Effect of plant-derived smoke on salinity tolerance of chili pepper seedlings

Faride Noroozi Shahri and Saeid Jalali Honarmand*

Department of Plant Production and Genetics, Razi University, Kermanshah, Iran

Abstract

Over the last two decades, the stimulatory and PGRs-like effects of plant-derived smoke (smoke-water: SW) have been noticed on seed germination rate and seedling development. In this study, SW-priming was investigated as a new method to stimulate seed germination and enhance the seedling vigor of chili peppers under salinity stress conditions. This experiment was conducted at the Faculty of Agricultural Sciences and Engineering of Razi University in 2024. The experimental factors included two levels of salinity stress (control and 80 mM of NaCl) and six levels of chili pepper seed priming (control, hydro-prime, and SW-prime at 0.001, 0.01, 0.1, and 1% concentrations. Results showed that salinity stress reduced SDW (11.29%), RL (51.71%), PL (26.38%), GP (9.16%), AC (34.36%), SLVI (51.24 %), and SWVI (19.12%), compared to the control treatment. SW-priming at a concentration of 0.1% increased SDW (13.88%), RL (42.24%), PL (19.64%), GP (32.76%), SLVI (75.58%), and SWVI (49.68%) compared to the control. SW-priming also caused MGT reduction in control and salinity stress conditions compared to the control. Therefore, SW-priming can potentially reduce the adverse effects of salinity stress at early stages of the chili pepper life cycle.

Keywords: *Capsicum annuum*, chili Pepper, germination, plumule, radicle, vigor

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Introduction

Salinity is one of the most significant edaphic challenges in many regions globally. The principal cause of soil salinity is NaCl. Cl^- and Na^+ ions constitute 62% of the sum of the anions and 60% of the sum of cations, respectively (Hadjadj et al., 2022). Soil salinity disrupts plant metabolic processes by reducing soil water potential and causing nutritional imbalances, negatively affecting plant development. Tolerance to salinity stress during early growth stages is critical for establishing plants in areas with saline soils. Therefore, proper management and efforts to enhance seed and seedling tolerance to salinity are very important. Priming seeds with water or low osmotic solutions acts as a stress reliever, leading to faster metabolic rate recovery compared to control seeds, ultimately enhancing both the germination rate and the vigor of the seedlings (Johnson and Puthur, 2021; Choudhary et al., 2024).

It has been reported that regulators and plant elicitors help improve plant tolerance to stresses by maintaining redox equilibrium and synthesizing osmolytes in plant cells (Singh et al., 2023). One of the elicitors that has gained attention in recent decades is plant-derived smoke. Plant-derived smoke is used in agriculture in two ways: as an aerosol and as smoke-water (SW) (Govindaraj et al., 2016). The biochemical and physiological responses of plants to bioactive compounds found

* *Corresponding Author*
E-mail Address: sjhonarmand@razi.ac.ir
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in SW indicate that these compounds either act directly as plant growth regulators (PGRs) or indirectly influence the metabolism of the endogenous PGRs, such as auxins, cytokinins, and gibberellins (Jain et al., 2008; Aremu et al., 2016). Previous studies indicate a significant interaction between SW and environmental conditions on the germination of plants (Moyo et al., 2022).

Hürkan and AKI (2024) observed that SW and SW-derived karrikinolide regulate the expression of certain genes encoding antioxidant enzymes in apricot (*Prunus armeniaca* L.) under salinity stress (100 mM NaCl). In their study, smoke at a concentration of 1:1000 v/v and karrikinolide at a concentration of 1 µM enhanced germination rate and increased the expression levels of catalase, superoxide dismutase, and glutathione peroxidase genes under salinity stress conditions. Meanwhile, root and stem length showed the best results at a concentration of 100 v/v (Hürkan and AKI, 2024). In another study, priming indica rice seeds variety (NIAB-IR-9) with different concentrations of SW significantly increased the germination percentage, seedling vigor, and fresh and dry weights compared to unprimed seeds (Jamil et al., 2014). This experiment investigated the effect of SW-priming on the improvement of germination rate and growth characteristics of red chili pepper (*Capsicum annuum* L.) during the early stages of its life cycle under salinity stress conditions.

Materials and Methods

This experiment was conducted as a factorial based on the completely randomized design with three replications in 2024 at the Faculty of Agricultural Sciences and Engineering of Razi University. The experimental factors included two levels of salinity stress (control and 80 mM of NaCl) and six levels of chili pepper seed priming (control, hydro-primed, and SW-primed at 0.001, 0.01, 0.1, and 1% concentrations). The SW used in the experiment was prepared according to the method of Noroozi Shahri et al. (2018). The experimental units were placed in an environment with a temperature of 25 °C and a 12:12 hours light-dark cycle for 12 days. The number of germinated seeds was counted and recorded daily. Seeds were considered germinated when the radicle was at least 2 mm long. Each experimental unit contained 50 seeds primed with distilled water or different concentrations of SW before the experiment was conducted and was designed to maintain a constant salt concentration throughout the experiment.

At the end of the experiment, the length of plumule (PL) and radicle (RL) of seedlings, seedlings dry weight (SDW), germination percentage (GP) (Equation. 1), mean germination time (MGT) (Eq. 2), allometric coefficient (AC) (Eq. 3), seedling length vigor index (SLVI) (Eq. 4) and seedling weight vigor index (SWVI) (Eq. 5) were measured (Metwally et al., 2022). In these relationships, N is the total number of seeds, n_i is the number of seeds germinated in the i^{th} time interval, and t_i is the time interval. All statistical analyses were conducted using the software SAS version 9.

(1) $GP = \left(\frac{n_i}{N}\right) \times 100$

(2) $MGT = \frac{\sum n_i t_i}{n_i}$

(3) $AC = \frac{RL\ (cm)}{PL\ (cm)}$

(4) $SLVI = GP \times (PL\ (cm) + RL\ (cm))$

(5) $\text{SWVI} = GP \times SDW\ (mg)$

Results

The analysis of variances showed that all investigated traits in the salinity stress condition were significantly lower compared to the control (Table 1). The highest SDW was in the control condition, with 30.54 mg while the lowest amount was obtained in the salinity stress, with 27.09 mg. RL and PL under the salinity stress (2.96 and 1.06 cm, respectively) were significantly lower compared to the control condition (6.13 and 1.44 cm, respectively). The highest GP was obtained in the control group (74%), and the lowest amount of this trait was obtained in salinity stress (67.22%). The control group achieved the highest AC of 4.22 while the salinity stress group recorded a

Table 1
ANOVA of germination traits in chili pepper seeds primed with different levels of SW under control and salinity conditions

SoV	DF	Mean Square							
		SDW†	RL	PL	GP	MGT	AC	SLVI	SWVI
Stress (S)	1	107.12**	90.28**	1.29**	413.44**	5.49**	18.82**	75.47**	168.77**
Priming (P)	5	10.58*	1.72*	0.04**	256.11**	0.71**	0.54ns	3.44**	43.00**
S × P	5	4.04ns	0.36ns	0.005ns	9.17ns	0.21*	0.26ns	0.26ns	2.52ns
Error	24	3.00	0.52	0.006	50.77	0.07	0.31	0.57	6.26
CV (%)		6.01	15.88	6.59	10.09	3.56	16.02	18.12	12.22

†SDW: Seedling dry weight; RL: Radicle length; PL: Plumule length; GP: Germination percent; MGT: Mean germination time; AC: Allometric coefficient; SLVI: Seedling vigor length index; SWVI: Seedling vigor weight index; ns, *, and ** are non-significant and significant at the probability levels 0.05 and 0.01, respectively.

Table 2
Mean comparison of germination traits in chili pepper seeds primed with different levels of SW under control and salinity conditions

Treatment		SDW† (mg)	RL (cm)	PL (cm)	GP (%)	MGT (day)	AC	SLVI	SWVI
Stress	Ctrl	30.54	6.13	1.44	74.00	7.52	4.22	5.64	22.64
	Salinity	27.09	2.96	1.06	67.22	8.30	2.77	2.75	18.31
LSD		1.19	0.49	0.05	4.90	0.19	0.38	0.52	1.72
Priming	Ctrl	26.65	3.74	1.12	59.00	7.98	3.17	2.99	15.88
	Hydroprime	28.78	4.25	1.25	69.66	7.71	3.24	3.88	20.16
	SW 0.001%	30.00	4.46	1.27	73.00	7.80	3.39	4.24	21.98
	SW 0.01%	28.91	4.82	1.34	74.00	7.83	3.45	4.65	21.39
	SW 0.1%	30.35	5.32	1.34	78.33	7.56	3.90	5.25	23.77
	SW 1%	28.21	4.68	1.21	69.66	8.55	3.82	4.17	19.68
LSD		2.06	0.86	0.09	8.49	0.33	0.66	0.90	2.98

†SDW: Seedling dry weight; RL: Radicle length; PL: Plumule length; GP: Germination percent; MGT: Mean germination time; AC: Allometric coefficient; SLVI: Seedling vigor length index; SWVI: Seedling vigor weight index. LSD: The least significant difference.

significantly lower AC of 2.77. The control group also recorded the highest SLVI (5.64) while the lowest SLVI was observed in the salinity stress group (2.75). Similarly, the control group had the highest SWVI (22.64) compared to the lowest SWVI (18.31) under salinity stress (Table 2).

The highest and the lowest SDW were obtained in SW at a concentration of 0.1% (30.35 mg) and control condition (26.65 mg), respectively. The highest RL and PL were observed with the application of SW 0.1% (5.32 and 1.34 cm, respectively) while the lowest RL and PL were observed in the control group (3.74 and 1.12 cm, respectively). SW-priming at a concentration of 0.1% of SW increased GP by 32.76% compared to control. The highest SLVI and SWVI were observed with the application of SW 0.1% (5.25 and 23.77, respectively). On the other hand, the lowest RL and PL were observed in the control group (2.99 and 15.88, respectively) (Table 2).

Table 3
Slicing ANOVA of chili pepper seed priming with different concentrations of SW in control and salinity conditions for MTG

SoV	DF	Mean Square
		MGT†
Ctrl	5	0.79**
Stress (S)	5	0.13ns

†MGT: Mean germination time; ns, *, and ** are non-significant and significant at the probability levels 0.05 and 0.01, respectively.

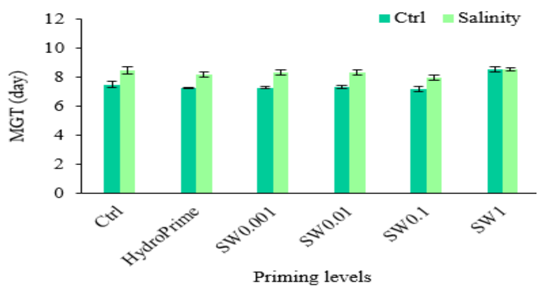

Fig. I. The interaction effect of stress × priming on MTG of chili pepper seeds; error bars show the standard error of the mean. MGT: Mean germination time

The analysis of the effects of Stress × Priming indicated that SW-priming at a concentration of 0.1% exhibited the lowest MGT under control conditions (7.16 days). However, different priming levels under stress conditions did not show statistically significant differences (Table 3 and Fig. I).

Discussion

Salinity stress causes an excessive uptake of ions into the cells, disrupting the physiological and metabolic processes of embryonic tissues, such as enzyme activities, cell division, phytohormones ratio (GA/ABA balance), and differentiation (Hadjadj et al., 2022). An imbalance of GA/ABA acid along with lower water potential in the medium around the seeds due to high ion concentration, slows down cell division and the embryonic axis growth. It also prevents the loosening of cell walls, which stops radicle emergence (Guja et al., 2013). In the current study, SW-priming has led to an increase in GP and a decrease in MGT. GA triggers the production of alpha-amylase, proteases, and β-glucanases in the seed embryo to hydrolyze stored food in the seed (Gimeno-Gilles et al., 2009). Given the confirmed effects of SW's GA-like properties in previous studies (Jain et al., 2008), it is likely that SW-priming causes a similar signaling process to GA in the embryo, activating germination even under salinity stress conditions.

Additionally, enhancing the accumulation of osmolytes in plant cells, along with an increase in the activity rate of antioxidant enzymes and the strengthening of the cellular defense mechanisms during seed priming, contribute to maintaining water balance and stability of plasma membranes when exposed to salinity stress. In addition, seed priming affects ion homeostasis and reduces salt toxicity by regulating sodium levels and improving the selectivity of the K^+/Na^+ ratio (Habibi et al., 2024). It has been declared that the bioactive compounds in SW, such as 3-methyl-2H-furo [2,3-c] pyran-2-one and other butanolides, may affect the channel proteins and aquaporins in the plasma membrane. Therefore, seeds treated with SW absorb water more quickly compared to the control group (Moyo et al., 2022).

The traits studied in our experiment showed a SW concentration-dependent effect. Increasing the concentration to 0.1% improved seed germination rate and seedling development while increasing the concentration to 1% decreased the measured traits, or the observed variations were not significantly different from those in the control group. The inhibitory effect of SW at high concentrations may be due to the activity of compounds such as 3,4,5-trimethyl furan-2(5H)-one, dihydroxy toluene, dihydroxy benzenes, 2-ferroic acid, and naphthalene at these levels (Flematti et al., 2013; Light et al., 2010).

The decrease in SDW, RL, and PL in seedlings during early growth stages under salt stress may be due to disruptions in physiological and biochemical systems, swelling of thylakoid membranes, and decreased chloroplast efficiency under non-biological stress conditions, which negatively affect photosynthesis (Singh et al., 2023). On the other hand, using SW and karrikins increases photosynthetic pigments, CO_2 concentration, stomatal conductance, transpiration rate, and photosynthetic performance. Proteomic reports indicate that smoke boosts the synthesis of RuBisCO activase and RuBisCO large/small subunits, which decrease under abiotic stresses (Shah et al., 2020; Komatsu et al., 2022; Singh et al., 2023).

Probably, at optimal concentrations, the cytokinin-like effects of SW enhance the rate of cell division, accelerate the emergence of the radicle, and lead to increased dry matter production (Aremu et al., 2016). SW contains growth regulatory compounds that can boost plant productivity even in salinity stress conditions (Jamil et al., 2014). The increase in the SDW, PL, RL, and GP ultimately enhanced the SLVI and SWVI in both control and stress conditions. Germination and establishing seedlings with high SLVI and SWVI under abiotic stress are vital factors in plant development. These factors significantly influence the plant's tolerance to stress and crop productivity (Ellouzi et al., 2024).

Conclusions

This research contributes to our knowledge-on the effects of SW application on plant growth as a sustainable and effective strategy for reducing the adverse effects of abiotic stress on crop production. SW-priming with a concentration of 0.1% reduced the negative effects of salinity stress during the chili pepper's early life-cycle stages. Therefore, these findings show the potential of innovative techniques such as seed priming and the application of SW to optimize seed germination under abiotic stresses.

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