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2024 Bayannur, China

Proceedings of the

21st International Sunflower Conference

August 20-24, 2024

Wuyuan, Inner Mongolia, China

Organizers

The International Sunflower Association (ISA)

The Inner Mongolia Sunflower Association (IMSA)

Inner Mongolia Agricultural University (IMAU)

Yuan Longping High-tech Agriculture Co., Ltd.

Co-organizers

The People’s Government of Wuyuan

Sanrui Agritec Co., Ltd.

Sunboy（Inner Mongolia） Food Group Co., Ltd.

Chacha Food Co., Ltd.

PREFACE

The 21st International Sunflower Conference was successfully held in Wuyuan, Inner Mongolia, China, from August 20-24, 2024. The 5-day conference consisted of plenary talks, scientific workshops and poster sessions. The conference centered around the theme of "**Fostering A Dialogue Between Confection and Oilseed Sunflower**" providing a dynamic platform for collaboration, and sharing of the latest achievements in breeding research and industry development. Topics of interest included Genetics and Breeding, Genomics and Biotechnology, Cultivation & Crop Production, Germplasm Resources and Utilization, Broomrape & Herbicide Resistance, Biotic Stress and Abiotic Stress, and other fields related to the entire sunflower industry chain. We believe that this event will strengthen the global scientific sunflower community, as well as, demonstrate the dynamic changes and significant accomplishments of the confection sunflower industry in China.

During the 5-day event, over 360 participants from 24 different countries attended the conference. The conference had 11 invited plenary talks and 43 oral presentations covering 8 different topical sessions. The organization committee received 95 abstracts with 35 poster presentations during the conference. Participants during the Conference had the opportunity to visit the impressive field demonstration trials of 139 elite sunflower varieties including 93 confection hybrids, 35 oil type hybrids, 2 dual-purpose hybrids, and 9 ornamental hybrids from 30 domestic and foreign research institutes or companies.

At the closing ceremony, the ISA awarded Prof. Philippe Debaeke, Dr. Vladimir Miklic, Dr. Begoña Pérez-Vich, and Prof. Loren Rieseberg the Pustovoit Award for their significant contributions to sunflower research. The organizing committee also presented the best poster awards to three graduate students: Jeslca Cespedes Martinez from National Institute of Agricultural Technology, Argentina; Yating Wang from the Inner Mongolia Agricultural University, China; Emrah Akpina from Trakya University, Turkey. Additionally, two young researchers, Yanke An from Sanrui Agritec Co. Ltd., China and Audrey Gantell from SOLTIS, France also received an honorable mention award for their excellent posters.

Finally, we are grateful for the International Sunflower Association (ISA) granting us the opportunity to organize and host such an important conference in China. Thank you to all the participants who came from all over world to be here and support the conference. Also, we greatly appreciate with the organizers, Inner Mongolia Sunflower Association, Inner Mongolia Agricultural University, the People's Government of Wuyuan, Yuan Longping High-tech Agriculture Co. Ltd., Sanrui Agritec Co. Ltd., Sunboy Food Technology Group Co. Ltd, Chacha Food Co. Ltd., Gansu Jiarui Seed Co. Ltd., and BASF Agriculture for their diverse roles and finical support for making this a very successful event.

In this golden autumn of August, let's build dreams for the future! We wish you all a successful career in sunflower research and industry development!

Chair: **Prof. Jun ZHAO**
President of ISA
The Scientific Committee

Chair: **Mr. Yongping ZHANG**
Co-Chair: **Dr. Jiuhuan FENG**
The Organizing Committee

21st International Sunflower Conference
Wuyuan, Inner Mogolia, August 20-24, 2024

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The 21st International Sunflower Conference
PROGRAM AGENDA

20 August (Tuesday)	
8:00-20:00	Registration at Hotel
18:30-20:30	**Dinner**
21 August (Wednesday)	
9:00-10:00	Opening Ceremony / Photo Taken
10:00-10:30	**Coffee Break**
10:30-12:00 (HALL A)	**INVITED PRESENTATIONS** **Chair by Vladimir Miklic** **Plenary 1**: Etienne Pilorgé (Terres Inovia, France) Sunflower in the global food system: situation and perspectives **Plenary 2**: Loren Rieseberg (University of British Columbia, Canada) Pervasive structural variation in Helianthus: implications for sunflower breeding **Plenary 3**: Li Lianshe (Sanrui Agritec Co., Ltd., China) Recent progress of confection sunflower breeding and production in China
12:00-14:00	**Lunch**
14:30-16:00 (HALL A)	**Oral Presentation 1: Genetics & Sunflower Breeding** **Chair by Chao-Chien Jan** 1. **Belén Fernández-Melero** (Institute for Sustainable Agriculture, Spain) Sunflower genes on chromosome 4 confer different resistances to the parasitic plant *Orobanche cumana* 2. **Huang Kaichi** (University of British Columbia, Canada) The genomics of linkage drag in inbred lines of sunflower 3. **Yu Yue** (University of British Columbia, Canada) Genomic insights into disease resistance in sunflowers: identifying key regions and candidate genes for *Verticillium dahliae* resistance 4. **Audrey Ganteil** (SOLTIS, France) Comparison of sunflower breeding strategies using a simulation approach 5. **Zhao Cunpeng** (Hebei Academy of Agriculture and Forestry Sciences, China.) Physical mapping and candidate gene prediction of fertility restorer gene of cytoplasmic male sterility in oil sunflower based on BSA-seq technology
14:30-16:00 (HALL B)	**Oral Presentation 2: Cultivation & Crop Production** **Chair by Nicky Creux** 1. **Vladimir Miklic** (Institute of Field and Vegetable Crops, Serbia) Production of sunflower seed in new conditions 2. **Olabisi Somefun** (Federal University of Agriculture, Nigeria) Agronomic responses of sunflower (*Helianthus annuus* L.) introduced at different dates into sesame-based intercropping system in a humid tropical location 3. **Zhang Xili** (Bayannur Modern Agricultural & Animal Husbandry Development Center, China) Current status of confectionery sunflower production in Bayannur China 4. **Fernando Turienzo Aguilar** (International Seed Sales Manager Valia Genetics- Argensun)

	Is it feasible to anticipate the chemical desiccation of female inbred lines in confection sunflower seed production?
16:00-16:30	**Coffee Break**
16:30-17:30 (HALL A)	**Oral Presentation 3: Genomics & Transcriptomics** **Chair by Nicolas Langlade** 1. **Huang Qixiu** (Xinjiang Academy of Agricultural Sciences, China) Preliminary exploration of the mechanism of confectionary sunflower resistance to broomrape based on RNA-seq 2. **Li Juanjuan** (Zhejiang University, China) *bZIP* genes identification and the signal functions to flowering in *Helianthus annuus* L 3. **Huang Jiaying** (Inner Mongolia Agricultural University, China) Identification of signal peptide secretory function of metalloprotease PhMEP1 from *Puccinia helianthi*n 4. **Hou Junbin** (Northwest A&F University, China) Identification and dual RNA-seq analysis of sunflower (*Helianthus annuus* L.) head rot pathogen 5. **Shao Ying** (Inner Mongolia Academy of Agricultural &Animal Husbandry Sciences) Transcriptome analysis reveals the potential molecular mechanism involved in fatty acids biosynthesis of sunflower
16:30-17:30 (HALL B)	**Oral Presentation 4: Germplasm Resources and Utilization** **Chair by Nada Hladni** 1. **Sujatha Mulpuri** (ICAR-Indian Institute of Oilseeds Research, India) Interspecific hybridization of cultivated sunflower with *Helianthus praecox* towards transfer of resistance to powdery mildew 2. **Dudhe Mangesh Yuwaraj** (ICAR-Indian Institute of Oilseeds Research, India) Current status, issues and challenges of sunflower genetic resources in India 3. **Tan Meilian** (Chinese Academy of Agricultural Science, China) Collection, preservation, identification and evaluation of sunflower germplasm 4. **Chang Min** (Sanrui Agritec Co., Ltd., China) Resistance of wild *Helianthus* species to the prevailing Chinese broomrape races and new gene identification 5. **Lv Jian** (Syngenta Biotech China) Haploid facultative parthenogenesis in sexual sunflowers
18:30-20:00	**Welcome Reception**
22 August (Thursday)	
8:30-9:30 (HALL A)	**INVITED PRESENTATIONS** **Chair by Loren Rieseberg** **Plenary 4**: Stéphane Muños (LIPM-INRAE, University of Toulouse, France) Recent advances in the resistance, diversity, and interaction of the *O. cumana*/ sunflower pathosystem **Plenary 5**: Sandra Cvejić (Institute of Field & Vegetable Crops, Serbia) Innovations in sunflower breeding for enhanced drought adaptation
9:30-10:00	**Coffee Break**

10:00-12:00 (HALL A)	**Oral Presentation 5: Broomrape & Herberside Resistance** **Chair by Maria Duca (1-4) & Begoña Pérez-Vich (5-8)** 1. **Leonardo Velasco** (Institute for Sustainable Agriculture, Spain) Genetic recombination of virulence alleles as a mechanism of racial evolution of sunflower broomrape in Spain 2. **Li Juanjuan** (Zhejiang University, China) Resistance potential of *HaCERK1* gene in response to *Orobanche cumana* infection in sunflower 3. **Elena Dangla** (Paul Sabatier University, France) The STIGO project: understanding the molecular mechanism for *Orobanche cumana* seed germination 4. **Wang Bing (**Institute of Chinese Academy of Sciences, China) Mechanism of strigolactone pathway and its roles in *Orobanche* resistance in sunflower and tomato 5. **Xu Ling** (Zhejiang Sci-Tech University, China) Mechanism study on 5-aminolevulinic acid enhancing sunflower resistance to *Orobanche cumana* Wallr. 6. **Boško Dedić** (Institute of Field and Vegetable Crops, Serbia) Resistance of IFVCNS inbred lines to race F with origin from Spain 7. **Zhang Jian** (Inner Mongolia Agricultural University, China) Genetic diversity analysis and physiological race identification of *Orobanche cumana* Wallr. collected from China and Eastern Europe countries 8. **Matthias Pfenning** (BASF SE, Germany) Combination of Prohexadione calcium and Imazamox provides synergistic control of *Orobanche cumana* in Clearfield® sunflower
10:00-12:00 (HALL B)	**Oral Presentation 6: Physiology and Abiotic Stress** **Chair by Yalcin Kaya** 1. **Maria Duca** (Moldova State University, Moldova) Drought tolerance mechanisms in sunflower: molecular physiological and morphological responses 2. **Aleksandra Radanović** (Institute of Field and Vegetable Crops, Serbia) Unravelling mechanisms of drought tolerance and stress recovery in sunflower 3. **Jing Bing** (Northwest A&F University, China) The Impact of drought and salt stress on leaf wax accumulation and cloning of wax alkane synthesis genes in sunflower (*Helianthus annuus* L.) 4. **Wu Yang** (Inner Mongolia Agricultural University, China) Research on drought-resistant mechanism and variety selection of sunflower (*Helianthus annuus* L.) 5. **Zhou Yingying** (Zhejiang University, China) Whole-genome identification and expression of lateral organ boundaries domain genes under biotic and abiotic stresses in *Helianthus annuus* L.
12:00-14:00	**Lunch**
14:30-15:30 (HALL A)	**INVITED PRESENTATIONS** **Chair by Stéphane Muños** **Plenary 6**: Norma Paniego (National Institute of Agricultural Technology, Argentina) Unraveling sunflower resistance to fungal pathogens in Argentine germplasm through genomic analysis **Plenary 7**: Chen Tao (Hangzhou Normal University, China)

	The T2T genome of confection sunflower and molecular mechanism of head domestication
15:30-16:00	**Coffee Break**
16:00-18:00 (HALL A)	**Oral Presentation 7: Disease Resistance & Pest Control** **Chair by Maria Joita-Pacureanu (1-4) & Norma Paniego（5-8）** **1. Nomfundo Shinga** (University of Pretoria, South Africa) Investigating the influence of plant growth regulators on mycelial growth and sclerotia production of *Sclerotinia sclerotiorum* **2. Yang Jianfeng** (Inner Mongolia Agricultural University, China) Unravel biological mechanism under decreasing disease severity of sunflower *Verticillium* Wilt via delaying sowing date **3. Thibault Roudaire** (University of Toulouse, France) Using multi-service cover crops to control sunflower soil-borne pests **4. Zhang Wenbing** (Inner Mongolia Agricultural University, China) Trapping effect of different colored sticky boards to *Frankliniella intonsa* in sunflower field **5. Qu Hui** (Inner Mongolia Agricultural University, China) Study on green control strategy of *Frankliniella intonsa* (Trybom) in sunflower field based on chemical ecology **6. Shi Li** (Inner Mongolia Agricultural University, China) Diversity of arthropod communities and population dynamics of *Frankliniella intonsa* of the sunflower field in Inner Mongolia **7. Lu Yan** (Inner Mongolia Agricultural University, China) Study on spore germination and infection process of *Puccinia helianthin*
16:00-18:00 (HALL B)	**Oral Presentation 8: Seed Quality & Processing** **Chair by Leonardo Velasco** **1. Yalcin Kaya** (Trakya University Engineering Faculty, Turkey) Quality determination in confectionery sunflower **2. Christophe Nguyen** (National Institute for Agriculture, Food, and Environment, France) Cadmium in sunflower seeds: different contents in hulls and kernels and consequences for food and feed industry **3. Nada Hladni** (Institute of Field and Vegetable Crops, Serbia) A novel-based technique for 3D shape acquisition of confectionery sunflower seeds and associated shape descriptions **4. Patrick Carré** (Terres Inovia, France) Explaining the variability of sunflower hullability **5. Yin Wenting** (Henan Technology University, China) Enzyme treatment combined with Maillard reaction modification of sunflower meal hydrolysates to prepare sunflower seed oil flavor enhancer
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Session 1: Genetics and sunflower breeding

Genetics and breeding of sunflower for oil quality

Yakov Demurin*

All-Russian Research Institute of Oil Crops V.S. Pustovoit (VNIIMK), Krasnodar, Russia

*yakdemurin@yandex.ru

Abstract: Sunflower is a relatively young field oilseed crop in the global agricultural production, but scientific research and breeding for oil quality have developed quite effectively. In this regard, an analytical review of the work done in this area seems interesting. The main biochemical characteristics of oil quality include the fatty acid composition of reserve triglycerides, the total content and composition of fat-soluble accompanying substances, such as tocopherols and phytosterols. The concept of “end-to-end characters” of oil quality is based on the sequential steps of their biosynthesis in fat-storing seed cells under field conditions and the transition to oil raw materials and food products, determining their optimal use. To date, three genetic sources of high-oleic sunflower are known. However, a mutation from the Pervenets variety has received wide breeding use. This dominant mutation in some genotypic environments may have incomplete penetrance, leading up to reversion to the wild type. Recessive mutations of high palmitic and stearic acid content allow to obtain semi-solid oil from sunflower seeds for the confectionery industry. The development of sunflower with low saturated acid level also attracts attention from the nutritional point of view. Recessive mutations of tocopherol composition, significantly changing the antioxidant potential of these minor substances, allow to create sunflower hybrids with significantly increased oil stability to oxidation. On the other hand, there is a possibility of genetic increase of total tocopherol content in seeds in order to enhance vitamin E potential of oil. The advisability of using genetic variation in the total content and composition of phytosterols, as well as triglyceride composition of oil, is not obvious. With regard to any genetic control of biochemical traits of oil quality by genotype of seed embryo, dominant or recessive, it is necessary to create both parental lines with the changed trait to maintain the desired level of oil quality in the raw material seeds of the hybrid. The use of molecular markers increases the efficiency of oil quality research, especially in case of speed-breeding.

Keywords: Fatty acid; Tocopherol; Mutation; Oil

Recent progress of confection sunflower breeding and production in China

Lianshe Li*, Yongping Zhang, Jiuhuan Feng, Xingchun Dong, Min Chang

Sunflower Research Institute, Sanrui Agritec Co., Bayannur, Inner Mongolia 015100, China

* 343442480@qq.com

Abstract: In the past two decades, China has made significant achievements in the production and breeding of confection sunflower through four phases of variety improvement: from traditional family farming, selection of conventional varieties, introduction of foreign hybrids, and development of domestic hybrids. The sunflower production area in China has remained stable at approximately 0.59 million hectares, with a yield of around 2.4 million tons, making it an important economic crop in regions such as Inner Mongolia, Xinjiang, and Jilin.

China primarily focuses on confection sunflowers, accounting for over 95% of the total area, which has made China a major producer of confection sunflowers globally. Currently, the cultivation of sunflowers is gradually evolving towards large-scale, standardized, and industrialized practices, to meet new challenges for the confection sunflower industry.

Over the past 20 years, many breeding institutions and seed companies have enhanced the collection and utilization of germplasm resources, including wild species, landraces, and cultivated varieties, significantly enriching the sunflower germplasm pool. Particularly, favorable genes for resistance to disease, such as *Orobanche* and herbicide derived from wild species have been widely used in breeding programs. The application of modern biotechnological methods such as molecular marker-assisted selection, genomic selection and gene editing has become imperative for achieving precise and efficient breeding.

Currently, a whole-genome SNP molecular marker platform has been utilized in sunflower breeding. Sanrui Agritec Ltd Co. has made great strides in resolving the "bottleneck" issues for confection sunflower breeding. A series of high yielding, premium quality and large seed size hybrids have been independently developed and released to the markets further enhancing the development of the sunflower industry. However, some challenges for hybrid breeding and production remain. Addressing these issues is of paramount importance for the future development of the domestic sunflower industry in China.

Keywords: Confection sunflower; Production; Germplasm resources; Molecular technology.

Funding: This study was funded by “Germplasm Utilization and Breeding of New Varieties in Sunflower (2022JBGS0034)”; and “National Joint Collaboration in Sunflower Breeding (YZ2023001)”.

Comparison of sunflower breeding strategies using a simulation approach

Audrey Ganteil, Benoit Bleys and Grégoire Marandel

SOLTIS, Mondonville, FRANCE

Abstract: In silico simulations of various breeding schemes have shown increased interest during the last decade. Germplasm of sunflower can be simulated and breeding strategies compared to promote long-term genetic gain. In this study, the real molecular profiles of 220 restorer lines, consisting of 12000 Single Nucleotide Polymorphism (SNP) markers, were used to simulate virtual founders. We focused the selection on a simulated quantitative polygenic trait. We performed eight different breeding schemes here, they differed by the number of starting crosses (50 or 100 crosses), the size of the F_5 populations generated from these crosses (100 or 200 plants per cross) and the effort of phenotyping in the F_5 generation (1000 or 2000 topcross hybrid progenies). Each breeding scheme was compared in terms of genetic gain and genetic diversity evolution. After 10 selection cycles, we observed that breeding schemes with 50 starting crosses had lower genetic gains than those with 100 starting crosses. Regarding the size of the F5 populations, few differences were observed between population sizes 100 and 200. At the testing cross stage, the scenarios with 2000 tested F_5 population had a higher genetic gain. This analysis highlighted the interest of simulations to compare the key parameters of a sunflower breeding scheme. In particular, the number of starting crosses had a great impact on long-term genetic progress. To optimize the scheme, this study could be supplemented by scenarios enabling other combinations of parameters, and by an economic analysis of the different scenarios.

Keywords: selection scheme; simulation; genomic selection

Funding: SOLTIS

Characterisation of loci Associated with anther colour in sunflower

Audrey Ganteil, Benoit Bleys and Grégoire Marandel

SOLTIS, Mondonville, FRANCE

Abstract: In cultivated sunflowers, the anthers vary in colour from yellow to dark brown. This study compared the molecular profiles of 198 inbreds of cultivated sunflower in which polymorphism in. anther colour was observed. We phenotyped 186 lines with brown anthers and 12 with blond anthers. Each line was genotyped using a 12000 Single Nucleotide Polymorphism (SNP) chip. To identify chromosomal regions differentiating the two groups of lines on the basis of anther colour, we performed a genetic differentiation analysis to compute the fixation index (FST) using PLINK software. Genetic differentiation analysis revealed 12 genomic regions on chromosomes 1, 4, 7, 10 and 15. From the list of annotated genes on the reference XRQv2 genome, we extracted the genes present in the various regions of interest. This list of genes allowed us to perform a functional enrichment analysis to highlight specific molecular functions or biological processes linked to the anther colour phenotype. In particular, our analysis highlighted genes associated with the molecular function of alkene reduction. These initial results suggested that anther colour in cultivated sunflowers is a polygenic trait. The development of a biparental population for the anther colour trait would be an interesting approach to validate these results.

Keywords: Anther colour; Genetic differentiation; Fixation index

QTL Mapping and Genetic Map for the Ornamental Sunflower in China

Jixia Liu[1], Junjian Shan[1], Ping Wang[1]*

[1]Agricultural and Forestry Academic Institution of Ninxia Autonomous Region

Abstract: Quantitative trait locus (QTL) mapping and genetic map are of great significance for ornamental sunflowers in China. In this study, a total of 956.50 Mbp data were obtained, the average Q30 was 93.76%, the average GC content was 42.43%, and the GC distribution of the parents and F2 population of the ornamental sunflowers was normal. At the same time, the double end comparison efficiency of control data was 90.28%, and the enzyme digestion efficiency was 92.01%. The Specific Locus Amplified Fragment (SLAF) library construction was normal. Furthermore, a total of 734,893 SLAF markers were obtained, among which 127,855 were polymorphic SLAF markers and 38,908 could be used for genetic map construction, and the effective polymorphism of the parents was 5.29%. Moreover, we constructed a total of 17 linkage groups, with 6181 markers in the QTL mapping, the total map distance was 2608.66 cM, the marker integrity in the figure above was 99%, the proportion of double exchange was 0.05, the sequencing depth of the parents was 42.455×, and the progeny was 9.24×. The relationship of traits (plant height, stem diameter, disk diameter, number of petals, leaf number, stigma color, petal color, petiole color) and QTL mapping was closely related to show the best of ornamental effect.

Keywords: QTL; Ornamental sunflower; SLAF traits; Genetic map

Mutation breeding on sunflower for resistance to broomrape and herbicide

Mehmet Ibrahim Yilmaz[1*], Necmi Beser[2], Yalcin Kaya[2]

[1]Trakya Agricultural Research Institute, Edirne, Türkiye; [2]Trakya University Engineering Faculty, Genetic and Bioengineering Dept, Edirne, Turkey

*yilmazmehmet22@hotmail.com

Abstract: Sunflower (*Helianthus annuus* L.) grows mainly for oil purposes because sunflower oil is among the most preferred vegetable oils in Turkey as well as in the world. Although it varies depending on the years, sunflower has been planted on approximately 750-800 hectares in recent years in Turkey. In this context, breeding sunflower varieties to be tolerant/resistant to biotic and abiotic stress factors will help prevent yield losses due to these limiting factors and enable economic cultivation in larger areas. Mutation-based breeding methods are favored by plant breeders when modifications of important yield traits, new genetic variations, or the development of new genes are needed. Mutation breeding is widely used by breeders in the world However, it is relatively limited in sunflower, especially in Turkey. In this study, line 2517 A (CMS) registered by Trakya Agricultural Research Institute and 2517 B, which is the maintainer of the sunflower parent line, was used. The general combination ability of the line is quite high, and it is used as the main line of registered sunflower varieties, namely Tarsan-1018, 08 TR 003, DERAY and KAYRA. The seeds of the 2517 B sunflower parental line were treated with Cesium-137 (Cs137), and the variations that occurred in the M_1 and M_2 generations would be investigated. It was aimed to determine new genotypes with superior yield and quality characteristics by determining the morphological characters of the plants in M_1 and M_2 offspring and their resistance to broomrape and herbicide, such as imazamox herbicide active ingredient (40 g/l) and 75% Tribenuron-methyl herbicide active ingredient. Based on first two years results of the study, the significant variations were observed, and some promising genetic materials with desired yield characteristics were also be detected and can be used for genetic breeding.

Keywords: Sunflower, mutation breeding, resistance to broomrape and herbicide

Sunflower genes on Chromosome 4 confer different resistances to the parasitic plant *Orobanche cumana*

Belén Fernández-Melero[1*], Lidia del Moral[1], Mireille Chabaud[2], Alberto Martín-Sanz[3], Stéphane Muños[2], Leonardo Velasco[1], Begoña Pérez-Vich[1]

[1] Instituto de Agricultura Sostenible (IAS-CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain;[2] Laboratoire des Interactions Plantes Microbes-Environnement (LIPME), Université de Toulouse, CNRS, INRAE, Castanet-Tolosan, France;[3] Syngenta España S.A., Carmona, Spain * present address: [2] Laboratoire des Interactions Plantes Microbes-Environnement (LIPME), Université de Toulouse, CNRS, INRAE, Castanet-Tolosan, France

*belen.fernandez-melero@inrae.fr

Abstract: Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the world. *Orobanche cumana*, commonly known as sunflower broomrape, causes significant yield losses in many sunflower producing areas from south-eastern Europe to central Asia and, more recently, in North Africa. *O. cumana* is a holoparasitic weed that grows exclusively on sunflower crops. It attaches to the sunflower roots, being fully dependent on sunflower for its nutrients. Breeding for genetic resistances has been the most efficient method for controlling this parasitic plant. Several resistance genes have been identified to control different sunflower broomrape races, since a gene-for-gene model governs the interaction between sunflower and *O. cumana*. Three new resistance genes, OrAnom1, OrDeb2 and OrSII, have been mapped in the upper part of chromosome 4 of the sunflower reference genome (HanXRQr2.0). This locus shows a structure containing highly similar genes in which a cluster of receptor-like kinases has been identified as candidate genes. It is noteworthy that these three resistance genes trigger different resistance mechanisms. While OrDeb2 prevents entering the cortex cells, OrAnom1 and OrSII trigger a later post-attachment resistance mechanism taking part after vascular connections development and even, in the second case, allowing the broomrape to emerge from the soil before it dies. This suggests that while closely linked or allelic variants of the same gene, the upper part of chromosome 4 of the sunflower genome contain *O. cumana* resistance genes conferring different recognition specificities and resistance mechanisms.

Keywords: sunflower broomrape; resistance genes; resistance mechanisms; genetic mapping

Genetic and molecular characterization of charcoal rot resistance in sunflower

Nemanja Ćuk[1], Sadra Cvejić[1], Aleksandra Radanović[1], Siniša Jocić[1], Dragana Miladinović[1], Vladimir Miklič[1], Milan Jocković[1], Jelena Jocković[1], Brankica Babec[1], Miloš Krstić[1], Boško Dedić[1]*.

[1]Institute od Field and vegetable Crops Novi Sad, National institute of the Republic of Serbia, 21400 Novi Sad, Maksima Gorkog 31.

*bosko.dedic80@gmail.com

Abstract: Charcoal rot, caused by *Macrophomina phaseolina*, is a soil-borne plant pathogen, and presents a significant threat to sunflower cultivation, impacting regions across the globe, particularly under drought stress. This disease can substantially decrease sunflower seed yields, with yield reductions of around 20% potentially reaching up to 90% in severe cases. Limited information about the inheritance of charcoal rot resistance is available to sunflower breeders. To study the inheritance of charcoal rot disease resistance in sunflower. We developed two distinct populations of totaling 200 genotypes from the F_2 generation of sunflowers for genetic and molecular screening. The first population originated from crossing inbred lines AB OR 8 and PB 21, while the second is from crossing VL A 8 PR and AB OR 8. Notably, AB OR 8 is classified as a highly susceptible line, whereas PB 21 and VL A 8 PR were resistant. The segregation ratio was investigated in F_3 populations using the cut-stem method of inoculation. Based on the obtained data, it was determined that the resistance to *M. phaseolina* in sunflower does not correspond to the theoretical segregation pattern, indicating the presence of more than two genes involved in inheritance of resistance. For the analysis of resistance QTLs, we exploited SSR markers that were equally distributed throughout the sunflower genome. The majority of significantly associated SSR resistance markers were not common to the two mapping populations, except for two, OR S995 and ORS1265, which displayed consistent efficacy across both populations, suggesting their potential as robust indicators. Future research should explore these markers further, as well as include more markers, particularly in larger population samples, to validate their utility comprehensively.

Keywords: Sunflower; Charcoal rot; SSR markers; QTLs

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Advances in sunflower breeding to increase oil content and drought resistance

Vladimir Miklič, Milan Jocković, Siniša Jocić, Sandra Cvejić, Nemanja Ćuk, Jelena Jocković, Aleksandra Radanović, Ana Marjanović Jeromela, Dragana Miladinović, Nada Grahovac

Institute of Field and Vegetable Crops, Novi Sad, Serbia

*jockovic@gmail.com

Abstract: Sunflower is a globally important oilseed crop mainly used for oil in human consumption, while secondary products have nutritional value in livestock feed. Neverthless there have been advancements in breeding sunflower genotypes with desirable traits, high yield, and disease resistance, further research is needed to address emerging challenges, such as climate resilience and sustainability. Bearing in mind that sunflower breeding over the last several decades has mainly foucused on biotic and abiotic resilience, sunflower oil content in hybrids has remained at the same level, 40-50%. Considering the trends of climate change, drought tolerance certainly represents an essential trait in breeding programs worldwide. The question arises whether it is time to further orient in sunflower breeding towards increasing oil content, bearing in mind that in light of increasingly frequent dry years. It is very difficult to make progress in seed yield. The aim of our study was to develop sunflower genotypes characterized by drought tolerance and increased oil content (>55%). Initial plant material was selected from a gene pool of the Institute of Field and Vegetable Crops, Novi Sad. We used a traditional approach by crossing highly drought tolerant inbred lines characterized by oil content ranging from 50-52%. Genetic material for drought tolerance was selected based on field trials and *in vitro* drought conditions, over six vegetations (3 vegetations in field conditions and 3 vegetations in *in vitro* conditions). Oil content was determined using nuclear magnetic resonance analyzer (NMR, Maran Ultra-10). The pedigree method of selection was used to develop new genetic material with desirable traits. As a result, we developed seven inbred lines designated as DO1 to DO7 characterized by high drought tolerance and high oil content ranging from 55.09% to 60.39%. Our results revealed that there is a possibility for significant improvement in sunflower oil content while simultaneously breeding for drought resistant. Further studies will include biotechnological tools in order to identify QTLs associated with drought tolerance and increased oil content and to develop markers associated with traits of interest in order to accelerate the breeding process.

Keywords: Sunflower; Drought tolerance; Oil content

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Physical mapping and candidate gene prediction of fertility restorer gene of cytoplasmic male sterility in oil sunflower based on BSA-seq technology

Cunpeng Zhao, Kaihui Wang, Dan Li, Junyi Geng, Suen Liu, Haina Zhang,
Xu Liu, Baosheng Guo*

Institute of Cotton, Hebei Academy of Agriculture and Forestry Sciences,
Shijiazhuang, 050051, Hebei, China.

*guobaosheng111@126.com

Abstract: Cytoplasmic male sterility (CMS) is a maternally inherited trait resulting in the failure to produce functional pollen. It plays a pivotal role in crop heterosis exploitation. The large-scale application of plant DNA re-sequencing has ushered in a new stage of gene discovery. The current study combined the bulked segregant analysis (BSA) with DNA resequencing to identify the candidate genes associated with the fertility restorer gene (Rf) in CMS oil sunflower. Illumina sequencing systematically investigated the parents. A segregating population comprising of 30+30 F_2 individuals was developed using 2012-2005A (female parent) as sterile and 2012-3010R (male parent) as restorer. The information analysis mainly included data quality control (removal of splints and low-quality data), comparison with reference genomes, variation detection and annotation (SNP, InDel), association analysis, and annotation of candidate SNPs and candidate genes. We annotated subsequent regions based on SNP-index analysis, ED association analysis, and whole-genome resequencing. The Clean Bases obtained by sequencing were 246.92 Gbp and Q30 reached 80 %. The average comparison efficiency between the sample and the reference genome was 98.61 %, the average coverage depth was 19.50X, and the genome coverage was 92.93 % (at least one base coverage). A total of 5,353,185 SNPs were obtained between parents, including 68,083 SNPs with non-synonymous mutations. A total of 1,753,094 SNPs were obtained between the mixed pools, and 12,606 SNPs caused non-synonymous mutations. InDel detection: A total of 973,013 Small InDel were obtained between parents; A total of 376,710 Small InDel were obtained between the mixed pools. respectively. Results of the intersection of SNP and InDel: The intersection of the association regions of SNP and InDel was taken, and a candidate region related to the traits was obtained, which was located on chromosome CM007902.1 with a total length of 2.99 Mbp. Forty-eight genes were annotated in the association region, including 29 non-synonymous mutant genes. In this study, associated markers identified by super-BSA could accelerate the study of CMS in oil sunflower, and as well as in other crops. Some of the 48 genes' preliminary characteristics provided useful information for further studies on CMS crops.

Keywords: CMS, High-throughput sequencing; Super-BSA; Oil sunflower

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Comparative transcriptome analysis in two contrasting genotypes for Sclerotinia Sclerotiorum resistance in sunflower

Mingzhu Zhao[1], Bing Yi[1], Xiaohong Liu[1], Dexing Wang[1], Dianxiu Song[1], Enyu Sun[1], Liangji Cui[1], Jingang Liu[1]*, Liangshan Feng[2]*

[1] Institute of Crop Research, Liaoning Academy of Agricultural Sciences, Shenyang, China;
[2]Liaoning Academy of Agricultural Sciences, Shenyang,China

* jingangliu2022@163.com and fenglsh@163.com

Abstract: *Sclerotinia sclerotiorum* as a necrotrophic fungus causes the devastating diseases in many important oilseed crops worldwide. The preferred strategy for controlling *S. sclerotiorum* is to develop resistant varieties, but the molecular mechanisms underlying *S. sclerotiorum* resistance remain poorly defined in sunflower (*Helianthus annuus*). Here, a comparative transcriptomic analysis was performed in leaves of two contrasting sunflower genotypes, disease susceptible (DS) B728 and disease resistant (DR) C6 after *S. sclerotiorum* inoculation. At 24 h post-inoculation, the DR genotype exhibited no visible growth of the hyphae as well as greater activity of superoxide dismutase activity (SOD), peroxidase (POD), catalase (CAT), glutathione-S-transferase (GST), ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDAR) than DS genotype. A total of 10151 and 7439 differentially expressed genes (DEGs) were detected in DS and DR genotypes, respectively. Most of DEGs were enriched in cell wall organisation, protein kinase activity, hormone, transcription factor activities, redox homeostasis, immune response, and secondary metabolism. Differential expression of genes involved in expansins, pectate lyase activities, ethylene biosynthesis and signaling and antioxidant activity after *S. sclerotiorum* infection could potentially be responsible for the differential resistance between two genotypes. In summary, these findings provide additional insights into the potential molecular mechanisms of *S. sclerotiorum*'s defense response and facilitate the breeding of Sclerotinia-resistant sunflower varieties.

Keywords: Differentially expressed genes; Molecular mechanisms; Sunflower (*Helianthus annuus*); *Sclerotinia sclerotiorum*; Transcriptomic

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Discovery of natural variation in genes related to haploid induction in sunflower

Jesica Cespedes Martinez[1], Daniel Alvarez[2], Nicolas Heinz[2], Agustín Mazzalay[2], Andrea Puebla[1], Verónica Lia[3,4], Mónica Fass[1+]*and Norma Paniego[1,3+]*

[1] Instituto de Agrobiotecnología y Biología Molecular (IABIMO), De los Reseros y N. Repetto s/n, Buenos Aires, Argentina; [2] Estación Experimental Agropecuaria Manfredi, INTA, Ruta Nacional Nº 9 - Km. 636 , Córdoba, Argentina; [3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [4] Facultad de Ciencias Exactasy Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. +equal contribution *fass.monica@inta.gob.ar

Abstract: Double haploids are a valuable tool for generating homozygous lines in a short time and at a reduced cost. The starting point is the development of a haploid induction system, followed by chromosome doubling. Although in vitro culture of haploid gametophytic tissues is still the most popular method for haploidization, the laborious protocols make haploid inducer lines a desirable in vivo system by simple crossing. In maize, the first haploid inducer line (Stock 6) was found among inbreds, suggesting the presence of natural inducers. The latest findings in plant reproduction relate variants of the gene centromere-specific histone 3 variant (CENH3) and variability in the gene family DOMAIN OF UNKNOWN FUNCTION 679 membrane protein (DMP) to haploid induction by uniparental genome elimination. The goal of this work is to screen genetic resources of the National Institute of Agricultural Technologies (INTA) in Argentina for variability in the genes *HaCENH3* and *HaDMP/DMP-like* 1 to 9. First, primers were designed for the HaCENH3 gene (comprising 1931 bp/3569 bp, 54%) and nine HaDMP/DMP-like genes (comprising 41% to 96% of each gene sequence, mean of 78%). Then, 48 inbred lines were selected from the Active Germplasm Bank of INTA Manfredi (AGB-IM) according to breeding knowledge and representing the variability in an association mapping population. Microfluidics-based multiplex PCR sequencing (mmPCR-seq) of these regions was performed on each genotype, combining Fluidigm and Illumina technologies. Next, a bioinformatic pipeline was established, using Bowtie2 for mapping to the reference (HanXRQv2) and Bcftools, with the mpileup and call tools, for variant identification. A total of 11 variants (7 SNPs and 4 indels) were found in the gene *HaCENH3*, all of them in intronic regions. These variants are present in only four of the 48 screened inbred lines. In the genes *HaDMP/DMP-like*, one to 26 variants were found in each gene, counting a total of 83 variants. Since *HaDMP/DMP-like* genes have no introns, all variants were found in coding regions. However, many of them produced synonymous substitutions. A detailed summary of the variants will be found in the poster. The number of variants compared to the reference in the different inbred lines varied from two to 34, with variants appearing in one to six genes per inbred line. This study represents the first survey of natural variants of the genes associated with haploid induction in sunflower. As expected, the *HaCENH3* gene was highly conserved among inbred lines, with variants restricted to intronic regions. Since the DMP-family is represented by nine genes, functional overlapping can be expected. Further exploration is required to assess the contribution of these genes to meiotic segregation and gametogenesis.

Keywords: *CENH3*, DMP, Haploid inducer lines.

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Exploration of genes and molecular regulatory mechanisms related to oleic acid synthesis in sunflower

Fei Zhou, Wenjun Wang, Pengyuan Xie, Jing Wang, Hongjiang Guan, Yan Liu,

Xutang Huang*

Institute of Industrial Crops, Heilongjiang Academy of Agricultural Sciences, Heilongjiang Harbin, 150086,

* huangxutang@126.com

Abstract: Sunflower oil is rich in unsaturated fatty acids, among which oleic acid as monounsaturated fatty acids, the higher the proportion of oleic acid, the higher the nutritional value and oxidation stability of edible oil. In the preliminary study, the key gene *HaFAD2-1* for oleic acid synthesis of sunflower seeds was discovered through multi-omics technology using high-oleic acid resources imported from abroad Heterologous overexpression of *HaFAD2-1* in *Arabidopsis Thaliana* confirmed the function of this gene involved in unsaturated fatty acid synthesis. In this study, the promoter sequence of *HaFAD2-1* gene (named proHaFAD2-1) was cloned, and predictive analysis showed that proHaFAD2-1 contained photoresponse, dehydration response, abscisic acid response elements, and binding sites of MYC and MYB transcription factors. A plant expression vector pBWA(V)HG-proHaFAD2-1: GUS was constructed, and transiently transformed into tobacco leaves and stably genetic transformed into *Arabidopsis thaliana*. The results showed that proHaFAD2-1 promoter could drive the expression of *GUS* gene, which verified the function of proHaFAD2-1 promoter. The yeast one-hybrid library was constructed. Using proHaFAD2-1 as the bait sequence, four transcription factors interacting with the proHaFAD2-1 promoter were screened. Yeast one-hybrid point-to-point experiments showed that proHaFAD2-1 could interact with the transcription factor HaDREB1D, and the interaction was strong. The dual-luciferase reporter gene assay showed that the promoter proHaFAD2-1 activity was significantly enhanced after co-transfection of the promoter proHaFAD2-1 and the transcription factor HaDREB1D compared with the control group (proHaFAD2-1 and empty vector co-transfection), indicating that the transcription factor HaDREB1D has an activation effect on the promoter proHaFAD2-1. JASPAR was used to predict that there were three motifs for the binding of transcription factor HaDREB1D to proHaFAD2-1. The results of gel migration experiment (EMSA) showed that the binding site of proHaFAD2-1 to transcription factor HaDREB1D was on the ' CCAAGTCGGCAAG ' motif. The results of this study provided the genetic resources and theoretical basis for the genetic improvement of quality in sunflower.

Keywords: Sunflower; Oleic acid; Gene excavation; Molecular regulation mechanism

Funding: Natural Science Foundation of Heilongjiang Province (LH2023C096); National Characteristic Oil Industry Technology System Project (CARS-14)

Transcriptome analysis reveals the potential molecular mechanism of fatty acids biosynthesis in sunflower

Ying Shao[1], Lingmin Zhao[1], Yanfang Zhang[2], Suping Li[1], Hui Nie[1], Yingnan Mu[1], Congzi Zhang[3], Chen Liang[1], Changrong Liu[1], Shuchun Guo[1,2,3]* and Haifeng Yu[1]*

[1] Institute of Crop Science, Inner Mongolia Academy of Agricultural &Animal Husbandry Sciences, Hohhot, 010031, China; [2] Department of horticulture and plant protection college, Inner Mongolia Agricultural University, Hohhot , China; [3] School of life sciences,Inner Mongolia University, Hohhot,China

*nkyyhf@163.com

Abstract: Sunflower (*Helianthus annuus*) is one of the most important oilseed crops worldwide. This study aims to reveal the molecular mechanism of biosynthesis and accumulation of major fatty acid components in sunflower, and to clarify the primary and secondary relationships between temperature, genes, and fatty acid synthesis in sunflower. We selected the sunflower inbred lines J9 and P50 with high and low oleic acids, respectively. RNA-Seq technology was utilized to sequence the different samples to obtain the gene expression data related to fatty acid synthesis for each sample. Five main fatty acids were detected, including oleic acid, linoleic acid, stearic acid, linolenic acid, palmitic acid and the relative contents of oleic acid and linoleic acid were negatively correlated, while the relative contents of linolenic acid and palmitic acid were lower, and showed a continuous decreasing trend during seed development. The crucial turning point in sunflower fatty acid synthesis occurs at 20 days after flowering (DAF) Notably, the fatty acid fractions and contents of high oleic sunflower lines were little affected by temperature, while low oleic lines were susceptible to temperature. A total of 81,676 unigenes were obtained by transcriptome sequencing. Based on the KEGG database, a total of 19 pathways associated with lipid metabolism, with a cumulative enrichment of 29 DEGs. Through the analysis of metabolic pathways, a total of 42 differentially expressed genes (DEGs) directly related to sunflower fatty acid metabolism were identified. These DEGs belong to 22 different types of enzymes, including PIGT, ACC, SAD, MFP, ACP, and FAB, which are key genes involved in the biosynthesis of unsaturated fatty acids and oil accumulation in sunflowers. We predicted that PIGT, ACC, SAD, MFP, ACP, and FAB were the key enzymes regulating by these genes in the fatty acid biosynthesis and oil accumulation in sunflower seed. These data lay the foundation for elucidating the molecular mechanisms of fatty acid biosynthesis and oil accumulation in sunflower.

Keywords: *Helianthus annuus L.*; Fatty acid synthesis; Transcriptomics

Funding: National Modern Agricultural Industrial Technology System (CARS-14-1-27); Science and Technology Program of Inner Mongolia Autonomous Region (2021GG0014).

KASP marker-based seed purity verification in sunflower

Yanke An, Bingyue Zhang, Zhiheng Wei, Min Chang, Xili Zhang, Jiuhuan Feng*

Sunflower Research Institute, Sanrui Agritec Co., Ltd., Bayannur, Inner Mongolia 015100, China

*srfengjh@lpht.com.cn

Abstract: Sunflower (*Helianthus annuus* L.) has become one of the most competitive cash crops suitable for less fertile soils mainly concentrated in the northwest region of China. Currently, sunflower is commonly produced with a “three-line hybrid” breeding system where seed purity identification is an important tool for internal seed quality control. In this study, a highly efficient method for seed purity identification based on KASP markers was developed for sunflower. DNA was extracted from the seed samples using SDS method, and the amplification of real-time PCR was conducted for genotyping. Among 1500 KASP markers developed from previous research, 20 high quality and highly polymorphic (PIC>0.48) markers were selected as the core primers for purity testing. A total of 29 batches of seeds from seven varieties (including SR1, SR9, SR10, SR11, SR373, SR376 and SR377) produced in 2022 and 2023, respectively, by Sanrui Agritec Co. Ltd. were tested. For 23 batches of seeds in 2022, except for two batches in which atypical individuals were detected (purity of 99.48% and 98.96%, respectively), the purity for the remaining 21 batches was 100%. These results were further confirmed by the field survey at the Hainan station, indicating the reliability of KASP-based molecular test. In 2023, seeds from the different seed production stations (the established and newly developed ones) were tested separately. Compared with the established station, the seed purities of most batches from the new station decreased, from 100% to 98.4%; while the results from the established station were still highly consistent, with all batches remaining at 100%. In conclusion, a KASP-based method for variety verification was established in confection sunflower characterized as accurate, efficient and time-flexible that can be widely used not only in quality control of hybrid breeding, but also provide technical support for marketing high quality sunflower seeds.

Keywords: Sunflower, Seed purity verification, KASP marker

Funding: This study was supported by the grant “Germplasm Utilization and Breeding of New Varieties in Sunflower (2022JBGS0034)”; and “National Joint Collaboration in Sunflower Breeding (YZ2023001)”.

Fingerprint construction for the predominant market varieties of confection sunflower based on SNP markers

Zhiheng Wei, Yiqing Zhao, Ke Wang, Yanke An, Min Chang, Jiuhuan Feng *

Sunflower Research Institute, Sanrui Agritec Co. Ltd., Bayannur, Inner Mongolia 015100, China

*srfengjh@lpht.com.cn

Abstract: Sunflower (*Helianthus annuus* L.) is one of the most important economic crops in northwest China, with an average annual planting of about 0.6 million hectares. In recent years, owing to breeding progress, many new sunflower varieties have been released in the market. However, high genetic homogeneity with similar genetic backgrounds among the varieties has become a common phenomenon. Molecular markers such as SSR markers or high-throughput SNP markers has been successfully used for variety identification. Here, by used 45 selected SNP markers, a fingerprint for 52 predominant varieties released in the market was constructed to provide a practical technical support for genetic pedigree analysis, genuine variety identification, and market supervision of confection sunflower.

In this study, a total of 52 confection sunflower varieties were selected, including 18 proprietary hybrids and 6 associated Essentially Derived Varieties (EDV) released by Sanrui Agritec. Co. Ltd, and 28 elite hybrids released by other seed companies. All 45 KASP markers used in this study successfully amplified distinct signals for the genotypes, clustering the 52 confection varieties into three groups. Class I 363 series: including SH363, SR3, SH383, XM8199, GEGL336, etc.; Class II 361 series: including SH361, GE1, XM1, JR5161, DS9, etc.; Other series of Class III: including LJ368, LJ366, SX6, JH9, SR2101, etc. Through cluster analysis, it was found that the initial varieties and their corresponding essentially derived varieties were clustered into the same group, such as SH363, and the associated broomrape resistance types SH363E and SH363F; SR9 and the broomrape resistance types SR9E and SR9F clustering together, etc. These results suggested that the target genes (broomrape resistance) were successfully introduced into the initial hybrids through the backcross process, and also showed the reliability of the KASP-based variety identification technology. In conclusion, by using selected core SNP markers, we have successfully constructed a fingerprint for 52 elite confection hybrids released in the Chinese market which provides practical technical support for genetic pedigree analysis, genuine variety identification, and market supervision of confection sunflower.

Keywords: Confection sunflower, SNP marker, Fingerprints, Variety identification

Funding: This study was supported by the grant “Germplasm Utilization and Breeding of New Varieties in Sunflower (2022JBGS0034)”; and “National Joint Collaboration in Sunflower Breeding (YZ2023001)”.

Haploid facultative parthenogenesis in sexual sunflowers

Jian Lv[1*], Dawei Liang[1], Eric Bumann[2], Virginie Mirleau Thebaud[3], Huaibing Jin[1], Changbao Li[2], Clemence Paris[3], Yinghui Dan[1], Chao Li[1], Xianxia Chen[1], David Szwerdszarf[4], Peter Wittich[5], Bobby Clegg[2], Agustin Tassara[6], May Dan[1], Xiaolong Tian[7], Jared Carter[2], Federico Bock[3] and Timothy Kelliher[2*]

[1]Syngenta Beijing Innovation Center; Beijing, China, [2]Syngenta Crop Protection, LLC; Research Triangle Park, USA, [3]Seeds France SAS; Saint-Sauveur, France, [4]Syngenta S.A.; Arica, Chile, [5]Syngenta Seeds B.V.; Enkhuizen, Netherlands, [6]Syngenta Agro S.A.; Camet, Argentina, [7]Zhongxin Technology Co., Ltd, Syngenta Group China; Beijing, China

*Jian.lv@syngenta.com, tim.kelliher@syngenta.com

Abstract: Flowering plant sexual reproduction requires double fertilization, yielding embryo and endosperm seed compartments: the latter supports embryo growth and seed germination. In an experiment to generate haploid embryos through inhibition of pollen phospholipase activity in sunflower (*Helianthus annus*), we serendipitously discovered that emasculated sunflowers spontaneously form parthenogenic haploid seed. Induction of doubled haploid (DH) plants can greatly accelerate plant breeding, but despite successful genetic engineering of haploid induction in many crops, few reported systems are commercially scalable. Here we report efficient methods of chemical emasculation and genome doubling to produce fertile plants and enable a scalable sunflower DH system.

Keywords: Haploid facultative parthenogenesis, haploid induction, doubled haploid (DH) pipeline

Funding: Syngenta

Note: Facultative parthenogenesis empowers switch between sexual and asexual parthenogenetic reproduction. In plants, Haploid facultative parthenogenesis is quite rare. We proved in some commercial sunflower varieties can go through haploid facultative parthenogenesis, which can develop into haploid seed without endosperm development. The phenomena is controlled by nuclear genome and varies in haploid seed setting among different germplasm. For lines with parthenogenesis phenomena, a straightforward DH pipeline is developed.

Gene actions and heritability of scuffing in confectionary type sunflower genotypes

Mazzalay[1], N. Heinz[1], I. Gatti [2], D. Alvarez[1]

[1] INTA Manfredi, 5988, Córdoba, Argentina; [2] CIUNR, Council of Researchers of the National University of Rosario, Rosario, Argentina.

*Mazzalay.agustin@inta.gob.ar

Abstract: Currently approximately 10% of the world's annual sunflower production is used for non-oil purposes. Market demands and production areas are increasing due to its nutritional value for human consumption, as well as for poultry and pets foods. Scuffing, or the loss of color due to friction, adversely affects the appearance of the shell and is a trait of great industrial importance, leading to penalties in the marketing of confectionary sunflower. This study aimed to develop an objective methodology using specific software to quantify scuffing and determine the genetic actions involved in the heritability. A trial was conducted by individually crossing of 8 male-sterile inbred lines (AMf1 to AMf8) and one hybrid material as a female parent (AMf9=AMf1*BMf2), with two restorers fertility lines (RMf1, RMf2), in a North Carolina II design during the 2017/2018 agricultural season. The trial was conducted at two locations: Huinca Renancó (-34.83, -64.371) and Manfredi (-32, -64) with two sowing dates at the latter location. The free software Tomato Analyzer 3.0 was used to analyze random samples from each material, scanned in .jpg format with 600 dpi. The genetic actions were determined, and the heritability was calculated using the free statistical software Genes version 2019.

The results indicated that the dominance component was superior to the additive component, accounting for 55% of the genetic variance. The RMf1 tester was the selected using the reduction selection criterion with AMf8 and the simple androsterile hybrid being the standout lines. The test cross AMf8*RMf1 showd the most promising results. Heritability in the strict sense was superior for the tester than for hybrids and lines. Therefore, it is important to study the crosses to be carried out in order to reduce scuffing in future breeding. In conclusion, this study successfully quantified the color loss due to friction using imaging analysis and genetic studies on the inheritance of this trait. The best performing hybrids and lines for further improvement process were identified.

Keywords: Confectionary Sunflower, Scuffing, imaging diagnosis

bZIP genes identification and the signal functions to flowering in Helianthus annuus L.

Juanjuan Li[1], Yingying Zhou[1], Ling Xu[2], Qian Huang[1], Bixian Shi[3], Weijun Zhou[1]*

[1] Institute of Crop Science and Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou 310058, China, [2] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou 310018, China, [3] Institute of Economic Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China

*wjzhou@zju.edu.cn

Abstract: *Helianthus annuus* L. (sunflower) is one of the four major oil crops cultivated throughout the world. However, *Orobanche cumana*, an obligate root parasite which specifically infects roots of sunflower caused severe yield and economic losses worldwide. Identification of key genes responding to *O. cumana* stress is important for sunflower resistance breeding. The basic leucine (Leu) zipper (bZIP) family of transcription factors (TFs) plays crucial roles in response to different environment stresses and has undergone significant expansion during plant evolution via gene duplications. In our study, we identified 117 sunflower *bZIP* genes and analyzed expression patterns of *HabZIP*s at various developmental stages as well as the key functions in flowering time regulation. Timely flowering is critical for plant reproductive success. Numerous plants rely on environmental signals, such as seasonal variations in day length (photoperiod), to regulate when they flower. Sunflower variety SH361 was used in this study. Plants were grown in growth chambers under long-day or short-day conditions with supplemental lighting. During inductive conditions, the FLOWERING LOCUS T (FT) protein is produced in the leaves. This FT protein acts as a mobile signal that can move to the shoot apex, triggering the flowering process. *HaFT1* and its orthologs (*HaFT2*, *HaFT3* and *HaFT4*) are produced in leaves and then transported through the phloem to the shoot apical meristem (SAM), where they physically interact with the bZIP transcription factor, FD, with the assistance of a 14-3-3 scaffold protein to form a florigen activation complex (FAC) in the nucleus to activate sunflower flowering. This interaction was predicted by AlphaFold3 and confirmed through biochemical analyses. Interestingly, transcriptome data analysis found that sunflower bZIP mRNAs could be transported to *O. cumana* stems. RNA-seq analysis found some sensitive *HabZIP* genes to abiotic stresses and broomrape-responses, with possible different mechanisms. This study provides an overall insight of this gene family and their potential involvement in growth, development and stress responses. This will facilitate further research on the *bZIP* gene family regarding their evolutionary history and biological functions.

Keywords: *bZIP* genes; Flowering time; FT; Biochemical analyses; RNA-seq analysis

Funding: This research was supported by the National Natural Science Foundation of China (32172429), Zhejiang Provincial Science and Technology Key Project (2022C02034), Xinjiang Natural Science Foundation for Distinguished Young Scholars (2022D01E59) and Program for Innovative Research Team in Universities of Inner Mongolia Autonomous Region (NMGIRT2320).

Session 2: Cultivation and crop production

Sunflower in the global food system: situation and perspectives

Etienne Pilorgé*

Terres Inovia, Av L. Brétignières, F78850 Thiverval-Grignon, France *e.pilorge@terresinovia.fr

Abstract: The communication is based on the analysis of long-term statistical series on sunflower products (seeds, oil, meals) production, uses and trade (OilWorld ISTA and FAO data) to give a picture of the past evolutions and present situation of the global sunflower sector, and cross analysis with qualitative information on innovation trends and evolutions of needs and challenges in the next decades related to food and carbon transitions.

Sunflower is the third oilseed produced in the world, the fourth vegetable oil and third oilseed meal among protein feed sources. The evolution of sunflower crop at global scale and over a long period is remarkable since the production grew twice as fast as the acreage since the mid 1970^{ies} to present, reflecting both a dynamic market and sustained technical progress. In a context of tough competition in the vegetable oils and meals markets, respectively driven by palm oil and soybean meal, the sunflower sector succeeded in maintaining its competitiveness and market share through continuous innovation in genetics and cropping practices.

As many spring crops, the sunflower performance is challenged by the climate change, which could lead to evolutions in the production areas, especially for rainfed crops.

The oil fraction remains the main valorization of oil type sunflowers, the vegetable oils prices remaining at high levels, supported by the uses as biofuels, even if sunflower oil is used today mainly as edible oil, with a segmentation between oleic and classical oils.

The dehulled sunflower meal is already well valorized as feed. Nevertheless, beyond the production of confectionary sunflower, a higher valorization of the protein fraction for food industries could be developed in the context of the increasing global need of food proteins and necessary food transitions to limit carbon emissions, but research efforts are still needed in processing technologies and food formulation.

The literature also shows that many works are carried out to valorize the oil and protein fractions in different ways than today, and also the other fractions of sunflower plants, such as fibers, that could gain in importance in case of growing resources scarcity.

Keywords: Sunflower; economy; competitiveness; edible oil market; sunflower seeds markets

Many opportunities could be open in future for sunflower, leading to an increased valorization of the whole plant through a biorefinery approach and a diversification of uses of oil (for food, food industry, biofuels, biomaterials), proteins (for feed, including aquaculture, food, potentially biomaterials), and even cellulosic fraction. The plasticity of sunflower makes it a crop relatively adaptable to different kinds of agricultural policies and resulting agricultural systems. The dilemma will remain in the repartition of research and innovation efforts towards different and multiple objectives, this issue being reinforced by the higher market segmentation.

The status of sunflower cultivation in the world in the past 10 years and the development prospects of sunflowers in China

Li Yang, Ren Xiaoci, Guo Rongqiu, Li Xiaowei, He Zhongguo, Yang Xiangyu, Chen Xiaoshu, Cao Tiehua, Wang Baizhong*

Institute of Peanut Research, Jilin Academy of Agricultural Sciences (Northeast Agricultural Research Center of China), Gongzhuling, 136100, China

*wbz.1975@163.com

Abstract: Sunflower is one of the four major oil crops in the world, with sunflower seed oil accounting for approximately 9.0% of the main vegetable oil consumption. From 2013 to 2022, the average planting area of sunflowers in the world was about 26.78 million hectares, with a total yield of about 49.279 million tons. Europe has the highest total sunflower seed production, accounting for 69.7% of the world's total, followed by Asia with 12.9%, the America accounts for 9.6%; Africa accounts for 4.7%. China is mainly cultivate confectionary sunflower, accounting for over 95.0% of the country's total area. In 2022, the total sunflower seed production ranked 8th in the world. In the past decade, sunflower seeds in China maintained a trade surplus as an oil product, with exports increasing from 190000 tons to 445000 tons, mainly exported to Asia and Europe. The export value increased from 310 million US dollars in 2013 to 550 million US dollars in 2022. The sunflower industry in China is currently undergoing a transformation towards high-quality developmental pattern. After the efforts on the selection and breeding of new varieties, the planting area of the domestic sunflower hybrids has successfully that of foreign hybrid varieties. However, there is still a certain gap between the breeding level of oil and confectionary sunflower. The development of agricultural machinery is the key driven force for the oil sunflower breeding. In terms of the current situation of sunflower breeding, there still has bottleneck between the combination of traditional and modern breeding technologies. It is necessary to fully utilize new modern breeding technologies, explore excellent germplasm resources, and combine gene sequencing and molecular marker assisted selection technology to promote breeding efficiency. In terms of the utilization of saline alkali land, oil sunflower is an important oil crop in northern China, with natural characteristics of salt alkali tolerance. Developing salt alkali tolerant oil sunflowers can avoid competing with grain crops for land, increase the variety of crops in saline alkali ecological areas, and broaden the path to solve the problem of comprehensive utilization of saline alkali land. In terms of planting technology, the inherent advantages of short growth period and strong adaptability of oil sunflowers can be used to develop efficient planting technology according to local conditions, take advantage of onion intercropping sunflower cultivation technology and wheat sunflower compound planting technology, so as to coordinate the symbiotic relationship between the two crops. At present, sunflower is one of the characteristic oil crops with development prospects in China, which integrates oil supply, utilization of saline alkali land, and export revenue generation.

Keywords: sunflower; cultivation; development prospects; China

Effects of different organic fertilizers on fatty acids, oil content and tocopherol in confectionery sunflower

Nada Hladni[1], Veljko Petrović, Sandra Cvejić[1], Vladimir Miklič[1], Siniša Jocić[1], Nada Grahovac[1], Dragana Miladinović[1]

[1] Institute of Field and Vegetable Crops (IFVCNS), Maksima Gorkog 30, 21000 Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Technical Sciences, 21000 Novi Sad, Serbia
*nada.hladni@ifvcns.ns.ac.rs

Abstract: Organic agriculture has to satisfy a growing need for food satety with maintaining soil fertility and ameliorating climate change effects. NS confectionary hybrids have good characteristics, uniform color and size and are suitable for machine dehulling.They are used for production of both large amounts of unrefined cold pressed oil and oilcake. Testing NS confenctinary hybrids under different production systems (conventional and organic) is important when breeding for hybrids with a wide degree of adaptability to different cultivation conditions The specific treatments were analyzed under organic cultivation condition. The application of the Guanito organic fertilizer at a dose of 0.5t ha^{-1} applied during the BBCH 01 stage, the application of Trainer at 3l ha^{-1} and Natur Plasma at 5l ha^{-1}, were applied during the BBCH 36 stage. This was compared to a purely conventional treatment with mixed NPK fertilizer (50:50:50), and a control cultivation in organic production with no treatment. Five different confenctionary hybrids were analyzed under above conditions, making for 25 unique test conditions. fatty acids, oil content and tocopherol in sunflower seed were measured under lab condition. The analysis firstly was performed with one level ANOVA on both hybrids and different treatments, as well as with two-level ANOVA. Differences between hybrids were found to be significant in the one-way ANOVA for 4 different kind offatty acids, and seed oil content. No ignificant was found with one-way ANOVA test among different treatments. Two-way ANOVA analysis for alpha-tocopherol has been confirmed the significant differences among tested hybrids., The significant interaction were detected between both hybrids and treatments for palmytic, stearic and linoleic content, as well as the significant differences for hybrids is only detected on oleic content. Due to there is no significant differences was found between different models with the Akaike Information Criterion, that's why Wilcox's robust two-way ANOVA analogue was used with n = 1000 for subsampling. A robust one-way ANOVA analogue was applied using Wilcox's algorithm and Welch's method with 20% trimmed means and 5000 subsampling steps. Significant results were found for stearic, oleic, linoleic and oil content. Given that these models have been validated with robust statistics, they were elected as suitable for *post hoc* analysis. Preliminary result analysis shows that there are no statistically significant differences between organic, control and conventional treatments. It detects no significant differences in tocopherol levels between different hybrids, but the available datashowed the significant (at a 0.001 level) differences in fatty acid and oil content. Further work should be developed to study the use of organic fertilizers on NS confenctionary hybrids so as to creat the organic growth conditions for sunflower.

Keywords: high protein sunflower, organic cultivation, fertilizer, fatty acids and tocopherol

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The "Robust Sunflower" dashboard, a decision support tool for sunflower crop establishment strategy

M. Abella[1]*, V. Jauvion[2]

[1] Terres Inovia – DRT - 110 chemin de la Côte Vieille, 31450 Baziège – France; [2] Terres Inovia – DTVG - 11, rue G. Monge – 33610 Canejan – France;

*m.abella@terresinovia.fr

Abstract: Crop establishment is a key stage in the success of sunflower cultivation. This sequence is decisive in obtaining a robust sunflower, which Terres Inovia defines as being able to express its yield potential by not being limited i-nor by its stand, since sunflowers do not have the ability to compensate for missing plants, ii-nor by its leaf area, which must avoid both exuberance and deficiency, iii-nor by its rooting, which must enable it to draw water and mineral resources from the largest possible volume of soil. The Institute's monitoring and support work with 3 groups of farmers, which were located in several French sunflower production zones, focusing on the theme of sunflower establishment between 2019 and 2022. This work enabled us to show that the margins for technical progress in all the operations, which were implemented by growers to establish sunflower substantially. Another finding was the lack of an operational tool for growers and advisors to manage sunflower establishment and evaluate its success. Facing these observations, the Institute mobilized its expertise to develop and propose the "Tournesol Robuste" dashboard, which can be used by all French agricultural advisors and growers, to promote successful sunflower establishment. Terres Inovia also drew on the monitoring and testing carried out by farmers within the framework of the abovementioned groups, thus leading a process of co-design of the tool with practitioners. The "Robust Sunflower" dashboard is a strategic tool designed to guide the technical decisions for crop establishment and help growers achieve robust sunflower status. Seven intermediate stages, called "key stages", decisive in reaching this final objective, have been defined. They are assessed throughout the production season using indicators derived from field observations, such as dates when certain key stages are reached, phenological indices or yield components, to be carried out by the practitioners themselves directly on the crop. The key stages are linked by cause-and-effect relationships, enabling a detailed analysis at the end of the campaign of the mechanisms that led to the successes and failures in obtaining a robust sunflower in each single field. The cultivation practices implemented by growers can then be assessed in terms of the extent to which key stages and the final objective have been achieved. The dashboard can also be used before planting, to help build a strategy based on the formalization of cause-and-effect links between practices, key stages and the final result. This tool was published in October, 2023, as part of a specific book dedicated to sunflower planting, compiling operational knowledge, decision rules and strategies, enabling cultivation techniques to be adapted to different situations, for successful planting and crop establishment.

Keywords: Robust sunflower; strategic management; evaluation of success; plant observations

Field evaluation of INTA's Association Mapping Population in Argentina and use of indices for drought tolerance in sunflower

N. Heinz.*[1]; A. Mazzalay.[1]; C. Filippi[2,3]; J. Di Rienzo[4]; N. Paniego.[2,3]; V. Lia.[2, 3]; D. Álvarez.[1]

[1] INTA EEA Manfredi, Argentina, [2] CONICET, Argentina, [3] INTA Instituto de Agrobiotecnología y Biología Molecular CICVyA Hurlingham, Argentina, [4] Universidad Nacional de Córdoba, Argentina

*heinz.nicolas@inta.gob.ar

Abstract: World population growth and climate change have intensified the global challenge of food security, where water deficit emerges as a critical factor limiting sunflower yield. In this context, a study was carried out in the Association Mapping Population (AMP) of INTA with the objective of characterizing the behavior against water deficit, generating data for association mapping studies and identifying new sources of tolerance to water deficit. 159 inbred lines were evaluated in three field trials with moderate water deficit at the Agricultural Experimental Station, Manfredi, Córdoba, Argentina (-31.85, -63.75). Planting was carried out according to a partially repeated block design with randomly distributed plots, and a progressive moderate water deficit was induced by means of agroethylene bands from the V6-V8 stage until the end of the grown season of sunflower. The irrigation condition was achieved by drip irrigation. Leaf temperature, stomatal conductance, radiation interception, plant height, stem and head diameter, seed weight, number of seeds per head, oil content, grain yield and oil yield were recorded. Subsequently, linear mixed models were used to obtain the adjusted means for each variable analyzed, which were used as the basis for generating the drought tolerance index (DTI) (Fernandez. 1992) and drought tolerance stability index (DTSI) (Bouslama & Schapaugh Jr. 1984). The DTI index showed significant differences ($p < 0.05$) among genotypes for all the descriptors evaluated except for plant height and stomatal conductance. As for the DTSI index, significant differences ($p < 0.05$) between genotypes were only found for fat yield. All the indices together allowed discriminating groups of genotypes and distinguishing those with better performance against water deficit, based on their resilience to water deficit and their productivity in environments with such stress. The data generated are being used for association mapping studies to identify new sources of tolerance to water deficit.

Keywords: Drought; tolerance; association mapping; breeding

Current status of confectionery sunflower production in bayannur

Zhang Xili, Wang Yujie*, Liu Yonggang, Li Ling, Yang Qian, An Xiaomin,

Wang Yue, Yang Pu

Modern Agriculture and Animal Husbandry Business Development Center, Bureau of Agriculture and Animal Husbandry, Bayannur City, Inner Mongolia, China 015000

*979140424@qq.com

Abstract: In recent years, the sunflower planting area in China has remained stable due to its increasing demand in both domestic and global markets. China has successfully developed a series of elite confection hybrids with high yield and superior quality, resulting in an average annual planting area of approximately 560,000 ha. The sunflower planting region are mainly concentrated in the northern latitudes, specifically between 35°and 50°N, including provinces such as Heilongjiang, Liaoning, Jilin, Inner Mongolia, Hebei, Shanxi, Shaanxi, Gansu, Ningxia, and Xinjiang. These provinces account for 2/3the total global confectionery sunflower planting area. Among them, Inner Mongolia has become the biggest producer of confectionery sunflowers in China, with the total planting area about 423,000 ha. Bayannur with the area approximately 65 thousand square kilometers located in the west part of Inner Mongolia, with a 135-day frost-free period, annual precipitation of 188 mm, and a 14-18°C difference in day-and-night temperature, accounts for approximately 70% of the planted area in Inner Mongolia and over 25% of the country's sunflower total production. Bayannur accounts for one-third of China's total output in terms of planting area and annual yield. This city is also the center of both confection sunflower planting and distribution. Furthermore, it has been recognized by the Ministry of Commerce as a national-level demonstration base for foreign trade transformation. The sunflower seeds produced here are known for their large kernal size and high levels of unsaturated fatty acids, vitamins, and trace elements. Thus, "Hetao Sunflower" has become a well-known national geographical brand. In the past five years, the sown area for confectionery sunflowers was increased, reaching to 300,853 ha. The total seed production ranged from 796,140 to 1,043,647 tons. Sunflower industry has been becoming the most important driven force to shake off the poverty for local farmers.

Keywords: confection sunflower; output and benefit; sunflower species; seeds industry

Perspectives on sunflower production in the Republic of Moldova

Rodion Domenco*, Steliana Clapco, Maria Duca

Moldova State University, Chisinau, Republic of Moldova *rodion.domenco@usm.md

Abstract: Due to the high nutritional content, sunflower (*Helianthus annuus* L.) is an economically valuable crop and a versatile source for oil and protein production. In the Republic of Moldova, sunflower is cultivated since the beginning of the 20th century. The planting area has grown gradually to approximately 135,000 hectares in 1980s. In the 1990s, the area planted with sunflower represented about 7.4% of the total agricultural areas in the country, followed by an increase up to 10.4% and, decreased around 17.1% in the following two decades.

In the period of 2011-2020, sunflower planting area has increased by more than 1.3 times compared to the previous decade, exceeding recommended planting area by approximately 2.5 times. Concomitantly, seed production has increased in each decade, excepting period of 1991-2000. On the other hand, the average yield revealed in the last two decades is lower compared to the values reported in the other countries. According to FAO data, in the top 10 sunflower seeds producing countries, the yield ranged from 1.8 to 2.8 t/ha in 2011-2021, while in Moldova it was 1,8 t/ha.

Seeds production decreased partially attributed to current climate change. The results of our analysis showed that climatic factors, especially precipitation during the growing season, explain a maximum of 30% of the variation in sunflower yield, indicating that crop productivity is preferentially determined by t soil fertility, planting date, hybrid type, and agricultural technologies etc. Temperatures exceeding 30°C during the flowering period cause significant effects on seed production and oil content reducing.

Considering the fact that area cultivated with sunflower increase continuously, while yield remains constant or increase slightly, it is on interest to analysed the degree of favourability (taking into consideration climate variables) of the territory of the Republic of Moldova for this crop. We calculated and analysed the correlation coefficients between the average values of sunflower yield and: temperature from April to August; precipitation during the vegetation period; precipitation during the cold period of the year, from the period of 2003-2021.

The formula for calculation of the degree of favourability was determined as follow:

$$Fv = P\_veget*0.375163 + P\_cold*0.46399 + T\_apr\text{-}aug *(- 0.498989), \quad (1)$$

where: Fv – degree of favorability; P_veget - precipitation during the vegetation period; P_rece - precipitation during the cold period of the year; T_apr-aug - average air temperature from April to August.

Using the formula and Geographical Information Systems, we found that the conditions in the north of the country are more favourable for sunflower growing than in the southern part. These findings could be used to diminish the negative effects of climate change in the agricultural sector.

Keywords: correlations; favourable areas; planting area; yield climate conditions, sunflower.
Funding: subprogramme 011101 Genetic and biotechnological approaches to the management of agroecosystems in the conditions of climate change, funded by the Ministry of Education and Research.

Production of sunflower seed under new climate conditions

Vladimir Miklič*, Nenad Dušanić, Branislav Ostojić, Velimir Radić, Miloš Krstić, Goran Jokić,
Daliborka Butaš, Jelena Ovuka, Igor Balalić, Siniša Jocić, Nada Hladni, Ana Marjanović Jeromela,
Sandra Cvejić, Milan Jocković, Nemanja Ćuk, Dragana Miladinović

Institute of Field and Vegetable Crops, Novi Sad, Serbia, *vladimir.miklic@nsseme.com

Abstract: Sunflower is the third largest oil crop and in 2023 it was grown on over 28 million hectares in the world. With the discovery of the CMS source in 1969, sunflower hybrids began to be created. The first commercial hybrids based on CMS were registered in Romania, in 1974 and in Yugoslavia, in 1978. It is estimated that hybrids are grown on around 85% of the total sunflower planting area today, and the remaining 15% are still landrace varieties, mostly of the confectionery type. Therefore, the requirment for hybrid sunflower seeds in the world today is around 120 million kg (about 12 million bags of 150,000 grains). To reach to this amount, the sunflower will be with an average yield of 700 kg/ha of processed seeds, it is necessary to plant sunflower with area about 170,000 ha. The total commercial hybrid sunflower seed production is estimated at USD 2 billion today. The production of hybrid sunflower seeds today is exposed to many new challenges. Global climate change facilitate warming environmentally, thus make drought and stormy winds occurrence. By monitoring the daily dynamics of bee visits, we determined that the maximum number of visits occurs at air temperatures between 20 and 28°C, and air humidity of 40-50%. In recent years, the average temperature in Serbia has increased by nearly 2°C compared to the multi-year average. This has a negative effect on the vitality of pollen and fertilization, as well as on the visit of bees, so the recommendations for supplying beehives have increased from 2 to 4/ha. Bees are the most important pollinators for sunflowerand on average make up 71% of the total visit (in some years over 90%), the insects is mainly from the Syrfidae family (19% of visits), bumblebees (8%) and butterflies (2%), whose populations are constantly decreasing. Due to the increase in the sunflower planting area, it is increasingly difficult to ensure spatial isolation of insects, it decreases to 500 m in many countries, and this already leads to increased foreign fertilization and the spread of wild sunflowers, which is especially dangerous if they carry some of the genes for resistance to herbicides. In the last 20 years, many herbicides, insecticides and fungicides have lost their registration, especially in Europe. Seed treatment makes sunflower production especially difficult, as well as the international trade of seeds, because the situation differs among different countries. With the announced cancellation of the license for Reglone desiccant, the situation is further aggravated and higher costs can be expected (primarily due to drying), but also a significantly drop in yield and seed quality. The potential substitutes techniques we tried to create (Kabuki, carfentrazone, pelargonic acid, glyphosate...) did not have good results. Problems with high fluctuations in mercantile prices and the increased price of energy and fertilizers, with a lack of labor and especially with the war in Ukraine, leads therestrictions on seed trade in Russia, significantly affect the sunflower production, thus leading to an increasing risk on the price of sunflower seeds in the future.

Keywords: sunflower, hybrid seed production, climate changes, pollinators, pesticides

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Optimizing leaf area index in sunflower-legume intercropping system

Brankica Babec[1*], Nemanja Ćuk[1], Srdjan Šeremešić[2], Nada Hladni[1], Miloš Krstić[1], Sonja Gvozdenac[1], Siniša Jocić[1], Vladimir Miklič[1] Sandra Cvejić[1]

[1] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia; [2] University of Novi Sad, Faculty of Agriculture

*nemanja.cuk@ifvcns.ns.ac.rs

Abstract: The leaf area index (LAI) is one of the most common used indice for describing the above-ground biomass of crops, which is useful for understanding the complex roles of different factors on affecting the leaf development. The photosynthetic capacity of the sunflower plant is defined, by the leaf area index as well as the dynamics of its change during the vegetation period, whereas, photosynthesis is directly responsible for the accumulation of nutrients and yields. The goal of the research was to analyze LAI differences in s intercropping systems between sunflower and legume. A four-year trial (2017-2020) was conducted in Serbia's agroecological conditions using a split-plot design. Two oil-type and one confectionary-type hybrid were intercropped with common vetch, red clover, and alfalfa. LAI was obtained using one direct and two indirect methods. The threshold for correlation coefficient indicating the statistical significance of the correlation in a matrix (critical r-value) for a probability of 95% (α 0.05) is 0.576, and for 99% probability (α 0.001) is 0.708.

In both of 2019 and 2020, LAI was obtained at the beginning of the bud phase in sunflower (LAI I) using METER's LP-80 ceptometer, then by measuring the leaf surfaces with the LI3100 area meter (LI-COR) device also in the bud phase (LAI IIa) and flowering phase (LAI IIb) and by remote sensing using a spectral camera and NDVI vegetation index in the bud phase (LAI III). In 2020, LAI was also obtained using METER's LP-80 ceptometer in the bud (LAI Ia) and flowering phase (LAI Ib), then by measuring the leaf area with LI3100 area meter also in the bud (LAI IIa) and flowering (LAI IIb) phases, and by remote sensing i.e. NDVI (LAI III), ExR and NGRD vegetation indexes in the bud phase.

A significant positive correlation was established between seed yield and LAI IIa and LAI IIb in intercropping of sunflower with red clover, alfalfa. In addition, seed yield and LAI I were positively correlated in sunflower and alfalfa intercropping. Significant negative correlations between the examined indexes and sunflower yield were not observed during 2019, while a significant negative correlation was observed in 2020 between yield and ExR vegetation index in both sunflower and alfalfa intercroppingand control. In conclude, LAI is a robust predictor todemonstrate the significant accuracy in modeling plant growth dynamically. This metric can effectively be employed for early-stage yield prediction, providing valuable agricultural management and optimization insights.

Keywords: LAI; sunflower; intercropping; legumes

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Agronomic responses of sunflower (Helianthus annuus L.) introduced at different dates into sesame-based intercropping system in a humid tropical location

Olabisi Somefun[1], Victor Olowe[2], Sunday Adigbo[2], Faucet Olasantan[3]

[1] Department of Plant Physiology and Crop Production, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, (FUNAAB), PMB 2240, Abeokuta, Nigeria. [2] Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, (FUNAAB), PMB 2240, Abeokuta, Nigeria. [3] Department of Horticulture, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, (FUNAAB), PMB 2240, Abeokuta, Nigeria.

*olowevio@funaab.edu.ng

Abstract: Enhanced agronomic output is very crucial for any cropping system to deliver the much needed, such as economic, social and environmental services. Therefore, two field trials were carried out during the late cropping season (July – Nov.) of 2018 and 2019 to determine the agronomic performance of sunflower (var. Favorit) introduced into sesame-based cropping system. The experiment was laid out in a randomized complete block design using a split-split plot arrangement with sesame varieties (E8 and Cameroun White), organic fertilizer (no fertilizer and organic fertilizer) and sunflower staggered sowing dates (C1- sown simultaneously with sesame, C2 - 10 days after sowing DAS, and C3 - 20 DAS) allocated to the main, sub and sub-sub plots, respectively. Consequently, twelve cropping systems were evaluated in this study. Data were collected on Agronomic Efficiency (AE), Partial Factor Productivity (PFP), Production Efficiency (PE) and grain yield. Cropping system significantly ($P<0.05$) affected grain yield and PE in both years, with sole sunflower grown on plots treated with organic fertilizer and sunflower introduced on C1 into E8 (V1OFC1), recording the highest grain yield and PF. Similarly, cropping system significantly ($P<0.05$) affected AE and PFP of sunflower on plots treated with organic fertilizer and the sole. On average, AE and PFP values of sunflower introduced into E8 were significantly ($P<0.05$) higher than the AE and PFP values of Cameroun White across the sowing dates in 2018. Among the cropping system, V1OFC1 recorded PFP values significantly ($P<0.05$) higher than the values under V2 mixtures in both years, except V2OFC1 in 2019. Sunflower sole yield in 2018 (1,346.2 kg/ha) and 2019 (1005.5 kg/ha) compared well with Africa average yield (1,037.8 kg/ha) and lower than the world average yield (1,855.4 kg/ha). Sunflower yield in mixtures were lower and ranged between 447.8 and 1,054.0 kg/ha in 2018, and 506.6 and 720.0 kg/ha in 2019. For enhanced agronomic mixture performance, sunflower can be introduced into sesame, especially E8 simultaneously or latest 10 DAS (C2) in the humid tropics.

Keywords: agronomic efficiency; grain yield; partial factor productivity; production efficiency; organic sunflower

Identification of promising confectionery sunflower hybrids from INTA breeding program in Argentina

Mazzalay, N. Heinz, D. Alvarez

INTA Manfredi, 5988, Córdoba, Argentina.

*mazzalay.agustin@inta.gob.ar

Abstract: Currently, approximately 10% of the world's annual sunflower production is allocated to non-oil purposes. Market demanding and sunflower production areas are increasing due their nutritional value for both human and animal consumption. While the breeding of confectionery sunflower breeding is similar to oil sunflower breeding, it has distinct objectives, including hull color, kernel size, reduced scuffing and other quality traits. Breeding programs leverage heterosis, typically found in hybrids derived from unrelated inbred lines. So, it is important to identify genotypes with high combining ability that are also responsive to environmental conditions.

This study aim to identify the most promising confectionery-type hybrids with economic potential from elite inbred lines within the INTA breeding program. We evaluated yield (kg/ha) and grain size (measured with a four-mesh sieve: 9.5 mm, 8.75 mm, 8 mm and 6.5 mm) over three seasons (2020/21, 2021/22, 2022/23) in Manfredi, Argentina (coordinates: -32, -64). The experimental unit consisted of two-row plots, each 5.10 m in length and spaced 0.70 m apart, in a randomized complete block trial with three replicates. The average yields for the 2020/21, 2021/22 and 2022/23 seasons were 1331.67 kg/ha, 2765.5 kg/ha and 1816.69 kg/ha, respectively, with hybrids 102-2.401, 369.455-1 and 333.455-1 showing the highest performance.

For the grain size measurements in the 2020/21 season, the average percentages for the hybrids were as follow: 40% (9.5 mm), 27% (8.75 mm), 11% (8 mm) and 8% (6.5 mm). Notably, BS58 had the highest percentage of grains larger than 9.5 mm, at 89%. In the 2021/22 season, the average percentages were 49% (9.5 mm), 26% (8.75 mm), 11% (8 mm) and 10% (6.5 mm), with hybrid 101.401 having the highest percentage (91%) for grains larger than 9.5 mm.

In summary, this study successfully differentiated confectionery hybrids based on the evaluated characteristics, revealing genetic variability. The findings allowed for the identification of the most promising lines for further breeding and the assessment of their combining ability with other lines from the INTA breeding program.

Keywords: confectionary sunflower, quality traits, hybrid.

Funding: This research was supported by Instituto Nacional de Tecnología Agropecuaria (PE I127, 111).

Quality determination in confectionery sunflower

Yalcin Kaya[1]*, Emrah Akpinar[1], Ufuk Bagci[2], Necmi Beser[1]

[1] Trakya University Engineering Faculty, Genetic and Bioengineering Dept, Edirne, Turkey
[2] Trakya University Engineering Faculty, Food Engineering Dept, Edirne, Turkey

*yalcinkaya22@gmail.com

Abstract: Confectionery sunflower is one of the most preferred and consumed snacks both in Turkey and also in the world. In Turkey, confectionery sunflower is cultivated on approximately 80,000 to 100,000 hectares, with an annual seed production of around 160,000 to 170,000 tons. These cultivation area are primarily located in the provinces of Denizli, Kayseri, Aksaray, Istanbul, Istanbul, Bursa and Konya (TUIK, 2021). However, since local populations such as İnegöl Alacası etc. are predominantly grown in Turkey, hybrid varieties with higher yield, quality, uniformity and coarse seeds should be incorporated in Turkish production compared to these local varieties. The reliance on local varieties leads to limited product development, economic losses, and high wastage due to low production and lack of uniformity during processing. Additionally, Turkey imports around $100 million worth of confectionery sunflower seeds annually from China, primarily due to consumer preference for the taste of these imported snack products. To date, no scientific studies have been conducted in Turkey or globally to investigate the profile of confectionery sunflower.

In the study, domestic commonly planted hybrids“white-striped İnegöl Alaca” ,developed by TRAGEN Arge Co., located in Trakya Technopark, and the highly demanded black colored varieties, imported from China, were used as materials. Based on the study results, seed yield of the varieties ranged from 1,280 - 4,010 kg/ha. Seed colors varied among black, variegated and white. Protein content ranged from 17.87 to 30.05%. In terms of fatty acids, linolenic acid (Omega 3) content ranged from 0.13% to 1.27%, while oleic acid content ranged from 31.25% to 48.25%. Sensory analysis was performed to evalute consumer preferance, ranking the varieties from lowest to the highest in taste as well as browning index. As a result, TG 400 variety stood out in several aspects and can be used as a standard variety in future evaluations of taste and other yield elements.

Keywords: Confectionery sunflower, quality characteristics, seed, taste, hybrids

Is it feasible to anticipate the chemical desiccation of female inbred lines in confection sunflower seed production?

Josefina Demicheli[1*], Ignacio Gaggioli[1], Fernando Turienzo Aguilar[1] and M. Ezequiel Barreto[1]
Valia Genetics (by Argensun Foods SA.) *josefina.demicheli@argensun.com.ar

Abstract: Application of desiccants on sunflower cultivation serves as a valuable tool for several purposes, including anticipating harvest, homogenizing cultivar stages and mitigating exposure to unfavorable conditions such as extreme weather, pathogens, or bird predation. This practice is particularly crucial for confection sunflower, where late sowing dates often results in harvesting during early autumn. During this period, cool and wet weather may reduce harvesting windows and create favorable conditions for certain diseases and root lodging. Desiccation is typically recommended when seed moisture reaches 28-30%. however, evidence suggests that physiological maturity in confection sunflowers occurs at higher moisture levels. Most commonly used desiccants, such as paraquat or diquat, are class II toxicity herbicides. Recently, saflufenacil has emerged as an alternative with a lower environmental and health impact, though its application in confection sunflower remains under explored.

This study aimed to determine the feasibility of advancing desiccant application in female confection sunflower inbred lines, enabling earlier harvests without compromising yield or seed quality. A two-year field trial was conducted in Hilario Ascasubi, Buenos Aires (38,78457° S, 62,27320° W), involving saflufenacil applications to a female line from 16 to 44 days after the end of flowering (R5.9). Grain humidity was measured at the time of application, along with the number of days require to achieve the target moisture level for harvest (8%). Germination rate (GR) and thousand-seed weight(P1000) were assessed to evaluate seed quality and yield. A linear model was fitted to the data to explore relationships between the variables. For data spanning multiple growing season, a mixed model was employed, treating the growing season as a random effect. Analysis of variance (ANOVA) was performed to assess the overall significance of the model and post-hoc comparisons using Tukey's test identified significant differences between groups ($p<0.05$).

Seed moisture at application ranged from 53.90% to 9.30%, with plots requiring 4 to 22 to reach the desired harvest moisture level. No significant correlation was found between the GR or P1000 and the timing of desiccant application. The GR consistently exceeded 95% surpassing commercial standards across all treatments and growing seasons. Additionally, P1000 averaged 122 ± 9g, with no apparent trends or correlations with any treatment. These results suggest that desiccant application in confection sunflower can be advanced to as early as 16 days after R5.9, or at grain moisture content is as high as 54%, without compromising seed quality or yield. This anticipated application facilitates an earlier harvest, reducing seed exposure to factors that could negatively affect yield (e.g., root lodging, bird predation) or seed quality (humidity, diseases associated with maturity). Moreover, chemical desiccation allows for the uniform maturation of the cultivar and creates optimal conditions for chemical fallow in subsequent crop. The evidence presented in this study suggests that the use of chemical desiccation with saflufenacil in confection sunflower inbred female lines can be significantly advanced, providing numerous advantages without compromising seed quality or yield. However, further comprehensive studies are necessary to validate these preliminary findings.

Keywords: Crop desiccation; confection sunflower; seed quality

Funding: This study was founded by Argensun Foods SA.

Validation of a putative stay green sunflower genotype and its yield stability under nitrogen fertilization condition

Melanie Corzo[1]*, Daniela Becheran[2], Edmundo Ploschuk[2], Sofia A. Bengoa Louni [1], Santiago Trukpin[3], Daniel Alvarez[4], Nicolas Heinz[4], Natalia Izquierdo[5], Carla Caputo[2], Sebastian Moschen[6], Danilo Centeno[7], Paula Fernandez[1]

[1] IABIMO- UEDD INTA-CONICET; [2] Facultad de Agronomía. Universidad de Buenos Aires; [3] IF-INTA Castelar.; [4] EEA-INTA Manfredi; [5] Facultad de Ciencias Agrarias. UNMdP Balcarce, [6] CONICET; 7Universidade Federal do ABC *corzo.melanie@inta.gob.ar

Abstract: Leaf senescence is characterized by a decrease in photosynthetic activity and the recycling of nutrients to regions of active growth. It is a biological process with economic influence, because the ability to maintain an active photosynthetic system, especially during grain filling phases, ultimately impacts the yield and oil content in seeds. It can be associated with different stresses or environmental conditions, manifesting itself widely in a context of climate change and limit yield, especially in crops of agronomic relevance.

The main objective of this work is to validate a sunflower genotype belonging to INTA National Breeding Program that exhibits a putative "stay green" phenotype. This study evaluated the yield stability of these genotypes in control conditions and under nitrogen(N) supply. Two inbred sunflower genotypes were sown in the field: R453 (early senescence) and B481–6 (late senescence), which have contrasting N supply in the soil but share the same ontogeny cycle length. Parameters measured included photosynthetic activity, leaf chlorophyll content, grain yield, oleic and linoleic fatty acids content and biomass percentage per organ. The relative gene expression of early leaf senescence candidate genes (NAC01, CAB) in leaves were measured by qPCR. According to the nutritional level, nitrogenous amino acids were analyzed in phloem exudates and metabolites distribution in leaves by GC-MS. The genotype B481-6 showed higher photosynthetic activity and leaf chlorophyll content, which was reflected in a higher percentage of grain oil and linoleic content, in contrast to genotype R453. Additionally, the relative gene expression is being validated through qPCR experiments of previously identified candidate genes. Regarding nutritional level, the genotypes behaved stably against differential nitrogen conditions, showing no variation in performance, TCR, biomass percentage per organ, plant height or head diameter. Additionally, this study addresses the integration of metabolites under different sampling time series to know their mobility and/or presence of nitrogenous compounds that can explain its stable behavior, as well as the analysis of nitrogenous amino acids in phloem exudates and leaves. These experiments have been carried out and are in the process of analysis.The results confirm genotype-dependent differences in the "stay green" phenotype, classifying the B481-6 genotype as a putative functional "stay green". This genotype was stable under differential nitrogen conditions, showing no variation in performance or other agronomic parameters. These findings suggest that the differences in leaf senescence are due to intrinsic genotype characteristics, making the B481-6 a valuable genotype for breeding programs in order to increase yield and diminish the gap between actual and potential ones.

Keywords: Sunflower; Yield; Stay green; Nitrogen

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Session 3: Genetic resources and germplasm utilization

The T2T genome of confection sunflower and molecular mechanism of head domestication

Chen Tao*

Hangzhou Normal University, China

*20190028@hznu.edu.cn

Abstract: Sunflower is an important crop species, which serves as oilseed and confection worldwide. Cultivated sunflower (Helianthus annuus) was believed to be domesticated from wild ancestors thousand years ago in North America; however, the genetic mechanism during artificial selection and domestication syndrome such as enlarged flower head remains unclear. We assembled a high-quality/T2T genome for an edible sunflower line (Shikui, SK), based on which a comprehensive variome was built from oil and edible lines. Population analyses revealed that cultivated sunflower shared a closer genetic relationship with the H. annuus lines from Texus. Genome-wide association with the large disc trait identified a same 1.5-Mb region on chromosome 10 in the oil and edible lines, the QTL region was narrowed to 280Kb, 11 genes included, base on separation population. Two transcription regulator genes, LH1 and LH2, were inferred as potential causal genes which might regulate head development in sunflower. By methods of genetics, biochemistry, molecular biology, physiology, haplotype analysis and genome-wide analysis, we confirmed the two TFs was responsible for head development. These findings were applied in field and accelerated molecular breeding of sunflowers.

Keywords: Sunflower; Genetic Mechanism; Molecular Breeding

Pervasive structural variation in Helianthus: implications for sunflower breeding

Loren Rieseberg*

Department of Botany and Biodiversity Research Centre, University of British Columbia, Canada

*lriesebe@mail.ubc.ca

Abstract: In plants, structural variants affect more base pairs than do single nucleotide polymorphisms and typically have outsized effects on phenotypic variation. However, only recently have we had the genomic tools needed to fully explore the impact of structural changes in wild and domesticated species. I will first discuss how large structural variants, such as chromosomal inversions, can aid environmental adaptation by suppressing recombination between locally favored mutations. I will illustrate this in wild sunflowers where the colonization of extreme habitats such as sand dunes has been made possible by massive (up to 100 Mbp) non-recombining "supergenes" (mainly caused by structural variants) that are associated with ecologically and agriculturally relevant developmental, physiological, and climate variation. This work has helped solved a longstanding evolutionary mystery of how some groups of organisms have been able to diversify in the face of rampant genetic exchange. Following my discussion of the role of structural variation in the diversification of wild sunflowers, I will shift my focus to the origin and impacts of structural variants for sunflower improvement. Structural variants hamper plant and animal breeding because they impede recombination and introgression and can be the cause of hybrid inviability or sterility. Focusing on the cultivated sunflower gene pool, I will show that gene presence/absence variation – which affects ~40% of the genes in the cultivated sunflower pan genome – is often associated with introgressions from wild species that were introduced by breeding programs. In many cases, genes are missing in the introgressed regions, which partly explains why such introgressions often are deleterious. I will further show that expression complementation of such gene presence absence polymorphisms in hybrids is an important contributor to heterosis in sunflower. Thus, hybrid production permits the use of wild relative alleles for improvement without sacrificing productivity.

Keywords: Genomics; introgression; inversions; structural variation; heterosis

Funding: Genome British Columbia, Genome Canada, International Consortium for Sunflower Genomics, Natural Sciences and Engineering Research Council of Canada, U.S. National Science Foundation

The genomics of linkage drag in inbred lines of sunflower

Kaichi Huang[1],†,*, Mojtaba Jahani[1],†, Jérôme Gouzy[2], Alexandra Legendre[2], Sebastien Carrere[2], José Miguel Lázaro-Guevara[1], Eric Gerardo González Segovia[1], Marco Todesco[1], Baptiste Mayjonade[2], Nathalie Rodde[3], Stéphane Cauet[3], Isabelle Dufau[3], S Evan Staton[1,4], Nicolas Pouilly[2], Marie-Claude Boniface[2], Camille Tapy[2], Brigitte Mangin[2], Alexandra Duhnen[2,] Véronique Gautier[5], Charles Poncet[5], Cécile Donnadieu[6], Tali Mandel[7], Sariel Hübner[7], John M. Burke[8], Sonia Vautrin[3], Arnaud Bellec[3], Gregory L. Owens[9], Nicolas Langlade[2],#, Stéphane Muños[2],#, and Loren H. Rieseberg[1,]#,*

[1] Department of Botany and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, V6R 2A5, Canada; [2] Laboratoire des Interactions Plantes Microbes-Environnement (LIPME), CNRS, INRAE, Université de Toulouse, Castanet-Tolosan, France; [3] INRAE, CNRGV French Plant Genomic Resource Center, F-31320, Castanet-Tolosan, France; [4] NRGene Canada Inc., Saskatoon, SK, S7N 3R3, Canada; [5] Gentyane Genomic Platform, INRAE, Clermont Ferrand, France; [6] GeT-PlaGe Genomic Platform, INRAE, Toulouse, France; [7] Galilee Research Institute (MIGAL), Tel-Hai Academic College, Upper Galilee, Israel; [8] Department of Plant Biology, University of Georgia, Athens, GA, 30602; [9] Department of Biology, University of Victoria, Victoria, BC, V8W 2Y2, Canada

*kaichi.huang@botany.ubc.ca; lriesebe@mail.ubc.ca

Abstract: Crop wild relatives represent valuable sources of alleles for crop improvement, including adaptation to climate change and emerging diseases. However, introgressions from wild relatives might have deleterious effects on desirable traits, including yield, due to linkage drag. Here, we analyzed the genomic and phenotypic impacts of wild introgressions in inbred lines of cultivated sunflower to estimate the impacts of linkage drag. First, we generated reference sequences for seven cultivated and one wild sunflower genotype, as well as improved assemblies for two additional cultivars. Next, relying on previously generated sequences from wild donor species, we identified introgressions in the cultivated reference sequences, as well as the sequence and structural variants they contain. We then used a ridge-regression best linear unbiased prediction model to test the effects of the introgressions on phenotypic traits in the cultivated sunflower association mapping population. We found that introgression has introduced substantial sequence and structural variation into the cultivated sunflower gene pool, including >3,000 new genes. While introgressions reduced genetic load at protein-coding sequences, they mostly had negative impacts on yield and quality traits. Introgressions found at high frequency in the cultivated gene pool had larger effects than low-frequency introgressions, suggesting that the former likely were targeted by artificial selection. Also, introgressions from more distantly related species were more likely to be maladaptive than those from the wild progenitor of cultivated sunflower. Thus, breeding efforts should focus, as far as possible, on closely related and fully compatible wild relatives.

Keywords: linkage drag; genome assembly; introgression; structural variation; crop improvement

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Current status, issues and challenges of sunflower genetic resources in India

M.Y. Dudhe* and R. K. Mathur

ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad-500 030, Telangana, India

*mangesh.dudhe@icar.gov.inx

Abstract: Sunflower crop is introduced in India during early 1970's. Till now, 3126 sunflower accessions are kept in the medium term storage of the Indian Institute of Oilseeds Research (IIOR) under the Germplasm Multiplication Unit (GMU). The ICAR-IIOR collection consists of the following: backcross converted lines (15), prebred lines, inbreds augmented from USDA, (360), genetic stocks (105), inbreds (350), populations (DRSF 5), exotic collection (350), genetic stocks (105), inbreds (350), and gene pools (GP) for high oil, yield, and autogamy (340). 2719 sunflower accessions are maintained at ICAR-National Bureau of Plant Genetic Resources (NBPGR). In 2018, a catalog of germplasm containing 3126 accessions was released, offering details on the accessibility of sunflower accessions that are preserved and cared for at ICAR-IIOR and NBPGR, Delhi. Breeders of sunflowers will find the descriptive material useful in identifying accessions, selecting novel materials for a variety of uses, and identifying potential parents to use in breeding. Enhancement of trait-specific germplasm, use of promising cultivar germplasm and wild *Helianthus* species in inbred development, development of maintainer/restorer gene pools, parental line improvement, and resistance breeding programs are the current areas of interest. Regarding the enhancement, multiplication, conservation, and use of sunflower genetic resources in the Indian breeding program. One of the biggest obstacles to expanding sunflower germplasm is getting access to international gene banks outside of the USDA. Another problem is the non-viability of the seed due to prolonged storage under normal conditions where viability is reduced up to 20 to 30% within one year of storage. To keep the germplasm intact, accessions with viability lower than 50% are regularly rejuvenated and multiplied. The insufficiency of large-scale multilocation evaluation limits the ability to identify and subsequently utilize potential accessions. Major barriers to genetic improvement through interspecific hybridization are differences in ploidy. It is urgently necessary to identify duplicates using specialized instruments and methods. The present paper discusses about the various activities carried out with regards to conservation, multiplication, evaluation, issues and challenges in the utilization of sunflower germplasm during past two decades.

Keywords: Genetic resources, sunflower, utilization, issues, India

Funding: This research received no external funding

Identification and evaluation of drought tolerance of sunflower genetic resource at seedling stage

Zirong Zhu, Wei Wang, Jiajun Wu, Xinrong Zhang, Ling Wang, Yuxiao Li, Lei Wang, Meilian Tan*

Oil Crops Research Institute, Chinese Academy of Agricultural Science, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, P. R. China, Hubei-Wuhan, 430062

*meiliantan@126.com

Abstract: Drought, a severe global abiotic stress, has significantly hampered crop production and food security, particularly in the main sunflower producing areas in China. Seasonal drought poses a serious threat to the growth and yield of sunflowers. Our current study aimed to assess the drought tolerance of various sunflower accessions and identify superior materials exhibiting robust drought resilience, so as to provide scientific basis for improving drought-tolerant and drought-tolerant varieties of sunflower. In this study, 19 sunflower varieties (lines) were used as materials, and a pot experiment was carried out to maintain 20% absolute soil water content in the control group. Before stress treatment, the treatment group poured water once to maintain the absolute soil water content to 20%±1%, and then stopped watering and let the soil dry naturally until the soil water content dropped to 6%±1%. The drought tolerance of 19 cultivar's seedlings under normal watering and drought stress was identified and evaluated by comparing 6 indexes, including relative plant height, relative stem diameter, relative leaf area, relative SPAD value, relative leaf water content and relative root-shoot ratio, combing with principal component analysis, comprehensive membership function and regression analysis and cluster analysis. The results showed that the identification method of drought-tolerance at seedling stage of sunflower was established, and 4 indexes, such as relative plant height, relative stem diameter, relative leaf area and relative root-shoot ratio, were identified as the key indexes to evaluate drought tolerance at seedling stage. Two strong drought-tolerant varieties and 4 drought-tolerant varieties were selected among the 19 varieties. The drought tolerance of 104 sunflower accessions was evaluated by using above method, and 1 drought-tolerant germplasm and 13 drought-tolerant germplasm were excavated, which provided materials for the subsequent research on drought stress response and breeding of drought-resistant varieties in sunflower.

Keywords：Sunflower; Evaluation of drought tolerance; Seedling stage; Comprehensive membership function method; Cluster analysis

Collection, preservation, identification and evaluation of sunflower germplasm

Meilian Tan*, Xingchu Yan, Zirong Zhu, Ling Wang, Lei Wang, Yuxiao Li, Xinrong Zhang, Wei Wang, Jiajun Wu

Oil Crops Research Institute, Chinese Academy of Agricultural Science, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, P. R. China, Hubei-Wuhan, 430062

*meiliantan@126.com

Abstract: As one of the most important oil crops in the world, sunflower is loved by people for its bright flowers and nutritious seeds. China is a major producer of sunflower, with an annual seeding area of 1million hectares and a total output of 2.93 million tons. In China, sunflower is mainly planted in Inner Mongolia, Xinjiang, Jilin, Hebei, Gansu ects., among them, Inner Mongolia has the largest sunflower planting area, accounting for 55% of the country's total production, followed by Xinjiang and Jilin, with 15% and 7%, respectively.

More than 3300 accessions including 70% edible sunflowers were collected and preserved through the collection, census, introduction and other ways. The genotype, important agronomic traits, quality characters, salt tolerance and drought resistance of some sunflower accessions were identified and evaluated, a rapid detection method of oil quality character and the identification method of salt and drought tolerance for sunflower were established. Genotype identification of more than 2000 accessions was completed by simplified genome sequencing. A number of elite materials with one or more excellent traits such as early maturity, salt tolerance, drought tolerance, high oil content and high oleic acid were discovered by many years of multi-point identification of some accessions. 19 loci and 14 candidate genes related to salt tolerance were excavated by combining the phenotype of salt tolerance identification with genotype and transcriptome data, which provided materials and technical methods for the breeding of sunflower new varieties.

Keywords: Sunflower；Germplasm resource；Collection and preservation；Identification and evaluation; Quality trait；Salt tolerance；Drought tolerance

Interspecific hybridization of cultivated sunflower with Helianthus praecox towards transfer of resistance to powdery mildew

Sujatha Mulpuri[1]*, Prathap Reddy Kallamadi[1], Helan B Thomas[1], Sreelekha Duruvasula[2], Ulaganathan Kandasamy[2], Meena Hari Prakash[1], Dudhe Y Mangesh[1]

[1]ICAR-Indian Institute of Oilseeds Research, Rajendranagar, India; [2]CPMB, Osmania University, Hyderabad 500 007, Telangana, India *mulpurishjata@yahoo.com

Abstract: Sunflower (*Helianthus annuus* L.) is one of the important edible oilseed crops in India. Powdery mildew incited by *Golovinomyces latisporus*, has become a serious problem limiting sunflower yields in the country since its appearance 15 years ago. Hence, attempts were made to identify reliable sources of resistance to this pathogen, characterize and understand pathogen infection process, study the inheritance of resistance, and develop appropriate mapping populations for identification of candidate genes and molecular markers to diagnose the reaction to the pathogen. Among the limited sources identified in wild *Helianthus* species, *Helianthus praecox* (PRA-1823) was considered for introgressive breeding as it is a diploid annual with a good cross ability and success rate in crosses with cultivated sunflower (Acc PS-2023). Morphological, molecular analysis using ITS primers and sequence analysis of the IGS region confirmed the fungi infecting sunflower including the wild species as *G. latisporus*. Light microscopic analysis of powdery mildew infection showed initiation of the infection process within 6 hours of inoculation with faster hyphal growth and penetration in the susceptible genotype (PS 2023) while there was no hyphal development untill 10 days of post inoculation in *H. praecox*. Proteomic studies were undertaken to understand the sunflower-powdery mildew infection process. *H. praecox*, exhibited 50% of differentially expressed proteins that were related to photosynthesis such as the light harvesting complex, photo system I, ATRAB7B, apocytochrome f and cytochrome b559 in high abundance. Inheritance of resistance showed dominance of the trait but owing to segregation and high variability for various characteristics such as, plant height, branching, petiole pigmentation, disc colour, capitulum size, etc in the F_2 population of the interspecific cross, the material was advanced till F_8 generation. From this stable interspecific recombinant inbred lines (IRILs), morphologically similar branched types but varying reaction to powdery mildew were isolated. The resistant and susceptible IRILs were easily distinguishable with 0 and 9 disease score, respectively on a 0-9 scale followed for the pathogen. The susceptible and resistant IRILs were subjected to molecular analysis, which resulted in the identification of a set of 14 sunflower specific SSR markers that could reliably distinguish both the phenotypes. Interestingly, this set of diagnostic markers when tested on a larger panel of cultivated sunflower accessions (245) and few wild *Helianthus* species (11) discriminated the resistant and susceptible cultivars reliably. Further, transcriptome analysis of the control and pathogen infected susceptible and resistant IRILs indicated the involvement of mildew locus (MLO) genes in conferring susceptibility to the pathogen and revealed 12 differentially expressed MLO genes between both the types. Sunflower is one of the crops which has been genetically upgraded through introgression of several desirable traits from wild sunflowers. This study is yet another example of successful use of a wild diploid annual *Helianthus* species which provided insights into the sunflower-powdery mildew infection process and also led to the identification of candidate genes along with a molecular marker set so as to facilitate the transfer of resistance into agronomically superior cultivars either through genome editing of MLO genes or via marker assisted breeding.

Keywords: Sunflower; Introgressive breeding; Molecular markers; Powdery mild

Resistance of Helianthus wild species to the prevailing Chinese broomrape races and genetic characterization

Min Chang, Liquan Yang, Jiuhuan Feng, Chao-Chien Jan

Sunflower Research Institute, Sanrui Agritec Co., Ltd., Bayannur, Inner Mongolia 015100, China

*srchangm@lpht.com.cn

Abstract: Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligatory and non-photosynthetic root parasitic plant that specifically infects sunflower (*Helianthus annuus L.*）. It is currently a major limiting factor for all sunflower production in China. Wild species of *Helianthus* possess valuable resistance genes that could be incorporated into cultivated sunflower through breeding programs. To identify more different sources of resistance to broomrape，41 wild *Helianthus* species including 14 annuals and 27 perennials were evaluated for the resistance to highly virulent broomrape in China. The results showed that all the wild *Helianthus* perennials used were immune against Chinese virulent broomrape. Among the 30 accessions of 14 wild annual species, 6 accessions were immune, 16 were intermediate showing segregation on resistance trait, and 6 were highly susceptible. Three wild annual species, *H. exilis* (PI 664628), *H. neglectus* (PI 435762), and *H. petiolaris* (PI 468842), were selected to be interspecific crossed with confection sunflower inbred line L22. Through backcrossing and selfing, three pre-breeding lines which were resistant to the high virulent sunflower broomrape were identified.

To dissect the genetic basis of resistance, the populations in BC_2F_1 and BC_2F_2 derived from the crosses between *H. exilis* and L22 were screened for resistance to broomrape and genotyped with selected 100 SNP markers in 17 LGs using resistant and susceptible bulks. A new resistance gene was identified and mapping in LG7. By genotyping 153 individuals of BC_2F_1 populations，a co-segregated SNP marker CP52 was located in 142344833 bp in reference genome HanXRQr2.0, which was about 1Mb to the proximal end of gene *HaOr7* which was previously reported. Further research is needed to determine whether the resistant gene from *H. exilis* identified in this study is the gene *HaOr7* as previously reported.

Keywords: Wild *Helianthus* species，Sunflower broomrape，SNP marker

Session 4: Disease resistance and pest control

The recent progress of the integrated control on sunflower soilborne diseases in China

Jun Zhao*

Department of Horticultural and Plant Protection, Inner Mongolia Agricultural Univeristy, Huhhot, Inner Mongolia, China

*zhaojun@imau.edu.cn

Abstract: Sunflower (*Helianthus annuus* L.) is the most important oil crop in China, its planting area is around 1 million hectares. Inner Mongolia (IM) is the biggest sunflower planting region, its planting area is over 60% total planting area in China, thus make it became the most important sunflower planting, seeds processing and distribution region. Due to the difficulty of the rotation, the successive planting of sunflower caused the soilbrone diseases, such as Sunflower White Mold (SWV), Sunflower Verticillium Wilt (SVW) and Sunflower broomrape (SB) became the bottleneck for the limiting the sunflower industry development in IM region. In this talk, we will review the most recently progress on the soilborne diseases study in China, including characteristics of the pathogens, occurrence and epidemic rule, and the techniques which were used for integrated control on SVW, SVW and SB. Finally, we also highlighted the basic research on the biological mechanism under each single techniques, such as delaying sowing date and rotation with crop ect. These results will lay a stone for providing the effective controlling strategy against sunflower soilborne diseases in the future.

Keywords: Sunflower, integrated control; soilborne diseases.

Funding: China Agricultural Research System (CARS-14); Basic research funds of Inner Mongolia Agricultural University（BR22-13-09) and Inner Mengolia Science & Technology Plan (2022JBGS0010).

The impact of different planting pattern on the population distribution of Frankliniella intonsa in sunflower

Hongxing Yang, Zerun Chuai, Hui Qu, Haiping Li *

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010019, China

*lihaiping5820@163.com

Abstract: *Frankliniella intonsa* primarily feeds on confection sunflower seeds causing sunflower seedcoat rust. The leaf of buckwheat is more attractive to *F. intonsa* than leaf of sunflower, and the flowering stage of buckwheat is longer than that of sunflower. Confection sunflower and buckwheat were selected to plant inner and outer layers in a field to control *F. intonsa* in two areas. Blue traps were hung to monitor the quantity of *F. intones*. The results showed the quality of *F. intonsa* in the buckwheat field was higher than that in confection sunflower field from R3 to R6 flowering stages of sunflower. There was a significant difference in the quantity of *F. intonsa* trapped on confection sunflower 1.4m, 2.8m and 4.2m away from the buckwheat during the R4 flowering stage. Moreover, as the planting distance to buckwheat increased, the quantity of *F. intonsa* in confection sunflower gradually decreased until the R7 flowering stage of sunflower.

Keywords: *Frankliniella intonsa*; sunflower; buckwheat; distance; quality

Investigating the influence of plant growth regulators on mycelial growth and sclerotia production of *Sclerotinia sclerotiorum*.

Nomfundo Shinga[1,5], Almuth Hammerbacher[2,5], Dirk Swanevelder[3], Markus Wilken[4,5] and Nicky Creux[1,5]

[1] Department of Plant and Soil Sciences, University of Pretoria. [2] Department of Zoology and Entomology University of Pretoria. [3] Agricultural Research Council (ARC)—Biotechnology Platform, [4] Department of Biochemistry, Genetics and Microbiology University of Pretoria. [5] Forestry and Agricultural Biotechnology Institute (FABI) University of Pretoria.

*nicole.creux@fabi.up.ac.za

Abstract: *Sclerotinia sclerotiorum* is the causal agent of one of the major diseases affecting the South African sunflower industry, known as sclerotinia headrot (SHR). SHR occurs when anthesis coincides with cool and wet conditions. Farmers use staggered planting as a management strategy for SHR. Staggered plantings are expensive, time consuming, and during cooler, wetter seasons multiple plantings can still be affected. Plant growth regulators (PGRs) are a potential strategy to shift anthesis timing to better align with non-conducive conditions. PGRs have also been reported to have mixed effects (promoting or inhibiting) on fungal pathogen development. This study aimed to investigate the feasibility of PGRs as an escape strategy from SHR by shifting the time of anthesis. The first objective is to determine the effect of different PGRs on mycelial growth and sclerotia production *in-vitro*. Mycelial plugs were cultured on media amended with different concentrations of abscisic acid (ABA), paclobutrazol, ethephone and 1-Naphthaleneacetic Acid (NAA). The number of sclerotia produced was counted and weighed. The findings indicate that ABA had no effects on mycelia growth or sclerotia production. All concentrations of paclobutrazol completely inhibited mycelial growth and sclerotia production. NAA inhibitory effects increased with increasing concentration ranging from 45-100%. Ethephone slowed down mycelial growth while increasing sclerotia production. ABA did not change *S. sclerotiorum* development, while Paclobutrazol and NAA reduced growth, suggesting these PGRs have potential and should be explored further for their effect on sunflower anthesis and SHR-sunflower interaction. This project may provide alternate options for control of SHR and provide insight into both the sunflower and *S. sclerotiorum* life cycles.

Keywords: Sunflower; Phytohormones; abscisic acid; 1-Naphthaleneacetic Acid and paclobutrazol; White mold

Genomic insights into disease resistance in sunflowers: identifying key regions and candidate genes for Verticillium dahliae resistance

Yue Yu[1], Jianfeng Yang[2], Jun Zhao[2], Loren Rieseberg[1]*

[1] Department of Botany and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, Canada; [2] College of Agronomy, Inner Mongolia Agricultural University

*loren.rieseberg@botany.ubc.ca

Abstract: Sunflower (*Helianthus annuus*) is a globally significant field crop, and disease resistance is crucial for ensuring yield stability and crop quality. *Verticillium dahliae* is a notorious soilborne pathogen that causes verticillium wilt and threatens sunflower production worldwide. In this study, we conducted a comprehensive assessment of *Verticillium dahliae* resistance across 287 sunflower cultivar lines, from the Sunflower Association Mapping (SAM) population. We employed *EMMAX* and ridge regression best linear unbiased prediction (*rrBLUP)* to identify genomic regions and candidate genes associated with *Verticillium dahliae* resistance. Our study reveals the genetic basis of *Verticillium dahliae* resistance in sunflowers, providing insights for breeding disease-resistant sunflower cultivars. This research contributes to ongoing efforts to enhance crop resilience and reduce yield losses due to *Verticillium dahliae*, ultimately benefiting sunflower growers and the agricultural sector.

Keywords: Sunflowers; *Verticillium dahlia*; Disease Resistance; GWAS

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC), China Scholarship Council (CSC).

Trapping effect of different colored sticky boards to Frankliniella intonsa in sunflower field

Wenbin Zhang, Xueliang Chen, Hui Qu, Zerun Chuai, Haiping Li *

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010019, China

*lihaiping5820@163.com

Abstract: The colored sticky board is widely used in the integrated management of agricultural and forestry pests. *Frankliniella intonsa* is a key pest causing sunflower seedcoat rust. This paper evaluated trapping effect of different colored sticky boards on *F. intonsa* in sunflower field. The results showed that the trapping effect of pink (R7: 6241.67/per board) and blue (R7: 5329.33/per board) sticky boards on *F. intonsa* were the best, followed by white sticky board (R7:3395.33/per board), and trapping effect of yellow and green sticky boards were the worst. The number of *F. intonsa* attracted by the stick board hanging in 2.4m was significantly higher than that in 1.2m. The population of *F. intonsa* in sunflower fields showed a trend of first increasing and then decreasing with the change of flowering period of sunflower, reaching its peak (8049.25/per board) around August 20 and gradually decreasing thereafter. Therefore, pink and blue sticky insect boards with a hanging height of 2.4m should be used to monitor and control thrips in sunflower field.

Keywords: sunflower; *Frankliniella intonsa*; colored sticky board; hanging height

Toxicity and effect of insecticides on Frankliniella intonsa in sunflower

Zerun Chuai, Xueliang Chen, Hui Qu, Wenbing Zhang, Haiping Li *

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010019, China

*lihaiping5820@163.com

Abstract: *Frankliniella intonsa* is a key pest causing sunflower seedcoat rust, and chemical control is the main method to control *F. intonsa* now. This paper primarily measured the toxicity and effect of eight pesticides on *F. intonsa* in sunflower fields. The results showed that toxicities of spinosad and spinetoram to *F. intonsa* nymphs and adults were the best among eight tested insecticides, and LC_{50} of spinosad to nymphs was 0.32 mg/L and 0.397 mg/L to adults, while the LC_{50} of spinetoram to nymphs was 0.3 mg/L and 0.313 mg/L to adults. Three insecticides were sprayed on the sunflower and buckwheat in the fields at the sunflower threshold bloom stage. The control effects of three insecticides on *F. intonsa* ranged from 44.6% to 77.7%. In the monoculture sunflower field, the incidence of sunflower seedcoat rust after spraying spinetoram was 17.22%, while 25.68% in the intercropping field of sunflower and buckwheat after spraying spinetoram only in the buckwheat planting area. The seedcoat rust incidence rate of insecticides sprayed sunflower was significantly lower than that of control.

Keywords: *Frankliniella intonsa*; sunflower seedcoat rust; insecticides; toxicity; effect

Study on green control strategy of Frankliniella intonsa (Trybom) in sunflower field based on chemical ecology

Hui Qu, Haiping Li*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010019, China.

*lihaiping5820@163.com

Abstract: *Frankliniella intonsa* (Thysanoptera: Thripidae) is a significant invasive pest that can damage numerous plants and crops, and spread the Tomato spotted wilt virus. In the chief sunflower production area in China, during the flowering period of sunflowers, *F. intonsa*-infested sunflower heads produce kernels with marked visual damage (otherwise known as the "seed scarring"), which reduces the quality and profit of sunflower seeds. Based on chemical ecology, traditional chemical ecology techniques (Y-tube olfactometer bioassays and GC-MS) were used to screen secondary plants and plant volatiles that were repellent or attractive to *F. intonsa* in the sunflower field. The study findings revealed that *F. intonsa* preferred confection sunflower of flowering stage to oilseed sunflower. In contrast, *F. intonsa* preferred buckwheat of flowering stage to confection sunflower of seedling stage. Among the 15 VOCs selected, *F. intonsa* were attracted by 6 VOCs (ethyl isovalerate, γ-terpinene, (R)-(+)-limonene, α-caryophyllene, verbenone, octane), 4 VOCs (1-nonene, geranyl bromide, α-pinene, β-pinene) had the effect of repelling *F. intonsa*, among them, ethyl isovalerate and geranyl bromide are the key volatiles that leads to the difference in the selection of *F. intonsa* to oilseed sunflower and confection sunflower. Finally, the field trapping effect of buckwheat on *F. intonsa* was verified by the sunflower/buckwheat intercropping experiment. Meanwhile, field verification revealed that intercropping confection and oilseed sunflowers could effectively control *F. intonsa*. Therefore, oilseed sunflower is a good repelling plant to *F. intonsa*, and buckwheat is a trap plant with the effect of trapping *F. intonsa*. Based on traditional chemical ecology techniques and combined with field verification, a new method for green control *F. intonsa* was provided in the sunflower field in this study.

Keywords: *Frankliniella intonsa*; Olfactometer bioassay; Volatile Organic Compounds; GC-MS; Chemical Ecology; Intercropping

Funding: This research was supported by the central government guides local science and technology development fund projects (No. 2022ZY0096); National modern agricultural industrial technology system (No. CARS-14)

Study on the biological characteristics, pathogenicity differentiation and genetic diversity of Sclerotinia minor isolated from different hosts

Baizhen Hao[1], Jian Zhang[1] and Jun Zhao[1*]

[1] College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot, China,

*zhaojun@imau.edu.cn

Abstract: *Sclerotinia* is a fungal disease caused by pathogenic fungi of the genus Sclerotinia. In this study, we took *Sclerotinia minor* strains isolated from both sunflower and potato in different regions of Inner Mongolia as the objects of research and studied their biological characteristics, pathogenicity differentiation, and genetic diversity.

Firstly, we isolated and purified 12 and 28 strains of *S.minor* separately from both potato and sunflower diseased plants, collected from Chahar Right-Wing Qian Banner of Ulaanchab City. The isolates were confirmed to be *S. minor* by Koch's postulate. Then, the biological characteristics and pathogenicity of the above samples were compared. The results suggested that the growth rate of the mycelium of *S. minor* from potatoes was faster than that of *S.minor* from sunflower, but mycelium of *S. minor* from potatoes grew rather sparsely on the PDA plate than that of isolates from sunflower. There was no significant difference in oxalic acid (OA) secretion and PG enzyme activity among all tested isolates. The results of cross-infection suggested that *S.minor* from potato had a higher pathogenicity on its isolated host potato than that of the *S.minor* from sunflower; but there is no difference in the pathogenicity of all tested isolates on sunflower. Subsequently, the mycelium compatibility group (MCG) of tested isolates of *S. minor* from sunflower showed that the 120 isolates were divided into 21 MCG groups, and the isolates collected from the same sunflower field were distributed within different MCG groups. Twelve isolates from potatoes were also classified into four different MCG groups. The results of mating type identification suggested that Inv- was the dominant type among all tested isolates. Phylogenetic analysis results indicated that 26 strains of *S. minor* from sunflower and 10 strains of *S. minor* potato s were interspersed in different genetic clusters, indicating the dramatic genetic difference of *S. minor* isolated from both sunflower and potato.

In summary, this is the first report of *S.minor* infesting potato in China; and genetic difference is rather dramatic among tested *S. minor* isolated from both sunflower and potato.

Keywords: Sclerotinia minor; Biological characteristics; Genetic diversity; Pathogenicity differentiation; Genetic transformation

Study on the mechanism of rotation with corn to decrease the severity of Sunflower Verticillium Wilt

Tie Li[1], Jianfeng Yang[1], Jian Zhang[1], Wenbing Zhang[1], and Jun Zhao[1]*

[1] College of Horticulture and Plant Protection, Inner Mongolia Agricultural University,
Hohhot, China,

*zhaojun@imau.edu.cn

Abstract: Sunflower is one of the important oilseed crops in China. The challenge of crop rotation difficulties has made Sunflower Verticillium Wilt (SVW) another significant soil-borne disease. In this study, we attempted to investigate the mechanism of sustaining the disease suppression effect of sunflower-corn rotation on the subsequent SVW. We measured the disease index (DI) of SVW and the number of microsclerotia of *Verticillium dahliae* in the soil collected from sunflower continuous cropping fields and fields rotated with maize, compared with the continuous field, the DI of SVW decreased by 59.54% after rotation with maize for 2 years; meanwhile, the amount of *V. dahliae* in soil decreased by 87.49%. Also, in exogenous watering sunflower with maize root exudates, the inhibition efficacy against SVW is 55.78%. A step further, the effect of maize root secretion compounds on suppressing the pathogen of Verticillium Wilt was also investigated via metabolomics. The root secretion components of both maize and sunflower were analyzed in pairs, 323 metabolites specifically secreted from maize roots were screened out, and five metabolites, including guaiacic acid, 5, 6-dimethylbenzimidazole, 5-hydroxyindole-3-acetic acid, guanosine, and MBOA were confirmed to have inhibition effects on both mycelium growth and spore germination. This study revealed the biological mechanism of sunflower- maize crop rotation in decreasing the severity of SVW.

Keywords: Sunflower Verticillium Wilt; Crop Rotation; Corn Root Exudates; Inhibitary effect

Funding: This research was funded by China Ministry of Agriculture, China Agricultural Research System (CARS-14).

The biocontrol mechanism of Bacillus velezensis XS142 in reducing soil-borne diseases of sunflower

Yating Wang[1], Jianfeng Yang[1], Jian Zhang[1], Wenbing Zhang[1], and Jun Zhao[1]*

[1] College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot, China,

*zhaojun@imau.edu.cn

Abstract: The difficulties in crop rotation facilitated the occurrence of different soil-borne diseases and affected the yield and quality of sunflower seriously. We isolated *Bacillus velezensis* XS142 from soil rhizosphere of potato, and it has antagonist effects on potato wilt, this promoted us to test its broad-spectrum effects on controlling sunflower soil-borne diseases.

Preliminary data indicated that XS142 exhibits a certain broad-spectrum antifungal activity against three pathogenic fungi which caused different soil-borne diseases, such as Sunflower White Mold (SWM), Sunflower Verticillium Wilt (SVW) and Sunflower Fusarium Wilt (SFW) on plates. The non-volatile metabolites produced by XS142 exhibited significant antifungal effects. Multiple antibacterial metabolites have been identified through LC/MS analysis. Indoor pot experiments showed that, when the roots of sunflower plants were pretreated with the suspension of XS142, followed by artificial inoculation with different pathogenic fungi, the control effect of XS142 against *Verticillium dahlia*, *Fusarium* spp. and *Sclerotinia sclerotirum* was 30.79%, 59.35% and 32.32% separately. Additionally, pretreatment of detached sunflower leaves and stems with the bacterial suspension XS142, followed by inoculation with *S. sclerotiorum* could inhibit the expansion of lesions effectively. Furthermore, the stable colonization of XS142 on the roots of sunflower was confirmed by using GFP tag. After pretreatment with XS142, accumulation of H_2O_2, and activity of defense-related enzymes (such as POD, SOD, PPO, etc.) in the roots of the sunflower were also detected accordingly.

In a word, we concluded that the *B. velezensis* XS142 strain showed antagonist effects on the occurrence of different soi-borne diseases of sunflower via producing antifungal metabolites, competing spatially with pathogenic fungi on the root surface, and promoting the establishment of resistance of sunflower.

Keywords: Sunflower; Soil-borne diseases; *Bacillus velezensis*; Biocontrol mechanisms

Funding: This research was funded by Basic research funds of Inner Mongolia Agricultural University (BR22-13-09), China Ministry of Agriculture, China Agricultural Research System (CARS-14).

Diversity of arthropod communities and population dynamics of Frankliniella intonsa of the sunflower field in Inner Mongolia

Hushuan Fan, Li Shi*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010018, China

*lirui2003@imau.edu.cn

Abstract: Sunflower is one of the important economic crops worldwide, and Bayannur City in Inner Mongolia is not only the most important sunflower production area in China, but also a serious area of damage from sunflower pests such as *Homoeosoma nebulellum* and *Frankliniella intonsa* in our region. The ground traps, blue sticky traps, and temperature recorders were used to survey the diversity of ground arthropod communities, population dynamics of *Frankliniella intonsa*, and heat production rhythm of sunflower discs in Wuyuan County to know the occurrence dynamics of thrips in a sunflower field and provide scientific data support for comprehensive pest control strategy in the sunflower field. The results showed that there were significant differences in community diversity of ground arthropods with different growth stages. The vertical distribution of thrips in sunflower plants had characteristics of the upper layer higher than the middle layer and the middle layer higher than the lower layer. The peak number of thrips on sunflower leaves and discs appeared in sunflower R4 and R6 stages, with 319.7 thrips per 10 plants and 169 thrips per 10 plants respectively. The spatial distribution patterns of *Frankliniella intonsa* populations at all fertility stages belonged to aggregated distribution. The peak activity of *Frankliniella intonsa* occurred from 12:00 to 14:00 in a day and the peak activity of thrips throughout the growing season occurred in early September. The intercropping of oilseed sunflower and confection sunflower could reduce the incidence of sunflower seedcoat rust to a certain extent. The heat generation rhythm of sunflower discs in the thrips zone was more obvious before flowering, and the temperature of discs reached the peak at 10:00 am or so, which may be one of the reasons for the high number of thrips during flowering.

Keywords: *Helianthus annuus*; community structure; thrips; population dynamics

Funding: The research was funded by the Inner Mongolia Science & Technology Plan Projects (No. 2021GG0027, 2022JBGS0010) and the project “Supported by Program for Innovative Research Team in Universities of Inner Mongolia Autonomous Region” (No. NMGIRT2320).

Diversity of insects in sunflower field in Bayannur City, Inner Mongolia

Qingsong Shi, Li Shi*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010018, China

*lirui2003@imau.edu.cn

Abstract: Bayannur City in Inner Mongolia is the largest area for sunflower cultivation in China. Sunflower pests cause economic losses, directly threatening the development of the sunflower industry. Through the investigation of natural enemies on sunflower, the families, genera, and species of natural enemies are known. It aims at using local natural enemies to control sunflower pests and increasing production and farmers' income. The malaise trap was used to collect insects in sunflower fields from Wuyuan County, Bayannur City. A total of 20,087 insects of 119 families of 8 orders were sorted. The order Diptera including 46 families with the highest number of individuals accounted for 65.92%, and the dominant families are Sciaridae, Phoridae, Chloropidae, Ceratopogonidae, Culicidae, Milichiidae, Anthomyiidae, and Muscidae. The order Hymenoptera with 34 families accounted for 11.56%, and the dominant families are Diapriidae, Mymaridae, Ichneumonidae, Braconidae, Megaspilidae, and Scelionidae. Among them, 36 species of 26 genera of 21 subfamilies of families Ichneumonidae and Braconidae were identified. The species *Orgilus wuyuanensis* sp. nov. is new to science. Four species——*Anomalon cruentatum* (Geoffroy, 1785), *Lissonota coracina* (Gmelin, 1790), *Dichrogaster kichijoi* (Uchida, 1940), *Mesoleius subroseus* Thomson, 1888——are newly recorded for China and twenty-one species are newly recorded for Inner Mongolia. The host ranges of these parasitoids involve in Lepidoptera, Hemiptera, Coleoptera, and Diptera and the parasitoids control sunflower pests as natural enemies. The thrip *Frankliniella intonsa* (order Thysanoptera) rose to be the main pest from 2021 to 2023 in Wuyuan County. Still, no reports showed that these parasitoids could be natural enemies for control thrips. The further research is needed to discover the parasitoids of thrips in sunflower fields.

Keywords: Hymenoptera; Diversity; new species; new record; sunflower

Funding: The research was funded by the Inner Mongolia Science & Technology Plan Projects (No.2021GG0027, 2022JBGS0010).

Preliminary study on the function of candidate effector P2 of Puccinia helianthi

Xiangjiu Kong, Xiaoyu Lian, Yan Lu, Xiaohan Cui and Lan Jing*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Huhhot Inner Mongolia, China 010011,

*jinglan71@126.com

Abstract: Sunflower rust, caused by *Puccinia helianthi*, is one of the most destructive diseases affecting sunflowers industry worldwide. As a biotrophic fungal pathogen, *P. helianthi* evolves rapidly, easily overcoming sunflower resistance. It is urgent to accelerate the deciphering of the virulence and variation mechanisms of *P. helianthi*, which represents a significant scientific challenge. Given the significant role of effector proteins in the interaction between pathogens and host plants, studying the function of effectors is crucial for unraveling the pathogenic mechanisms of *P. helianthi*.

In this study, a candidate effectors (named P2) was selected from a predicted pool of 497 *P. helianthi* effectors identified previously, based on its prominent effector characteristics, and a preliminary functional analysis of this protein was performed. It was found that P2 has an N-terminal signal peptide, excluding transmembrane domains, typical nuclear localization signals, Pfam domain, and anchoring points. *P2* gene was cloned using *P. helianthi* cDNA as a template. An expression vector, pGR107, carrying the target gene with signal peptide (P2) or signal peptide removed ($P2^{\Delta SP}$), was constructed. The *Agrobacterium* transient expression system in *Nicotiana benthamiana* leaves revealed that neither P2 nor $P2^{\Delta SP}$ induces cell necrosis, but could inhibit the hypersensitive necrosis response triggered by mouse pro-apoptotic protein BAX in tobacco. Besides, a fusion expression vector of P2 and GFP was constructed and expressed in *N. benthamiana* to observe subcellular localization, implying that P2 targets to host cell nucleus and cell membrane. Furthermore, quantitative real-time PCR analysis of *P2* gene revealed that its expression peaked at 24 hours post-inoculation, approximately 14 times higher than the urediospores stage. Our study will lay the foundation for a deeper understanding of the molecular mechanisms underlying the interaction between sunflowers and *P. helianthi*, providing strong evidence for further disease-resistant breeding.

Keywords: Sunflower rust, *Puccinia helianthin*, effectors, virulence, interaction

Funding: The study was funded by National Natural Science Foundation of China (NO: 32160642), the Program for Innovative Research Team in Universities of Inner Mongolia Autonomous Region (NMGIRT2320), and the Natural Science Foundation of Inner Mongolia Autonomous Region (NO: 2024QN03057).

Study on spore germination and infection process of Puccinia helianthi

Yan Lu, Jiayun Wang, Xiangjiu Kong, Jiaying Huang and Lan Jing*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Huhhot Inner Mongolia, China 010011

*jinglan71@126.com

Abstract: Sunflower rust, caused by *Puccinia helianthi*, is one of the most important diseases of sunflower in China and worldwide. The germination of spores is a prerequisite for successful invasion. However, the suitable conditions for the germination of teliospores and aeciospores of *P. helianthi* are not yet clear. Therefore, studying the suitable conditions for the germination of teliospores and aeciospores can help understand the disease occurrence regularity. At present, there have been reports on the ultrastructure of the development stage of urediospores of *P. helianthi*, but no systematic study on the entire development process and related ultrastructure of the five types of spores of sunflower rust. In this study, we screened the optimal germination conditions for teliospores and aeciospores, and the development of *P. helianthi* on the host was observed by electron and optical microscopies. It will provide a theoretical basis to elucidate the complete life history of *P. helianthi*. We found that the collected fresh winter spores could germinate only after being stored at room temperature for more than 180 days. Storage at -20°C had a better germination effect, with a germination rate of 39.45% after 180 days, and by 420 days, the activity of teliospores was lost. Teliospores could germinate on water agar medium at 15°C for 12 hours, reaching a peak germination rate of 31.89% after 48 hours. 20°C and pH 7 were the conditions for the best germination of aeciospores. Light didn't affect the germination of teliospores but promoted the germination of aeciospores. The sunflower began to produce chlorotic spots six to seven days after being infected by urediospores of *P. helianthi*. After 14 days, urediospores broke through the leaf epidermis and formed uredia. Urediospores were elliptical, with dense thorny protrusions on the surface. When leaves aged, teliospores were produced, which had two cells and a smooth surface. Teliospores germinated to produce basidiospores. Five or six days after inoculation with basidiospores, chlorotic spots began to appear on the upper surface of the leaves. After 14 days, orange pycnia formed, which was in flask shape, producing a large number of pycniosporophores and periphyses inside, and honeydew started appearing on the pycnia around 18 days. Aecia began to form six or seven days after applying honeydew, which was cylinder in shape and covered with coglike ornaments. Seven days after inoculating aeciospores on sunflowers, chlorotic spots began to appear on the surface of the leaves, and urediospores were formed after 14 days.

Keywords: Sunflower rust; Teliospores; Aeciospores; Ultrastructure; Fluorescence microscopy

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Genetic diversity of Vericillium dahliae isolates infecting sunflower in argentina

Aguilera, P.[1]*; Colombo, D. [2]; Ben Guerrero, E. [1]; Montecchia, J.F.[1]; Troglia, C.[3]; Corro Molas A. [4]; Maringolo, Carla[5], Deperi, S.[5]; Lia, V.[1]; Martínez, M. C.[5]; Paniego, N.[1]

[1] Instituto de Agrobiotecnología y Biología Molecular (IABIMO), UEDD INTA-CONICET, Bs. As., Argentina. [2] EEA INTA Anguil "Guillermo Covas", La Pampa, Argentina. [3] Agencia de Extensión Agropecuaria INTA Balcarce, Buenos Aires. [4] Agencia de Extensión Agropecuaria INTA General Pico.[5] EEA INTA Balcarce, Bs. As., Argentina.

*aguilera.pablo@inta.gob.ar

Abstract: *Verticillium dahliae* is a soil-borne pathogenic fungus that infects sunflower productive areas worldwide and can significantly reduce crop productivity. Although resistant hybrids are available in the Argentine market, the pressure exerted by climatic events on the pathogens requires monitoring of *V. dahliae* populations in sunflower growing areas as well as the resistance performance of commercial cultivars. In Argentina, four pathogenic races of *V. dahliae* have been described in sunflower (Clemente et al., 2017), but their genetic diversity and population structure remain unknown.

To contribute to the genetic characterization of *V. dahliae* isolates infecting sunflower, 20 isolates from symptomatic plants (2001-2022), two isolates from France and one from the USA, were studied. A panel of 16,471 single nucleotide polymorphism (SNP) markers was obtained by double digestion sequencing (ddRADseq). The MAT genes were amplified by PCR. The resulting allele data were subjected to phylogenetic analyses, which revealed a new lineage of *V. dahliae* linking Argentinean and French isolates to the reference isolate 85S (Deppotter et al., 2019). This lineage is divided into four subgroups that correspond to those described by Clemente et al (2017). The profile of MAT locus distinguishes a sub-group carrying the MAT-1-2 idiomorph from the remaining three corresponding to the Mat-1-1 idiomorph.

These results contribute to our knowledge of the genomic diversity of *V. dahlia*e in sunflower-growing areas in Argentina and provide valuable resources for epidemiological surveillance and the development of resistant cultivars.

Keywords: Sunflower; Verticillium Wilt; Fungal Diversity

Funding: I085/083 INTA Host-Pathogen Interaction Poject, PICT 1689-2019 National Agency for Research, Development and Innovation (ANPCyT).

Diaporthe helianthi identified as the primary causal agent of Phomopsis Stem Canker in the sunflower main growing region in Argentina

Denis Colombo[1]*, Ricardo Comerio[1], Andrés Corró Molas[2] and Norma Paniego[3]

[1] Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Anguil (6326). [2] Instituto Nacional de Tecnología Agropecuaria (INTA) AGR General Pico (6360) / Facultad de Agronomía-Universidad Nacional de La Pampa, Argentina.[3] Instituto de Biotecnología, IABIMO. UDD INTA-CONICET, Buenos Aires, Argentina

*colombo.denis@inta.gob.ar

Abstract: Diaporthe-Phomopsis diseases lead to important yield losses in agricultural production worldwide. In Argentina, Phomopsis stem canker caused by *Diaporthe* spp reappeared in sunflower in 2015 and spread rapidly in the southern growing region. This work aimed to investigate the etiology of the disease. During the 2023/2024 season, 41 commercial fields in the southeastern region of Buenos Aires were surveyed and a regional prevalence of 88% was detected. The incidence of Phomopsis stem canker averaged 5.2%, with a maximum incidence of 40%. In the semiarid Pampa region, the study included 50 commercial fields in La Pampa and western Buenos Aires during the 2023/24 season. The prevalence of stem canker was 68%, with a maximum incidence of 5%. Samples were taken from symptomatic sunflower stems. Infected stems were sterilized with 70% (v/v) ethanol for 30 seconds and 1% (w/v) sodium hypochlorite for one minute. Tissue fragments were aseptically cut from the edge of the internal lesion, transferred to potato dextrose agar (PDA), and incubated at 25°C for 14 days. A total of 220 isolates were obtained, showing expanded colonies with whitish to brownish mycelium, scattered stromata, and dispersed pycnidia. Morphometric characteristics consistent with *Diaporthe helianthi* Munt-Cvetk., Mihaljč. & M. Petrov were observed. After obtaining the isolates, DNA was extracted using the CTAB method. The intergenic ribosomal spacer region (ITS) and translation elongation factor 1-α (EF1-α) were amplified and sequenced using ITS1/ITS4 and EF1-728F/EF1-986 primers. The sequences obtained presented more than 99% similarity to the ITS and EF1-α regions of the *D. helianthi* type material. These results indicate that *D. helianthi* is the predominant causative agent of sunflower Phomopsis stem canker in the main growing region in Argentina. Regular monitoring of the pathogen in these regions will play a role in the development of resistant sunflower hybrids.

Keywords: Diaporthe; Sunflower; stem canker; incidence

Advancements in sunflower multiparental population phenotyping for Verticillium Wilt using UAV-based multispectral imagery

Matías Domínguez[1], Denis Colombo[2], Alexandra Dillcheneider[2], Javier Lavandera[1], Andrés Corro Molas[3], Carolina Troglia[4] and Norma Paniego[5*]

[1] INTA, EEA Pergamino, Argentina; [2] INTA, EEA Anguil, Argentina; [3] INTA, AER General Pico, Argentina; [4] INTA, EEA Balcarce, Argentina; [5] INTA, Instituto de Agrobiotecnología y Biología Molecular, Argentina. *paniego.norma@inta.gob.ar

Abstract: Here we present progress in phenotyping a sunflower Multiparent Advanced Generation Inter-Crosses (MAGIC) population for Verticillium wilt (VW), one of the most important sunflower diseases in Argentina. In addition, the implementation of high-throughput phenotyping (HTP) using unmanned aerial vehicles (UAV) is being explored to complement manual phenotyping and integrate it into breeding pipelines. A subset of 349 F2-MAGIC families was studied during the 2020/21 summer season in a VW-infested field in the EEA INTA Balcarce (37°50′ 0″ S, 58°15′ 33″ W, Argentina). Eighty F5-MAGIC contrast families for VW were selected from the 2020/21 phenotyping trial and phenotyped in another VW- infested field in the EEA INTA Anguil (36° 32′17″ S, 63° 59′ 20″ W) in the 2023/24 summer season. VW incidence, severity and disease severity index (DSI) were recorded for each plot (one row of 5 m length). In the 2020/21 season, the trial was flown once during the flowering period (R5) using a Parrot Disco-Pro Ag drone with a Parrot Sequoia camera with 4 spectral bands, including green (G) (550nm ± 40nm), red (R) (660nm ± 40nm), red edge (RE) (735nm ± 10nm) and near infrared (NIR) (790nm ± 40nm). The flight altitude was 50 m. In the 2023/24 season, we used a Phantom 4 drone with a multispectral camera with five bands, including the blue (B) (450nm ± 16nm), G (560nm ± 16nm), R (650nm ± 16nm), RE (730nm ± 16nm) and NIR (840nm ± 26nm). The flight altitude was 40 m and the trial was flown four times during the flowering and grain-filling period from R1 to R9. The image processing was done with Agisoft Metashape for building the orthomosaics and with QGIS for creating the grid plot, extracting the reflectance and the vegetation indices (VIs) values. The Normalized Difference Vegetation Index (NDVI), the Normalized Water Vegetation Index (NWVI), the Optimized Soil-Adjusted Vegetation Index (OSAVI), and the Leaf Chlorophyll Index (LCI) VIs were estimated for the 2020/21 season. For the 2023/24 season, the NDVI, the Green Normalized Difference Vegetation Index (GNDVI), the Enhanced Vegetation Index (EVI), the Normalized difference red edge index (NDRE), the Green Red Vegetation Index (GRVI), the Green Leaf Index (GLI), the Plant Senescence Reflectance Index (PSRI), the Differenced Vegetation Index (DVI), the Visible Atmospherically Resistant Index (VARI) and the Chlorophyll Index Red Edge (CIRE) were extracted from each flight. Using the information from the spectral bands and the VIs, different machine learning models (MLM) were applied to classify each plot as susceptible or resistant to VW using the CARET library in R. The results confirmed the phenotypic variability of the MAGIC population for VW. Thirty resistant MAGIC F5 families exhibiting a DSI below 5 % were identified as valuable candidates for future breeding purposes. The MLM achieved a prediction accuracy of about 65 % in both trials, with the XGBoost model showing better prediction performance. Overall, the results highlight the potential of HTP for sunflower disease phenotyping and its applicability in sunflower breeding programs.

Keywords: MAGIC, Machine learning, disease phenotyping, prediction

Funding: I111 INTA oil crops research project, PICT 2523-2017 National Agency for Research, Development and Innovation.

Classification of the agromyzids (Diptera: Agromyzidae) in sunflower fields

Xinting Fu, Li Shi*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010018, China

*lirui2003@imau.edu.cn

Abstract: The pest damage is one of the major constraints for sunflower (*Helianthus annuus* L.) production. Among them, the harm of agromyzids is easily ignored just because the damage symptoms by larvae are insignificant and get no attention. The 3,375 species of 39 genera of family Agromyzidae in Diptera are known in the world and 169 species of 21 genera occur in China. The seventeen species are sunflower miners with thirteen species damaging leaves, two species harming stems and carrying bacteria or fungi, and two species destructing seed production. At present, there are few studies on sunflower miners in China with only three species Liriomyza sativae, Liriomyza trifolii, Phytomyza horticola as sunflower miners. In this study, the agromyzids were collected by the malaise trap in the sunflower fields of Wuyuan County, Bayannaoer City, Inner Mongolia. The comparative morphology and anatomy were used to classify species of seven genera (Amauromyza, Ophiomyza, Agromyza, Melanagromyza, Liriomyza, Phytomyza, Calycomyza) of the family Agromyzidae. The results showed that two species Amauromyza wuyuanensis sp. nov. and Ophiomyia neimengguensis sp. nov. are new to science, and two species Amauromyza karli and Agromyza kincaidi are newly recorded for China, with twenty-one plates of adults and male genitalia. The keys to three genera and thirty-five species are provided. All examined specimens are deposited in the entomological museum of Inner Mongolia Agricultural University (IMAU). This study aims at providing scientific data on control of agromyzids and exploiting domestic parasitoids on sunflower miners and reducing the economic damage to sunflower production in the future.

Keywords: leaf-mining flies; taxonomy; agromyzids; sunflower

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Identification and dual RNA-seq analysis of sunflower head rot pathogen

Junbin Hou,[1,2] Zhuo Wang,[1,2] Xu Ran,[1,2] Siqi Tang,[1,2] Wei Zhang,[3] Hongliang Lei,[3]

Zhonghua Wang[1,2*]

[1] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, Northwest A&F University, Yangling, Shaanxi 712100, China; [2] College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100, China; [3] Erdos Ecological Environment Career Academy, Erdos, Inner Mongolia 017010, China

*zhonghuawang@nwafu.edu.cn

Abstract: Sunflower (*Helianthus annuus* L.) is the important oil economic crop in China. But in recent years, many kinds of sunflower diseases are seriously threatening the development of the sunflower industry. In 2022, a large number of sunflower head rot was found in the experimental plot of Northwest A & F University. Through the isolation and identification of pathogens in the infected tissues, it was found that the head rot was caused by *Rhizopus oryzae* (syn. *Rhizopus arrhizus*). In order to further explore the pathogenic mechanism of *R. oryzae* on sunflower, transcriptome sequencing was used to analyze the gene expression profile changes of *R. oryzae* and sunflower during the infection. As a result, 2676 differential genes (DEGs) of *R. oryzae* were obtained, including 699 up-regulated genes and 1977 down-regulated genes, and there were 6868 DEGs in sunflower, including 3380 up-regulated genes and 3488 down-regulated genes. Then by using the Blast tools, these differential genes were grouped with the non-redundant Protein Database (NR), Swiss-Prot, Cluster of Orthologous Groups Database (COG), euKaryotic Orthologous Groups Database (KOG) for annotation, Gene Ontology Database (GO), and Kyoto Encyclopedia of Genes and Genomics Database (KEGG). It was found that sunflower inoculated with *R. oryzae* resulted in significant up-regulated expression of genes involved in lipid metabolic process, oxidoreductase activity, biosynthesis of unsaturated fatty acids, and fatty acid metabolism suggesting that *R. oryzae* increased the synthesis of lipid and fatty acids in sunflowers to provide energy for itself. Also, a series of plant defense-related genes were down-regulated, including transcription factors associated with plant defense, plant hormone signal transduction, MAPK signaling pathway, and plant-pathogen interaction, suggesting that *R. oryzae* may inhibit these genes and pathways to weaken sunflower defense to benefit infection. On the other hand, DEGs in *R. oryzae* were mainly enriched in genes related to metabolic pathways and carbon metabolism, which suggested that during infection, the metabolic process in *R. oryzae* is absolutely animated. This work reveals the dynamic alterations in *R. oryzae* genes during its infection and the impact on the transcriptional levels of sunflower genes. We hope that this work will contribute to the theoretical foundation for uncovering the molecular mechanisms underlying sunflower disease resistance and for the development of disease-resistant sunflower cultivars.

Keywords: sunflower head rot; Rhizopus oryzae; pathogenic mechanism

Variation of resistance of some Downy Mildew resistance genes to Downy Mildew races in Trakya region, Turkey

Mehmet İbrahim Yilmaz[1*], Veli Pekcan[1], Samet Saglam[1], Bayram Serkan Cabar[1], Göksel Evci[2],
Yalçın Kaya[3]

[1] Trakya Agricultural Research Institute, Edirne, Türkiye; [2] Trakya Birlik, Edirne, Türkiye;
[3] Trakya University Engineering Faculty, Genetic and Bioengineering Dept, Edirne, Turkey

*yilmazmehmet22@hotmail.com

Abstract: Sunflower is the most important oil crop in the Balkans and Blacksea region which has over 60% of world sunflower production. Biotic stresses are the main limiting factors reducing sunflower yield. Broomrape parasite and sunflower downy mildew (*Plasmopara halstedii* (Farlow) Berlese & de Toni) are the main problems in sunflower production areas in this region. Downy mildew fungal disease has developed over twenty races in sunflower and exists in almost all parts of the world. Therefore, resistance to downy mildew is one of the main targets in sunflower breeding programs. It has been determined that there are some races of downy mildew in the Trakya region which is European part of Turkey and has more than 50% of sunflower planted areas of Turkey and resistant varieties have been developed and planting widely in the region. However, in recent years, mildew disease has started to be seen again in planting varieties widely used in the region. This study aimed to determine the changes in resistance status of the Pl_6, Pl_8 and Pl_{13} resistance genes, which provide resistance to downy mildew races in the Trakya Region of Turkey, in recent years (2012-2023). In this study, HA 335 lines containing the $P1_6$ gene, RHA 437 lines containing the $P1_8$ gene and HAR5 lines containing the $P1_{13}$ gene were used. In the HA 335, RHA 437 and HAR 5 lines, 12.6%, 2.3% and 0% symptoms were observed in 2012, respectively, while 96.7 %, 47.9% and 5.3% of the disease symptoms were observed in 2023. As a result, it could be concluded that new downy mildew races have started to appear in the Trakya Region and the resistance of the Pl_{13} gene continues to the new races in the region.

Keywords: Sunflower; hybrid breeding; resistance; downy mildew; races

Identification of signal peptide secretory function of metalloprotease PhMEP1 from *Puccinia helianthi*

Jiaying Huang, Yan Lu* and Lan Jing*

College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Huhhot Inner Mongolia, China 010011

*luyan820918@126.com; jinglan71@126.com

Abstract: Sunflower rust, caused by *Puccinia helianthi*, is one of the most important diseases threatening sunflower production, and that can also reduce seed quality and oil content. However, the key disease-associated genes of sunflower rust are still not clear, so, there is no specific targets for prevention. Identifying the pathogenic factors and mechanism of rust is essential for disease control strategies. Our team found that the extracellular metalloprotease PhMEP1 plays an important role in the pathogenesis of disease. To further clarify its pathogenic mechanism, in this study, we first identified the sites where the protein interacts with the host. The transport of proteins depends on a signal sequence with complex physiological functions. The signal peptide (SP) of PhMEP1 is a signal sequence that can be cleaved, but the protein transport depends on it. The signal peptide sequence of PhMEP1 was analyzed for structure and function using bioinformatics software, and the results suggested that the signal peptide was the front 22 amino acids of the ORF region. Then, the secretory function of the signal peptide was verified by using the yeast secretion system. The results showed that the yeast transformants of the signal peptide fusion protein could grow normally on YPRAA medium, the secretion of invertase and the hydrolysis of raffinose into monosaccharides were also confirmed with TTC, which reacts with monosaccharides to form the insoluble TPF. To determine whether the signal peptide has a secretory function in the pathogen-plant interaction, western blot was used to detect the expression of the signal peptide fusion protein in tobacco cells. The results showed that the signal peptide fusion protein was detected in tobacco apoplastic fluid, indicating that the fusion protein could be transferred to the intercellular with the signal peptide of PhMEP1. These results suggest that the signal peptide of PhMEP1 has secretion activity, and the protease was released to extracellular space to perform the function.

Keywords: *Puccinia helianthi*; Metalloproteinases; Signal peptide; Secretion activity

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Unravel Biological Mechanism Under Decreasing Disease Severity of Sunflower Verticillium Wilt Via Delaying Sowing Date

Jianfeng Yang[1], Jian Zhang[1], Tie Li[1], Wenbing Zhang[1], and Jun Zhao[1]*

[1] College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot, China,

*zhaojun@imau.edu.cn

Abstract: Verticillium Wilt (VW), caused by *Verticillium dahliae* Kleb. is particularly challenging due to the soil-borne nature of *V. dahliae*. Our research, conducted over multiple years and across various locations, indicates that delaying the sowing date can effectively reduce disease incidence. However, the underlying biological mechanisms driving this agronomic response remain unclear.

Preliminary data suggest that delaying the sowing date results in a decrease in the number of microsclerotia detected through both Q-PCR and NP-10 selection medium. Correspondingly, the total biomass of *V. dahliae* colonizing sunflower roots was reduced. High-throughput sequencing of soil samples collected at different sowing dates revealed an increased abundance of bacterial genera, such as Pseudomonas, Azoarcus, and Bacillus, in soils from later sowings. By integrating bacterial isolation data from early and late sowing soils with microbial diversity analyses, we identified five bacterial strains that were more enriched in late-sown soils and exhibited antagonistic activity. These strains were incorporated into synthetic communities (SynComs) and added to the soil, resulting in a noteworthy disease suppression efficacy of 33.12% following SynCom application. Additionally, the physical and chemical properties of soils at different sowing dates were analyzed using a handheld soil rapid testing platform. The most significant differences observed were in soil temperature and humidity. We subsequently focused on soil temperature to investigate its effects on the growth of *V. dahliae* and the associated changes in host plant resistance. As expected, increased temperatures correlated with a decrease in pathogen growth. Conversely, higher temperatures promoted more rapid growth of the sunflower root system, along with enhanced accumulation of callus and lignin. This was accompanied by increased expression of resistance-related genes (HanXRQr2_Chr03g0133841, HanXRQr2_Chr10g0420821, HanXRQr2_Chr03g0133841, etc.) and elevated activity of resistance-associated enzymes (PAL, SOD, POD, etc.). In conclusion, our study demonstrates that higher soil temperatures suppress the growth of *V. dahliae* while enhancing crop resistance. The altered composition of key soil microorganisms further influences the pathogen's ability to colonize the host root system, thereby reducing disease incidence.

Keywords: Sunflower Verticillium Wilt; Delaying Sowing Date; Disease Reduction; Biological Mechanism.

Funding: This research was funded by Basic research funds of Inner Mongolia Agricultural University (BR22-13-09), China Ministry of Agriculture, China Agricultural Research System (CARS-14).

Study on the control effect of different pesticides on the westernflower thrips of sunflower

Mu Jianguo[1,2], Yi Guanghui[2], Chen Guihong[3], Potakuzi Ayidenbek[2], Zhang Xuekun[5], Chen Yanfang[2], Cui Jiawei[2], Xing Qianqian[2], Mei Zeyu[2], Wan Sumei[1], Liu Yantao[4]*, Wang Peng [4]*

[1] Tarim University, Alar City, Xinjiang 843300, China; [2] Agricultural Science Research Institute of the 10th Division, Xinjiang Production and Construction Corps, Beitun 836000, China; [3] Agricultural Technology Extension Station of the 10th Division of Xinjiang Production and Construction Corps, Beitun City, Xinjiang 836000, China; [4] Crop Research Institute, Xinjiang College of Reclamation Science, Shihezi, Xinjiang 832000, China;
[5] Shihezi University, Shihezi City, Xinjiang 832000

*125034325@qq.com

Abstract: The damage caused by the western flower thrips (*Frankliniella occidentalis*) to sunflowers is increasingly serious, and its host range is wide, especially damaging chrysanthemum crops. The western flower thrips feed on the tender seed coat of sunflower during the flowering periods, causing damage to the appearance of sunflower seeds as a commercial product. In this study, 8 different pesticides were used to control western flower thrips in the budding and flowering periods of sunflowers. The thrips population, residual insects, and seed weight after spraying for 3 days and 5 days were measured. The results showed that spraying 5% methyl abamectin in the budding period had the best control effect on western flower thrips, with a control effect of more than 60% after spraying for 3 days and 5 days and an increase of 55.56% in seed weight of sunflowers, and the hazard rating of rust spots were all in level 1. Spraying 60g/L ethiprole in the flowering period had good persistence in reducing the thrips population and control effect for 3 days and 5 days with an increase of 46.67% in seed weight, and the hazard rating of rust spots were all in level 0-1. This study can provide green and safe pesticide control technology for the control of rust spots caused by western flower thrips in sunflowers, and provide a theoretical basis for the healthy and sustainable development of the sunflower industry in Xinjiang.

Keyword: Edible sunflower; western flower thrips; control effect

Using multi-service cover crops to control sunflower soil-borne pests

Neila Ait Kaci Ahmed[1], Thibault Roudaire[2], Mireille Chabaud[2], Sophie Smith Caillault[2], Stéphane Muños[2*], and Célia Seassau[1,3*]

[1] AGIR, Université de Toulouse, INRAE, Castanet-Tolosan, France; [2] Laboratoire des Interactions Plantes Microbes Environnement (LIPME), CNRS, INRAE, Université de Toulouse, Castanet-Tolosan, France; [3] INP-PURPAN, Université de Toulouse, Castanet-Tolosan, France

*stephane.munos@inrae.fr ; Celia.Seassau@purpan.fr

Abstract: In France, sunflower cultivation is generally carried out in short rotations following a long fallow season when the soil is left bare. This practice not only causes nutrient leaching but also increases the phytosanitary pressure exerted on the crop. It notably leads to an increase in the soil seed bank of *Orobanche cumana* and inoculum of pathogenic fungi like *Verticillium dahliae*, which can remain in the soil for several years waiting for favorable germination conditions. Yield losses caused by these organisms are currently limited thanks to sunflower varietal resistance and chemical pesticides. However, this resistance can be overcome by the evolution of pathogen races, and the use of chemical pesticides must not become the norm. Biofumigation of selected multi-service cover crops implemented before the sunflower cultivation could be a promising method to regulate soil-borne pests thanks to the natural toxic compounds they release. By simulating artificial biofumigation under controlled conditions using plant grinds, we evaluated the ability of three Brassicaceae species (*Brassica juncea*, *Brassica rapa* ssp. *oleifera*, *Raphanus sativus*) and one Fabaceae species (*Vicia benghalensis*) to inhibit the development of *O. cumana* and *V. dahliae*. These plants significantly inhibited the development of both pests compared to the control, with *Brassica juncea* showing the most pronounced effects achieving up to 100% inhibition. These results pave the way for a novel agroecological control method for sunflower soil-borne pests, which could complement ongoing breeding efforts for generating resistant sunflower varieties. The identification of the molecules responsible for this inhibition is currently under investigation.

Keywords: Multi-Service Cover Crops; *Helianthus annuus* L.; *Orobanche cumana*; *Verticillium dahliae*; biofumigation.

Funding: PlantAlliance; ANR; Plant2Pro; MAS Seeds

Session 5: Physiology and abiotic stress

Recuperating from stress – How does sunflower recover from drought?

Aleksandra Radanović[1*], Boško Dedić[1], Laura Verena Junker-Frohn[2], Anna Galinski[2], Sandra Cvejić[1], Milan Jocković[1], Srđan Bursać[1], Nemanja Ćuk[1], Jelena Jocković[1], Siniša Jocić[1], Kerstin A. Nagel[2], Dragana Miladinović[1]

[1] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, 21000 Novi Sad, Serbia, [2] IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany,。

*aleksandra.radanovic@ifvcns.ns.ac.rs

Abstract: Climate change profoundly affects crop production. Under field conditions, there is a cycle of rainfall and rainless periods. The intervals between rainfalls have become prolonged in certain growing areas due to climate change leading to a significant reduction in crop production. The ability of crops to recover from drought stress depends on the species, genotype, and the duration and severity of drought stress. Sunflowers are particularly sensitive to drought at the germination stage. Therefore, this study aimed to develop a test to assess the capacity of sunflower to recover from stress at the initial developmental stage. The test was condected in rhizotrons to monitor root growth in controlled conditions. After 7 days of drought stress, plants were re-watered to reach the control's gravimetric water content (65% qwc). Re-watering in rhizotrons can be challenging due to the surface depth ratio, which hinders the uniform distribution of water. To address this, we introduced a novel slow re-watering method, facilitating a more homogeneous increase in soil water content throughout the rhizotron. For developing this test, we selected a drought-sensitive sunflower line, subjected it to drought conditions (50% gwc), and then re-watered it to observe its recovery capacity. Root and shoot traits between control and treatment were analyzed, including total, primary and lateral root length, root system width and depth, leaf area, and fresh and dry shoot weight. This test will provide valuable insights into how sunflowers recover from drought stress, which will be very helpful in breeding programs.

Keywords: *Helianthus annuus* L., drought, re-watering, root phenotyping, rhizotron

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Unravelling mechanisms of drought tolerance and stress recovery in sunflower

Aleksandra Radanović[1]*, Sandra Cvejić[1], Boško Dedić[1], Milan Jocković[1], Srđan Bursać[1], Nemanja Ćuk[1], Jelena Jocković[1], Siniša Jocić[1], Dragana Miladinović[1]

[1] Institute of Field and Vegetable Crops, National Institute of Republic of Serbia,
21000 Novi Sad, Serbia,

*aleksandra.radanovic@ifvcns.ns.ac.rs

Abstract: Drought is a global threat to food security and is a major abiotic factor limiting crop production. Enhancing drought tolerance in crops is therefore a critical goal in breeding programs worldwide. Despite being considered a moderately drought-tolerant crop, sunflower's production is still affected by drought. Drought tolerance is a complex trait, that triggers numerous responses at morphological, physiological and molecular levels. Hence, a comprehensive approach is needed to decipher the underlying mechanisms in sunflower.

At the Institute of Field and Vegetable Crops (IFVCNS), we have created a broad sunflower panel of nearly 50 IFVCNS inbred lines, that were tested under *in vitro* conditions. Through comprehensive phenotyping, we identify traits associated with drought tolerance. The most drought-tolerant and sensitive genotypes were identified and subjected to further testing in pot experiments to validate the *in vitro* results and to examine sunflower responses to drought stress at later development stages on transcriptomic and epigenetic levels. Additionally, the recovery capacity of the genotypes is being examined. Current efforts are focused on determine the key mechanisms involved in drought tolerance by analyzing gene expression, transcriptome and epigenome variations. The goal of this research is to identify stable drought-induced transcriptomic and epigenetic variations, as well as target genes and epiQTLs, that can be used in marker-assisted breeding.

Keywords: *Helianthus annuus* L., abiotic stress, transcriptome, non-coding RNAs, epiQTLs

Funding: This work is supported by the European Commission through projects CROPINNO, grant number 101059784, Horizon Europe HelEx N 101081974 funded by the European Commission, the Science Fund of the Republic of Serbia, through IDEAS project "Creating climate smart sunflower for future challenges" (SMARTSUN), grant number 7732457, by the Ministry of Science, Technological Development and Innovations of Republic of Serbia, grant number 451-03-66/2024-03/200032, and by the Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia. Moreover, it is a part of COST Actions "Reproductive enhancement of CROP resilience to extreme climates" (RECROP) – CA22157 and "Epigenetic Mechanisms of Crop Adaptation to Climate Change" (EPI-CATCH) – CA19125.

Root Morphological Differences and Drought Resistance Identification of confectionary Sunflower Hybrids under Drought Stress at Seedlings Stage

Bing Yi*, Mingzhu Zhao, Xiaohong Liu, Dexing Wang, Dianxiu Song, Enyu Sun, Liangji Cui, Jingang Liu, Liangshan Feng

Liaoning Academy of Agricultural Sciences, Donglin Rd, No.86, Shengyang, China

*yibing8749@126.com

Abstract: Studying the effect of drought stress on sunflower root morphology and biomass, so as to identify drought resistance sunflowers is of great significance for drought resistance breeding and water-saving cultivation of sunflower. In this study, 17 confectionary sunflower hybrids were used, applying a 1/2Hoagland nutrient solution hydroponic method with PEG-6000 to simulate drought stress. The response of root morphology and biomass at seedling stage to drought stress was analyzed, and a comprehensive evaluation of the drought resistance in these hybrids was conducted.

The results showed that under drought stress at seedling stage, the total root length and number of root tips mainly increased, while root surface area, root volume and root average diameter decreased. Root dry weight and root-to-shoot ratio increased, whereas shoot dry weight and overall plant biomass were inhibited. The root morphology of confectionary sunflower at seedling stage is more sensitive to drought stress than biomass. The sensitivity ranking of root morphology to drought stress was as follows: root volume, average root diameter, number of root tips, total root length, root surface area. The sensitivity ranking of biomass was shoot dry weight, root-to-shoot ratio, plant dry weight, and root dry weight. The comprehensive drought resistance capacity (D value) was highly significant positively correlated with total root length, root dry weight, plant dry weight, root tips number and root surface area range, and significantly positively correlated with shoot dry weight. Confectionary sunflower AR7-6660, Chi 2008 and Xinsikui No.7 demonstrated strong comprehensive drought resistance at seedling stage.

Keywords: Confectionary sunflower; Drought stress; Root morphology; Drought resistance identification

Funding: Supported by China Agriculture Research System of MOF and MARA(CARS-14); Shenyang Municipal Science and Technology Plan: Seed Industry Innovation Special Program (22-318-2-19).

The Sunflower Kinome: Genomic and Transcriptomic Analysis Under Biotic and Abiotic Stresses

Ningning Yan[1], Jian Zhang[1], and Jun Zhao[1]*

[1]College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Zhaowuda Rd.306, Saihan Distr. Hohhot, China,

*zhaojun@imau.edu.cn

Abstract: Protein kinases play crucial roles in plant responses to biotic and abiotic stresses, as well as in growth and development processes. While the kinome has been extensively investigated in crops like Arabidopsis, soybean, common bean, and cotton, studies on protein kinases in sunflower remain limited. Our objective is to explore protein kinases in sunflower, aiming to fill this research gap and contribute to the understanding of protein kinase functions in this particular crop. Using HMMER tools, we conducted a screening of protein kinases in sunflower, and further validated the results through phylogenetic analyses and reference to the Pfam database. This analysis yielded a total of 2,583 protein kinases, which were subsequently categorized into 22 families and 121 subfamilies. The largest family identified was RLK-Pelle, consisting of 1,967 protein kinases, representing 76.15% of the total count. Following that, we proceeded to investigate the chromosome distribution, molecular weight, isoelectric point, as well as the structural and evolutionary diversity of protein kinases in sunflower. Through collinearity analysis, it was revealed that sunflower underwent a whole-genome duplication (WGD) event approximately 32.72 million years ago (MYA). These findings strongly suggest that the expansion of HaPKs in the sunflower genome is primarily driven by the WGD mechanism. Remarkably, this mechanism contributed to the expansion of 15 out of the 22 HaPK families. To investigate the involvement of protein kinases in sunflower's response to diverse stresses, we analyzed transcriptome data from sunflower plants subjected to eight abiotic stresses and two biotic stresses. By conducting an extensive analysis of these transcriptome datasets, we identified 73 significant protein kinases, whose functions were annotated. This enabled us to preliminarily determine the role of HaPKs in sunflower's adaptive growth. Our objective is to make a valuable contribution to sunflower resistance breeding, leveraging the insights gained from this research.

Keywords: Sunflower; Protein kinase; Biotic stress; Abiotic stress

Funding: This research was funded by Basic research funds of Inner Mongolia Agricultural University (BR22-13-09), China Agricultural Research System (CARS-14).

The Impact of Drought and Salt Stress on Leaf Wax Accumulation and Cloning of Wax Alkane Synthesis Genes in Sunflower (Helianthus annuus L.)

Zhengyang Wei[1, 2], Xueqi Wang[1], Weiwei Li[1], Xiali Zhou[2], Zhonghua Wang[1], Bing Jing[1*]

[1] Northwest Agriculture and Forestry University, Yanglin, Shannxi; [2] Zhumadian Academy Sciences of Agricultura, Zhumadian, Henan *jingbing@nwafu.edu.cn

Abstract: Drought and salt stresses significantly affect the growth and development of sunflower. Plant cuticular wax, which covers the surface of plant tissues, plays a crucial role in reducing water loss and enhancing the plant's adaptability to water-deficient environments. This study investigates the effects of drought and salt stresses on wax accumulation in sunflower leaves. The leaf cuticular wax content and components of a selected oilseed sunflower inbred line were measured and analyzed under drought, salt and PEG6000-induced stresses using gas chromatography-mass spectrometry. Candidate genes associated with the synthesis of alkanes were identified through bioinformatics analysis. The expression patterns of two candidate genes under salt and PEG6000 treatments were examined using quantitative real-time PCR (qRT-PCR). The function of *HaCER3-1* was investigated via heterologous expression in *Arabidopsis thaliana*. The findings are as follows:

The main components of the leaf epidermal wax in sunflower seedlings include primary alcohols, alkanes and fatty acids. After 10 days of exposure to drought and salt stresses, the total amount of epidermal wax in sunflower leaves increased by more than 8.0% compared to the control group. Specifically, the contents of alkane and fatty acid increased by 62.5% and 45.0%, respectively, following the drought treatment, and by 47.0% and 49.0%, respectively, following the salt treatment.

Six candidate genes involved in alkane synthesis were identified and the phylogenetic tree showed that they are categorized into two groups: *CER1* homologs and *CER3* homologs. Among the three *CER3* homologous genes, *HaCER3-1* exhibited the highest similarity to the Arabidopsis *CER3*. Transcriptomic analysis indicated that *HaCER1-1* and *HaCER3-1*, potentially involved in alkane synthesis, were highly expressed in sunflower leaves. Sequencing results showed that the coding regions of *HaCER1-1* and *HaCER3-1* are 1869 bp and 1674 bp in length, encoding proteins of 622 and 557 amino acids, respectively. qRT-PCR results revealed that *HaCER1-1* and *HaCER3-1* were significantly up-regulated after salt and PEG6000 treatment. Twelve hours after processing, the expression of *HaCER1-1* increased by more than 30-fold, and that of *HaCER3-1* increased by more than 10-fold, indicating that these genes are induced by drought and salt stresses. Overexpression of *HaCER3-1* increased the alkane content in the rosette leaves of transgenic plants and reduced leaf permeability. A water-deficit experiment demonstrated that overexpression of *HaCER3-1* decreased the wilting rate of transgenic plants under water-deficient conditions.

Keywords: Sunflower; Cuticular wax; Alkane; salt stress; drought

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Drought Tolerance Mechanisms in Sunflower: molecular, physiological, and morphological responses

Maria Duca, Steliana Clapco*, Angela Port, Ana Mutu, Rodica Martea

Moldova State University, Chisinau, Republic of Moldova,

*steliana.clapco@usm.md

Abstract: Sunflower (*Helianthus annuus* L.) faces substantial challenges due to changing climatic conditions, particularly the escalating threat of drought stress. This study employs a comprehensive approach to evaluate drought tolerance in sunflower hybrids, integrating both field and laboratory screening with molecular analyses. The research focuses on analyzing plants phenotypic traits and transcriptional changes in cotyledons and roots of sunflower hybrids exposed to different levels of drought stress.Forty experimental hybrids were screened under natural drought conditions during the dry year of 2020 and under osmotic stress induced by PEG-6000 at different concentrations The study examined productivity-associated traits, as well as of seeds germination indices. Results revealed significant variations of 14 studied morpho-physiological parameters between stressed and non-stressed plants, leading to the classification of hybrids as either tolerant or sensitive. The germination stress tolerance index and root length stress index were identified as the most important indicators in genotypes differentiation at the germination and seedling stage, making them useful for preliminary screening of sunflower germoplasm. As a result, eight hybrids exhibited superior drought tolerance and could be integrated into breeding programs.

To further understand sunflower responses to drought stress, the expression of specific drought-induced genes (*Rab18-like, Xero1* and *COR47-like*) was analyzed in a model system including both a drought-tolerant and drought-sensitive hybrid subjected to progressive hydric stress. Investigation of dehydrins (DHNs) accumulation provided insights into their differential expression patterns in response to induced hydric stress. The drought-tolerant hybrid indicated increased expression levels of DHNs genes, suggesting their potential use as markers for estimating drought resistance and improving pre-screening trials in breeding programs.

In conclusion, the integration of laboratory screening, seed germination analysis, and molecular insights contributes to a comprehensive understanding of drought tolerance in sunflower hybrids. The identified tolerant genotypes and molecular markers offer valuable inputs for enhancing breeding programs aimed at developing sunflower varieties resilient to water-deficit stresses.

Keywords: sunflower; abiotic stress; hydric stress; dehydrins; germination

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Study on the effect of melatonin on saline-alkali tolerance transcriptome and metabolome of different types of oil sunflower

Changyan Zhao[1,2], Yantao Liu[1*], Xiuping Jia[3], Shengli Liu[1], Peng Wang[1], Hongye Dong[1], Zengshuai Lv[1] and Wei Duan[1]

[1]Crop Research Institute, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, Xinjiang Uygur Autonomous Region, China;[2]College of Agriculture, Tarim University, Alar, Xinjiang 843300, China; [3]Crop Research Institute of, Gansu Academy of Agricultural Sciences, Lanzhou, Gansu,730070, China

*ziheng1979@126.com

Abstract: Seedling stage is a critical period for sunflower, characterized by the weakest tolerance to salts, and saline-alkali stress, which significantly affect growth, development and yield formation. In this study, the salt-tolerant germplasms were identified, and then the important metabolic genes and pathways were determined involved in the response to exogenous melatonin under saline-alkali stress. Through transcriptome and metabolome analysis, we investigated the effects of melatonin on sunflower with varying salt tolerance levels. Transcriptome analysis revealed 530 common genes (37 up-regulated genes and 493 down-regulated genes) in leaves under melatonin+salt stress and salt stress treatments. These genes were primarily involved in fatty acids metabolism, diterpenoid biosynthesis, linolenic acid metabolism, and cysteine and methionine metabolism. Metabolome analysis showed 60 common genes (17 up-regulated and 43 down-regulated), associated mainly with tryptophan metabolism, amino acid biosynthesis, secondary metabolite biosynthesis, and other metabolic pathways. The integrated transcriptome and metabolome analysis found that melatonin increased the content of metabolites such as spermine and spermidine in sunflower under salt stress by regulating the pathways like β-alanine metabolism, monoterpene biosynthesis, and glutathione metabolism, thereby improving the salt tolerance of sunflower.

Keyword: Sunflower; Abiotic stress; Multi-omics

Transcriptomics and Genome-Wide Association Analysis reveal salt tolerance genes in sunflower

Xinrong Zhang, Yuxiao Li, Lei Wang, Jiajun Wu, Wei Wang, Ling Wang, Xingchu Yan, Meilian Tan*

Oil Crops Research Institute, Chinese Academy of Agricultural Science,
Wuhan, Hubei Province, 430062

*meiliantan@126.com

Abstract: Soil salinization, an increasingly severe environmental issue exacerbated by global warming, considerably limits the production of the oilseed crop sunflower (*Helianthus annus* L.). To develop efficient salt-tolerant varieties, a better understanding of the mechanisms regulating salt tolerance is essential, and identifying salt tolerance genes is crucial for breeding efforts. However, the regulation mechanisms of salt tolerance at the transcriptomic level in sunflower remains unclear, and the genes regulating salt tolerance in sunflower are still in progess.

In this study, we present an integrated study combining transcriptomics and genome-wide association (GWA) analysis to identify salt-tolerance genes in sunflower. Based on morphological, physiological and biochemical indicators, it was found that the growth inhibition of salt-tolerant variety (HZ001) under salt stress was weaker than that of salt-sensitive variety(Y5A×YJMR2), which may be related to the stronger antioxidant activity and osmotic regulation mechanism in HZ001.

The profiles of gene expression between HZ001 and Y5A×YJMR2 under 250 mmol/L NaCl stress for 7 and 14 days revealed significant enrichment in the MAPK signaling pathway and plant hormone signal transduction pathway in response to salt stress. Within these pathways, one gene encoding abscisic acid (ABA) receptor (*LOC110884474*), four genes encoding peroxidase-related enzymes (*LOC110894758, LOC110894649, LOC110872274, LOC110894757*), four genes encoding *PP2C* transcription family members (*LOC110873889, LOC110877093, LOC110877945, LOC110886507*), and one gene of the *WRKY* transcription family (*LOC110915516*) were identified as putative salt-tolerance genes.

In addition, a genome-wide association analysis was performed based on the data of 124 varieties grown in the environment with and without salt stress, and 21 SNP loci highly associated with salt tolerance were identified. The regions adjacent to the physical segments of these loci were excavated and analyzed, with 56 genes obtained, among which five genes (*LOC110917577, LOC110915949, LOC110877624, LOC110873143, LOC110922246*) were identified as potential salt-tolerance genes. Our study not only contributes to the understanding of salt tolerance mechanisms at the transcriptomic level, but also provides valuable candidate genes for salt tolerance breeding.

Keyword: Sunflower; Salt tolerance; Transcriptomics

Research on drought-resistant mechanism and variety selection of sunflower (Helianthus annuus L.)

Yang Wu, Liuxi Yi*

College of Agronomy, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

*yiliuxivip@163.com

Abstract: Sunflower is characterized by barrenness tolerance, drought resistance, salinity resistance as well as significant absorption of heavy metals, so it has low soil requirements and is highly valued in saline areas. Most of the concentrated sunflower growing areas in China are arid areas, and the biggest problem sunflower faces is drought stress. Drought stress can cause serious impacts in various growth stages of sunflower, such as the seedling stage affecting the germination rate, the flowering stage affecting the fruiting rate, and so on, which will eventually lead to a significant reduction in the oil yield and fruiting rate, causing losses to the local sunflower industry. In this study, 118 drought resistance related genes were obtained from the sunflower germplasm population by linkage analysis and association analysis, and HaABI5 gene was found to be significantly associated with drought resistance in sunflower by transcriptome table analysis, and its cloning and functional validation showed that HaABI5 gene encoded 390 amino acids. the sequence of HaABI5 protein was highly homologous to the ABI5 protein of the Asteraceae family. The expression of HaABI5 was higher in sunflower leaves than in roots after drought stress. Subcellular localization revealed that the gene is a nuclear regulated protein. An overexpression vector was constructed and HaABI5 was transfected into tobacco for overexpression through Agrobacterium-mediated transfer, and it was initially hypothesized that HaABI5 could enhance drought resistance of tobacco by maintaining osmotic stress stability and reducing oxidative damage.

Keywords: Sunflower; drought-resistance; HaABI5 gene

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Whole-genome identification and expression of lateral organ boundaries domain genes under biotic and abiotic stresses in Helianthus annuus L.

Yingying Zhou[1], Juanjuan Li[1], Ahsan Ayyaz[1], Qian Huang[1], Kangni Zhang[1], Ling Xu[2], Weijun Zhou[1]*

[1] Institute of Crop Science and Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou 310058, China; [2] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou 310018, China

*wjzhou@zju.edu.cn

Abstract: The lateral organ boundaries domain (LBD) protein, a plant-specific transcription factor family, is essential for plant development, phytohormone induction, and response to diverse abiotic stressors. However, the *LBD* gene family in sunflower (*Helianthus annuus* L.) remains unexplored. In this study, a comprehensive analysis was conducted to identify a total of 47 *LBD* genes in the *H. annuus* whole-genome. These genes were subsequently phylogenetically grouped into two distinct clusters (Class I and Class II) along with LBDs from *Arabidopsis* and *Oryza sativa*. It was observed that these LBD genes were not evenly distributed across the 17 chromosomes of *H. annuus*. Evolutionary analysis revealed that *HaLBD* genes could be classified into two groups, Class I and Class II, based on their phylogenetic relationship with genes in *Arabidopsis*. Collinearity analysis identified 30 pairs of segmentally duplicated genes, highlighting the significant contribution of segmental duplication events to the expansion of the *HaLBD* gene family. The expression profiles of *HaLBD* genes at various developmental stages of sunflowers exhibited organ-specific patterns. Additionally, RNA-seq re-analysis identified *HaLBD11* and *HaLBD25* as crucial genes in the genetic module involved in drought stress response, with *HaLBD10* playing a key role in the genetic response to parasitism by *O. cumana*. By employing the cutting-edge structural prediction model AlphaFold3, we have conducted a systematic investigation into the structures and functions of the *LBD* gene family in sunflowers. Network analysis performed via Weighted Gene Co-expression Network Analysis (WGCNA) and enrichment analysis conducted through Kyoto Encyclopedia of Genes and Genomes (KEGG) have indicated that the *LBD* gene family demonstrates significant involvement in regulating the growth, development, and abiotic stress responses of sunflowers through distinct gene networks or biological processes. These finding will facilitate introducing targeted regulators and their corresponding gene networks into sunflower lines.

Keywords: *HaLBD*; Abiotic stress; *Orobanche cumana;* WGCNA; Sunflower

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Innovations in sunflower breeding for enhanced drought adaptation

Sandra Cvejić[1]*, Boško Dedić[1], Aleksandra Radanović[1], Milan Jocković[1], Olivera Lazić[2], Sonja Gvozdenac[1], Nemanja Ćuk[1], Srđan Bursać[1], Jelena Jocković[1], Siniša Jocić[1], Dragana Miladinović[1]

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia;[2]Faculty of Technical Science, University of Novi Sad, Serbia *sandra.cvejic@ifvcns.ns.ac.rs

Abstract: Among the effects of wide-ranging climate change, drought presents a significant threat to global agricultural production. Drought reduces yield quantity and quality of crops, particularly in the semi-arid and arid regions. Sunflower is known for its moderate tolerance to water stress conditions thanks to its well-developed root system and ability to grow in different agroecological conditions. However, prolonged exposure to drought significantly reduces sunflower seed and oil yields, impacting global oil quality. Drought stress in sunflower mostly occurs during the seedling stage, vegetative phase or reproductive phase. Understanding the mechanisms and factors influencing drought tolerance is essential for developing resilient sunflower capable of sustaining agricultural productivity in the face of climate variability and water scarcity. Therefore, breeding for drought tolerance is an economical, feasible, and environmentally friendly control method However, it requires the use of the unexploited approach in sunflower research due to the complexity of the trait. Initially, determination of the genetic diversity existing within and between sunflower species remains the basis for elucidating of the genetic structure and for improvement of traits, including drought tolerance. Manipulation of this diversity to improve drought tolerance among sunflower genotypes may be achieved through selection of adaptive mechanisms that include drought escape, avoidance and tolerance. The breeding tools and technologies for drought tolerance are based on so-called “-omics” techniques, including phenomics, genomics, transcriptomics, proteomics, metabolomics, and epigenomics. Phenomics refers to a quantitative description of the plant's morphological, anatomical, ontogenetic, physiological, and biochemical properties. Recent advancements in phenomics, facilitated by high-throughput phenotyping tools, empower researchers to explore multivariate phenotypic information. Utilizing mainly non-invasive methods, phenotyping facilitates the measurement of complex plant traits, pivotal for comprehending plant growth, development, and interactions with the environment across various scales. Image-based techniques significantly enhance the scale and efficiency of plant phenotyping activities, necessitating the transformation of images into reliable and accurate phenotypic measurements. With phenomics insights into plant traits, researchers can optimize phenotypes tailored to specific climatic conditions and agronomic practices, with root traits playing a central role in identifying features beneficial to crop performance and yield.

Keywords: Sunflower; Phenomics; Drought tolerance; Breeding

Funding: This research was supported by the Science Fund of Serbia (SmartSun project, IDEJE, no. 7732457), the Ministry of Science, Technological Development and Innovation of Serbia (no. 451-03-47/2023-01/200032), the European Commission (no. 101059784 and HelEx no. 101081974).

Identification of lethal gene involved in photosynthesis on sunflower

Ludovic Chauchard, Audrey Ganteil, Benoit Bleys

SOLTIS, 6 Chemin. de Panedautes, 31700 Mondonville (France)

*ludovic.chauchard@soltis-research.com

Abstract: An observation of a segregation of white cotyledons character have been done on F2 population in Mondonville, France, in 2023. The plants loss the ability for photosynthesis and this character is lethal for the plants. The ratio of segregation has been done within population containing 384 individuals, among them 94 individuals are carrying the white cotyledon phenotype. This ratio 3:1 suggest a simple genetic determinism with a single recessive gene. A bulk segregant analysis approach (BSA) has been used to genotype 93 bulks of 4 plants with a 12 000 SNP markers array. A Genome Wide Association Study (GWAS) with a single marker test was performed to identify SNP (Single Nucleotide Polymorphism) related to the white cotyledon character. Four SNP strongly linked with the trait have been identified and they are located on chromosome 14, between 44894167 and 96759457 bp on HanXRQr2.0 reference genome. A study on gene annotation bright to a light the rpoC2 gene which have a strong similarity in the identified region. This gene is involved in albino phenotype on ornamental chimeric plants and chlorophyll structure. This discover can be deeply studied for a better understanding of the mechanism link with the photosynthesis of sunflower.

Keywords: Sunflower; White Cotyledons; Photosynthesis; Lethal Gene

Session 6: Broomrape and herbicide resistance

Recent advances in the resistance, diversity, and interaction of the O. cumana/sunflower pathosystem

Stéphane Muños,

Laboratoire des Interactions Plantes-Microbes-Environnement (LIPME), INRAE, France

*stephane.munos@inrae.fr

Abstract: Sunflower broomrape (*Orobanche cumana*) is one of the main pests that cause yield losses in sunflower crops. This is particularly true in the countries around the Black Sea, where the most virulent populations are found. Indeed, *O. cumana* evolves quickly by overcoming the resistance genes introduced in cultivated hybrids. Wild relatives are a reservoir of resistances and are used to introgress the genomic regions containing the resistance genes into the cultivated hybrids.

Several major resistance genes have been identified. Interestingly, these genes encode proteins similar to those known as resistance proteins to microorganism pathogens. Moreover, the same resistance functions against other parasitic plants have been found.

However, the resistance genes to *O. cumana* do not provide resistance to all *O. cumana* populations. It has been shown that the resistances act according to a gene-for-gene model, meaning that the avirulence proteins interact with specific resistance proteins. One of the main objectives now is to identify the genes encoding avirulence proteins. Colleagues from the IAS-CSIC (Spain) have been able to map and characterize the evolution of the virulence, which will enable their identification in the near future. At the cellular level, it is important to understand how the broomrape can enter sunflower roots. New biocontrol methods are also being developed.

I will give a short review of the main results on this pathosystem.

Keywords: sunflower; broomrape; resistance genes;

Broomrape in Serbia

Boško Dedić[1]*, Jun Zhao[2], Dragana Miladinović[1], Siniša Jocić[1], Vladimir Miklič[1], Aleksandra Radanović[1], Sandra Cvejić[1], Lan Jing[2], Hongyou Zhou[2], Jian Zhang[2], Zhiwei Zhang[2], Jiale Yang[2]

[1] Institute of Field and Vegetable Crops, Novi Sad, Serbia; [2] Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China

*bosko.dedic@ifvcns.ns.ac.rs

Abstract: Broomrape (*Orobanche cumana*) is a continuous threat to sunflower production. Awidely used method to control this parasitic plant is the development of resistant hybrids. The control method itself requires knowledge of parasite virulence present in the particular region. Virulence determination is based on the reaction of differential sunflower genotypes and several genotypes are in use by researchers to determine broomrape race labeled with capital Latin letters. In Serbia, broomrape is present in some sunflower planting regions. Recently, researched limited number of broomrape populations indicated a sparse presence of race F. In this research over 200 sunflower fields were surveyed for the presence of the parasites. Broomrape plants were collected, air dried and labeled. Parasite virulence was determined using inbred lines AD-66, LC1002, LC1003, LC1093, NR5, and P96, to identify race type based on the reaction of sunflower to broomrape. Experiments were conducted in semi- controlled greenhouse conditions by growing differential lines in pots containing substrate with broomrape seeds. Reaction of each line was determined based on broomrape presence on the host root. Broomrape was found in 88 sunflower fields. In majority of surveyed fields, parasite was found sparsely ranging from few individuals to small patches of infested sunflower plants. Results have confirmed the presence of race F based on the susceptibility of line NR5 and the presence of race G based on the susceptibility of line P96.

Further research will include increased number of genotypes to assess variability in broomrape virulence and experiments for genetic diversity determination of parasitic populations of various geographical origin.

Keywords: sunflower; broomrape; virulence; race G

Funding: This work is supported by Project of scientific-technological cooperation between the Republic of Serbia and the PR China “Sunflower Broomrape - comparative research on virulence and diversity of parasite population from China and Serbia - SBD (Sunflower Broomrape Diversity)” 451-02-818/2021-09/02, by the European Commission through projects CROPINNO, grant number 101059784, the Science Fund of the Republic of Serbia, through IDEAS project “Creating climate smart sunflower for future challenges” (SMARTSUN), grant number 7732457, and by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, grant number 451-03-66/2024-03/20032, and by the Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops – Climate Crop, Institute of Field and Vegetable Crops, Novi Sad, Serbia.

Resistance of IFVCNS inbred lines to race F with origin from Spain

Boško Dedić[1]*, Dragana Miladinović[1], Siniša Jocić[1], Vladimir Miklič[1], Aleksandra Radanović[1], Sandra Cvejić[1], Ana Belén García-Carneros[2], Leire Molinero-Ruiz[2]

[1] Institute of Field and Vegetable Crops, Novi Sad, Serbia; [2] Sosteinable Agriculture Institute, Cordoba, Spain

*bosko.dedic@ifvcns.ns.ac.rs

Abstract: Broomrape (*Orobanche cumana*) is one of the most important constraints for sunflower croping. Parasite populations are segregating on virulence criterion and different races are described. Breeding efforts directed for development of resistant genotypes face challenges such as genetically diverse broomrape populations and broomrape of different virulence. One strategy to minimize the impact of broomrape virulence variability in breeding for resistance is to test sunflower genotypes resistance with broomrape with different origins. In this study, a selection of 24 IFVCNS inbred lines based on resistance to dominant broomrape populations in Serbia was tested to broomrape race F, collected in a sunflower field of Andalusia region in Spain. Six-week greenhouse pot experiments were conducted in CSIC, Spain. The line B117, used as susceptible control, had broomrape on all tested plants. For 13 genotypes no broomrape was present on roots, 10 genotypes had broomrape attached on part of tested plants, and broomrape incidence on one genotype was 100%. Inbred lines were analyzed on the basis of genetic background and importance in breeding. In the group of AB-OR lines, with origins from *Helianthus divaricarus,* 7 out of 9 lines were completely resistant. Inbred line HA-267, with a single recessive mode of resistance inheritance, was confirmed resistant. Inbred lines, RUB-3 and SOL-SU-26 were also completely resistant to broomrape. The results indicate the importance of using different broomrape populations and conducting multi-location experiments to enhance the breeding process together with using various sources of resistance. Selection of resistance genotypes will be further tested for their resistance to broomrape populations and resilience to adverse enviromental conditions.

Keywords: sunflower; broomrape; resistance; race F

Funding: This work is supported by the European Commission through projects CROPINNO, grant number 101059784, the Science Fund of the Republic of Serbia, through IDEAS project "Creating climate smart sunflower for future challenges" (SMARTSUN), grant number 7732457, and by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, grant number 451-03-66/2024-03/20032, and by the Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops – Climate Crop, Institute of Field and Vegetable Crops, Novi Sad, Serbia.

Resistance potential of HaCERK1 gene in response to Orobanche cumana infection in sunflower

Juanjuan Li[1], Yingying Zhou[1], Fakhir Hannan[1], Bixian Shi[2], Qian Huang[1], Ling Xu[3], Weijun Zhou[1]*

[1] Institute of Crop Science and Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou 310058, China, [2] Institute of Economic Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China, [3] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou 310018, China

*wjzhou@zju.edu.cn

Abstract: Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic weed that relies on the roots of sunflower (*Helianthus annuus* L.) for water and nutrients, resulting in considerable crop yield loss globally. To effectively manage this issue, it is vital to comprehend the specific defense mechanisms of the host plant during the parasitic process underground. CERK1 (Chitin elicitor receptor kinase 1) is a protein that plays a crucial role in plant immunity. It belongs to the family of receptor-like kinases (RLKs) and it has been implicated in a number of processes such as plant development, cell elongation, and stress response. The function of *CERK1* is realized by triggering a signaling cascade that leads to the activation of plant defense responses, such as the production of reactive oxygen species (ROS) and the expression of defense-related genes. In this research, we conducted computational prediction of protein structure and structural interaction of HaCERK1 protein with downstream protein by AlphaFold3, and further performed preliminary identification of their interactions *in vivo* and *in vitro* by biochemical means. The study of CERK1 and its signaling pathway is for understanding host and parasitic plant interactions and for developing strategies to enhance plant resistance to *O cumana*. We also applied the *in situ* hybridization technique to study the role sites and expression patterns of *HaCERK1* gene in the interaction process of *O. cumana* infestation. Seed-soak agroinoculation (SSA) technique was developed, and recombinant vectors of the tobacco rattle virus were designed to produce RNA interference (RNAi) inducers, resulting in virus-induced gene silencing (VIGS) in sunflower and the host target gene *HaCERK1* was systematically silenced in both the leaf and root tissues. Further, the cut-dip-building (CDB) gene delivery system was adapted to achieve instantaneous overexpression. Results revealed that Agrobacterium rhizogenes in sunflower SH361 achieved a high transformation efficiency. Our findings suggest that *HaCERK1* gene play a crucial role in the sunflower immune response to *O. cumana* infection, demonstrating that SSA and CDB methods can be used for genetic transformation, thus opening up an avenue for genetic engineering of sunflower plants.

Keywords: *HaCERK1* gene; Plant-pathogen interactions; virus-induced gene silencing (VIGS); cut-dip-building (CDB) system

Funding: This research was supported by the National Natural Science Foundation of China (32172429), Zhejiang Provincial Science and Technology Key Project (2022C02034) and Program for Innovative Research Team in Universities of Inner Mongolia Autonomous Region (NMGIRT2320).

Genetic relationships between Orobanche cumana (Wallr.) populations infesting sunflower in different countries

Maria Duca*, Steliana Clapco, Angela Port, Ana Mutu, Ina Bivol
Moldova State University, Chisinau, Republic of Moldova *maria.duca@usm.md

Abstract: The broomrape (*Orobanche cumana* Wallr.) is a root parasitic plant that has encountered in sunflower culture for more than 150 years. It is spread in the majority of sunflower-growing countries, developing several races with increased virulence. Investigation of genetic divergence and phylogenetic relationships among different populations is essential for understanding the evolutionary history of this parasitic plant.
The present study was focused on the assessment of intra- and interpopulation genetic relationships of thirty-three populations of broomrape, which parasites sunflower in the Republic of Moldova, Bulgaria, Romania, Serbia, Turkey and China, belonging to races ≤E, F, G and H.

Diversity-based statistics of microsatellite data obtained using 26 markers (ISSR and SSR), clustering and Principal Component Analysis (PCA) were applied to infer population structure.

Some specific alleles that differentiated populations by country were found, suggesting the long independent evolution of these groups of *O. cumana* within different geographical regions. ISSR markers revealed specific alleles for Turkish populations, while SSRs showed alleles with major contributions to genetic differentiation of populations from Moldova, China, and Serbia. Multivariate analysis of SSR data showed three distinct groups which comprised populations from China, Serbia and Turkey, respectively, as well as one heterogeneous group including the populations from Moldova, Romania and Bulgaria.

According to the PCA based on allele frequencies from all 26 genetic markers, the broomrape populations from China, Serbia and Turkey are small and isolated, while those from the Republic of Moldova, Romania and Bulgaria can be considered as a large, continuous population in which many local populations are spread.

Based on the correlations between common alleles and their interactions with specific and rare alleles within principal components of the differentiation model, the formation of groups that relate to race G and H was revealed, also reflected the associations with the country of origin.

The information resulting from the genetic relationship analysis suggested that the genetic drift as a micro-evolutionary process in the broomrape populations of the countries from the Black Sea region could randomly change allele frequencies in the next generations and that the direction of its action can't be pre-established.

Keywords: *Orobanche cumana*; sunflower; microsatelite markers; genetic diversity; intra-; interpopulational diversity
Funding: subprogramme 011101 Genetic and biotechnological approaches to the management of agroecosystems in the conditions of climate change, funded by the Ministry of Education and Research.

The STIGO project: understanding the molecular mechanism for Orobanche cumana seed germination

Elena Dangla[1, 2], Jan Gielen[1], Clotilde Claudel[1], Alexandra Legendre[2], Mireille Chabaud[2], Clothilde Boubée de Gramont[1], Jean-Bernard Pouvreau[3], Sabine Delgrange[3], Julien Affholder[3], François-Didier Boyer[5], Philippe Delavault[3], Alexandre De Saint Germain[4], Catherine Rameau[4], Ludovic Cottret, Stéphane Muños[2]*

[1] Innolea SAS, [2] INRAe LIPME Auzeville, [3] US2B, ijpb, [4] ICSN

*stephane.munos@inrae.fr

Abstract: *Orobanche cumana* is an obligate parasitic plant specific to sunflower crops. The STIGO project is a consortium of 4 French public labs and one private company aiming to provide a better understanding of the molecular dialog for *O. cumana* seed germination. The main goal of this project is to identify the germination stimulants (GS) and the genes involved in their biosynthesis pathway. The project aims to characterize the *O. cumana* Germination Stimulants receptors as well. Finally, the long-term purpose is to develop new control methods. Using wild sunflower genome sequences, we used the «MetExplore» tool to identify genes encoding for the biosynthesis of the GS. Because a deficiency in some mineral nutrients can impact the biosynthesis of root GS, we evaluated the capacity of a susceptible line to induce the germination of *O. cumana* seeds. After 6 weeks of growth, a susceptible line grown on a poor medium induced more germination than a rich medium. Using transcriptomic analyses on roots, we hope to reveal the genes involved in the biosynthesis of the GS. The candidate genes will be validated by qRT-PCR. Finally, in addition to producing new resistant hybrids, the STIGO project will give new insights into the specific GS of sunflower roots.

Keywords: *Orobanche cumana*; root exsudates; RNAseq; germination stimulants; receptors

Funding: STIGO RNA Project

Genetic diversity analysis and physiological race identification of Orobanche cumana Wallr. collected from China and Eastern Europe countries

Jian Zhang[1], Yukuan Zhang[1], Wenbing Zhang[1] and Jun Zhao[1]*

[1] College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot, China

*zhaojun@imau.edu.cn

Abstract: *Orobanche cumana* Wallr., a fully parasitic seed plant, infests sunflower root systems, significantly impacting yield and quality. It became a major challenge to global sunflower production. In this study, we utilized both ISSR and SSR molecular markers to analyze the genetic diversity of broomrape samples collected from China, Moldova, and Serbia. The race type was identified using race differentials lines, and virulence differentiation were investigated among the same race type which were collected from different locations. The results indicated that 14 SSR primer pairs and 13 ISSR primers were screened out from 54 SSR primer pairs and 112 ISSR test primers. The selected SSR primer can detect 112 loci and 77 polymorphic bands with the polymorphism rate 68.75%; ISSR primer detected 186 loci, 153 polymorphic bands with polymorphism rate 82.26%. Higher genetic diversity in Gansu population was detected by SSR markers, with Shannon index 0.6±0.02, followed by Hebei and Serbia populations, with the Shannon index 0.35±0.06 and 0.44±0.07 respectively. Genetic analysis with ISSR markers showed varied genetic diversity among samples collected from different regions, the samples collected from both Inner Mongolia and Hebei Province exhibited higher diversity; whereas, the samples from Moldova and Yunnan showed lower genetic diversity. Genetic clustering is tightly correlated with geographical origin, samples from both Serbia and Moldova formed subgroup I, while, samples from Inner Mongolia, Xinjiang, Hebei, and Shanxi were clustered into subgroup II, displaying higher polymorphism. Five race types (A, D, E, F, G) were identified among Chinese samples, and race F is the dominant type (39.08%). Race D is the dominant type in Gansu population (21.8%); whereas, races E (19.63%) and G (18.39%) were prominent in northern Shanxi Province and western Inner Mongolia; the race A is only identified in samples collected from Hebei Province. In East Europe population, race G is the dominant one for both Serbia (51.4%) and Moldova population (42.8%). Pathogenicity differentiation was confirmed among the same race types, but originally collected from different regions. However, the same race type collected from China showed much higher pathogenicity than that from Moldova.

Keywords: *Orobanche cumana*; Genetic diversity; Physiological race identification; Pathogenicity differentiation

Mechanism study on 5-aminolevulinic acid enhancing sunflower resistance to Orobanche cumana Wallr.

Zishu XU[1], Yi DUAN[1], Juanjuan LI[2], Qian HUANG[2], Hui LIU[3], Bixian SHI[4*], Weijun ZHOU[2*], Ling XU[1*]

[1] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou 310018, China; [2] Institute of Crop Science, Zhejiang University, Hangzhou 310058, China; [3] Faculty of Science, The University of Western Australia, WA 6009, Australia; [4] Institute of Economic Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China

*shibixian@xaas.ac.cn (B. Shi); wjzhou@zstu.edu.cn (W. Zhou); lxu@zstu.edu.cn (L. Xu)

Abstract: Sunflower (*Helianthus annuus* L.) is an essential oilseed crop, but its production is severely affected by the parasitic weed broomrape (*Orobanche cumana* Wallr.), causing significant economic losses. Preliminary research indicated that the growth regulator 5-aminolevulinic acid (ALA) could effectively enhance sunflower resistance to *O. cumana*, though its mechanism remains unclear. By comparing two different sunflower varieties, TY0409 and Tonghui 15, with different resistance levels, before and after treatment with ALA, we measured physiological and biochemical indicators such as biomass, chlorophyll content, and levels of peroxidation. The results showed that under *O. cumana* stress, ALA application could significantly increase the above ground biomass of TY0409 and Tonghui 15 by 15.15% and 26.67%, and dramatically increased total chlorophyll content by 15.54% and 32.71%, respectively. There was a significant decrease in reactive oxygen species (ROS) content of sunflower under *O. cumana* infection after ALA spraying. Meanwhile, ALA could obviously ameliorate the cell ultrastructure of sunflower under *O. cumana* invasion. Transcriptome analyses revealed that ALA spraying treatment triggered the high expression of photosynthetic and ABA pathway-related genes in sunflower under *O. cumana* stress. Under GSEA analysis, the gene sets after ALA treatment were also mainly enriched in photoperiod-related and plant hormone-related pathways. Then, the contents of 24 hormones including ABA in sunflower were detected for further association analysis. RNA-seq and WGCNA analyses unraveled the underlying mechanisms of ALA in sunflower under *O. cumana* stress and identified the light-responsive key transcription factor *HaHY5.1* and the key protein kinase *HaSnRK2.1*. The results of subcellular localization of these two genes were consistent with the prediction of bioinformatics, *HaHY5.1* was mainly distributed in the nucleus, and *HaSnRK2.1* was distributed in all parts of the cell. In addition, the *HaSnRK2.1* interaction genes were explored through yeast sieve library, and 18 high-expression interaction genes were identified according to the transcriptome analyses. We are conducting further functional studies and molecular regulatory network of *HaSnRK2.1*. This research would elucidate the physiological and molecular mechanisms by which ALA enhances sunflower resistance to *O. cumana*, and provide a theoretical foundation for improving new varieties of sunflowers resistant to *O. cumana*.

Keywords: *Helianthus annuus* L.; Transcriptome; 5-aminolevulinic acid (ALA); WGCNA; *Orobanche cuma* Wallr.

Funding: Xinjiang Uygur Autonomous Region (2022D01E59) & China Scholarship Council (CSC)

Genetic recombination of virulence alleles as a mechanism of racial evolution of sunflower broomrape in Spain

Velasco, L.[1*], Fernández-Melero, B.[1], Del Moral, L.[1], Martín-Sanz, A.[2], Pérez-Vich, B.[1]

[1] Instituto de Agricultura Sostenible – Consejo Superior de Investigaciones Científicas (IAS-CSIC), Córdoba, Spain; [2] Syngenta España, S.A., Carmona, Spain.

*lvelasco@ias.csic.es.

Abstract: The parasitic sunflower broomrape (*Orobanche cumana* Wallr.) has traditionally been considered a self-pollinated plant. However, previous research has demonstrated the existence of cross-pollination that can reach up to 30% under experimental conditions. There have traditionally been two gene pools of sunflower broomrape in Spain, well separated geographically and genetically, one in Cuenca province in central Spain (named CU) and the other in the Guadalquivir Valley in the south (GV). Both gene pools remained isolated until a decade ago when populations containing mixtures of both pools were observed for the first time. In recent years, we have observed that the complexity of the broomrape populations in both Spanish areas has risen drastically, paralleled by an increase in virulence compared to the traditional populations classified as race E or F. To clarify the current situation of sunflower broomrape in Spain, we have studied seven populations from the GV and ten populations from CU, most showing increased intrapopulation diversity and virulence.

We have documented the hybridization between CU and GV gene pool individuals in both areas. The process is more advanced in the populations collected in the GV, in which homozygous individuals of the original gene pools or even F_1 plants are very scarce. Conversely, in the CU area, the populations still conserve homozygous individuals that have not hybridized yet with individuals of the other gene pool and F_1 plants, thus suggesting that the hybridization process is occurring nowadays. Since the hybridization between individuals of both CU and GV gene pools has been unequivocally associated with a new form of virulence that surpasses the resistance of race-F resistant hybrids carrying the *Or*$_7$ allele, named race G_{CU} and G_{GV} tentatively, we hypothesize that the new virulence has been the result of genetic recombination between the virulence alleles present in the original homozygous populations of races F_{CU} and F_{GV}. Preliminary results of comparative genetic studies support our hypothesis.

Our research findings highlight for the first time the pivotal role of genetic recombination in the creation of more aggressive races in sunflower broomrape. This discovery not only deepens our understanding of the parasite's evolution but also underscores the criticality of controlling the movement of broomrape seeds between geographical areas.

Keywords: *Orobanche cumana*; race evolution; genetic recombination; genetic diversity.

Funding: The study was funded by research project PID2020-117286RB-I00 of the Spanish Ministry of Science and Innovation (co-funded with EU FEDER Funds)

Combination of Prohexadione calcium and Imazamox provides synergistic control of Orobanche cumana in Clearfield® sunflower

Matthias Pfenning[1]*

[1] BASF SE, Agrarzentrum Limburgerhof, Germany;

*matthias.pfenning@basf.com

Abstract： Sunflower broomrape (*Orobanche cumana*) is a holoparasitic plant and one of the most important pests in sunflower production. Specifically higher infection causes severe yield loss and endanger the cultivation of sunflower, particular in summer dry regions from the Mediterranean to China. The first step in the life cycle of *O. cumana* is seed germination, which is induced by Strigolactones – plant hormones released by the host plant into the soil. They play an important role in regulating plant architecture, as well as promoting germination of root parasitic weeds. Prohexadione-calcium (PhCa) is a multi-functional systemic plant bioregulator which inhibits gibberellin biosynthesis and triggers pathogen resistance and transiently alters the spectrum of flavonoids and their phenolic precursors. Orobanche control using the herbicide imazamox in Clearfield® sunflower is an established and effective control strategy and complements the genetic resistance against *broomrape*.

Pot experiments showed that Prohexadione calcium can retard *O. cumana* tubercle formation and development. Newer field studies contacted with the combination of the two distinct different crop protection products imazamox and PhCa with different modes of action showed in their combined activity a synergistic effect on *O. cumana* by reducing the total number of broomrape attachments and consequently increasing yield. In the present study, the effect of sublethal dose of imazamox combined with PhCa was investigated.

Keywords: *Orobanche. cumana*； prohexadione - calcium； imazamox, Clearfield®； *Helianthus annuus*

Effects of two herbicides on AHAS enzyme activity and the main agronomic traits of clearfiled oil sunflower

Zengshuai Lv, Peng Wang*, Hongye Dong, Yantao Liu, Wei Daun, Shengli Liu *

Crop Research Institute, Xinjiang Academy of Agricultural Reclamation Sciences, shihezi xinjiang 832000

*495437597@qq.com

Abstract: In order to explore the effects of applying two different types of herbicides on changing AHAS enzyme activity spraying and affecting the on main agronomic traits of sunflower, randomized block design was arranged and the results showed that clearfiled sunflower had a low degree of tolerance to sulfosulfuron, it can significantly inhibite plant height, reduced seed setting rate, and caused yield loss. Imidazolinone herbicides can be used as safe post-emergence herbicides for clearfiled sunflower. Both imazethapyr and rimsulfuron significantly affected the AHAS enzyme activity of plant, and the variation of sunflower main characteristics were also changed significantly. Through the correlation analysis of the main agronomic traits after applying two herbicides, the high correlation was revealed between yield and kernel rate single plate filled grain number after herbicide treatment. The kernel rate was positively correlated with the number of grains per plate, whereas, the plant height, kernel weight and the shell weight was negatively correlated with the yield and kernel rate. The decomposition analysis of yield structure showed that the increase of 100-grain weight after spraying sulfosulfuron was significantly correlated with the increase of 100-shell weight.

Keyword: Oil sunflower; herbicide; AHAS enzyme activity; agronomic traits

Mechanism of the strigolactone pathway and its roles in Orobanche resistance in sunflower and tomato

Yanrong Hao[1], Xinwei Ban[1], Jinhua Yang[1], Qingliang Hu[1], Jiayang Li[1,2] and Bing Wang[1]*

[1]Key Laboratory of Seed Innovation, and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China;
[2]Yazhouwan National Laboratory, Sanya, Hainan 572024, China

*bingwang@genetics.ac.cn

Abstract: The phytohormone strigolactones (SLs) play essential roles in plant development and the interactions of host plants with symbiotic fungi and parasitic weeds. SL signaling requires the hormone-dependent interaction of the SL receptor DWARF14 (D14) with the F-box D3 and the repressor protein D53. D53 can regulate plant architecture through repressing the transcriptional activity of IPA1 in rice. Importantly, we synthesized the SL analog GR24^{4DO}, which can specifically stimulate SL response, and systematically identified SL responsive genes in *Arabidopsis thaliana* and rice. The D53 homologs in Arabidopsis, SUPPRESSOR OF MAX2-LIKE 6, 7 and 8 (SMXL6, 7 and 8), can directly bind their own promoters and repress transcription, demonstrating that SMXL6, 7 and 8 serve as autoregulated-transcription factors to maintain homeostasis of SL signaling. We also found that the NODULATION SIGNALING PATHWAY 1 (NSP1) and NSP2 are essential transcription factors in the process of low-phosphorus-induced SL biosynthesis in rice. Recently, we performed antiparasitic assays using a tomato population including ~150 accessions in the field infested by *Orobanche* in Xinjiang Province. GWAS and genetic analyses indicated that SL transport from roots to the rhizosphere is important for *Orobanche* resistance in tomato. We further set up an experimental system to analyze the *Orobanche*-resistant phenotypes of sunflower in the greenhouse and explored the genetic transformation system of sunflower, which is necessary for functional genetic analyses in sunflower.

Keywords: Strigolactones, Sunflower, Tomato, *Orobanche* resistance, Genetic transformation

Session 7: Processing technology for seed quality products

Enzyme treatment combined with Maillard reaction modification of sunflower meal hydrolysates to prepare sunflower seed oil flavor enhancer

Shuai Xu, Wenting Yin, Qing Guo, Yuxuan Jin, Runyang Zhang, Huamin Liu*, Zhao Qin, Xuede Wang

College of Food Science and Engineering & Institute of Special Oilseed Processing and Technology, Henan University of Technology, Zhengzhou 450001, China

*liuhuamin5108@163.com

Abstract: Currently the processing of flavor oil adopts traditional production technology. Take sunflower oil as an example. Usually, the whole sunflower seeds are roasted at high temperature for oil pressing, the main purpose of which is to improve the oil yield and make the oil fragrant. However, controlling the process of producing oil is often not stable and the high temperature pressing is relatively easy to produce carcinogens, and the Maillard aroma source reaction can overcome this disadvantage. Here we propose a simple two-step process of enzymatic hydrolysis of sunflower seed meal (SSM) and reheating. In other words, SSM was enzymatically hydrolyzed by different enzymes to obtain sunflower seed reducing sugar and amino acids enzymatic hydrolysates. The hydrolysate was then heated with primary refined sunflower seed oil (SSO) for Maillard aroma source reaction to produce low-temperature fragrant SSO. In this study, low-temperature fragrant SSO was prepared from four different enzymatic hydrolysates, and the aroma profiles among prepared SSO were holistically compared via SAFE-GC–MS/O-based flavoromics. Furthermore, the SSM hydrolysates color, free amino acids, reducing sugars, and browning intensity were determined before-and-after roasting. Results showed that the fluorescence intensity of the enzyme-treated SSM was increased. The change of macromolecular spatial conformation at high temperature resulted in the destruction of primary structures such as glycoside bond and peptide bond, and the infrared absorption tended to be flat. The highest increase in valine (3-fold) and rhamnose content (10-40-fold) after enzymatic hydrolysis. Glucose and valine showed the highest reduction rates after roasting. Additionally, a total of 49 volatile compounds were identified, of which 16 were identified as important odorants with odor-activity values (OAVs) greater than 1. 7 potential markers were identified to distinguish by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), including ethyl-pyrazine, trimethyl-pyrazine, 2,6-diethyl-pyrazine, 2,3,5-trimethyl-6-ethylpyrazine, 2,3,5-trimethyl-6-isopentylpyrazine, 2-acetyl-2-methyltetrahydrofuran, pyrrole, were proposed to constitute the aroma boundaries of different enzyme-treated SSO. As for aroma harmony, SSO-RVA (viscozyme L + alcalase) outstood due to a pleasant nutty note. This work provides new information on enhancing fragrant sunflower oil's flavor without high temperatures that should be useful for the industrial production of edible oils.

Keywords: Sunflower seed meal; Maillard reaction; Enzymatic flavoring

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About low temperature mechanical extraction of sunflower kernels

F. Rousseau[1,2], P. Carré[1]*, R. Savoire[2], T. Gouyo[1], V. Jauvion[1]

[1] Terres Inovia – DTVG - 11, rue G. Monge – 33610 Canejan – France;[2] CBMN, Groupe CLIP'IN, CNRS, Université de Bordeaux, Bordeaux INP, 33600 Pessac, France. *p.carre@terresinovia.fr

Abstract: It is well known in the profession that fully dehulled materials like sunflower kernels are difficult material for mechanical extraction, especially at low temperature. The aim of this work was to contribute to understanding the poor performance of screw-presses with these high-oil and low-fibre materials for enabling the production of protein meals with high nutritional value. The usual explanation given for the poor performances of screw presses is the lack of friction caused by the absence of the fibrous envelopes of the seeds. This work aims at supporting an alternative hypothesis postulating that resistance to filtration is the main cause of the press problematic behaviour. It was carried out at two scales: with a uniaxial compression cell and an instrumented screw-press of a design is similar to that of industrial presses. The cell of uniaxial compression had a diameter of 40 mm and a perforated bottom allowing the oil outflow. A piston of the same diameter slides into the upper part and can be moved at controlled speed while recording the force opposed to its displacement. It was used to study the effect of piston speed, on the rate of compression reached at the maximum possible pressure without extrusion of solid material through the orifices of the cell. The results showed that high compression speed was leading to a reduction of the rate of compression caused by the higher filtration resistance of low fibre matrices. The pressure rises because the oil flow is limited by the cake porosity. Higher compression speed requires higher oil flow rate in a limited channel. Moreover, higher pressure could lead to the collapse in part of the capillary network reinforcing the filtration resistance. Observations on the screw-press with uncooked kernels were made à two rotating speeds (4 and 8 rpm). The studied parameters were the flow rate of oils and cakes, the oil content of the cake at the outlet of the press and in samples picked after stopping the press in steady state and opening the cage, the radial pressure measured along the press cage, and the temperature of the cage. A strong difference in behaviour was observed between 4 and 8 rpm. At the low speed, the radial pressure was increasing progressively from the entry to the outlet of the press, the oil content of the cake was progressively diminishing, and the temperature increased. At 8 rpm, the oil flow was limited to the first two sections of the cage, the pressure measured in these sections ware higher than at 4 rpm but in the last part of cage, no oil was expressed, the oil content of the cake remained steady, the pressure as the temperature were remaining low. This change could be explained by the higher speed of compression at 8 rpm which reached the critical point where the oil filtration became impossible leading to a change in the cake structure, a lack of cohesion allowing it to pass through the throttling parts of the press with a resistance insufficient to build up the pressure required to express the oil. With nondehulled seeds, the hulls presence is helping the filtration capacity of the cake to withstand higher pressure. The design of screw presses should be made to avoid excessive compression speed to cope with the limited filtration capacity observed in dehulled sunflower.

Keywords: Mechanical extraction; Kernels; Filtration resistance; Friction; Compression

Session 8: Others

Cadmium in sunflower seeds: different contents in hulls and kernels and consequences for food and feed industry

Christophe Nguyen[1], Sylvie Dauguet[2*], Jean-Philippe Loison[3], Céline Motard[2], Vincent Jauvion[2]

[1] UMR Ispa INRAE and Bordeaux Sciences Agro - CS 20032 33882 Villenave d'Ornon - France,
[2] Terres Inovia– DTVG - 11, rue G. Monge – 33610 Canejan – France,
[3] ITERG - 11, rue G. Monge – 33610 Canejan – France

*s.dauguet@terresinovia.fr

Abstract: Context: Contamination of sunflower seeds by soil-derived cadmium is an important issue for food and feed， due to this crop strongly accumulates the toxic metal. Diffuse contamination of agricultural soils by Cd, which is mainly derived from soil pedogenesis or agricultural inputs and atmospheric fallouts, is a publica concern issue. Cd diffuse result in the contamination of harvests seeds, thus do not comply with the regulation for contaminants in food products. The deadline of Cd content in sunflower seeds is at 0.5 mg/kg (EC 2023/915). In the crushing process, Cd is mainly concentrated in the meal, while little Cd is found in oil. For animal feed, raw materials made by sunflower seeds should not contain more than 1mg/kg (Directive 2022/32/EC). De-hulling the seeds before crushing results in a higher protein content in meals, but if Cd is more concentrated in kernels or hulls of the seeds, it can increase the risk of nonconformity of the meal. There are also some prospects to valorize sunflower meals as proteins for humans, but this may be hampered by the Cd concentration over the deadline in pressed kernels. The goals of this work were (1) to study the repartition of Cd between seed hulls and kernels, so as to evaluate the consequences for the Cd content in meals and (2) to evaluate the genetic variability for Cd repartition between hulls and kernels as a perspective for breeding. The results showed that kernels were richer in Cd than the hulls. The size of the kernel, relative to that of the hull, may affect the concentration of Cd in kernel tissues and there might be genetic variability for the capacity of transfer of Cd from the hull to the kernel. This study will opens the perspective of breeding sunflower genotypes that accumulate less Cd in kernels, so as to increase the food/feed safety.

Keywords: cadmium; sunflower seed; genetic variability

A novel AI-based technique for 3d shape acquisition of confectionery sunflower seeds and associated shape descriptors

Nada Hladni[1], Branislav Kisačanin[2], Veljko Petrović[3], Ashmit Dutta[4], Vladimir Miklič[1], Dragana Miladinović[1]

[1] Institute of Field and Vegetable Crops (IFVCNS), Maksima Gorkog 30, 21000 Novi Sad, Serbia; [2] Nvidia Corporation and The Institute for Al R&D of Serbia; [3] University of Novi Sad, Faculty of Technical Sciences, 21000 Novi Sad, Serbia; [4] University of Illinois at Urbana-Champaign, USA

*nada.hladni@ifvcns.ns.ac.rs

Abstract: Evaluating the qualities of sunflower hybrids is partially based on laboratory analysis which is amenable to strict quantification and statistical and computer analysis. However, the insignificant amount of information is not gleaned from direct visual observation which is partially qualitative and where it is quantitative, it is laborious and prone to human error. To remedy this state of affairs, we have begun work on an automated, AI-powered analysis workflow for visual confectionary sunflower seed evaluation. We foresee that these advances can be used both to increase efficiency of dehulling via model machine and helping to generatenovel varieties with novel seed shapes. This advancement should be possible by combining automated shape analysis, genomic analysis, and the development of generative AI models which create plausible simulations of seed geometry under known genetic and environmental conditions. In this paper we address the first step of 3D shape analysis: 3D shape acquisition. Traditional 3D shape acquisition techniques require relatively expensive equipment, consisting of multiple carefully calibrated cameras. To reduce cost and guarantee the potential for ubiquity of this approach, we propose an alternative approach based on cheap image acquisition technology and AI-powered 3D geometry reconstruction. We employ Neural Radiance Fields (NeRF) which take on the order of a hundred photographic 2D images of an object, taken from a variety of random angles and distances, computer likely camera positions, and then calculates a radiance field, which can be used to generate completely new views of the objectfrom different angles and distances. This image can be used to reconstruct the 3D geometry of the photographed objects. We confirm our hypothesis: acquiring high-definition 3D shapes of confectionery sunflower seeds using a NeRF-based approach is possible, and it does not require multiple calibrated cameras. We have demonstrated that sufficient fidelity via using this approach, even with uncalibrated inexpensive cameras. Resolution is no barrier to adequate geometry reconstruction with resolutions in the vicinity of 1 megapixel (1280x720) showing the best results. This in turn helps curb prohibitive VRAM requirements for GPU accelerated NeRF computation. Further, we subjected to analysis 3D model formats and 3D shape descriptors that can be computed from reconstructed geometry. One limit of the approach is lacking of reliable scale in the reconstructed geometry. In order to resolve this problem, we introduced an object of known dimensions into the 3D scene and derived the scale from it. We expect this novel technique can be applied in breeding programs to accelerate the confectionery sunflower breeding speed, so as to creat the new lines and hybrids with elite traits.

Keywords: 3D shape analysis; 3D shape acquisition; NeRF; confectionery sunflower seeds
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Explaining the variability of sunflower hullability

P. Carré[1*], J.P. Loison[2], A. Cavaco-Soares[2], V. Jauvion[1]

[1] Terres Inovia – DTVG -11, rue G. Monge-33610 Canejan-France, [2] ITERG-11, rue G. Monge, 33610 Canejan, France. *p.carre@terresinovia.fr

Abstract: Increasing the oil content of sunflower seeds is a recurring goal of plant breeding. To achieve this goal, achenes hull content was reduced from 40-45% to 20-25%. This change has made seeds of sunflower difficult to shell. At present, the suitability for hulling is not the subject of breeding, so there is a wide variability at the phenotypic level. It is therefore difficult to remove more than 70% of the hulls due to the presence of achenes that are resistant to shelling. The purpose of this study was to focus on this phenomenon of resistance to hulling. 30 commercial hybrids were grown in 2021 and 2022 at four locations in France. The seeds were shelled after equilibrating their moisture content in the presence of a saturated ammonium nitrate solution. Approximately 10 g of achenes were impacted 3 times at a peripheral velocity of 21 m.s -1 , sieved at 2 mm and separated on an air column. The results show a very wide range of extracted hull rates ranging from 0 to more than 25% of achene mass. The correlation analysis result shows that the trait "extracted hulls" (EH) is only weakly correlated with the proximal and dimensional characteristics of achenes. The highest R values found are, in order of importance, for oil content, protein expressed on defatted dry matter, and bulk density (-0.34, -0.28, -0.26 respectively). On the global dataset, the analysis of variance shows that the Cultivar effect (CV), although significant, but it can only explain 14.1% of the variability, the effects of Year is 11.3% and the location (Loc) effects is 19.0% (sum of the squares type III). Only 2 growing sites were identical for the two years. By restricting the analysis of variance to this subset of data to test the Loc * Year interaction, the 'EH = Year + Loc + CV + Loc*Year' model explains 59% of the variability, including 24% for the CV effect, 17% for the Loc effect and 17% for the interaction. The effect of the Year*CV interaction is significant and explains nearly 23% of the variability. To better understand these differences, an analysis of the structure of achenes was performed by image analysis. Cross-sections were made on achenes from a subset of samples with contrasting dehulling behaviors. The variation were observed on achene surface, the area surface delimited by the inner limit of the pericarp, the surface of the embryo. By calculation, the surface of the pericarp, its thickness, and the surface of the area between the pericarp and the outer edge of the embryo. A step-by-step multiple regression determined that the EH variable was explained at 93% by only two variables, the thickness of the pericarp and the void rate between the hull and the kernel. These results allow us to formulate a hypothesis which the hulling ability is determined by a combination of genetic factors, controlling the potential size of the pericarp and its thickness, and pedoclimatic factors conditioning the expression of the size, especially at the beginning of flowering, and determining the filling of the embryo. Stress at the beginning of flowering could limit the size of the achenes and if the conditions at the end of the cycle are favourable, the volume delimited by the pericarp becomes limiting, forcing the embryo to occupy all the available volume, thus causing adhesion phenomena between shells and kernels.

Keywords: Dehulling resistance; achene structure; pericarp; breeding; physiology

Correlation analysis and comprehensive evaluation of edible sunflower seed phenotypes and nutritional composition

Yingying Cai, Zhonghua Lei, Lijun Xiang, Jiakang Shao, Li Zhang, Qixiu Huang*

Institute of Cash Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091

*13899525177@163.com

Abstract: In order to deeply study the relationship between sunflower seed phenotypes and their nutrient compositions, commodities harvested from 19 edible sunflower varieties generated via different parental lines were examined and analyzed for seed kernel traits, such as 100-kernel weight, kernel length, and total fat, as well as ten nutrient compositions indexes, by using a variety of statistical methods (correlation analysis, principal component analysis, and cluster analysis). The results showed that each index exhibited significant differences among tested varieties ($p < 0.01$), indicating the diversity and complexity of sunflower edible varieties. Grain length showed highly significant positive and negative correlation with oleic acid, and linoleic acid separately. Soluble protein showed significant positive correlation with chlorogenic and stearic acids. Also, the negative correlation between vitamin E and hydrogen peroxide was extremely significant. Notably, the negative correlation between oleic acid and linoleic acid was also detected, with a correlation coefficient as high as 0.994. Further principal component analysis and cluster analysis grouped fourteen indicators into five principal components, with a cumulative variance contribution of 79.969%. Among all tested varieties, JSK21 commodities showed the most outstanding performance in the comprehensive evaluation. This study not only revealed the complex interactions between sunflower seed traits and nutrients factors, but also lay a stone for quality breeding of sunflower.

Keywords: sunflower; quality; fatty acid; nutrient content; correlation analysis

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ID	Full Name	Institution	Country	Registered E-mail
1	Fernando Turienzo Aguilar	Argensun S.A.	Argentina	fturienzo@argensun.com.ar
2	Pablo Diaz Colodrero	Argensun S.A.		
3	Maximo Ezequiel Barreto	Argensun S.A.		
4	Norma Paniego	National Institute of Agricultural Technology	Argentina	paniego.norma@inta.gob.ar
5	Santiago Jorge Renteria	Advanta SEMILLAS S.A.I.C.	Argentina	santiago.renteria@advantaseeds.com
6	Victor Javier Abertondo	Advanta Seeds	Argentina	victor.abertondo@advantaseeds.com
7	Anibal Sergio Fernandez	Sundance Seed SA	Argentina	asf@sundanceseed.com.ar
8	Ana Virginia Dalossi Olivato	Heliagro Agricultura Ltda	Brazil	anavirginia@heliagro.com.br
9	Loren Rieseberg	University of British Columbia	Canada	loren.rieseberg@botany.ubc.ca
10	Yue Yu	University of British Columbia	Canada	yue.yu@ubc.ca
11	Kaichi Huang	University of British Columbia	Canada	huangkch5@mail.sysu.edu.cn
12	Ashish Bhan	BASF Agro	China	ashish.bhan@basf.com
13	Biaofeng Sui	BASF Agro	China	biaofeng.sui@basf.com
14	Etienne Pilorgé	TERRES INOVIA	France	e.pilorge@terresinovia.fr
15	Vincent Jauvion	TERRES INOVIA	France	v.jauvion@terresinovia.fr
16	Laetitia Devedeux	The International Sunflower Association	France	l.devedeux@terresinovia.fr
17	Alexandra Legendre	INRAE	France	alexandra.legendre@inrae.fr
18	Belen Fernandez Melero	INRAE	France	belen.fernandez-melero@inrae.fr
19	Elena Dangla	INRAE	France	elena.dangla@inrae.fr

20	Nicolas Langlade	INRAE	France	nicolas.langlade@inrae.fr
21	Stephane Munos	INRAE	France	stephane.munos@inrae.fr
22	Thibault Roudaire	INRAE	France	thibault.roudaire@inrae.fr
23	Audrey Ganteil	SOLTIS	France	audrey.ganteil@soltis-research.com
24	Ludovic Chauchard	SOLTIS	France	ludovic.chauchard@soltis-research.com
25	Federico Carlos Pablo Bock	Syngenta	France	federico.bock@syngenta.com
26	Dennis Reckwell	KWS SAAT SE	Germany	dennis.reckwell@kws.com
27	Dilan S Rajapakshsa Patiranage	KWS SAAT SE	Germany	dilan.patiranage@kws.com
28	Matthias Adolf Pfenning	BASF Agro	Germany	matthias.pfenning@basf.com
29	Kristóf Poór	KWS SAAT	Hungary	kristof.poor@kws.com
30	Péter Stréb	KWS SAAT	Hungary	peter.streb@kws.com
31	Tibor Mátrai	Agromag Kft	Hungary	matrai.tibor@agromag.hu
32	Mangesh Yuwaraj Dudhe	ICAR-Indian Institute of Oilseeds Research	India	mangeshdudhe@gmail.com
33	Sujatha Mulpuri	ICAR-Indian Institute of Oilseeds Research	India	mulpurisujata@yahoo.com
34	Hideaki Ikeguchi	Takii	Japan	hideaki-ikeguchi@takii.co.jp
35	Aoike Hitomi	Takii	Japan	hideaki-ikeguchi@takii.co.jp
36	Maria Duca	Moldova State University	Moldova	mduca2000@yahoo.com
37	Steliana Clapeo	Moldova State University	Moldova	mduca2000@yahoo.com
38	Nadezhda Dolmatova	National Research University Higher School Of Economics	Russia	dna@aonsa.ru
39	Alina Chernova	Oilgene	Russia	Alin.chernova@gmail.com
40	Boldyrev Stepan	Oilgene		
41	Elena Rylova	Private Entrepreneur Aleksey Evgenevich Volkov	Russia	rylova.ea@smart74.ru
42	Sergei Pikunov	Private Entrepreneur Aleksey Evgenevich Volkov		

43	Alexandr Shurakov	Private Entrepreneur Aleksey Evgenevich Volkov		
44	Galina Tereschenko	Private Entrepreneur Aleksey Evgenevich Volkov		
45	Yakov Demurin	VNIIMK	Russia	yakdemurin@yandex.ru
46	Maksim Shikin	Martin LLC	Russia	Artur-saakuan1@mail.ru
47	Artur Saakian	Martin LLC	Russia	
48	Khakhaeva Viktoriia	Martin LLC	Russia	
49	Luxita Risnoveanu	Bucharest University of Economic Studies	Romania	luxita_risnoveanu@yahoo.co.uk
50	Anton Florin Gabriel	National Agricultural Research and Development Institute, Fundulea	Romania	gabi22mai@yahoo.com
51	Maria Joita-Pacureanu	National Agricultural Research and Development Institute, Fundulea	Romania	mariapacureanu2@yahoo.com
52	Elisabeta Sava	The State Institute for Variety Testing and Registration	Romania	mariapacureanu2@yahoo.com
53	Boško Dedić	Institute of Field and Vegetable Crops	Serbia	bosko.dedic@ifvcns.ns.ac.rs
54	Sandra Cvejić	Institute of Field and Vegetable Crops	Serbia	sandracvejic03@gmail.com
55	Aleksandra Radanović	Institute of Field and Vegetable Crops	Serbia	aleksandra.dimitrijevic@ifvcns.ns.ac.rs
56	Vladimir Miklič	Institute Of Field & Vegetable Crops	Serbia	miklicv@gmail.com
57	Nada Hladni	Institute Of Field & Vegetable Crops	Serbia	nada.hladni@ifvcns.ns.ac.rs
58	Veljko Jocić	Feed & Seed	Serbia	veljko.jocic@feed-seed.co.rs
59	Leonardo Velasco	Institute for Sustainable Agriculture, CSIC	Spain	lvelasco@ias.csic.es
60	Begoña Pérez-Vich	Institute for Sustainable Agriculture, CSIC	Spain	bperez@ias.csic.es
61	Jose R Perez Monreal	Hibrisol	Spain	hibrisol@gmail.com
62	Maria Dolores del Valle Campos	Hibrisol	Spain	hibrisol@gmail.com

63	Nicole Marie Creux	FABI, University of Pretoria	South Africa	nicole.creux@fabi.up.ac.za
64	Nomfundo S Shinga	GLOBAL SEEDS	South Africa	m.grishin@gl-seeds.com
65	Emrah Akpinar	Trakya University	Turkey	emrahakpinar@trakya.edu.tr
66	Yalcin Kaya	Trakya University	Turkey	yalcinkaya22@gmail.com
67	Wei Zhou	Turkish Genetic Oguz	Turkey	15810118877@139.com
68	Chao-Chien Jan	USDA-ARS (Retired)	USA	chaochien.jan@gmail.com
69	Grace Wei	USDA-ARS (Retired)		
70	Jinguo Hu	USDA-ARS (Retired)	USA	jhu69@yahoo.com
71	E Liu	USDA-ARS (Retired)	USA	
72	Brent S Hulke	USDA-ARS	USA	brent.hulke@ndsu.edu
73	Bilig Bater	New Century Seeds LLC	USA	biligbater@yahoo.com

Chinese Participants

No.	Name in Chinese	Name	Institution	E-mail
1	张希吏	Zhang Xi-Li	巴彦淖尔市现代农牧事业发展中心	979140424@qq.com
2	白盼盼	Bai Pan-Pan	巴彦淖尔市关尔农业发展有限责任公司	2356177845@qq.com
3	李军	Li Jun	巴彦淖尔市农牧业科学研究所	lijun.6606@163.com
4	邬雪瑞	Wu Xue-Rui	巴彦淖尔市农牧业科学研究所	
5	王刚	Wang Gang	巴彦淖尔市农牧业科学研究所	
6	杜瑞霞	Du Rui-Xia	巴彦淖尔市农牧业科学研究所	369275165@qq.com
7	刘春晖	Liu Chun-Hui	巴彦淖尔市农牧业科学研究所	liuchunhui67@163.com
8	张玉忠	Zhang Yu-Zhong	巴彦淖尔市西亚农业科技发展有限公司	326789414@qq.com
9	刘刃生	Liu Ren-Sheng	北京天葵立德种子科技有限公司	liurensheng@tiankuiseeds.com
10	郭爱华	Guo Ai-Hua	北京天葵立德种子科技有限公司	guoaihua@tiankuiseeds.com
11	刘鸿福	Liu Hong-Fu	北京天葵立德种子科技有限公司	liuhongfu@tiankuiseeds.com
12	梁普政	Liang Pu-Zheng	昌达商贸责任有限公司	905318594@qq.com

13	孙逊	Sun Xun	昌吉州西亚种子有限责任公司	1575930914@qq.com
14	谭丽萍	Tan Li-Ping	赤峰市农牧科学研究所	cfnkytlp@163.com
15	张玮	Zhang Wei	赤峰市农牧科学研究所	120285017@qq.com
16	张云彤	Zhang Yun-Tong	赤峰市农牧科学研究所	972692033@qq.com
17	薛伟	Xue Wei	赤峰市农牧科学研究所	158490108@qq.com
18	毕经伟	Bi Jing-Wei	赤峰市农牧科学研究所	48308983@qq.com
19	雷红量	Lei Hong-Liang	鄂尔多斯生态环境职业学院	1076953622@qq.com
20	张伟	Zhang Wei	鄂尔多斯生态环境职业学院	975408133@qq.com
21	刘彤	Liu Tong	甘肃成田种业有限公司	532175407@qq.com
22	张晓荣	Zhang Xiao-Rong	甘肃嘉瑞种业有限责任公司	zxr8788@126.com
23	徐金花	Xu Jin-Hua	甘肃嘉瑞种业有限责任公司	jiaruiseed@163.com
24	张晓虎	Zhang Xiao-Hu	甘肃嘉瑞种业有限责任公司	jiaruiseed@163.com
25	郭双龙	Guo Shuang-Long	甘肃嘉瑞种业有限责任公司	jiaruiseed@163.com
26	喻小龙	Yu Xiao-Long	甘肃科锋种业有限责任公司	13893788840@163.com
27	卞伟勋	Bian Wei-Xun	甘肃七禾瑞种业	
28	王兴珍	Wang Xing-Zhen	甘肃省农业科学院作物研究所	luoluo1668@126.com
29	贾秀苹	Jia Xiu-Ping	甘肃省农业科学院作物研究所	gsjxp666@163.com
30	卯旭辉	Mao Xu-Hui	甘肃省农业科学院作物研究所	wd-mxh@163.com
31	王振锋	Wang Zhen-Feng	甘肃省庆阳市环县种子管理站	abc3968481@126.com
32	金志琴	Jin Zhi-Qin	甘肃同辉种业有限责任公司	jzq2536578451@163.com
33	张圆菲	Zhang Yuan-Fei	甘肃同辉种业有限责任公司	zhangyf@nwafu.edu.cn
34	姜华	Jiang Hua	甘肃同辉种业有限责任公司	condymxd@126.com
35	李建华	Li Jian-Hua	贵州省油料研究所	
36	杨航	Yang Hang	贵州省油料研究所	hangy0508@163.com
37	奉斌	Feng Bin	贵州省油料研究所	672596450@qq.com
38	李慧琳	Li Hui-Lin	贵州省油料研究所	355523836@qq.com
39	赵存鹏	Zhao Cun-Peng	河北省农林科学院棉花研究所	410111767@qq.com
40	王凯辉	Wang Kai-Hui	河北省农林科学院棉花研究所	kaihwang@126.com
41	张海娜	Zhang Hai-Na	河北省农林科学院棉花研究所	82599500@qq.com
42	吕晓艳	Lv Xiao-Yan	河北省农林科学院棉花研究所	zms67@163.com
43	王海燕	Wang Hai-Yan	河北省农林科学院棉花研究所	ndwanghaiyan@163.com

44	党继革	Dang Ji-Ge	河北双星种业股份有限公司	297411781@qq.com
45	高文娇	Gao Wen-Jiao	河北双星种业股份有限公司	cherry@shuangxingseed.com
46	王文军	Wang Wen-Jun	黑龙江省农业科学院经济作物研究所	xpyhaas@outlook.com
47	谢鹏远	Xie Peng-Yuan	黑龙江省农业科学院经济作物研究所	xpyhaas@outlook.com
48	孟庆林	Meng Qing-Lin	黑龙江省农业科学院植物保护研究所	mqlhlcn@126.com
49	李慧英	Li Hui-Ying	吉林省白城市农业科学院	leahuiy@163.com
50	牛庆杰	Niu Qing-Jie	吉林省白城市农业科学院	niuqingjie@126.com
51	张雷	Zhang Lei	吉林省白城市农业科学院	BCZHANGL@163.com
52	张曼	Zhang Man	吉林省白城市农业科学院	18844696509@163.com
53	王祉诺	Wang Zhi-Nuo	吉林省白城市农业科学院	wznwxy0309@163.com
54	何中国	He Zhong-Guo	吉林省农业科学院（中国农业科技东北创新中心）	zg_h@163.com
55	郭容秋	Guo Rong-Qiu	吉林省农业科学院（中国农业科技东北创新中心）	821402132@qq.com
56	王佰众	Wang Bai-Zhong	吉林省农业科学院（中国农业科技东北创新中心）	Wbz.1975@163.com
57	李洋	Li Yang	吉林省农业科学院（中国农业科技东北创新中心）	548553062@qq.com
58	王士红	Wang Shi-Hong	江苏丘陵地区南京农业科学研究所	10301541@qq.com
59	王勇	Wang Yong	江苏丘陵地区南京农业科学研究所	728409652@qq.com
60	陈涛	Chen Tao	江苏沿海地区农业科学研究所	1453647042@qq.com
61	任昂彦	Ren Ang-Yan	江苏沿海地区农业科学研究所	
62	依兵	Yi Bing	辽宁省农业科学院	yibing8749@126.com
63	赵明珠	Zhao Ming-Zhu	辽宁省农业科学院	zhaomingzhu23@163.com
64	曹瑀轩	Cao Yu-Xuan	辽宁省农业科学院	caoyuxuan991003@163.com
65	王东亮	Wang Dong-Liang	内蒙古蒙东农业科技有限公司	342661350@qq.com
66	刘慧	Liu Hui	内蒙古蒙东农业科技有限公司	
67	刘鹏	Liu Peng	内蒙古蒙疆望日种业有限责任公司	171251663@qq.com
68	张键	Zhang Jian	内蒙古农业大学	zhj19890128@imau.edu.cn
69	张文兵	Zhang Wen-Bing	内蒙古农业大学	zhangwenbing@imau.edu.cn
70	杨剑锋	Yang Jian-Feng	内蒙古农业大学	jianfengyang89@126.com

71	王娅婷	Wang Ya-Ting	内蒙古农业大学	wangyating0901@163.com
72	史钰辰	Shi Yu-Chen	内蒙古农业大学	3364302064@qq.com
73	杨红星	Yang Hong-Xing	内蒙古农业大学	773646697@qq.com
74	啜泽润	Chuai Ze-Run	内蒙古农业大学	17614987559@163.com
75	付鑫婷	Fu Xin-Ting	内蒙古农业大学	1078689130@qq.com
76	张婷	Zhang Ting	内蒙古农业大学	2890483987@qq.com
77	渠汇	Qu Hui	内蒙古农业大学	1398845584@qq.com
78	石青松	Shi Qing-Song	内蒙古农业大学	1256975471@qq.com
79	李海平	Li Hai-Ping	内蒙古农业大学	lihaiping5820@hotmail.com
80	史丽	Shi Li	内蒙古农业大学	lirui2003@imau.edu.cn
81	孔祥久	Kong Xiang-Jiu	内蒙古农业大学	xjkong0528@126.com
82	景岚	Jing Lan	内蒙古农业大学	jinglan71@126.com
83	郭爽	Guo Shuang	内蒙古农业大学	2563634505@qq.com
84	崔晓晗	Cui Xiao-Han	内蒙古农业大学	1207301978@qq.com
85	杨婉秋	Yang Wan-Qiu	内蒙古农业大学	18102456552@163.com
86	黄家英	Huang Jia-Ying	内蒙古农业大学	jyhuang666@126.com
87	路妍	Lu Yan	内蒙古农业大学	luyan820918@126.com
88	赵妍	Zhao Yan	内蒙古农业大学	767252832@qq.com
89	李艳艳	Li Yan-Yan	内蒙古农业大学	liyanyan@imau.edu.cn
90	赵君	Zhao Jun	内蒙古农业大学	zhaojun02@hotmail.com
91	伊六喜	Yi Liu-Xi	内蒙古农业大学	yiliuxivip@163.com
92	邬阳	Wu Yang	内蒙古农业大学	357236067@qq.com
93	郭建光	Guo Jian-Guang	内蒙古五原县丰收 向日葵种业有限责任公司	bmwygjg6103@163.com
94	白敏	Bai Min	内蒙古五原县丰收 向日葵种业有限责任公司	bm0146@126.com
95	杨建国	Yang Jian-Guo	内蒙古西蒙种业有限公司	
96	刘谨	Liu Jin	内蒙古西蒙种业有限公司	
97	杨荐均	Yang Jian-Jun	内蒙古西蒙种业有限公司	
98	郭昌欣	Guo Chang-Xin	内蒙古欣广农业有限责任公司	961995786@qq.com
99	齐轩	Qi Xuan	内蒙古轩丰农业科技有限公司	denise@xuanda-food.com
100	梅婷	Mei Ting	内蒙古轩丰农业科技有限公司	denise@xuanda-food.com

101	张君	Zhang Jun	内蒙古自治区农牧业科学院	64359906@qq.com
102	侯建伟	Hou Jian-Wei	内蒙古自治区农牧业科学院	hjw19860627@126.com
103	白全江	Bai Quan-Jiang	内蒙古自治区农牧业科学院	283973470@qq.com
104	杜磊	Du Lei	内蒙古自治区农牧业科学院	215551501@qq.com
105	田晓燕	Tian Xiao-Yan	内蒙古自治区农牧业科学院	283973470@qq.com
106	张轶敏	Zhang Yi-Min	内蒙古自治区农牧业科学院	1305070717@qq.com
107	云晓鹏	Yun Xiao-Peng	内蒙古自治区农牧业科学院	283973470@qq.com
108	苏雅杰	Su Ya-Jie	内蒙古自治区农牧业科学院	m15247533925@163.com
109	李素萍	Li Su-Ping	内蒙古自治区农牧业科学院	lisuping640129@163.com
110	郭树春	Guo Shu-Chun	内蒙古自治区农牧业科学院	200114050@163.com
111	于海峰	Yu Hai-Feng	内蒙古自治区农牧业科学院	nkyyhf@163.com
112	聂惠	Nie Hui	内蒙古自治区农牧业科学院	nh52004@sina.com
113	牟英男	Mu Ying-Nan	内蒙古自治区农牧业科学院	422762786@qq.com
114	赵令敏	Zhao Ling-Min	内蒙古自治区农牧业科学院	1844632035@qq.com
115	何文寿	He Wen-Shou	宁夏大学	hews818@163.com
116	刘继霞	Liu Ji-Xia	宁夏农林科学院农作物研究所	liujixia_2008@163.com
117	王平	Wang Ping	宁夏农林科学院农作物研究所	wp_1001@163.com
118	齐文增	Qi Wen-Zeng	青岛百纳德信息咨询有限公司	fqi@qd-bnd.com
119	王瑞静	Wang Rui-Jing	青岛百纳德信息咨询有限公司	fqi@qd-bnd.com
120	丁浩	Ding Hao	青岛百纳德信息咨询有限公司	tding@qd-bnd.com
121	赵瑞英	Zhao Rui-Ying	青岛百纳德信息咨询有限公司	tding@qd-bnd.com
122	冯九焕	Feng Jiu-Huan	三瑞农业科技股份有限公司	srfengjh@lpht.com.cn
123	常敏	Chang Min	三瑞农业科技股份有限公司	srchangm@lpht.com.cn
124	王科	Wang Ke	三瑞农业科技股份有限公司	wke1020@163.com
125	安燕珂	An Yan-Ke	三瑞农业科技股份有限公司	srnkayk@163.com
126	魏志恒	Wei Zhi-Heng	三瑞农业科技股份有限公司	srweizh@lpht.com.cn
127	杨立全	Yang Li-Quan	三瑞农业科技股份有限公司	sryanglq@lpht.com.cn
128	李生晔	Li Sheng-Ye	三瑞农业科技股份有限公司	13992036861@163.com
129	张耀	Zhang Yao	三瑞农业科技股份有限公司	263504475@qq.com
130	康国栋	Kang Guo-Dong	三瑞农业科技股份有限公司	1109324213@qq.com
131	陈海军	Chen Hai-Jun	三瑞农业科技股份有限公司	srchenhj@lpht.com.cn

132	尚嘉欣	Shang Jia-Xin	三瑞农业科技股份有限公司	704750126@qq.com
133	李联社	Li Lian-She	三瑞农业科技股份有限公司	srlils@lpht.com.cn
134	董兴春	Dong Xing-Chun	三瑞农业科技股份有限公司	343442480@qq.com
135	高丽军	Gao Li-Jun	山西福莱沃种业有限公司	380263685@qq.com
136	王怿国	Wang Yi-Guo	山西福莱沃种业有限公司	
137	郑洪元	Zheng Hong-Yuan	山西农业大学经济作物研究所	18935439365@163.com
138	王文浩	Wang Wen-Hao	山西农业大学经济作物研究所	wangwh696@163.com
139	刘文俊	Liu Wen-Jun	山西农业大学经济作物研究所	Wenjun_5257@163.com
140	宋敏	Song Min	山西农业大学经济作物研究所	jzsshyk@163.com
141	王鹏冬	Wang Peng-Dong	山西农业大学经济作物研究所	wangppdd@126.com
142	雷伟	Lei Wei	山西农业大学农学院	563872891@qq.com
143	李华	Li Hua	五原县世达向日葵种业有限责任公司	460712756@qq.com
144	王利民	Wang Li-Min	五原县世达向日葵种业有限责任公司	
145	唐思琦	Tang Si-Qi	西北农林大学	tangsiqi2024@163.com
146	王中华	Wang Zhong-Hua	西北农林科技大学	zhonghuawang@nwafu.edu.cn
147	冉旭	Ran Xu	西北农林科技大学	xran0188@gmail.com
148	景兵	Jing Bing	西北农林科技大学	
149	曹艺萌	Cao Yi-Meng	西北农林科技大学	caoyimeng@nwafu.edu.cn
150	侯俊斌	Hou Jun-Bin	西北农林科技大学农学院	houjunbin123@163.com
151	吕建	Lv Jian	先正达生物科技(中国)有限公司	jian.lv@syngenta.com
152	曹莹莹	Cao Ying-Ying	先正达生物科技（中国）有限公司	ycao_3@163.com
153	刘胜利	Liu Sheng-Li	新疆农垦科学院	495437597@qq.com
154	柳延涛	Liu Yan-Tao	新疆农垦科学院	519293988@qq.com
155	吕增帅	Lv Zeng-Shuai	新疆农垦科学院	2830697273@qq.com
156	段维	Duan Wei	新疆农垦科学院	419015884@qq.com
157	董红业	Dong Hong-Ye	新疆农垦科学院	644212760@qq.com
158	陈方圆	Chen Fang-Yuan	新疆农业科学院	13319911276@163.com
159	黄启秀	Huang Qi-Xiu	新疆农业科学院	13899525177@163.com
160	魏良民	Wei Liang-Min	新疆农业科学院经济作物研究所	13999991108@163.com
161	雷中华	Lei Zhong-Hua	新疆农业科学院经济作物研究所	leizh@xaas.ac.cn

162	石必显	Shi Bi-Xian	新疆农业科学院经济作物研究所	122614591@qq.com
163	崔新菊	Cui Xin-Ju	伊犁哈萨克自治州农业科学研究所	2635467830@qq.com
164	朱增芳	Zhu Zeng-Fang	伊犁哈萨克自治州农业科学研究所	zhuzengfang95@163.com
165	曲志华	Qu Zhi-Hua	张家口市农业科学院	qxcqxy2020@163.com
166	胡杨	Hu Yang	张家口市农业科学院	huyang_2015@126.com
167	李娟娟	Li Juan-Juan	浙江大学	12116017@zju.edu.cn
168	周盈莹	Zhou Ying-Ying	浙江大学	12207155@zju.edu.cn
169	许玲	Xu Ling	浙江理工大学	lxu@zstu.edu.cn
170	陈珊宇	Chen Shan-Yu	浙江省农业科学院	22894186@qq.com
171	阮关海	Ruan Guan-Hai	浙江省农业科学院	13906520484@163.com
172	潘研研	Pan Yan-Yan	郑州市鼎盛机械制造有限公司	everbest09@dingmachinery.com
173	吴嘉琦	Wu Jia-Qi	郑州市鼎盛机械制造有限公司	everbest03@dingmachinery.com
174	高乐旋	Gao Le-Xuan	中国科学院分子植物科学卓越创新中心	lexuan@sippe.ac.cn
175	王冰	Wang Bing	中国科学院遗传与发育生物学研究所	bingwang@genetics.ac.cn
176	郝彦蓉	Hao Yan-Rong	中国科学院遗传与发育生物学研究所	haoyanrong@genetics.ac.cn
177	汪魏	Wang Wei	中国农业科学院油料作物研究所	wangwei03@caas.cn
178	谭美莲	Tan Mei-Lian	中国农业科学院油料作物研究所	meiliantan@126.com
179	张欣融	Zhang Xin-Rong	中国农业科学院油料作物研究所	zhangxinrong@caas.cn
180	张克诚	Zhang Ke-Cheng	中国农业科学院植物保护研究所	zhangkecheng@sina.com
181	左成山	Zuo Cheng-Shan	中国种子集团有限公司	chengshan.zuo@syngentagroup.cn
182	马宗兴	Ma Zong-Xing	中国种子集团有限公司	zongxing.ma@syngentagroup.cn
183	李静	Li Jing	中国种子集团有限公司	jing.li-1@syngentagroup.cn
184	何晓明	He Xiao-Ming	中化现代农业（内蒙古）有限公司	363583940@qq.com
185	刘桂军	Liu Gui-Jun		18811030718@139.com