways to enhance flavour. Because of this it is important to understand what the chemical profile of the herbs is and how and why it varies, since the flavour is the key attribute of these plants.

For this project three herbs were selected: Rosemary (*Rosmarinus officinallis*), Coriander (*Coriandrum sativum var* Cruiser) and Basil (*Ocimum basilicum* var Sweet Genovese). For each of these herbs, different types of production were analysed (pot grown, soil grown under polytunnel protection, outside field grown and hydroponic production under glass) and also different locations in the UK. Fresh samples were used for the head-space volatiles analysis and this was done using solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Relative abundance was calculated using an internal standard and statistical analysis was done to determine the significance of variation in volatiles compounds attributed to production method, location of production and season.

Complex and diverse aroma volatile profiles were observed for each of the herbs. Rosemary was the species with the most aroma volatile compounds detected, followed by basil and then coriander, however, basil was the one with the most common compounds across all samples. There were significant differences between the different sample origins for all the herbs that were attributed to the different types of production, pot produced herbs were significantly different form the other types of production. In some cases, significant differences were associated with geographical location however, other variables might be causing these differences, such as type of soil, irrigation, fertilizers.

In summary, there are many variables involved in the production of herbs, consequently influencing the chemical profile. Further analysis needs to be done, in order to have a clear understanding of how pre-harvest variables influence the aroma volatile content and how this relates to consumer perception.

Biodiversity and ecogeography of wild *Lactuca* species in Hungary

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Hungary is a very diverse country from a geomorphological and climatic point of view, can be divided into six main areas according to the prevailing conditions on them, and can be characterized as a country with a transitional climate. In climate maps, it is possible to see a gradual transition from oceanic climate to continental. In the Pannonian area, we find one significant border of two vegetation strips. It is a boundary between wet and semi-arid areas, ie deciduous forests and forest-steppes. Forest-steppes have more Mediterranean than continental character. When comparing temperature and precipitation, the climate is similar to that of the Black Sea region. According to the literature (Flora of Hungary), we can find *L. perennis* L., *L. viminea* (L.) J. et C. PRESL., *L. quercina* L., *L. saligna* L., *L. serriola* L. and *L sativa* L. in Hungary.

The main aim of our study was to examine biodiversity and ecogeography of genus *Lactuca* in Hungary. Data were obtained during field expeditions realized by the staff of the Department of Botany of Palacky University in Olomouc in the period 1999 to 2017 (232 locations were processed). The primary goal was to describe the surface of Hungary from a geographical and climatic point of view, applicate collected data into maps and analyse if it correlated with literature data.

The occurrence of four species of the genus *Lactuca* was recorded. Namely *L. perennis L.*, *L. viminea* (L.) J. et C. PRESL, *L. saligna* L. and *L. serriola* L. The basic geographic and ecologic characteristics and attributes of the locations where the *Lactuca* representatives were found in Hungary were processed. The most common species, *L. serriola* f. *serriola*, were recorded on 183 locations, most occurring along roads and in urban areas. A single specimen was found on sand dunes. These findings which were obtained confirms the literature, that this species is a commonly occurring plant in Hungary. Among the observed *L. serriola*, only form *serriola* was found. The form *integrifolia* has not been found, which is confirmed by the fact that the main area of its occurrence is southwestern Europe. However, it is possible that this form may occur in Hungary, for example in the Great Danube Plain around watercourses, where environmental conditions can meet its demands for a warmer environment with plenty of moisture. The average altitude of all localities of *L. serriola* f. *serriola* was 178 m.

L. saligna was recorded in 47 localities, found were var. *saligna*, var. *saligna* s.l., var. *runcinata* and some samples were not further determined. Samples were found in the Great Danube Plain and the North Hungarian Highlands with an average altitude of 169 m above sea level. The typical habitat of this plant is also roadsides and urbanized areas. *L perennis* was observed only once, on Pillis Mountain in 1999 in the Transdanubia Central Mountains. This knowledge agrees with the information in Flora of Hungary. Since then, this finding of *L. perennis* has not been confirmed in this area.

The results from individual habitats support the previously obtained information on the characteristics and the distribution of individual species of the *Lactuca* genus in Hungary. In the next phase of this work, we would to monitor higher number of localities on the larger territory of Hungary.

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Evaluation of the existence of a new race of *Bremia lactucae* in Turkey

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According to Turkish statistics of 2018, 487.543 tons of Lettuce with various varieties is produced in fields, green houses and under plastic tunnels. Downy mildew occurs sporadically everywhere where lettuce is grown in the fields in Turkey. In order to grow and breed resistant varieties, a project work was started to identify the races of *Bremia lactucae* in Turkey, since there is no information about the races of lettuce downy mildew. For this aim, surveys of lettuce growing areas in Ankara were undertaken during growing seasons and disease samples were collected from fields grown Toscanas and Cartagenas lettuce varieties in Beypazarı, Ankara, in 2018. Samples were stored at -20 °C and -80 °C. In order to find races of two isolates (TR-1201 and BC-1), they were inoculated to IBEB differential cultivars. The inoculated series of petri plates and seedlings of the differentials were kept in darkness for 24 hours and then with a 12h photoperiod in the climate chamber at 15-17 °C until sporulation. Reactions of the differentials were evaluated based on IBEB scale, the final scoring being 14 days after inoculation. Sextet codes calculated for two isolates did not match any of the present races listed by IBEB which could be a new race.

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Nutritional characterization of mineral elements and total protein content in the leaf tissues of *Amaranthus*

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Amaranthus is an underutilised and neglected leafy vegetable widely grown in tropical and sub-tropical regions of the world. It is one of an orphan crop that have been reported to be rich in macro and micro-nutrients as well as protein contents and hence able to contribute significant quantities to human diets. Extensive nutritional phenotyping of *Amaranthus* species will enable the identification of breeding lines that are rich in specific nutritional traits. This will then facilitate the identification and selection of candidate parents with desired nutritional profiles for *Amaranthus* nutritional quality breeding.

Hence, it is important to profile the nutritional traits in the leaves of *amaranthus* to enable the selection for breeding. The study was conducted at the Roodeplaat research farm of the Agricultural Research Council (ARC) in South Africa.

The analysis of variance showed significant differences for all mineral elements indicating that wide genetic differences existed among the test genotypes. The concentration of mineral elements (mg 100 g^{-1}) determined were in the ranges Na (54.90-519.780), K (257.00-4089.46), Mg (1304.55-2468.67), Ca (2051.340-3875.59), Mn (4.55-13.96), Fe (63.57-681.28), Cu (0.89-2.10), Zn (3.61-7.49), P (460.80-765.80), and Al (149.54-976.65). The protein content varied from 17.10 to 28.97%. A high level of heritability coupled with the genetic advance was obtained for most of the traits, which would result in successful advancement and crosses for those nutritional traits in the breeding programme towards utilization of underutilized plant species for biofortification for food, nutritional and health security.

Acknowledgements

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Selection of cowpea genotypes [*Vigna unguiculata* (L.) Walp.] for nutritional compositon in the fresh leaves for nutritional quality breeding

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Cowpea is indigenous to the African continent and is grown for its leaves, immature green pods and grain in different countries of the world. The objective of this study was to assess the genetic variability of nutritional composition in the fresh leaves of selected cowpea genotypes grown across South Africa. Twenty-five cowpea genotypes were evaluated for two cropping seasons at the field condition and the young leaf samples were collected for nutritional analysis.

The analysis of variance for the combined mean values showed substantial variation in the nutritional composition evaluated among the tested genotypes. The mean values of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn varied from 2.23 to 3.69 mg kg^{-1}; 6.96 to 14.15 mg kg^{-1}; 311.30 to 1049.95 mg kg^{-1}; 1.13 to 1.74 mg kg^{-1}; 0.36 to 0.70 mg kg^{-1}; 130.08 to 186.42 mg kg^{-1}; 126.62 to 307.87 mg kg^{-1}; 0.27 to 0.39 mg kg^{-1}; and 27.76 to 43.55 mg kg^{-1}, respectively. The total protein content varied from 21.39 to 33.45%. The correlation analysis revealed significant degree of trait associations nutritional compositions. The genetic biometrical analysis revealed that the phenotypic variances were higher than the genotypic variances. High values of heritability estimates was also observed for most of the evaluated nutritional traits. The principal component analysis showed that the first three principal components contributed 71.93% of total variation among the entire nutritional traits for the test genotypes. The study therefore, showed that there is ample genetic differences existed that can be exploited for use in breeding for nutritional quality in cowpea leaves.

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Prickly lettuce in Czech herbals from the first half of 20th century

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Prickly lettuce (*Lactuca serriola* L.) is morphologically very variable species, and on the base of the character of cauline leaves, two forms are distinguished, i.e. *L. serriola* f. *serriola* (divided cauline leaves), *L. serriola* f. *integrifolia* (entire cauline leaves). Leaf form (divided *vs* entire) is regarded as a plant adaptation to specific ecological and climatic conditions on different areas, and this trait is genetically fixed. Only plants with divided leaves were observed by members of the Department of Botany (Palacký University in Olomouc) during field trips organized in the Czech Republic since 1980.

The main aim of our study was to examine the leaf form of prickly lettuce in Czech Lands in 19th and first half of 20th centuries, and to track species eco-geographic distribution. Our study was focused on historical documentation of prickly lettuce in living herbals from the territory of Czech Lands. The set of 260 herbarium items from 14 herbarium collections in the Czech Republic covering period of 1853 – 2014 has been analysed. Main goal of this study was to verify the taxonomic status of plants and to perform the intraspecific determination of items representing *L. serriola*.

From the set of 260 items there were 239 from the the territory of recent Czech Republic, remaining items originated from another countries or their original location could not be determined. Three items were re-determined (*Senecio* sp., *Sonchus* sp., member of Poaceae), 8 items were represented by species *Lactuca viminea* L., *Lactuca saligna* L., *Lactuca perennis* L., *Lactuca quercina* L., *Lactuca sativa* L. a *Lactuca virosa* L. Out of 231 items of *L. serriola* from the Czech Republic 86 items were acquired in the years 1853 – 1950, and 145 items between years 1951 and 2014.

Analysed plants *L. serriola* were collected from altitude of 150 - 610 m a.s.l., what corresponds to literature data on species distribution in Europe. Similarly data on habitats, available for 149 items, are typical for prickly lettuce (e.g. along roads, in ruderal sites). Only one original habitat (district of Třebíč), i.e. wet margin of the forest, was unusual.

In the set of items obtained in 1853 - 1950, 70 items were determined as *L. serriola* f. *serriola*, one item as *L. serriola* f. *integrifolia* and for 12 items the leaf form could not be determined. Between items from 1951 - 2014, 126 items were determined as *L. serriola* f. *serriola*, 3 items as *L. serriola* f. *integrifolia* and 13 items *L. serriola* L. were not determined on subspecific level.

Even this relatively small number of four items of *L. serriola* with entire leaves is significant. Further field research and study of herbarium items will continue to study this phenomenon.

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Defining, predicting and delivering 'fresh' in the context of leafy salads

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Current methods of predicting shelf-life in fresh vegetables and salad are dubious, evidenced by the proportion of wastage in this category; fresh vegetables and salad account for almost a quarter of all avoidable food waste by weight. Confusion between types of date marking is often cited as reasons why food is thrown away. Date markings are often very conservative or tenuous in the in which they are derived.

The initial phase of the project has been focused on defining post-harvest changes that occur during domestic refrigerated storage post-retail and benchmarking these empirical data from observations made within the pack. Physiological, biological and microbiological measurements are being taken throughout different growing seasons and regions from July 2018 through to July 2021, for Iceberg lettuce (IL), Rocket and Spinach. For the first trial of IL, which was carried out on pre-processed ready-to-eat bags, grown during July of 2018 in the UK. Fifteen bags were analysed on days: 1, 3, 7, 11, 14 and 21 post-processing from samples stored at 4 °C. On each sampling day the leaves were removed from their packaging and images were captured for colour analysis, the samples were then frozen and freeze-dried. Freeze-dried samples were analysed for sugar, organic acid, nitrite, nitrate, ammonia, chlorophyll and total carotenoid content. Of all the variables measured, visible discolouration in the form of development of pink and brown pigments and nitrate were the only ones to show any significant differences over the trial period. Nitrate content decreased by 65% ($p < 10^{-6}$) and discolouration significantly increased by 176% ($p = 2.7 \times 10^{-10}$). In the case of Nitrates there were significant differences after three days, whereas with discolouration, significant differences were only observed after seven days, which is after its stated end-of-life.

The other variables did not give any indication of a change in quality, which is contrary to what was expected based on the literature. Future experiments, conducted at different points in the growing season and different countries, may demonstrate changes in some of the other parameters, as in the case of the July 2018 trial the growing conditions were optimal when this crop was produced.

High-throughput phenotyping of drought stress responses of lettuce genotypes using the optical imaging techniques

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High throughput phenotyping enables to test a high number of genetic resources and it is very well designed for assess the variable morphological forms and interesting nutritional health-promoting substances of lettuce (*Lactuca sativa* L.). A high number of different forms of lettuce pose a challenge in selecting suitable genotypes for cultivation. The classical destructive analysis of plant material is slow, and time-consuming and often expensive. Currently, modern approaches based on the optical signals from plants provide opportunities for fast characterization and selection of appropriate biological material. In our study, the adaptive responses twelve different genotypes of lettuce plants to progressive drought were studied in a pot experiment at environmentally controlled conditions using the PlantScreenTM phenotyping platform in the AgroBioTech Research Center of the Slovak University of Agriculture in Nitra, Slovakia. Genotypes profiling was complemented with the measuring of water content and biochemical compounds (chlorophylls, carotenoids, anthocyanins, polyphenols and proline content). A high-throughput phenotyping method based on automated digital image analysis of fluorescence, RGB and hyperspectral signals was used under whole vegetation period to accurately measure the growth and physiological and nutritional responses of lettuce genotypes to water shortage. Non-invasive automatic RGB and VNIR hyperspectral imaging of plants allowed specifically divided genotypes based on leaf coloring into three groups (light green, dark green and red leaves). Morphometrically analysis shows the water shortage resulted into reduction of plant growth measured as a planar plant area with significant genotype differences. On a physiological level, plant water stress has been accompanied by a pronounced reduction of leaf water content and proline accumulation the maximal and actual photochemical efficiency of PSII has been observed higher in red lettuce genotypes. This observation was confirmed by the evaluation level of the photochemical reflectance index. Utilization scanning of optical

signals from plants has been potential for discriminating lettuce cultivars with different colors and physiological traits and can be successfully used as a basic automatic genotype selection.

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Morphological and genetic variation of lettuce from Egypt

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Egypt is considered as secondary place of origin of cultivated lettuce (*Lactuca sativa* L.). The diversity of local lettuce landraces and the richness of wild *Lactuca* species are not adequately known, described and explored. This is the first more detailed study focused on lettuce material originating from Egypt.

A set of 48 accessions representing thirty-five lettuce samples from Egypt (*L. sativa* Cos type and/or Oilseed type, *L. serriola*), three germplasm accessions of *L. sativa* Cos type from Gene Bank in the Great Britain, one commercial variety of *L. sativa* Cos-type, one commercial variety of *L. sativa* Butterhead type, six *L. serriola* samples from Europe and Near East, and an outgroup of *L. virosa* and *L. saligna* (one sample each) was cultivated in the greenhouse of Palacký University in Olomouc in 2018. Nineteen morphological traits and three developmental characteristics were assessed continuously during the whole vegetation period. Taxonomic status of samples and *L. sativa* morphotypes were verified. Genetic analyses are based on 713 AFLP markers generated by twelve selective primer combinations.

Neighbor-Joining clustering revealed, beside an outgroup of *L. virosa* and *L. saligna* samples, two main clusters were observed: large clade consisted of three well defined clusters (A, B, C) representing exclusively samples of cultivated lettuce and separate cluster D containing exclusively *L. serriola* samples. Cluster A was represented by 18 samples of *L. sativa* from Egypt, from this number 14 samples had traits of Oilseed type, and remaining 4 samples were classified, according to the morphological traits as Cos type. Cluster B consisted of 16 *L. sativa* Cos type samples from Egypt. Plants bore traits of less advanced landraces, and did not form heads under long day. Three germplasm accessions *L. sativa* Cos type from Great Britain and two commercial varieties (i.e. Cos type „Valmaine“ and Butterhead type “Lednický”) fell in cluster C. All these samples represent more advanced cultivars, exhibiting tendency to form heads even under the condition of long day. Position of seven *L. serriola* samples in the cluster D is linked to their geographic origin (Europe – Near East – Africa).

With the exception of the four Cos type samples in the cluster A, morphological traits, taxonomic classification and data on geographic origin of samples were in a good relation to the results of molecular analyses.

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Lettuce lines with potyvirus resistance: differential set for study of strains and new genitors of resistance

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Lettuce mosaic virus (LMV; genus *Potyvirus*) is distributed worldwide. This potyvirus could be destructive for lettuce crops and some strains are seed-transmitted. Two resistance alleles of the *mo1* gene were identifed: *mo1*^{1} from Gallega de Invierno was used in European breeding and *mo1*^{2} from PI 251245, a wild Egyptian lettuce, was used in breeding in the USA. Among different LMV isolates collected in Europe, both alleles conferred a partial resistance and untransmissibility by seeds to the common isolate LMV-0, and were overcome by isolates LMV-E and LMV-13. However, these genes showed a differential reaction to isolates LMV-1 and LMV-9, since only *mo1*^{2} was resistant to isolates LMV-1 and LMV-9. To study new LMV isolates or new potyviruses, the set of differential hosts includes butterhead genotypes for susceptibility and for *mo1*^{1} and iceberg genotypes for *mo1*^{2}. The growth of these lettuce types could be different; moreover iceberg lettuce is more difficult for inoculation and symptom observation due to the leaf structure and head shape. Besides that material, a new resistance gene (*Mo3*), efficient against all LMV isolates, has been identified in *L. virosa* PIVT1398 and introgressed into butterhead-type lettuce. Projects were developed to create a set of near-isogenic butterhead lettuce lines useful to characterize LMV and potyvirus pathotypes.

To created near-isogenic lines with *mo1*, a backcrossing programme was used for introducing the *mo1*^{1} gene from the butterhead variety Mantilia or the *mo1*^{2} gene from the iceberg variety Salinas88 into LMV-susceptible Girelle and Mariska butterhead cultivars. Between backcrosses (BC), resistance tests were made on F_2 plants with LMV-0 for *mo1*^{1} screening and LMV-9 for *mo1*^{2}. With this method, four lines with butterhead phenotypes were obtained after four or five BC for *mo1*^{1} and eight BC for *mo1*^{2}; these lines are named Girellemo1^1, Girelle-mo1^2, Mariska-mo1^1 and Mariska-mo1^2. That material could be useful for research on new LMV isolates or new potyviruses.

The *Mo3* was introgressed from *L. virosa* into two butterhead lettuce by backcrosses after interspecific F_1 hybrids (Maisonneuve et al, 2018. Plant Pathol). The screenings for *Mo3* were made by tests of resistance to LMV-13, LMV-0 or LMV-9. The two near-isogenic lines created, named Girelle-Mo3 and Mantilia-Mo3, were very useful to demonstrate the efficiency of *Mo3* against a new potyvirus present in South-East France: lettuce Italian necrotic virus (LINV). Some physiological disorders (necrotic streaks on stem or corky stem basis), resulting probably from interspecific crosses, were still observed in bolting and flowering despite seven or nine BC by lettuce cultivars. Nevertheless these lines could be interesting for research and as genitors for breeding. All *mo1*$^{+}$, *mo1*^{1}, *mo1*^{2} and *Mo3* near-isogenic lines are released for research laboratories.

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Validation of differentials for new *Fusarium oxysporum* f. sp. *lactucae* race 4 (Fol: 4) - Lettuce

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Fusarium oxysporum f. sp. *lactucae* is used as a DUS criteria for registration and protection of varieties, for its race 1. A new race of *Fusarium oxysporum* f. sp. *lactucae* race 4 (Fol: 4), was described, and several varieties were used to differentiate it from the previously identified races (Giraldi et al, 2016). Therefore, a new table for differentials has been proposed at the ISF DRT Working group for publication. But the variety Patriot, used as differential and known to be susceptible to races 1, 2 and 3 of Fol had not been described in the publication of Giraldi and was tested by one of the ISF Disease Resistance Terminology working group (ISF DRT WG) participants and found to not be clearly susceptible. This study was planned at ISF DRT group to validate by an interlaboratory test, the newly identified differentials and race for use in resistance claims. The interlaboratory test was coordinated by GEVES.

A collection of several isolates from the four races of Fol has been built up. Isolates were tested on the four differentials described by at least 3 participants. The test was run according to UPOV guidelines and based on a harmonized test performed at IBEB. Results were consistent among laboratories for Fol: 1 but were very variable between isolates and laboratories for the other three races. The variety Patriot expected susceptible to all races was observed resistant to two isolates whereas other differentials as Costa Rica or Romabella were observed susceptible.

Based on these results, ISF DRT Working group decided that the differentials table was not ready to publish, and more research was needed for describing Fol: 2, 3, 4 races for resistance claims. ISF decided to organize a new ring test. Isolates giving results closer to the expected results have been selected for a second interlaboratory test. Panel were made up of the same controls than in first ring test and run in the same form as the first one with same UPOV guidelines and same conditions. Results of these 2 ring tests for characterization of races and definition of differentials will be presented.

Measuring sugars in a mapping population of salad rocket (*Eruca sativa*) at two different locations

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Rocket leaves are ready-to-eat salads (RTE) and are increasing in popularity, with 40 million bags consumed in the UK annually. There are two common species of rocket that are commercially cultivated, wild rocket (*Diplotaxis tenuifolia*) and salad rocket (*Eruca sativa*). Although rocket has many important phytochemicals such as glucosinolates, flavanols, vitamins and minerals that are thought to benefit human health, due to its hot, pungent flavour rocket is not widely accepted by all consumers. Sugars play an important role in determining taste and flavour as they can mask the bitterness and pungency of rocket.

The aim of this study was to quantify the abundance of different types of sugars in a mapping population of salad rocket (*Eruca sativa*) grown in two different locations; Italy and the UK.

Sugar analysis was conducted on a mapping population developed in collaboration with Elsoms Seeds Ltd. (Spalding, UK). Field trials on 142 lines of the F6 generation were carried out in two different locations, Italy and the UK, to evaluate the impact of different environmental conditions on sugar concentrations. Sugars analysis was carried out using Agilent 1260 infinity II HPLC system, equipped with a quaternary pump, degasser, auto-sampler, thermostat controlled Column Compartment and IR detector. A Bio-Rad Aminex column with a guard column was used to achieve separation with an isocratic gradient of 10 mM sulfuric acid. Samples were quantified using standards and analysed with Agilent ChemStation software.

We originally hypothesised that sugar concentration would be higher in the Italian trial, compared to the UK one, whereas we found an opposite trend in our results. The UK grown trial showed two-fold higher average concentrations of sugars compared to the Italy grown trial across the mapping population (UK average =80.23 mg. gDW^{-1}; Italy average =39.56 mg. gDW^{-1}). However, a 6-fold variation was observed across all the lines within the Italian trial (range from 11.5 to 67.3 mg. gDW^{-1}) as compared to the UK grown trial which showed a 2.5 fold variation (range from 53 to 132 mg. gDW^{-1}) across the lines within the mapping population.

It is widely known that sweetness reduces consumer perception of bitterness in foods. Results from the present study will help to identify genetic markers for sugar. By combining this knowledge with genetic and chemical information, it will be possible to breed rocket for increased consumer acceptance, while maintaining the maximum health benefits associated with the crop.

Effects of some nitrogen fertilizers on growth parameters and nitrate accumulatio in lettuce (*Lactuca sativa* L.)

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Inorganic and organic fertilizers are applied to maintain the nutritional condition of different cropping systems. Nitrogen is an essential element for plant growth and development , vegetable crops have main role in healthy and diet of people but it accumulates large quantities of nitrate in its leaves, due to the environmental pollution, that threatened public health.

For the reason an experiment was accomplished to assess the effect of some nitogen fertilizers on growth and yield properties especially nitrate accumulation of lettuce. The experiment was conducted in open field; it was carried out using three varieties of lettuce. The treatment applied comprised two nitrogen fertilizers (Ammonium nitrate, Urea) with a control. The data collected included plant fresh weight, plant dry weight, leaves chlorophyll fluorescence, plant height and nitrate content.

Results indicated significant differences in growth parameters amongst treatments compared to control. Head fresh and dry weight in cultivars grown with the nitrogen fertilizers differed. All cultivars fertilized with urea had relatively higher average number of plant weight and height and they accumulate more nitrate than cultivars treated with ammonium nitrate. By comparison, nitrate concentration showed a great variability between varieties. Furthermore, nitrate distribution and chlorophyll fluorescence on the leaves are closely related.

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Breeding lettuce for resistance to *Impatiens necrotic spot virus*, Verticillium wilt, and tipburn in California

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The lettuce breeding program at the USDA-ARS in the Salinas Valley, California focuses on development of breeding lines with improved resistance to a wide array of soilborne and foliar fungal and bacterial pathogens, insect vectored and soilborne viruses, several abiotic stresses, and crop quality. Presented here is an overview of the research on resistances to *Impatiens necrotic spot virus* (INSV), Verticillium wilt, and tipburn. INSV has become more widespread and severe in the Salinas Valley in recent years, associated with large populations of its thrips vector. Initial symptoms of the thrips-vectored orthotospovirus, INSV, appear as small brown lesions or spots that expand into larger necrotic sections on infected leaves. The wide range of host species that can be infected by INSV complicates disease management, but there is evidence of host plant resistance to INSV in lettuce. *Verticillium dahliae* poses a serious, long-term threat to lettuce production in Salinas and Pajaro Valleys; the pathogen is long lived in soil. Two races of the pathogen are currently known with a possible third race being investigated. Symptoms of Verticillium wilt on lettuce, include lower leaf wilting and yellowing, dark green discoloration along the vascular bundles in the taproot, formation of microsclerotia and shrinking and collapse of the mature head. The disease is most destructive on iceberg lettuce, where total yield losses may occur. Methyl bromide soil fumigation is effective for control, but is no longer an option due to restrictions on its use related to atmospheric ozone depletion. Tipburn is a physiological defect manifested as necrotic or "burned" areas on leaf margins on spring and summer harvested lettuce. Tipburn can occur on all lettuce types, typically on inner leaves within enclosed heads where the damage is not readily observed. Some companies reject entire fields when tipburn incidence is >5%, and lettuce processed into salad requires even higher stringencies. Tipburn occurs during a narrow period of time immediately preceding harvest and is induced by factors that either decrease transpiration from the leaf surface or promote rapid growth. Lettuce plants symptomatic for INSV, Verticillium wilt, and tipburn are not marketable; high incidence results, therefore, in substantial economic losses to growers in the Central Coast region of California. Our long-term objective is to develop lettuce germplasm resistant to these stressors. Results of preliminary efforts are presented.

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Identifying lettuce for phosphorus use efficiency

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In the United States, lettuce (*Lactuca sativa* L.) is a 3-billion-dollar business annually. The crop represents 80 million dollars for field growers at the Everglades Agricultural Area (EAA) in Florida. Lettuce is also cultivated in hydroponics, aquaponics, and vertical farms, an emerging industry throughout the state. The increase of fertilizer applications for crop production and environmental concerns in runoffs into water canals, especially for Phosphorus (P), creates the need to enhance P use efficiency in lettuce. The identification of P efficient cultivars could minimize P applications and reduce costs of lettuce cultivation in the EAA and in protected environments, benefiting farmers and stimulating a more sustainable production. The objective of this study was to identify lettuce cultivars adaptive to low P inputs. In 2018 and 2019, we tested 64 cultivars, breeding lines, and plant introductions (PIs) of romaine, iceberg, butterhead, leaf and a group of unique cultivars in field conditions under three different phosphorus rates: total recommended dose (224 kg/ha), half dose (122 kg/ha), and no P application (0 kg/ha). Yield and other horticultural traits were measured at harvest maturity. Tissue and soil samples were collected to determine P content following protocols for total P and Mehlich 3 (M3). In 2018, we identified 5 romaine, 4 iceberg, 4 butterhead, 3 leaf, and 1 Latin type varieties that presented high and similar yield under total and half P rates. Cultivars 'Okeechobee' and 'Honcho II' performed similarly under half and total doses of P in both years and are good candidates for genetic studies. Cultivars 'Coyote', 'Beacon', and 'Cooper' had the highest yield in 2019 with half P dose. The greatest tissue P content was observed in PI '342440' and breeding line '70096' with half P dose in 2018 and 2019, respectively, possibly indicating greater P uptake efficiency. Overall, the extractable M3 phosphorus was positively correlated ($P<0.05$) to the total soil P content, and a weak yet significant correlation ($P<0.05$) was found between M3-P and yield. An additional experiment will be conducted in the 2019-20 season to confirm these results. Cultivars selected will be used to develop mapping populations for further genetics studies of lettuce adaptive to low P inputs.

A future-proof differential set for *Peronospora effusa* in spinach

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Downy mildew (*Peronospora effusa*) is an important disease of spinach. In the past 18 years, the International Working Group for Peronospora in Spinach (IWGP), administered by Plantum in Gouda, The Netherlands, has characterized many new isolates of this pathogen on a set of differential spinach lines and hybrids. The reaction phenotypes of the differentials are recorded in an annual inventory of the new isolates. Reaction phenotype patterns associated with a significant breakdown of resistance, which are found in more than one spinach growing region and in more than one year may be selected by IWGP as candidates for a ringtest and potential denomination of a new race. Reference isolates with a stable performance in multi-lab ringtest are needed to define a new race. After careful evaluation of the inventory list and stability tests, the IWGP will denominate a new race and announce the existence of the new race in a press release simultaneously in the US and the EU. Importantly, only after the publication date of the press release, the new race can be mentioned in seed catalogues and in other market communication.

The IWGP has taken responsibility for the production of seed of differentials needed for isolate evaluations. Naktuinbouw has distributed the seed to IWGP members and other stakeholders on request. Over the years, the differential set has gradually increased in size, reflecting the increasing number of resistance factors that are used by breeders. Recently, the differential set was revamped by replacing six F1 hybrids by six near-isogenic lines (NILs) with the same, presumably single-gene resistances. The new NIL differentials were validated by verifying the expected reaction phenotypes with all reference isolates. More NIL differentials will become available in the near future to replace (most of) the remaining F1 differentials.

The collaborative effort of IWGP to improve the differential set by replacing F1 hybrids with NILs creates a strong, future-proof foundation for defining the potentially changing breeding targets that spinach breeders pursue, and for advancing genetic theory.

ISF guidelines on the nomination of novel plant pest races

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Disease resistance is a major goal in breeding new varieties and plays a key role in vegetable crop production and integrated pest management practices. It is also carefully described to differentiate new varieties from older ones on the market. The objective of the Working Group on Disease Resistance Terminology at International Seed Federation is to promote the consistent use of terminology in relation to disease resistance.

The Working Group codes pathogens for which companies claim resistance in their varieties, promotes harmonized terminology across the industry to avoid any liability due to miscommunication, develops host differentials, and establishes procedures based on peer-reviewed scientific publication and industry practices to identify pathogen races/strains.

To support the decision process associated with the nomination of novel races, the Working Group has formulated recommended guidelines. The recommendations constitute the minimum criteria that should be fulfilled in order to nominate a novel race for a specific plant pest. One of the most important selection criteria is the relative economic importance of the emerging resistance breaking event. The pest should have caused significant economic damage at least once, the geographical extent of this event should be of significance, and the event should be recurrent in time having been observed over multiple growing seasons and/or years. The guidelines include basic rules for nomination and numbering of novel races. The nomination criteria and examples of numbering and differentials will be presented.

https://www.worldseed.org/wp-content/uploads/2018/07/Guidelines-on-race-nomination_18.06.2018_FINAL_edited.pdf

Identifying changes in the flavour profile *Apium graveolens* harvested at different maturities

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Apium graveolens, or celery, is a biennial crop belonging to the Apiaceae family. It is consumed globally and used as a staple ingredient throughout cooking for its strong distinct flavours; forming the basis of many soups, stocks and sauces as well as eaten raw in salads. A range of volatile compounds including terpenes, phthalides and alcohols all contribute differently to the characteristic aroma and consequently, the flavour of celery. The project aims to assess the development of the flavour profile of celery over time, allowing for flavour compounds that are key contributors to the typical celery aroma to be identified.

Using 11 parental celery lines, sampling was completed 2 weeks before the maturity harvest date, on the harvest date and 2 weeks after the harvest date. Solid-Phase MicroExtraction Gas Chromatography Mass Spectrometry was used to analyse the volatile profile in the headspace of each extract. More than 40 volatile compounds were identified in the headspace of celery extract including sedanenolide, γ-terpinene, limonene and hexanal in varying abundances. Volatile compounds known as phthalides including sedanenolide and 3-*n*-butylphthalide are character impact compounds contributing to the distinctive celery flavour and bitter tasting notes.

Completion of further analysis, including assessing the changes in sugars (glucose, fructose, sucrose and mannitol) and phenolic acid (chlorogenic acid and apigenin) contents over time using liquid chromatography techniques respectively, will help identify changes in taste characteristics as maturity develops.

Carrying out ANOVA, followed by Tukey's HSD on aroma compounds and their abundancies in the three timeframes, identified a significant difference *(P=<0.05)* between phthalides and terpene compounds as maturity increases and that some parental lines change more significantly than others. Furthermore, using principle component analysis, identified significant relationships between compounds, particularly between phthalides, monoterpenes and sesquiterpenes.

Genetic analysis of cadmium tolerance and accumulation in lettuce (*Lactuca sativa L.*)

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Cadmium (Cd) is a metal pollutant that accumulates in cultivated soils. It has detrimental consequences in terms of food safety. Among the vegetable species used for food, lettuce (*Lactuca sativa* L.) has a high capacity to accumulate Cd in its tissues.

Our objectives are to characterize mechanisms controlling cadmium accumulation in this species and identify the genetic determinants underlying these mechanisms, ultimately aiming at breeding lettuce for cadmium under-accumulation.

This work analyzed cadmium tolerance and accumulation in lettuce at both the physiological and genetic levels. These traits were evaluated in 18 *Lactuca* accessions representing a large genetic diversity. Cd tolerance and accumulation in roots and shoots as well as Cd translocation from roots to the shoot varied independently and in a large range. Analyses of F_1 progenies of crosses between cultivars with contrasted phenotypes showed that high tolerance to Cd, low Cd accumulation and low root-to-shoot Cd translocation were recessive traits. Analyzes of F_2 progenies suggested that root Cd concentration and root-to-shoot Cd translocation were under a complex genetic determinism. This work thus revealed that limit both Cd accumulation and root-to-shoot Cd translocation in lettuce is possible and depends on recessive loci. Differences in the ability to accumulate Cd in roots in the long term could not be linked to differences in short-term ^{109}Cd uptake into, or efflux from roots. In contrast, the root-to-shoot Cd translocation varied similarly in the short and long terms between selected cultivars. Our perspectives are to identify the genes controlling cadmium

accumulation combining a “QTL mapping” and “candidate gene” approach, and we could appreciate to collaborate on these topics.

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Chlorophyll fluorescence imaging in assessment of the drought stress tolerance in lettuce parental lines

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Chlorophyll fluorescence imaging is considered to be very promising tool for screening the vitality and the photosynthetic functions of the plants exposed to various environmental conditions. It enables to recognize the spatial heterogeneity as well as the severity of the effects. In our study, we tested opportunity to apply CFI to recognize drought sensitivity in lettuce genotypes. Eight genetically distinct parental lines of cultivated lettuce (*Lactuca sativa* L.) and one drought resistant wild lettuce (*Lactuca serriola* L.) were cultivated in a growth chamber under limited/non-limited water supply. At the end of the experiment, plants were exposed to severe drought stress by withholding of irrigation for 3 more days. CFI was recorded regularly in light exposed plants at the actinic light intensity set on the ambient level. Depending on genotypes, total dry mass in drought stressed plants decreased by 20-50% compared to control; the relative plant dry mass decrease (DMD) was used as a measure of drought sensitivity of genotypes. CFI analyses have shown a significant decrease in the apparent electron transport rate, ETR, in all genotypes (having the same trend as the efficient quantum yield, Φ_{PSII}). However, contrary to expectations, the moderate drought stress led to negligible decrease or even a slight increase of ETR, which did not correspond to the observed decrease of photosynthetic performance. It indicates that the electron transport was efficiently re-directed to alternative energy-consuming pathways, such as photorespiration and others. On the other hand, we observed a significant decrease of steady-state fluorescence intensity (F_s), both in moderate and severe drought. The steady-state fluorescence signal (F_s) reflected well the effects of water deficit on the photosynthetic apparatus better than parameter ETR, which is often referred as the most useful parameter for assessment of the photosynthetic functions. This is an important information emphasizing the need of testing the techniques in individual crops and different stress scenarios.

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