



International Conference on Biodiversity and Molecular Plant Breeding

02.-06.10.2022.



International Conference on Biodiversity and Molecular Plant Breeding

2nd – 6th October 2022.

Novigrad, Croatia

Programme and Book of Abstracts

Editors:

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Europska unija
Zajedno do fondova EU



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Operativni program
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Projekt je sufinancirala Europska unija iz Europskog fonda za regionalni razvoj.

Sadržaj materijala isključiva je odgovornost

Znanstvenog centra izvrsnosti za bioraznolikost i molekularno oplemenjivanje bilja (ZCI CroP-BioDiv).



Welcome note

Dear colleagues,

On behalf of the Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia, we are pleased to invite you to the International Conference on Biodiversity and Molecular Plant Breeding. The conference will take place in Novigrad, Croatia, from 2 to 6 October 2022.

The conference will be an excellent opportunity to meet with researchers from all over the world who are working on different aspects of analysis, conservation and use of plant diversity in plant breeding programmes and different approaches in the development and implementation of new technologies in molecular plant breeding.

The Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv) is a research-oriented network designed to provide both knowledge and results in direct support of agricultural research progress. The research team consists of scientists from three faculties (University of Zagreb, Faculty of Agriculture; J. J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek; University of Zagreb, Faculty of Science) and three institutes (Institute of Agriculture and Tourism Poreč, Agricultural Institute Osijek, Institute for Adriatic Crops and Karst Reclamation Split) and thus includes the leading scientists from all higher education institutions as well as all research institutes in the field of agriculture in Croatia.

The objectives of the CoE include:

- Increasing the benefits derived from the use of plant genetic resources for food and agriculture,
- Identifying important plant traits through field trials and laboratory analysis and optimising protocols for phenotyping,
- Optimising genotyping protocols by introducing common standardized lab analysis procedures, and (d) introducing new statistical methods. The research is conducted on eight model species representing the most economically important and the most promising crops in Croatia: Maize, Wheat, Soybean, Grapevine, Olive, Brassicas/Alliums, Common bean and Dalmatian pyrethrum/Salvias.

The main objective of the conference is to promote new approaches to plant diversity conservation and molecular plant breeding by providing participants with the opportunity for a stimulating and inspiring exchange of knowledge, skills and experiences.

We look forward to seeing you in Novigrad!



International Conference on Biodiversity and Molecular Plant Breeding

02.- 06.10.2022.



ORGANIZER



CroP-BioDiv

ZNANSTVENI CENTAR IZVRSNOSTI ZA BIORAZNOLIKOST I MOLEKULARNO OPLEMENJIVANJE BILJA
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Programme

Monday, October 3th 2022

REGISTRATION	9:30-11:00		
OPENING	11:00 - 11:10		
	11:10-11:30	Zlatko Šatović	Centre of Excellence for Biodiversity and Molecular Plant Breeding
	AUTHORS		TITLE
	11:30 - 12:00	Rina Kamenetsky-Goldstein	Garlic (<i>Allium sativum</i> L.) breeding and crop improvement: recent achievements and outlook
COFFEE BREAK	12:00-12:15		
<i>Session1</i>	AUTHORS		TITLE
Moderator: Z. Liber	12:15-12:30	Gabriela Vuletin Selak, Mira Radunić, Josip Tadić, Maja Jukić Špika, Mirella Žanetić, Marina Raboteg Božiković, Slavko Perica	Genetic and phenotypic diversity of Croatian olive germplasm
	12:30-12:45	Zlatko Liber, __Gabriela Vuletin Selak, Mira Radunić, Zlatko Šatović, Monica Marilena Miazzi, Alenka Baruca Arbeiter, Dunja Bandelj, Josip Tadić, Marina Raboteg Božiković, Maja Jukić Špika, Katja Žanić, Tatjana Klepo, Višeslav Glavinović, Slavko Perica	Genetic diversity of olives in Croatia
	12:45-13:00	Zoe Andrijanić, Nelson Nazzicari, Hrvoje Šarčević, Aleksandra Sudarić, Ivan Pejić	Genetic diversity and population structure of Crop-BioDiv soybean germplasm collection
	13:00-13:15	Filip Varga, Zlatko Liber, Zlatko Šatović, Ivan Radosavljević, Nina Jeran, Martina Grdiša	Genetic diversity of Croatian Dalmatian pyrethrum as revealed by newly developed genomic SSRs
	13:15-13:30	Brunilda Hasanbelli, Filip Varga, Zlatko Šatović, Zlatko Liber,	Genetic diversity of natural Dalmatian sage (<i>Salvia</i>



	Alban Ibraliu	<i>officinalis</i> L.) populations from Albania
13:30-13:45	<i>Discussion</i>	
13:45-15:30	Lunch break	
<i>Session 2</i>	AUTHORS	TITLE
Moderator: K. Carović Stanko	15:30-15:45 Smiljana Goreta Ban, Josipa Perković, Iva Bažon, Nikola Major, Mario Franić, Igor Lukić, Danijela Poljuha, Zlatko Šatović, Dean Ban	Biodiversity of <i>Allium</i> species cultivated in Croatia: morphological, biochemical and molecular aspects
	15:45-16:00 Martina Grdiša, Filip Varga, Nina Jeran, Ante Turudić, Zlatko Liber, Zlatko Šatović	Influence of bioclimatic conditions on the pyrethrin profiles of Dalmatian pyrethrum natural populations
	16:00-16:15 Jerko Gunjača, Monika Vidak, Boris Lazarević, Klaudija Carović-Stanko	"Fingerprinting" the Croatian beans - An overview of the research on Croatian common bean collection
	16:15-16:30	<i>Discussion</i>
COFFEE BREAK	16:30-16:45	
<i>Session 3</i>	AUTHORS	TITLE
Moderator: J. Karoglan Kontić	16:45-17:00 Igor Lukić, Karolina Brkić Bubola, Sanja Radeka, Marijan Bubola, Marin Krapac, Sara Godena, Ivana Horvat, Marina Lukić, Mirella Žanetić, Irena Budić-Letoc, Silvia Carlind, Urska Vrhovsek	Volatilome and phenolome profiling for differentiation of Croatian monovarietal wines and olive oils and elucidation of their particular typical sensory attributes
	17:00-17:15 Iva Šikuten, Petra Štambuk, Ivana Tomaz, Jasminka Karoglan Kontić,	Metabolomic discrimination of genetic and geographical groups of grapevine



	Edi Maletić, Darko Preiner	varieties (<i>Vitis vinifera</i> L.)
17:15-17:30	Zvezdana Marković , Petra Štambuk, Darko Preiner, Iva Šikuten, Edi Maletić, Jasminka Karoglan Kontić	<i>In vitro</i> tissue culture, a tool for long-term conservation of Croatian grapevine genetic resources
17:30-17:45	<i>Discussion</i>	

Tuesday, October 4th 2022

Session 4	AUTHORS	TITLE
Moderator: G. Vuletin Selak	9:30-10:00 Luca Sebastiani	Abiotic factors and related stresses: assess the olive germplasm variability for the identification of tolerant varieties
	10:00-10:30 Luciana Baldoni, Maria Cristina Valeri	The fascinating mechanism of inter- and self-incompatibility of the olive varieties
	10:30-10:45 Josip Tadić, Gvozden Dumičić, Maja Veršić Bratinčević, Sandra Vitko, Sandra Radić Brkanac	Response of Wild (<i>Olea europaea</i> subsp. <i>europaea</i> var. <i>sylvestris</i>) and Cultivated Olive to Salinity and Drought
	10:45-11:00 Marija Polić Pasković	Cultivar dependant variations of olive leaf oleuropein concentrations
	11:00-11:15 <i>Discussion</i>	
COFFEE BREAK	11:15-11:30	
Session 5	AUTHORS	TITLE
Moderator: M. Grdiša	11:30-11:45 Igor Pasković, Katarina Vogel-Mikuš, Mitja Kelemen, Primož Vapetić, Primož Pelicon, Paula Žurga, Nikolina Vidović, Marija Polić Pasković, Smiljana Goreta Ban, Igor Lukić, Paula Pongrac	Bulk concentrations and tissue-specific macronutrients allocation in the leaves of two olive cultivars



	11:45-12:00	Iva Bažon, Dean Ban, Martina Grdiša, Zlatko Šatović, Smiljana Goreta Ban	Morphological diversity of Croatian garlic (<i>Allium sativum</i> L.) genetic resources
	12:00-12:15	Tvrtko Karlo Kovačević, Nina Išić, Josipa Perković, Iva Bažon, Dean Ban, Marta Sivec, Smiljana Goreta Ban, Nikola Major	Does above sea level elevation affect phytochemical profile of wild garlic?
	12:15-12:30	Nikola Major, Josipa Perković, Igor Palčić, Iva Bažon, Ivana Horvat, Dean Ban, Smiljana Goreta Ban	The antioxidant and nutritional aspect of shallots
	12:30-13:00	Jernej Jakše, Ajay Kumar Mishra, Jaroslav Matoušek, Tanja Guček, Nataša Štajner, Sebastjan Radišek	CBCVd viroid in Slovenian hop production
	13:00-13:15	<i>Discussion</i>	
LUNCH BREAK	13:15-15:00		
Session 6		AUTHORS	TITLE
Moderator:	15:00-15:30	Marek Živčák, Marián Brestič, Andrej Filaček, Mária Barboričová, Marek Kovár, Pavol Hauptvogel	Phenotyping of wheat genetic resources using non-invasive methods
B. Lazarević	15:30-15:45	Andrej Filaček, Mária Barboričová	Use of non-invasive methods for evaluation the effect of high temperature and acclimation mechanisms on photosynthetic processes of wheat (<i>Triticum sp. L.</i>)
	15:45-16:00	Tomislav Javornik, Boris Lazarević, Klaudija Carović-Stanko	Monitoring drought stress in common bean using chlorophyll fluorescence and multispectral imaging
	16:00-16:15	Nina Išić, Mario Franić, Marta Sivec, Dean Ban, Smiljana Goreta Ban	Photosynthetic and spectral response of garlic landraces under drought stress
	16:15-16:30	Dominika Mlynáriková Vysoká,	Phenotyping of lettuce



		Marek Živčák, Marián Brestič, Andrej Filaček, Mária Barboričová, Marek Kovár	genetic resources exposed to drought using imaging methods
	16:30-16:45	<i>Discussion</i>	
COFFEE BREAK	16:45-17:00		
Session 7	17:00-17:15	Mária Barboričová, Andrej Filaček	Monitoring the impact of climate extremes on different wheat varieties using fluorescence methods
Moderator:			
E. Maletić			
	17:15-17:30	Boris Lazarević, Jerko Gunjača, Toni Safner, Monika Vidak, Tomislav Javornik, Klaudija Carović-Stanko	Non-destructive quantification of nutrient deficiency symptoms in common bean
	17:30-17:45	Petra Štambuk, Darko Preiner, Iva Šikuten, Zvezdana Marković, Edi Maletić, Ivana Tomaz, Jasminka Karoglan Kontić	Phenotyping of Croatian native grapevine (<i>Vitis vinifera</i> L.) varieties in susceptibility to <i>Plasmopara viticola</i>
	17:45-18:00	Mate Čarija, Silvija Černi, Tomislav Radić, Emanuel Gaši, Katarina Hančević	Relative expression dynamics of GFkV, GVA and GPGV in grapevine host plants
	18:00-18:15	Magdalena Matić, Rosemary Vuković, Karolina Vrandečić, Ivana Štolfa Čamagajevac, Jasenka Ćosić, Ana Vuković, Krešimir Dvojković, Dario Novoselović	The effect of <i>Fusarium</i> inoculation and nitrogen fertilization on phenolics and phenylalanine ammonia-lyase
	18:15-18:30	<i>Discussion</i>	
SOCIAL DINNER	20:00-22:00		

Wednesday, October 5th 2022

<i>Session 8</i>			
		AUTHORS	TITLE
Moderator:			
D. Šimić	9:00-9:30	Nelson Nazzicari	Genomic selection in legumes: the experience at CREA
	9:30-9:45	Ivana Plavšin, Jerko Gunjača, Dario Novoselović	Factors affecting the accuracy of genomic selection for quality traits within a biparental wheat population
	9:45-10:15	Zetzsche Holger	Resistances to major fungal pathogens - breeding progress and challenges in wheat and barley
	10:15-10:45	Violeta Andjelkovic, Vojka Babic, Natalija Kravic	Utilisation of Maize Genetic Resources for Agro-Biodiversity Enhancement
	10:45-11:00	<i>Discussion</i>	
COFFEE BREAK	11:00-11:15		
<i>Session 9</i>			
Moderator:			
D. Novoselović	11:15-11:30	Domagoj Šimić	On the cusp of Breeding 4.0: Molecular plant breeding and interdisciplinarity
	11:30-11:45	Vlatko Galić, Josip Spišić, Tatjana Ledenčan, Antun Jambrović, Zvonimir Zdunić, Domagoj Šimić	The usability of proximal sensing in modern maize breeding programs
	11:45-12:00	Bruno Rajković, Ana Lovrić, Marko Maričević, Dario Novoselović, Hrvoje Šarčević	Allelic impacts of SNP markers on pre-harvest sprouting resistance in a panel of winter wheat (<i>Triticum Aestivum</i> L.) cultivars
	12:00-12:30	Ana Margarida Sampaio, Letice Gonçalves, Ana Margarida	Novel genetic factors controlling seed quality in



	Rodrigues, Carla António, Maria Carlota Vaz Patto	grass pea revealed by metabolic genome wide association study
12:30-12:45	Zoe Andrijanić, Nelson Nazzicari, Hrvoje Šarčević, Aleksandra Sudarić, Ivan Pejić	Genome-wide association study for seed quality traits in soybean [<i>Glycine max</i> (L.) Merr.]
12:45-13:00	Ante Turudić, Zlatko Liber, Martina Grdiša, Jernej Jakše, Filip Varga, Zlatko Šatović	Topics on the standardisation of chloroplast DNA sequence data
13:00-13:15	<i>Discussion</i>	
<i>CLOSING</i>	13:15 - 13:30	
<i>LUNCH</i>	13:30-14:30	
<i>TEHNICAL VISIT - IPTPO and POREČ</i>	15:00 - 19:00	

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Genomic selection has high potential for accelerating variety improvement on a wide spectrum of species. It works on either allogamous or autogamous organisms, doesn't require a reference genome and in the right conditions genomic prediction models can reach nearly optimal accuracy.	32
As of today, genomic selection has been applied at Italian CREA institute for seven years on different legume plants used both for human and animal consumption. From the point of view of the bioinformatics analysis, several patterns emerged regardless of the specific plant and target phenotype. During this talk we'll discuss in general terms what makes the ideal trait for genomic selection, what is the required number of molecular markers, the break-even point where genomic selection becomes economically preferable to traditional selection and the genotyping techniques to be preferred in this context.....	32
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Garlic (*Allium sativum*) breeding and crop improvement: recent achievements and outlook

Rina Kamenetsky-Goldstein

Agricultural Research Organization, The Volcani Center, Rishon-LeZion, Israel

Abstract

Commercial garlic varieties are propagated only vegetatively due to their complete sterility. Although wild relatives of *Allium sativum* produce seeds, wild ancestor of this species was not found. Therefore, new garlic varieties have been selected only from existing living collections, natural or induced mutations. The demand for garlic products with specific characteristics and constant increase in garlic production require breeding of this crop and its adaptation to different climatic conditions. Garlic fertility has been restored in the last decades, and research and breeding have undergone rapid progress. Currently, breeding in this crop is developing in three main directions: (1) conventional vegetative selection from variable germplasm collections; (2) breeding and selection from sexually-reproduced populations; and (3) employment of biotechnological tools. Novel methods of genome editing and marker-assisted breeding are not available in garlic yet, and therefore, fertility restoration, hybridization, and seed production are the most important goals in future breeding. The variability of seed-producing garlic lines is already available, but breeding and propagation from seed are still far from the commercial stage. Large investments are required for the development of seed-propagated garlic and breeding via hybridization, but the advantages of this approach for the future improvement of modern garlic are evident. Cleaning from viruses and diseases and *in vitro* propagation of outstanding varieties can improve the existing garlic cultivars

Keywords

Allium sativum, bulb, clonal propagation, fertility restoration, Hybridization, *In vitro* propagation, sexual reproduction



Genetic and Phenotypic diversity of Croatian olive germplasm

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Abstract

The research activities of the project "Biodiversity and Molecular Plant Breeding" were conservation, phenotyping and genotyping of olive genetic resources in Croatia. During the field expeditions, nine populations of wild olives were selected and included in the study together with cultivars from the field collections at Institute for Adriatic Crops and from the production orchards. Qualitative and quantitative traits of leaf, fruit, stone and flower were assessed at 97 wild genotypes, 28 autochthonous and 35 foreign cultivars. Fruit and stone traits showed high discriminatory capacity between wild olives and cultivars. The samples of 223 genotypes were molecularly characterized using 12 SSR (Simple Sequence Repeats) markers. The results showed high genetic variability of the Croatian germplasm separating the wild genotypes from the cultivated ones. Chemical and sensory profiling of 54 monovarietal extra virgin olive oils showed that fatty acids, phenolic content and sensory profile are predominantly characteristics of a cultivar. According to obtained results the plant material was propagated and used for establishment of new collection orchard and in greenhouse experiments to assess the sensitivity of different genotypes to abiotic and biotic stresses. The alternate bearing of Oblica, the main Croatian olive cultivar was studied in production orchard. The trees with different fruit load showed differences in flower and fruit morphology as in fruit and oil yields. The olive research group at Institute for Adriatic Crops will continue to work in selection, evaluation and preservation of olive autochthonous and wild genotypes.

Keywords

Wild olives, cultivars, morphological characteristics, SSR markers, olive oil

Genetic diversity of olives in Croatia

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Abstract

The olive tree (*Olea europaea* L.) is an important fruit tree species in all Mediterranean countries, including Croatia, which is characterized by a fair number of indigenous, locally grown varieties, and by a rich heritage of wild olive trees still found on numerous islands along the Adriatic coast. In the framework of the project "Biodiversity and Molecular Plant Breeding", the samples of 223 olive varieties and wild olives were collected from different locations in the olive growing area and molecularly characterized using 12 SSR (Simple Sequence Repeats) markers. The results showed a unique profile for most cultivars and wild trees, except for three cases of synonymy, probably attributable to misnaming. Overall, the Croatian germplasm showed high genetic variability. The neighbor-joining dendrogram separated a group of wild genotypes originated mostly from the middle and southern Dalmatian islands. Cultivated genotypes mostly clustered together but they also showed relationship with wild germplasm. The results obtained underlined the richness of the genetic resources of the olive tree in Croatia, providing information for the protection of genetic material and monovarietal oils.

Keywords

Genotyping, cultivars, wild olive, olive diversity

Genetic diversity and population structure of Crop-BioDiv soybean germplasm collection

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Abstract

Knowledge of the genetic structure and diversity of germplasm collections is critical for sustained genetic improvement through hybridization programs and rapid adaptation to changing breeding objectives. The objective of this study was to determine the genetic diversity and population structure of 221 Crop-BioDiv accessions using single nucleotide polymorphism (SNP) microarray markers for potential utilization. These genotypes, representing early and very early breeding material suitable for cultivation in SE Europe, were divided into groups by country of origin, maturity group, and decade of release. Several approaches were used to estimate population structure. Cluster and principal component analysis revealed genetic diversity among 221 accessions classified into two stratifications. The same clustering pattern was seen in the Bayesian population structure analysis, which divided the 221 accessions into two groups. Genetic diversity was highest in genotypes imported from North America and Austria, also as for genotypes of maturity group 000. The average F_{st} was highest according to the origin of the accessions. The highest F_{st} was found between genotypes originating from Serbia and Switzerland, and the lowest between genotypes originating from the 1990s and 2000s. Analysis of molecular variance revealed 9% of genetic variance among breeding programs (countries) and 91% of genetic variance within the breeding programs. Our study provides information on the genetic structure and diversity of Crop-BioDiv soybean accessions, allowing use of these genotypes in soybean improvement programs, especially parent selection in breeding programs.

Keywords

Soybean, genetic diversity, population structure

Genetic diversity of Croatian Dalmatian pyrethrum as revealed by newly developed genomic SSRs

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Abstract

Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevis.) Sch. Bip.) is an endemic species of the eastern Adriatic from the family *Asteraceae*. The plant produces pyrethrin, a secondary metabolite that has been shown to be an effective natural insecticide with little or no adverse effects on humans.

To assess genetic diversity, samples were collected from 10 natural populations of Dalmatian pyrethrum along the Adriatic coast and on the islands representing the species' range in Croatia. Genetic diversity analysis was carried out using 12 newly developed microsatellite markers.

A total of 121 alleles with an average of 10.08 alleles per locus were detected in the studied populations, indicating high genetic diversity. Allelic richness (N_{ar}) varied from 2.86 (Biokovo) to 5.02 (Zlarin). A total of 36 private alleles were observed in 10 populations with the highest number observed in Konavle (7). Evidence of bottlenecks was found only in the Biokovo population. Analysis of molecular variance showed that most of the genetic diversity can be explained by differences between individuals within populations (87.03%). Bayesian analysis of population structure revealed the existence of two gene pools, the mainland gene pool and the island gene pool. At $K = 3$, the Biokovo population formed a separate gene pool. Spatial analysis revealed that only a small proportion of genetic differentiation was due to isolation by distance (0.7%), while 40.6% of genetic differentiation was due to isolation by environmental distance.

The results of this research will contribute to the development of future breeding programmes and hopefully commercial varieties of *T. cinerariifolium* to revive Dalmatian pyrethrum production in Croatia and the region. Furthermore, these results will help to improve conservation strategies for the species.

Keywords

Tanacetum cinerariifolium (Trevis.) Sch. Bip., genomic SSR, plant genetic resources, genetic diversity

Genetic diversity of natural Dalmatian sage (*Salvia officinalis* L.) populations from Albania

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Abstract

Salvia officinalis L. occupies an important place in the Albanian market for aromatic medicinal plants due to the high content and composition of its essential oil and is the most economically important species for the country.

The aim of this study was to investigate the genetic diversity and structure of eight populations of Dalmatian sage from Albania, using eight microsatellite markers (SSRs) previously developed for this species. All eight-microsatellite markers yielded unique and easily detectable amplified fragments that revealed a high level of polymorphism (average of 15 alleles per locus). Overall values of expected heterozygosity (H_E) ranged from 0.608 to 0.818, with a mean of 0.721. The highest gene diversity was found in northern Adriatic populations, decreasing towards the southern limit of the species range. Multilocus estimates of Wright's inbreeding coefficient within populations (F_{IS}) showed no significant deviation from Hardy-Weinberg equilibrium. Analysis of molecular variance showed that most of the total genetic diversity was attributable to differences between individuals within populations (83.14 %) which is to be expected due to outcrossing nature of the species. Bayesian analysis of population structure revealed the existence of two gene pools, the northern gene pool, and the southern gene pool. At $K = 3$, the Dibër population (the easternmost population in the sample) formed a separate gene pool.

As commercial collection of Dalmatian sage in the wild has a negative impact on biodiversity, these results will form the basis for of plant genetic resource conservation and plant breeding programmes for this valuable species.

Keywords

Lamiaceae, genetic diversity, genomic SSRs, population structure

Biodiversity of *Allium* species cultivated in Croatia: morphological, biochemical and molecular aspects

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Abstract

Species of the genus *Allium* are among the most important representatives of vegetables grown all over Croatia, as well as around the world. *Alliums* are highly valued foods of high nutritional value and their beneficial effect on a number of conditions and diseases in humans is known. Due to high content of certain secondary metabolites, certain types of *Alliums* are also used for plant protection as ecological preparations. The identification and characterization of local ecotypes and traditional cultivars with the aim of preserving genetic diversity and traditional production has in recent years become increasingly important on a global scale. The best results of such studies are achieved by applying a multidisciplinary approach that combines insights from agronomic, biological and biochemical points of view. In the territory of Croatia there is a significant biological and morphological diversity of cultivated and wild *Allium* species. A few years ago, the Institute of Agriculture and Tourism launched an extensive program of collecting and characterizing cultivated onions with an emphasis on garlics and shallots. The collection currently consists of 122 garlic accessions from all over the world and Croatia and 35 accessions of shallots from Croatia. A high morphological, biological, molecular and biochemical variability of the studied species has been determined, which can be used for the selection of accessions with desirable properties for breeding programs and cultivation.

Key words: *Allium sativum*, *Allium cepa* var. *Agregatum*, *Allium x cornutum*, bioactive compounds, SSR

Influence of bioclimatic conditions on the pyrethrin profiles of Dalmatian pyrethrum natural populations

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Abstract

Pyrethrin is a leading plant biopesticide that is highly effective against numerous pests and has little or no negative impact on human health and the environment. It is a specialized metabolite from Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevis.) Sch. Bip.; Asteraceae), a plant species native to the eastern Adriatic coast. Pyrethrin consists of six compounds, pyrethrin I and II, cinerin I and II, and jasmolin I and II, with pyrethrin I and II accounting for the largest proportion of total pyrethrins. In order to determine the intraspecific biochemical variability and to investigate the influence of different ecological conditions on the accumulation of the individual compounds, 15 natural Dalmatian pyrethrum samples were collected. Ultrasound-assisted extraction was used for the pyrethrin extraction, followed by their qualitative and quantitative analysis on HPLC-UV-DAD. Total pyrethrin content ranged from 0.82 % to 1.27% of flower dry weight (DW). Populations were clustered in five bioclimatic groups; A (Northern Adriatic populations and one coastal population from central Adriatic), B (two central Adriatic island populations), C (two central Adriatic island populations), D (Southern Adriatic island populations), E (inland Southern Adriatic populations, populations from Bosnia and Herzegovina and Montenegro), each characterized by a unique chemical profile. The highest average values for pyrethrin I (53.07% of total pyrethrin), total pyrethrin content (1.06 % DW) and the ratio of pyrethrin I and II (1.85) were found in the bioclimatic group E. The observed variability was partially explained by significant correlation found between the levels of some pyrethrin compounds and bioclimatic variables (e.g., BIO03 Isothermality and BIO04 Temperature seasonality). The results will support the selection of populations for breeding programs aimed at producing cultivars with desirable biochemical properties and adaptation to different bioclimatic conditions.

Keywords

Biopesticides, biochemical variability, pyrethrin, *Tanacetum cinerariifolium*

"Fingerprinting" the Croatian beans - An overview of the research on Croatian common bean collection

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Abstract

The collection of Croatian common bean accessions, predominantly consisting of traditional cultivars (landraces), was established at the University of Zagreb Faculty of Agriculture. During the last decade it has provided a valuable material for the research on several scientific projects. The collection was first genotyped for 26 SSR markers in order to assess its genetic diversity and structure. The grouping of accessions into three major clusters corresponded well with their phaseolin type, as well as with their Mesoamerican or Andean origin. Selected set of accessions was then grown in a field trial and scored for their nutrient content. Results revealed some differences in mineral content between cultivars of a different origin, but generally mineral levels were high enough (compared to those from other authors) to consider the collection as a valuable resource for plant breeding. Further genotyping based on 6311 SNP markers provided additional confirmation of earlier results obtained using SSR markers and resolved some earlier doubts. Finally, collected data were submitted to GWAS, which resulted in discovering 22 QTNs associated with N, P, Ca, Mg and Zn content.

Keywords

Traditional cultivars, genetic diversity, molecular markers, population structure, GWAS

Volatilome and phenolome profiling for differentiation of Croatian monovarietal wines and olive oils and elucidation of their particular typical sensory attributes

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Abstract

Despite the long tradition and significant anecdotal knowledge of experts and professionals acquired through practice, the concept of varietal typicity of wine and olive oil has been poorly investigated. This study sought to determine objective and measurable indicators of varietal typicity of wines and olive oils from Croatian native varieties by determining their typical sensory attributes and tentatively identifying corresponding sensory-active chemical compounds behind them, on the basis of which wines or olive oils of different varieties can be recognized and differentiated.

Samples of monovarietal wines and extra virgin olive oils were collected from Croatian producers and subjected to quantitative descriptive sensory analysis and in-depth volatilomic and phenolic profiling by GC-MS, GC×GC-TOF-MS, and UPLC-MS/MS, respectively. Numerous volatile and phenolic compounds were identified, among which many served as potent markers useful for differentiation according to variety. The results of sensory and chemical analysis were put in relation and diverse typical sensory attributes were tentatively ascribed to particular variations in chemical composition. For example, higher ruby red color intensity with purple reflexes considered typical for Teran red wine coincided with higher concentration of anthocyanins, while typical pungency of Istarska bjelica olive oil, described as immediately strong and burning, was assumed to originate mainly from oleocanthal.

The obtained results should contribute to better understanding of typical sensory attributes of Croatian products and could be exploited for obtaining wines and olive oils with more pronounced varietal character.

Keywords

Varietal typicity, wine, olive oil, aromas, phenols

Metabolomic discrimination of genetic and geographical groups of grapevine varieties (*Vitis vinifera* L.)

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Abstract

Grapevine germplasm is highly variable and classified into geographical groups. Polyphenolic and volatile organic compounds (VOCs) have crucial role in winemaking industry due to their influence on wine quality. The aim of the research was to investigate the polyphenolic and volatile profiles of 50 grapevine varieties from different GEN-GEO groups: C2 (varieties from Italy and France), C7 (varieties from Croatia), C8 (varieties from Spain and Portugal). The most abundant class of polyphenols were anthocyanins, flavan-3-ols and flavonols, while carbonyls, alcohols and sesquiterpenes were the most abundant class of VOCs. Using discriminant analysis, the GEN-GEO groups were clearly separated by their polyphenolic and volatile profiles. Polyphenolic compounds contributing the most to the discrimination of groups belong to classes of hydroxycinnamic acids, flavan-3-ols, and flavonols. Furthermore, some of the compounds contributing to discrimination are found in relatively small amounts. Regarding the discrimination based on volatile profiles, GEO groups were discriminated by their overall volatile profile. C2 group contains higher amounts of carbonyl compounds and alcohols, while C8 group contains higher amounts of sesquiterpenes and acids. Group C7 is characterized by low content of VOCs. This data demonstrates that geographical origin, combined with genotype, influences the overall polyphenolic and volatile profiles.

Keywords

Secondary metabolites, grapevine varieties, GEN-GEO groups, discriminant analysis



***In vitro* tissue culture, a tool for long-term conservation of Croatian grapevine genetic resources**

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Abstract

In vitro culture obtain the tools to perform specific studies that it would be impossible to carry out with the whole plants grown on field or under greenhouse conditions. Cryopreservation presents the long-term conservation without a metabolic changes of plant material and cryotherapy can be efficient in virus elimination. Tested cryopreservation protocols did not gave sufficient regeneration. Preculture media of cryopreservation profotocols was evaluated with Croatian grapevine cultivars ('Graševina', 'Plavac mali' and 'Pošip') with different antioxidants (salicylic acid, ascorbic acid and glutathione). The highest growth *in vitro* was achieved on the medium with the addition of glutathione and the lowest on the medium with addition of salicylic acid. The growth *in vitro* and regeneration after cryopreservation was depended on genotype and sanitary status. A reliable protocol for the regeneration after cryopreservation (within cryotherapy) should be defined for each grapevine cultivar.

Virus elimination was tested by meristem tip culture with 18 Croatian cultivars, whereas, regeneration *in vitro* was between 6.82 and 53.22%, but virus elimination was achieved only with three cultivars and was very low (23.8%). Additionally, the method of conservation of grapevine cultivars by cultivation in isolated conditions of small glass containers (2L), was tested. Two cultivars were grown in containers in the growth chamber under controlled conditions. This study has revealed that the plants can be conserved this way for more than 2 years (ongoing trial). Plants grown this way can be the source of plant material for new *in vitro* introduction and multiplication. Overall, the presented methods should be further evaluated for broad application.

Keywords

Vitis vinifera L., methods of conservation, preculture with antioxidants, salicylic acid, regeneration



Abiotic factors and related stresses: assess the olive germplasm variability for the identification of tolerant varieties

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Abstract

In the Mediterranean ecosystem, olive trees continuously interact with a multitude of environmental factors and related stresses that require complex and variable mechanisms for plant survival and growth. Main abiotic factors include water availability, temperature, mineral nutrients balance, and salinity which interact each other and vary in time and space inducing negative physiological consequences. In olive, the combination of adaptation and acclimation processes have contribute to the overall tolerance of this species to the Mediterranean abiotic stresses. However, both climate change than orchards plantation in sub-optimal regions of the world have exposed olive plants to the negative impact of abiotic stresses. Moreover, olive cultivation changes towards high-density and high-input growing systems might receive a strong negative impact on both yield and quality form plant stresses. In this work, the state of art on olive germplasm variability to abiotic stresses will be analyzed and discussed in order to disclose some of the tolerance mechanisms in olive trees. The information highlighting genotypic variability in olive will be contextualized in the framework of the strategy for the identification of tolerant varieties. These varieties will be highly necessary to improve olive plant responses to multiple environmental stresses and to efficiently achieve resilient olive cultivation to climate change environmental scenario predicting rising temperatures, precipitation abnormalities and salinization.

Keywords

Cultivar, drought, *Olea europaea*, salinity, water deficit



The fascinating mechanism of inter- and self-incompatibility of the olive varieties

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Abstract

Recent advances in the study of the genetic and molecular mechanisms underlying inter- and self - incompatibility in olive have shown a peculiar system, only observed in other Oleaceae species. Studies were conducted by means of stigma-test on a large number of olive varieties, to verify the germination of pollen on the stigmas of two certainly inter-compatible varieties. It was observed that the pollen of each variety germinated only on one of the two varieties and never on the other, allowing to divide all varieties into two groups. This incompatibility system, defined as diallelic self-incompatibility (DSI), is controlled by a single locus with two alleles and with only two possible genotypic combinations (Ss and ss), respectively corresponding to only two incompatibility groups (IG): G1 and G2, where all varieties of the G1 group are inter-incompatible with each other and compatible with all varieties of the G2 group, and vice versa.

Markers linked to the incompatibility group have been identified, able to screen any olive cultivar for its IG.

At open field level, it was confirmed that fertilization occurs exclusively between cultivars with different IG, but some of them showed greater pollination efficiency than others, highlighting that it is not enough that varieties are inter-compatible to also play as good pollinators, able to fertilize and produce fruits.

Further studies are needed to explain the occurrence of self-fertility observed in some varieties.

Keywords

Incompatibility, flower, stigma test, *Olea europaea*, cultivars



Response of wild (*Olea europaea* subsp. *europaea* var. *sylvestris*) and cultivated olive to salinity and drought

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Abstract

In the face of climate change, water deficit and increasing soil salinity pose a challenge to olive cultivation in the Mediterranean basin. Croatian wild olive genotypes were evaluated and compared with cultivars (cv.) 'Oblica', 'Leccino' and 'Koroneiki'. Plants were exposed either to 150mM NaCl or 300mM mannitol treatment for 3 weeks. The goal of the research was to determine the impact of abiotic stresses to the morphological, physiological and biochemical changes. The morphometric parameters of shoot length, dry mass and leaf area are significant indicators of the level of drought stress. Na⁺ and Cl⁻ in the shoot leaves of cv. 'Oblica' are probably involved in maintaining the osmotic pressure because, despite the high values of these ions, the other results did not indicate serious signs of stress. The relative content of K⁺ in the roots decreased significantly in almost every genotype. The increased values of Ca²⁺ absorption proved to be the most energetically efficient response in a short period of time. Changes in SOD enzyme activity and the content of chlorophyll *a* can serve as indicators of stress, while proline can be viewed in the context of a signal molecule. The cv. 'Koroneiki' did not have a significantly increased activity of antioxidant enzymes or osmolyte accumulation. Wild olive genotypes LA 13 and PLJ 18, with satisfactory results from morphometric and biochemical analyses, deserve research based on genotypic characterization for future breeding programs.

Keywords

Salinity, wild olive, osmotic stress, ion accumulation, antioxidative enzyme

Cultivar dependant variations of olive leaf oleuropein concentrations

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Abstract

Olive leaf is one of the traditional herbal remedies, used since the time of ancient Egypt. One of the most important biological components of olive leaves are phenols, the most represented by secoiridoid oleuropein. Is to be assumed that different olive cultivars can differ by their leaf oleuropein content.

Thus, the aim of this work was to investigate the difference in oleuropein concentrations in olive leaves of five Croatian autochthonous cultivars: Buža puntoža, Buža, Istarska bjelica, Karbonaca, Rošinjola and Italian Leccino as one of the most common allochthonous olive cultivar in Croatian olive groves. Sampling was conducted at a growth season 2017/2018 in olive grove located in Istria during two terms, harvest and pruning. Each of the six cultivars was represented by 3 trees.

Selected cultivars had shown significant differences in oleuropein concentrations in both sampling periods. During harvest, the highest concentration of oleuropein in the leaf was measured in Leccino cv. leaves 4962,29 (mg/100 g DW), and the lowest in Rošinjola cv. 500,8 (mg/100 g DW). During pruning, the highest concentration of oleuropein in the leaf was measured in Buža cv. 8064,04 (mg/100 g DW) and the lowest was Karbonaca cv. 1627,73 (mg/100 g DW).

Due to leaf oleuropein concentration differences in selected Croatian olive cultivars further trials with more cultivars and different locations need to be conducted.

Keywords

Oleuropein, *Olea europaea* L., Phenols, Leccino cv., Buža cv.

Bulk concentrations and tissue-specific macronutrients allocation in the leaves of two olive cultivars

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Abstract

The olive (*Olea europaea* L.) leaves, as central part of the olive tree metabolism, has important role in determination of olive nutrient status and fertilizers requirements. Thus, the aim of our study was to define differences in leaf phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations between Leccino and Istarska bjelica cultivars.

The field experiment included total of 18 olive trees per cultivar, equally distributed in six locations all along Croatian Adriatic coast. Sampling for leaf bulk analysis was conducted during harvest, winter and pruning period (2017/2018) (n=54). In the greenhouse experiment, with 150 one-year old olive seedlings per cultivar, leaves were sampled for bulk and micro-PIXE analysis in November 2019 (n=3). Field experiment results shown that Leccino cv. leaves has higher bulk P, Ca and Mg concentrations when compared to Istarska bjelica cv.. In greenhouse experiment total Ca and Mg leaf concentrations were higher in Leccino cv. with elevated K concentration in Istarska bjelica cv..

Micro-PIXE analysis revealed that larger Ca and Mg bulk concentrations in Leccino cv. leaves can be linked to higher Ca concentration in epidermis and in leaf blade tissues as well as with higher Mg concentration in mostly all leaf tissues, with exception the bundle sheath cells and the main vascular bundle. While P concentration was higher in epidermis and spongy mesophyll of Leccino cv. leaves, K concentration didn't differ between selected cultivars in any of the leaf tissues examined.

Keywords

Phosphorous, calcium, magnesium, Leccino cv., Istarska Bjelica cv., micro-PIXE.



Morphological diversity of Croatian garlic (*Allium sativum* L.) genetic resources

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Abstract

Garlic (*Allium sativum* L.) is a worldwide grown crop, used for culinary and medicinal purposes. Garlic landraces are adapted to local agro-ecological conditions and can have specific morphological traits. The aim of this study was to evaluate morphological diversity of garlic from the Croatian garlic gen-bank collection. Seventy-seven accessions were described two months after harvest. Seven qualitative and four quantitative bulb traits were described according to ECPGR descriptor for *Allium* on ten bulbs per accession. The diversity among accessions within region and average diversity within accessions were the highest for Istrian accessions for skin color of the clove. Most phenotypic diversity of the analysed accessions was attributable to variation within accessions and among accessions within region, while the rest could be attributed to differences among regions. Skin colour of the clove and bulb structure type had a strong association with qualitative bulb traits: shape of mature dry bulbs, shape of mature garlic bulb, outer skin colour of compound bulb and shape of the compound bulb in horizontal section. Two morphotypes were determined. The first morphotype grouped accessions with yellow and light brown cloves, irregular structure, elliptic shape and no ability to flower. Whereas, the second morphotype grouped accessions with violet cloves, regular structure, circular shape and ability to flower. The results can ensure more effective collection management and use of garlic in breeding programs.

Keywords: clove, colour, descriptors, gene-bank, phenotyping

Does above sea level elevation affect phytochemical profile of wild garlic?

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Abstract

Allium ursinum L., otherwise known as wild garlic, is a wild relative of onion and garlic along with many other species of the numerous *Allium* genus. Wild garlic is a wild edible plant which usually grows in forests, preferably in shade and sometimes in vicinity of water streams. It is prevalent throughout Europe and western Asia, from lowlands to highlands. Being described as a wild plant, the plant itself has adapted to various environmental conditions. In the past, wild garlic was used as a remedy which refers to wild garlic having compounds that promote good health. Those properties are mostly attributed to the organosulfur compounds and antioxidants produced in plants as a secondary metabolites. *Allium* species are characterized by an abundance of organosulfur compounds which are directly responsible for the distinct flavor and pungent odor, while polyphenols represent a group of numerous compounds that possess antioxidant properties. The aim of this study was to investigate the differences in phytochemical profiles between bulbs and leaves of wild garlic originating from 8 different locations, namely differences in antioxidant capacities, total polyphenol contents, volatile profiles, and amino acid. Moreover, effect of above sea elevation on antioxidant capacities, total polyphenol contents, volatile profiles, and amino acid levels in bulbs and leaves of wild garlic was investigated as well. Antioxidant capacities and total polyphenol contents were determined by spectrometric methods (DPPH, FRAP, ORAC, TPC) while volatile profile and amino acid levels were determined using GC-MS and HPLC, respectively. In total, 23 volatile compounds were determined in leaf samples, while in bulb samples 28 volatile compounds were determined. Also, 12 amino acids were quantified in leaf samples, while in bulb samples 14 amino acids were quantified. Furthermore, statistical analysis (factorial ANOVA) showed how the quantity of investigated compounds is not only dependent on the plant's organ, but on above sea level elevation as well. Therefore, significant interaction between plant organ and elevation was established in 56.5% of determined compounds. In leaves of wild garlic, the effect of elevation was not significant in most of the investigated compounds, while in bulb samples that grew on lower elevation, higher antioxidant capacity and amino acid content was observed. Meanwhile, bulbs from higher elevation exhibited higher level of volatile compounds compared to bulbs from lower elevation.

Keywords: *Allium ursinum*, amino acid, antioxidant capacity, aroma, altitude, flavor, organosulfur compounds, plant organ, (poly)sulfides, secondary metabolites

The antioxidant and nutritional aspect of shallots

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Abstract

Shallots are a perennial plant from the Alliaceae family classified within the common onion under the name *Allium cepa* *Aggregatum* group. The term shallot is also used for diploid and triploid viviparous onions known as *Allium* × *proliferum* (Moench) Schrad and *Allium* × *cornutum* Clementi ex Vis. The nutritional composition of 34 shallot accessions was analysed. Shallot accessions belonging to the *A.* × *cornutum* and *A.* × *proliferum* groups are characterized by high dry matter content (around 25%) of which a little less than 50% is of inulin-type sugars, polysaccharides considered an excellent prebiotic with beneficial effects on human health. On the other hand, accessions belonging to the *A.* *cepa* *Aggregatum* group have lower dry matter content and as a result lower pungency (measured as pyruvic acid content) making them more acceptable for fresh consumption by a broader range of consumers but at the same time abundant in phenolic compounds especially in quercetin and isorhamnetin glycosides. We also observed a greater biodiversity among accessions within the *A.* *cepa* *Aggregatum* group in all analysed physico-chemical parameters compared to the other shallot groups. The investigated shallot accessions have excellent *in vitro* antioxidant capacity as well as excellent nutritional properties.

Keywords: shallot, flavonoid, inulin, FOS, antioxidant activity

CBCVd viroid in Slovenian hop production

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Abstract

In 2007, hop farmers in the Savinja Valley in Slovenia reported the appearance of stunted hop plants. In the following years, this phenomenon spread rapidly in hop gardens suggesting mechanical means and planting material between farms. Classical diagnostic methods and molecular testing techniques were unable to detect a new pathogen. Therefore, NGS of total RNA and small RNAs from healthy plants and those with symptoms was used to identify the new pathogen. Bioinformatic analysis resulting in the identification of a new sequence of Citrus bark cracking viroid (CBCVd) in the stunted hop plants, which has never been reported before in hops. In addition, a controlled transmission experiment confirmed CBCVd as the causal agent of severe hop stunt disease. The new viroid challenged European hop producers and authorities to prevent its spread and eradicate infected plants, as well as to develop a rapid and reliable method for its detection. A reliable one-step multiplex method RT-PCR (mRT-PCR) was developed to simultaneously detect the four known viroids infecting hops. On the other hand, the discovery of new viroid with severe symptoms represents an interesting model to study the pathogenesis and hop-viroid interactions. To investigate the possible gene silencing in viroid-infected plants by viroid-derived small RNAs, *in silico* prediction of target transcripts for viroid-derived small RNAs was performed. The prediction models revealed that many hop transcripts have nucleotide homologies viroid-derived small RNAs and therefore could be silenced by RNA interference. Analysis of the response of hop microRNA genes to CBCVd infection was initiated. More than a hundred miRNA genes were identified from the hop genome. Some of them were differentially expressed in response to CBCVd infection, demonstrating the importance of miRNAs in the innate immune response of hop plants. The presented efforts to identify and better understand the molecular mechanisms of viroid disease could initiate new strategies for prevention and help in the search for possible genetic resistance.

Keywords:



Phenotyping of wheat genetic resources using non-invasive methods

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Abstract

Exploitation of genetic resources in genebanks is limited by insufficient phenotypic data, including information on the stress tolerance. The aim of the study was to assess the techniques for rapid evaluation of the traits associated with drought tolerance. As a material, we used a collection of diverse winter wheat genotypes selected from accessions of Slovak Genebank, covering well a diversity of leaf traits. To examine the links between the target traits and drought stress responses, we performed the phenotyping experiment using a whole collection of genotypes. Plants were grown in pots outdoors and in a growth phase of stem elongation, a half of the plants were exposed to long-term moderate water deficit by limiting water supply. The growth responses to drought were assessed by measurements of chlorophyll content (SPAD), chlorophyll fluorescence, leaf temperature (IR thermal imaging) and VNIR hyperspectral records were performed in 3-4 days interval to assess the effect of drought stress on photosynthetic apparatus. The measurements were followed by the analyses of aboveground biomass and grain yield. We observed a high diversity in the level of reduction of the leaf area, leaf chlorophyll content, spectral reflectance and parameters derived from fast chlorophyll fluorescence records indicating unequal effects of drought on the photosynthetic apparatus of diverse wheat genotypes. Moreover, we identified a close relationship between the changes of leaf traits and leaf optical properties measured by the hyperspectral reflectance records. The study was supported by the national grants VEGA 1-0683-20, VEGA 1-0664-22, and APVV-18-465.

Keywords: wheat, chlorophyll fluorescence, spectral reflectance, phenomics



Use of non-invasive methods for evaluation the effect of high temperature and acclimation mechanisms on photosynthetic processes of wheat (*Triticum sp. L.*)

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Abstract

To deepen our understanding of the complex mechanisms underlying plant response to environmental stimuli, quantitative measurement of plant growth response under different environmental conditions is important. Non-invasive methods play an important role in this. This study aimed to provide physiological evidence for the effect of long-term temperature acclimation on wheat photosynthetic responses to an episode of severe heat stress, specifically targeting genotype and acclimation-related responses observed at the level of PSII photochemistry. To test this experimental design, we used three contrasting genotypes of *Triticum sp.* (These, Roter Samtiger Kolbenweizen and ANK32A). The pot experiment was realized in outdoor conditions. The plants were exposed to full sunlight and watered regularly to prevent dehydration. Acclimation was induced by moving half of the plants to a polyethylene foil tunnel where they were exposed to elevated temperature (30°C) for 14 days. Plants from both variants (nonacclimated - NA; temperature acclimated - TA) were subsequently moved to a growth chamber with artificial actinic lighting (Osram Fluora, PAR intensity 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and exposed to severe heat stress (45 °C) for ~12 h. Short-term exposure to high temperature led to a significant non-stomatal limitation of photosynthesis. To analyze the processes specifically affected by heat stress, we also used the analysis of some specific fluorescence parameters, which are derived from the JIP-test. Acute heat stress led to a decrease in the number of active reaction centers (RC/ABS), the maximum quantum yield of PSII (Fv/Fm) and the total activity of PSII (PI_{ABS}) in all genotypes, but the efficiency of electron transport between the two photosystems (ψREo) was negatively affected only in non-acclimated variants. The group of non-acclimated plants of genotype ANK32A showed the most sensitive reaction to the effect of high temperature among all genotypes. However, significant differences between genotypes were eliminated by previous acclimation of plants to elevated temperature. The increase in temperature resistance was associated with a significant increase of parameters derived from fast fluorescence kinetics related to PSI activity; this finding supports the hypothesis that increased PSI activity, especially cyclic electron transport, may play a key role in protecting the chloroplast membrane structure from the adverse effects of acute heat stress.

Keywords: wheat, heat stress, acclimation, non-invasive methods, photosynthesis



Monitoring drought stress in common bean using chlorophyll fluorescence and multispectral imaging

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Abstract

Drought is a significant constraint in bean production, as about 60% of beans are grown in regions with water shortages. In this study, we used high throughput phenotyping (chlorophyll fluorescence imaging, multispectral imaging, 3D multispectral scanning) to continuously monitor the development of drought-induced morphological and physiological symptoms of water deficit. This experiment aims to select the most drought-sensitive plant phenotypic traits which could be used for early detection of drought stress and screening for drought tolerance among common bean genotypes. The experiment consisted of four treatments with ten plants each. Plants were grown in control (fully irrigated sand) and three water deficit treatments (irrigated with 70 mL, 50 mL, and 30 mL of distilled water, respectively). All measurements were performed on five consecutive days, and the last measurement was on the 8-th day of the experiment. The earliest detected symptoms were found on the fourth day of the experiment when a reduction in digital volume, total leaf area, and leaf area index was determined in treatments with 30 mL and 50 mL compared to control and 70 mL. In addition to morphological changes on the fourth day of the experiment, drought-induced treatments caused significant changes in several multispectral traits (specific green and saturation). Chlorophyll fluorescence traits showed to be less affected by drought, and significant changes were found only after prolonged drought stress.

Keywords: drought, common bean, chlorophyll fluorescence, multispectral imaging



Photosynthetic and spectral response of garlic landraces under drought stress

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Abstract

Drought is a major obstacle in agricultural production, limiting plant growth and yield. There is a need for a long-term solution to climate change threats, which can be found in cultivars better adapted to a lack of water. Garlic is an important vegetable crop, and despite its wide use and production, there is lack of research on its physiological response to water stress. The wide spectrum of garlic diversity in Croatia provides a possible level of variation in its tolerance to drought. In this research, 36 garlic landraces were analyzed in their morphological and physiological responses to water stress by measuring gas exchange (LI-6800, LI-COR, USA) and multispectral traits (PlantEye Microscanner, Phenospex, Netherlands). Drought led to a significant decrease in gas exchange parameters. Transpiration rate in drought stressed plants decreased for 48% compared to well-watered plants, and other photosynthetic parameters followed. Interaction between landrace and drought stress was found in assimilation and transpiration indicating differences in landrace response. The results showed a change in spectral vegetation indices, suggesting increased stress in drought exposed garlic plants compared to well-watered ones. Differences among garlic landraces in drought stress tolerance could be used for selection of more adapted cultivars. Combining morphological and spectral traits with photosynthetic response to water shortage can give an insight into garlic adaptations to drought stress.

Keywords: *Allium sativum* L., abiotic stress, gas exchange, NDVI, NPCI



Phenotyping of lettuce genetic resources exposed to drought using imaging methods

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Abstract

In our study, we test the adaptive responses to progressive drought in 12 leafy lettuce genotypes differing in leaf color. Plants were cultivated and tested by the Plantscreen phenotyping platform at SAU Nitra, Slovakia. Structural responses of all genotypes were simultaneously complemented with measuring other biochemical (leaf pigments and proline content), photochemical (PSII efficiency), hyperspectral reflectance (vegetation indices), and leaf water status. We also assessed the contents of total flavonoids, anthocyanins, and total phenolics, including their noninvasive assessment. Imaging analyses of water-stressed plants revealed plant development and senescence of the oldest leaves. At a physiological level, plant water stress has been accompanied by a pronounced reduction of leaf water content and osmotic potential resulting in a genotype-specific level of osmotic adjustment. Genotypes with higher drought tolerance maintained a high level of chlorophyll content and increased leaf carotenoids, anthocyanins, and flavonoids. The analyses have shown a tight correlation between the parameters derived from the fluorescence analyses and the flavonoid and anthocyanin content. The anthocyanin contents can be well estimated using hyperspectral indicators. The study identified the parameters enabling recognition of the drought stress level in individual genotypes that can be used in lettuce breeding programs. The study was supported by the national grants VEGA 1-0683-20, VEGA 1-0664-22, and APVV-18-465.

Keywords: lettuce, noninvasive methods, stress, phenomics



Monitoring the impact of climate extremes on different wheat varieties using fluorescence methods

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Abstract

The aim of our work was to characterize specific phenotypic manifestations of genetic resources and modern varieties of wheat, their tolerance during the interaction of drought and high temperature (36 °C), at the level of various physiological and biochemical parameters. To examine this reliability under conditions of drought and high temperature, we tested the set of 35 of summer wheat winter form (*Triticum species*) with various ploidies and tolerance, which were grown in pots. Non-invasive methods were used to identify the physiological properties of plants; for evaluation of chlorophyll fluorescence parameters characterizing the structure and state of photosystem 2 (PSII) (Handy Pea, Hansatech, GB), specific photosynthetic parameters derived from absorbance and fluorescence measurements (PhotosynQ, USA) and determination of relative water content (RWC). The problem of tolerance to environmental stress is very complex. The relative water content (RWC) in the leaves of individual genotypes exposed to dehydration and high temperature shows variability in values from 65 to 80%, which results in differences in the regulation of the water regime under stress conditions. Based on the analyses of fluorescence measurements, our results show that the effect of stressed factor was highly significant for the parameters of maximum photochemical efficiency of PSII (Fv/Fm) and especially non-photochemical quenching (NPQt). The final stress caused the inhibition of linear electron transport (LEF) and the number of active PSI reaction centers (RC/ABS parameter). The stated conditions caused an increase in variable fluorescence, measured by the rapid kinetics of chlorophyll fluorescence, in a time of 0.3 ms (Wk). Thus, the analyses applied in our study seem to be useful as a valuable and reliable tools for monitoring the climate extremes in wheat varieties exposed to stress conditions (interaction of drought and high temperature).

Keywords: wheat, enviromental stress, drought, high temperature, chlorophyll fluorescence.

Non-destructive quantification of nutrient deficiency symptoms in common bean

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Abstract

High yields in crop production rely on a constant supply with high amounts of plant nutrients. Thus, inappropriate nutrient supply can cause significant economic losses. Nutrient deficiency alters plant morphological and physiological traits, i.e. cause specific symptoms which could be detected by non-destructive methods, using spectral reflectance analyses. This study aimed to describe and quantify the development of the nutrient deficit (nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and iron (Fe)) in common bean (*Phaseolus vulgaris* L.) using multispectral and chlorophyll fluorescence imaging. Plants were grown in a control nutrient solution and in solutions lacking N, P, K, Mg or Fe and were analyzed every three days during 12 days of growth.

Irrespective of measured traits, earliest symptoms and most pronounced changes were detected for K and N deficiency, whereas slowest and least pronounced symptoms development were found in Fe deficiency. From the fluorescence parameters, the earliest and most pronounced response was detected in electron transport rate, which decreased after six days for all treatments compared to control, except for the Fe deficiency. Of the morphological traits, the earliest response reduction in leaf area, found at sixth day in all treatments compared to the control. From multispectral traits, the earliest and most significant differences compared to control were reduction in green leaf index and increase in specific green reflection. These results show that nutrient deficiency could be detected and traced nondestructively using multispectral and chlorophyll fluorescence analysis.

Keywords: common bean, nutrient deficiency, multispectral analysis, chlorophyll fluorescence, symptom development

Phenotyping of Croatian native grapevine (*Vitis vinifera* L.) varieties in susceptibility to *Plasmopara viticola*

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Abstract

A long history of grapevine cultivation in diversified geographical regions in Croatia gave rise to a high number of native varieties. In the era of sustainable production, there is a growing demand to define their differences in susceptibility to downy mildew.

By applying leaf disc bioassay in controlled laboratory conditions, it has been found that native varieties react differently to the infection of *Plasmopara viticola*. Therefore, they were ascribed to classes of resistance according to the OIV descriptor 452-1 [Leaf: degree of resistance to *Plasmopara* (leaf disc test)]. The leaves were analysed using high-performance liquid chromatography (HPLC) to define if their chemical background, i.e., polyphenolic composition, is responsible for native varieties' different levels of resistance. The leaf disc test is a simple method to perform, and it brings about trustworthy results when genotypes with a known level of resistance are comparatively evaluated. Polyphenolic compounds proved to be responsible for the discrimination of varieties among the OIV classes of resistance. It has been found that the innate constitutive polyphenolic profile contributes to the separation of susceptible OIV classes (1, 3, and 5) into three groups. The content of resveratrol-3-*O*-glucoside and total stilbenes discriminated non-infected and infected samples, whereas the content of piceatannol and total stilbenes discriminated completely resistant OIV class 9 (*V. riparia*) and the remaining OIV classes.

Less susceptible grapevine varieties that belong to OIV class 5 (Malvazija istarska, Ranfol, Teran) could be interesting to use in breeding programs aiming to produce high-quality genotypes resistant to main fungal diseases.

Keywords: *Vitis vinifera* L., downy mildew, biotic stress, polyphenols



Relative expression dynamics of GFkV, GVA and GPGV in grapevine host plants

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Abstract

With more than 80 viruses and virus-like agents described, grapevine is a perennial plant species seriously affected by virus diseases. Mixed infections occur naturally in grapevine host plants and contrary to the well-known grapevine viruses (leafroll and infective degeneration causal agents), limited information is presented on relative expression of grapevine fleck virus (GFkV), grapevine virus A (GVA) and grapevine pinot gris virus (GPGV).

In this study, grapevine indicator plants were green grafted with 4 inoculums containing GFkV, GVA and GPGV in different combinations. Cortical scrapings were collected in 3 sampling periods: at dormancy phenological stage (5 and 16 months post inoculation) and the beginning of bud swelling (8 months post inoculation). Total RNA was extracted using commercial kit followed by cDNA synthesis. Using SYBR green based qPCR, relative expression of the virus genes was determined by normalizing their Ct values with those of housekeeping genes.

This study showed that relative expression of GVA was decreasing continuously in all three sampling periods. Relative expressions of GPGV and GFkV significantly decreased in bud swelling stage compared to the first dormancy stage, but not from the second. In conclusion, relative expression of virus genes varied significantly in different sampling periods and it was virus dependent.

Keywords: grapevine viruses, GVA, GFkV, GPGV, Qpcr



The effect of *Fusarium* inoculation and nitrogen fertilization on phenolics and phenylalanine ammonia-lyase

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Abstract

Wheat (*Triticum aestivum* L.) is exposed to a variety of abiotic and biotic stresses that have a significant impact on its yield and quality. *Fusarium* head blight and inadequate nitrogen fertilization can cause numerous biochemical changes in wheat. The main aim of this study was to determine the effects of *Fusarium* inoculation and a broader range of different nitrogen fertilization on the defense response in the spikes of four wheat varieties that differ in their susceptibility to FHB. Total soluble phenolics content (PHE) and phenylalanine ammonia-lyase (PAL) activity were determined as indicators of defense response. In both growing seasons, *Fusarium* inoculation resulted in an increase in PHE content in the partially resistant varieties (Apache and Graindor), indicating the involvement of PHE in the defense response and better disease tolerance in the more resistant varieties. Furthermore, positive correlations were found between PHE content and PAL activity in the Graindor variety, suggesting that both PAL and PHE contribute to better FHB tolerance in more resistant varieties. In conclusion, breeding wheat varieties with increased PHE synthesis could be a promising strategy for FHB management.

Keywords: wheat, fusarium head blight, nitrogen, phenols, defense response



Genomic selection in legumes: the experience at CREA

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Abstract

Genomic selection has high potential for accelerating variety improvement on a wide spectrum of species. It works on either allogamous or autogamous organisms, doesn't require a reference genome and in the right conditions genomic prediction models can reach nearly optimal accuracy. As of today, genomic selection has been applied at Italian CREA institute for seven years on different legume plants used both for human and animal consumption. From the point of view of the bioinformatics analysis, several patterns emerged regardless of the specific plant and target phenotype. During this talk we'll discuss in general terms what makes the ideal trait for genomic selection, what is the required number of molecular markers, the break-even point where genomic selection becomes economically preferable to traditional selection and the genotyping techniques to be preferred in this context.

Factors affecting the accuracy of genomic selection for quality traits within a biparental wheat population

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Abstract

Genomic selection is one of the recently deployed approaches to breeding which aims to reduce the potential costs of phenotyping that occur in the classical breeding process. The basic principle of genomic selection consists of the estimation of marker effects using phenotypic and genotypic data of a training population that will be later used to calculate genomic-estimated breeding values (GEBV) of a validation population. The success of genomic selection depends on the obtained prediction accuracy which can be affected by many factors. The present research aimed to assess the impact of marker density, training population size, and chosen model on prediction accuracy when predicting quality traits within a biparental population. The population comprised of 153 recombinant inbred lines (RILs) derived from a cross between Monika and Golubica cultivars. The experiment was conducted for 3 consecutive years at Osijek and Slavonski Brod (Croatia) and predictions were done across all six environments. Obtained phenotypic data included grain protein content (GPC), test weight (TW), and time required for optimal dough development (midline peak time; MPT). To assess the effect of marker density on prediction accuracy two sizes of marker dataset ($N_M = 2231$ and $N_M = 1123$) were used in predictions, while the assessment of training population (TP) size was done using 50%, 60%, 70% and 80% of the population as TP (70, 83, 97, 111 RILs). Prediction models used in the present study were RR-BLUP, BayesLASSO, Elastic Net, and Random Forest. Broad-sense heritabilities for all traits were high ($H^2 \geq 0.78$). A positive impact of the increase in population size and marker density was noticed for all investigated traits. The highest mean prediction accuracies were obtained using BayesLASSO, RR-BLUP, and Random Forest for GPC, TW, and MPT, respectively. In overall, the highest prediction accuracies were observed for MPT ($r = 0.43 - 0.57$), followed by GPC ($r = 0.39 - 0.49$) while genomic selection for TW seems to be least effective ($r = 0.29 - 0.37$).

Keywords: wheat, quality, biparental population, genomic selection, prediction accuracy

Resistances to major fungal pathogens - breeding progress and challenges in wheat and barley

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Abstract

In wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) an average of 20 to 25% of the potential harvest is destroyed worldwide by fungal and viral pathogens as well as by insects. Therefore and by the changing abundance of pathogens due to climate change, as well as in the context of a more sustainable crop-production to conserve natural resources, resistance breeding is of particular importance.

With the comprehensive BRIWECS study, based on commercially successful wheat varieties in Western Europe over the last five decades, we showed that breeding improved variety performance under optimal but also at production systems with reduced agrochemical input levels. Genetic variants with favorable effects on yield-determining parameters such as photosynthetic and nutrient use efficiency have been incrementally accumulated in new varieties while grain quality could be maintained. Yield progress has been accompanied by improved disease resistance, leading to enhanced yield stability. Our genetic analyses imply that improved resistance largely based on quantitative, durable resistance loci that have been enriched in modern varieties.

To continue such genetic gain it will be necessary to exploit resistances from genetic resources such as MAGIC populations, land races and crop-wild relatives. Therefore, the JKI and partner institutions regularly assess the resistance status of large diversity panels against various pathogen isolates to identify potential resistance donors. By using high-throughput phenotyping technologies, improved genotyping technologies and full genome sequence information as well as recent statistical methods we assume that the resistance level of wheat and barley elite lines can be further enhanced.

Keywords: wheat, barley, fungal pathogens, resistance progress



Utilisation of Maize Genetic Resources for Agro-Biodiversity Enhancement

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Abstract

Maize Research Institute „Zemun Polje“ gene bank preserves *ex-situ* an active collection (5806 accessions). Local landraces are the most significant, since they were created by natural selection and adapted to local growth conditions. In the last 15 years, in MRIZP gene bank was conducted identification of superior genotypes for specific traits and drought tolerant and improved grain quality core collections were established. Moreover, exchange of inbred lines from MRIZP gene bank and Agricultural Institute Osijek working collections, genotyping and phenotyping of inbreds, and statistical analysis of molecular and agronomic data, is conducted through ongoing Project “Harmonization of methods for phenotyping, genotyping and management of genetic resources in maize”. Although the accessions conserved in gene banks are valuable sources for breeding varieties more nutritious, productive and resilient to pests, diseases and climatic changes, there is a limited information of their agronomic characteristics. To overcome this, the European Evaluation Network (EVA) was established in 2019 by the European Cooperative Programme for Plant Genetic Resources (ECPGR). All activities within the EVA networks are intended at the pre-breeding stage, to increase the knowledge about publicly available crop germplasm, with aim to introducing these into breeding programmes. Through crop-specific public–private partnerships, maize EVA is generating standardized evaluation data (both the phenotypic and the genotypic) for numerous accessions and landraces available in European gene banks. During the project, a total of 95 MRIZP accessions will be genotyped and evaluated in multilocation experiments for further use by breeding companies.

Keywords: core collection, EVA, gene bank, pre-breeding, *Zea mays* L.



On the cusp of Breeding 4.0: Molecular plant breeding and interdisciplinarity

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Abstract

Thirty years ago, molecular plant breeding as an application of molecular biology tools in agriculture, predominantly included molecular biologists and agronomists. To make plant breeding more efficient, molecular biologists were focused on genotype using methods of genetic transformations and, recently, gene editing. On the other hand, agronomists, i.e. plant breeders were mostly interested in how molecular plant breeding could bridge the genotype-phenotype gap applying marker assisted selection (QTL mapping, GWAS or genomic selection). This breeding era is also known as Breeding 3.0 and it is the current state of the art characterized by inexpensive (pan)genome-wide data. However, it was shown that typical reductionist approach in genomics had limited potential for enhancing selection for quantitative traits in crops. The most important question is still how to adapt crops to better fit agricultural environments. It implies a (re)assessment of genotype \times environment interaction on closing the genotype-phenotype gap. Moreover, traditional discipline-centered approaches should be converted to the integrative genotype \times environment \times management (G \times E \times M) framework to search the full spectrum of G \times E \times M combinations forming the adaptation landscape. It entails integration of vast data sets including high-throughput genomics, phenomics and other omics data applying also new paradigms such as precision agriculture and machine learning.

Keywords: breeding eras, crops, genomics, genotype-phenotype gap, molecular plant breeding

The usability of proximal sensing in modern maize breeding programs

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Abstract

Besides breeder's notes, modern maize breeding programs take only two types of objective information for selection of favorable progenies: genomic data and yield performance, overlooking the underlying biological complexity. Understanding the process of yield formation implies the need for increased information density during the vegetation period. We developed a novel low-cost proximal sensing node with reflectance reads at six adjusted wavelengths (610, 680, 730, 760, 810 and 860 nm). The nodes were set to multiple breeding trials in 2021 and 2022 growing seasons and the measurements were collected throughout the flowering and grain filling stages. It was shown that the reflectance reads and the derived vegetation indices can be used for monitoring of crop physiological state through modeling of plant photosynthetic efficiency. However, to utilize such proximal sensing nodes, the collected phenotypic data should be integrated with data on molecular level, such as SNP reads, thus increasing the information density on the whole system level. The possibilities for such integration and prospects of use for the developed proximal sensing node in a modern maize breeding program in the era of machine learning will be discussed.

Keywords: proximal sensing, remote sensing, maize breeding, SNP, machine learning

Allelic impacts of SNP markers on pre-harvest sprouting resistance in a panel of winter wheat (*Triticum Aestivum L.*) cultivars

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Abstract

Selection for pre-harvest sprouting (PHS) resistance in wheat is often costly and time-consuming, especially in early segregating generations when a large number of lines are phenotyped. The objective of this study was to evaluate the phenotypic variation in PHS resistance of 200 winter wheat cultivars adapted to growing conditions in SE Europe and to investigate the allelic impact of 38 KASP (SNP) markers on their PHS resistance. Cultivars were examined for their PHS phenotype based on germination index (GI) in four environments. The combined ANOVA across environments revealed that genetic (G), environmental (E), and G × E interaction variance accounted for 46, 22, and 27% of the total phenotypic variance, respectively, with an estimated broad-sense heritability of 0.86. After excluding markers with an extremely high rate of missing data and those that are monomorphic, a total of 21 polymorphic SNP markers were analyzed for their effects on GI, considering only homozygous genotypes that have either a tolerant or a susceptible allele. Analysis of variance across environments revealed that GI was significantly affected by environment (E) for 21 markers, genotype of the marker (G) for 12 markers, and G × E interaction for eight markers. Of the 12 markers that showed a significant genotypic effect on GI, two were from chromosome 3A, two from 3B, six from 4A, and one each from chromosomes 5A and 7B. The functional marker TaMKK3, developed from sequence polymorphisms in allelic variants of the mitogen-activated protein kinase kinase 3 (MKK3) gene on chromosome 4A, showed the greatest impact on PHS resistance. The results of the present study may be useful in the selection of markers for marker-assisted selection for PHS resistance in wheat.

Keywords: wheat, germination index, KASP markers, TaMKK3

Novel genetic factors controlling seed quality in grass pea revealed by metabolic genome wide association study

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Abstract

With plant-based eating habits increase, grain legumes are becoming more popular. Legumes are rich in protein and metabolites with health promoting effects. However, not all grain legumes are equal in terms of quality and limited data is available on some promising species, hampering their use and improvement. Being an important source of protein and calories in drier areas, with ability to respond to climate challenges, *Lathyrus sativus* (grass pea) is one such understudied example.

To reduce this knowledge gap, the primary metabolism of a worldwide grass pea collection of accessions was characterized, and their genetic basis and environmental influence analyzed. Seeds were harvested from a three years repeated field trial and milled into flour.

Using GC-TOF-MS primary metabolites were relatively quantified. Forty-eight primary metabolites were identified, including amino acids, sugars, organic acids, among others. Clear environmental differentiation was observed based on the metabolomic profiles, and 43 of the 48 metabolites revealed significant differences between genotypes. Nevertheless, only two metabolites showed a GxE interaction variance component superior to the genotypic.

To unravel the genetic basis of these metabolites content, adjusted means were combined with 5,651 SNPs through GWAS. Sixty-seven genomic regions were associated with the 43 metabolites variation, with six regions associated with multiple traits. Candidate genes are involved in solute transport, RNA processing/biosynthesis, among other functions.

The generated knowledge on metabolite variation and associated genomic regions will support precision breeding efforts to answer consumers' nutritional quality concerns and increase the use of grass pea in diets.

Keywords: *Lathyrus sativus*, legumes, mGWAS, primary metabolism, seed quality.

Genome-wide association study for seed quality traits in soybean [*Glycine max* (L.) Merr.]

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Abstract

The soybean [*Glycine max* (L.) Merr.] is the world's leading oil and protein crop, recognized as a high-quality food and feed source. The value of soybean comes from the chemical composition of the seed, which is rich in proteins, oils, and essential amino acids. Due to the great potential of soybean as a food crop and to meet market demands, intensive work is being done worldwide to improve the genetic basis for seed quality. Using a whole-genome association study, it is possible to detect quantitative trait loci (QTL) associated with desired traits and accelerate their introgression into breeding lines through marker-assisted selection compared to the traditional approach. To investigate the genetic basis of variation in oil and protein content in soybean seeds, a diverse collection of 192 mainly European soybean cultivars was grown over two years and at two locations under field conditions. The mapping panel was phenotyped using near-infrared spectroscopy and genotyped by the SoySNP50K beadchip. Using principal components and the fixed and random model circulating probability unification (BLINK), positive signals for protein content were detected on chromosomes 12, 16, 17, and 20, some of which have been reported in previous studies. Regions related to oil content were detected on chromosomes 2, 7, 10, 17, and 18. Soybean is deficient in sulfur-containing amino acids compared with animal proteins. In this study, methionine signals were detected on chromosomes 3 and 18 and cysteine on chromosomes 17 and 20, respectively. The identified QTLs and desirable germplasm (donors) could be used for further improvement of soybean seed quality traits in breeding programs.

Keywords: soybean, GWAS, protein, oil, amino acids

Topics on the standardisation of chloroplast DNA sequence data

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Abstract

The number of available DNA sequences in public genetic databases is constantly increasing. In plants, this is particularly evident in the amount of available complete chloroplast genomes, which are widely used in phylogenetic research. Chloroplast DNA genomes are circular and most have a four-part structure caused by two copies of a large inverted repeat (IR). We investigated inconsistencies in publicly available chloroplast genome sequence data regarding how stored public data account for structure.

Our results show that there is no standardization in the storage of chloroplast genome sequences with respect to the structure of inverted repeats, as sequences are stored in different orders. Furthermore, there are many sequences in the public data without annotated inverted repeats, although these repeats are expected. In reviewing specialized chloroplast annotation tools, we found that there is no uniform method for identifying inverted repeats. Each tool analyzed takes a different approach and covers different specific situations.

These results show that there is a need to standardize formats when it comes to storing data of specific types such as chloroplast sequences. Our results suggest that the existing public chloroplast data should be revised in terms of standard storage format and missing data. In addition to stored data, we found that specialized chloroplast annotation tools need improvement regarding the detection of inverted repeats.

Keywords: chloroplast genome, standardization, annotation, inverted repeats, repeat identification