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RESEARCH

Seed priming with cold plasma improved early growth, flowering, and protection of *Cichorium intybus* against selenium nanoparticle

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Abstract

Plasma as a rapidly evolving technology has been succeeded to widely exploit in various industrial fields. We attempt to address the short- and long-time effects of seed priming with cold plasma in *Cichorium intybus*. The seeds were subjected to plasma (dielectric barrier discharge). The post-reactions of the plasma-primed seedlings were monitored in response to different concentrations (0, 2, and 10 mgl^{-1}) of selenium nanoparticle (nSe). The plasma treatments enhanced seedling early growth in both shoot and roots. Besides, the simultaneous treatments of nSe of 2 mgl^{-1} and plasma synergistically improved seedling growth (mean = 78%). The plasma treatments mitigated the nSe10-associated phytotoxicity. The plasma and/or nSe treatments induced the enzyme activities of catalase (mean = 35%) and peroxidase (mean = 30%). In a complementary experiment, the long-time effects of plasma priming were monitored in plants grown under soil condition. The seed priming with cold plasma led to significant increases in shoot fresh mass (mean = 32%) and root biomass (mean = 26.8%). Moreover, the plasma-primed seedlings produced higher numbers of flowers (mean = 41.5%) and enhanced flower fresh weights (mean = 24%). The findings underline this hypothesis that exposure to plasma may associate with the activation of plant defense machinery and long-time modification in plant growth and development.

Keywords Cold plasma · Nanoparticle · Seed priming · Selenium · Toxicity

Introduction

Extensive efforts have been made to facilitate the emergence of seedlings [1, 2], reduce microbial contamination of seeds [3–5], modify cellular metabolism [6], and improve seedling protection under stress conditions [1, 7, 8]. Plenty of physical and chemical seed priming procedures have been employed to promote the early performance of seedlings, in particular, crops under stress conditions [1]. However, the appropriate seed priming strategy depending on plant species and priming methods can be varied. Moreover, following the initiation of seed germination, growth and performance of seedling are a crucial developmental phase for the establishment of cultivating plants and most sensitive to physicochemical stresses [3, 4]. Besides, the recent findings underline this opinion that plasma science and technology with an economically safe and eco-friendly nature offers new opportunities to achieve agricultural goals [1–5]. Taking seed technology into account, cold plasma technology appears to be an emerging eco-friendly approach for the control of seed-borne pathogens [9]. Several lines of evidence exhibited potential advantages of cold plasma on seed germination, growth, morphology, anatomy, and physiology in different plant species, like *Momordica charantia* [2], sunflower [10], *Astragalus fridae* [3, 4], soybean [11], *Capsicum annum* [12, 13], *Oryza sativa* [7], and hemp [14]. Owing to the plasma-derived productions of bioactive reactive nitrogen and oxygen active species (RNS and ROS) as well as UV radiation, exposure to plasma may trigger stress signaling, thereby upregulating complex defense machinery in living cells, like plant cells [15]. Moreover, recent studies underline the plasma-triggered modifications in the cellular transcription program of genes [4, 8, 14, 16], concentrations of hormones [10, 17], and proteome [10]. Recent scientific reports [1, 3, 4, 7, 15, 16] further suggest

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that plasma priming may be epigenetically associated with long-term effects on plant growth, metabolism, protection, and productivity. The considerable functions of plasma on seed germination, decontamination, and early seedling performance have been well illustrated [5, 16]. However, few studies have reported the long-term effects of cold plasma application on plants at different developmental stages, such as flowering [2] and yield [11]. Moreover, the potential benefits of plasma priming techniques have been experimentally displayed in laboratory conditions. However, how the plasma-primed seeds perform under the nature of the soil matrix remains unclear and obscure. Therefore, more studies in this concern should be carried out to gain insight into the plasma priming procedure.

Cichorium intybus L. (chicory) belonging to the Asteraceae family has gained a lot of concern as a pharmaceutically valuable plant species. It is cultivated for its numerous functions, like vegetables, extraction of inulin (applied as food fiber and a low-calorie sweetener), and its outstanding medical functions (especially hepatoprotection) [18]. In addition to cereals, vegetables (an important component of human diet) may undergo bio-fortification with essential elements like zinc (Zn), iron (Fe), and selenium (Se), taking nutrient requirements of humans into account [1, 6, 19]. Among these micronutrients, Zn and Fe are essential for plant species, whereas Se requirement among plant kingdom is controversial and it associates with phytotoxicity at high doses [1, 6]. Several recent works support the beneficial functions of nSe in plants to improve growth, yield, and resistance against stress factors [1, 6, 20–22]. On the other side, the likelihood of contamination of water, soil, and food chain is rapidly increasing, mainly owing to the increasing production and usage of nanoscale products [1, 6]. Hence, the presence of these nano-contaminants, especially unnecessary minerals, like nSe may restrict seed germination and seedling growth.

In this research, we attempt to address three main questions: (I) Does plasma treatment have the potential to mitigate the possible phytotoxicity associated with nSe? (II) Is the response of plasma-primed seedlings different in a petri dish or soil conditions? (III) Does plasma treatment have long-term effects and can it affect the flowering phenomenon at the reproductive stage?

Material and methods

Experimental design and conditions

The seeds of chicory were soaked for 24 h prior exposure to cold plasma generated by dielectric barrier discharge apparatus (DBD; Model PS200, Nik Fanavaran Plasma Co., Iran). The DBD design and plasma diagnostic data have represented in our other recent report [8]. DBD-derived productions of diverse oxygen and nitrogen-excited species and UV generation were confirmed by optical emission spectroscopy represented elsewhere [8]. During this experiment, details of the plasma generation were as follows: a functional gas: argon with a gas flow rate of 1 L min^{-1}; power: 80 W; frequency of 13 kHz; and the surface power density: 0.84 W/cm^2. The behaviors of the plasma-primed seedlings were monitored in two independent experiments under petri dish and pot conditions.

Experiments in petri dish condition

To answer the first research question, the first experiment was designed in petri dish conditions. The chicory seeds were surface-disinfected with 0.5% (v/v) sodium hypochlorite for 15 min and washed with water three times. The disinfected seeds were soaked in water for 24 h prior exposure to plasma. The applied plasma-generating device was dielectric barrier discharge (DBD) (PS200, Nik Fanavaran Plasma Co., Iran). Taking the plasma diagnostic data into account, we previously represented the optical emission spectroscopy-based spectrum, confirming generations of UV, oxygen, and nitrogen-related species [8]. The seeds were subjected to plasma treatments (DBD; power: 80 W; frequency: 13 kHz; a functional gas: argon; gas flow rate: 1 $Lmin^{-1}$; and surface power density: 0.84 Wcm^{-2}) with three exposure times of 0, 60, and 120 s.

The plasma-treated seeds were grown in a petri dish containing a filter paper wetted with few ml of 1/4 strength Hoagland nutrient solution. After 24 h, 20 ml of 1/4 strength Hoagland solution supplemented with three concentrations of nSe (0, 2, and 10 mgl^{-1}) were poured inside each petri dish. All treated samples were incubated under the same controlled environmental condition (temperature of 24 °C; photo-intensity of 30 µmol photon m^{-2} s^{-1} (16/8 h of light/dark)). The treatment groups and experimental descriptions are displayed in Table 1. Seven days after the nSe treatments, the seedlings were harvested and subjected for the further biochemical analysis.

Quantifications of activities of catalase and peroxidase

Seedlings were well-grounded in liquid nitrogen, then homogenized by applications of extraction buffer (100 mM phosphate buffer containing ascorbate and Na2EDTA; pH 7.1), and finally centrifuged at 4 °C. The supernatant as an enzyme extract was stored in −80 °C until analysis. The activity of the peroxidase enzyme was spectrophotometrically quantified according to absorbance difference in min at 530 nm [23]. Also, the activity of catalase enzyme was estimated

Table 1 Treatment groups and experimental descriptions

Treatment groups	Treatment description
C	Control: seeds were grown in petri dish containing Whatman filter paper and 20 ml of 1/4 strength Hoagland nutrient solution
P60	Seeds were treated with plasma for 60 s and grown in petri dish containing Whatman filter paper and 20 ml of 1/4 strength Hoagland nutrient solution
P120	Seeds were treated with plasma for 120 s and grown in petri dish containing Whatman filter paper and 20 ml of 1/4 strength Hoagland nutrient solution
nSe2	Seeds were grown in petri dish containing Whatman filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 2 mgl^{-1} (nSe treatment 24 h after the plasma treatment)
nSe10	Seeds were grown in petri dish containing Whatman filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 10 mgl^{-1} (nSe treatment 24 h after the plasma treatment)
P60 + nSe2	Plasma (60 s)-treated seeds were grown in petri dish containing filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 2 mgl^{-1} (nSe treatment 24 h after the plasma treatment)
P120 + nSe2	Plasma (120 s)-treated seeds were grown in petri dish containing filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 2 mgl^{-1} (nSe treatment 24 h after the plasma treatment)
P60 + nSe10	Plasma (60 s)-treated seeds were grown in petri dish containing filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 10 mgl^{-1} (nSe treatment 24 h after the plasma treatment)
P120 + nSe10	Plasma (120 s)-treated seeds were grown in petri dish containing filter paper and 20 ml of 1/4 strength Hoagland solution supplemented with nSe at 10 mgl^{-1} (nSe treatment 24 h after the plasma treatment)

according to the method of Moghanloo et al. [3] using a spectrophotometer.

Pot experiment

A second experiment was designed to address the second and third research questions of this study. The seed disinfection procedure and the protocol of plasma treatments were similar to those described above for the petri dish experiment. The plasma-primed seeds were planted in a pot containing loam soil. Treatments groups were called C (untreated control), P60 (plasma of 60 s), and P120 (plasma of 120 s). Finally, the 180-day-old seedlings were harvested to evaluate the long-term effects of plasma priming on biomass and flowering in the plasma-treated plants grown under pot condition containing soil.

Statistical analysis

Statistical analysis on all data was performed using SPSS software. Results were displayed as mean ± standard error (SE) values of three replications. The significant mean differences among the treatment groups were evaluated based on Duncan's multiple range test at the significance level of $P \leq 0.05$.

Results

Petri dish experiments

The results confirmed that the seed priming with cold plasma had the potency to improve plant protection against stress caused by nSe of 10 mgl^{-1} (Fig. 1). The individual treatments of plasma led to an increase in seedling height by an average of 42% when compared to the untreated control (Fig. 2). The nSe of 2 mgl^{-1} slightly increased this character by 25.5% over than the control (Fig. 2). The simultaneous treatments of nSe and plasma synergistically improved seedling growth by an average of 78% relative to the control (Fig. 2), while the nSe of 10 mgl⁻1 displayed an adverse impact on seedling growth and reduced seedling height by 40% (Fig. 2). Interestingly, the plasma treatments considerably relieved the growth-reducing effects of nSe at the high dose (Fig. 2). To elucidate possible mechanisms contributed to the plasma-mediated plant protection, the plasma-associated changes in the activities of peroxidase and catalase (two vital antioxidant enzymes as a part of plant defense machinery) were monitored. The individual seed treatments with plasma resulted in the induction in the catalase activity by approximately 21.8%, compared to the control. The nSe2 and nSe10 treatment groups individually enhanced the catalase activity by 28% and 16.7%, respectively. Moreover, the simultaneous applications of nSe and plasma were the most effective treatments to stimulate the catalase activity by a mean of 75.4% over than the control. The P60, P120, nSe2, and nSe10 treatments slightly upregulated the peroxidase activities by 16.2%, 22%, 25%, and 21.5%, respectively. However, the mixed

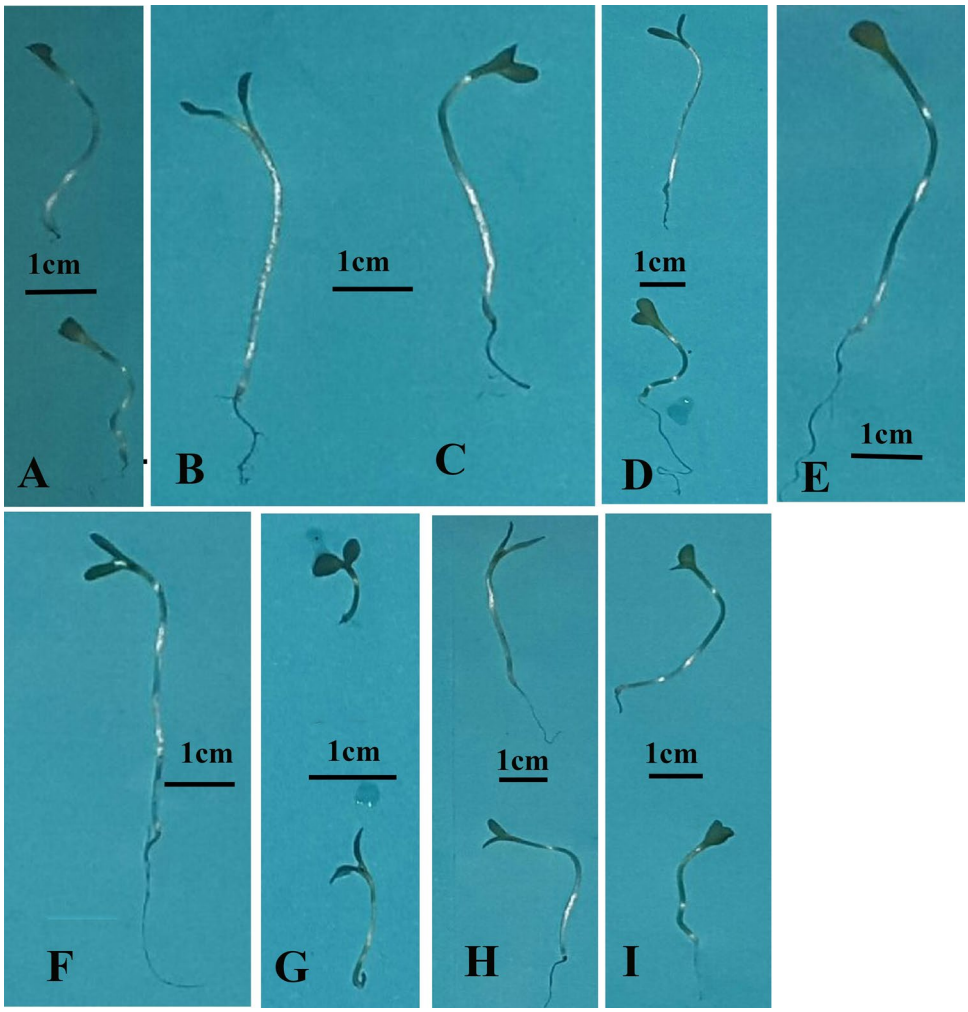

Fig. 1 The effects of seed priming with cold plasma on the early growth of chicory seedlings grown in petri dish containing ¼ strength Hoagland nutrient solution and the post-responses of plasma-primed seedlings to nSe of 2 and 10 mgl^{-1}. The exposures to the different nSe concentrations were carried out 24 h after the plasma priming. A. control; B. plasma of 60 s; C. plasma of 120 s; D. nSe of 2 mgl^{-1}; E. plasma of 60 s and nSe of 2 mgl^{-1}; F. plasma of 120 s and nSe of 2mgl^{-1}; G. nSe of 10 mgl^{-1}; H. plasma of 60 s and nSe of 10 mgl^{-1}; I. plasma of 120 s and nSe of 10 mgl^{-1}

treatments of nSe and plasma led to a drastic increase in peroxidase activity by approximately 67% in comparison with the control (Fig. 2).

Pot experiment

The cold plasma treatments caused significant ($P \leq 0.05$) increases in shoot fresh mass (mean = 32%) in comparison with the control (Fig. 3). However, difference between the P60 and P120 groups was not statistically significant. With a similar trend, seed priming with plasma significantly ($P \leq 0.05$) enhanced the root fresh mass by a mean of 26.8% (Fig. 3), while there was no statistically significant difference between the plasma-treated seedlings. Moreover, the plasma-primed seedlings produced significantly ($P \leq 0.05$) higher flowers by approximately 41.5% when compared to the control (Fig. 3). The flower fresh weights in the plasma-treated seedlings were also significantly ($P \leq 0.05$) higher by a mean of 24% than the control (Fig. 3).

Discussion

Exposure to the cold plasma considerably reinforced the root system which is of significant role during seedling early establishment. Consistent with our results, the modifications in root development following exposure to cold plasma have been introduced as an important mechanism by which plant growth and protection may be enhanced [2, 3, 12, 16, 24]. The phytotoxicity of nSe at high doses has been addressed in several recent reports [1, 6, 20]. Our results are in a good agreement with the findings of Babajani et al. [1] who reported the plasma-associated plant protection against high concentrations of nano-products. Furthermore, the cold plasma treatments relieved toxicity associated with cadmium [25] and salinity [8] in wheat. Taking involved mechanisms into account, several recent works confirmed plasma-mediated modifications in endogenous levels of signaling agents (especially H2O2 and NO) [25], expressions of genes [4, 8, 14, 16, 25], phytohormones [10], activities of defensive enzymes [1, 7, 8, 16], and differentiation of conducting xylem tissues [2–4]. For instance, the plasma

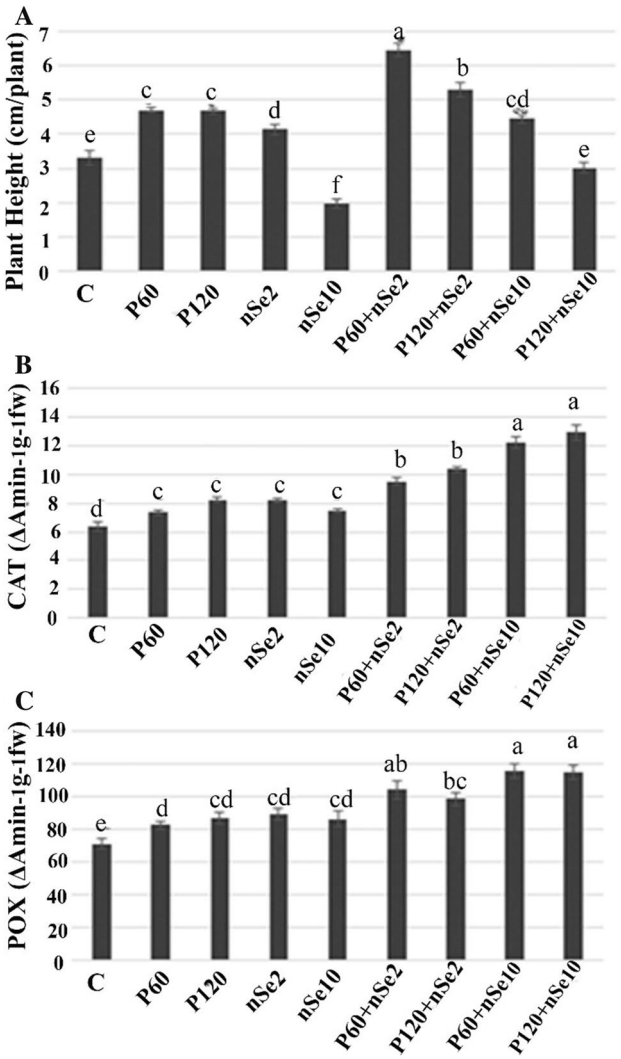

Fig. 2 Plasma and nSe-associated changes in **a** seedlings height, **b** activity of catalase (CAT) enzyme, and **c** activity of peroxidase (POX) enzyme. Data are mean ± SE of three independent replications. Different letters (a, b, etc.) on columns indicate statistical difference according to the Duncan test ($P \leq 0.05$). Treatments: C—control; P60—plasma of 60 s; P120—plasma of 120 s; nSe2—nSe of 2 mgl^{-1}; nSe10—nSe of 10 mgl^{-1}; P60 + nSe2—plasma of 60 s and nSe of 2 mgl^{-1}; P60 + nSe10—plasma of 60 s and nSe of 10 mgl^{-1}; P120 + nSe2—plasma of 120 s and nSe of 2 mgl^{-1}; P120 + nSe10—plasma of 120 s and nSe of 10 mgl^{-1}

application mediated changes in endogenous H2O2 and NO concentrations in wheat plants exposed to cadmium heavy metals [25]. In this study, seed priming with plasma stimulated the activity of catalase and peroxidase which are two major enzymes that contributed to the cellular antioxidant machinery. These results are in agreement with the findings of Sheteiwy et al. [7], Babajani et al. [1], Kabir et al. [25], and Iranbakhsh et al. [8, 12]. In wheat, the plasma treatments led to upregulation in the expressions of CAT and SOD genes (2 key antioxidant enzymes) and activities of corresponding enzymes [25]. It has been recently reported that cold plasma treatments were associated with alterations in expression of WRKY1 transcription factor, implying the plasma efficiency to alter nuclear transcriptional program [14]. In *Oryza sativa*, seed priming with non-thermal plasma improved salinity tolerance through the plasma-mediated modifications in photosynthetic gas exchange, photosynthesis efficiency, K^+ and Ca^{2+} levels, antioxidant system, and membrane integrity [7]. Furthermore, the non-thermal plasma application induced adaptive reactions in *Melissa officinalis* [1] and pea [15]. Several lines of evidence [1, 3, 4, 7, 8, 10, 12, 14–16, 25] supported this hypothesis that plasma may associate with the activation of adaptive defensive machinery through which plasma priming may improve plant tolerance to stress condition. Our hypothesis on the potential advantage of cold plasma to improve plant tolerance to nSe at high dose, therefore, was confirmed.

In the second experiment, the plasma-primed seedlings had higher biomass in both root and shoot. Moreover, the plasma treatments improved the quality and quantity of the produced flowers, indicating the long-time responses to the plasma treatments. The long-time responses to plasma may be attributed to the plasma-mediated changes in the cellular transcription program [4, 8, 14, 16, 25], hormones [10], proteome [10], and tissue differentiation [2–4]. This hypothesis may be supported by several recent reports of transcriptional changes in expressions of genes, like heat shock factor A4A (HSFA4A) [8], DAT [16], universal stress protein (USP) [4], CAT, and SOD [25]. Moreover, Mildažienė et al. [10] have provided convincing data on the plasma-mediated alterations in phytohormones which are the main regulators of plant growth program. Furthermore, several lines of anatomical evidence confirmed the plasma-associated reinforcement in the differentiation of xylem-conducting tissue [2–4, 12], which in turn can improve nutritional status, growth, and yield quality. Cold plasma treatments enhanced nitrogen and phosphate concentrations in tomato plants [26]. The non-thermal plasma enhanced growth indexes and biomass accumulation in the root system in zoysia grass stolon cuttings [27]. The considerable effectiveness of cold plasma application on improving grain yield in wheat has also been reported by Saberi et al. [28]. Besides, cold plasma treatment in bitter melon not only enhanced vegetative growth but also influenced flowering at the reproductive stage [2].

Conclusion

This study provides a better insight into the potential advantages of cold plasma toward improving plant early growth, protection, and productivity. Our findings underline this opinion that plasma can improve plant tolerance to stress conditions through activation of defense system, especially

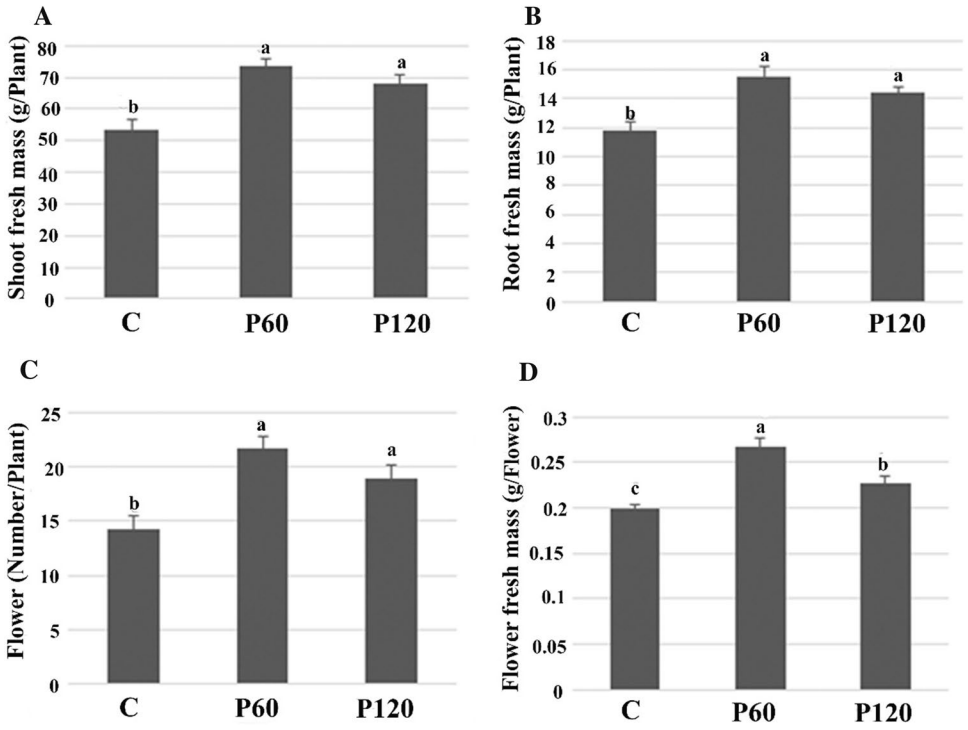

Fig. 3 The plasma-mediated long-time changes in growth and flowering in *C.intybus*. Data are mean ± SE of three independent replications. Different letters (a, b, etc.) on columns indicate statistical difference according to the Duncan test ($P \leq 0.05$). C—control; P60—plasma of 60 s; P90—plasma of 90 s

antioxidant machinery. Moreover, the plasma priming along with nSe at low optimum dose can be introduced as an effective protocol to promote plant growth, biochemistry, and protection. Taking long-time responses into account, the results manifest this hypothesis that seed priming with plasma not only can improve early seedling performance but also it can associate with long-term responses in plants. It appears that seed priming with cold plasma can mediate modification in flowering processes. Plasma as a rapidly evolving technology has been succeeded to widely commercialize in various industrial fields, particularly medicine, food, and agriculture. However, considering agriculture and its challenges, there is a great need for basic convincing researches.

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