Applied potential of cold plasmas for seed processing

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CA19110
Plasma applications for smart and sustainable agriculture

IAEA
Atoms for Peace and Development

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What is seed?

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

- "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."**

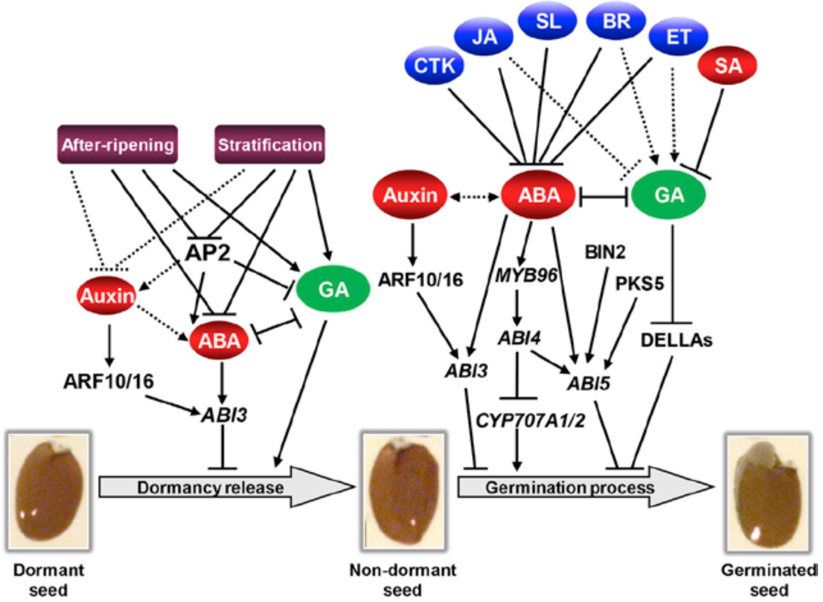
Lorence O. Copeland, Miller M. McDonald, Principles of seed science and technology, 4th Edition, 2001, Springer Science + business media, LLC

Obtaining high yields in agricultural production starts with planting seeds that germinate in high percentages and produce robust plants:

The key importance of seeds for agriculture

Seeds - complex and dynamic biological systems

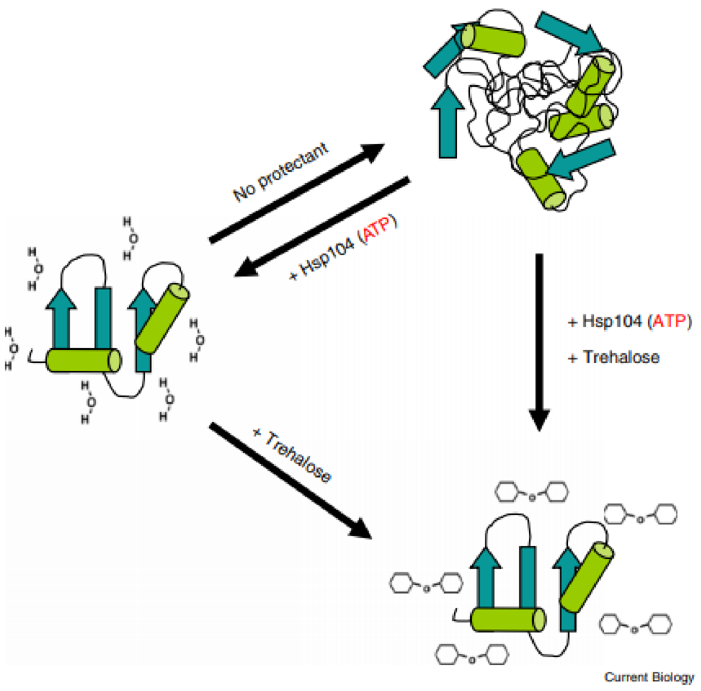
- Every seed undergoes different stages of development: embryo growth, seed filling, dessication, dormancy, after ripening, germination;
- In recent years there has been noticeable progress in understanding of the physiological and molecular mechanisms that regulate seed germination.
- Seed germination is governed by complex interplay between phytohormonal pathways determining germination and ROS metabolism. Many details in a sequence of events have not yet been clarified.
- Knowledge on seed physiology and biochemistry is an ultimate pre-requisity for understanding the effects of plasma or other stressors on seed germination and plant development.



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.

Anhydrobiotic state (5-10% water) affords high resistance to environmental factors and aging:

- 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.

For stimulation of seed germination artificial dormancy breaking agents are used: physical, chemical, and biological (phytohormones, etc.)

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

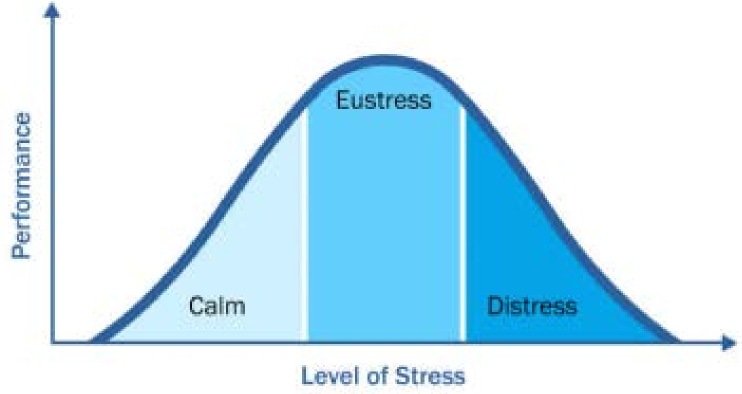
High atmospheric pressure/Vacuum

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

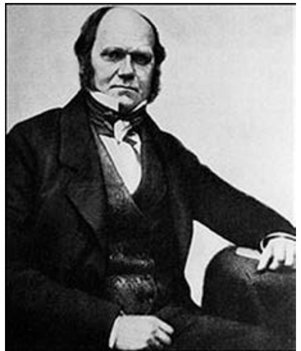
The Encyclopedia of Seeds– Science, Technology and Uses
J. Derek Bewley, Michael Black, Peter Halmer, eds, CABI, 2006

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 plant species and treatment dose



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 potential of seed irradiation with low temperature plasmas (cold plasmas, CP) for application in agriculture is under intensive investigation, as a green alternative to conventional chemicals (fertilizers, pesticides and herbicides).

Effects of seed irradiation with plasma:

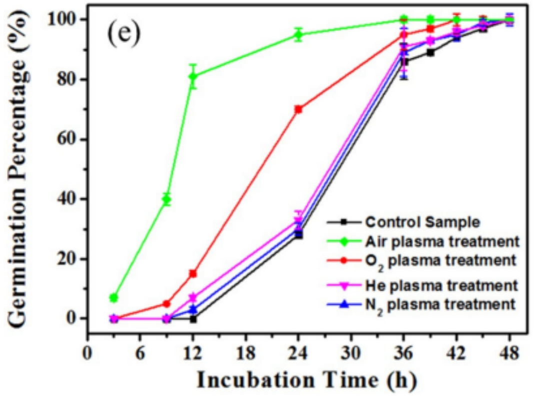
Seed

- ➢ Microbial seed decontamination
- ➢ Stimulated germination (rate and/or maximal germination)

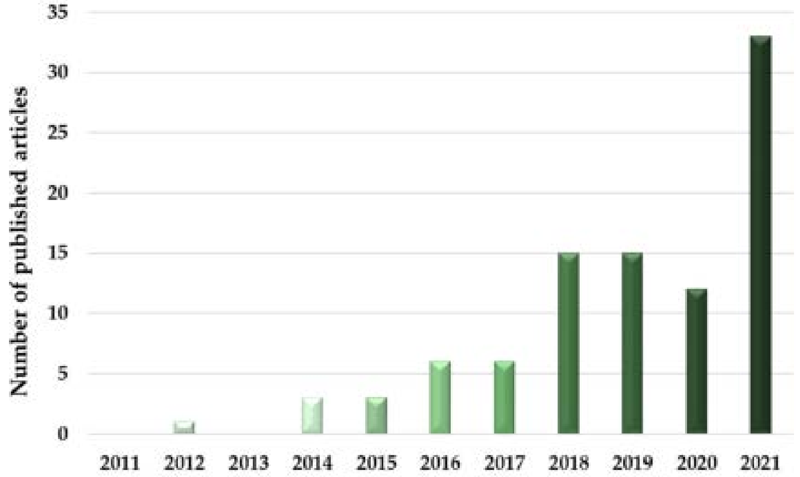
Plant

- ➢ Stimulated early growth of seedling (better seedling establishment)
- ➢ Changes in plant metabolism and physiological processes (photosynthesis
- ➢ Improved stress resistance
- ➢ Increased plant biomass gain
- ➢ Promoted seed production

Increased harvest



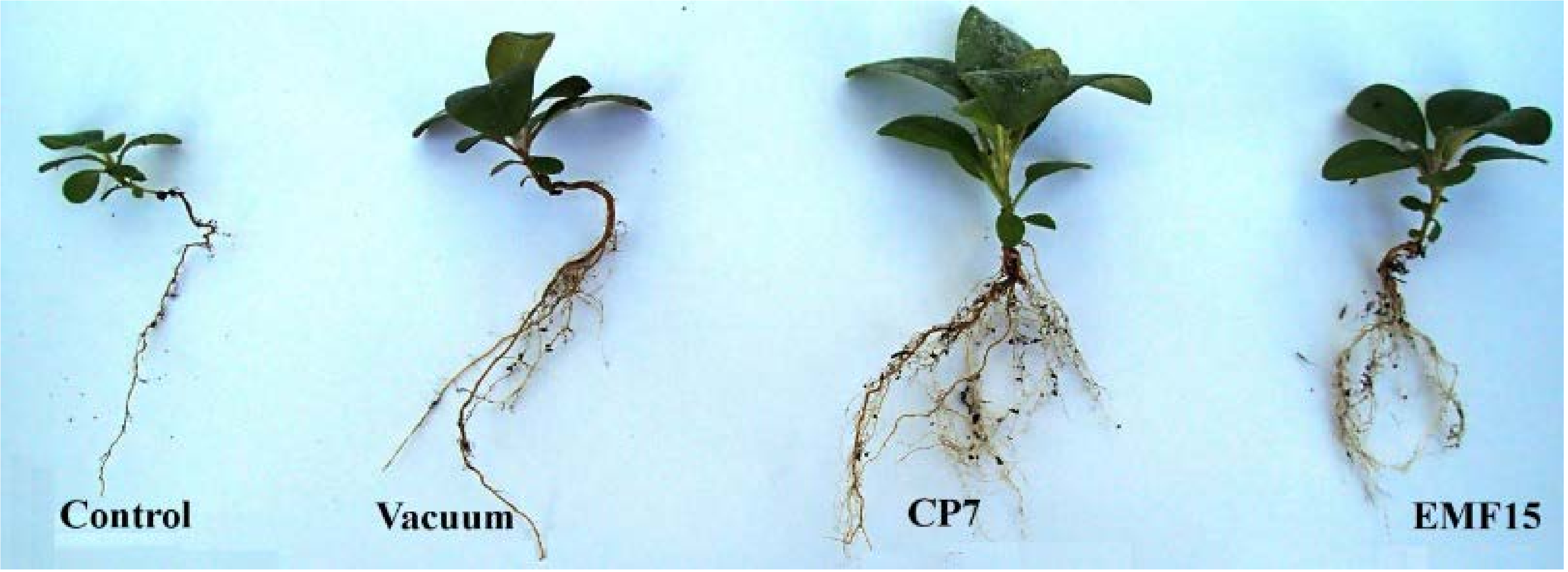
Yan et al.. Plasma 2022, 5, 98–110.



Leti et al. The Modulatory Effects of Non-Thermal Plasma on Seed's Morphology, Germination and Genetics—A Review. Plants 2022, 11, 2181.

First our results (in 2015):

Effects of seed treatments on growth of Smirnov's rhododendron, *Rhododendron smirnowii* Trautv.



Mildaziene et al. Response of Perennial Woody Plants to Seed Treatment by Electromagnetic Field and Low-Temperature Plasma // Bioelectromagnetics. 2016, 37, 536-548. DOI: 1002/bem.22003

The challenge for research

The complexity behind the interaction of biological systems with plasma

Plasma is a complex stressor

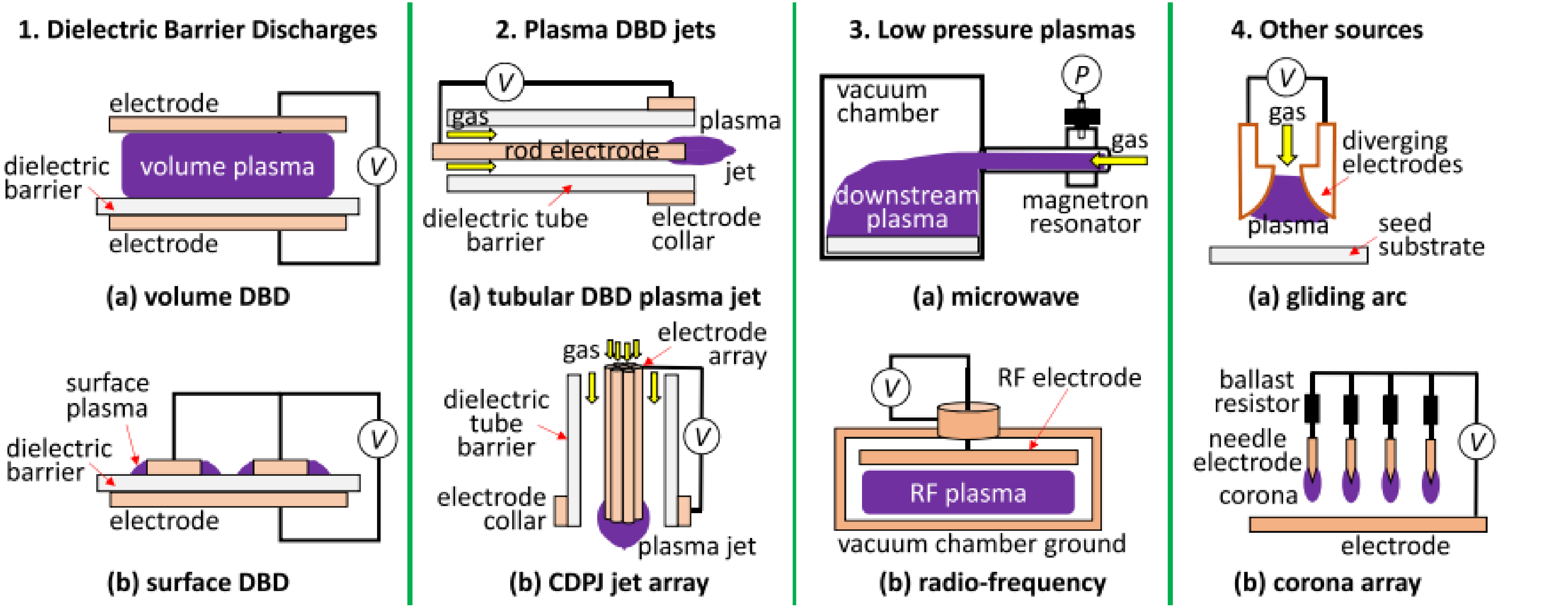
Different equipment

- Construction, geometry, energies and treatment exposure
- Aggressive particles: ROS species, ions, electrons, etc.
- UV
- Electrostatic/electromagnetic components of discharge
- Pressure (± vacuum)
- Temperature changes

Biological systems are complex

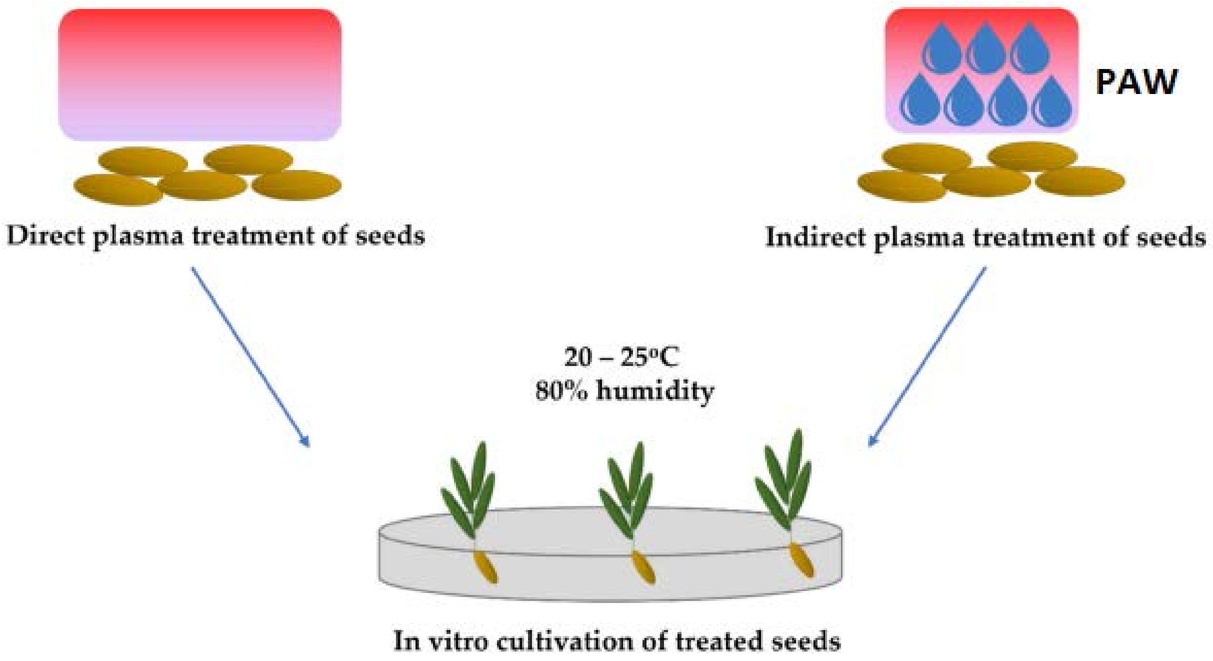
- Dependence on plant species
- Genetic polymorphism
- Hierarchical structure
- Physiological status, e.g. seed dormancy or seed/plant
- Dose dependent response: eustress/distress
- The molecular mechanisms for signal perception and transduction --- not yet established

Different plasma reactors are used for seed treatments and the research still has to be performed on standardizing the effective treatment conditions



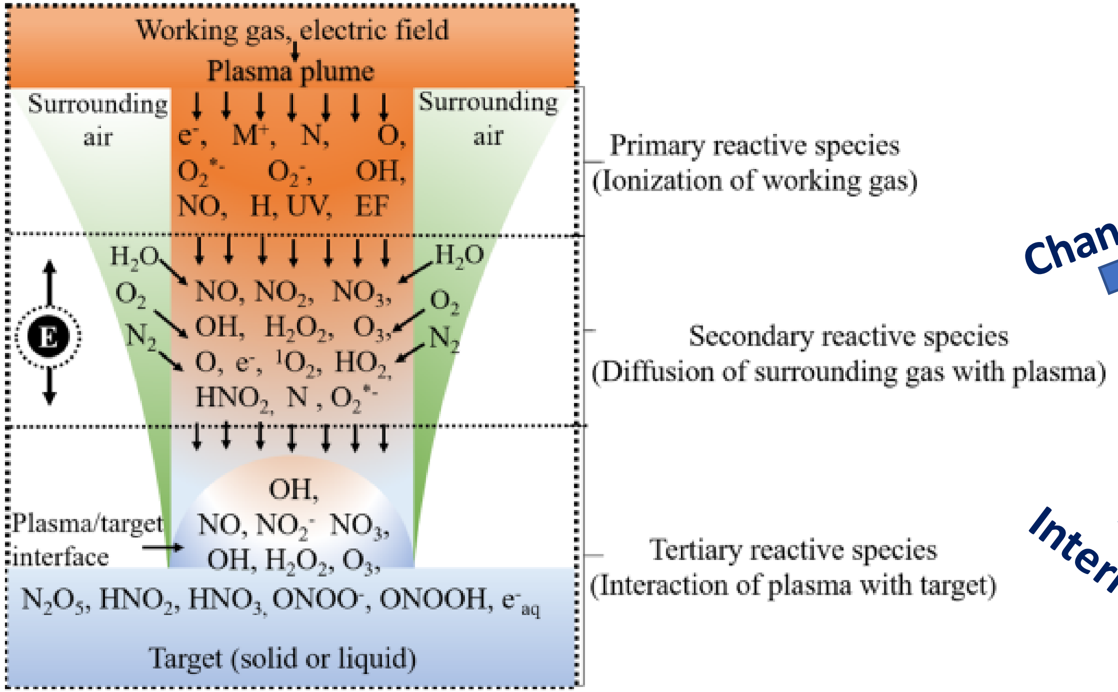
Waskov A. et al., Entering the plasma agriculture field: An attempt to standardize protocols for plasma treatment of seeds. Plasma Process Polym. 2021;e2100152 www.plasma-polymers.com | 1 of 22 https://doi.org/10.1002/ppap.202100152

Two modes of plasma treatment - direct and indirect (PAW, plasma activated water) can be applied for seeds

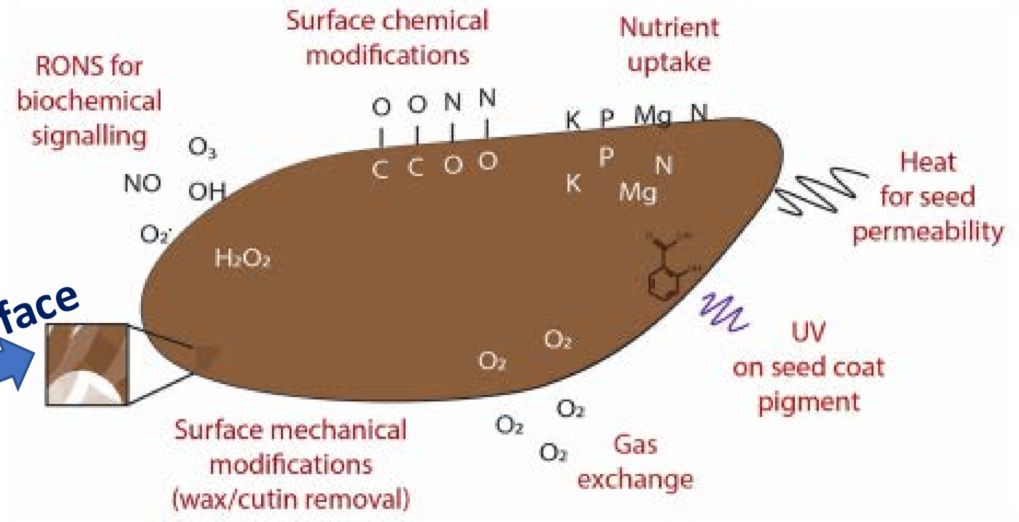


Leti et al. The Modulatory Effects of Non-Thermal Plasma on Seed's Morphology, Germination and Genetics—A Review. Plants 2022, 11, 2181.

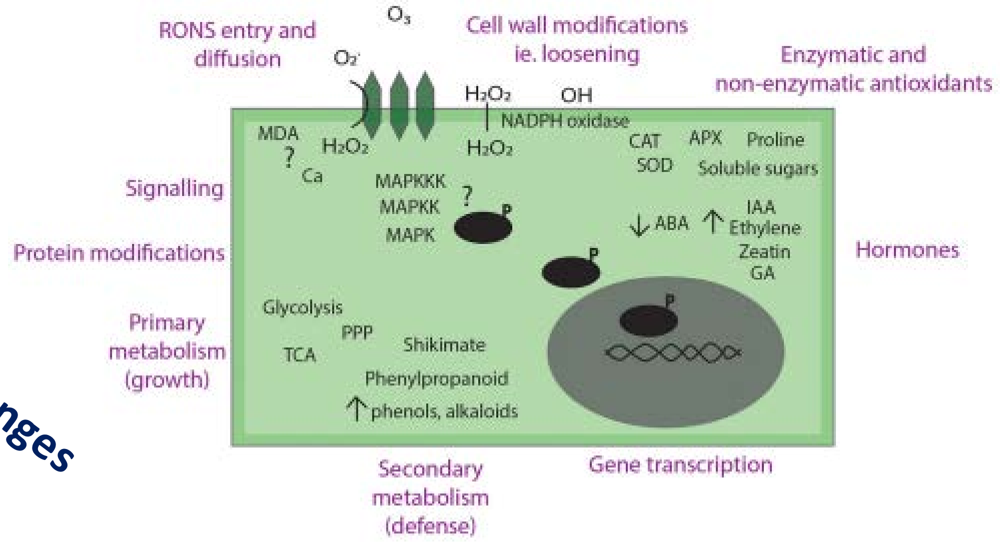
In both cases, it is considered that different reactive particles generated by plasma are responsible for the effects



Changes on seed surface



Internal molecular changes



Barjasteh, A. *et al.* Recent Progress of Non-thermal Atmospheric Pressure Plasma for Seed Germination and Plant Development: Current Scenario and Future Landscape. *J Plant Growth Regul* **42**, 5417–5432 (2023). https://doi.org/10.1007/s00344-023-10979-0

Waskow A, Howling A and Furno I (2021) Mechanisms of Plasma-Seed Treatments as a Potential Seed Processing Technology. Front. Phys. 9:617345. doi: 10.3389/fphy.2021.617345

Positive effects of seed treatments have been demonstrated for numerous (at least >60) plant species

- *Allium sativum*
- *Andrographis paniculata*
- *Arabidopsis thaliana*
- *Arachis hipogaea L.*
- *Astragalus fridae*
- *Avena sativa*
- *Betula pendula Roth*
- *Brassicaceae*
- *Brassica juncea L.*
- *Brasicca napus L.*
- *Cannabis sativa L.*
- *Capsicum annuum*
- *Carthamus tinctorium L.*
- *Catharanthus roseus*
- *Cichorium intybus*
- *Chenopodium album agg.*
- *Chenopodium quinoa*
- *Coriandum sativum*
- *Cucumis melo*
- *Cucumis sativus*
- *Cucurbita maxima L*
- *Cynara scolimus L.*
- *Daucus carota sativus L.*
- *Echinacea purpurea*
- Eremochloa ophiuroides (Munro.) Hack.
- *Erythrina velutina*
- *Helianthus annuus*
- *Hordeum vulgare*
- *Glycine max L. Merr.*
- *Galega virginiana*
- *Gossypium spp.*
- *Fagopyrum esculentum*
- *Lavatera thuringiaca L.*
- *Lens culinaris*
- *Lupinus angustifolius*
- *Lycopersicon esculentum L.*
- *Melilotus albus*
- *Melissa officinalis*
- *Mimosa caesalpiniafolia*
- *Moringa oleifera*
- *Morus nigra*
- *Ocimum basilicum*
- *Oryza sativa L.* Phaseolus vulgaris L.
- Picea abies
- Pinus silvestris
- Pisum sativum
- Raphanus sativus
- *Rhododendron smirnowii* Trautv.
- Robinia pseudoacacia L.
- Salvia nemorosa L.
- *Stevia Reboudiana*
- Solanum lycopersicum
- Spinacia oleracea L.
- *Stevia Reboudiana*
- Trifolium pratense L.
- Trifolium alexandrinum L.
- Trigonella foenumgraecum
- Triticum aestivum
- Vigna radiate
- Vitis vinifera
- Zea mays L.
- Zoysia willd.

The effects are dependent on numerous biological factors:

- Plant species
- Seed dormancy
- Year of seed harvest
- Plant cultivar
- Plant genotype
- Seed color

TABLE 2 Summary of the changes induced by seed treatment with CP in seedlings of seven genetic Norway spruce families

	Growth		Chl $a+b$		Carotenoids		TPC		
Half-sibs family	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	Total points
457	+1[a] CP2	+1 CP2	0	+2	0	0	+2	−1 CP2	5
463	+1 CP1	0	−1 CP1	−1 CP2	−1 CP1	+1 CP2	+2	+2	3[b]
477	0	+1 CP2	+2	+1 CP2	+1 CP2	−1 CP1	+2	+1 CP1	7[b]
541	0	0	−1 CP1	0	−1 CP1	0	0	+2	0
548	0	0	+1 CP1	+1 CP2	+1 CP1	+2	0	+1 CP1	6
577	0	+1 CP2	0	0	0	−1 CP2	+2	0	2
599	+1 CP1	+1 CP1	0	0	0	0	+2	−1 CP2	2

Abbreviations: CP, cold plasma; TPC, total phenolic content.

[a]Positive effect is scored by +2 points if both CP1 and CP2 treatments were effective, and by +1 point if only one CP treatment duration (duration indicated next to the number) was effective; the absence of statistically significant effect—by 0 points, negative effect by −1 point when 1 CP duration (indicated in the corresponding line) was effective.

[b]Combination of the positive response of growth and increase in phenolics.

Sirgedaite-Šežiene, V. et al. Long-term response of ˙ Norway spruce to seed treatment with cold plasma: Dependence of the effects on the genotype. Plasma Processes Polym. 2021, 18, 2000159

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 'Futura 75' plants, 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/. Vacuum increased amount of non-psichotropic cannabinoid CBDA
- Effects on growth of common buckwheat and industrial hemp in 2020 were qualitatively **reproduced** using different CP and EMF equipment. DBD plasma stimulated growth of 'Santica 27' cultivar by 30%, but inibited growth of 'Futura 75' cultivar plants...

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

Why changes induced in secondary metabolism are important?

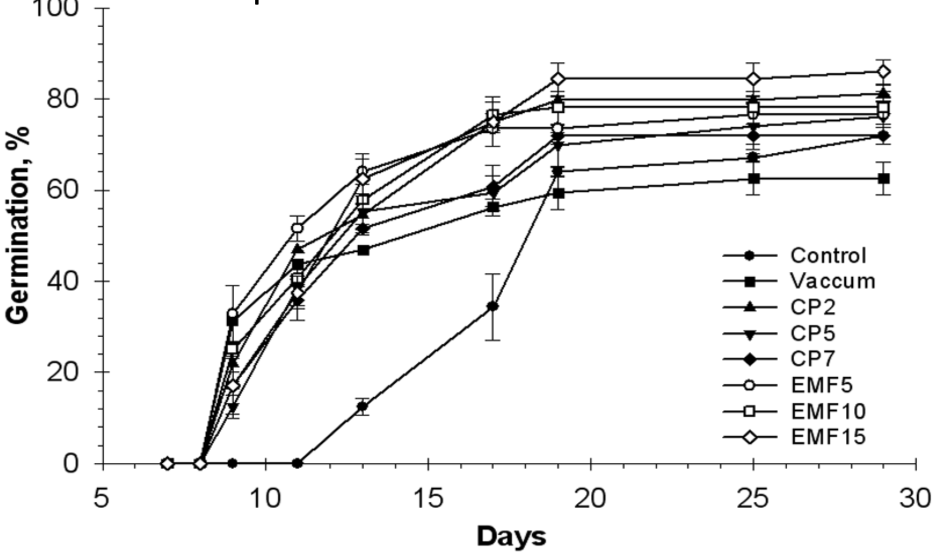
Secondary metabolites (SM) are:

- 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 or other plant growth promoting bacteria, PGPR;
- biologically active substances with numerous beneficial effects on health of animals consuming plants for food, including humans.

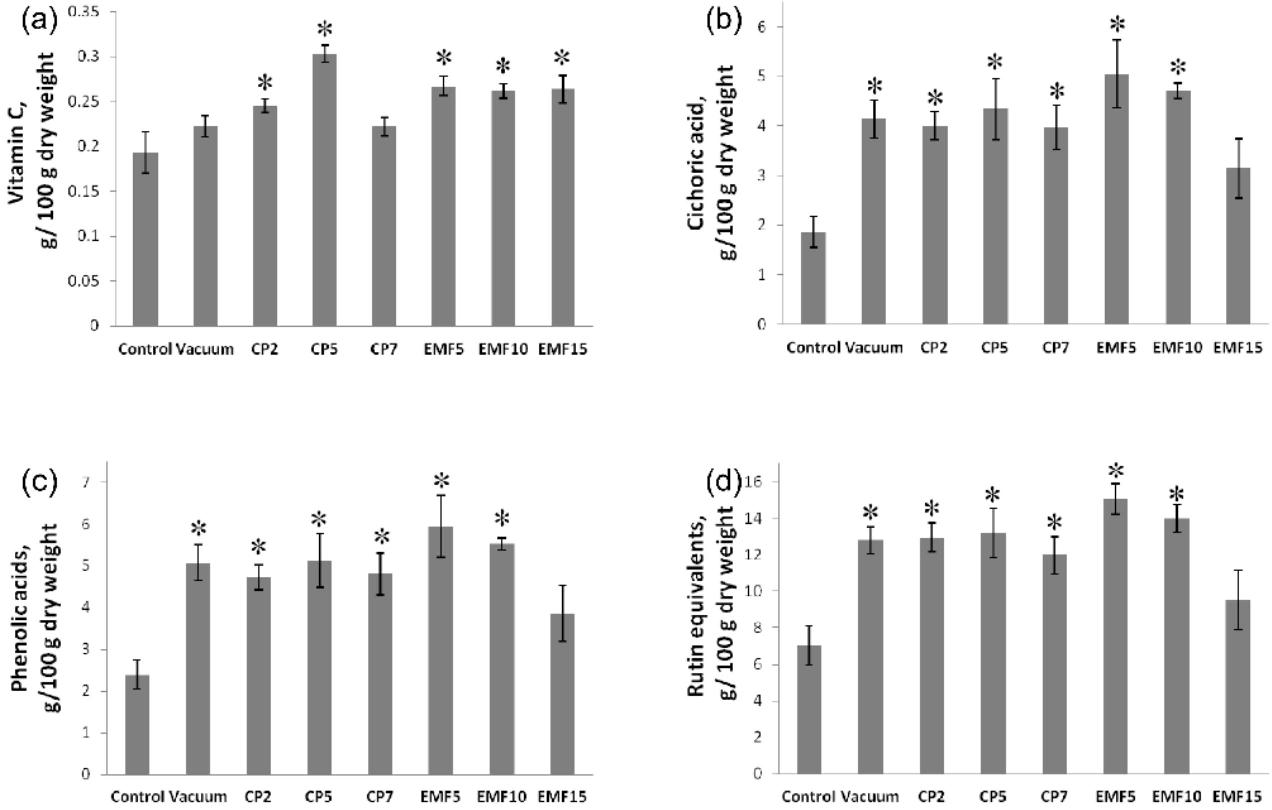
E.purpurea:

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



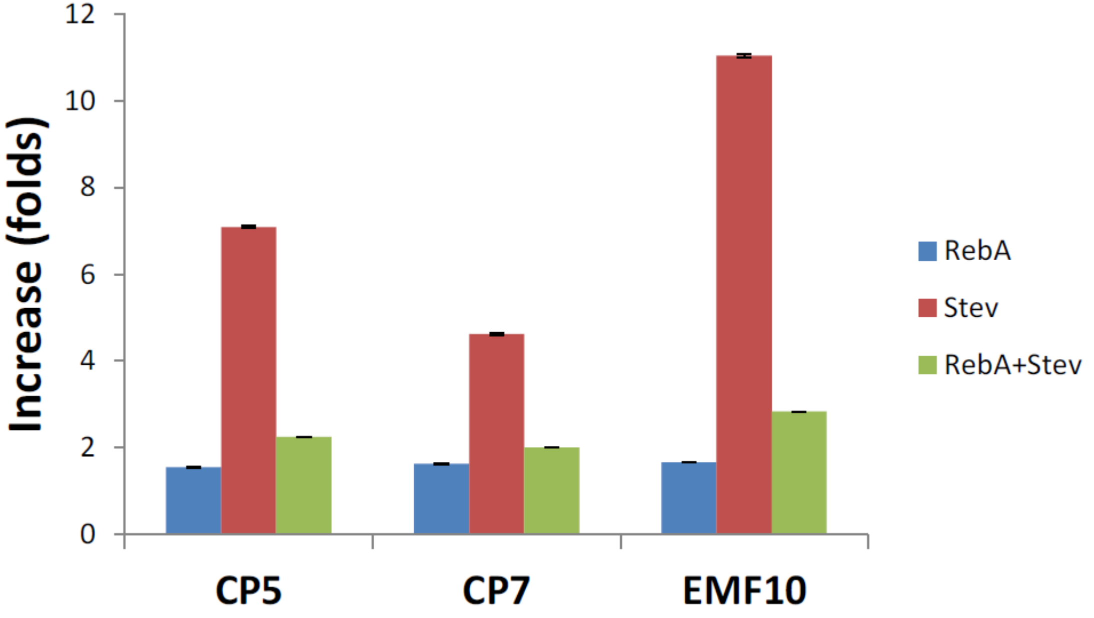
CP, vacuum and EMF effects on the leaf content of vitamin C, phenolic acids, and antioxidant activity

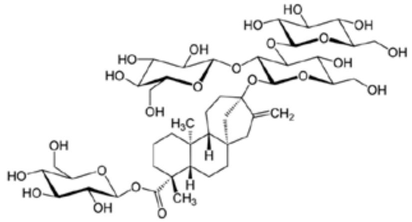
Judickaite et al., The Potential of Cold Plasma and Electromagnetic Field as Stimulators of Natural Sweeteners Biosynthesis in *Stevia rebaudiana* Bertoni. Plants 2022, 11, 611.

Effect of seed treatment with CP and EMF on content of steviol glycoside content mg/1g DW and ratio in *Stevia rebaudiana* leaves

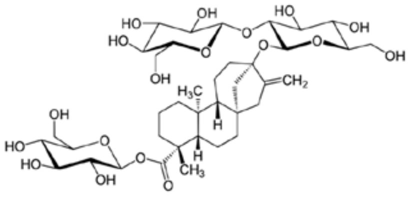
	RebA	Stev	RebA+Stev	RebA/Stev	RebA/(RebA+Stev)	Stev/(RebA+Stev)
Control	36.71 ± 3.10	5.27 ± 1.63	41.98 ± 4.71	8.35 ± 1.62	0.88 ± 0.02	0.12 ± 0.02
CP5	56.63 ± 9.07 *	37.35 ± 8.83 *	93.99 ± 17.89 *	1.86 ± 0.24 *	0.64 ± 0.03 *	0.36 ± 0.03 *
CP7	59.58 ± 9.12 *	24.35 ± 4.14 *	83.93 ± 13.25 *	2.50 ± 0.07 *	0.71 ± 0.01 *	0.29 ± 0.01 *
EMF10	60.77 ± 0.33 *	58.15 ± 0.15 *	118.93 ± 0.18 *	1.05 ± 0.01 *	0.51 ± 0.00 *	0.49 ± 0.00 *

* statistically significant difference compared to control ($p < 0.05$).



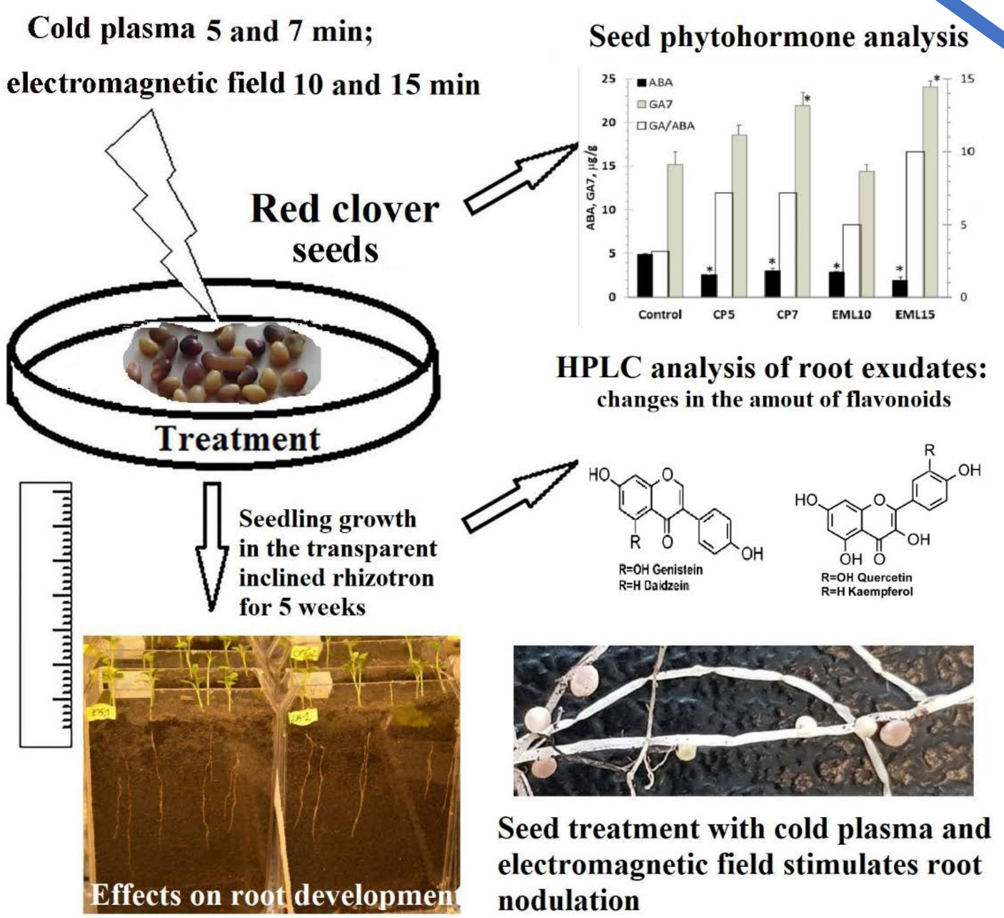


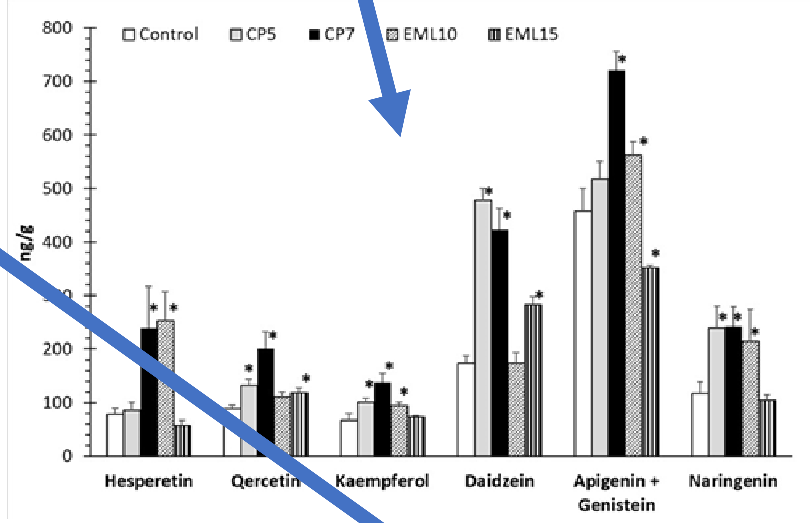
Rebaudioside A



Stevioside

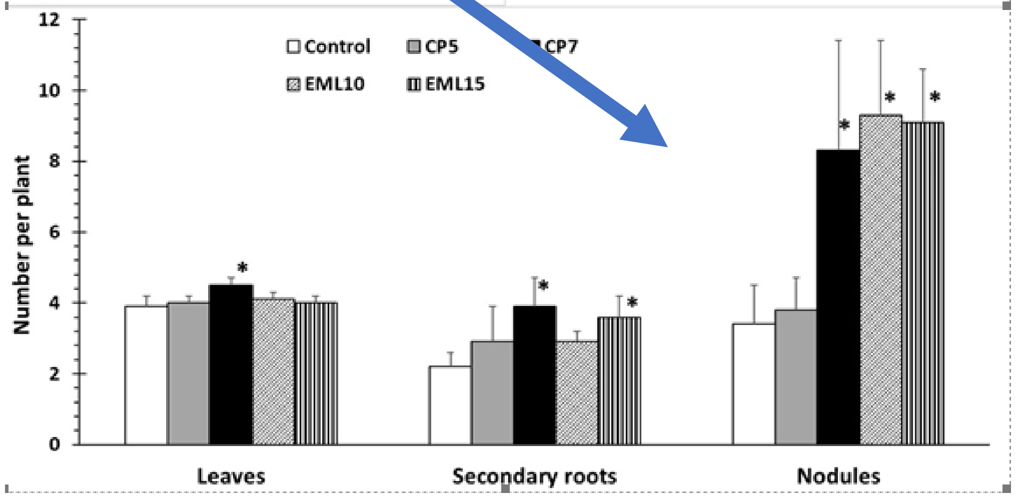
Pre-sowing seed treatments increased the amount of isoflavonoids in root exudates and the number of nodules in red clover roots





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:



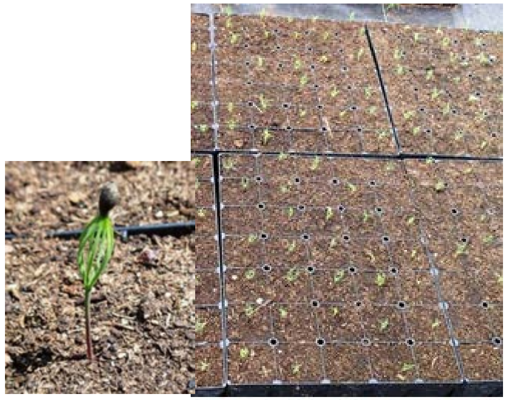
Stimulation of nodulation ≈ plant N-fertilisation → reduced need for chemical fertilisers

How persistent can be seed treatment effects?

The observation of seed treatment effects on Norway spruce growth for 9 years:

2014-2022

2014



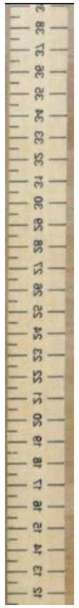
cassetes

2016

pots

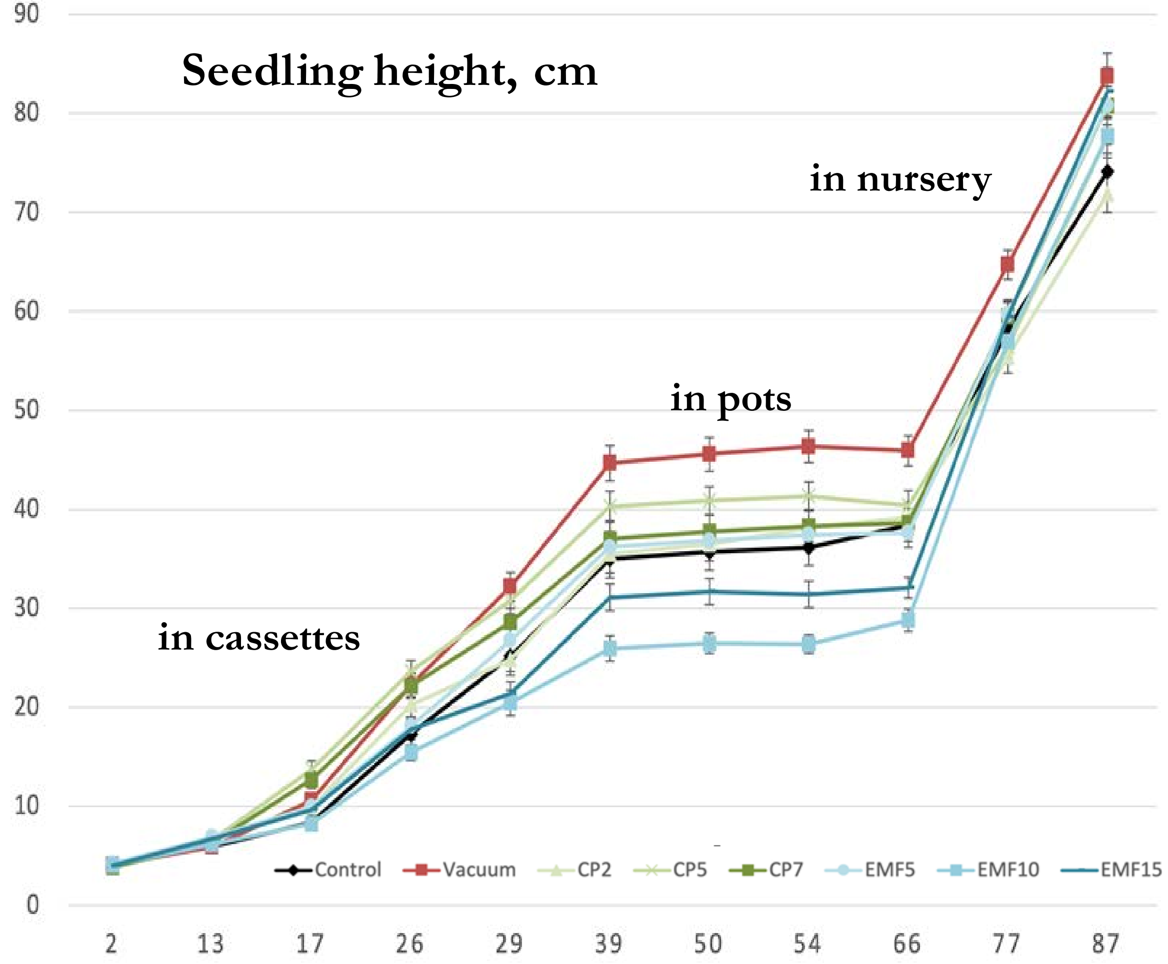
2020

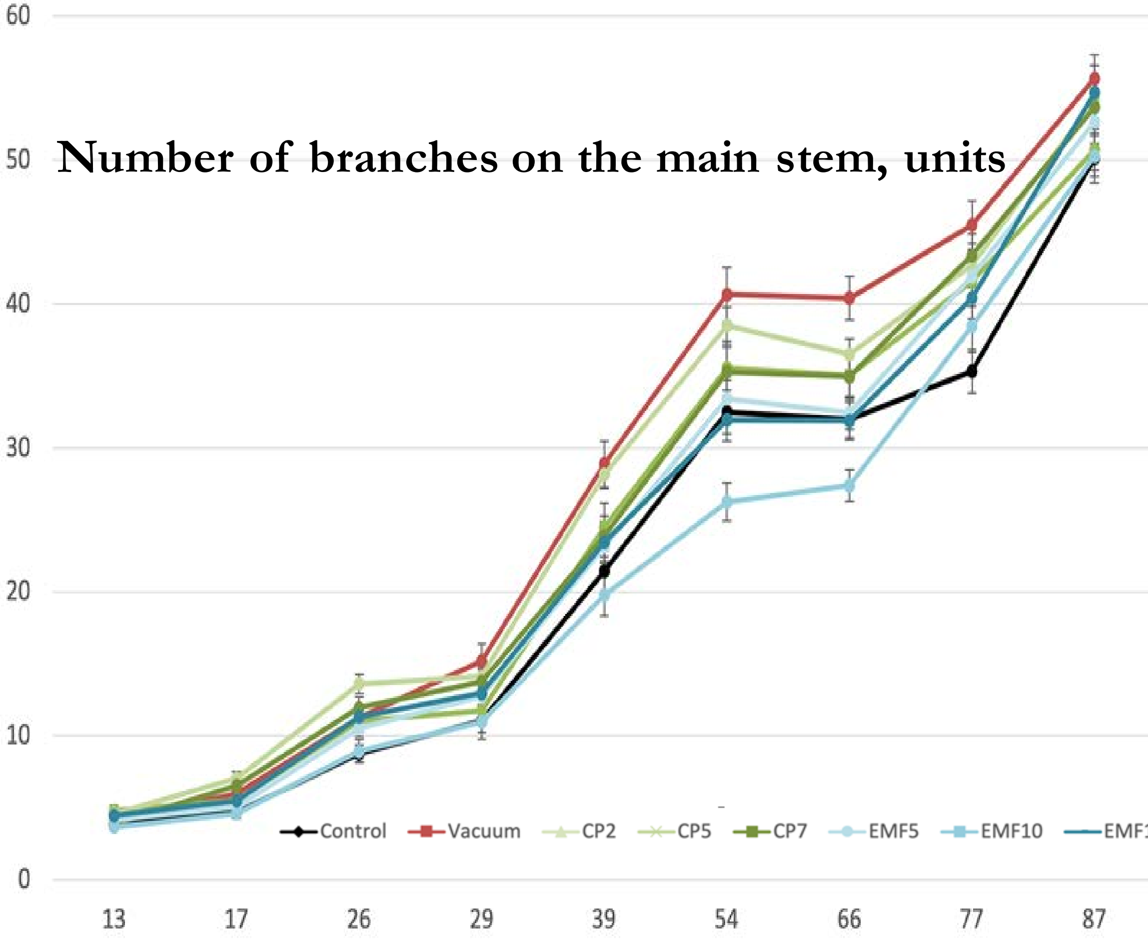
field (nursery)



40-50 plants per experimental group.
The total size of collection – over 400 seedlings…

Growth dynamics of Norway spruce seedlings





Months after sowing

- 17 month-age seedlings grown from CP (5 min) and CP (7 min) treated seeds, had 50–60% larger height and 40–50% increased branching in comparison to the control.
- In 39-month period after sowing plants grown from vacuum and CP (5 min) treated seeds were higher (by 26 and 17%, respectively) and had larger (by 33 and 21%, respectively) number of branches, as compared to the control.
- After planting to the nursery, growth of seedlings from all EMF groups exceeded the growth of other seedlings so that only in CP2 and EMF10 groups morphometric parameters were not different from the control, in the rest groups seedlings performed better compared to control. Seedlings in vacuum group still showed the best result

Antioxidative activity and content of phenols and flavonoids in the needles of Norway spruce seedlings in 2021 and 2022

Sampling date	Treatment	ABTS radical scavenging activity, µmol/g	DPPH radical scavenging activity, µmol/g	Total phenols content (TPC), mg g^{-1}	Total flavonoids content (TFC), mg g^{-1}
		Average±SE			
September, 2021	Control	1309.20±19.68	374.64±7.96	7.25±0.11	24.30±1.07
	Vacuum	**1199.64±28.01***	382.22±6.42	**6.76±0.16***	23.62±0.75
	CP2	**1196.97±41.28***	344.86±12.07	**6.50±0.19****	**21.39±0.63***
	CP5	1190.23±56.19	352.31±8.54	**6.11±0.18*****	**20.89±0.86***
	CP7	**1126.33±50.05***	**334.69±5.97****	**6.38±0.21****	**20.93±0.86***
	EMF5	**1091.66±41.04*****	**294.54±10.78*****	**5.70±0.15*****	**17.82±0.59*****
	EMF10	1244.52±35.14	376.96±5.40	7.33±0.22	23.01±0.63
	EMF15	**1129.03±24.46*****	**346.61±6.62***	**6.15±0.12*****	**19.16±0.15****
June, 2022	Control	792.49±46.02	185.88±6.43	4.69±0.21	14.36±0.52
	Vacuum	**450.44±23.69*****	**130.09±4.34*****	**3.31±0.06*****	**9.78±0.19*****
	CP2	**390.31±18.20*****	**118.54±6.81*****	**3.23±0.17*****	**9.69±0.18*****
	CP5	**416.86±18.00*****	**115.43±5.93*****	**3.29±0.16*****	**9.43±0.18*****
	CP7	**370.02±30.42*****	**89.52±5.75*****	**2.66±0.14*****	**7.79±0.13*****
	EMF5	**316.03±8.40*****	**78.61±8.81*****	**2.29±0.12*****	**6.59±0.07*****
	EMF10	**597.05±66.28***	**134.19±10.31*****	**3.31±0.10*****	**9.67±0.40*****
	EMF15	**527.18±23.76*****	**116.19±8.17*****	**2.65±0.09*****	**8.66±0.26*****

*The asterisk (*) indicates the statistical significance of the difference between the treatment group and the control in Norway spruce needle extracts (*p<0.05; **<0.005; ***p<0.001).*

Both in Sept 2021 and Jun 2022, seed treatments resulted in decreased antioxidative activity and amounts of TPC and flavonoids in seedling needles

Compared to June 2022, effects in Sep 2021 were much smaller (25-60 % versus 7-27 %)

The largest negative effects were observed in EMF5, CP7 and EMF15 groups; the smallest effects – in vacuum group.

Possible explanation – trade-off effects – the balance in distribution resources between growth and defence?

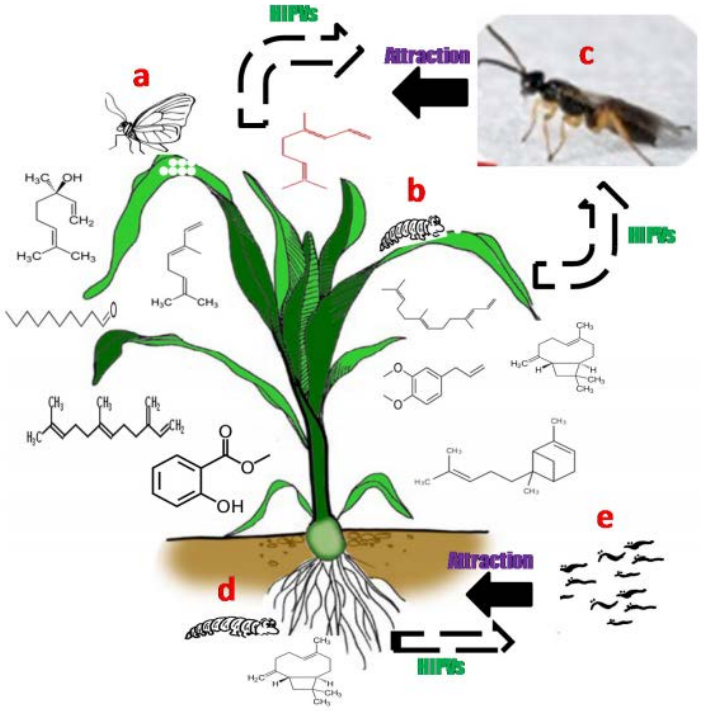
- The effects of seed treatments with CP (2-7 min), vacuum (7 min), EMF(5-15 min) on growth and biochemical traits of Norway spruce seedings are persistent for at least **for 9 years** (the entire period of observation);
- The dynamics of the observed effects on growth is complex and depends on the conditions of seedling cultivation;
- The effects on the secondary metabolism and antioxidative capacity are persistant as well;
- The effects on the composition of the communities of plant associated microorganisms (i.e fungal diversity) are also persistant.

What is applied potential of seed treatment with CP for agriculture?

Effect	Result	Implication	Economical importance	
Microbial seed decontamination	Elimination of pathogens	Reduced risk of plant diseases	➢ Reduced loss of agro-production	
Stimulated germination	More seedlings or faster emergence		➢ Increased production	
Stimulation of primary metabolism and photosynthesis	Faster growth and biomass gain	Enhanced harvest	➢ Increased production ➢ Improved food quality	
Stimulated secondary metabolism	Increased production of defensive molecules	•Improved resistance to biotic and abiotic stress (pathogens, draught, salinity, etc.); •Stimulated N-fixation in legumes	➢ Increased agro-production under unfavorable conditions ➢ Enhanced production of biologically active phytochemicals ➢ (e.g. antioxidants, drugs, steviosides, etc.) ➢ Reduced need for chemical N-fertilizers	

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.**



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Perspectives?

- **Further research on the physiological, biochemical, and molecular mechanisms of stress tolerance in plasma-treated seeds or plants;**
- **Selection of the most responsive plant species, varieties, genotypes;**
- **Standardizing and optimizing of treatment conditions**
- **Applied technologies for farming? Upscaling: technical designs and large-scale field studies;**
- **…**

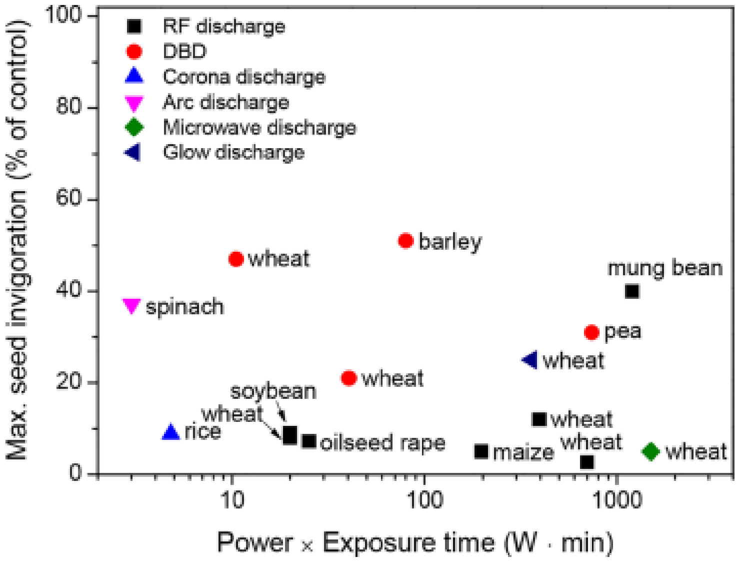

FIGURE 2 | Maximum seed invigoration (% of control) as a function of the power (W) and exposure time (min) of the plasma treatments for a wide range of crops, which were described in the literature presented in **Table 1**.

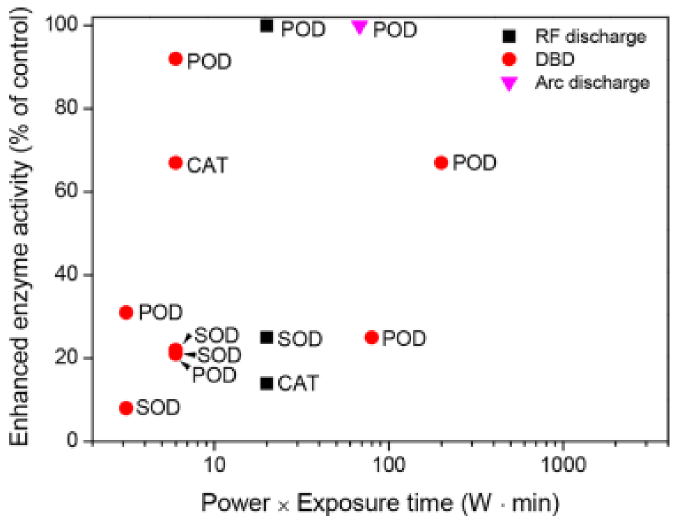

FIGURE 4 | Enhanced enzyme activity (% of control) as a function of the power (W) and exposure time (min) of the plasma treatments in seeds (or seedlings), which were described in the literature presented in **Table 3**.

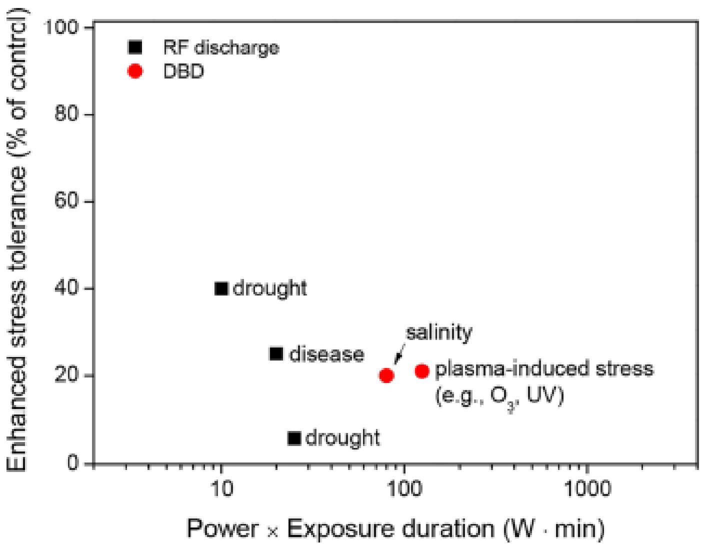

FIGURE 6 | Enhanced stress tolerance (% of control) as a function of the power (W) and exposure time (min) of the plasma treatments on seeds (or seedlings), which were described in the literature presented in **Table 4**.

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