Plasma application in agriculture: seed treatment with plasma as an alternative method for increasing plant productivity

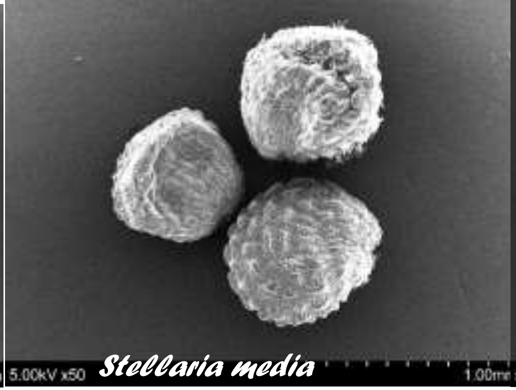
The project implemented by Vytautas Magnus University (VMU), Kaunas, Lithuania and Leibniz Plasma Research and Technology Institute (INP), Greifswald, Germany in 2020 is supported by the Baltic-German University Liaison Office is supported by the German Academic Exchange Service (DAAD) with funds from the Foreign Office of the Federal Republic German

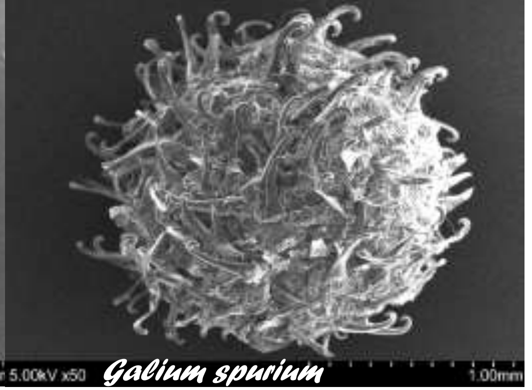
The project aims to establish interdisciplinary collaboration between research groups with complementary expertise: plant biology/biochemistry (Lithuania) and plasma physics as well as plant biology and microbiology (Mecklenburg-Vorpommern, Germany) and to establish the basis for joint writing and submission of international grant proposals dealing with the application of plasma in agriculture.

Digitalized public lectures:

Lecturers:

- **dr. H. Brust “Application of Plasma Technologies for Plant Food Production”**
- **prof. V. Mildažienė “Lessons of Seed Processing with Plasma: How does high stress resistance combine with sensitivity to environmental changes”?**



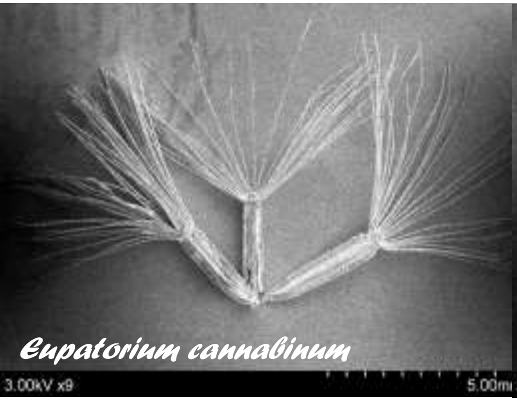


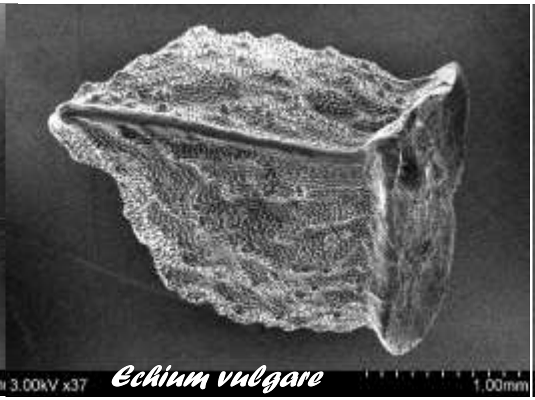
PUBLIC LECTURE

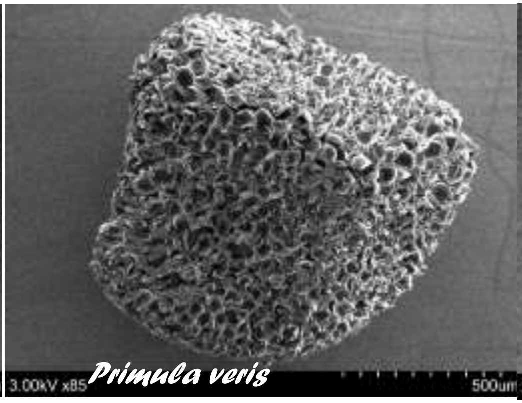
Lessons of Seed Processing with Plasma: How does high stress resistance combine with sensitivity to environmental changes?

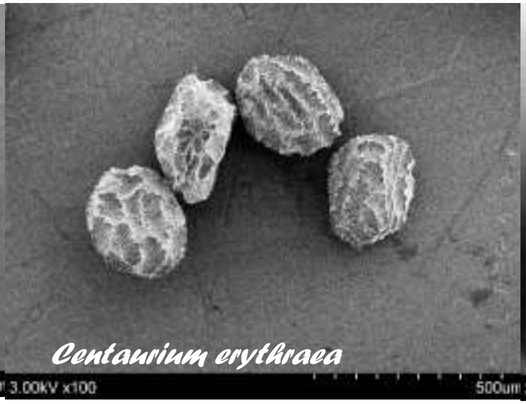
Vida Mildaziene

Vytautas Magnus university, Kaunas, Lithuania









Lecture outline ɞɞɞ

- Seeds – definition, function, structure, diversity;
- Seed longevity and stress resistance;
- Seed sensitivity to environmental signals revealed in studies of plant response to seed treatment with cold plasma;

Why seeds?

SEEDS:
Planting material

High quality seeds – improved production

Food & Forage
Textile
Construction materials
Energy
Medicines
Landscape
Environment

What is seed? Small embryonic plant enclosed in a covering called the seed coat (or testa), usually with some stored food

SEEDS
TIME CAPSULES OF LIFE:
https://doorofperception.com/2019/03/seeds-time-capsules-of-life/

- “In many ways, the seed is a microcosm of life itself. The seed is a neatly mapped package containing a living organism capable of exhibiting almost all of the processes found in the mature plant.”
- “While humankind no longer prays to the goddesses of grain (Demeter the Greek, and Ceres the Roman), we have a long way to go to unravel all the mysteries of the seed.”

Lorence O. Copeland, Miller M. McDonald, Principles of seed science and technology, 4th Edition, 2001, Springer Science + business media, LLC

The life of mighty tree begins from the small seed

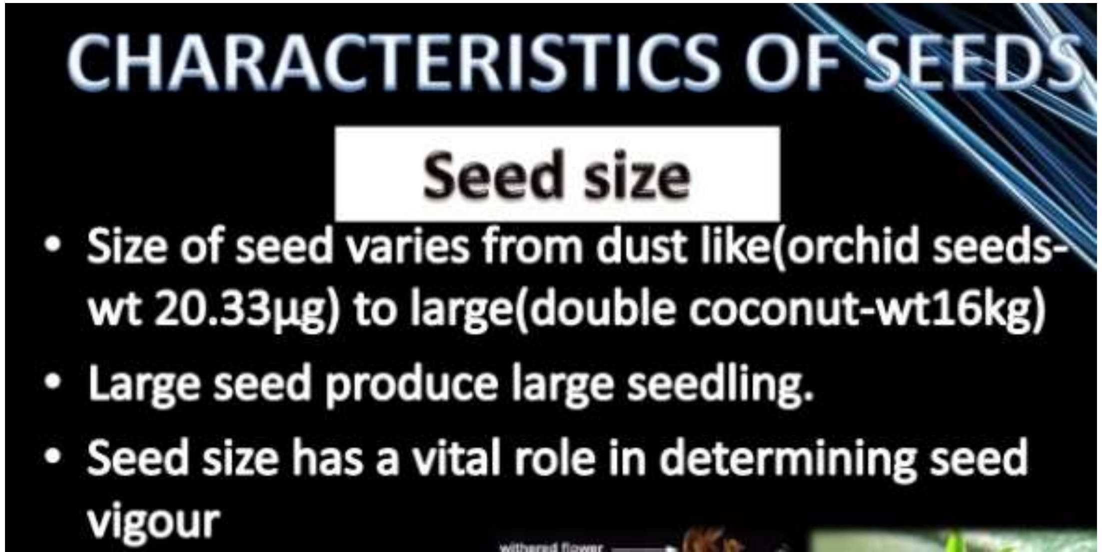
Seed and tree of
Giant sequoia, *Sequoiadendron giganteum*,

How it works?

What is inside?

SEED FUNCTION: reproduction – central process in plant life cycle

- **Seeds** – the most important means of reproduction for numerous plants;
- The only mobile form in plant life cycle. Function – transfer and distribution of genetic plant material in time and space.
- Seeds are the dispersal and propagation units of the Spermatophyta (seed plants): gymnosperms (conifers and related clades ~750 species) and angiosperms (flowering plants, ~250 000 species).
- Large variability in size, shape, colour, external and internal structure.
- Variability in seed shape also exists within a given species and is referred to as seed polymorphism. Characteristic of polymorphic seeds is that they differ not only in shape or colour, but also in their germination behaviour and dormancy. Every seed lot = seed population.

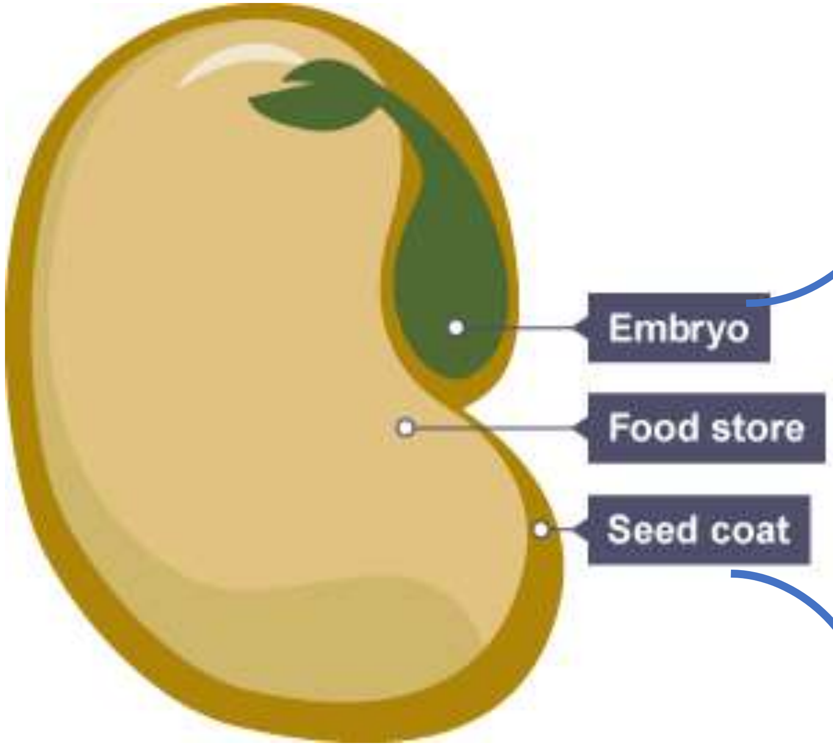


Seed types by dessication resistance

- Develops after the fertilization of female gamete by male gamete in the embryo sac of the ovule in ovary of the flower;
- Undergoes development, maturation and ripening;
- **Orthodox seeds** survive drying and acquire high stress resistance due to dessication (water content ~5%, vitrification);
- **Recalcitrant** or dessication sensitive **seeds** – do not survive drying and cannot be stored for long periods can lose viability (e.g. oak, coconut seed);
- Seeds of most plants species are dormant – special biochemical mechanism ensures that seeds do not germinate immediately after dispersal;
- Seed is very dynamic organism state. They undergo biochemical changes. During 6 months of storage biochemical reactions take place and genes (>400) change their expression.

Seed structure is complex and extremely diverse:

Simplified scheme: 3 main parts



Contains all information for plant development

Nutritive reserves reserves for seed germination: carbohydrates, proteins and lipids.

Seed coat or testa – separates and protects internal seed content from the environment. Coat pigments determine seed colour.

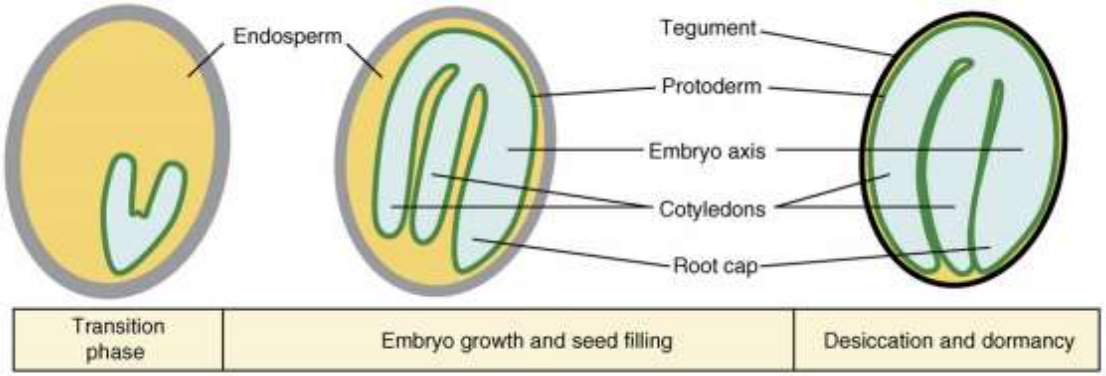
Why are seeds advantageous for plants?

- maintain dormancy until better environmental conditions arise;
- afford protection to young plant at vulnerable developmental stage;
- contain adequate food supply until photosynthesis is possible;
- dispersal of plants.

Stages of seed development
morphogenesis and maturation

As a result of fertilization the embryo is formed.

L. Gutierrez et al., Combined networks regulating seed maturation.
TRENDS in Plant Science 2007, 12, 294-300.



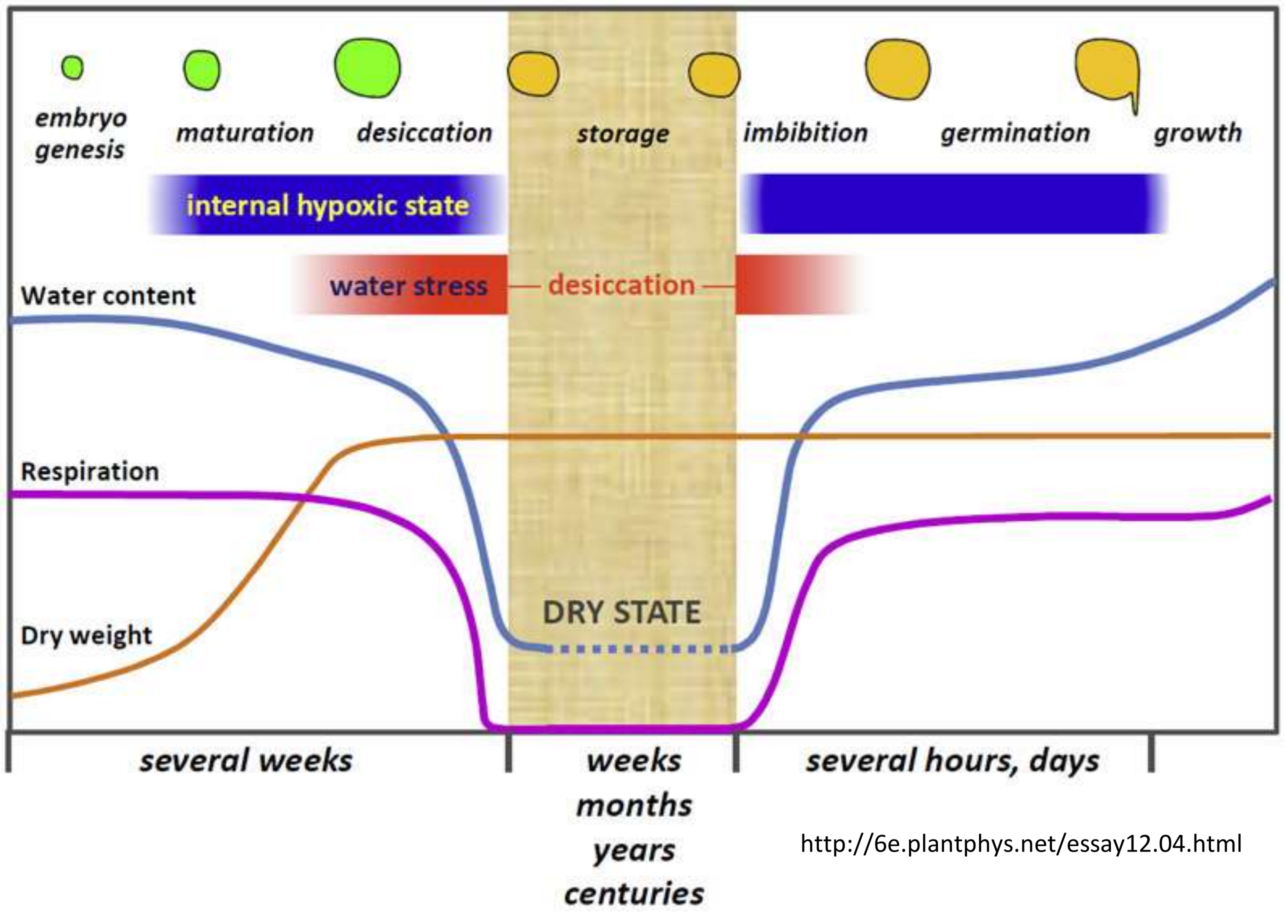
Maturation takes place in the fruit on the parent plant

- In short, seed maturation begins – (1) seed enters the transition phase - switch from maternal to autonomical (filial) controls occurs;
- (2) After the transition phase, the seed undergoes a period of **embryo growth** during which storage products accumulate in embryo and surrounding tissues (seed filling). The **histodifferentiation** occurs – seed tissues are formed;
- (3) Then embryo ceases to grow, testa layers issued from maternal integuments differentiate to form the protective tegument;
- (4) Maturation ends with seed **desiccation** and **dormancy induction**.

Dessication (maturation drying). Ortodoxic seeds are anhydrobiotes.

Anhydrobiosis is a vital property of seeds.

Moisture content in dry seeds is less than 0.1g H_2O/g dry weight



http://6e.plantphys.net/essay12.04.html

In the dry state, seeds are:

(1) metabolically inactive (water is not available for biochemical reactions);

(2) Number of organelles per cell falls, vacuoles deflate;

(3) Food reserves become dense crystalline bodies;

(4) Highly tolerant to environmental conditions;

(5) Can remain viable for long periods of time (2000-year-old date seeds actually produced healthy plants; Sallon et al. 2008).

When appropriate conditions (water, temperature, oxygen) are met, and in the absence of dormancy, seeds rapidly resume metabolism upon imbibition and shift to a new developmental program leading to germination and seedling establishment.

The maintenance of biological structures surviving desiccation requires cellular adaptations at the molecular level. The mechanisms involve a concerted array of protective and repair mechanisms, including the accumulation of membrane and macromolecule protectants (sugars, stress proteins), the control of metabolism and oxidative load.

SEED longevity

Museum botanists in Canada reported the germination of lupin seeds that had been buried deep in a Canadian peat bog for an estimated 10,000 years (Porsild and Harrington 1967). Germinating Indian lotus seeds from a Manchurian lake bed were first estimated to be 120 to 400 years old (Ohga 1926), but were later found, using radiocarbon dating, to be over 1000 years old (Libby 1951).

The two oldest and bestknown studies are those founded by Beal in 1879 at Michigan State University and Duvel of the United States Department of Agriculture in 1902. In both studies, seeds buried in soil continue to germinate demonstrating the remarkable life span of many seeds

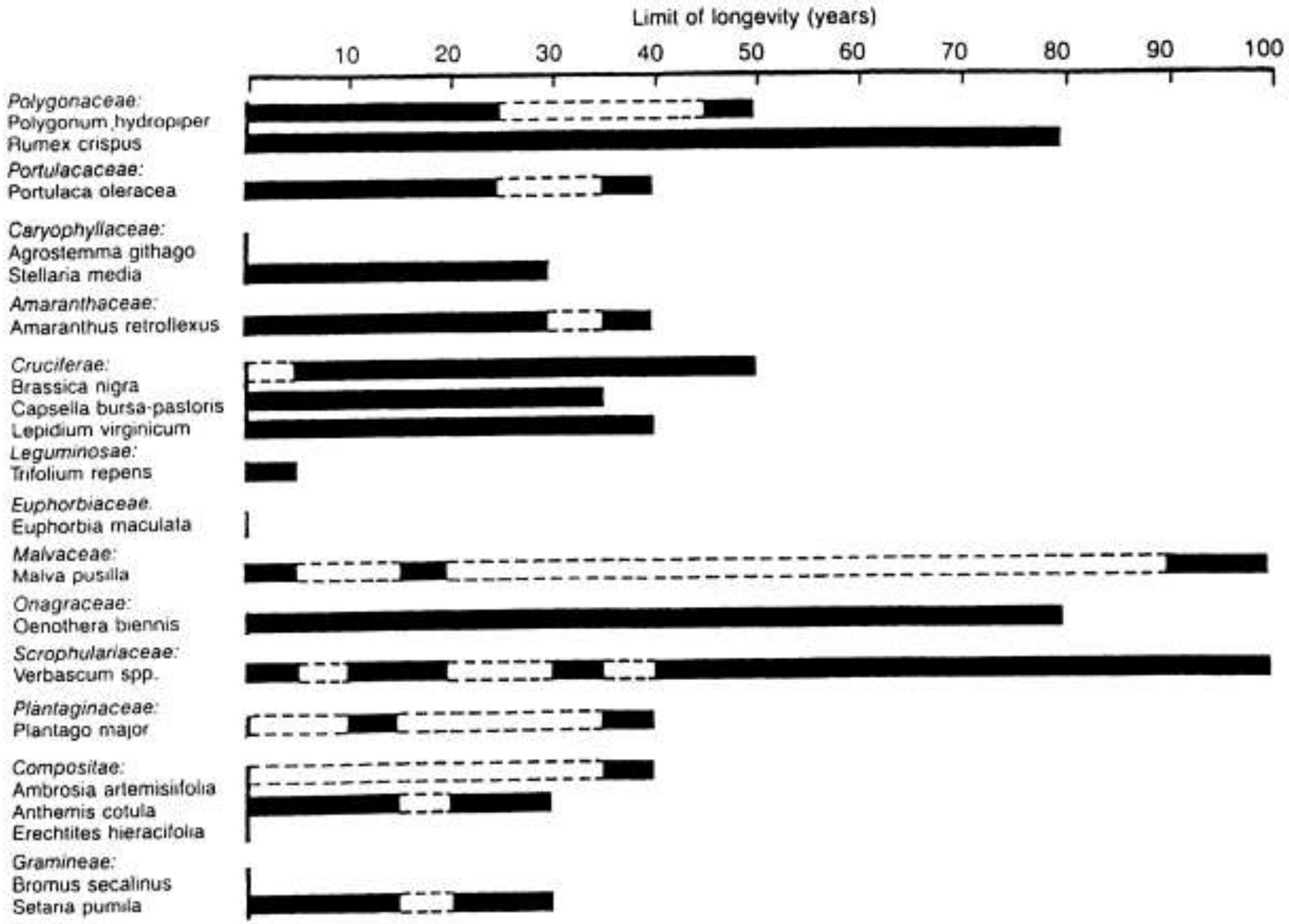


Figure 9.1. Limits of longevity for seeds in Beal's burial experiment. Germination tests were performed every five years for the first 40 years and every 10 years thereafter. Dashed lines indicate test periods when no positive germinations were noted. "Verbascum spp." indicates V. thapsus *and/or* V. blattaria *(From Priestley 1986).*

The Judean date palm

is a date palm (*Phoenix dactylifera*) which was spruted in 2005 from a preserved 2000-year-old seed. The palm, a male tree named Methuselah reached 3.5 meters (3.8 yards) in February, 2020.

The Judean Date Palm at Ketura, Israel, nicknamed Methuselah.
https://en.wikipedia.org/wiki/Judean_date_palm

https://www.indefenseofplants.com/blog/2015/11/4/germinating-a-seed-after-32000-years

“What you are looking at are plants that were grown from seeds buried in permafrost for nearly 32,000 years. The seeds were discovered on the banks of the Kolyma River in Siberia”.
” Using micropropagation techniques coupled with tissue cultures, researchers were able to grow and flower the 32,000 year old seeds. What they discovered was that these seeds belonged to a plant that can still be found in the Arctic today. It is a small species in the family Caryophyllaceae called *Silene stenophylla*.

https://alchetron.com/Silene-stenophylla

Seed dormancy

a **state in which seeds are prevented from germinating even under environmental conditions favorable for germination.**

Baskin and Baskin, 2004: A **dormant seed** does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination, i.e. after the seed becomes non-dormant.

- The special physiological mechanism (hormonal regulation) ensures that seeds do not germinate immediately after maturation.
- Preharvest sprooting or vivipary is an example of not properly functioning dormancy

Shu K., Liu X.-d., Xie Q., and He Z.-h. (2016). Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. Mol. Plant. 9, 34–45.

Pre-harvest sprouting of crops often occurs when mature plants encounter prolonged rainfall and high humidity during the harvest season, which decreases yields and grain quality and also causes problems in industrial process. Red arrows indicate sprouting seeds on panicles.

Importance of Seed Dormancy

1. It allows the storage of seeds for later use by animals and man.
2. It helps in the dispersal of the seeds through the unfavourable environment.
3. Dormancy induced by the inhibitors present in the seed coats is highly useful to desert plants.
4. Allows the seeds to continue to be in suspended animation without any harm during cold or high summer temperature and even under drought conditions.
5. Dormancy helps seeds to remain alive in the soil for several years and provides a continuous source of new plants, even when all the mature plants of the area have died down due to natural disasters.

Classification of of seed dormancy

Nikolajeva 1977; Baskin&Baskin 1998, 2004; Black&Bewley, 1980.

http://www.seedbiology.de/dormancy.asp

Exogenous or coat imposed dormancy

Physical dormancy (PY):

- physical growth constraint;
- limited permeability to water and oxygen
- limited release of inhibitors from endosperm and seed coat

Endogenous or embryo dormancy

- Morphological dormancy (MD) – under-developed or immatured embryo;
- Physiological dormancy (PD) – physiological inhibition
- Morphophysiological dormancy (MPD)

PY+PD = Combinational dormancy;

ND = Non dormant seeds

PD is the most abundant form in seeds of gymnosperms and all major angiosperm clades (~80% of plants).
PD is also the major form of dormancy in most seed model species, including *Arabidopsis thaliana*, *Helianthus annuus*, *Lactuca sativa*, *Lycopersicon esculentum*, *Nicotiana* spp., *Avena fatua*, and several cereals.

Dormancy maintenance and breaking

Seeds in a dormant state are characterized by

- Suspended growth and development.
- Lowered metabolic rate.
- **Resistance to adverse environmental conditions.**
- Ability to maintain viable for long periods of time.

Germination requires

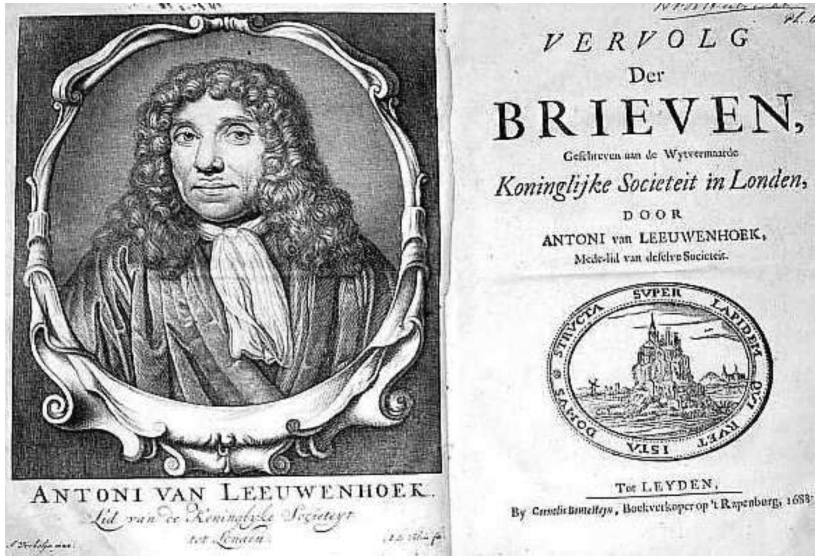
- Seeds require moisture and the right temperature to germinate.
- In addition, some seeds germinate only after dormancy breaking by:

(A) certain environmental signals
- ➢ Temperature (period of cold or heat) - stratification
- ➢ Disruption of the seed coat – scarification
- ➢ Chemical and physical agents
- ➢ Priming – short imbibition followed by drying;

(B) after ripening

What makes seeds resistant to environmental conditions?

- Dessicated seeds enter anhydrobiotic state: water content less than 0.1g H_2O/g dry weight
- **Anhydrobiosis** is a cryptobiotic (or suspended animation) state: metabolic processes are nearly halted;
- Due to the lack of water and stimulated synthesis of carbohydrates (sucrose or trehalose) cell cytoplasm acquires *glassy state* or "*vitrificates*"
- Anhydrobiosis is common among bacteria;
- Dry yeasts are in anhydrobiotic state;
- In animal kingdom only rotifers, water bears (*Tardigrade*) and nematodes can enter such state;
- Seeds and dessication tolerant plants



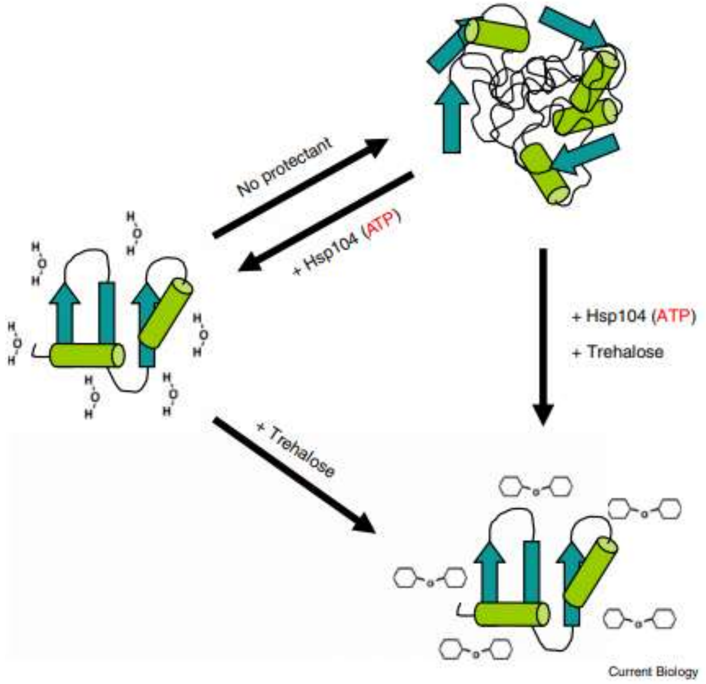
Antoni van Leewenhoek, who hirst described anhydrobiosis of rotifers

Normal and anhydrobiotic state of Water bear (*Tardigrade*)

Anhydrobiotic state affords high resistance

Resistance to environmental factors:

- Heating;
- Freezing;
- Pressure;
- Anoxia;
- Vacuum;
- Ionising radiation;
- Organic solvents



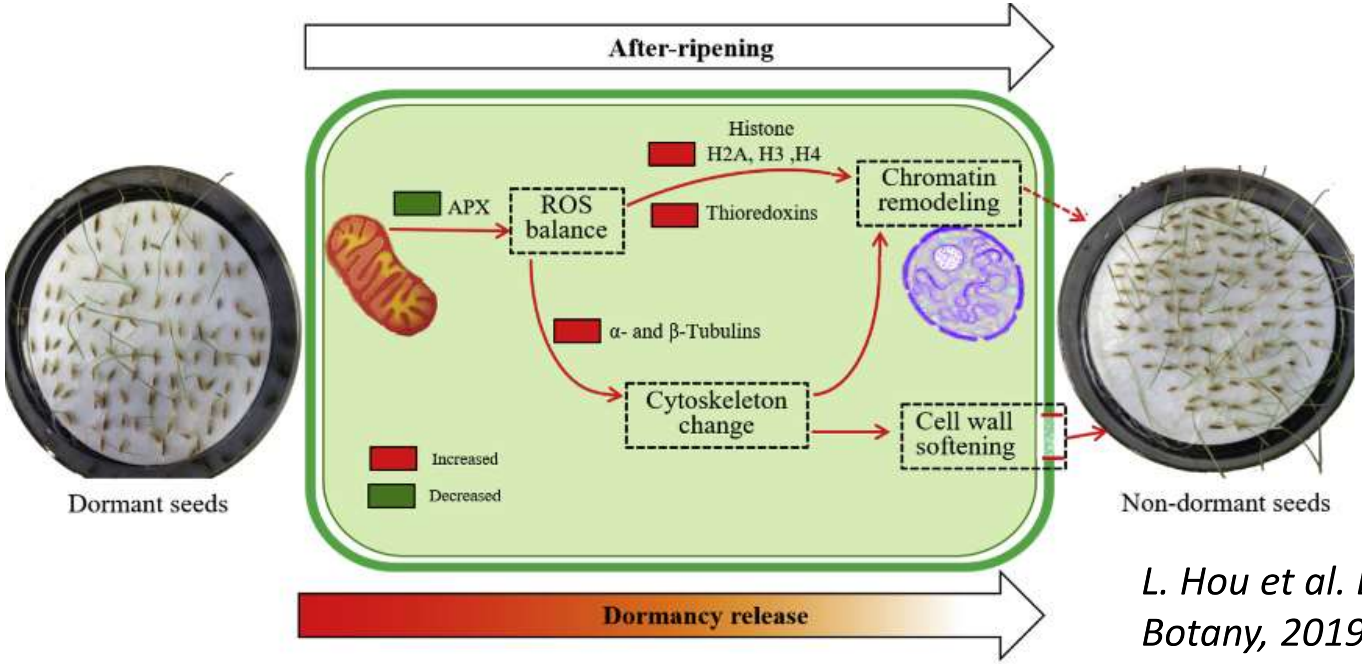
Reasons:

- In a glassy state, the most labile biopolimers such as proteins, RNA, or membranes are stabilized due to formation of hydrogen bonds with sugars instead of water;
- Supplemental protection is provided by cryoprotector proteins (LEA proteins and molecular chaperones);
- Slow metabolism leads to reduced ROS production.

Morano K.A. Anhydrobiosis: Drying Out with Sugar. Current Biology, 2014, 24, R1121-R1123.

The natural breaking of seed dormancy is called after-ripening:

transition between dormancy and germination leads to dormancy release and promotion of germination which occurs during seed storage after harwest. Dessicated seeds slowly undergo dormancy alleviation and acquire “germination competent state”.



L. Hou et al. Environmental and Experimental Botany, 2019, 162, 95-102.

Seed after-ripening is characterized by change in the expression of >400 genes, resulting in:

A decrease in ABA level and sensitivity and an increase in GA sensitivity or loss of GA requirement.

A loss of light-requirement for germination in seeds that do not germinate in darkness.

An increase in seed sensitivity to light in seeds that do not germinate even with light.

An increase of germination velocity.

Promotion of coat rupture and endosperm rupture.

For artificial dormancy breaking environmental dormancy breaking agents are used:

Temperature:
- Alternating temperatures (cold/warm cycles);
- Chilling (cold stratification);
- Warming (warm stratification);

Light:
- Alternating light (light/dark cycles);
- Single doses of light;
- Laser light

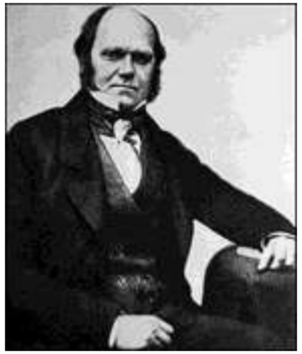
Chemicals:
- Smoke (fire, NO, butenolide)
- Inorganic;
- Organic (including allelochemicals).

Scarification:
- Mechanical;
- Chemical
- Enzymatic
- Percussion?

Ultrasound

High atmospheric pressure/Vacuum

Radiation:
- infrared or gas plasma (glow discharge, etc.) radiation,
- Low temperature plasma
- radio frequencies and ultrahigh frequency (microwave) electromagnetic fields,



Charles Darwin was the first scientist who described positive effects of the pre-sowing seed treatment with salt solutions http://www.npr.org/templates/story/story.php?storyId=6105541

The Encyclopedia of Seeds– Science, Technology and Uses
J. Derek Bewley,Michael Black,Peter Halmer, eds, CABI, 2006

Carol C. Baskin,Jerry M. Baskin. **Seeds– Ecology, Biogeography, and, Evolution of Dormancy and Germination, Elsievier, 2014 , 2nd ed.**

Eustress or distress effects are strongly dependent on species and dose

Germination - the emergence of a new plant from a seed. It starts with water uptake by the dry seed and is complete when the elongating radicle traverses the seed coat.

Emergence of the embryo, usually a radicle (embryonic root) is called *sensu stricto* germination.

Factors affecting germination

- **Water -** germination occurs when the seed absorbs enough water to rupture the seed coat.
- Rehydration of tissues - dilution of inhibitors

Hydration of a seed, which is called imbibition (*imbibere* in Latin means *to drink*), is an essential step for seed germination.

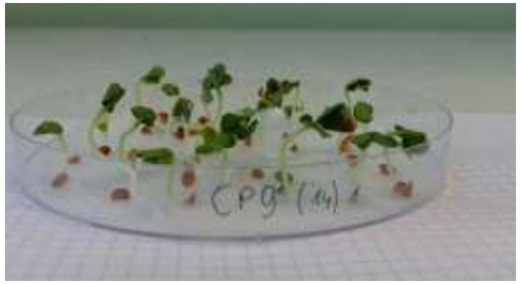
- **Oxygen**
  Aerobic respiration
- **Warmth**
  Enzyme controlled processes
- Some seeds may require exposure to light or high temperatures (fire).

Germination leads to the elongation of the embryonic axis from a seed, allowing subsequent seedling emergence. Elongation of emerged roots and seedling development are referred to as post-germination events

Concluding remarks for part I

- Despite small size, seeds are very complex and dynamic biological systems;
- Every seed undergoes different stages of development: embryo growth, seed filling, dessication, dormancy, after ripening, germination;
- In recent years there has been noticeable progress in understanding of the physiological and molecular mechanisms that regulate seed germination.
- Seed germination is governed by complex interplay between hormonal pathways determining germination and ROS metabolism, and many details have not yet been clarified.
- Knowledge on seed physiology is an ultimate pre-requisity for understanding the effects of plasma or other stressors on seed germination and plant development.

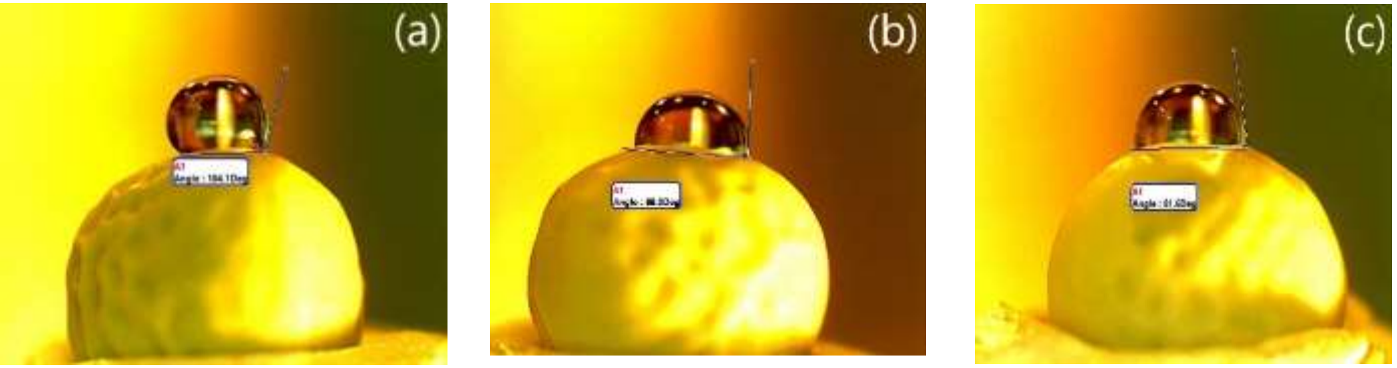
One of the applications of cold plasma (CP) in agriculture – pre-sowing seed treatment



CP treatments increase seed quality due to:

- (1) microbial decontamination of seeds;
- (2) stimulation of seed germination.

The majority of studies were limited by effects on seed surface (wettability), seed germination and early seedling growth



Positive effects on seed decontamination, germination and seedling growth are regarded as evidence for the usefulness of plasma application in agriculture

Since 2014 we started research on seed treatment with cold plasma (CP, duration 2-7 min), vacuum (5-7 min) and electromagnetic field (EMF, duration 5-15 min)

- **We performed long time observations of treatment effects on plant growth in the field that lasted from 2 to 6 years for perennials:**

  Norway spruce, *Picea abies*;

  Purple coneflower, *Echinacea purpurea* (L.) Moench;

  Red clover, *Trifolium pratense* L.;

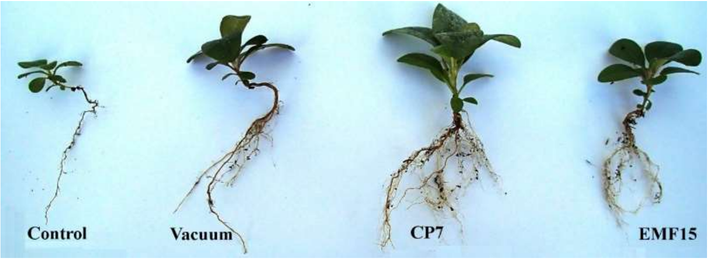
  Smirnov's rhododendron, *Rhododendron smirnowii* Trautv.

or 4-5 months for annual plants:

  Common buckwheat, *Fagopyrum esculentum*

  Hemp, *Cannabis sativa L.*

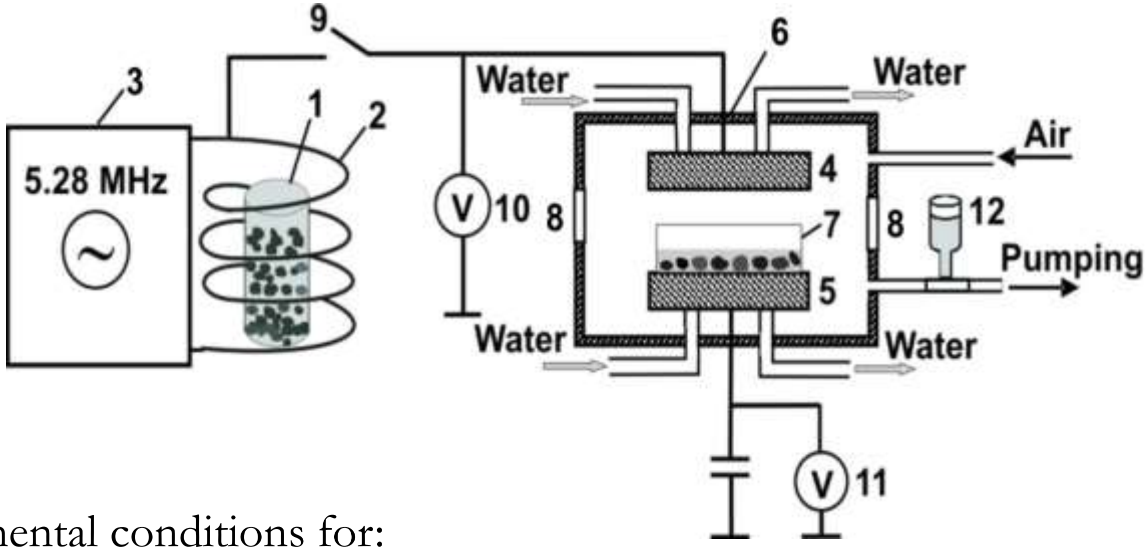
R. Smirnowii **- 1 year and 5 years old**



Methods

Experimental setup for seed treatment with RF EMF and CP – capacitively coupled plasma

in B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus:



Experimental conditions for:

EMF treatment

➢**Atmospheric pressure**

➢**H = 590 A/m; E = 12,7 kV/m**

➢**Inductor 5.28 MHz**

➢**Exposure – 5, 10, 15 min**

CP treatment:

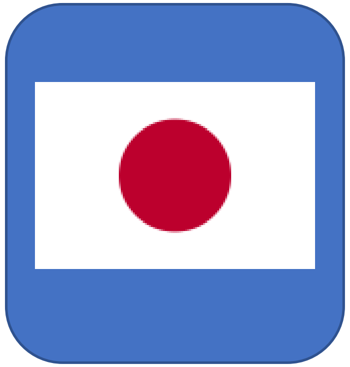
➢**5.28 MHz air plasma,**

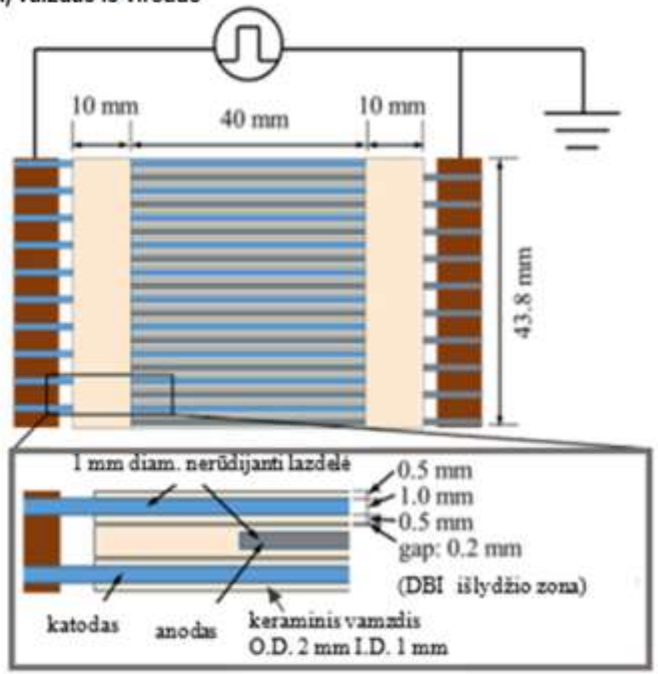
➢**gas pressure – 40 Pa**

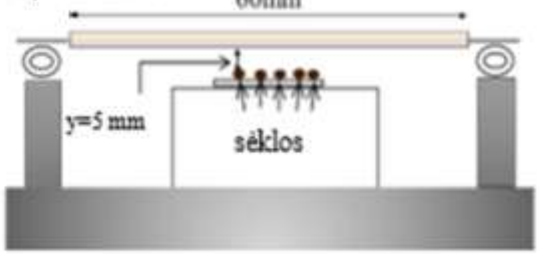
➢**the specific RF power – 0.1 - 0.6 W/cm3**

➢**exposure – 2, 5, 7 min**

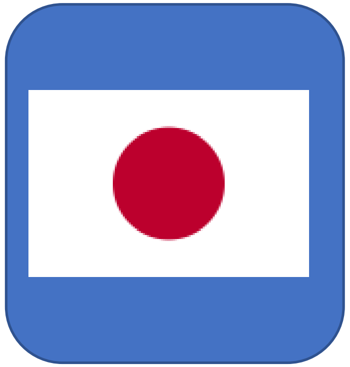
Atmospheric DBD (*dielectric barrier discharge)* plasma device was installed in Nov2017 in Kaunas by prof. M. Shiratani from Kyushu universitety (Japan)





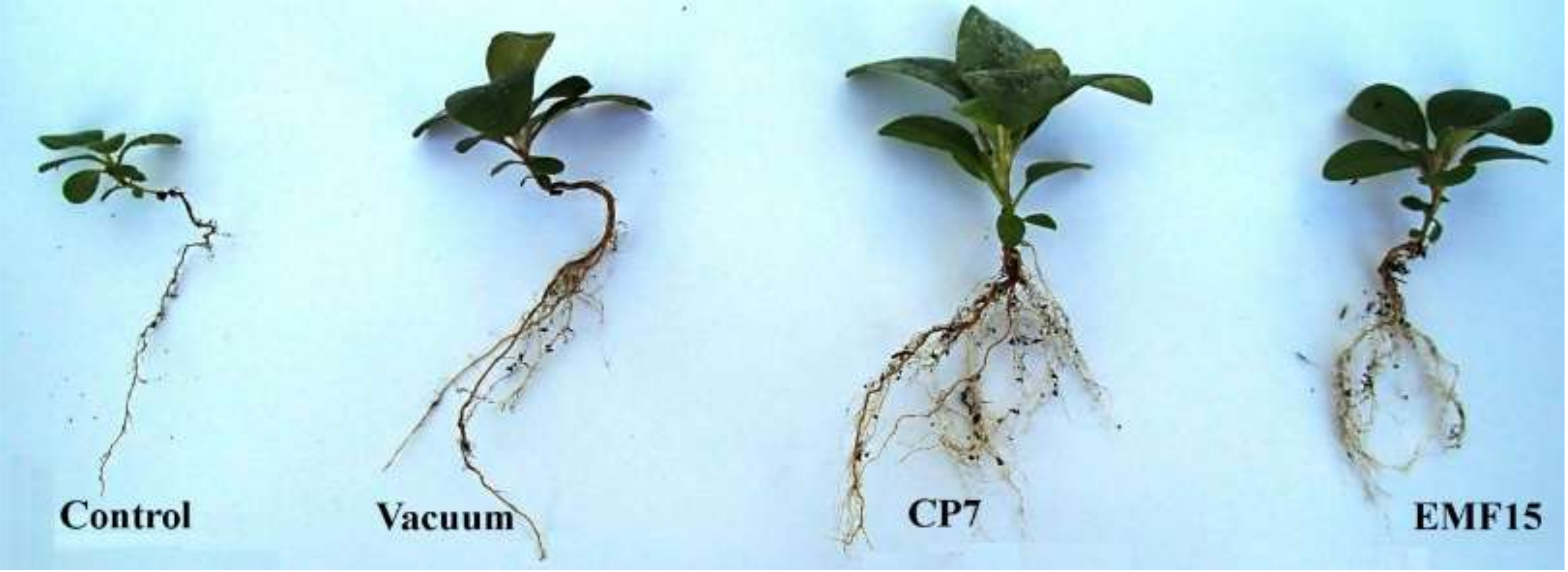


Capacitively coupled low pressure plasma device with controllable gaseous phase was installed in Kaunas by Japanese partners in 2020, March 4-6: just in time...



VGTU, Dr. V. Novickij EMF treatment device

Smirnov's rhododendron, *Rhododendron smirnowii* Trautv., 13 months after sowing



The experiment on Norway spruce started in 2014 and still continues

The size of collection – 400 seedlings…

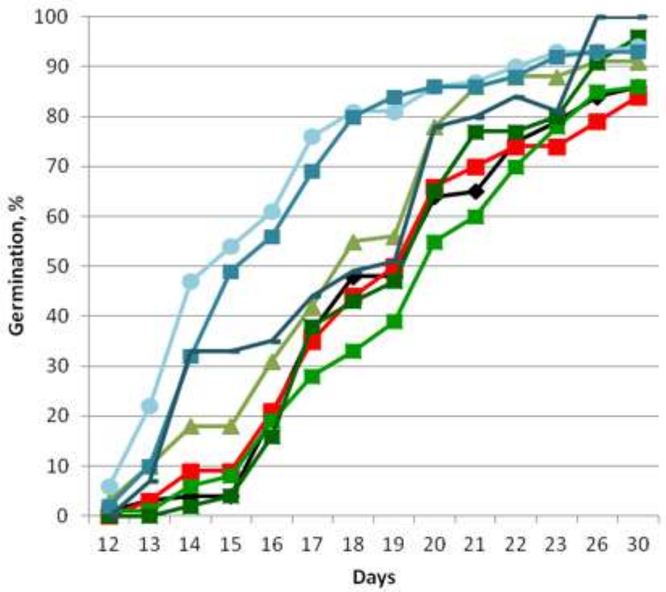
2014

2019

In March, 2020 this collection was transferred from pots to the arboretum of the Lithuanian institute of Forestry for further observations

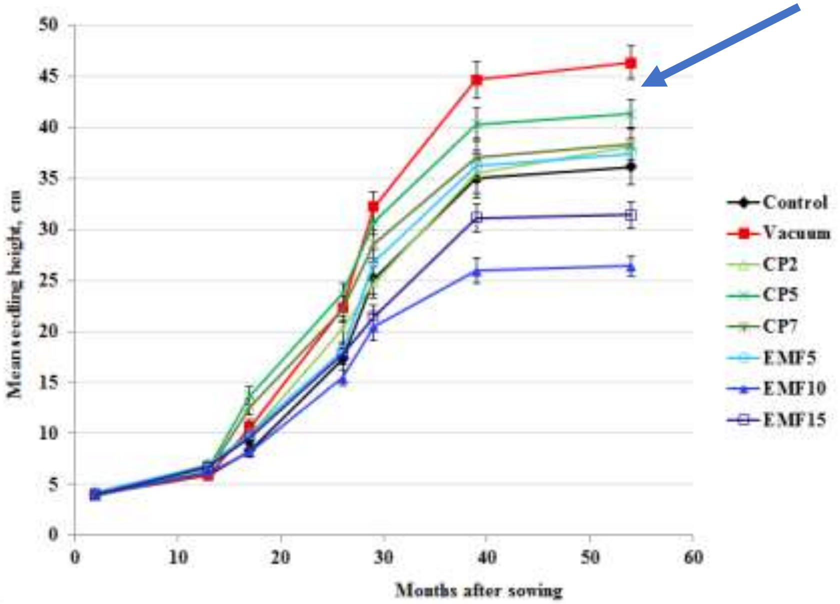
How long the effects of seed treatment (2-15 min) on plant growth can persist?

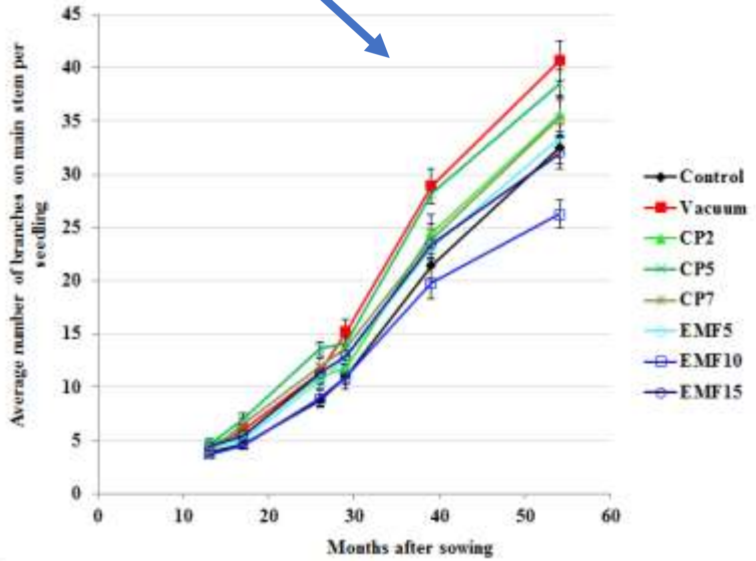
At least 5 years after sowing!!!



Effects on seed germination in 2014

Effects on seedling height and number of branches (2014-2020)





What effects on agro-production yield (harvest) can be achieved by seed processing (without use of chemical fertilizers)?

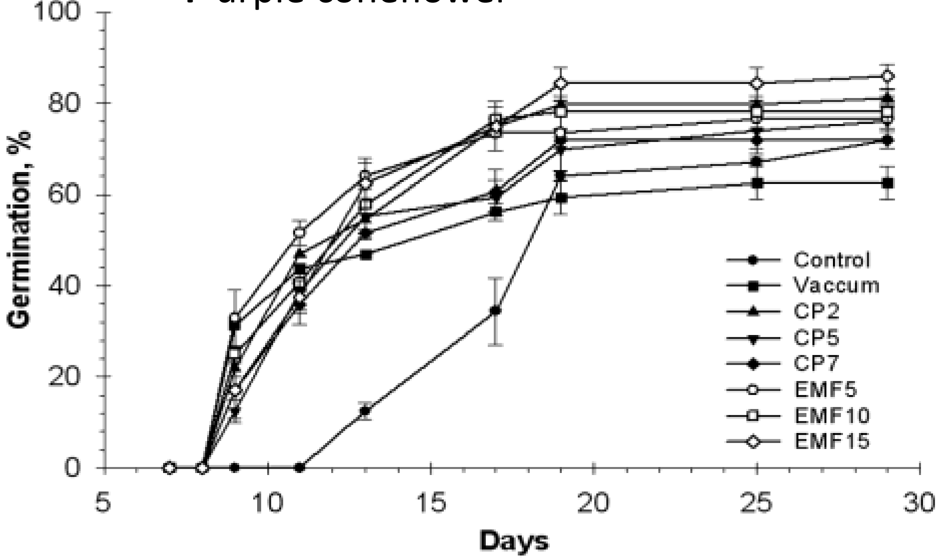
- **Red clover:** biomass production increased up to 49% /grown in the field for 5 months/
- **Buckwheat:** strong positive effect on biomass (up to 97%) and on the seed yield (up to 85%) /grown in the field for 4 months/
- **Industrial hemp:** In female plants, EMF treatment induced positive changes in weight of the above ground part (66%) and number of inflorescences (70%). CP 5 min. treatment decreased the average weight of female plants by 27% but increased the weight of male plants 1.4-fold. /grown in the field for 4 months/

Dr. Laima Degutytė-Fomins,
Dr. Giedre Paužaitė Maskeliūnienė,
Dr. Danuta Romanovska

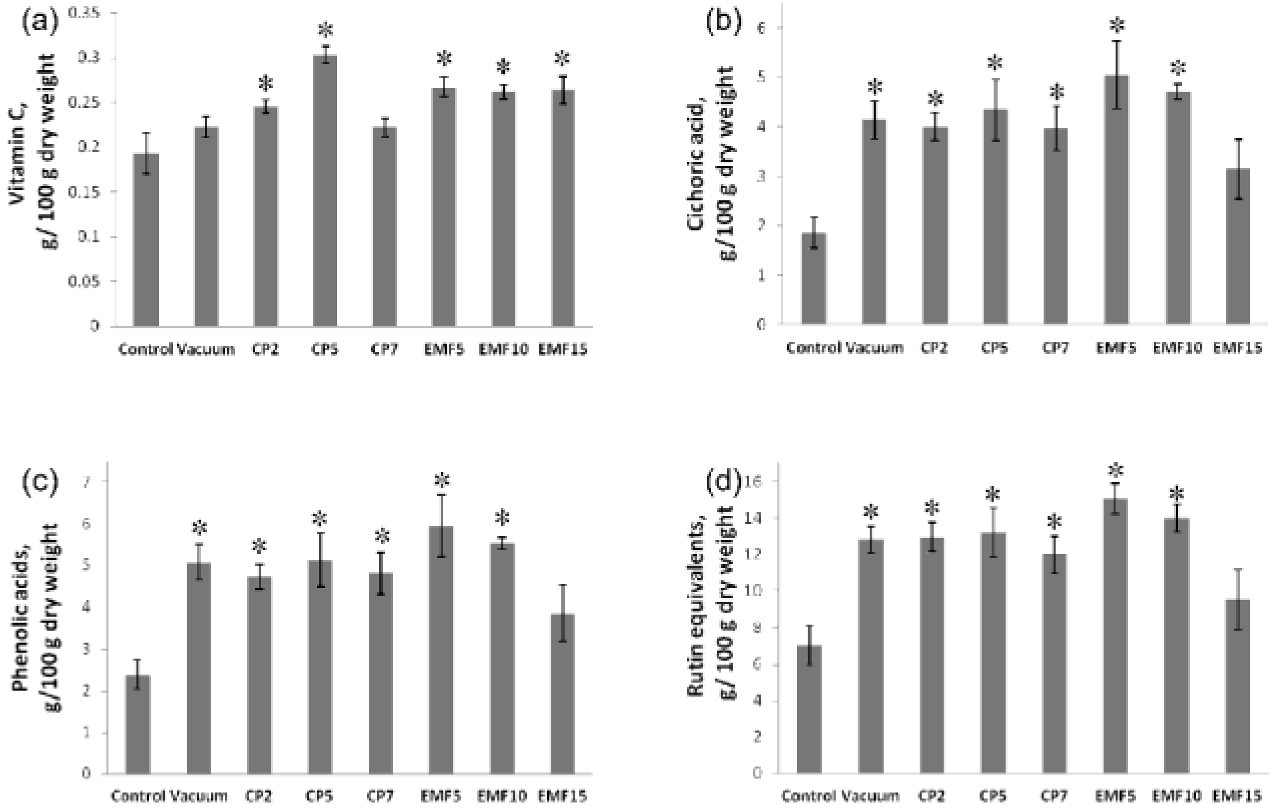
E.purpurea:

Pre-sowing seed treatment with EMF and CP induced increase in the amount of secondary metabolites, vitamin C and radical scavenging activity in plant leaves

Purple coneflower



CP, vacuum and EMF effects on germination



CP, vacuum and EMF effects on the leaf content of vitamin C, phenolic acids, and antioxidant activity

Why changes induced in the secondary metabolism are important?

Secondary metabolites (SM) are:

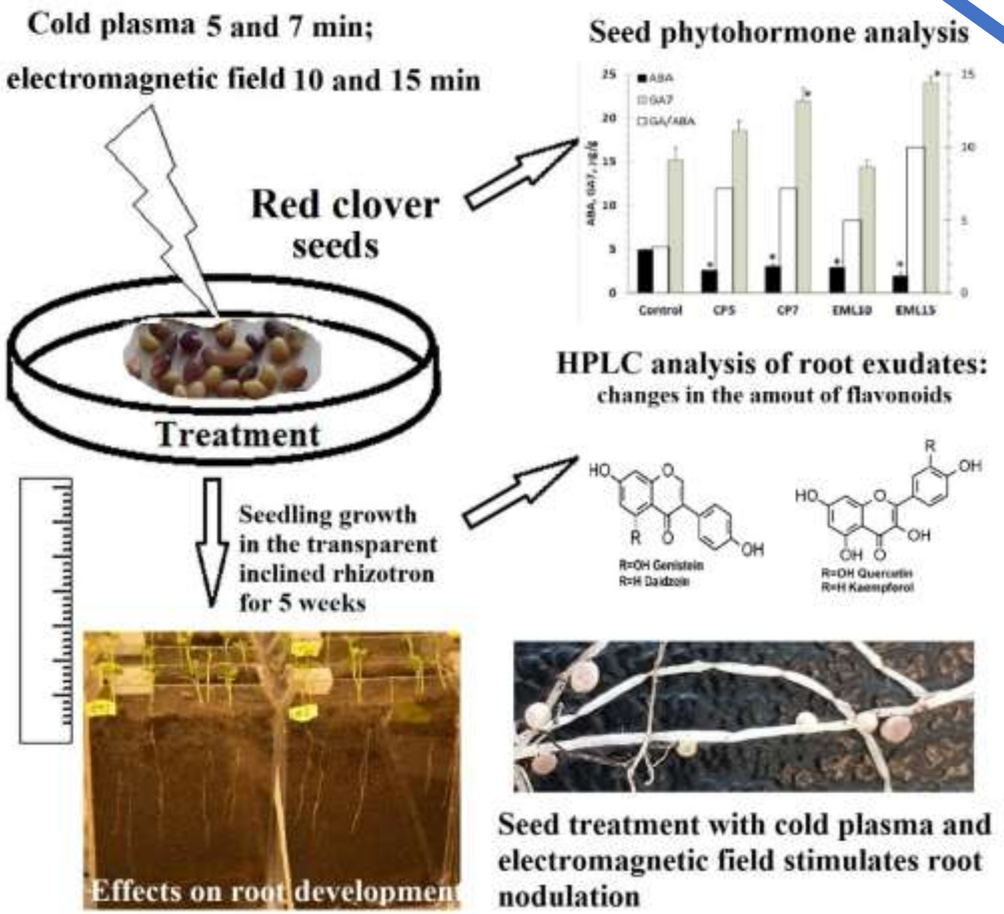
- important for plant stress response, fitness and protection mechanisms, determine plant resistance to biotic and abiotic stress including diseases;
- means of plant communication with microorganisms, including N-fixating soil rhizobacteria;
- biologically active substances with numerous beneficial effects on health of animals consuming plants for food, including humans.

Glossary:

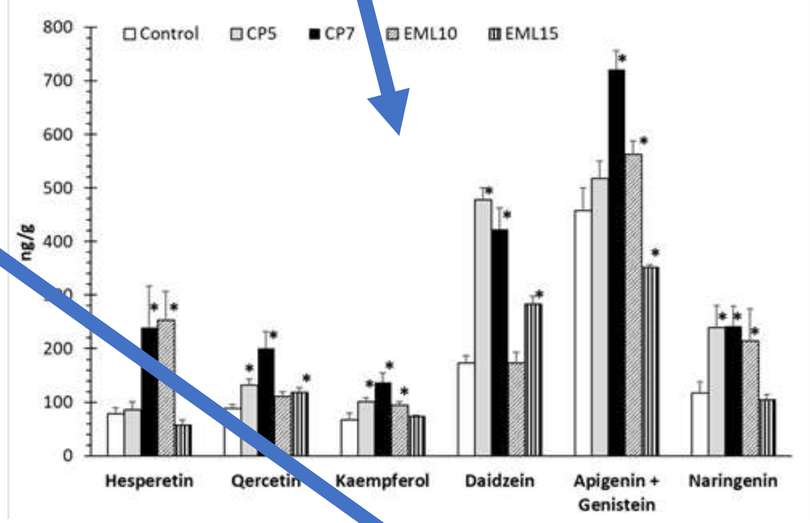
SMs: *The term "secondary" implies that while primary metabolites are present in every living cell capable of dividing, the SM are present only incidentally and are not of paramount significance for organism's life. Though SMs are derived from primary metabolism, they do not make up basic molecular skeleton of the organism. SMs absence does not immediately curtail the life of an organism, a feature contrary to primary metabolite, but survival of the organism is impaired to a larger extent.*

Root nodules: structures on the roots of plants (primarily legumes) formed by plant and symbiotic nitrogen fixing bacteria.

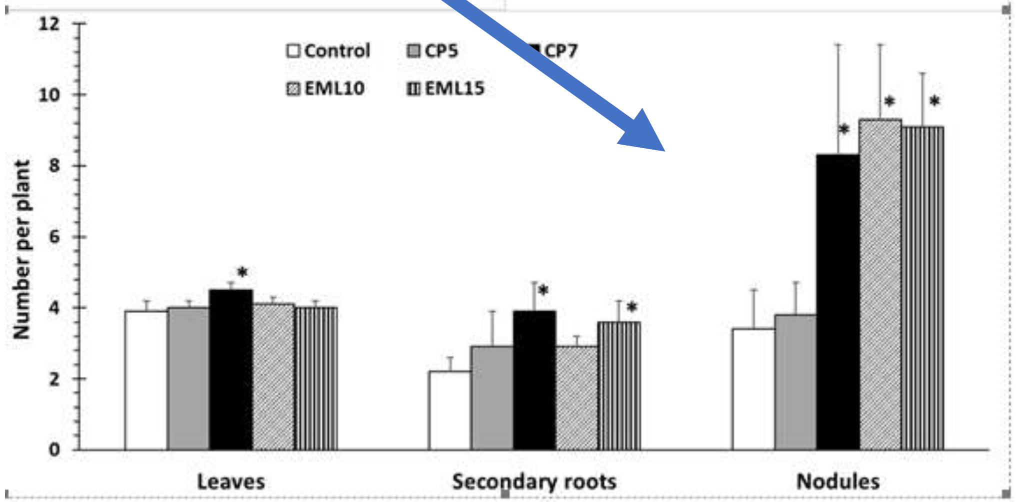
Pre-sowing seed treatments increased the amount of isoflavonoids in root exudates and the number of nodules in red clover roots



Mildažienė V., et al. Seed treatment with cold plasma and electromagnetic field induces changes in red clover root growth dynamics, flavonoid exudation, and activates nodulation. Plasma Proc. Polym. 2020, 18(2), 2000160



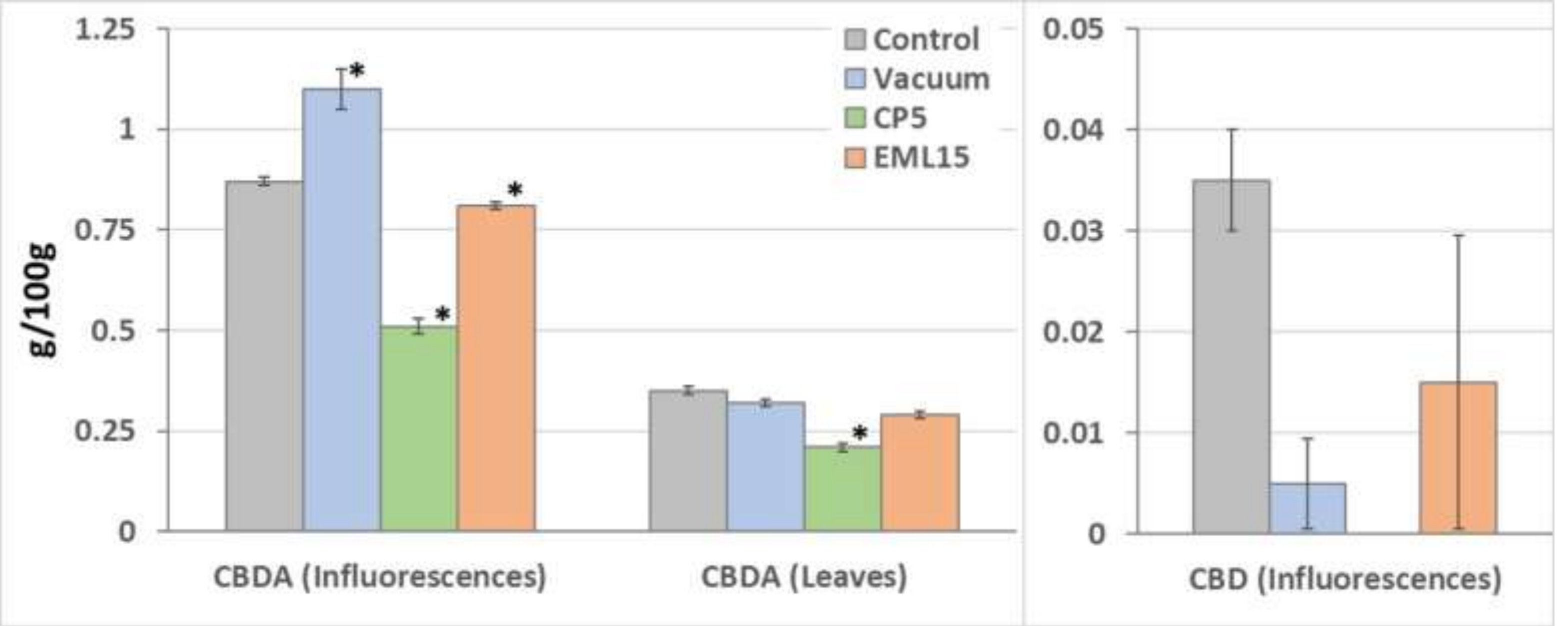
CP7 increased the number of nodules 2.4 times, EMF – 2.7 times:



Stimulation of nodulation ≈ plant N-fertilisation → reduced need for chemical fertilisers

Increased production of biologically active metabolites
Changes induced in the amounts of non-psychotropic cannabinoids CBDA and CBD in industrial hemp

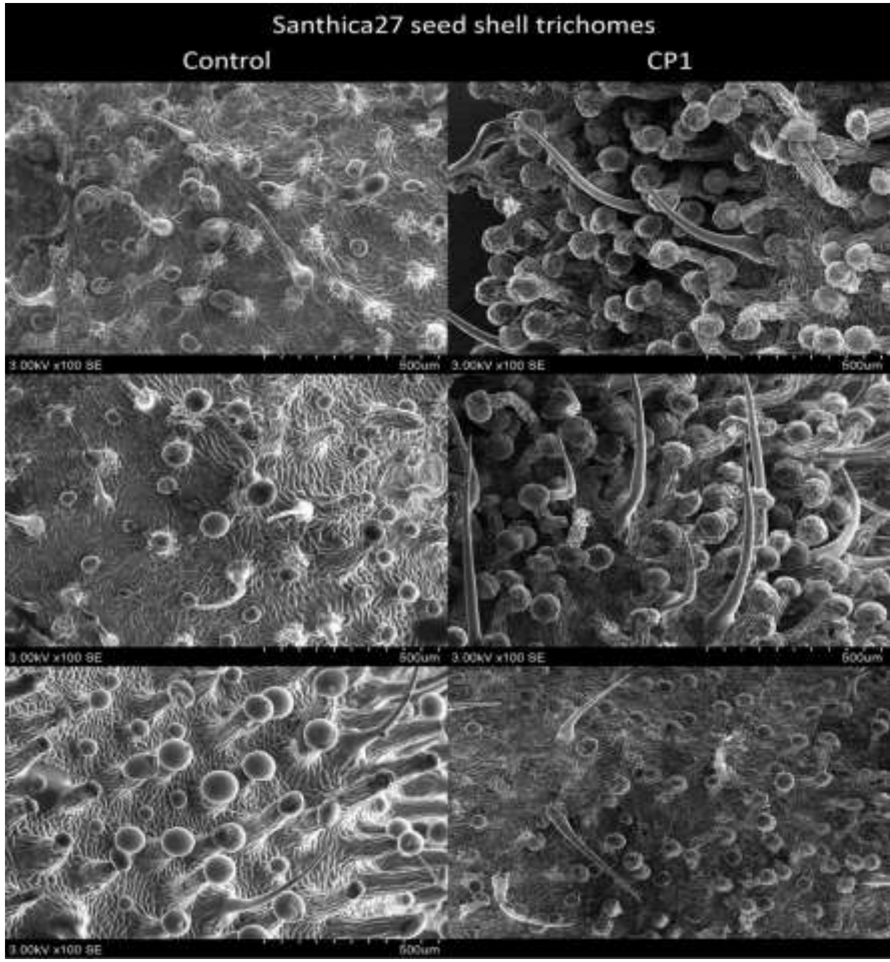
The amounts of CBDA and CBD in hemp female plant leaves and influorescences (g/100g)



Vacuum treatment increased the amount of CBDA in the inflorescences by 27%.
The amount of CBDA in inflorescences was decreased by CP5 (-41%) and EMF15 (7%).

Glossary:
Trichomes: fine structures or appendages on different plant surfaces. Certain protective SMs are synthesized or accumulated in trichomes.

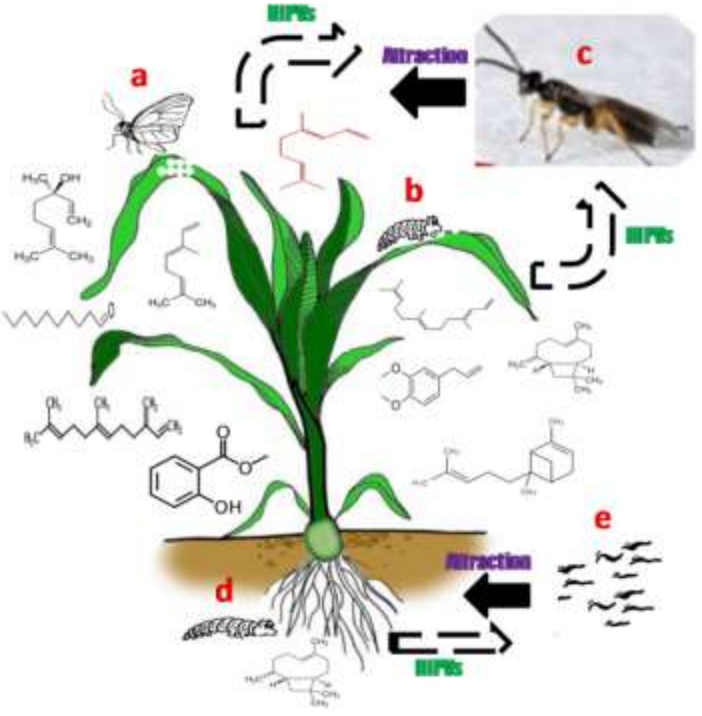
Hemp trichomes (on SEM pictures):



The density of staminate globular trichomes increased more than twice in CP1 group compared with control

Conclussion: seed treatment effects behind germination are of importance for sustainable agriculture

- **The effects of seed treatments on plant growth persist for the long time and have complex dynamics. Long-term observations are required to estimate the response of plants to seed treatment with physical stressors.**
- **Seed treatments for few minutes can substantially increase plant biomass and seed harvest – relevant for agro-production;**
- **Seed treatment induced stress is followed by the long lasting changes in secondary plant metabolism - relevant for production of medicines and for better plant fitness including resistance to pathogens;**
- **Due to the induced changes composition of root exudations seed treatments can activate nodulation in legume roots, and that results in stimulated N-fixation. That has potential for reducing the need for chemical fertilizers – relevant for sustainable agriculture;**
- **Certain secondary metabolites function as feeding deterrents for herbivores, therefore such treatments have potential to be used for reducing the need for pesticides.**

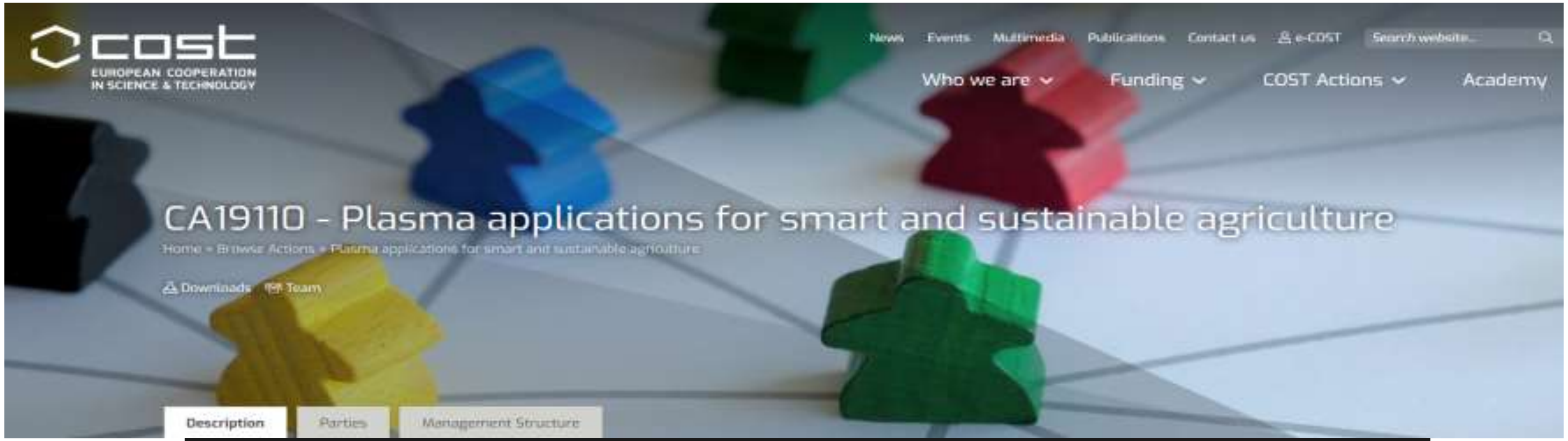


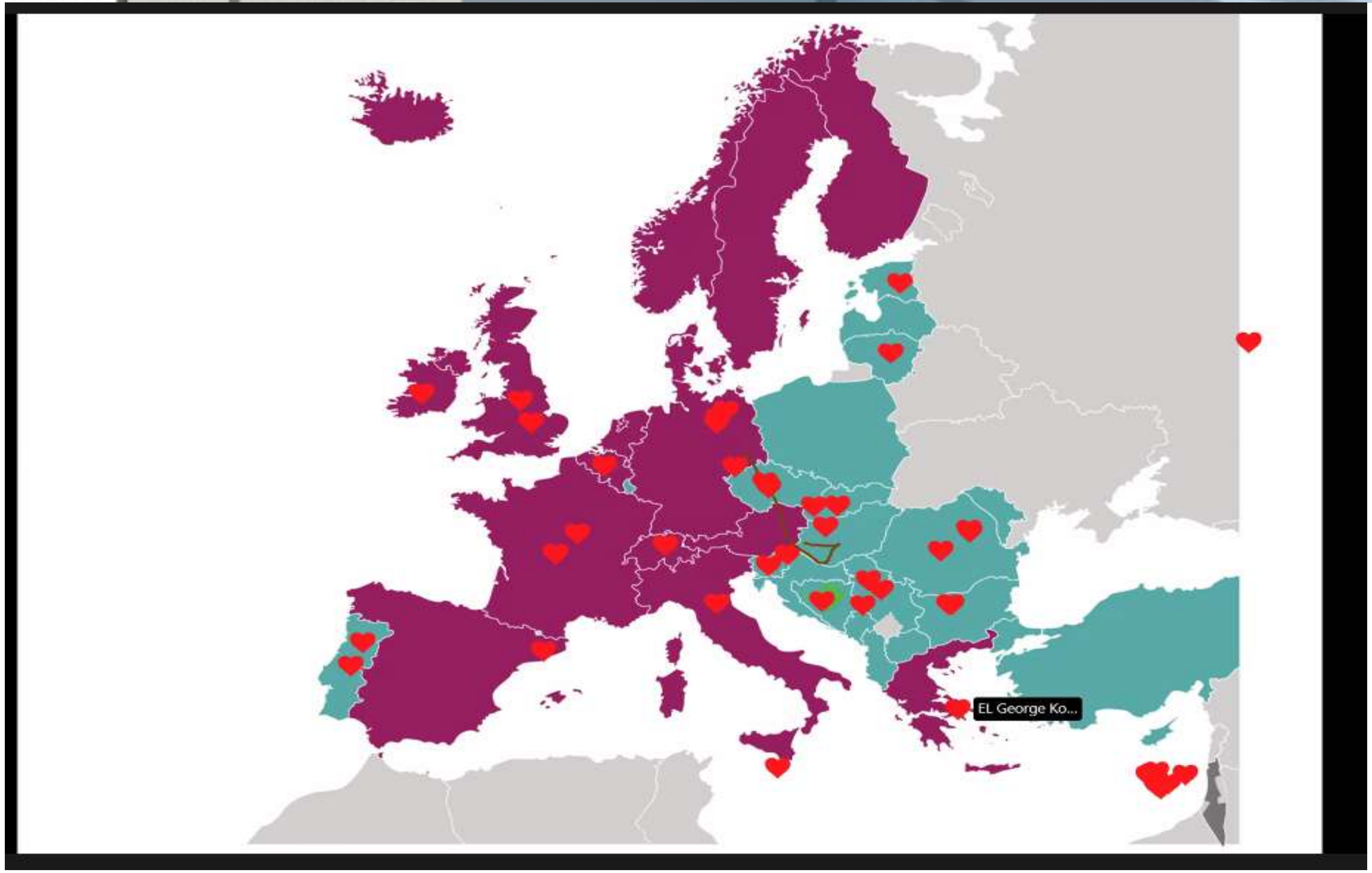
Tamiru and Z. R. Khan. Volatile Semiochemical Mediated Plant Defense in Cereals: A Novel Strategy for Crop Protection. Agronomy 2017, 7, 58

https://www.paulwagner.com/tag/highly-sensitive-person/

SUPER POWERS OF SEEDS: highly resistant // very sensitive and responsive!!!

- Due to anhydrobiotic state seeds are resistant to environmental factors. That results in seed longevity and is of key importance for plant reproduction and survival.
- Plants have developed universal molecular mechanisms in seeds for: (i) sensing the environmental changes that could be dangerous for the survival of seedlings, as well as for (ii) responding to such signals by mobilizing internal resources and the defensive potential, leading to an improved plant fitness and competitiveness (stimulated growth, defence and reproduction).
- The complexity of such response is only beginning to be understood, and detailed knowledge of these mechanisms needs to be gained to apply them in the development of innovative technologies in sustainable agriculture





Start of Action
06/10/2020

Partners
27 EU countries

Aknowledgements

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Thank you for attention!!!