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Evaluating the impact of Cold plasma on Seedling Growth properties, seed germination, and soybean antioxidant enzyme activity

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Abstract

Cold atmospheric pressure plasma (CAP) has garnered significant attention in recent years for its potential applications in biomedical, environmental, and agricultural fields. Cold plasma treatment exhibits a variety of effects in agricultural applications, including impacts on seed germination and seedling growth; however, further research is required. Soybean serves as a fundamental source of nutrients for both animals and humans. Soybean seeds possess impermeable and thick testae, which results in prolonged germination times and suboptimal germination rates. The soybeans exhibit low uniformity. As a result, poor crop establishment and yield reduction are inevitable outcomes. Therefore, the purpose of this study was to examine the effects of Iranian soybean cultivars, such as Sari, Saba, Arian, Katoul, and Williams, on seedling growth properties, seed germination, and antioxidant enzyme activity, using argon at time intervals of 30, 60, 180, 300, and 420 s. Cold plasma treatment significantly enhanced germination potential from 1.18 to 66.97%, germination index from 0.50 to 60.09%, germination rate from 1.78 to 32.17%, seedling length from 2.70 cm to 78.13 cm, root length from 2.87 cm to 56.13 cm, and seedling dry weight from 1.80 g to 36.63 g. Additionally, CAT activity increased from 0.88- to 4.40-fold, SOD activity from 0.86- to 5.89-fold, and APX activities from 0.40- to 4.01-fold compared to the control treatment. The findings indicated that the samples exhibited optimal results at treatment durations of 60 and 180 s. The influence of plasma on the antioxidant responses of seedlings, seed germination, and growth characteristics was contingent upon the duration of treatment. Cold plasma, when applied for an appropriate duration, may enhance soybean seedling growth characteristics and seed germination.

Keywords DBD cold plasma, Catalase (CAT), Superoxide dismutase (SOD), Ascorbate peroxidase (APx), Optical emission spectroscopy

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Introduction

Soybean is an economically import oilseed crop which is mainly cultivated for its high protein (40%), oil content (20%) and other valuable nutritional materials. This crop belongs to Leguminosae family which has the ability of reinforcement of soil due to nitrogen biologically fixation following by productivity and stability enhancement of soil texture and reduction of chemical fertilizer usage for next crops in rotation [1]. In the world, the various consumptions of soybean are referred to food (for human and livestock), medical usages and industrial products which cause the increase need for its production. The top five superior countries with the highest rate of soybean production consists of United states, Brazil, Argentina, China and India with total production area under cultivation of 129, 523,964 ha and production rate of 353,370,766 t [2].

Soybean seeds have thick and impermeable testae, resulting in poor germination, long germination time and low germination uniformity, which subsequently leads to poor establishment and yield reduction [3]. Promoting seed germination is the most direct way to improve soybean production. Methods for promoting seed germination are physical methods (magnetic treatment, sunlight, ultraviolet light and hot water soaking) and chemical methods (chemicals, fungicides and hormones) [4–6]. Although these methods can promote germination to a certain extent, they are time consuming, labor-intensive and produce chemical residues [7].

Plasma technology is nonthermal technique applied to foods in recent years [8]. Plasma treatment of seeds is a new approach that is being proposed to assist germination and survival [9]. Plasma is formed by a discharge in a gas, and in the case of an air plasma consists of ions, energetic electrons, neutral species, reactive oxygen species (ROS) and reactive nitrogen species (RNS) and produces electromagnetic radiation such as UV. Preliminary investigations have confirmed that plasma pre-treatment of seeds of important agricultural crops is an effective tool for improvement of germination, shoot, and root growth [10–12]. The plasma treatments provide good fungicidal and bactericidal effects, and increased water permeability through surface coat etching and stimulation of germination and seedlings growth [11, 13].

Various types of plasma sources have been created in the last 20 years such as dielectric barrier discharge (DBD), jet plasma, microwave discharge, radiofrequency (RF) discharge, and corona discharge. The seed germination was shown to vary depending on the plasma sources, seed type, treatment period, feed gas, and humidity [14]. Dielectric barrier discharge (DBD) is a new technique of cold plasma generation that has excellent treatment flexibility, and low power requirements, therefore, a great deal of biological goals can be achieved through this technique [15–17].

Numerous examinations have confirmed the positive effect of cold plasma on plant growth performance. The mechanism of this approach is to activate physiological functions in plasma-treated seeds. One study showed that cold plasma increased the expression of essential enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase from tomato seeds [18].

In another study, the sunfower seeds treated for 7 min before sowing increased germination, leaf weight, and seedling growth. In addition, gibberellin and abscisic acid production was modifed to increase germination [19]. In addition, the study by Renáta Švubová et al. (2021) showed that cold plasma increased the activity of succinate dehydrogenase in soybean, proving the switching of the germinating seed metabolism from anoxygenic to oxygenic. The results also showed that SOD, and GSH activity increased compared to the control group [20].

This study is motivated by the significance of global plant production, particularly in the context of climate change and the increasing food demands of human populations. Evaluating eco-agricultural technologies, including cold plasma, is crucial for enhancing seed quality, promoting initial plant growth and development, and ultimately increasing yield in crop production. Although some researches are performed in the area of cold plasma and plant seedlings, no research about the impact of plasma on germination of Iranian soybean cultivars have been examined so far. Hence, this study was performed to inspect the effect of cold plasma treatment on seedling growth, seed germination, and antioxidant enzyme activity of important soybean cultivars in Iran including Sari, Saba, Arian, Katoul, and Williams under time intervals of 30, 60, 180, 300, and 420 s of DBD using argon.

Materials and methods

Plant materials

The soybean seeds utilized in this study were sourced from Karaj Seed and the Seedling Registration and Certification Research Institute. This study utilized seeds from five distinct cultivars: Sari, Saba, Arian, Katoul, and Williams. Each cultivar comprised two categories: Registered seeds and Certified seeds. Registered seeds are the offspring of foundation seeds cultivated by specific farmers. The mean germination rate (MGR) of various soybean cultivars was 80%. The seeds were cultivated to maintain genetic identity and purity. Certified seeds are defined as seeds that originate from a recognized variety and are produced under stringent seed certification standards to ensure varietal purity. The soybean cultivars suitable for cultivation in Iran are listed in Table 1. The size and weight of soybean seeds varied significantly among different cultivars (Table 1). Katoul cultivars represent the

Table 1 Characteristics of studied soybean cultivars

Cultivar	Class (within cultivar)	The size of each seed (cm)	Weight of each seed (gr)
Sari	Registered	4.55	0.196
	Certified	4.25	0.175
Saba	Registered	5.25	0.165
	Certified	5.05	0.146
Arian	Registered	5.35	0.249
	Certified	5.15	0.219
Katoul	Registered	4.95	0.198
	Certified	4.80	0.154
Williams	Registered	4.75	0.161
	Certified	4.60	0.132

primary soybean cultivars in Golestan province, Sari, and are also the predominant cultivar in Mazandaran province. In Ardabil province (Moghan), the key soybean cultivars include Williams, Saba, and Arian. Golestan and Ardabil provinces rank first and second, respectively, in cultivation area; however, in terms of grain production, Ardabil ranks first and Golestan ranks second.

Plasma treatment

The dielectric barrier discharge (DBD) plasma, developed by High Tech Company (0045, Tehran, Iran), utilized two flat aluminum electrodes measuring 45 cm in length, 6.5 cm in width, and 0.02 cm in thickness. A mica-derived insulating sheet is placed between the electrodes to restrict the current flow to 1 μA. The dimensions of this sheet are 60 cm in length, 12 cm in width, and 0.01 cm in thickness. A plexiglass shield measuring 0.03 cm in width was installed between the electrodes. The value in the current study was measured using a thermocouple in electrodes, with the temperature recorded at approximately 40 °C. The cold plasma system was enclosed within an acrylic chamber equipped with gas inlet and outlet ports. The distance between the tip of the plasma plume and the seeds was maintained at 20 mm during plasma treatments. The existing supply was linked to an alternating current with a primary voltage of 220 V. The applied voltage and frequency on the plasma plume were 5 kV and 8 kHz, respectively, with a power output of 100 W. The feed gas utilized was argon, with a flow rate of 2 L/min. The seeds were subjected to cold plasma treatment for durations of 30, 60, 180, 300, and 420 s at the Physics Research Center of Azad University, Research and Sciences Branch, Tehran (Fig. 1).

Germination and growth parameters

A total of 25 seeds were subjected to cold plasma treatment for time intervals of 30, 60, 180, 300, and 420 s on sterile petri dishes. The seeds were subsequently placed in a germinator maintained at a constant temperature of 25 °C for a duration of seven days. The germinated seeds were counted and documented daily throughout the experiment. The criterion for seed germination was approximately 3 mm in root length. During the experiment, 5 ml of distilled water was added daily to the Petri dishes to maintain humidity for germination. The seeds were incubated in a 25 °C light incubator, and the germination percentage was recorded daily for seven days. Germination of the seeds should occur when the length of the radicals reaches half the length of the seed [21] Root and seedling lengths were measured on the seventh day, and to assess dry weight, ten seedlings were randomly selected from each repetition and dried in an oven for 48 h at 70 °C. Seedlings were placed in an oven at

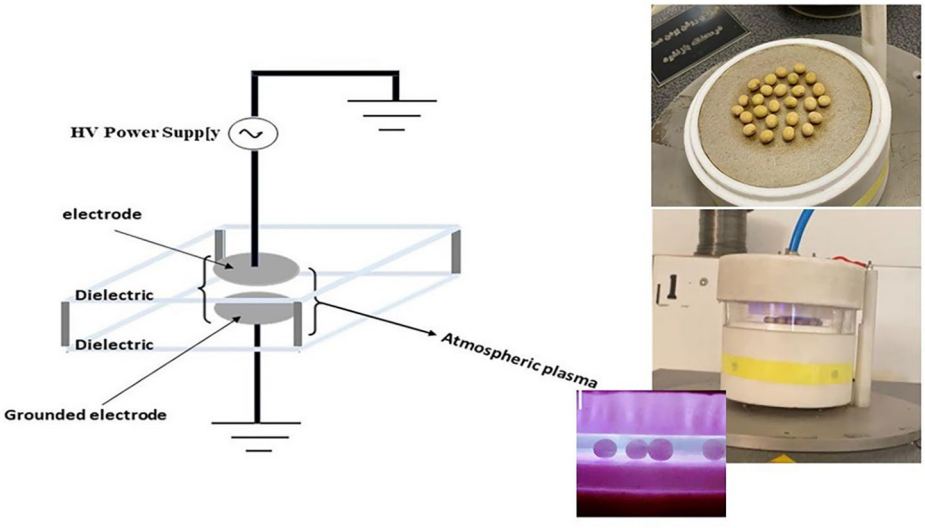

Fig. 1 Schematic of DBD treatment chamber

70 °C for 48 h to determine dry weight. The germination index (GI), germination potential (GP), and germination rate (GR) were assessed following the methodology outlined by Ling et al. (2014) [22].

Germination potential (%) = (Number of seeds germinated in 3 days /total number of seeds) × 100
Germination rate (%) = (Number of seeds germinated in 7 days /total number of seeds) × 100
Germination index = Σ (G_t/D_t)

Where, D_t shows germination days, and G_t shows the number of germinated seeds on the t day.

Enzyme extraction

The enzyme extraction was conducted following the methodology of Kar and Mishra (1976), with certain modifications [23]. Initially, 200 mg of fresh leaf tissue was weighed, cut into small pieces, and subsequently ground into a powder with a Chinese mortar with the aid of liquid nitrogen. Subsequently, 3 ml of 100 mM potassium phosphate buffer at pH 6.8 was added and thoroughly mixed. The sample was transferred to a 5 ml microtube and centrifuged at 17,000 g for 15 min at 4 °C. The supernatant was subsequently transferred to 2 ml microtubes for protein extraction and stored at -20 °C.

Enzymatic assays

Catalase (CAT)

The activity of CAT (EC 1.11.1.6) was assessed in a reaction mixture containing 50 mM K-PO_4 buffer (pH 7.0), distilled water, 15 mM hydrogen peroxide (H_2O_2), and 0.1 ml enzyme extract solution (extinction coefficient of 40 $mM^{-1}cm^{-1}$, and shown as $\mu M\ g^{-1}$). The absorbance was measured at 240 nm for 60 s [24].

Superoxide dismutase (SOD)

The activity of the superoxide dismutase (SOD) enzyme (EC 1.15.1.1) was assessed using a colorimetric method, including the measurement of formazan crystals. This was done by mixing 50 mM potassium-phosphate (K-PO_4) buffer solution (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 82.5 µL nitroblue tetrazolium (NBT), 2.2 µM riboflavin, 14.3 mM methionine, and the 200 µL of enzyme extract. The absorbance of the reaction mixture was then measured at 560 nm. Then, the negative control sample containing the above solution except the cell-free extract was placed under the fluorescent light as a control for comparison and final calculation, reporting results in U/min/mg/protein [25].

Ascorbate peroxidase (APX)

The activity of the Ascorbate peroxidase (APX) enzyme (EC 1.11.1.11) was measured based on the Asado and Nakano methodology [26]. This was done by mixing 50 mM potassium-phosphate (K-PO_4) buffer solution (pH 7.0), 0.1 mM EDTA, 0.5 mM Ascorbate, 0.1 mM H_2O_2, 10 µL enzyme extracts. The absorbance was measured at 290 nm for 60 s.

Optical emission spectroscopy

Optical emission spectroscopy (OES, Ocean Optics, HR4000CG-UV-NIR, USA) was employed to analyze the UV–visible emission spectrum linked to the generated free radicals. Voltage measurements were obtained using a high voltage probe (P6015A Tektronix HV), while current was measured with a digital oscilloscope (Tektronix MSO4032) paired with a TCP202 Tektronix current probe (5 kV) [11].

SEM (scanning Electron microscope)

Plasma-treated seeds and control seeds were examined using a scanning electron microscope (FEI S50, FEI Technologies Inc., Oregon, United States) to analyze alterations in seed structure, surface topology, and overall surface characteristics. SEM analysis was conducted on both non-treated and plasma-treated seeds, with treatment durations of 60 and 240 s. Images were captured at microscopic magnifications of 80X, 2500X, and 5000X using an accelerating voltage of 20 kV and secondary electron scanning mode, with distances of 1 mm, 50, and 20 µm [11].

Statistical analysis

The statistical analysis of the data was conducted using SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA). The data were reported as the mean ± standard error of the mean (SEM). The data underwent statistical analysis using a one-way analysis of variance (ANOVA). Given the exploratory character of the investigation, statistical significance was determined by considering probability values (P) that were less than or equal to 0.05.

Results

Seed germination and seedling properties

The results of the studied cultivars exhibited significant differences in seed germination and seedling characteristics, highlighting considerable genetic diversity among the cultivars. Plasma treatment demonstrated significant differences across all traits. The interaction effect of cultivar × time and class × time was significant, as it demonstrated the varying responses of cultivars and classes at different durations of plasma treatment (Table 2).

Seed germination properties

Germination potential (GP)

The results demonstrated a significant improvement in GP after plasma treatment ($P \leq 0.05$). The findings

Table 2 Results of analysis of variance for seed germination and seedling growth characteristics in soybean

		Means of Square					
S.O.V	**df**	**Germination percentage**	**Germination Rate**	**Germination index**	**Root length**	**Seedling length**	**Seedling dry weight**
Time	5	330.708**	255.667**	278.876**	72.995**	707.616**	0.253**
Cultivar	4	501.853**	516.071**	809.065**	106.986**	528.487**	1.455**
Cluster	1	700.057**	209.162**	1229.971**	458.587**	269.765**	0.350**
Time × Cultivar	20	36.085**	35.910**	47.429**	3.42	9.214**	0.042**
Cultivar × Cluster	4	182.91**	97.278**	228.877**	41.671**	80.426**	0.043*
Time × Cluster	5	85.203**	30.441**	65.252**	5.169**	39.672**	0.021^{ns}
Time × Cultivar × Cluster	20	34.985**	50.799**	28.567**	4.708**	17.241**	0.031**
Error	180	13.394	12.043	13.259	1.333	3.679	0.015
CV (%)	-	3.94	3.73	6.23	7.27	5.42	8.74

$^{ns, **, *}$: Not significant, significant at 1% and 5% levels

revealed that the maximum and minimum GP were 98.13±0.66% and 54.50±2.22%, respectively, as reported in the Arian-Certified report for the control treatment (Table 3). The results indicated that Williams and Sari exhibited the highest percentage of GP associated with plasma treatment at the 30th, 60th, and 180th s. The control group in Saba and Arian cultivars exhibited the lowest germination potential, as shown in Table 3. The GP for all control cultivars increased by 1.18–66.97% after 7 days of plasma treatment. The highest GP for Arian-Certified plasma treatments occurred at 60 s, while the lowest increase in germination potential compared to all control cultivars was noted for Katoul-Certified at 420 s.

Germination Rate (GR)

The results demonstrated a significant improvement in GR after plasma treatment ($P \leq 0.05$). The results indicated that the highest germination rate was 98.13±0.47%, while the lowest was 71.88±2.05%, both associated with Arian-Certified in the control treatment (Table 3). The lowest and highest percentages of increase in germination speed compared to all control cultivars were 1.78% and 32.17%, respectively, associated with Katoul-Certified and Arian-Certified treatments at the 420th and 60th s. Sari-Certified (0.93) exhibited the slowest germination speed, requiring 420 s for treatment, while Arian-Registered demonstrated the fastest rate at 6.65% treatment efficiency.

Germination index (GI)

The findings indicated a notable enhancement in GI following plasma treatment ($P \leq 0.05$). The highest GI index, exhibiting a significant difference, was associated with plasma treatment durations of 60 and 180 s for Williams and Sari cultivars. The control treatment in the Arian report exhibited the lowest GI, which was statistically significant. The cultivars exhibited a germination index ranging from 66.19±1.03% to 37.01±1.31%, with the latter value associated with Arian-Certified in the control treatment (Table 3). The seeds of Registered Arian and Certified Arian exhibited the lowest and highest percentage increases in the GI, at 3.6% and 60.09%, respectively, following 300- and 60-s treatments compared to the control. The lowest decrease in germination index, recorded at 2.44%, was observed in Arian after 420 s of plasma treatment, relative to the control group.

Seedling growth properties

Root length

The findings indicated that the Katoul cultivar exhibited the greatest root length, with a statistically significant difference observed in plasma treatment durations of 60 and 180 s. The control group in the Sari cultivar exhibited the lowest root length, demonstrating a significant difference. The minimum and maximum root lengths measured were 10.20±0.14 cm and 21.17±0.17 cm, respectively (Table 4). The lowest and highest percentages of root length increase relative to all control cultivars were 2.87% and 56.13%, attributed to Certified-Williams and Katoul-Registered, respectively, under cold plasma treatment for 420 and 180 s. Given that five cultivars were employed across two distinct classes of soybean seeds, it appears improbable that the variation in root length range can be attributed to genetic differences among the seeds.

Seedling length

The findings indicated that the Williams and Katoul cultivars exhibited the greatest seedling length, with a statistically significant difference, following plasma treatment at 30, 60, and 180 s. The control treatment exhibited the lowest seedling length with a statistically significant difference in both Sari and Arian cultivars. The minimum and maximum seedling lengths were reported as 22.00±0.19 cm and 45.20±0.82 cm, respectively (Table 5). The lowest and highest percentages of seedling length increases, 2.7% and 78.13% respectively, compared

Table 3 Effect of cold plasma on seed germination properties in soybean

Cultivar	Class (within cultivar)	Plasma treatment	Germination potential (%)	Germination Rate (seed day – 1)	Germination index
Sari	Registered	0	85.50 ± 1.26f-m	92.75 ± 0.63a-k	57.12 ± 1.18 g-p
		30	96.25 ± 0.85ab	98.13 ± 0.43a	66.19 ± 1.03a
		60	96.00 ± 1.22abc	98.00 ± 0.61a	63.94 ± 1.22a-d
		180	98.13 ± 0.66a	98.13 ± 0.66a	64.31 ± 1.25abc
		300	93.75 ± 0.75a-e	94.88 ± 0.37a-h	62.85 ± 0.69a-f
		420	95.25 ± 1.80a-d	97.13 ± 1.39a-d	63.51 ± 1.49a-d
	Certified	0	88.25 ± 1.11d-l	94.13 ± 0.55a-h	58.34 ± 1.06d-m
		30	94.50 ± 0.29a-d	97.25 ± 0.14abc	62.44 ± 0.29a-g
		60	95.75 ± 0.48abc	97.88 ± 0.24a	63.69 ± 0.48a-d
		180	95.75 ± 1.49abc	97.38 ± 0.97abc	63.69 ± 0.48a-d
		300	95.50 ± 1.50a-d	97.25 ± 1.11abc	63.69 ± 0.48a-d
		420	90.00 ± 1.29b-i	93.25 ± 1.44a-j	63.69 ± 0.48a-d
Saba	Registered	0	72.75 ± 3.66p	82.38 ± 2.38n	63.69 ± 0.48a-d
		30	85.75 ± 3.50f-m	88.88 ± 3.33 g-m	55.40 ± 2.81j-r
		60	88.75 ± 1.93c-k	92.38 ± 1.76a-l	57.48 ± 1.84f-o
		180	91.00 ± 1.29a-h	94.00 ± 1.47a-h	59.41 ± 1.15c-l
		300	81.75 ± 1.44k-o	87.88 ± 1.71i-n	51.91 ± 1.15o-t
		420	84.50 ± 1.76 h-n	90.25 ± 2.03e-l	49.99 ± 0.62rst
	Certified	0	79.50 ± 1.76mno	86.75 ± 2.11k-n	50.61 ± 2.10q-t
		30	86.50 ± 1.55f-m	92.25 ± 1.76a-l	55.67 ± 1.15i-q
		60	85.50 ± 1.19f-m	91.25 ± 0.92c-l	54.88 ± 1.46j-r
		180	84.75 ± 1.80 g-n	89.88 ± 1.83f-m	54.18 ± 1.62k-s
		300	88.25 ± 3.17d-l	94.63 ± 2.78a-h	56.53 ± 3.09i-p
		420	87.25 ± 3.12e-l	90.38 ± 2.55e-l	56.84 ± 2.20 h-p
Arian	Registered	0	84.50 ± 0.65 h-n	90.25 ± 0.66e-l	54.05 ± 0.66 L-s
		30	90.75 ± 2.14a-h	93.88 ± 1.51a-i	59.90 ± 1.88b-j
		60	92.00 ± 2.12a-g	96.00 ± 1.06a-f	62.31 ± 1.09a-h
		180	92.25 ± 1.65a-f	94.63 ± 1.25a-h	61.15 ± 1.42a-i
		300	85.50 ± 4.33f-m	87.63 ± 3.13j-n	56.00 ± 3.27i-q
		420	81.50 ± 3.38k-o	84.25 ± 3.90mn	52.73 ± 2.01 m-t
	Certified	0	54.50 ± 2.22q	71.88 ± 2.05 o	37.01 ± 1.31v
		30	84.75 ± 0.75 g-n	91.00 ± 0.84d-l	48.52 ± 3.64t
		60	91.00 ± 2.71a-h	95.00 ± 1.85a-g	59.26 ± 2.39c-l
		180	89.75 ± 1.55b-j	91.25 ± 3.44c-l	57.81 ± 0.66e-n
		300	86.50 ± 4.09f-m	91.25 ± 3.44c-l	52.80 ± 2.07 m-t
		420	82.50 ± 3.62j-o	88.75 ± 3.26 h-m	52.61 ± 2.43n-t
Katoul	Registered	0	81.25 ± 0.75 L-o	90.25 ± 0.43e-l	54.89 ± 0.44j-r
		30	94.75 ± 1.65a-d	97.38 ± 0.83abc	63.44 ± 1.51a-d
		60	96.25 ± 0.95ab	98.13 ± 0.47a	63.44 ± 0.29a-d
		180	93.75 ± 1.93a-e	96.38 ± 1.46a-e	62.08 ± 1.63a-h
		300	90.25 ± 5.19b-i	94.63 ± 2.55a-h	63.76 ± 0.81a-d
		420	94.00 ± 2.45a-e	96.00 ± 1.78a-f	63.76 ± 1.37a-d
	Certified	0	85.50 ± 1.26f-m	92.75 ± 0.63a-k	43.63 ± 1.03u
		30	83.00 ± 2.92i-o	91.50 ± 1.46b-l	51.76 ± 2.21p-t
		60	83.00 ± 2.92i-o	91.50 ± 1.46b-l	54.21 ± 1.69k-s
		180	76.50 ± 2.33op	82.75 ± 2.38n	48.89 ± 1.31st
		300	78.00 ± 2.16nop	86.50 ± 2.25lmn	48.91 ± 1.29st
		420	85.50 ± 1.26f-m	92.75 ± 0.63a-k	52.49 ± 1.72n-t
Williams	Registered	0	83.25 ± 1.38i-o	89.63 ± 0.69 g-m	59.73 ± 4.74b-k
		30	96.00 ± 1.29abc	97.50 ± 0.91ab	64.63 ± 1.06abc
		60	96.00 ± 1.87abc	97.50 ± 1.40ab	64.26 ± 1.59abc
		180	96.75 ± 0.48ab	97.88 ± 0.66a	65.01 ± 0.41abc
		300	96.75 ± 1.31ab	98.38 ± 0.66a	65.31 ± 0.90ab

Table 3 (continued)

Cultivar	Class (within cultivar)	Plasma treatment	Germination potential (%)	Germination Rate (seed day – 1)	Germination index
		420	95.25 ± 2.59a-d	96.13 ± 2.75a-e	64.76 ± 1.27abc
	Certified	0	85.50 ± 1.26f-m	92.75 ± 0.63a-k	54.25 ± 1.73k-s
		30	95.50 ± 1.04a-d	97.75 ± 0.52a	63.76 ± 1.11a-d
		60	96.75 ± 0.75ab	98.38 ± 0.38a	64.19 ± 0.63abc
		180	96.50 ± 0.87ab	97.75 ± 0.92a	64.76 ± 0.56abc
		300	94.75 ± 1.93a-d	96.88 ± 1.46a-d	63.34 ± 1.46a-e
		420	93.75 ± 1.31a-e	95.88 ± 1.16a-f	63.24 ± 1.00a-e

The data were expressed as the mean ± standard error of mean (Se). Different letters within a column indicate significant differences as determined by the Duncan test ($P \leq 0.01$) and ($P \leq 0.05$)

to the control group, were observed in Saba-Certified and Saba-Registered at 30 and 60 s of treatment.

Seedling dry weight

The results indicated that the highest seedling dry weight was observed in the 30, 60, and 180 s plasma treatments for the Williams and Katoul cultivars. The control treatment exhibited the lowest seedling dry weight in both Sari and Saba cultivars. The minimum and maximum seedling dry weights recorded were 1.07 ± 0.08 g and 1.83 ± 0.04 g, respectively (Table 4). The lowest and highest percentages of seedling dry weight increase were observed in the 30- and 300-s treatments, respectively, compared to the control group (1.4% and 36.6%). These increases were associated with the Registered-Sari and Registered-Katoul classes (Table 4). The Saba-Certified exhibited the lowest and highest percentages of seedling dry weight loss at 1.47% and 9.61%, respectively, observed during the 300 and 420 s of treatment.

The activity of antioxidant enzymes

Catalase enzyme (CAT)

The results indicated that the highest CAT activity was observed in the Katoul cultivar at the 60 s treatment, whereas the lowest values were recorded in the Saba-Certified, Arian- Registered, and Arian- Certified cultivars following 300 and 420 s of plasma treatment. The range of CAT activities varied from −0.88 to 4.40 times greater than the control condition. The findings indicated that the CAT varied from 20.19 ± 0.76 to 148.97 ± 0.51 μM g-1 FW min^{-1} (Table 6). The results indicate that Sari-Registered exhibited the highest CAT activity at the 60 s mark of treatment compared to the control group. Conversely, the lowest values were observed in Saba-Certified, Arian-Registered, and Arian-Certified at the 0, 300, and 420 s intervals of plasma treatment, respectively. The antioxidant activity of catalase varied from −0.88 to 4.40 times greater than that of all control cultivars.

Superoxide dismutase (SOD)

The findings indicated that the highest SOD activity was associated with the 60 s treatment of the Williams cultivar. Conversely, the lowest SOD activity, with a significant difference, was observed in the Arian and Saba cultivars during the 420 s treatment and control groups. The SOD activity varied from 9.11 ± 1.16 to 87.91 ± 1.32 μMg^{-1} FW min^{-1} across all samples (Table 6). Additionally, the highest SOD activity was recorded in Katoul-Registered, Katoul-Certified, and Williams-Registered under the 60 s treatment compared to control cultivars, while the Sari-Certified treatments at 0, 300, and 420 s exhibited the lowest SOD activity. An increase in SOD activity was noted, ranging from 0.86 to 5.89 times greater than that of all control cultivars.

Ascorbate peroxidase (APX)

The findings indicated that the Sari and Katoul cultivars exhibited the highest and lowest APX values, respectively, observed at 60 and 420 s of treatment. The APX values varied from 16.31 ± 0.82 to 97.84 ± 0.81 μMg^{-1} FW min^{-1} across all samples (Table 6). The highest levels of APX were observed in Sari-Registered and Saba-Registered across the treatments of 30, 60, and 180 s. The lowest antioxidant activity of ascorbate peroxidase was observed in Katoul-Certified during the control at 420 s of treatment. The antioxidant activity of APX was reported to be approximately 0.40 to 4.01 times greater than that of all control cultivars.

Optical emission spectroscopy

The results indicated that the species identified in the optical emission spectrum included OH (309 nm), N2I (380 nm), N2II (405.79 nm), oxygen atoms (755 and 842 nm), and argon (from 696 to 861.8 nm), as represented by the horizontal bar in Fig. 2. The results indicated that the N2 generated by Plasma significantly enhanced biological processes in seeds.

Scanning Electron microscope (SEM)

The results indicated that the maximum and minimum morphological characteristics of the seed surface were observed at 60 and 420 s, respectively. Results indicated that there were significant differences in the levels of seeds treated for 420 s when compared to those treated

Table 4 Effect of cold plasma on seeding properties in soybean

Cultivar	Class (within cultivar)	Plasma Treatment	Root length (cm)	Seedling length (cm)	Seedling dry weight (gr)
Sari	Registered	0	10.20 ± 0.14z	23.52 ± 0.45y	1.07 ± 0.08y
		30	14.67 ± 0.13o-s	34.35 ± 75n-u	1.17 ± 0.03u-y
		60	13.92 ± 0.35q-v	34.17 ± 0.52p-u	1.16 ± 0.03u-y
		180	14.87 ± 0.38o-r	34.57 ± 0.61 m-u	1.35 ± 0.02k-u
		300	13.75 ± 0.86q-v	30.75 ± 0.63vw	1.08 ± 0.04xy
		420	11.47 ± 0.54y	27.25 ± 0.37x	1.13 ± 0.07v-y
	Certified	0	12.1 ± 0.12w-y	22.57 ± 0.5y	1.27 ± 0.04p-y
		30	14.02 ± 0.25p-v	34.22 ± 0.22q-u	1.22 ± 0.03r-y
		60	15.17 ± 0.22 n-q	36.37 ± 0.46k-p	1.36 ± 0.04k-u
		180	14.97 ± 0.22 o-r	35.10 ± 0.31 m-t	1.22 ± 0.03r-y
		300	14.00 ± 0.32q-v	34.72 ± 0.39 m-u	1.22 ± 0.03r-y
		420	13.67 ± 0.28r-n	33.17 ± 0.19s-v	1.22 ± 0.03r-y
Saba	Registered	0	14.87 ± 0.94o-r	22.97 ± 0.69y	1.22 ± 0.03r-y
		30	16.82 ± 0.26i-l	36.00 ± 0.37 L-r	1.24 ± 0.05q-y
		60	21.17 ± 0.17a	40.92 ± 0.75c-f	1.32 ± 0.02 m-w
		180	20.60 ± 0.34ab	40.15 ± 0.35d-g	1.35 ± 0.05 L-u
		300	20.52 ± 0.62ab	39.57 ± 0.39d-i	1.16 ± 0.09u-y
		420	20.15 ± 0.65abc	39.47 ± 0.69d-i	1.28 ± 0.06o-x
	Certified	0	11.87 ± 0.15xy	32.50 ± 0.81t-w	1.23 ± 0.04q-y
		30	13.30 ± 0.17s-w	32.22 ± 0.14r-v	1.20 ± 0.08t-y
		60	14.87 ± 0.21o-r	34.60 ± 0.42 m-u	1.31 ± 0.04 m-w
		180	13.87 ± 0.21q-v	34.82 ± 0.16 m-t	1.29 ± 0.03n-w
		300	13.62 ± 0.17r-w	34.32 ± 59n-u	1.21 ± 0.02s-y
		420	13.30 ± 0.18s-w	33.32 ± 0.42q-v	1.11 ± 0.05wxy
Arian	Registered	0	12.60 ± 0.29v-y	22.05 ± 0.37y	1.28 ± 0.050-x
		30	15.75 ± 0.60 L-o	34.50 ± 1.04 m-u	1.62 ± 0.10b-h
		60	14.25 ± 0.09p-u	32.76 ± 0.29t-v	1.54 ± 0.04c-l
		180	14.70 ± 0.12o-s	31.97 ± 0.23 m-u	1.35 ± 0.07 L-u
		300	14.03 ± 0.09 p-t	30.77 ± 0.07vw	1.39 ± 0.08i-t
		420	14.00 ± 0.03q-v	29.97 ± 0.14w	1.30 ± 0.06 m-w
	Certified	0	13.7 ± 0.46r-v	22.00 ± 0.19y	1.31 ± 0.03 m-w
		30	18.52 ± 0.25d-h	36.70 ± 0.22j-p	1.65 ± 0.09a-f
		60	18.97 ± 0.13c-f	37.10 ± 0.14 h-n	1.62 ± 0.11b-g
		180	19.75 ± 0.20bcd	37.87 ± 0.12 g-l	1.67 ± 0.09a-e
		300	18.22 ± 0.21e-h	36.00 ± 0.24 L-r	1.58 ± 0.10c-i
		420	17.77 ± 0.35fk	35.90 ± 0.18 L-s	1.60 ± 0.08b-h
Katoul	Registered	0	12.65 ± 0.66v-y	30.82 ± 0.61vw	1.31 ± 0.03 m-w
		30	16.42 ± 0.46k-n	40.55 ± 0.85c-g	1.47 ± 0.11f-o
		60	18.02 ± 0.67e-i	41.95 ± 0.51b-e	1.61 ± 0.05b-h
		180	19.75 ± 0.92bcd	43.94 ± 0.94abc	1.73 ± 0.03abc
		300	18.37 ± 1.13d-h	42.15 ± 0.52bcd	1.79 ± 0.08ab
		420	17.62 ± 0.61 g-k	39.35 ± 0.47e-i	1.50 ± 0.11d-m
	Certified	0	16.50 ± 0.37 j-n	30.77 ± 0.42vw	1.38 ± 0.03j-t
		30	18.85 ± 0.89c-h	39.82 ± 0.52d-h	52.35 ± 0.04a
		60	18.80 ± 0.10c-h	39.22 ± 0.15f-i	1.74 ± 0.04abc
		180	19.30 ± 0.44bcd	40.17 ± 0.97d-g	1.69 ± 0.04a-d
		300	18.68 ± 0.50d-h	38.35 ± 0.58f-l	1.73 ± 0.07abc
		420	17.47 ± 0.47 h-k	36.02 ± 0.29 L-q	1.69 ± 0.03a-d
Williams	Foundation	0	16.00 ± 0.34 L-o	31.42 ± 1.02vw	1.31 ± 0.06 m-w
		30	16.80 ± 0.16i-m	44.03 ± 0.18ab	1.49 ± 0.04e-n
		60	18.65 ± 0.31d-h	44.50 ± 0.30ab	1.61 ± 0.04b-h
		180	19.1 ± 0.37c-f	45.20 ± 0.82a	1.42 ± 0.10 g-r
		300	18.45 ± 0.53d-h	42.90 ± 0.92abc	1.47 ± 0.04f-p

Table 4 (continued)

Cultivar	Class (within cultivar)	Plasma Treatment	Root length (cm)	Seedling length (cm)	Seedling dry weight (gr)
		420	17.85 ± 0.42f-j	40.60 ± 1.23c-g	1.57 ± 0.06c-j
	Certified	0	12.82 ± 0.43u-y	30.62 ± 0.39vw	1.23 ± 0.03q-y
		30	15.45 ± 020 m-p	39.05 ± 0.40f-k	1.55 ± 0.03c-k
		60	14.2 ± 0.21p-u	37.25 ± 0.16 h-m	1.43 ± 0.06 g-q
		180	13.80 ± 0.22q-v	37.00 ± 0.15i-o	1.58 ± 0.04c-i
		300	13.32 ± 0.15s-w	37.27 ± 0.16 h-m	1.46 ± 0.05f-p
		420	13.90 ± 0.16t-x	37.10 ± 0.17 h-n	1.41 ± 0.05 h-s

Table 5 Results of analysis of variance for catalase enzyme (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX)

		Means of Square		
S.O.V	df	CAT	SOD	APX
Time	5	33827.191**	24030.046**	28339.375**
Cultivar	4	8173.209**	3072.797**	851.359**
Cluster	1	3563.15**	1841.181**	6188.065**
Time × Cultivar	20	370.274**	304.585**	173.278**
Cultivar × Cluster	4	2564.238**	233.108**	314.454**
Time × Cluster	5	1142.240**	208.07**	145.001**
Time × Cultivar × Cluster	20	432.366**	21.678**	92.415**
Error	180	14.452	15.216	7.632
CV (%)	-	6.25	8.58	4.99

**: significant at 1% probability level

for 60 s and the untreated seeds. The seed structure exhibited minor alterations at 60 s relative to the control group. Significant changes in the level of treated seeds are observed in SEM images at 420 s. The results indicated that plasma significantly altered the morphological properties during the 420 s treatments (Fig. 3). The growth and germination parameters appeared to be influenced by the duration of plasma treatment and the type of plasma discharge. A reduced duration correlates with enhanced outcomes for seed germination.

Discussion

This study demonstrated that cold plasma significantly influenced soybean seed germination. The findings indicated that cold plasma treatment enhanced antioxidant activity and promoted soybean seed germination. The cold plasma technique has the potential to modify surface roughness and chemical structure, resulting in significant alterations to the seed surface that may influence water uptake and enhance germination (Fig. 2). The current research demonstrated that cold plasma treatment significantly increased seed weight, consistent with the findings of Šerá et al., 2021 [27], Guo et al., 2018 [28], and Ling et al., 2014 [7].

Plasma treatment durations of 60 s and 180 s enhanced germination potential, germination index, and germination rate relative to the control group. Ling et al. (2014) observed a significant increase in germination potential, with a rise of 14.7% relative to the control treatment, following 15 s of 80 W plasma treatment [29]. Li et al. (2017) demonstrated that the application of dielectric-barrier plasma in an air atmosphere for 7 min resulted in a 26.7% increase in germination potential in wheat following 7 min of DBD plasma treatment [30]. Fereydoni and Alizadeh (2022) found that the germination percentage potential of chickpea cultivars increased by 2% following 30 s of plasma treatment and by 11% after 60 s, relative to the control treatment [31]. Additionally, a study conducted by Pizá et al. (2018) investigated the exposure of soybeans to dielectric-barrier plasma at atmospheric pressure and a frequency of 50 Hz, utilizing nitrogen and oxygen gas sources for durations of 1, 2, and 3 min. A significant stimulating effect on germination potential was observed. The findings of these studies were consistent with the current research [16]. This study observed a maximum percentage increase in germination potential of 66.97% at treatment durations of 30, 60, and 180 s, in comparison to all control treatment figures, indicating a notable enhancement.

Švubová et al. (2021) reported germination inhibition following nitrogen plasma treatment for durations of 90 and 120 s [20]. In other treatments (30 and 60 s), a higher germination percentage was noted compared to the control [32], aligning with the findings of the current study. This study indicated that plasma treatment durations of 30, 60, and 180 s across all examined cultivars resulted in the highest germination potential. The germination potential decreased by 3.5% relative to the control treatment associated with Arian - Plasma treatment was

Table 6 Effect of cold plasma on the activity of antioxidant enzymes in soybean

Cultivar	Class (within cultivar)	Plasma Treatment	CAT activity ($\mu M\ g^{-1}\ FW\ min^{-1}$)	SOD activity ($\mu M\ g^{-1}\ FW\ min^{-1}$)	APX activity ($\mu M\ g^{-1}\ FW\ min^{-1}$)
Sari	Registered	0	33.82 ± 0.7v	17.99 ± 0.49tuv	36.42 ± 1.02np
		30	130 ± 1.1c	72.86 ± 0.94ef	96.03 ± 0.7ab
		60	148.97 ± 0.51a	77.48 ± 0.58cde	97.48 ± 0.81a
		180	133.72 ± 1.06b	73.47 ± 0.78def	95.31 ± 0.80ab
		300	34.6 ± 0.78v	16.4 ± 0.48uv	36.45 ± 0.84np
		420	31.89 ± 0.62 ±	15.40 ± 0.42vw	33.47 ± 0.40o-r
	Certified	0	39.07 ± 0.44u	10.65 ± 0.41wx	27.15 ± 0.94uvw
		30	75.45 ± 0.27klm	56.90 ± 0.28 lm	76.98 ± 0.69e-j
		60	79.69 ± 0.69j	62.76 ± 0.37 h-k	79.81 ± 0.47e
		180	74.14 ± 0.16 lm	57 ± 1.15 lm	77.87 ± 0.62e-j
		300	39.75 ± 1.44tu	10.75 ± 0.67wx	26.58 ± 0.63vw
		420	39.53 ± 1.27tu	9.11 ± 1.61x	24.98 ± 0.56w
Saba	Registered	0	39.24 ± 0.41u	20.00 ± 0.67s-v	54.8 ± 0.89 m
		30	72.45 ± 0.84mn	58.19 ± 1.03jkl	94.78 ± 0.54ab
		60	78.15 ± 0.50jk	60.03 ± 0.86i-l	93.97 ± 0.59b
		180	76.89 ± 0.17jkl	58.46 ± 0.29jkl	95.16 ± 0.65ab
		300	38.71 ± 1.17u	22.58 ± 0.87r-t	21.93 ± 1.12x
		420	38.83 ± 0.73u	21.80 ± 0.78r-u	25.30 ± 0.55w
	Certified	0	22.32 ± 0.32w	24 ± 0.47rs	33.84 ± 0.62o-q
		30	61.87 ± 0.5q	51.38 ± 1.10 m	74.77 ± 0.55f-k
		60	67.10 ± 0.52op	57.27 ± 0.72kl	77.31 ± 0.36e-l
		180	62.55 ± 0.5q	51.53 ± 0.83 m	73.31 ± 1.15jk
		300	22.78 ± 1.47w	27.20 ± 0.77r	32.94 ± 1.29p-s
		420	21.86 ± 1.85w	25.32 ± 0.81r-u	21.53 ± 1.17x
Arian	Registered	0	22.93 ± 0.97w	17.06 ± 0.72uv	35.61 ± 0.79np
		30	74.66 ± 1.94 lm	63.58 ± 0.977 h-j	74.41 ± 0.85f-k
		60	78.13 ± 1.08jk	68.17 ± 0.62fgh	75.89 ± 0.48f-j
		180	74.61 ± 0.94 lm	62.98 ± 0.87 h-k	74.18 ± 0.43 h-k
		300	23.23 ± 1.40w	20.96 ± 1.02s-v	35.87 ± 0.49np
		420	20.19 ± 0.76w	21.09 ± 0.64s-v	31.48 ± 0.90q-t
	Certified	0	21.12 ± 0.67w	16.4 ± 0.83uv	29.79 ± 1.45s-v
		30	64.77 ± 0.71pq	41.84 ± 0.25n	72.13 ± 0.85k
		60	69.94 ± 0.5no	64.43 ± 1.4 g-i	77.94 ± 0.5ef
		180	64.74 ± 1.52pq	54.25 ± 0.39 lm	73.49 ± 0.45jk
		300	22.28 ± 0.92w	18.21 ± 0.36tuv	30.19 ± 1.49p-u
		420	21.12 ± 1.2w	18.02 ± 0.75tuv	31.48 ± 0.9q-t
Katoul	Registered	0	51.90 ± 0.9r	20.33 ± 0.70s-v	36.63 ± 0.42no
		30	114.61 ± 1.62e	80.6 ± 1.09bc	74.30 ± 1.083 g-k
		60	118.05 ± 1.09d	87.91 ± 1.32a	77.73 ± 0.32e-h
		180	114.45 ± 1.87e	82.30 ± 0.74bc	74.83 ± 0.76f-k
		300	54.03 ± 0.86r	25.75 ± 0.93rs	37.01 ± 2.13no
		420	53.10 ± 0.48r	24.39 ± 1.16rs	38.02 ± 1.07n
	Certified	0	44.28 ± 0.99s	21.33 ± 0.71r-v	16.31 ± 0.82y
		30	95.57 ± 0.59 g	77.44 ± 0.45cde	62.77 ± 0.52 L
		60	99.39 ± 1.0f	85.2 ± 0.43ab	65.52 ± 0.40 L
		180	95.37 ± 0.65 g	79.64 ± 1.35bc	63.40 ± 0.75 L
		300	45.08 ± 1.27s	25.23 ± 0.79rs	19.64 ± 1.38x
		420	42.47 ± 0.77st	24.49 ± 1.03rs	19.29 ± 1.39xy
Williams	Registered	0	33.26 ± 1.36v	35.26 ± 0.68np	35 ± 26 ± np
		30	84.24 ± 0.65kl	86.03 ± 0.32d	86.03 ± 0.32d
		60	90.14 ± 0.93j	89.65 ± 0.29c	89.26 ± 0.29c
		180	86.02 ± 1.16kl	87.93 ± 0.46 cd	87.93 ± 0.46 cd

Table 6 (continued)

Cultivar	Class (within cultivar)	Plasma Treatment	CAT activity (μM g^{-1} FW min^{-1})	SOD activity (μM g^{-1} FW min^{-1})	APX activity (μM g^{-1} FW min^{-1})
		300	34.33 ± 0.85v	36.15 ± 1.42np	36.15 ± 1.42np
		420	32.74 ± 0.72v	35.04 ± 1.03np	35.04 ± 1.03np
	Certified	0	34.70 ± 0.69v	32.79 ± 1.33q	28.9 ± 0.21tuw
		30	75.93 ± 1.21i	67.22 ± 1.73gh	73.96 ± 0.51ijk
		60	79.54 ± 1.2 h	69.87 ± 1.07 fg	75.78 ± 0.84f-j
		180	76.13 ± 1.83i	66.87 ± 0.35gh	73.4 ± 0.18jk
		300	34.63 ± 0.70v	35.12 ± 1.34opq	26.69 ± 2.32vw
		420	33.44 ± 0.92v	34.14 ± 0.61pq	29.83 ± 1.07

The data were expressed as the mean ± standard error of mean (Se). Different letters within a column indicate significant differences as determined by the Duncan test ($P \leq 0.01$)

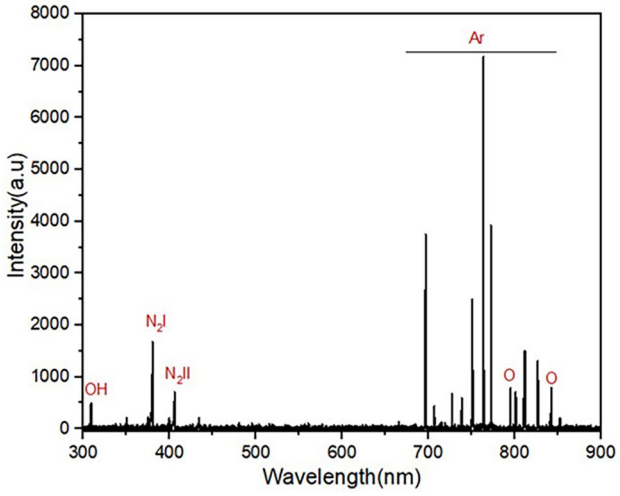

Fig. 2 Optical emission spectra of DBD plasma

registered, with a reported duration of 420 s, attributed to its destructive effects.

The impact of plasma irradiation duration on grain morphology was examined over 2- and 5-min intervals in a separate study. The results indicated an increase in seed pattern intensity, likely due to the removal of upper cuticle layers that may have been coated with wax during the 2 min plasma treatment. Extended exposure of seeds to plasma influenced the internal structure of the cuticle, resulting in damage or rupture in certain areas of the cuticle. Also, based on the results, a 5-min plasma treatment exhibited a destructive effect [33].

The SEM images obtained in this study indicated significant alterations in the surface of seeds subjected to cold plasma treatment for 60 s, resulting in the formation of cracks and porosity. These changes significantly influenced the water penetration of the seeds when compared to the control treatment. This results in enhanced seed germination. The results of the plasma treatment at 420 s demonstrated significant alterations in seed morphology, specifically evidenced by cracking in portions of the seed coat. Plasma treatment lasting less than 420 s significantly affected germination, growth, and enzyme activity traits. Increased plasma irradiation duration results in a higher formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) [34–37].

Extending the exposure of seeds to plasma may yield diverse outcomes. More significant alterations in the seed coat, particularly in the regions where it is detached from the peripheral sides, are observed. In certain cases, particularly at an exposure time of 420 s, seed splitting and significant changes in seed coat morphology are noted. Plasma interacts with biological material in various ways [38]. Excessive exposure likely results in structural degradation, volatilization, and cellular etching. Consequently, an increase in exposure time correlates with a greater overall effect. The alterations in the surface structure of seeds are likely attributable to the effects of gases produced, such as NO_2, O_3, and NO, as prolonged plasma irradiation may induce nitrogen stress and excessive oxidative conditions [39]. This may explain the reduced germination energy and capacity observed after the extended exposure of seeds to plasma.

Li et al. (2017) demonstrated that the application of dielectric barrier plasma in an air atmosphere for 7 min resulted in a 9.1% increase in wheat germination rates [40]. Švubová et al. (2021) subjected soybean seeds to plasma treatment in oxygen, nitrogen, and ambient atmospheres for durations of 30, 60, 90, and 120 s. The rate of seed germination increased by 20% in all treatments except the nitrogen plasma treatment at 90 and 120 s. This is likely because the plasma improved the activity of enzymes that are important in the early stages of germination [20]. The current research demonstrated that the germination rate increased by up to 32.17% in the 60-s treatment, representing the highest rate among all control cultivars. This represents a notable increase relative to the outcomes observed in other experiments. Additionally, the electron microscope images in the studies by Hosseini et al. (2018) demonstrated that plasma treatment induced micrometric fractures in the hard coating of artichoke, which ultimately enhanced the germination rate by increasing water absorption and facilitating

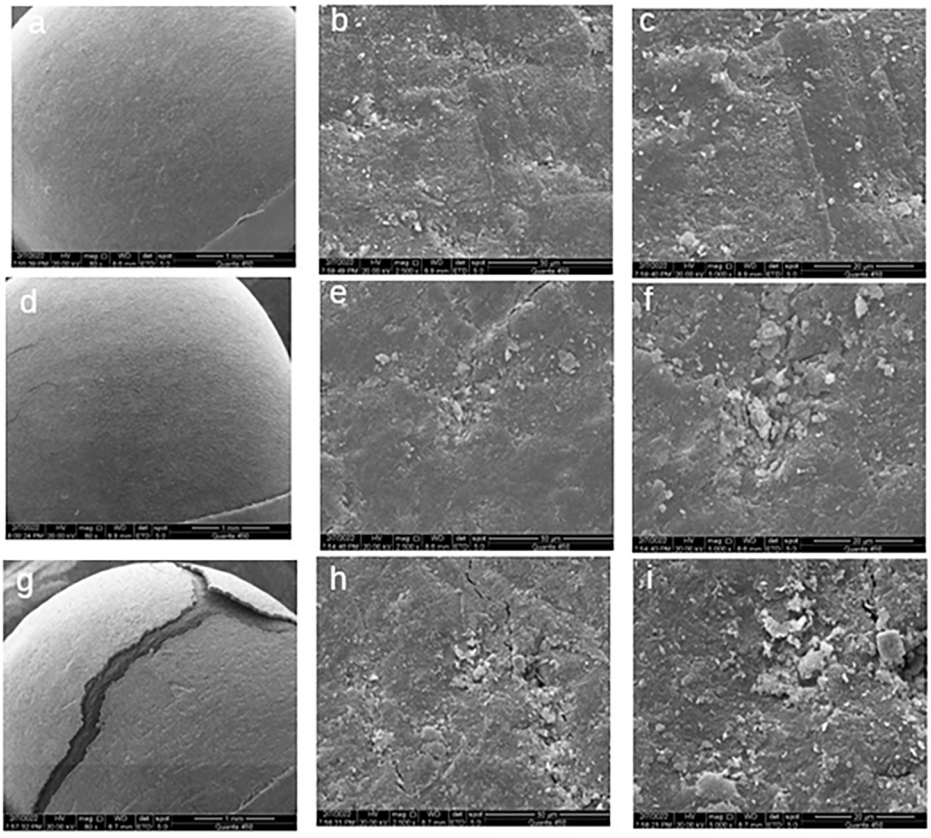

Fig. 3 SEM images of the seeds surface at different scales, before treatment (**a-c**), after 60 s plasma treatment (**d-f**), and after 420 s plasma treatment (**g-i**)

the breaking of the seed shell [41]. Gómez-Ramírez et al. (2017) posited that the observed increase in germination rate may be attributed to plasma etching on the seed coat, which disrupts the macromolecules present on the seed surface. This disruption facilitates the release of nitrate functional groups from the seed coat into the seed [42].

The mechanism for enhancing germination rates is associated with the seed surface structure, the capacity to rupture the seed coat, water absorption, and the release of chemically active species, such as reactive oxygen species (ROS), generated by atmospheric cold plasma through the seed shell. The absorption of water by the seed facilitates the movement of gibberellic acid hormones to the aleurone layer of the endosperm, resulting in enhanced expression of the alpha-amylase enzyme. The resulting alpha-amylase hydrolyzes nutrients stored in the endosperm, including starch, into sugars. The sugar generated as an energy source enhances the growth of the embryo during the germination phase [43]. Li et al. (2017) observed that exposure of dielectric barrier plasma to air for 7 min resulted in a 16.9% increase in the germination index of wheat [40]. Guo et al. (2018) observed a 13.9% enhancement in the germination index of wheat plants subjected to an 11 kV treatment for 4 min, relative to the control group [28]. The results of the present study indicated that the highest percentage increase in the germination index among various cultivars reached 60.09% after 60 s of treatment, in comparison to the control figures, representing a significant enhancement.

The oxidation processes of reactive plasma species enhance water absorption capacity by improving the wettability of the seed coat. They may also be associated with gas exchange and electrolyte leakage in the seed. Cold plasma may effectively alter the dormancy of rigid seeds by influencing seed permeability and initiating subsequent processes. Cold plasma positively influences seed germination and growth, subsequently enhancing seedling traits. It can reduce the stiffness associated with mechanical dormancy in dark legume species, including blue lupine, alfalfa, green peas, *Mimosa* sp., and *Trifolium* sp. Cold plasma can be employed to disinfect the surfaces of plant seeds and leguminous products. Legumes exhibit a high tolerance to the specified physicochemical treatment, and the mild tension generated by plasma has a beneficial effect on them. Changes in physiological factors can enhance both the quantity of plants in the field and their performance [44].

Multiple studies have reported a significant increase in root length following cold plasma treatment in soybean,

wheat, sesame, and chickpea, consistent with the findings of this study [45–47]. The current investigation revealed a significant increase in average root length across all cold plasma treatments when compared to the control measurements. Ling et al. (2014) observed that a 15-s treatment of 80 W cold plasma resulted in a notable increase in soybean seedling size by 13.8% relative to the control group [29]. Studies have documented a notable increase in seedling length following cold plasma treatment in wheat [30, 48, 49], *Catharanthus roseus* [50], and *sesame* [51], which aligns with the findings of the current study. Filatova et al. (2014) indicated that molecular nitrogen generated by plasma enhances root length, seedling length, and enzyme activity. Studies have demonstrated that plasma significantly boosts the oxidation of chemical compounds in seed shells by producing oxygen molecules, which in turn stimulates biochemical processes that aid in seed germination [52]. This study observed the highest percentage increase in seedling length, reaching 78.13% compared to the control at the 60-s treatment mark. Ling et al. (2014) observed that, relative to the control group in soybeans, an 80 W cold plasma treatment for 15 s resulted in a significant increase in shoot dry weight by 21.9% [7]. Li et al. (2017) observed that exposure to dielectric-barrier plasma in an air atmosphere for 7 min improved both the fresh weight and dry weight of wheat seedlings [40]. The findings indicated that the 300-s treatment resulted in the highest percent increase in dry-weight seedlings, measuring 36.6% relative to the control group. Zhang et al. (2018) observed an enhancement in peroxidase and catalase activity following DBD-argon plasma treatment of soybean seeds [53].

The findings of Pizá et al. (2018) indicated that plasma treatment of soybeans using various gas sources (nitrogen and oxygen) for durations of 1, 2, and 3 min resulted in a 29% reduction in catalase activity in healthy seeds, while superoxide dismutase activity showed no significant difference between treated and control seeds. The findings indicated that the low levels of catalase activity, glutathione content, and lipid peroxidation in plasma-treated seeds suggest that hydrogen peroxide (H_2O_2) generated during plasma treatment did not stimulate the antioxidant defense mechanisms nor inflict damage on the cell membrane. Hydrogen peroxide that is produced can function as a signaling molecule in the induction of germination [16]. In 2021, Švubová et al. did a study on cumin seeds that were treated with cold plasma for 5 and 10 min. They found that there were no significant differences in the levels of catalase enzymes and superoxide dismutase between control plants and those that were treated with plasma for 5 min. However, the enzymes ascorbate peroxidase and glutathione reductase exhibited a significant reduction in comparison to the control group. A 10-min plasma treatment resulted in an increase in superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase enzymes compared to the control [20]. A study by Hosseini et al. (2018) investigated the effects of cold plasma on soya utilized various gas sources (nitrogen, air, and oxygen) for durations of 30, 60, 90, and 120 s. They showed that the activity of superoxide dismutase increased following all plasma treatments, with the exception of nitrogen plasma at 90 and 120 s, when compared to the control group [41]. Rasooli et al. (2021) demonstrated that a 5-min plasma treatment for seed pretreatment enhanced nutrient uptake and increased the germination index, seedling height, root length, and total dry weight of cumin seeds. The results also showed that priming seeds with plasma for 10 min increased the activities of SOD, CAT, APX, and glutathione reductase enzymes compared to the control group, which is similar to the results of this study [54]. This study demonstrated that cold plasma treatment for durations of 60 and 180 s significantly influenced seed germination characteristics and seedling development. Furthermore, the plasma treatment significantly influenced the activity of SOD, ranging from 0.86 to 5.89, in contrast to CAT, which ranged from 0.88 to 4.40, and APX, which ranged from 0.40 to 4.01, at 60 s. In the 420-s treatment, the activity of all three enzymes studied exhibited a significant decrease relative to the control group.

Extended exposure duration of CP leads to progressive seed damage. This suggests that the agricultural sector should consider CP treatment as an innovative strategy. CP serves as an effective treatment for seed stimulation and also enhances the plant's defense system against diseases. Recently, plant pathologists have encountered challenges in creating alternative, chemical-free methods for the control of postharvest fungi at a commercial scale. CP represents a promising novel postharvest treatment, exhibiting no known adverse effects on fresh crops or the environment while effectively controlling bacterial infections that pose food safety risks [55].

Conclusion

The results of the present study indicate that cold plasma seed priming enhanced GP, GR, GI, and various growth characteristics, including root length, seedling length, and seedling dry weight, in comparison to the control sample. Furthermore, it positively influenced the activity of CAT, SOD, and AXP enzymes. This study yielded three novel findings: Different cultivars and classes within cultivars exhibited notable responses to the cold plasma pretreatment. The variation in plasma treatment duration resulted in notable differences in seedling growth characteristics and seed germination rates. The treatments lasting 60 and 180 s demonstrated the most favorable impact on the examined traits. Cold plasma, when applied within an optimal time frame, is an effective method for

enhancing seed germination and seedling development in agricultural crops. Priming soy seeds with cold plasma may represent an effective strategy for mitigating environmental stress in soybean crops.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12896-024-00921-x.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Author contributions
Khadijeh Sayahi conceived and carried out the experiments. Amir Hossein Sari, Aidin Hamidi, Bahareh Nowruzi, Farshid Hassani analyzed the data. Amir Hossein Sari, Aidin Hamidi, Bahareh Nowruzi, Farshid Hassani wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Data availability
This research was done according to Ling et al. 2014, with the modification of seed number per replicate. All data generated or analysed during this study are included in this published article (supplementary information files: S1, S2, S3, S4 and S5).

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Consent for participate
Not applicable.

Ethics approval and consent for participant
All local, national or international guidelines and legislation, including Act of Plant Varieties Registration, Control and Plant Material of Islamic Republic of Iran were adhered to in registration of studied cultivars and their seeds production and certification to the production of this study.
The seeds of soybean varieties used in this research were obtained free of charge from the Seed and Plant Certification and Registration Institute (SPCRI) in Iran.
The research was conducted by use of 5 soybean improved common commercial cultivars entitled in Iran Registered Plant Varieties National List seeds which are certified by Iranian national plant varieties registration and seed certification focal point, Seed and Plant Certification and Registration Institute (SPCRI) based on the act of plant varieties registration, control and certification of seed and plant material Islamic Republic of Iran.
The studied soybean seeds were not collected, they were obtained from the research institute.

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