Sunflower genetic resources: pre-breeding experience from Serbia

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20th International Sunflower Conference, Novi Sad

The sunflower plant

- From the first varieties in Russia in 1880, in the relatively short period of time, sunflower reached the second place on the list of oilseeds, right after soybeans - in 1970

- Intensive work on breeding resulted with the first sunflower hybrids in the 70's, and sunflower was developed into a high-yielding plant with relatively low requirements

Revisiting the ancestors

1916: T. Sazyperow crosses H. annuus and H. argophyllus for rust resistance

1935: Pustovoit, disease resistance introduction through interspecific crosses

1945: Heiser, large scale interspecific crosses focused on taxonomy

1955: Georgieva-Todorova, crosses with *H. laetiflorus*

1957: Putt, *Puccinia helianthi* resistance from a *wild H. annuus x Armavirsky 3497*

1969: Leclercq, first source of cytoplasmic male sterility in a cross with *H. petiolaris*

1970: Vranceanu, Downy mildew pl 1 gene from material developed on the basis of wild sunflowers

1978: VNIIMK, varietal populations on the basis of *H. tuberosus*.Varieties Yubilejnaya 60, Progress, October, incorporated genes of resistance to *Puccinia helianthi*, *Plasmopara helianthi*, *Phoma* sp., and *Orobanche cumana*.

1978: Škorić, restorer lines resistant to downy mildew based on *H. tuberosus*

Diseases can significantly lower sunflower seed yield

Wild sunflower species can be used as a source of resistance

1979: INRA Versailles, new F.A.O. subnetwork established: **Collection, evaluation and conservation of wild species and their use in sunflower breeding programmes**

IFVCNS designated as Liaison center of this subnetwork

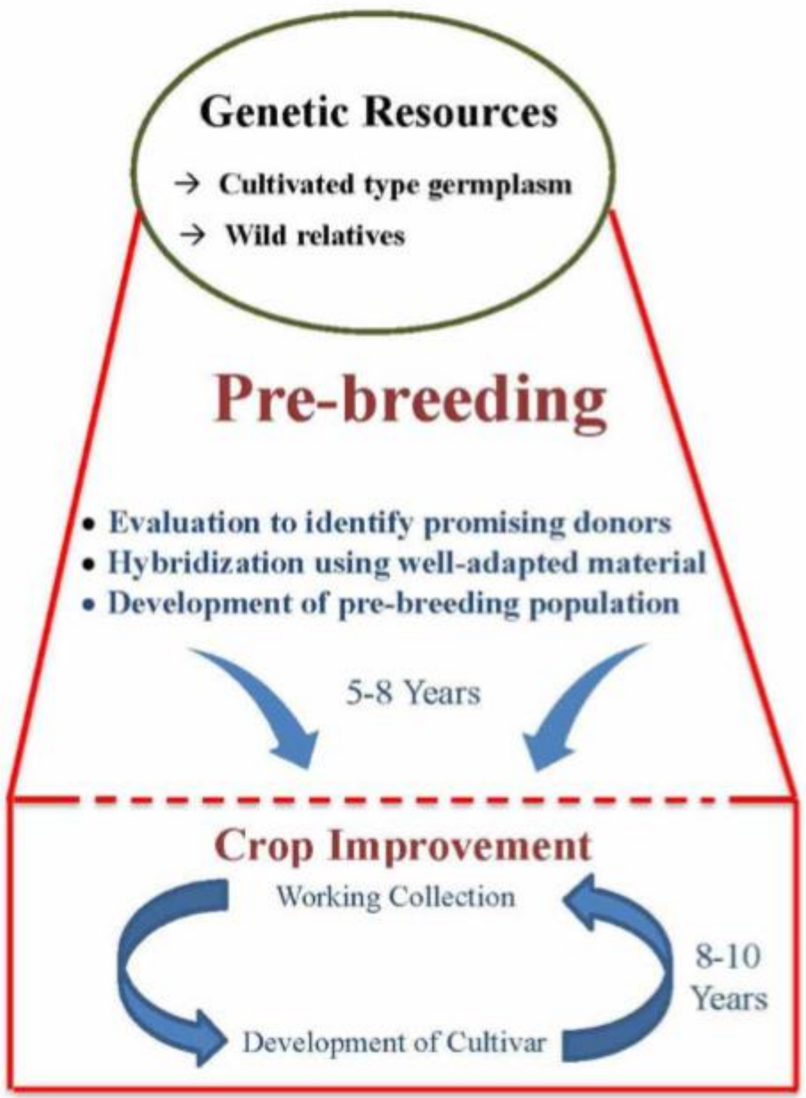
1962: Industrial crops department established

1978: IFVCNS among the first to introduce sunflower hybrids for commercial production

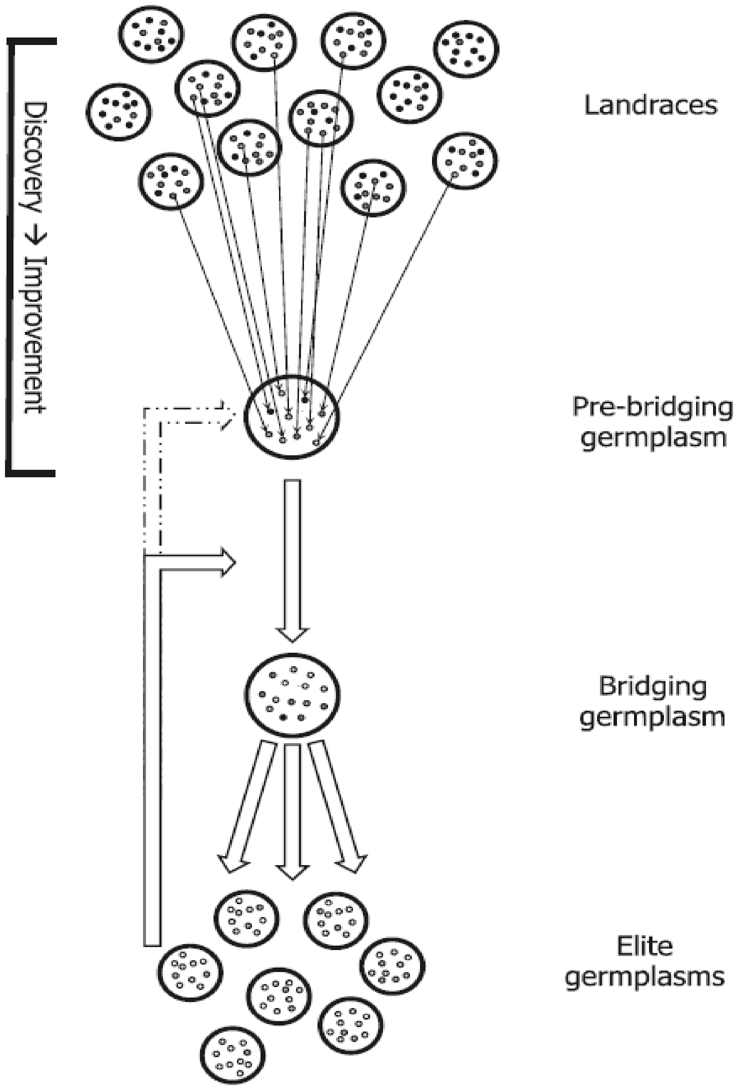
1980: IFVCNS collection of wild *Helianthus* species founded

Helianthus maximiliani

Pre-breeding



Sharma et al. 2013 Grain legumes pre-breeding…



Gorjanc et al. 2016 Maize pre-breeding…

1. Collecting
2. Maintenance
3. Characterisation
4. Biotechnology
5. Interspecific crosses

Helianthus

Section *Helianthus*

H.annuus
H.anomalus
H.argophyllus
H.bolanderi
H.debilis
H.deserticola
H.neglectus
H.niveus
H.paradoxus
H.petiolaris
H.praecox

Section *Agrestes*

H.agrestis

Section *Ciliares*

- **Series *Ciliares***: *H.arizonensis*, *H.ciliaris*, *H.laciniatus*
- **Series *Pumili***: *H.cusickii*, *H.gracilentus*, *H.pumilus*

Section *Divaricati*

- **Series *Corona-solis***: *H.californicus*, *H.decapetalus*, *H.divaricatus*, *H.eggertii*, *H.giganteus*, *H.grosseserratus*, *H.hirsutus*, *H.maximiliani*, *H.mollis*, *H.nuttallii*, *H.resinosus*, *H.salicifolius*, *H.schweinitzii*, *H.strumosus*, *H.tuberosus*
- **Series *Microcephali***: *H.glaucophyllus*, *H.laevigatus*, *H.microcephalus*, *H.porteri*, *H.smithii*
- **Series *Atrorubens***: *H.atrorubens*, *H.occidentalis*, *H.rigidus*, *H.silphioides*
- **Series *Angustifolii***: *H.angustifolius*, *H.carnosus*, *H.floridanus*, *H.heterophyllus*, *H.longifolius*, *H.radula*, *H.simulans*

Infrageneric classification of *Helianthus* (*Compositae*), Schilling, E. E., Heiser, Ch. B., (1981)

Sunflower germplasm explorations – 1976-2004:

Joint efforts between the USDA-ARS and:

Food and Agriculture Organization of the United Nations (FAO)

Wild Species Working Group, The International Plant Genetic Resources Institute, European Cooperative Program for Genetic Resources (IPGRI/ECPGR)

European Cooperative Research Network (ESCORENA)

US Agency for International Development (USAID), USDA Office of International Cooperation and Development (OICD)

The National Bureau of Plant Genetic Resources (NBPGR) New Delhi, India

Expeditions (1980-1991)

Collected by	Year	No. of states	No. of species	No. of accessions
Seiler, G. (USDA-ARS) Ćuk, L. (YUGIFVC)	1980	21 (USA)	37	384
Marinković, R. (YUGIFVC) Miller, J. (USDA-ARS)	1984	1 (Canada)	7	88
Škorić, D. (YUGIFVC), Seiler, G. (USDA-ARS) Rooth, W. (USDA-ARS)	1985	12 (USA)	13	88
Seiler, G. (USDA-ARS), Pomeroy, J. (USDA-ARS) Marinković, R. (YUGIFVC)	1987	6 (USA)	7	52
Dozet, B. (YUGIFVC), Seiler, G. (USDA-ARS) Pomeroy, J. (USDA-ARS), Gavrilova, V. (SUNWIR)	1989	6 (USA)	12	84
Dozet, B. (YUGIFVC) Marinković, R. (YUGIFVC)	1990	1 (Montenegro)	1	81
Marinković, R. (YUGIFVC), Seiler, G. (USDA-ARS) Stauffer, C. (USDA-ARS), Duhoon, S. (NBPGR)	1991	7 (USA)	9	140

Exchange

Prior to 1980: Exchange of breeding material

1980: IFVCNS **received**:

59 samples of 36 wild species from France

9 samples of 9 wild species from Bulgaria

1981: 24 samples of 24 wild species from Romania

8 samples of 7 wild species from Bulgaria

5 samples of 2 wild species from Argentina

1981: Wild sunflower species **sent** to other collections:

11 samples of 11 wild species to France

12 samples of 7 wild species to Bulgaria

Maintainance

The collection of wild sunflower species in Novi Sad (1984)

Dense populations, 2008

Weed control fabric - since 2010

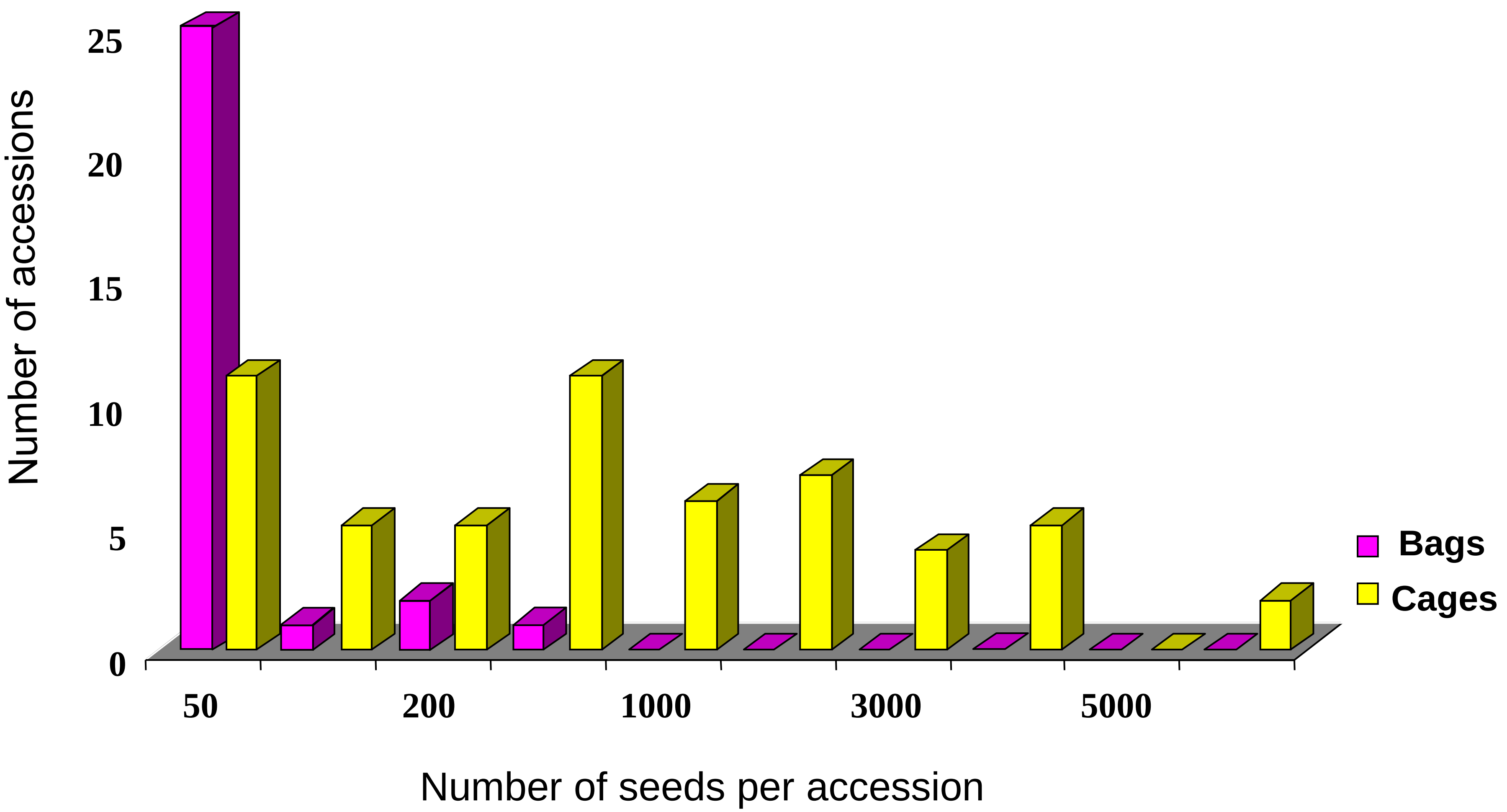
The IFVCNS collection of annual wild species (7/14)

Species	Number of accessions		Number of seeds
	in gene bank data base	with seed reserves	
H.annuus	323	74	10-2539
H.petiolaris	80	25	80-9130
H.praecox	27	26	335-10710
H.debilis	25	24	33-5490
H.argophyllus	23	6	1616-10396
H.neglectus	18	17	1564-4838
H.niveus	12	8	259-5910

The IFVCNS collection of perennial species (21/37)

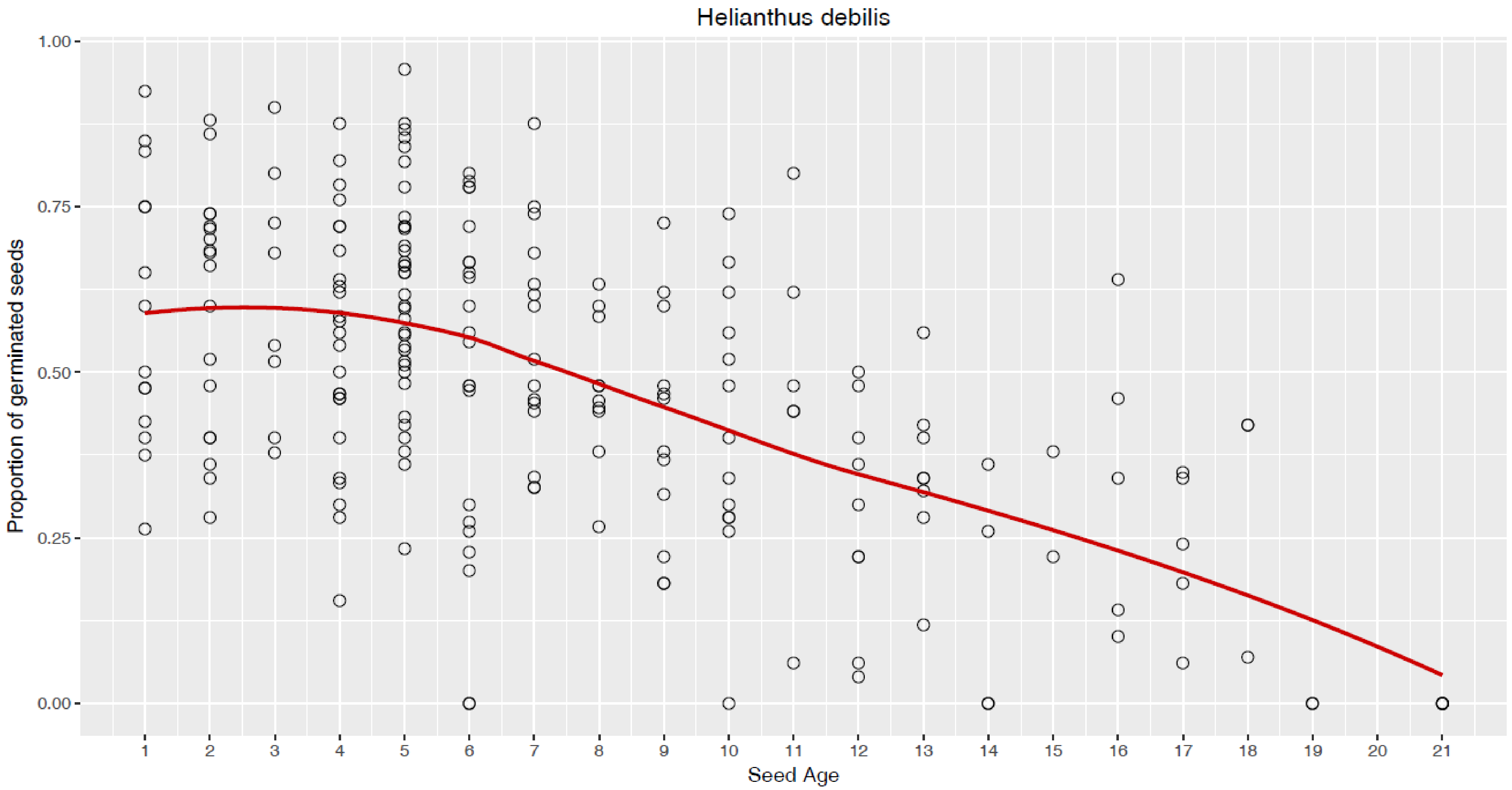
Species	Number of accessions		Number of seeds	Number of accessions in the field
	in gene bank data base	with seed reserves		
H.tuberosus	**158**	**20**	1-650	**112**
H.maximiliani	**48**	**36**	1-8630	**36**
H.grosseserratus	**44**	**20**	2-399	**32**
H.strumosus	**40**	**12**	2-354	**21**
⋮				
H.silphioides	**2**	**1**	13	**1**
H.salicifolius	**2**	**1**	20	**2**
H.glaucophyllus	**1**	**1**	38	**1**
H.californicus	**1**	**1**	1	**1**

The seed production of annual species in relation with the type of isolation



Cage pollination with bumblebees...

Seed longevity in short to medium-term storage conditions 4±2ºC and 55±5% RH



Terzić, unpublished results

The effect of seed scarification on germination

Perennial species	Number of accessions	Germination of non scarified seeds (%)	Germination of scarified seeds (%)	Increase (%)
H.grosseserratus	1	4	38	34
H.tuberosus	1	0	30	30
H.maximiliani	2	4	29,5	25,5
H.atrorubens	2	11	32	21
H.strumosus	1	18	30	12
H.mollis	3	27	36	9,3
H.hirsutus	2	0	9	9
H.rigidus	2	0	8	8
H.laevigatus	1	6	12	6

Average increase = 17,2%

Conserving F_1 hybrids with perennial species in the field

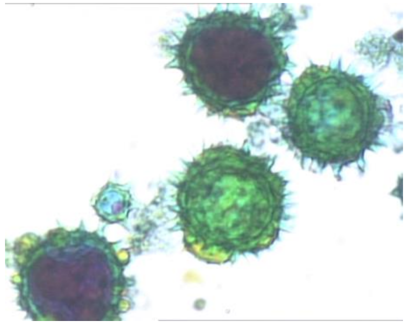
Hybrid plants keep the ability of vegetative reproduction in F_1 generation

Conserved in the field for 30 years under the same growing conditions as wild species

F1 EGG 1626

0%
pollen viability

Chromosome configurations
$32^{II}1^{IV}$; $28^{II}3^{IV}$



28%
pollen viability

Chromosome configurations
$32^{II}1^{IV}$; $30^{II}2^{IV}$

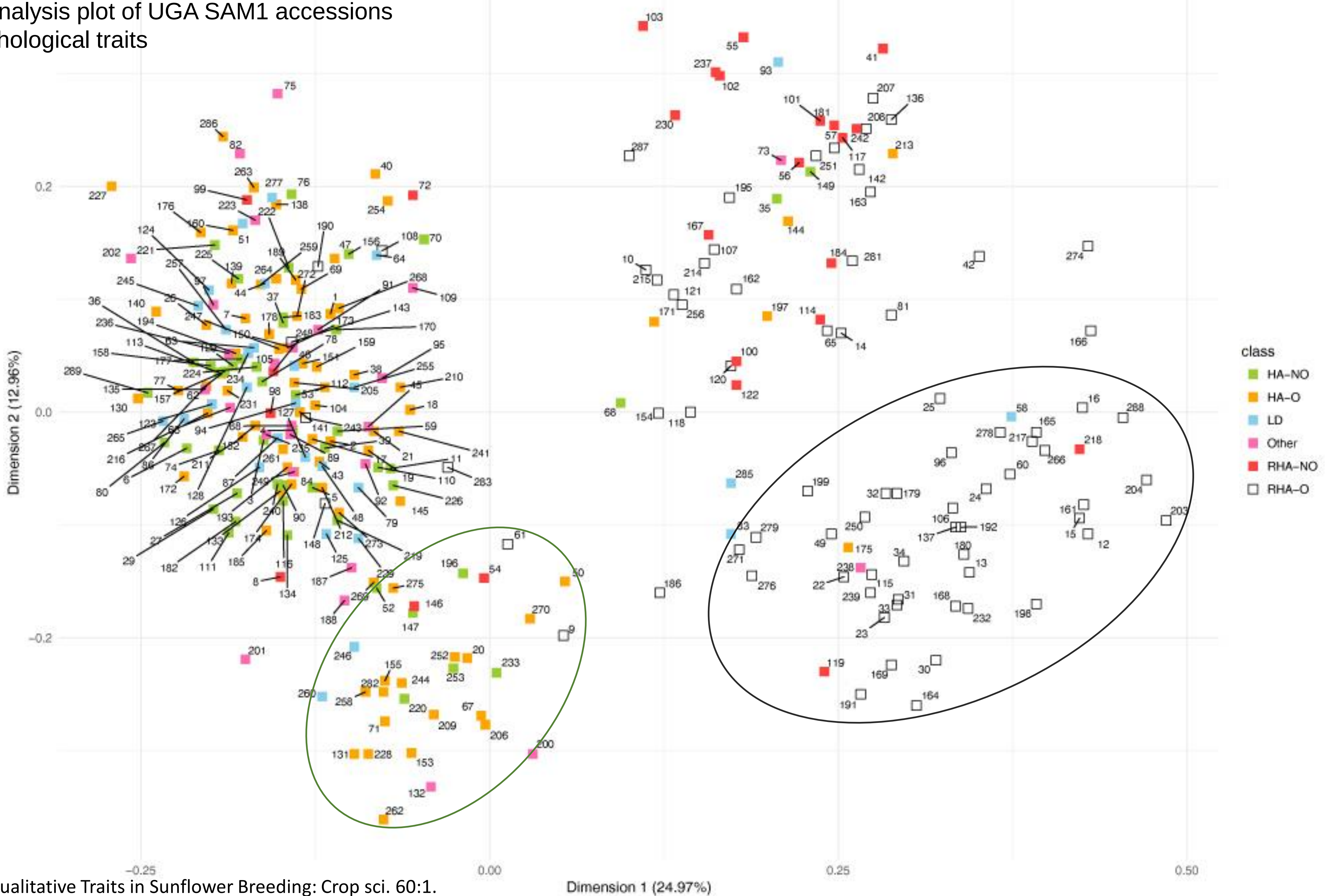
F1 RES 1545

Atlagić et al. 2012. Proc. 18th International sunflower conference.

Characterisation

59% of worldwide use of CWR for pest and disease resistance
13% abiotic stress tolerance
11% quality improvement
10% yield increase
4% sterility and fertility restoration

Homogeneity analysis plot of UGA SAM1 accessions based on morphological traits



Terzić et al. (2020) Qualitative Traits in Sunflower Breeding: Crop sci. 60:1.

Orphan/alternative crops...
Helianthus tuberosus

- 141 accessions
- Morphological comparison
- Mineral composition of tubers and leaves
- Total tuber sugar content
- Photoperiod and vegetation phases
- Climatic variables and yield components
- 2 cultivars registered
- New national DUS test guideline developed

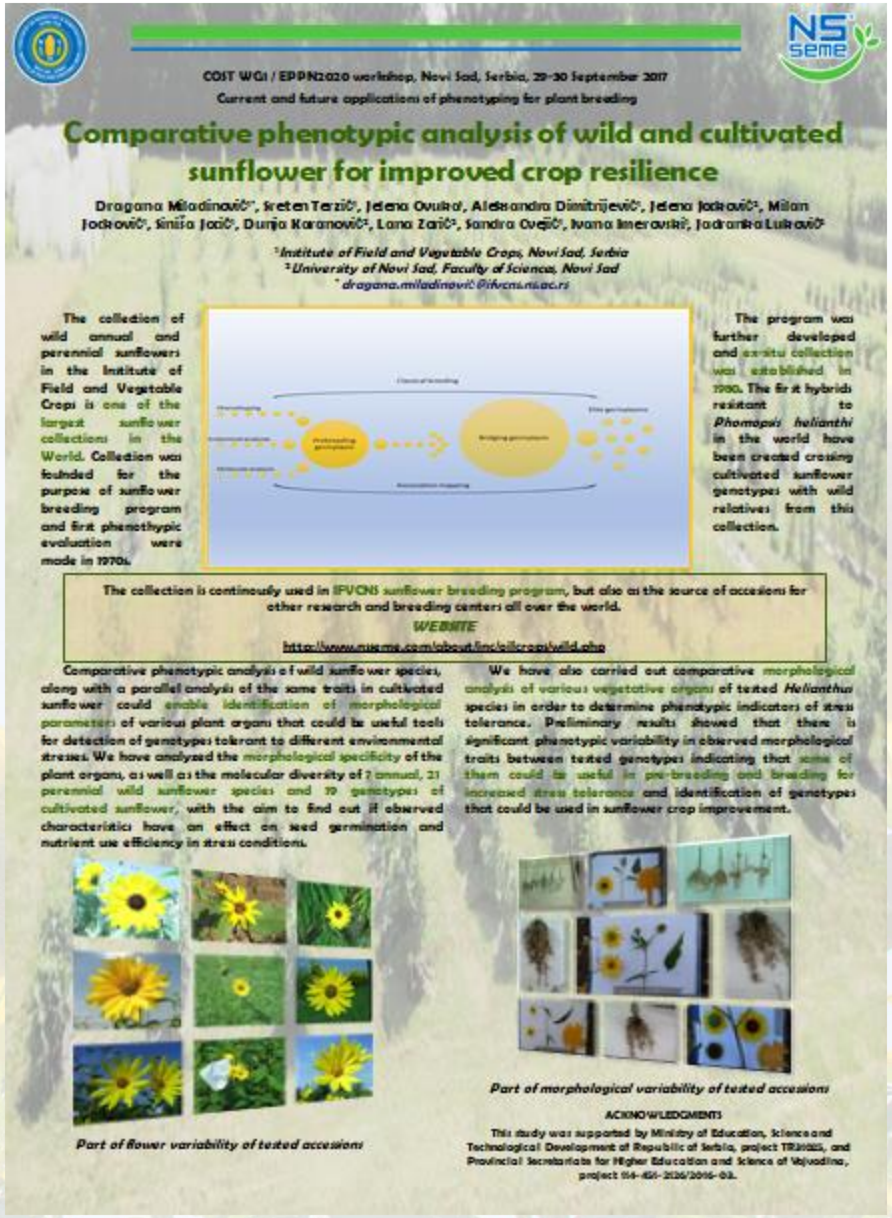
Provincial project, 2017-2020
Anatomic characterization of wild sunflower collection as a potential genepool for cultivated sunflower breeding

7 annual, 21 perennial species;
19 cultivated sunflower inbred lines of various background

All accessions phenologically screened and evaluated for oil content upon introduction to the collection

Phenotypic diversity

Root phenotyping - EPPN^{2020}



Agronomic uses and traits in US CWR

Taxon	Trait	Taxon	Trait
Corylus americana	Eastern filbert blight resistance and other traits	*Juglans major*	Rootstock for high pH soil
Helianthus anomalus	Fertility restoration genes	*Juglans microcarpa*	Rootstock for high pH soil
Helianthus argophyllus	Downy mildew resistance	*Juglans nigra*	Anthracnose resistance
Helianthus bolanderi	Genetic stock	*Malus fusca*	Graftstock
Helianthus debilis	Powdery mildew resistance	*Prunus andersonii*	Graftstock
Helianthus deserticola	Downy mildew resistance	*Prunus pumila*	Graftstock
Helianthus divaricatus	Broomrape resistance	*Prunus rivularis*	Graftstock
Helianthus giganteus	Fertility restoration genes	*Ribes nigrum*	Pest and disease resistance. Other useful agronomic traits.
Helianthus grosseserratus	Broomrape resistance	*Ribes uva-crispa*	Gall mite resistance
Helianthus hirsutus	Fertility restoration genes	*Solanum stoloniferum*	Late blight resistance
Helianthus maximilianii	Broomrape resistance	*Tripsacum dactyloides*	Corn leaf blight resistance
Helianthus neglectus	Fertility restoration genes	*Vitis acerifolia*	Graftstock
Helianthus paradoxus	Salt tolerance	*Vitis aestivalis*	Graftstock
Helianthus pauciflorus	Cytoplasmic male sterility	*Vitis cinerea*	Graftstock
Helianthus petiolaris	Verticillium resistance	*Vitis cinerea* var. *helleri*	Graftstock
Helianthus praecox	Downy mildew, rust, verticillium wilt and broomrape resistance	*Vitis labrusca*	Cold tolerance
Helianthus resinosus	Fertility restoration genes	*Vitis monticola*	Graftstock
Helianthus strumosus	Fertility restoration genes	*Vitis mustangensis*	Graftstock
Helianthus tuberosus	Broomrape resistance	*Vitis riparia*	Phylloxera vitifoliae resistance

Khoury et al. 2015. Partnering in CWR research

Disease incidence observed in a seven year period on **perennial *Helianthus* species** in a naturaly infected field

Helianthus species	Number of acc.	Powdery mildew	Alternaria leaf spot	Stem canker	Sclerotinia head rot
H. californicus	1				
H. decapetalus	8	1	1		1
H. divaricatus	10	1			
H. eggertii	2	1			
H. giganteus	16	11	2		
H. glaucophyllus	1				
H. grosseserratus	32	20	8		
H. hirsutus	4	3			
H. laevigatus	6	2			
H. maximiliani	37	25		3	1
H. microcephalus	2				
H. mollis	9	6	1		
H. x multiflorus	1				
H. nuttallii	23	17	5		
H. rigidus	14	11			
H. resinosus	2				
H. salicifolius	2				
H. silphioides	1				
H. smithii	2				
H. strumosus	21	9		1	
H. tuberosus	141	135		2	1
Total:	**336**	**242**	**17**	**6**	**3**

Disease incidence observed in a seven year period on **annual *Helianthus* species** in a naturaly infected field

Annual species	Total acc.	Powdery mildew *Erysiphe chichoracearum*	Black stem *Phoma macdonaldii*	Sclerotinia rot *Sclerotinia sclerotiorum*	Stem canker *Phomopsis Helianthi*	Charcoal rot *Macrophomina phaseolina*	Alternaria leaf spot *Alternaria helianthi*	Broomrape *Orobanche cumana*
ANN	121	34	104	32	96	10	4	75
ARG	8	3	7	2	4			1
DEB	29	7	13	3	4	1	5	
DES	1							
NEG	17	3	11	2	4	1	3	
NIV	8	1			2		2	
PET	34	5	22	4	22		1	7
PRA	26	7	14	4	2	4	1	1
Total:	244	60	171	47	134	16	16	84

Find new sources of resistance genes to the new broomrape races

Long-term program (wild relatives)

Short-term program (inbred lines in gene bank)

Testing wild species

Testing F_1 hybrids

Backcrossing F_1 with cultivated line
Sowing F_1 and producing F_2....

- Wild species evaluated in a long term characterization program since 1990s
- Greenhouse controlled conditions and infested fields
- Most recent resistance test included 7 annual *Helianthus* species and 182 accessions
- Highest resistance found in *H. petiolaris and H. niveus*

Helianthus spp.	**Total No. of accessions**	**Resistant accessions within species**	**Resistant plants within accessions**
H. petiolaris	31	0.81	0.98
H. niveus	6	0.67	0.94
H. argophyllus	6	0.67	0.86
H. debilis	24	0.58	0.85
H. praecox	25	0.52	0.84
H. neglectus	17	0.47	0.77
H. annuus	73	0.14	0.37
Susceptible check AD66		0	0

- F1 interspecific crosses with perennial species had almost 100% resistance
- Interspecific crosses with annuals species used to develop pre-breeding genepools

Terzić et al. 2010. Screening wild sunflower species and F1 interspecific hybrids for resistance to broomrape. Helia 33, No. 53, 25-30.

White head rot (*Sclerotinia sclerotiorum)*

Mid-stalk rot, head rot
Artificial inoculation
Screening of wild species in the field

Tolerance to capitula rot found in sunflower species *H. debilis* and *H. argophyllus*

Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.)

The results indicated that *H. annuus*, *H. tuberosus* and *H. petiolaris* can be used as a source of resistance to *M. phaseolina*

Downy mildew (*Plasmopara Halstedii)*

According to Seiler (2012), complete resistance to the downy mildew pathogen can be found in several annual and perennial wild sunflower species

When the donor lines are used in commercial breeding programs they have to be crossed with inbred lines which have desirable agronomical traits (Jocić et al. 2010)

IFVC trial:
6 annual species, 29 accessions
Plasmopara race 4 (730)
Resistance: *H. annuus* and *H. argophyllus*

Terzić et al. 2007. Transferring Plasmopara halstedii resistance from annual wild into cultivated sunflower. Helia 30, No. 47. 199-204.

Black stem disease (*Phoma macdonaldii*)

Tested in controled conditions, high level of resistance confirmed

Phomopsis Stem canker (*Phomopsis helianthi*)

Tolerance to Phomopsis stem cancer was found in the perennial species *H. maximiliani, H. pauciflorus, H. hirsutus, H. resinosus* and *H. tuberosus* (Škorić 1985; Dozet 1990)

141 accessions of *Helianthus tuberosus* from the IFVCNS collection were tested

Accessions TUB2046, TUB2062 and TUB CG 65 were most tolerant

The effects of a full tretment with Express total herbicide (active ingredient - tribenuron methyl) 7 annual species, 73 accessions tested

Sensitive plants of
Helianthus annuus

Resistant plants of
Helianthus argophyllus

Biotechnology

Natural interspecific hybridisation in the genus *Helianthus*...

GENETIC DISTANCE

- Difference in chromosome number
- Difference in chromosome structure

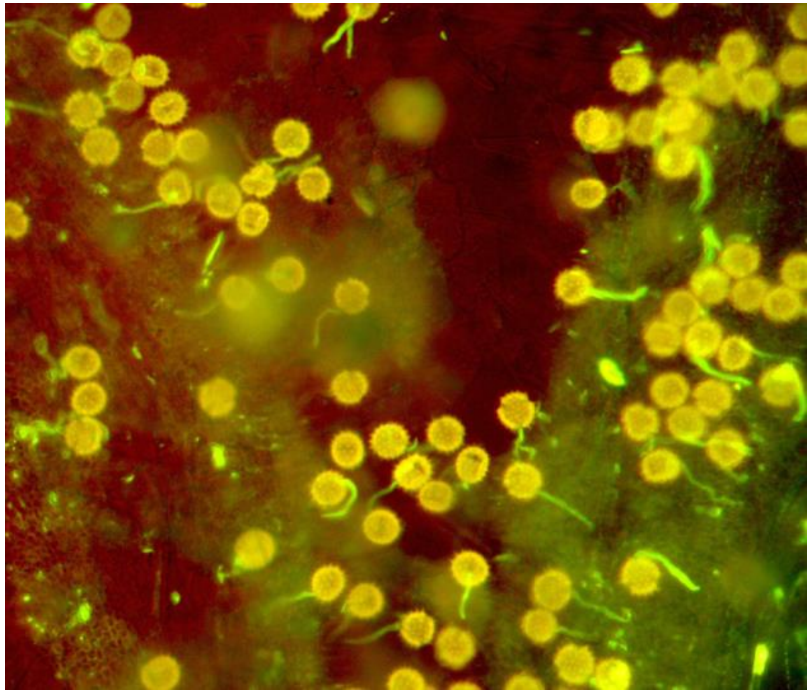
PROBLEMS

- Poor viability of seeds of wild species
- Long growing season and non uniform flowering
- Auto sterility
- Incompatibility
- Abortion of hybrid embryo
- Sterility of F_1 and F_2 interspecific hybrids

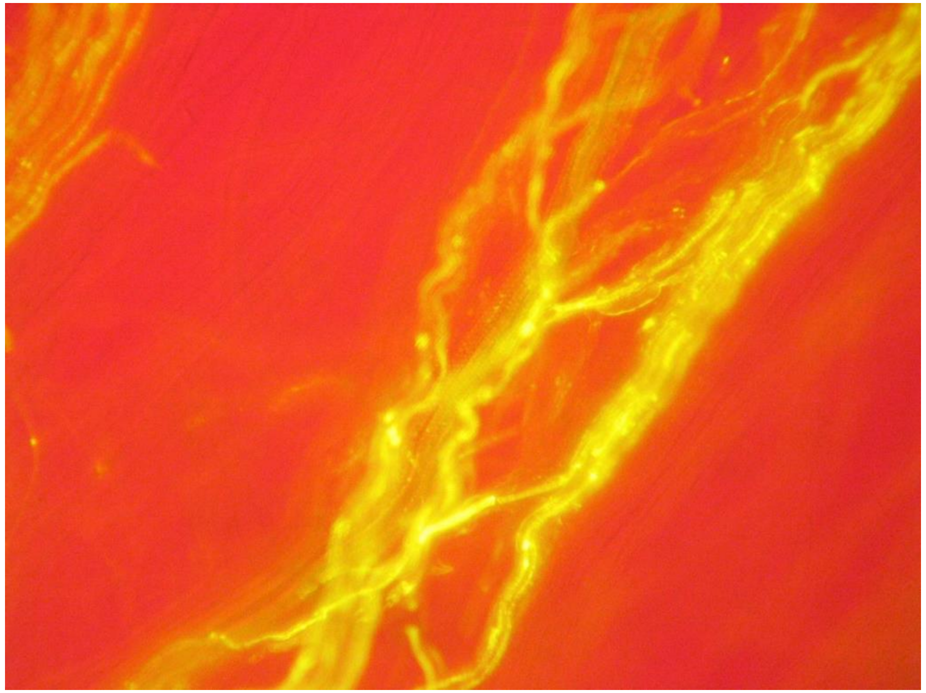
SOLUTIONS

- Chromosome doubling
- Tissue culture
- Embryo culture

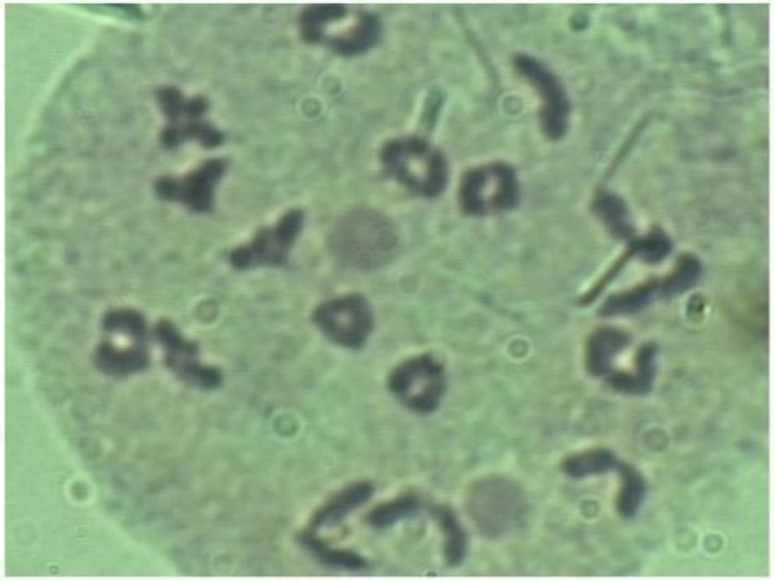
Fluorescent microscopy



Pollen germination

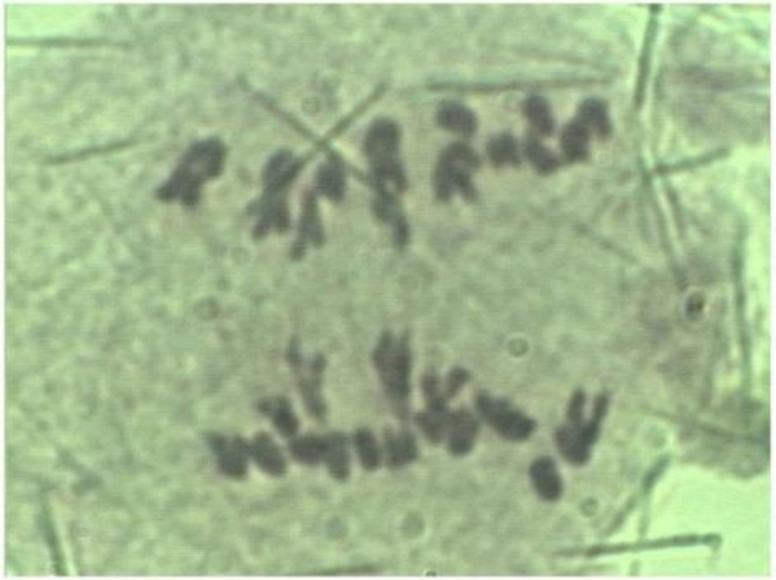


Pollen tube growth



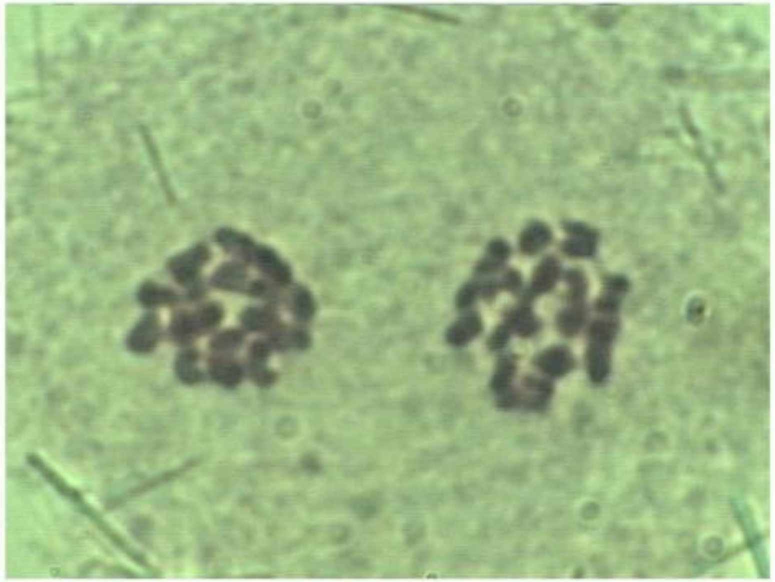
A) DIAKINESIS

B) METAPHASE I

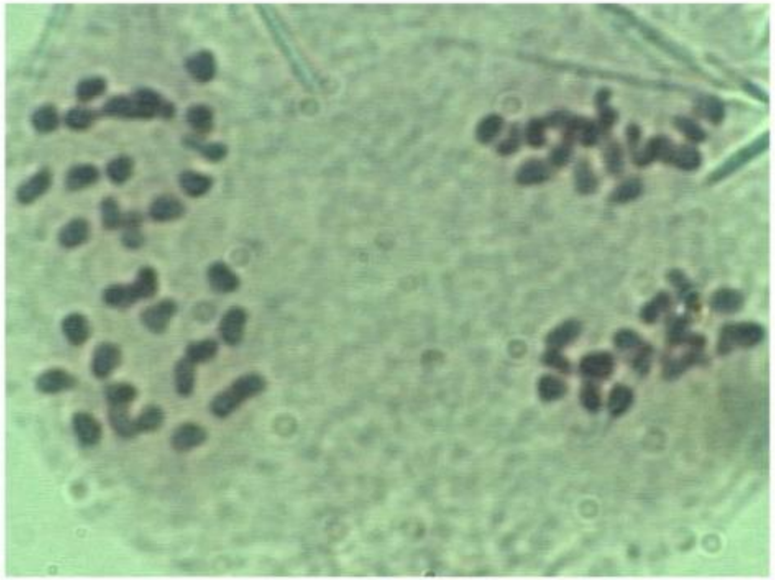


C) ANAPHASE I

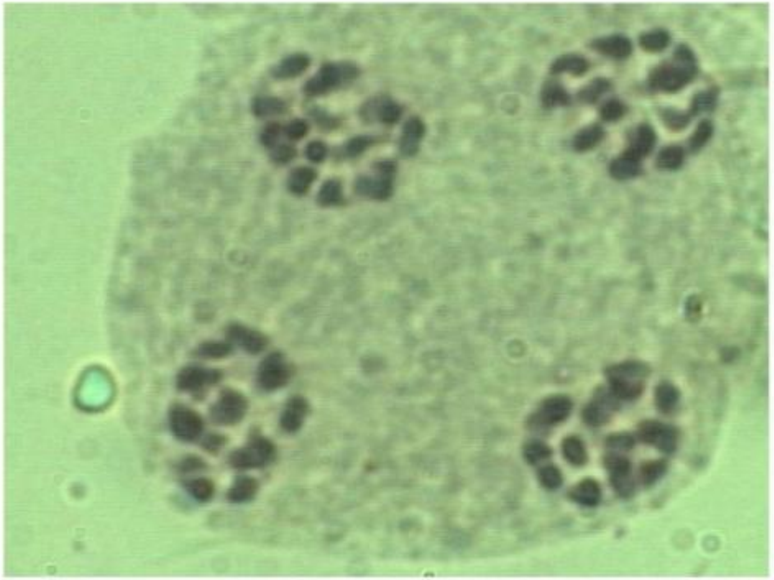
Regular phases of meiosis



D) TELOPHASE I

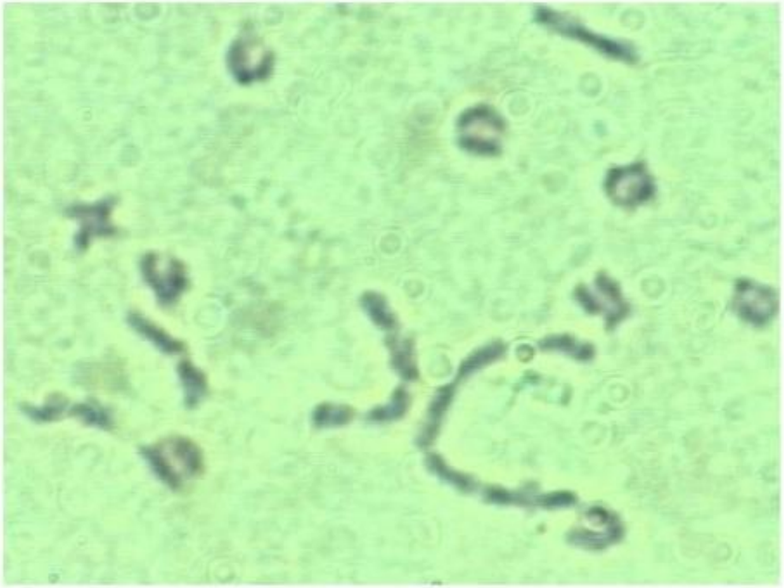


E) ANAPHASE II



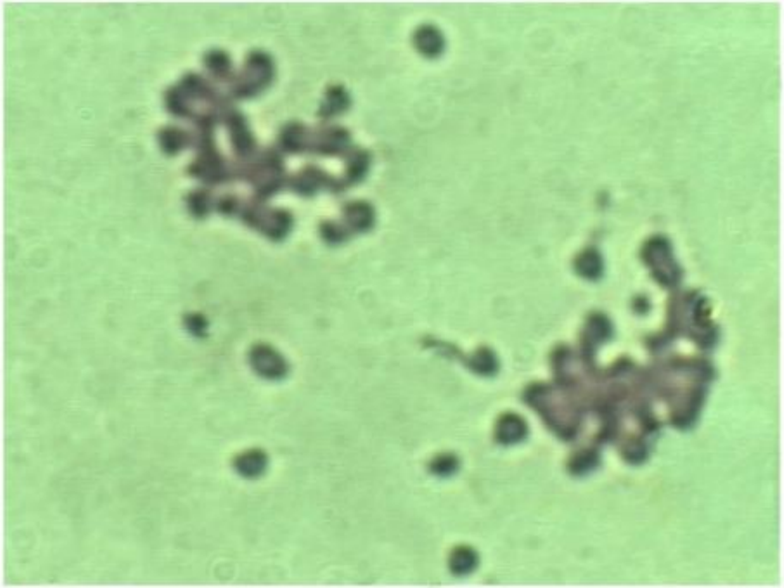
F) TELOPHASE II

Irregular phases of meiosis



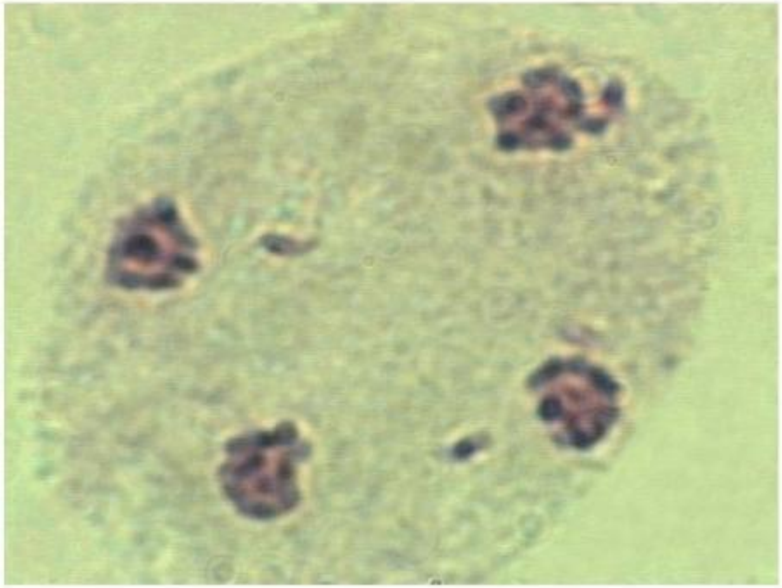
A) Diakinesis with a quadri and a hexavalent

B) Metaphase I with fast chromosomes



C) Anaphase I with lagging chromosomes

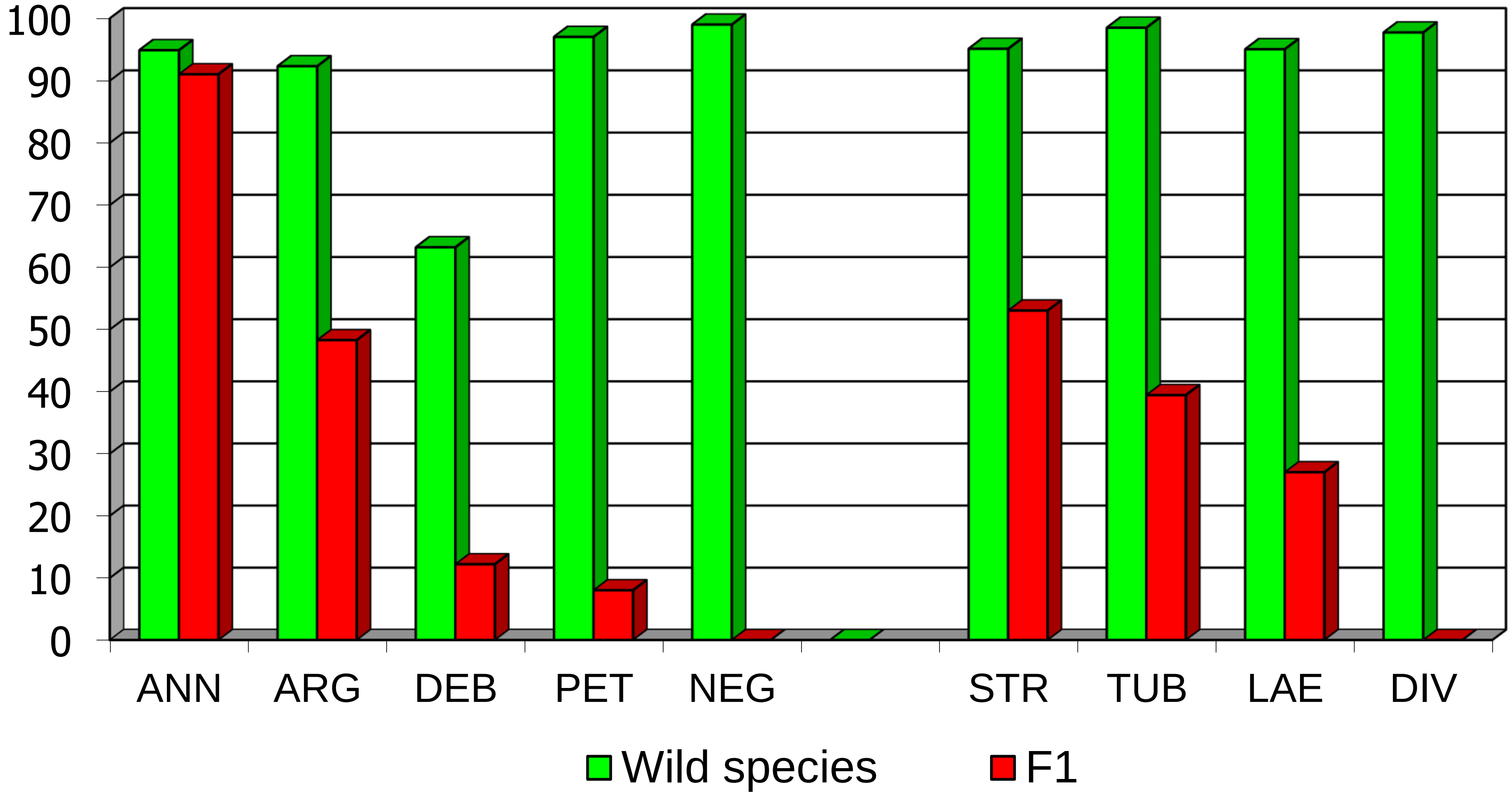
D) Anaphase I with a chromosome bridge

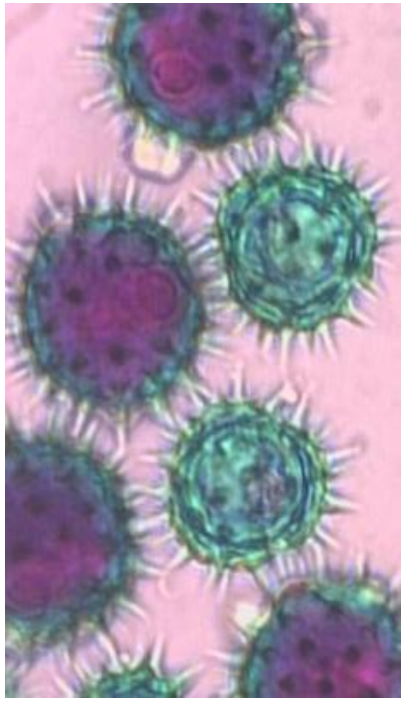


E) Telophase II with laging chromosomes

F) Telophase II with chromosome bridges

Pollen viability:





Genotyping in sunflower - Mapping traits, describing the diversity of germplasms and genomic prediction

Sequencing the sunflower genome - XRQ inbred line
Described in Nature 546, 148–152 (2017)

To date, three high quality genomes are available: XRQ, HA412 and PSC8

In 2019, a pan genome (gene content variation across the cultivated gene pool) based on re-sequencing of 287 cultivated sunflower lines (UGA SAM1) 17 Native American landraces and 189 wild accessions across three gene pools:

Primary: *H. annuus*
Secondary: *H. petiolaris, H.neglectus, H. argophyllus, H. anomalus, H. debilis, H. paradoxus and H. praecox*
Tertiary: *H. divaricatus, H. grosseserratus and H. giganteus*

The International Consortium on Sunflower Genomics, grouping 4 public research institutes and 8 private partners, aims to sequence reference genomes for wild sunflowers and wild relatives

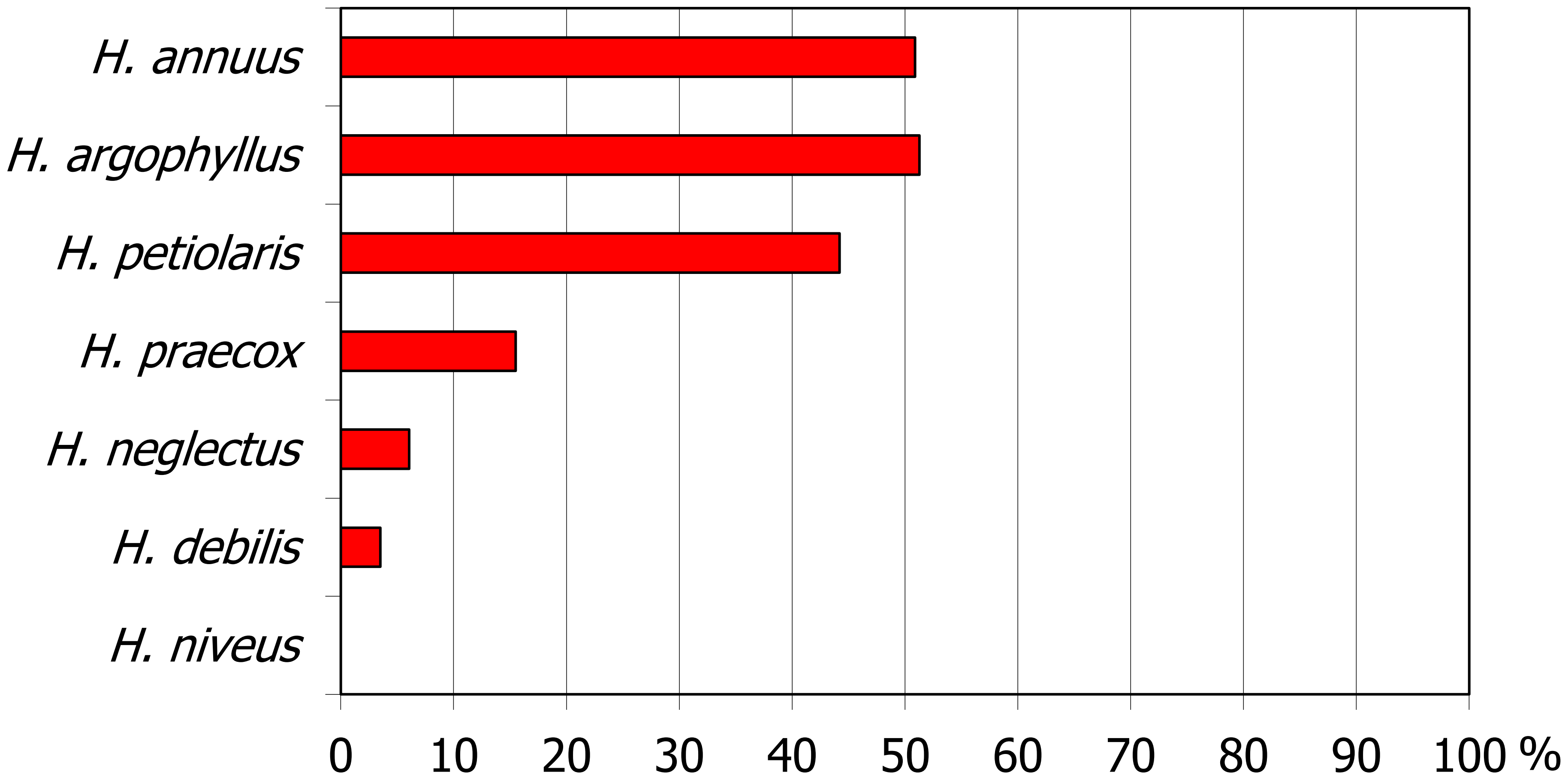
Interspecific crosses

Interspecific crosses:

Introduction of unwanted phenotypic traits can not be avoided in classical interspecific crosses

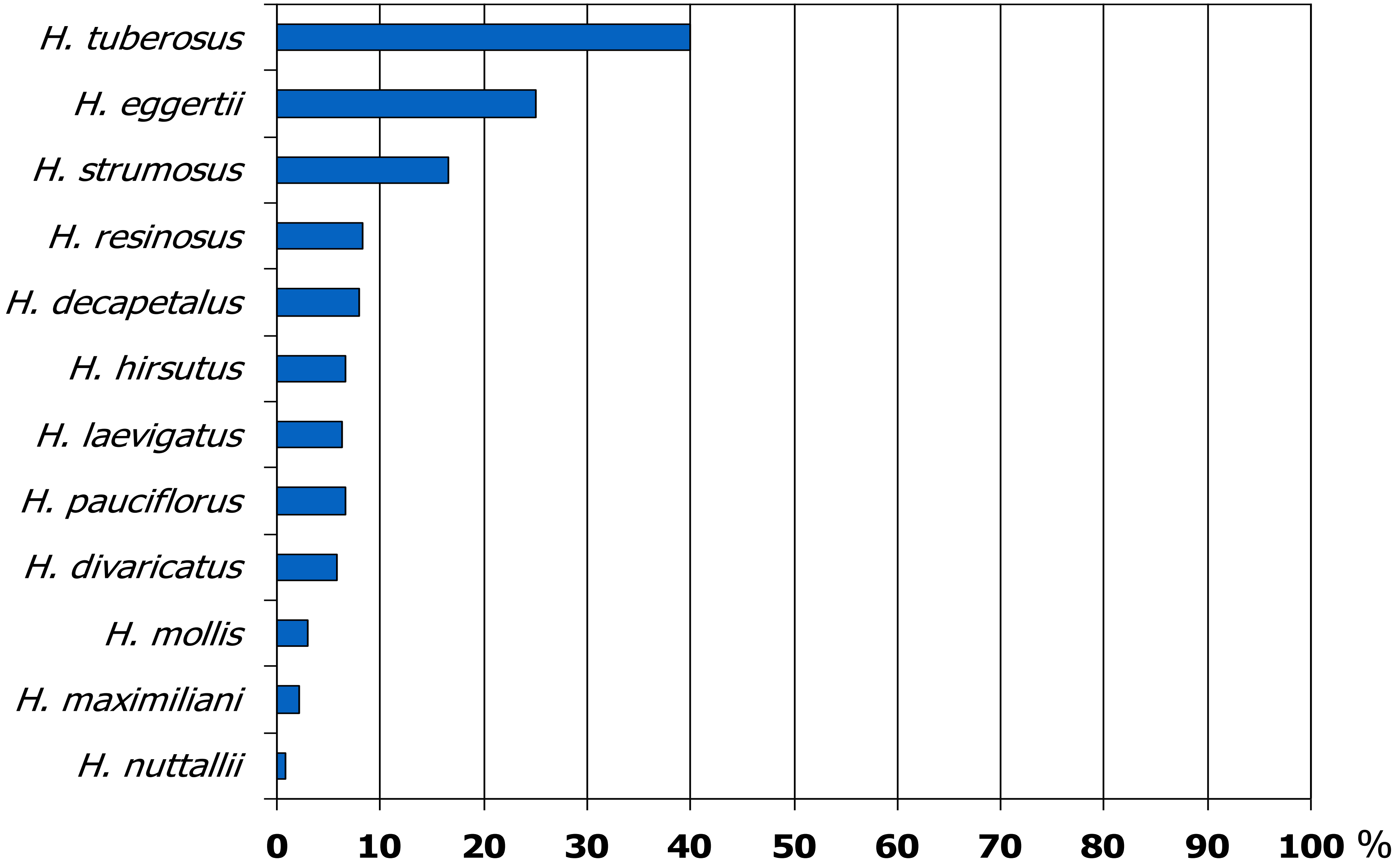
- Hybridization methods: classical-pollination, protoplast fusion, somatic hybridisation. . .
- 7 annual and 16 perennial species crossed with cultivated sunflower at IFVCNS
- Perennial species are harder to cross, but interspecific hybrids are obtainable

The success of interspecific crosses with annual species



Percentage of inflorescences with seed in comparison to the total number of pollinated

The success of interspecific crosses with perennial species



Percentage of inflorescences with seed in comparison to the total number of pollinated

H. rigidus

F_1 hybrid

Cultivated line

Confirmed and potential "breeding uses" of the 10 crops with most "breeding use" citations in the literature, categorized in trait classes

5 Agronomic traits

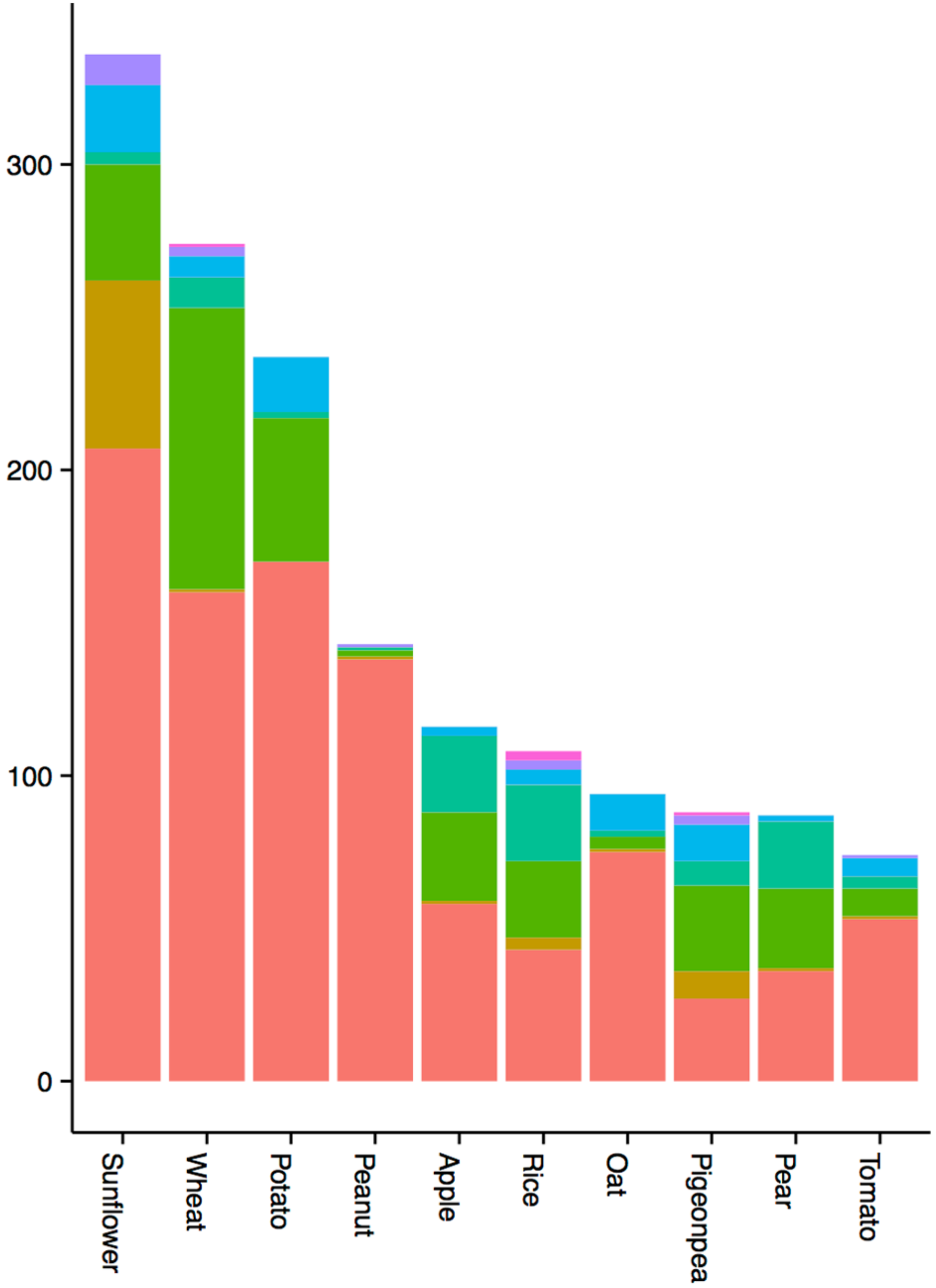
10 Phenological traits

25 Quality characters

40 Abiotic stresses

60 Male fertility restoration

210 Biotic stresses



Dempewolf et al. (2017) Past and future use of wild relatives in crop breeding. Crop Sci 57: 1070–1082.

High-throughput phenotyping – Low information sharing!?

Accessions are populations, not inbred lines...!
Species diverse in vegetation phases, habitus... Making automated phenotyping demanding
Greenhouse platforms, unmanned aerial or ground based vehicles...

Information portals should be used more to share the results/improve the usage of CWR

Eurisco (The European Search Catalogue for Plant Genetic Resources)
AEGIS (A European Genebank Integrated System)

U.S. National Plant Germplasm System

France: The PhenoDB INRAE database https://sunrise.toulouse.inra.fr/phenoDB/

Prebreeding – specific traits

***H. mollis* – sessile leaves**

cms9 x *H. mollis*
Sterile F_1
2 back crosses with rf line

Pedigre selection producing new lines

Crosses with testers and paralel tests for sclerotinia resistance

Shorter leaf petiole
Oil content variable

Inflorescence not inclining…

Broomrape resistance

1. Annuals and perennials, F_1 hybrids with perennials
2. Annual wild acc, F_1 with annual species
3. BC_1 produced and tested
4. BC_2 followed by selfing and further tests

Highest percentage of resistant plants in F_1 and BC_1F_1

Sclerotinia – head rot tolerance

1. 44 interspecific progenies with 6 annual wild
2. Field test and selfing of selected plants, 3-4 cycles
3. Pedigre selection producing new lines

Material from *H. debilis* with highest tolerance

Interspecific lines obtained from USDA, Seiler (1991)

6-7 years, pre-breeding

RES-834-1 (cms HA 89*2/H. resinosus 834) F5)
PRA-RUN-1329-1 (nms P21*3/H. praecox 1329) F3)
DEB-SIL-367-2 (cms HA 89*3/H. debilis 367) F3)
DES-1474-2 (cms89*2/H. deserticola 1474)//RHA 274 F4)

3 years of selfing

Field observations and pedigry selection

16 selected male fertile lines

H. resinosus: 1RES, 2RES, 5RES
H. debilis : 8DEB-SIL, 9DEB-SIL 10DEB-SIL
H. praecox : 11PRA-RUN, 12PRA-RUN, 13PRA-RUN, 14PRA-RUN
H. deserticola: 16DES, 17DES D, 18DES D, 19DES D, 20DES

8 years
CMS form

Three IFVC Rf lines with good GCA utilized as testers

Confectionary sunflower lines

Interspecific lines produced at IFVCNS, Terzić (2006)

HA-26 (hand emasculation)

×





- *H. debilis* 1848
- *H. praecox* 1341
- *H. petiolaris* 2119
- *H. annuus* 2168
- *H. neglectus* 1181

3-5 years

3-5 years

B-lines

Pedigree selection 4–6 years

6–8 years

Prebreeding material → New genotypes → Cms form

<u>Prebreeding material</u>

F1 S1 DEB 1848
F1 S1 DEB 1848
F1 S1 PRA 1341
F1 S1 PRA 1341
F1 S1 PRA 1341
BC2 PET 2119
BC2 PET 2119
BC2 S2 ANN 2168
BC2 S2 ANN 2168

3-6 years → Pedigree selection

RHA lines

Ts-s-1-17
Ts-s-1-22
Ts-s-1-192
Ts-s-1-200
Ts-s-1-202
Ts-bc-30
Ts-bc-31
Ts-bc-44
Ts-bc-45

Oil sunflower lines

Future prospects

The evolving field of phenomics will help accelerate sunflower CWR exploitation for introgression breeding by increasing the number of evaluated traits and accessions – thus more understanding their diversity

Existing sunflower collections could benefit from closer cooperation to improve the quality and availability of materials conserved: tackle issues like local gene bank standards, germplasm availability and characterisation data, genetic drift, pollination... *Gene bank peer review - AEGIS*

Intelectual property rights imposed by the funding body sometimes put further constraints... But international cooperation is possible and for major tasks like Sclerotinia and broomrape resistance it is necessary!

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Gene banks for wild and cultivated sunflower genetic resources[☆]

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Thank you for your attention !!!