

Radiation Physics and Engineering 2024; 5(4):41–45

# Analysis of flower phenology, flowering and stigma performance of saffron as a result of indirect treatment with DBD surface plasma under salt stress

Razieh Khalilzadeh<sup>a</sup>, Elnaz Khalilzadeh<sup>a,\*</sup>, Zohreh Deghani<sup>b</sup>, Alireza Pirzad<sup>c</sup>, Mahnaz Abdollahi<sup>b</sup>, Nayereh Abdollahi<sup>b</sup>, Narges Beigmohammadi<sup>b</sup>

<sup>a</sup>Department of Plant Production and Genetics Engineering, Faculty of Agriculture, Lorestan University, Khorramabad, Iran

<sup>b</sup>The Plasma Physics and Fusion Research School, Nuclear Science and Technology Research Institute, Tehran, Iran

<sup>c</sup>Department of Plant Production and Genetics, Faculty of Agriculture and Natural Resources, Urmia University, Urmia, Iran

## HIGHLIGHTS

- Applications of non-thermal plasma-based techniques are increasingly investigated in the field of agricultural science.
- Flowering beginning in saffron seems to be influenced by the combination of plasma and salinity stress.
- Saffron plants exposed to different time of plasma affect cellular physiology and improving immunity and plant growth.
- Plant exposed to plasma for 10 min can be an option to improve the saffron stigma DW in medium salinity level.

## ABSTRACT

Saffron (*Crocus sativus* L.) is cultivated in environments with very different climatic conditions and with very different corm rates from place to place, and it is appreciated for its red dried stigmas used as cooking spice and flavoring agent. To enhance the flower morphological and colorimetric traits under environmental stresses, the applications of non-thermal plasma based techniques are increasingly being investigated in the field of agricultural science as an alternative to conventional pre-germination treatments. Therefore, a field study was conducted to compare four levels P<sub>1</sub> (no treatment), P<sub>2</sub> (5 minute treatment), P<sub>3</sub> (10 minute treatment), P<sub>4</sub> (20 minute) and the salinity stress factors in three levels were 2, 4 and 6 dS.m<sup>-1</sup> according a factorial design with 3 replications. Flowering beginning in saffron seems to be influenced by the combination of plasma and salinity stress. High concentration of salinity (6 dS.m<sup>-1</sup>) resulted in a lower flower and stigma production. The highest number of leaf per plant was found when corms treated to P<sub>4</sub> and 2 dS.m<sup>-1</sup> 6 salinity stress condition. Exposure of saffron corm to plasma treatments of 20 minutes showed the most stimulating effect regarding the flower phenology and stigmas yield in saffron under salinity stress.

## KEYWORDS

Cold plasma  
Flowering  
Saffron corm  
Salinity  
Stigma yield

## HISTORY

Received: 22 July 2024

Revised: 26 August 2024

Accepted: 7 September 2024

Published: Autumn 2024

## 1 Introduction

Climate change has emerged as a significant threat, impacting the performance of plants through the occurrence of abiotic stresses. Soil salinity is a detrimental factor for many crops worldwide, greatly affecting plant production and derived products. It is estimated that 33% of irrigated arable soils are affected by salinization. Salinity primarily affects plant production by interfering in the absorption of water and nutrients, as well as toxicity in plant cells. Soil salinity occurs through two processes of salinization known as primary and secondary salinization. In primary salinization, the accumulation of salts is caused by nat-

ural phenomena such as weathering processes that have occurred throughout the history of the planet, or the deposition of oceanic salts carried by wind and rain. On the other hand, secondary salinization of soils occurs due to anthropogenic influences, such as the alteration of the hydrological balance of irrigation water, which is caused by the replacement of perennial vegetation for annual crops and by irrigation systems that use water with salts or have poor drainage (Hopmans et al., 2021). The extensive Iranian drylands, partly intensively populated today, are characterized by an ongoing aridification trend. Globally, these drylands are between those being most affected by soil salinization (Arabasadi et al., 2024). Soil salinization

\*Corresponding author: [e1.84111005@aut.ac.ir](mailto:e1.84111005@aut.ac.ir)

<https://doi.org/10.22034/rpe.2024.467848.1221>

is one of the important limiting factors for the growth of plants. A saline soil is defined as one, which has an electrical conductivity of  $2 \text{ dS.m}^{-1}$  and above. A significant decline in the yield of corn was observed when electrical conductivity was increased to  $1 \text{ dS.m}^{-1}$  in sandy loam and to  $2 \text{ dS.m}^{-1}$  in clay loam soil (Hopmans et al., 2021).

Saffron (*Crocus sativus* L., Iridaceae) is cultivated in Iran, India, Greece, Italy, Spain and France. It has been reported that saffron production in Iran has decreased significantly, possibly due to effect of weather (Eftekhari et al., 2023). There is a growing need to enhance the activity of plant defense mechanisms and promote plant growth and development, in order to adapt to these changing environmental conditions (Hopmans et al., 2021). Saffron, the most expensive cultivated herb, also known as Red Gold, has extensively been used as an antidepressant, antitumor, anticonvulsant, and antiinflammatory agent (Kothari et al., 2021). Despite the fact that this crop has been grown in the Iran region for an extensive span of time, there has not been much development in the technology used for its production or processing. At present, there exist no strategies to mitigate the adverse impacts of climate variability (Eftekhari et al., 2023).

The application of Cold Plasma (CP) has a profound impact on diverse developmental and processes in plants (Amini et al., 2017). In CPs, a wide variety of reactive oxygen and nitrogen species are produced through a variety of chemical reactions. Through electric discharge in a gas, which is often defined as a partially ionized gas containing both charged and neutral particles, CPs are generated. In CPs, ions, radicals, and excited molecules are also found along with photons emitted from dissociating electrically excited molecules. CPs can generate short-lived species with half-lives in the  $\mu\text{s}$  range ( $\text{H}_2\text{O}_2$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{O}_3$ ) and relatively long-lived species with half-lives in the millisecond range ( $\text{O}$ ,  $\text{OH}$ ,  $\text{O}_2^-$ ,  $^1\text{O}_2$ ,  $\text{NO}$ ,  $\text{NO}_2$ ) (Laroussi, 2020; Shelar et al., 2022). Plasmas have been applied to improve the water absorption capacity of seeds, avoiding seed dormancy effects and seed borne diseases and increasing seed resistance toward abiotic stresses. In this regard, very often, the improvements in germination rate and plant growth have been linked to an increase in seeds water uptake capacity upon their exposure to plasmas. It has been also highlighted that plasma generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (in general RONS) affects the biochemistry, the enzymatic activity, or even the gene expression processes of seeds and plants. However, the role of ROS or RNS and even their chemical nature is not yet completely clarified or understood, mainly because their short lifetimes make their detection difficult. Peroxo- and superoxide-like,  $\text{H}_2\text{O}_2$ , and  $\text{NO}_x$  species have been claimed as possible chemical species, which formed on the surface of plasma-treated seeds and then diffused to their interior, contributing to trigger a series of biochemical and gene regulation processes crucial for the germination and the development of plants. Among others, these processes may produce an increase in the activity of antioxidant enzymes, as well as osmotic adjustment substances (e.g., proline, soluble sugar) as well as a modification in the level of signaling

phytohormones such as the growing factor abscisic acid (Perea-Brenes et al., 2023; Starič et al., 2020).

Various types of cold plasma such as Dielectric Barrier Discharge (DBD), plasma jet and cold low pressure plasma such as radiofrequency (RF) and microwave (MW) discharge, have been used in food science and industry (Kim et al., 2017).

According to the high economic value of saffron, in this study, phenology, growth, and yield of saffron were examined in different plasma treatments to investigate the feasibility of cultivating saffron in saline soils.

## 2 Materials and Methods

### 2.1 Site description and climatic characteristics

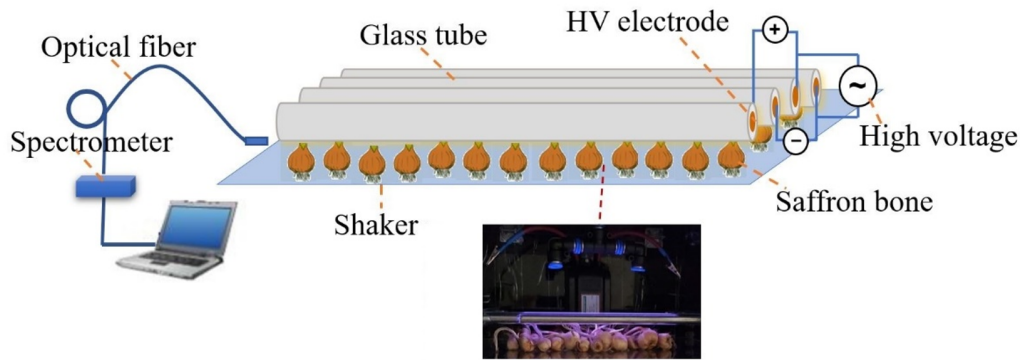
A field study (2024) was conducted at Agricultural Research Farm of Urmia University (latitude  $37^\circ 33' 09''$  N,  $45^\circ 05' 53''$  E and 1362 m above sea level), Iran. The elevation of the area ranges from 1300 to 1800 mm and the level of the groundwater table is greater than the adjacent areas. This region enjoys a semi-arid climate.

### 2.2 Experimental setups and plasma treatment process

The experiment was laid out in a factorial experiment based on completely randomized design with three replications for each treatment. The three levels of salinity (2, 4, and  $6 \text{ dS.m}^{-1}$ ) were applied for saffron plants as the first factor. Four levels of plasma treatments ( $P_1$ ,  $P_2$ ,  $P_3$ ,  $P_4$ ) were considered as the second factor.

The plasma source is the surface non-thermal dielectric barrier discharge (DBD) shown in Fig. 1. The plasma device is made of Pyrex glass dielectric tubes (diameter 14 mm, thickness 1.5 mm and length 320 mm). Steel rod electrodes with a diameter of 10 mm and a length of 250 mm are located in the center of the glass tubes. The distance between the rod electrodes is 16.7 mm. The AC power supply was an alternating current with a discharge voltage 10 kV and a frequency of 14 kHz and air was inserted into the reactor vessel as the carrier gas with a gas-flow rate of  $1.5 \text{ L.min}^{-1}$ .

To choose the type of plasma source and its required parameters for the treatment of seeds and agricultural products, several basic factors including their type of material and dimensions should be considered. In general, the treatment can be done directly or indirectly using different plasma sources. Studies show that direct and indirect plasma treatment leads to acceptable effects on agricultural seeds. Indirect plasma treatment has a distinct advantage that the treated seeds is preserved because the products are only exposed to radicals (not exposed to strong gas discharges). On the other hand, direct plasma treatment is faster because the concentration of active species in plasma is more significant. Results of previous studies display that the different plasma sources with the different parameters used for seed treatment can be effective. As an important result, it is very difficult not only to compare the results but also to determine the optimal plasma source for seed treatment due to the variety of



**Figure 1:** Schematic diagram of the DBD plasma system for seed treatment.

cold plasma producing devices. For example, the results obtained from wheat seeds treatment by different plasma sources are summarized in (Yarami and Sepaskhah, 2015; Attri et al., 2021), which confirm this issue. In the present work, due to the fact that the outer shell of the saffron corn is straw-like and also the size of the corns is large, indirect treatment has been used.

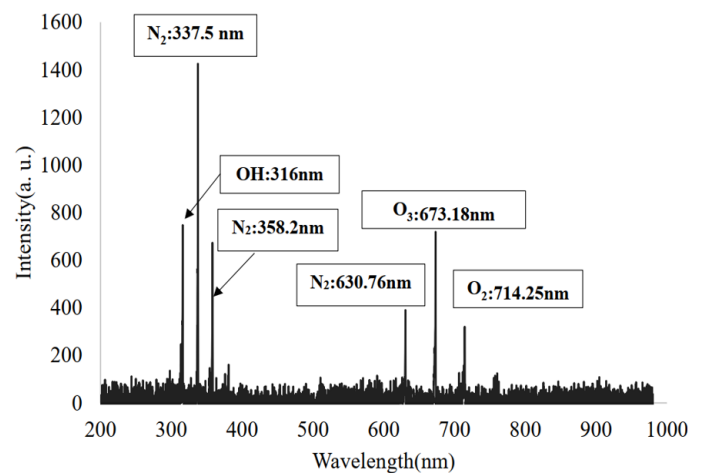
As it is clear in the Fig. 1, the treatment is of an indirect type, which was chosen according to the size of the saffron corns and the more effective plasma treatment. The saffron corns are placed on a shaker plate which is 50 mm away from the glass dielectric tubes. Plasma discharge was performed at different treatment times 5, 10, and 20 minute.

### 2.3 Optical Emission Spectroscopy (OES) analysis

A computer-controlled Stellar V900 spectrometer (manufactured in Iran) was used to determine the different active species in the air discharge inside the DBD plasma reactor. OES spectra were recorded in the ultraviolet-visible spectroscopy (UVVIS) wavelength range of 200 to 900 nm, with a spectral resolution of 2 nm and an integration time of 5 s. Figure 2 shows the plasma OES spectrum with emission lines of reactive oxygen and nitrogen species (RONS). It has been found in various studies before that ROS have an impact on signaling pathways. As a result of the interaction of reactive species with various signaling molecules (due to oxidative stress or reduction), a number of processes, such as differentiation, iron hemostasis, and DNA and nucleic acid cycles, are affected.

### 2.4 Statistical analysis

Statistical analysis and means comparison of data was performed with SAS Statistical Package Program (SAS Institute, Cary, NC). The Least Significant Difference (LSD) test was used to analyze the differences between means comparison of the main effects and interactions.



**Figure 2:** The emission spectrum of air plasma.

## 3 Results and discussion

It is noteworthy that  $S_1P_4$  obtained higher leaf dry weight (0.035 g per plant) and the lowest of it observed in  $S_3P_1$ . Leaf number of saffron grown in low salinity stress was also significantly higher than in high salinity (6 dS.m<sup>-1</sup>) when plant exposed to plasma treatment for 20 minutes. In effect, leaf number per plant was higher by 38% in  $S_3P_4$  compared to  $S_3P_1$  (Table 1). Moreover, there was a great variability of in dry weight of flower and stigma obtained in  $P_4$  plasma treatment. Dry weight of flower and stigma were higher in  $S_1P_1$  (0.033 and 0.01 g respectively) (Table 1). In saffron, plasma treatments play a role in flowering induction and flower appearance. In addition, the plant exposed to long-term plasma duration for flower initiation lies in the range of 122-125 days, but short exposure to these plasma treatment results in delayed flowering which was the 130 days (Table 1). The utilization of cold plasma as functional stimuli to enhance plant tolerance to salinity stress represents a burgeoning field of research that has already yielded promising outcomes.

Saffron flower yields are directly related to the environmental conditions, especially soil salinity (Scholtz et al., 2019). Our results indicated that the sensitivity of different saffron parts to salinity were not the same, as stigma

**Table 1:** The effect of different time of plasma on some characteristics of saffron under salinity stresses. In this table, ns, \*, and \*\* show non-significant and significant differences at 0.05, 0.01 probability level, respectively. Also, S<sub>1</sub> is 2 dS.m<sup>-1</sup>, S<sub>2</sub> is 4 dS.m<sup>-1</sup>, S<sub>3</sub> is 6 dS.m<sup>-1</sup>, P<sub>1</sub> is control, P<sub>2</sub> is 5 min, P<sub>3</sub> is 10 min, and P<sub>4</sub> is 20 min.

Treatments		Leaf dry weight (g per plant)	Leaf number (per plant)	Dry weight of stigma (per plant)	Dry weight of flower (per plant)	Start of flowering (number of days after planting)
S <sub>1</sub>	P <sub>1</sub>	0.025 <sup>d</sup>	8 <sup>g</sup>	0.0085 <sup>c</sup>	0.028 <sup>c</sup>	125 <sup>c</sup>
	P <sub>2</sub>	0.017 <sup>f</sup>	9 <sup>f</sup>	0.0090 <sup>bc</sup>	0.026 <sup>d</sup>	130 <sup>b</sup>
	P <sub>3</sub>	0.032 <sup>b</sup>	13 <sup>b</sup>	0.0097 <sup>ab</sup>	0.031 <sup>b</sup>	122 <sup>d</sup>
	P <sub>4</sub>	0.035 <sup>a</sup>	14 <sup>a</sup>	0.010 <sup>a</sup>	0.033 <sup>a</sup>	122 <sup>d</sup>
S <sub>2</sub>	P <sub>1</sub>	0.017 <sup>f</sup>	8 <sup>g</sup>	0.0044 <sup>e</sup>	0.024 <sup>e</sup>	130 <sup>b</sup>
	P <sub>2</sub>	0.02 <sup>e</sup>	10 <sup>e</sup>	0.0088 <sup>bc</sup>	0.024 <sup>e</sup>	130 <sup>b</sup>
	P <sub>3</sub>	0.030 <sup>bc</sup>	12 <sup>c</sup>	0.0094 <sup>abc</sup>	0.025 <sup>de</sup>	122 <sup>d</sup>
	P <sub>4</sub>	0.031 <sup>b</sup>	14 <sup>a</sup>	0.0097 <sup>ab</sup>	0.030 <sup>d</sup>	122 <sup>d</sup>
S <sub>3</sub>	P <sub>1</sub>	0.012 <sup>g</sup>	8 <sup>g</sup>	0.0022 <sup>f</sup>	0.018 <sup>g</sup>	130 <sup>b</sup>
	P <sub>2</sub>	0.014 <sup>g</sup>	8 <sup>g</sup>	0.0022 <sup>f</sup>	0.021 <sup>f</sup>	132 <sup>a</sup>
	P <sub>3</sub>	0.025 <sup>d</sup>	9 <sup>f</sup>	0.0044 <sup>e</sup>	0.020 <sup>f</sup>	125 <sup>c</sup>
	P <sub>4</sub>	0.028 <sup>c</sup>	11 <sup>d</sup>	0.0064 <sup>d</sup>	0.025 <sup>de</sup>	125 <sup>c</sup>
Salinity (S)	**	**	**	**	**	**
Plasma (P)	**	**	**	**	**	**
S × P	**	**	**	**	**	**

dry weight of stigma as economic yields were more sensitive; however; leaf yield and corm number were less sensitive to the soil salinity. The results of different studies are mainly dependent on types of plant species, plant developmental stage, plasma generating devices, exposure time, and feed gases and (Iranbakhsh et al., 2017; Zhou et al., 2016). Iranbakhsh et al. investigated that cold plasma could mediate plant growth of plant under stress condition by the activity of stress signaling genes, antioxidants, phytohormones, and histone modifications (Iranbakhsh et al., 2017). These results reveal that salinity can be an effective factor in flowering stimulation documented in various researches (Feizi et al., 2021; Shayganfar et al., 2021), and salinity stress at 4 dS.m<sup>-2</sup> is the most effective needs to be investigated further.

## 4 Conclusions

Consequently, it is concluded that saffron plants exposed to different time of plasma affect cellular physiology and improving immunity and plant growth. This study shows that plant exposed to plasma for 10 min can be an option to improve the saffron stigma DW in medium salinity level.

## Acknowledgements

Authors would like to thank the Plasma Technology Development Company for financial support.

## Conflict of Interest

The authors declare no potential conflict of interest regarding the publication of this work.

## References

- Amini, M., Ghoranneviss, M., and Abdijadid, S. (2017). Effect of cold plasma on crocin esters and volatile compounds of saffron. *Food Chemistry*, 235:290–293.
- Arabasadi, M., Ebrahimi, A., Amerian, M.-R., et al. (2024). The amelioration of salt stress-induced damage in fenugreek through the application of cold plasma and melatonin. *Plant Physiology and Biochemistry*, 207:108382.
- Attri, P., Koga, K., Okumura, T., et al. (2021). Impact of atmospheric pressure plasma treated seeds on germination, morphology, gene expression and biochemical responses. *Japanese Journal of Applied Physics*, 60(4):040502.
- Eftekhari, M., Javid, M. G., Aliniaiefard, S., et al. (2023). Alteration of flower yield and phytochemical compounds of saffron (*Crocus sativus* L.) by application of different light qualities and growth regulators. *Horticulturae*, 9(2):169.
- Feizi, H., Moradi, R., Pourghasemian, N., et al. (2021). Assessing saffron response to salinity stress and alleviating potential of gamma amino butyric acid, salicylic acid and vermicompost extract on salt damage. *South African Journal of Botany*, 141:330–343.
- Hopmans, J. W., Qureshi, A., and Kisekka, I. o. (2021). Critical knowledge gaps and research priorities in global soil salinity. *Advances in Agronomy*, 169:1–191.
- Iranbakhsh, A., Ghoranneviss, M., Oraghi Ardebili, Z., et al. (2017). Non-thermal plasma modified growth and physiology in *Triticum aestivum* via generated signaling molecules and UV radiation. *Biologia Plantarum*, 61:702–708.
- Kim, J. E., Oh, Y. J., Won, M. Y., et al. (2017). Microbial decontamination of onion powder using microwave-powered cold plasma treatments. *Food Microbiology*, 62:112–123.
- Kothari, D., Thakur, R., and Kumar, R. (2021). Saffron (*Crocus sativus* L.): Gold of the spicesA comprehensive review. *Horticulture, Environment, and Biotechnology*, 62(5):661–677.

Laroussi, M. (2020). Cold plasma in medicine and healthcare: The new frontier in low temperature plasma applications. *Frontiers in Physics*, 8:74.

Perea-Brenes, A., Garcia, J. L., Cantos, M., et al. (2023). Germination and First Stages of Growth in Drought, Salinity, and Cold Stress Conditions of Plasma-Treated Barley Seeds. *ACS Agricultural Science & Technology*, 3(9):760–770.

Scholtz, V., Šerá, B., Khun, J., et al. (2019). Effects of non-thermal plasma on wheat grains and products. *Journal of Food Quality*, 2019(1):7917825.

Shayganfar, A., Mohammadparast, B., Rostami, M., et al. (2021). Salt stress causes a significant increase in anti-cancer crocins content of saffron stigma. *South African Journal of Botany*, 143:61–68.

Shelar, A., Singh, A. V., Dietrich, P., et al. (2022). Emerging cold plasma treatment and machine learning prospects for seed priming: a step towards sustainable food production. *RSC Advances*, 12(17):10467–10488.

Starič, P., Vogel-Mikuš, K., Mozetič, M., et al. (2020). Effects of nonthermal plasma on morphology, genetics and physiology of seeds: A review. *Plants*, 9(12):1736.

Yarami, N. and Sepaskhah, A. R. (2015). Saffron response to irrigation water salinity, cow manure and planting method. *Agricultural Water Management*, 150:57–66.

Zhou, R., Zhou, R., Zhang, X., et al. (2016). Effects of atmospheric-pressure N<sub>2</sub>, He, air, and O<sub>2</sub> microplasmas on mung bean seed germination and seedling growth. *Scientific Reports*, 6(1):32603.

©2024 by the journal.

RPE is licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/) (CC BY-NC 4.0).



#### To cite this article:

Khalilzadeh, R., Khalilzadeh, E., Dehghani, Z., Pirzad, A., Abdollahi, M., Abdollahi, N., Beigmohammadi, N. (2024). Analysis of flower phenology, flowering and stigma performance of saffron as a result of indirect treatment with DBD surface plasma under salt stress. *Radiation Physics and Engineering*, 5(4), 41–45. doi: 10.22034/rpe.2024.467848.1221

DOI: [10.22034/rpe.2024.467848.1221](https://doi.org/10.22034/rpe.2024.467848.1221)

To link to this article: <https://doi.org/10.22034/rpe.2024.467848.1221>