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RESEARCH

Transcriptional responses following seed priming with cold plasma and electromagnetic field in *Salvia nemorosa* L.

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Abstract

This study was conducted to monitor the plant responses to seed priming with electromagnetic fields (0, 4, or 6 mT) or cold plasma (0, 80, or 100 s) in *Salvia nemorosa*. The cold plasma or electromagnetic field treatments significantly increased shoot fresh weight (49%), root fresh weight (41%), and root length (56%). The results highlighted that seed priming with cold plasma or the electromagnetic field is an effective method to modify seedling growth. The electromagnetic field and plasma treatments upregulated the *AREB1* gene (mean = 3.9-fold). Except for the electromagnetic field of 4mT, the other treatments stimulated expressions of the *WRKY1* gene by an average of 6.7-fold relative to the control. The cold plasma or electromagnetic field also induced the expression of cinnamoyl-CoA reductases (CCR2) gene (mean = fourfold). These treatments also changed the expression of the rosmarinic acid synthase by an average of sixfold. These findings may improve our knowledge of plant reactions to cold plasma and electromagnetic field for possible functions in seed technology.

Keywords Applied physics · Cold plasma · Electromagnetic field · Gene expression · Seed priming

Introduction

Plant members of the *Salvia* genus, like *S. nemorosa*, are significant sources of diverse isoprenoid and phenolic secondary metabolites. Taking their medicinal functions into account, these bioactive substances display promising antiproliferative and anticancer properties [1]. Therefore, the induction of secondary metabolism in this plant species is of critical importance [2]. In the past decade, plasma and electromagnetic fields offer highly competitive functions for utilization in a multitude of industries, such as seed and agriculture [3–5]. Different kinds of signaling agents, including UV radiation, active oxygen and nitrogen species, and some other free radicals are produced during plasma formation, many of which are capable of triggering signals in living tissues [6]. There are two different kinds of plasma (the fourth material state) dependent on temperature; I—thermal plasma and II—non-thermal (cold) plasma. Among these plasmas, cold plasma provides a great eco-friendly safe and economic opportunity in seed technology, especially for seed decontamination [4], germination [7], biofortification [6], and seedling early establishment [8, 9]. Moreover, seed priming with cold plasma can mediate long-term effects on plant productivity [10, 11]. In addition to these potential advantages, several lines of evidence manifest this hypothesis that seed or organ treatments with cold plasma may associate with an increase in plant tolerance to stress conditions [6, 8, 9, 12–14]. However, ongoing convincing experiments are required to illustrate the contributed mechanisms and improve our knowledge on this kind of interaction. The electromagnetic field approach is another physical treatment, exhibiting beneficial potentials toward the biological system, and recently, its application in agriculture is under investigation. The potential benefits of magnetic field applications have been underlined by several lines of evidence in various plant species [15, 16]. However, there are limited comparative data on the functions of cold plasma and electromagnetic fields in agriculture.

Plants as immobilized organisms can adapt to changes in environmental factors through changes in transcriptions of genes [8, 9, 17]. Furthermore, molecular data on plasma/electromagnetic field-triggered alterations is rare. To address the plasma-mediated changes in gene expression pattern,

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we monitored the possible modification in the expression of genes following seed priming with cold plasma or electromagnetic field. In the plant cells, several families of transcription factors manage the expression of genes in response to diverse environmental factors. Hence, the expression patterns of *WRKY1* and *AREB1* transcription factors were monitored. Moreover, lignin (a polymer of phenolic monolignols) is a secondary metabolite deposited in the plant cell wall. Lignin deposition on the cell wall is also a part of the plant defense system through which physical strength is increased. The productions of monolignols are mediated through the catalytic actions of several enzymes among which cinnamoyl-CoA reductases (CCRs) are of importance [18]. Hence, we monitor the possible changes in the expression of *CCR2* in response to cold plasma or electromagnetic field treatments. Besides, in the Lamiaceae family, one of the pharmaceutical important substance is rosmarinic acid, which is synthesized by the catalytic action of rosmarinic acid synthase (RAS) enzyme. So, we selected the *RAS* gene as another target gene involved in secondary metabolism. Therefore, the plasma or electromagnetic field-mediated changes in transcriptions of *CCR2* and *RAS* (two key genes in plant secondary metabolism) were also explored. Our experiment was designed to find out about two following questions: (I) Is plasma treatment associated with changes in genes of *WRKY1* and *AREB1* (two important transcription factors)? (II) Does plasma induce the expression of *CCR2* and *RAS* genes involved in secondary metabolism? Therefore, we aimed to investigate the possible changes in growth and expressions of target genes following seed priming with electromagnetic fields and cold plasma in *S. nemorosa*.

Materials and methods

Experimental apparatus and treatments

The *S. nemorosa* seeds were provided by PakanBazr company (Isfahan, Iran). In this experiment, dielectric-barrier discharge (DBD; Model PS200, Nik Fanavaran Plasma Co., Iran) and a solenoid (50 cm length and 10 cm diameter) were applied to treat seeds with cold plasma or electromagnetic field, respectively. The DBD descriptions are represented elsewhere [9]. The seeds were primed with DBD-originated plasma with the surface power density of plasma was 0.84 Wcm^{-2} for 0, 80, and 100 s. The intensity of the electric current and electromagnetic field current was controlled by an ammeter and a tesla meter, respectively. For an electric current generated in the solenoid, the power supply (ED-345BM, China) with 60 Hz input current and 10 A output current was applied. The seeds were subjected to electromagnetic fields at different intensities (0, 4, and 6 mT) for 30 min.

The plasma/electromagnetic field-primed seedlings were planted in the pots containing coco peat and perlite in the ratio of 1:1 and watered with Hoagland nutrient solution and water every other day. Thirty-day-old seedlings were subjected to growth and molecular analysis.

Expression of target genes

In leaves, expressions of *SnWRKY1*, *SnAREB1*, *SnCCR2*, and *SnRAS* as target genes were evaluated. Trizol-based RNA extraction was performed on the liquid nitrogen-grounded leaves. After that, complementary DNA (cDNAs) was synthesized using Kit. The sequences of forward and reverse primers for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), *SnWRKY1*, *SnAREB1*, *SnCCR2*, and *SnRAS* genes are shown in Table 1. In this study, GAPDH was used as a housekeeping gene. The expressions of target genes in the plasma/electromagnetic field-primed seedlings were quantified using the real-time quantitative RT-PCR method and a commercial kit. The relative expression levels of the target genes were presented in a fold change unit [17].

Table 1 Forward (F) and reverse (R) primer sequences for *GAPDH* (housekeeping gene), *SnRAS*, *SnWRKY1*, *SnAREB*, and *SnCCR2* as target genes

Amplicon (bp)	Tm	length	Sequence (5′–3′)	Primer name
174	65	20	TCGCCGGCCAACTACCACAC	*SnRAS*-F
	61	21	GTTGCAATCGATCTCAAGGCG	*SnRAS*-R
107	60	20	CTTCTCCCCTGCTCCTCCAA	*SnWRKY1*-F
	61	20	GGCTGTGGCAATTGCAATGG	*SnWRKY1*-R
131	61	20	TTACCGAGGACGCTTAGCCA	*SnAREB*-F
	61	20	TCACCCAAAGTAGGCTGCCT	*SnAREB*-R
93	60.8	20	TGGACCATCTGCCAAGGACT	*SnGAPDH*-F
	61	20	ACTTTGCCGACAGCCTTAGC	*SnGAPDH*-R
98	60	20	AAGCTCTGTGCCAAATCCCG	*SnCCR2*-F
	61	20	CACGCCCGATTTTTGCTTCG	*SnCCR2*-R

Statistical analysis

A factorial experiment based on a completely randomized design with three replicates was performed. ANOVA results were conducted by using SPSS software and mean comparisons were carried out using Duncan's multiple range test at a probability level of 0.05.

Result and discussion

In comparison with the control, the seed priming with cold plasma or electromagnetic field treatments increased shoot fresh mass (Fig. 1a). With a similar trend, the plasma- or electromagnetic-treated seedlings had significantly higher amounts of root fresh weight by averages of 50.9% and 32.85%, respectively (Fig. 1b). Likewise, the EMF4 (47.5%), EMF6 (52.5%), CP80 (85%), and CP100 (41%) treatments significantly enhanced the root length (Fig. 1c). However, the difference in root length among CP100, EMF4, and EMF6 was not a significant change (Fig. 1c). Our results showed that the seed priming with cold plasma or electromagnetic field treatments increased root length, shoot, and root fresh mass. However, seed priming with cold plasma was more efficient than the electromagnetic field to affect seedling early growth. These findings are in line with several other recent reports on growth-promoting roles of cold plasma [8, 10, 12, 13, 19] and electromagnetic field [3, 15, 20]. Contrary to these studies, a few reports highlighted this hypothesis that high exposure to plasma may associate with a delay in growth rate [4, 5]. The plasma-associated alterations in metabolism [8], phytohormones [10, 15], and xylem-conducting tissues [8, 10, 13] are considered as two main mechanisms by which this priming technique can improve plant growth. Likewise, it has been exhibited that the electromagnetic field application associated with increase in nutrition [20], hormones [15], and proteome [15] by which plant growth may be influenced. Abedi et al. [21] also indicated that seed priming with cold plasma modified flowering in *Cichorium intybus*.

The EMF of 6 mT led to moderate induction in the expression of the *WRKY1* gene (Fig. 2a). Similarly, the cold plasma treatments drastically upregulated the *WRKY1* gene by approximately 5.26- and 8.18-fold in CP80 and CP100 treatments, respectively (Fig. 2a). Besides, EMF4, EMF6, CP80, and CP100 treatments displayed the higher expression rates of *AREB1* about 2.18-, 3.97-, 5.34-, and 4.2-folds, respectively, relative to the control (Fig. 2b). Moreover, seed priming with the cold plasma of 80 and 100 s significantly stimulated the expression of *CCR2* gene by 5.07- and 5.84-folds. Likewise, EMF4 and EMF6 moderately transcriptionally upregulated *CCR2* by an average of 2.7-folds compared with control (Fig. 2c). Besides,

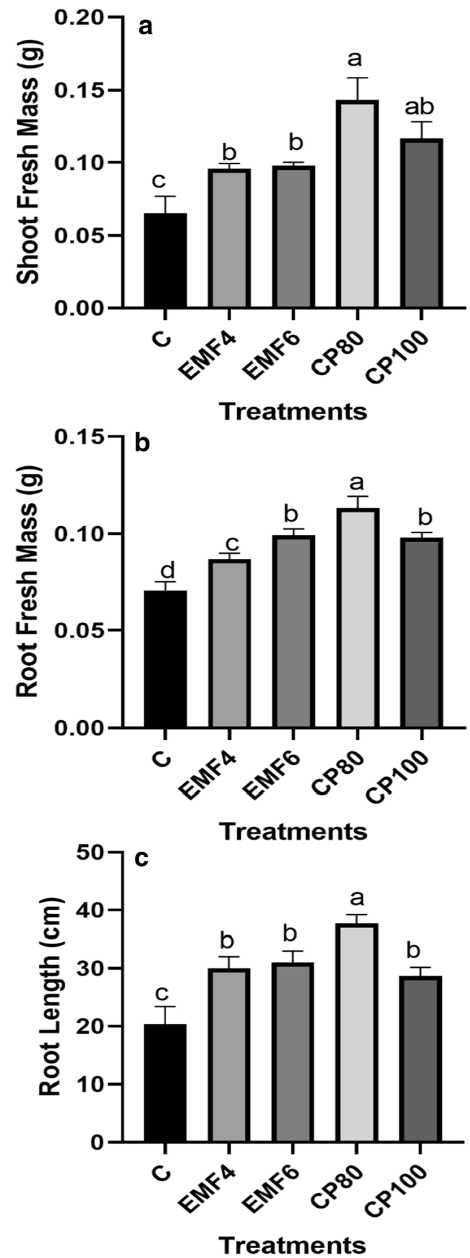

Fig. 1 The effects of seed priming with cold plasma or electromagnetic field on different growth-related parameters, including shoot fresh weight (**a**), root fresh weight (**b**), and root length (**c**) in *Salvia nemorosa*

cold plasma treatments enhanced the expression of the *RAS* gene about 6.5-folds (Fig. 2d). Similarly, the applied electromagnetic field made a significant increase in the expression of this gene (Fig. 2d). Our findings indicated that both the electromagnetic field and cold plasma had the potency to affect cellular transcription. However, cold plasma priming displayed higher efficacy to modify transcriptional programs over the electromagnetic priming. In line with our results, cold plasma-mediated induction in

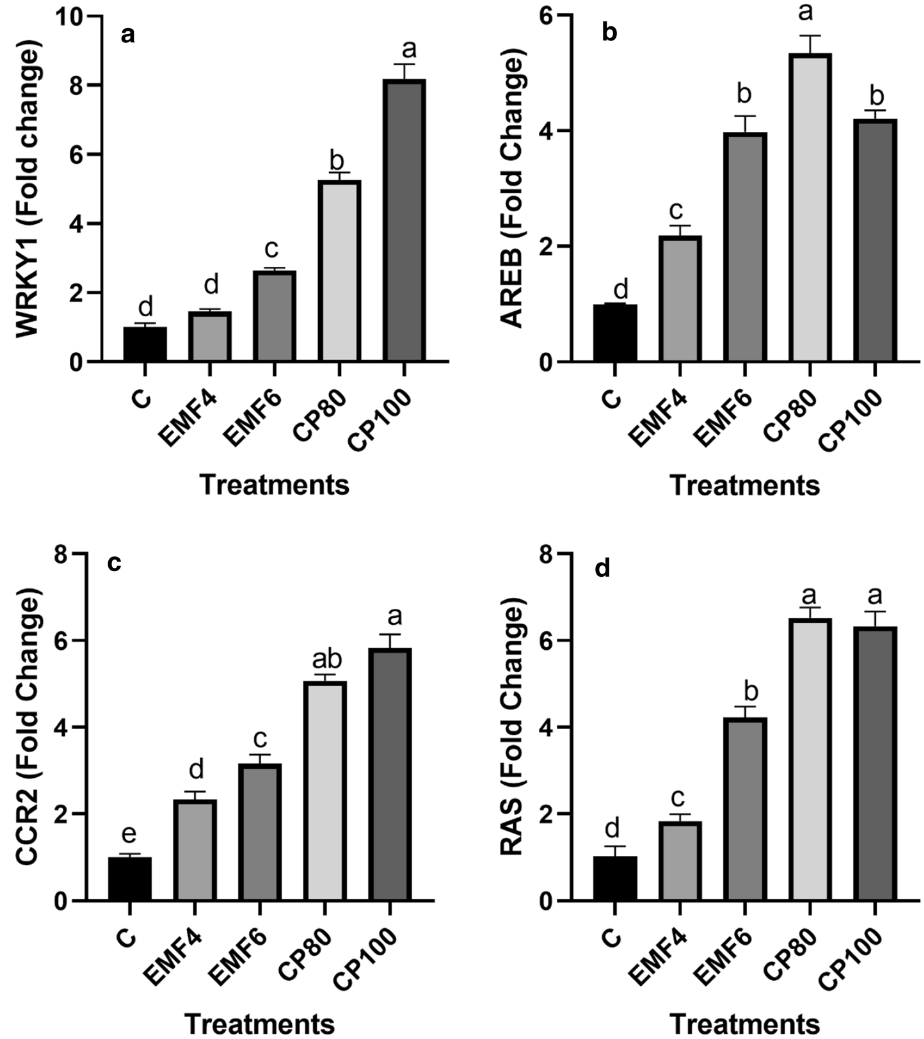

Fig. 2 The cold plasma/electromagnetic field-mediated changes in expressions of several genes, including *WRKY1* (**a**), *AREB1* (**b**), *CCR2* (**c**), and *RAS* (**D**) in *Salvia nemorosa*

the *WRKY1* transcription factor in canabis [22] has been recently reported. WRKYs are involved in the modification of transcriptional reprogramming of stress-responsive genes and protein functions [23] through interacting with the promoter region. Several experimental reports have confirmed that plasma application can enhance plant resistance to stress conditions [12–24]. Pre-sowing seed treatment with electromagnetic field or cold plasma was associated with long-time impact on leaf gene expression, especially inducing expression of proteins implicated in photosynthetic processes and their regulations [15], which is consistent with our result. According to our results, it seems that seed priming with electromagnetic field or cold plasma, especially the latter, can stimulate secondary metabolism and defense system, thereby improving plant protection. It has been reported that the upregulation in *CCR-2* can improve plant tolerance against stress conditions in different plant species, like *Leucaena leucocephala* [25] and soybean [26]. Zhang et al. [27] revealed that atmospheric cold plasma jet may cure the fungus-infected leaves and reduce the spread of infection. Cold plasma, therefore, is an attractive tool and chemical-free approach for plant disease management. It has been reported that there is a correlation between rosmarinic acid production and expression of the *RAS* gene [28]. UV along with diverse bioactive signaling substances, especially nitric oxide (NO) and ozone (O_3), are generated during plasma which all can individually or synergistically act as an elicitor, activate signaling routes, and modify transcriptions of responsive genes. In agreement with our results, cold plasma associated with differences in transcriptions of several genes, including *USP* [13], *WRKY1* [22, 29], and *HSFA4A* [9]. Furthermore, Döring et al. [30] showed that ozone upregulated *RAS* expression in *Melissa officinalis*. The analysis of variance confirmed that the effects of the electromagnetic field and cold plasma, especially the latter, on the evaluated traits were statistically significant (Table 2). Herein, we provided molecular evidence through which cold plasma or electromagnetic field priming may associate with an increase in plant tolerance to

Table 2 Analysis of variance for cold plasma and electromagnetic field for different evaluated traits

	F value						
Factor	Shoot fresh mass	Root fresh mass	Root length	*AREB*	*CCR2*	*WRKY1*	*RAS*
Cold Plasma	28.2**	69.2**	30.5**	30.4**	50.6**	47.1**	38.8**
Electromagnetic field	18.98**	46.9**	11.1**	13.5**	12.8**	26.2**	17.5**

$^*p \leq 0.05$

$^{**}p \leq 0.001$

unfavorable environmental conditions. However, the involved mechanisms remain obscured and need ongoing studies [6, 12, 14].

Conclusion

For the first time, comparative evidence on the cold plasma or electromagnetic field-associated changes in the early growth, biomass accumulation, and expressions of *SnWRKY1*, *SnAREB1*, *SnCCR2*, and *SnRAS* genes was provided in *S. nemorosa*. Herein, we introduced the plasma or electromagnetic field-mediated alterations in the expression of *WRKY1* and *AREB1* genes as a key mechanism by which these treatments may improve plant protection under stress. Moreover, seed priming with cold plasma or electromagnetic field influenced the expressions of *SnCCR2* and *SnRAS* genes, which are involved in secondary metabolites. These findings may improve our knowledge of plant reactions to cold plasma or electromagnetic field for possible functions in seed and agriculture technologies.

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References

1. Bahadori, M.B., Eskandani, M., Mieri, M.De, Hamburger, M., Nazemiyeh, H.: Anti-proliferative activity-guided isolation of clerodermic acid from *Salvia nemorosa* L.: geno/cytotoxicity and hypoxia-mediated mechanism of action. Food Chem. Toxicol. **120**, 155–163 (2018)
2. Kuzma, L., Wysokinska, H.: Production of secondary metabolites in shoots of *Salvia nemorosa* L. cultured in vitro. Biotechnologia **4**, 154–159 (2003)
3. Tien, P., Wang, S.R.: The influences of extremely low frequency AC magnetic fields at 60 Hz on mung beans growth. J. Am. Sci. **5**, 49 (2009)
4. Safari, N., Iranbakhsh, A., Ardebili, Z.O.: Non-thermal plasma modified growth and differentiation process of *Capsicum annuum* PP805 Godiva in in vitro conditions. Plasma Sci. Technol **19**, 055501 (2017)
5. Iranbakhsh, A., Ghoranneviss, M., Ardebili, Z.O., Ardebili, N.O., Tackallou, S.H., Nikmaram, H.: Non-thermal plasma modified growth and physiology in *Triticum aestivum* via generated signaling molecules and UV radiation. Biol. Plant. **61**, 702–708 (2017)
6. Babajani, A., Iranbakhsh, A., Ardebili, Z.O., Eslami, B.: Seed priming with non-thermal plasma modified plant reactions to selenium or zinc oxide nanoparticles: cold plasma as a novel emerging tool for plant science. Plasma Chem. Plasma Process. **39**, 21–34 (2019). https://doi.org/10.1007/s11090-018-9934-y
7. Šerá, B., Zahoranová, A., Bujdáková, H.: Šerý M Disinfection from pine seeds contaminated with Fusarium circinatum Nirenberg & O'Donnell using non-thermal plasma treatment. Rom. Rep. Phys. **71**, 701 (2019)
8. Moghanloo, M., Iranbakhsh, A., Ebadi, M., Satari, T.N., Ardebili, Z.O.: Seed priming with cold plasma and supplementation of culture medium with silicon nanoparticle modified growth, physiology, and anatomy in *Astragalus fridae* as an endangered species. Acta Physiol. Plant. **41**, 54 (2019)
9. Iranbakhsh, A., Ardebili, N.O., Ardebili, Z.O., Shafaati, M., Ghoranneviss, M.: Non-thermal plasma induced expression of heat shock factor A4A and improved wheat (*Triticum aestivum* L.) growth and resistance against salt stress. Plasma Chem. Plasma Process. **38**, 29–44 (2018)
10. Seddighinia, F.S., Iranbakhsh, A., Ardebili, Z.O., Satari, T.N., Soleimanpour, S.: Seed priming with cold plasma and multi-walled carbon nanotubes modified growth, tissue differentiation, anatomy, and yield in bitter melon (*Momordica charantia*). J. Plant Growth Regul. **39**, 87–98 (2020). https://doi.org/10.1007/s00344-019-09965-2
11. Gao, X., Zhang, A., Héroux, P., Sand, W., Sun, Z., Zhan, J., Wang, C., Hao, S., Li, Z., Guo, Y.: Effect of Dielectric Barrier Discharge Cold Plasma on Pea Seed Growth. Agric. Food Chem. **67**, 10813–10822 (2019)
12. Sheteiwy, M.S., An, J., Yin, M., Jia, X., Guan, Y., He, F., Hu, J.: Cold plasma treatment and exogenous salicylic acid priming enhances salinity tolerance of *Oryza sativa* seedlings. Protoplasma **256**, 79–99 (2019)
13. Moghanloo, M., Iranbakhsh, A., Ebadi, M., Ardebili, Z.O.: Differential physiology and expression of phenylalanine ammonia lyase (PAL) and universal stress protein (USP) in the endangered species *Astragalus fridae* following seed priming with cold plasma and manipulation of culture medium with silica nanoparticles. Biotech **9**, 288 (2019)
14. Iranbakhsh, A., Ardebili, Z.O., Ardebili, N.O., Ghoranneviss, M., Safari, N.: Cold plasma relieved toxicity signs of nano zinc oxide in *Capsicum annuum* cayenne via modifying growth, differentiation, and physiology. Acta Physiol. Plant. **40**, 154 (2018)
15. Mildažienė, V., Aleknavičiūtė, V., Žūkienė, R., Paužaitė, G., Naučienė, Z., Filatova, I., Lyushkevich, V., Haimi, P., Tamošiūnė, I., Baniulis, D.: Treatment of common sunflower (*Helianthus annus* L.) seeds with radio-frequency electromagnetic field and cold plasma induces changes in seed

phytohormone balance, seedling development and leaf protein expression. Sci. Rep. **9**, 6437 (2019)
16. Vashisth, A., Nagarajan, S.: Effect on germination and early growth characteristics in sunflower (Helianthus annuus) seeds exposed to static magnetic field. J. Plant Physiol. **167**, 149–156 (2010)
17. Sotoodehnia-Korani, S., Iranbakhsh, A., Ebadi, M., Majd, A., Ardebili, Z.O.: Selenium nanoparticles induced variations in growth, morphology, anatomy, biochemistry, gene expression, and epigenetic DNA methylation in *Capsicum annuum*; an in vitro study. Environ. Pollut. **265**, 114727 (2020). https://doi.org/10.1016/j.envpol.2020.114727
18. Yan, X., Liu, J., Kim, H., Liu, B., Huang, X., Yang, Z., Lin, Y.C., Chen, H., Yang, V., Wang, J.P., Muddiman, D.C.: CAD 1 and CCR 2 protein complex formation in monolignol biosynthesis in *Populus trichocarpa*. New Phytol. **222**, 244–260 (2019)
19. Liu, B., Honnorat, B., Yang, H., Arancibia, J., Rajjou, L., Rousseau, A.: Non-thermal DBD plasma array on seed germination of different plant species. J. Phys. D Appl. Phys. **52**, 025401 (2018)
20. Radhakrishnan, R., Kumari, B.D.: Pulsed magnetic field: a contemporary approach offers to enhance plant growth and yield of soybean. Plant Physiol. Biochem. **51**, 139–144 (2012)
21. Abedi, S., Iranbakhsh, A., Ardebili, Z.O., Ebadi, M.: Seed priming with cold plasma improved early growth, flowering, and protection of *Cichorium intybus* against selenium nanoparticle. J. Theor. Appl. Phys. **14**(2), 113–120 (2020)
22. Iranbakhsh, A., Ardebili, Z.O., Molaei, H., Ardebili, N.O., Amini, M.: Cold plasma up-regulated expressions of WRKY1 transcription factor and genes involved in biosynthesis of cannabinoids in Hemp (*Cannabis sativa* L.). Plasma Chem. Plasma Process. **40**, 527–537 (2020)
23. Rajaee Behbahani, S., Iranbakhsh, A., Ebadi, M., Majd, A., Ardebili, Z.O.: Red elemental selenium nanoparticles mediated substantial variations in growth, tissue differentiation, metabolism, gene transcription, epigenetic cytosine DNA methylation, and callogenesis in bittermelon (*Momordica charantia*); an in vitro experiment. PLoS One **15**(7), e0235556 (2020). https://doi.org/10.1371/journal.pone.0235556
24. Ling, L., Jiangang, L., Minchong, S., Chunlei, Z., Yuanhua, D.: Cold plasma treatment enhances oilseed rape seed germination under drought stress. Sci. Rep. **5**, 13033 (2015)
25. Srivastava, S., Vishwakarma, R.K., Arafat, Y.A., Gupta, S.K., Khan, B.M.: Abiotic stress induces change in Cinnamoyl CoA Reductase (CCR) protein abundance and lignin deposition in developing seedlings of *Leucaena leucocephala*. Physiol. Mol. Biol. Plant. **21**, 197–205 (2015)
26. So, H.A., Chung, E.S., Cho, C.W., Kim, K.Y., Lee, J.H.: Molecular cloning and characterization of soybean cinnamoyl CoA reductase induced by abiotic stresses. Plant Pathol. J. **26**, 380–385 (2010)
27. Zhang, X., Liu, D., Zhou, R., Song, Y., Sun, Y., Zhang, Q., Niu, J., Fan, H., Yang, S.Z.: Atmospheric cold plasma jet for plant disease treatment. Appl. Phys. Lett. **104**, 043702 (2014)
28. Babajani, A., Iranbakhsh, A., Ardebili, Z.O., Eslami, B.: Differential growth, nutrition, physiology, and gene expression in Melissa officinalis mediated by zinc oxide and elemental selenium nanoparticles. Environ. Sci. Pollut. R. **26**, 24430–24444 (2019)
29. Ghasempour, M., Iranbakhsh, A., Ebadi, M., Ardebili, Z.O.: Seed priming with cold plasma improved seedling performance, secondary metabolism, and expression of deacetylvindoline O-acetyltransferase gene in Catharanthus roseus. Contrib. Plasma Phys. **60**, e201900159 (2020). https://doi.org/10.1002/ctpp.201900159
30. Döring, A.S., Pellegrini, E., Della Batola, M., Nali, C., Lorenzini, G., Petersen, M.: How do background ozone concentrations affect the biosynthesis of rosmarinic acid in *Melissa officinalis*? J. Plant Physiol. **171**, 35–41 (2014)