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Seed Priming Treatments to Improve Heat Stress Tolerance of Garden Pea (*Pisum sativum* L.)

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Abstract: Heat stress seriously affects the production of cool-season food legume crops such as garden peas. Seed priming is a widely used technique that increases germination and improves plant growth and development, resulting in better field performance and higher yield of crops. In the current study, we investigated three seed priming treatments—hydropriming (dH_2O), osmopriming (2.2% w/v $CaCl_2$), and hormopriming (50 mg L^{-1} salicylic acid—SA)—and their effect on germination, initial seedling development, and physiological traits of two novel garden pea cultivars, under optimal conditions and heat stress. Seed priming with H_2O, $CaCl_2$, and SA enhanced garden pea performance under both optimal and stress conditions via significant improvements in germination energy, final germination, mean germination time, mean germination rate, seedling vigor index, shoot length, root length, fresh seedling weight, dry seedling weight, shoot elongation rate, root elongation rate, relative water content, chlorophyll content, and membrane stability index, as compared to control. The highest effect on the examined parameters was achieved by osmopriming and hormopriming in both cultivars, suggesting that these treatments could be used to improve the heat stress tolerance of garden pea, after extensive field trials.

Keywords: Seed quality; germination performance; priming treatments; hydropriming; $CaCl_2$; salicylic acid; optimal conditions; heat stress

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1. Introduction

The rapid growth of the world's population demands an increase in the food supply. In this regard, the situation is drastically aggravated due to rapid global climate changes. Serious threats to agriculture are posed by abiotic stresses, such as drought, extreme temperatures, salinity, and others. Among these factors, high-temperature stress has become the most important limiting factor to crop productivity. According to IPCC [1], by the end of the 21st century, the Earth's climate is predicted to warm by an average of 2–4 °C. Heat stress imposes the most prolonged effects on plant development, accompanied by a severe reduction in the yield potential of many crop species, especially at a temperature much above 30 °C [2,3]. Previous studies reported yield losses of many crops by more than 50% due to heat stress [4–6]. Heat stress reduces the germination potential of the seeds, resulting in poor germination and stand establishment [5], as well as the reduction in fertility of many species [7], net assimilation rate [8], and the total number of grains and grain weight [9]. Moreover, many physiological processes, such as reduction in water content and root conductance [5], reduced activity of nitrate reductase [10], increased membrane permeability, inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, and protein degradation have been affected by elevated temperatures [3,11].

Garden pea (*Pisum sativum* L.), belonging to the family *Fabaceae*, is a cool-season food legume crop. It is one of the nutritionally most important vegetable crops because

it contains a high percentage of proteins, essential amino acids, and a significant content of vitamins, minerals, and carbohydrates [12]. Global production of garden (green) pea has reached over 2.5 million hectares with a yield of 7.8 t ha^{-1} [13]. As a cool-season food legume, garden pea is susceptible to temperature variations [3]. Even a one-degree increase in temperature can be considered heat stress in cool-season food legumes, which has serious implications for their growth and biochemical functions [14]. Higher temperatures and lack of moisture in the spring lead to slower, non-uniform germination, and delay the emergence of garden pea by 3 to 4 weeks. As the temperature rises above optimal (approximately 20 °C), the intensity of garden pea germination decreases. Higher temperatures above 25 °C are depressing, especially during and immediately after flowering, while plants stop growing at 35 °C. In the vegetative phase, high temperatures do not allow optimal growth, and the plants remain significantly shorter and move more quickly into the generative phase [15]. Further, high temperatures accelerate technological maturity, which contributes to poorer grain and seed quality. In this regard, germination energy and sowing depth greatly mitigate the harmful effects of high temperatures and drought [16].

Many approaches have so far been applied to overcome the deleterious effects of heat stress. Breeding the garden pea cultivars more tolerant to heat stress, mapping genomic regions for complex traits, and using markers for genetic dissection have been used as genetics and genomics approaches in order to enhance pea productivity under the changing climate conditions [2,14,17]. Additionally, many agronomic strategies have been employed to prevent huge crop losses under heat stress, including foliar spraying with nutrients [18], changes in the sowing method and time [19], implementation of plant growth regulators [20], etc. Among various agronomic approaches, seed priming stands out as a quick, easy, low-cost, and effective strategy for improving germination, plant growth, yield-related parameters and subsequent grain yield, and overall plant defense against abiotic stresses in many crops [21,22]. It is defined as the pre-sowing seed treatment in which seeds are fully immersed in water or solution of any chemical agents (inorganic salts, organic compatible solutes, antioxidants, plant growth regulators, natural extracts, etc.) and dried back to storage moisture levels until further use [21–23]. Depending on the type of chemical agent, seed priming techniques are classified as hydropriming, hormopriming, halopriming, osmopriming, nutripriming, and redox priming [24]. Hydropriming or soaking seeds in water is the simplest, most eco-friendly, and most cost-effective technique of seed priming [25]. During osmopriming, seeds are soaked in an osmotic solution, such as polyethylene glycol (PEG), glycerol, sorbitol, mannitol, and different inorganic salts ($CaCl_2$, KCl, K_3PO_4, KNO_3, KH_2PO_4, NaCl, $MgSO_4$). Priming with salt solutions is also referred to as halopriming. Due to the low water potential of the osmotic solution, seeds achieve approximately 10 to 20% of full hydration, resulting in earlier germination and seedling emergence and a positive response to stress conditions [26,27]. Hormopriming, or, i.e., hormonal priming, is also one of the commonly used priming techniques in which seeds are soaked in essential phytohormones with a direct impact on seed metabolism, such as auxins (IAAs), cytokinins (CKs), gibberellins (GAs), abscisic acid (ABA), salicylic acid (SA), and ethylene (ET) to improve seed germination and seedling growth, and crop yield in adverse conditions [28].

Iqbal et al. [22] reported that various exogenous elicitors, such as plant growth regulators (SA) and inorganic salts ($CaCl_2$), have different efficacy during seed priming. For instance, SA proved to be an excellent agent in mitigating the effects of abiotic stresses such as water deficit, chilling, salinity, and high temperatures in maize, wheat, rice, smooth vetch, and faba bean [28–32]. Moreover, positive effects of seed priming with $CaCl_2$ in improving plant response to abiotic stresses, including salt, drought, and low temperature, have been observed in rapeseed, sorghum, and other crops [33,34]. So far, the most beneficial effects of seed priming on the morpho-physiological and yield characteristics of pea plants under drought stress conditions have been reported using the carrot extract, *Bacillus thuringiensis* and silicone [35], while hydro- and osmopriming with PEG and KNO_3 have had a beneficial effect on germination and initial growth of garden pea under saline

stress [36]. However, there is a lack of information on the impact of hydro-, osmo-, and hormopriming on the garden pea characteristics and tolerance to heat stress conditions. Understanding germination, growth, and physiological traits associated with heat adaptation can be useful for the genetic improvement of crops. Crop failures may be avoided by the use of resistant or tolerant cultivars and appropriate priming seed treatments. We assumed that the newly developed cultivars of garden pea differ in their tolerance to heat stress, and that priming treatments can be of great importance in overcoming this stress, especially considering future scenarios of climate change and issues of global food supply. Thus, the present study has been carried out so as to evaluate the impacts of H_2O, $CaCl_2$, and SA priming on germination, seedling growth and development, and physiological traits of two garden pea cultivars under optimal and high-temperature stress conditions.

2. Materials and Methods

2.1. Plant Material

Two garden pea (*Pisum sativum* L.) cultivars, coded as cv. 1 (very early variety) and cv. 2 (early variety), were chosen as plant material. Both cultivars were recently developed at the Department of Vegetable and Alternative Crops, Institute of Field and Vegetable Crops (IFVCNS), Novi Sad, Serbia. The seeds of the selected pea cultivars were produced on a chernozem soil at the Rimski Šančevi (Vojvodina Province, Serbia) experimental field of IFVCNS (N 45°19′, E 19°50′) in 2021. The average temperature and precipitation sum during seed production at the study site were 11.8 °C and 156 mm, respectively. The soil had the following properties: pH 8.08, $CaCO_3$ (%) 5.34, organic matter (%) 2.43, and total N (%) 0.181. In terms of the content of macroelements, this soil belongs to the medium provided by available phosphorus and potassium, having 12.9 mg and 21.8 mg per 100 g of soil, respectively.

2.2. Seed Priming

Seeds of the garden pea cultivars were firstly disinfected with 5% (*w*/*v*) sodium hypochlorite (Sigma Aldrich, St. Louis, MO, USA) for 5 min, and then rinsed thoroughly three times with sterile distilled water. The seeds were primed using the following priming techniques: hydropriming—seeds soaked in distilled water, osmopriming—seeds soaked in 2.2% $CaCl_2$ (Sigma Aldrich, St. Louis, MO, USA) solution, and hormopriming—seeds soaked in 50 mg L^{-1} salicylic acid (Sigma Aldrich, St. Louis, MO, USA) according to Iqbal et al. [22]. The seeds were fully immersed in solutions for 18 h at 25 °C under dark aseptic conditions, keeping the ratio of seed weight and solution volume 1:5 (*w*/*v*) [22]. Non-primed seeds were used as the control. Thereafter, the seeds were thoroughly rinsed with distilled water and air-dried on sterile filter paper at room temperature for 72 h close to the original weight.

2.3. Germination Test

The experiment was conducted in the Laboratory for Seed Testing of IFVCNS. The working sample consisted of 100 seeds per replicate. The experiment was set up as a two-factorial design: cultivar × treatment, in three replicates. Primed and non-primed seeds were sown in plastic boxes 240 × 150 mm with double-layer filter paper moistened with sterile distilled water. The experiment comprised a total of 48 boxes, grouped into two sets, containing 24 boxes for optimal conditions and 24 boxes for heat stress (2 cultivars × 4 treatments in 3 repetitions). One set of samples was germinated under optimal conditions for garden pea (20 °C) according to the ISTA Rules [37], while another set of samples was exposed to heat stress within the temperature regime 30/20 °C (altering temperature regime of 20 °C for 16 h in the dark and 30 °C for 8 h in the light), given that a daily temperature of 30 °C is considered limiting for peas [3]. Both sample sets were placed in the germination chamber for 8 days [37].

Determination of Germination and Germination-Related Parameters

The germinated seeds were counted on a daily basis. The germination energy, defined as the percentage (%) of seeds in a given sample that germinated within a definite period, was determined five days after sowing by counting only the seedlings with well-developed essential structures such as 10 mm primary root, shoot axis, and cotyledons [37]. Final germination (%), defined as the percentage of seedlings with a healthy and well-developed root and shoot system, and abnormal seedlings (%), defined as the percentage of seedlings which do not show potential for further development into satisfactory plants when grown in good quality soil and under favorable conditions of moisture, temperature, and light, were determined eight days after sowing [37].

Seedling vigor index (*SVI*) was calculated using the formula [38]:

$$SVI = SL \times FG,$$

where *SL*—seedling length (cm), *FG*—final germination (%).

All determinations were performed in three repetitions.

2.4. Determination of Seedling Growth, Biomass Accumulation, and Growth-Related Parameters

For the estimation of growth, 25 seeds of garden pea cultivars per replicate were placed in moistened filter paper and incubated in the germination chamber at optimal (20 °C) and heat stress (30/20 °C) conditions, in the same conditions as for germination test. Shoot length and root length of 10 normal seedlings per replication were determined using a ruler on the same days as germination energy (5th day) and seed germination (8th day). Furthermore, the fresh weight of seedlings was determined on the day of seed germination (8th day) using the analytical balance (Kern 770-13, KERN & Sohn GmbH, Balingen, Germany). Finally, the seedlings were oven-dried at 80 °C for 24 h so as to obtain the dry weight.

Shoot elongation rate (*SER*) and root elongation rate (*RER*) were calculated using the following formulas [6]:

$$SER = \frac{SLE - SLS}{TE - TS},$$

$$RER = \frac{RLE - RLS}{TE - TS},$$

where *SLS*, *SLE*—shoot length (mm) at the start (5th day) and at the end (8th day) of a measurement period; *RLS*, *RLE*—root length (mm) at the start (5th day) and at the end (8th day) of a measurement period; *TE-TS*—time duration (days) between two measurements.

All determinations were performed in three repetitions.

2.5. Determination of Membrane Stability Index, Relative Water Content, and Chlorophyll Content

The membrane stability index was calculated according to the protocol established by Sairam [39]. Two sets of test tubes containing 0.1 g of fresh leaves and 10 mL of distilled water were set up. One set of test tubes was heated in a water bath (VIMS elektrik, WKP-14, Tršić, Serbia) at 40 °C for 30 min, and electrical conductivity (C1) was determined using a professional laboratory bench meter (Laboratory Research Grade Benchtop EC/TDS/Salinity/Resistivity Meter—HI5321, Hanna Instruments, Woonsocket, RI, USA). To obtain electrical conductivity (*C*2), a second set of test tubes were heated in a water bath at 100 °C for 15 min. The membrane stability index (*MSI*) was determined using the following formula:

$$MSI = [1 - (C1/C2)] \times 100,$$

where *C*1—electrical conductivity of the samples heated at 40 °C for 30 min; *C*2—electrical conductivity of the samples heated at 100 °C for 15 min.

Relative water content was determined by the method explained by Farooq et al. [40]. Approximately 0.5 g of fresh leaves were weighed, rinsed, and put in water-containing test tubes until complete saturation and weighed. The saturated leaves were dried at 80 °C for 24 h and weighed. Relative water content (*RWC*) was determined using the following formula:

$$RWC = (Wf - Wd)/(Ws - Wd) \times 100\%,$$

where *Wf*—weight of fresh leaves (g); *Ws*—weight of saturated leaves (g); *Wd*—weight of dry leaves (g).

Chlorophyll content was determined according to the method explained by George et al. [41]. For the determination of chlorophyll, 0.1 g of leaf sample was added in a test tube containing 10 mL of 80% ethanol, stirred at vortex for 5–10 s, and then heated in a water bath at 100 °C for 3–5 min. Reading of the obtained extract solution was performed at 666 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-VIS Spectrophotometer, Waltham, MA, USA), while chlorophyll content (*Chl*) was determined using the following formula:

$$Chl\ (mg/g\ of\ FW) = (Abs\ -\ 0.01) \times 1/92.6474 \times 10/FW\ (g)$$

where *FW*—fresh weight (g); *Abs*—absorbance at 666 nm.

All analyses were performed in three repetitions.

2.6. Statistical Analysis

The experiments were set up in a completely randomized design (CRD) with three replications. The obtained data were processed statistically, using analysis of variance (ANOVA), followed by mean separation according to Duncan´s multiple range test (DMRT) ($p \leq 0.05$). The data were statistically processed by using STATISTICA 10.0 software (StatSoft Inc., Tulsa, OK, USA). The relationship between parameters was determined by Pearson's correlation analysis using R 4.2.2 software (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

The effects of cultivar, treatment, and their interactions are presented in Table 1. The obtained results clearly showed that all garden pea parameters were significantly influenced by seed priming treatments, except for abnormal seedlings, both under optimal and stress conditions (Table 1a,b). The cultivar had a significant effect on all examined parameters, except on chlorophyll content under optimal conditions (Table 1a), abnormal seedlings, chlorophyll content, and membrane stability index in heat-stress conditions (Table 1b). Moreover, cultivar × treatment interaction significantly altered germination energy, final germination, shoot and root length, fresh and dry seedlings weight, shoot and root elongation rate, seedling vigor index, and chlorophyll content under optimal conditions (Table 1a); shoot and root length, fresh and dry seedling weight, shoot and root elongation rate, seedling vigor index, relative water content, chlorophyll content, and membrane stability index of heat-stressed pea seeds (Table 1b).

Generally, heat stress caused a decrease in seed germination, seedling growth and development, and physiological parameters of two garden pea cultivars (Tables 2–4). The significant and positive effects of priming treatments were observed in relation to the control for all examined germination parameters, except germination energy and final germination of cv. 2 under optimal conditions, as well as abnormal seedlings of cv. 1 and cv. 2 in normal and heat-treated seeds (Table 2a,b). In addition, only SA treatment led to a significant increase in the seedling vigor index of cv. 1, as compared to the control, under both conditions (Table 2a,b). Furthermore, a prevalence of osmopriming and/or hormopriming over hydropriming was notable in seedling vigor index of cv. 2 under both conditions ($CaCl_2$ and SA vs. H_2O) (Table 2a,b); germination energy of cv. 1 and final germination of both cultivars under stress (SA vs. H_2O) (Table 2b). Interestingly, significant differences between $CaCl_2$ and SA treatments were observed only in the case of seedling

vigor index of cv. 2 under optimal conditions (Table 2a), as well as final germination and seedling vigor index of both cultivars under heat stress (Table 2b).

Table 1. Analysis of variance for parameters of two garden pea cultivars after hydropriming (H_2O), osmopriming ($CaCl_2$), and hormopriming (SA) under different laboratory conditions. (a) Optimal conditions; (b) heat stress.

Traits		Factors							
		(a) Optimal Conditions				(b) Heat Stress			
		Cultivar (C)	Treatment (T)	C × T	Error	Cultivar (C)	Treatment (T)	C × T	Error
Germination Energy	*df*	1	3	3	16	1	3	3	16
	MS	672	36.9	20.3	1.3	840	50.1	3.6	1.8
	p	0.00	0.00	0.00		0.00	0.00	0.16	
Final Germination	*df*	1	3	3	16	1	3	3	16
	MS	513	14.6	10.2	1.4	828	47.5	1.6	1.3
	p	0.00	0.00	0.00		0.00	0.00	0.34	
Abnormal Seedlings	*df*	1	3	3	16	1	3	3	16
	MS	18.4	0.15	0.49	0.54	0.17	0.33	1.61	0.83
	p	0.00	0.84	0.46		0.66	0.75	0.16	
Shoot Length	*df*	1	3	3	16	1	3	3	16
	MS	313	29.6	22.4	0.43	405	68.6	25.1	0.84
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Root Length	*df*	1	3	3	16	1	3	3	16
	MS	11,726	676	592	7.8	14,970	751	572	5.8
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Fresh Seedlings Weight	*df*	1	3	3	16	1	3	3	16
	MS	9.81	1.16	0.31	0.00	7.50	0.58	0.40	0.00
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Dry Seedlings Weight	*df*	1	3	3	16	1	3	3	16
	MS	0.08	0.01	0.00	0.00	0.08	0.00	0.00	0.00
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Shoot Elongation Rate	*df*	1	3	3	16	1	3	3	16
	MS	2.94	1.48	0.36	0.04	3.60	3.19	0.93	0.04
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Root Elongation Rate	*df*	1	3	3	16	1	3	3	16
	MS	212	35.2	4.77	0.45	386	14.3	7.35	0.21
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Seedling Vigor Index	*df*	1	3	3	16	1	3	3	16
	MS	2,051,443	111,435	64,489	1269	2,165,740	106,413	84,531	648
	p	0.00	0.00	0.00		0.00	0.00	0.00	
Relative Water Content	*df*	1	3	3	16	1	3	3	16
	MS	26.3	8.4	0.9	0.4	2.5	106.5	23.3	0.6
	p	0.00	0.00	0.11		0.05	0.00	0.00	
Chlorophyll Content	*df*	1	3	3	16	1	3	3	16
	MS	0.00	0.10	0.02	0.00	0.01	0.10	0.01	0.00
	p	0.70	0.00	0.03		0.13	0	0.03	
Membrane Stability Index	*df*	1	3	3	16	1	3	3	16
	MS	4.6	8.0	0.4	0.8	1.4	18.3	7.1	0.5
	p	0.03	0.00	0.73		0.13	0.00	0.00	

Under optimal conditions, seed priming with H_2O, $CaCl_2$, and SA increased germination energy by 8.3%, 8.3%, and 9% in cv. 1, and by 2%, 1%, and 1% in cv. 2, as compared to the control (Table 2a). Moreover, hormopriming led to the highest increase in germination energy of cv. 1 and cv. 2 (8.7% and 4.5%), followed by osmopriming (6.4% and 3.2%) and hydropriming (5% and 2.2%), over control in heat stress (Table 2b). Beneficial effects of priming treatments on final germination were also observed, with the following enhancements in cv. 1 and cv. 2 as compared to the control: H_2O (4.6% and 0.3%), $CaCl_2$ (5% and 0.3%), and SA (6.6% and 0.6%) under optimal conditions (Table 2a); H_2O (3.7% and 3.3%), $CaCl_2$ (4.7% and 3.7%), and SA (8% and 5.7%) under stress conditions (Table 2b). Moreover,

the highest increase in seedling vigor index of cv. 1 was obtained by hormopriming (10.5% and 22.9%), whereas osmopriming had the best effect in cv. 2 (50.9% and 87.3%), under optimal and heat stress conditions, respectively (Table 2a-b).

Table 2. Effect of seed priming treatments on germination energy (GE), final germination (FG), abnormal seedlings (AS), and seedling vigor index (SVI) of garden pea cultivars under optimal conditions (a) and heat stress (b).

Treatments	Traits							
	GE (%)		FG (%)		AS (%)		SVI	
	cv. 1	cv. 2	cv. 1	cv. 2	cv. 1	cv. 2	cv. 1	cv. 2
				Optimal Conditions (a)				
Control	77.0 c	93.0 a	84.7 c	97.7 a	3.00 a	0.67 b	760 f	1061 d
Hydropriming (H_2O)	85.3 b	95.0 a	89.3 b	98.0 a	2.67 a	0.67 b	820 ef	1392 c
Osmopriming ($CaCl_2$)	85.3 b	94.0 a	89.7 b	98.0 a	2.67 a	1.00 b	824 ef	1601 a
Hormopriming (SA)	86.0 b	94.0 a	91.3 b	98.3 a	2.00 a	1.00 b	840 e	1530 b
Average	83.4 B	94.0 A	88.8 B	97.8 A	2.59 A	0.84 B	804 B	1396 A
				Heat Stress (b)				
Control	69.3 e	83.8 b	72.3 f	85.0 c	1.00 a	2.00 a	375 f	648 d
Hydropriming (H_2O)	74.3 d	86.0 a	76.0 e	88.3 b	1.33 a	1.67 a	417 ef	1047 c
Osmopriming ($CaCl_2$)	75.7 cd	87.0 a	77.0 e	88.7 b	2.33 a	1.67 a	373 f	1214 a
Hormopriming (SA)	78.0 c	88.3 a	80.3 d	90.7 a	2.33 a	1.00 a	461 e	1119 b
Average	74.3 B	86.2 A	76.4 B	88.2 A	1.75 A	1.59 A	406 B	1007 A

Data are represented as mean (n = 3). Differences between treatments were analyzed using Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different.

Table 3. Effect of seed priming treatments on shoot length (SL), root length (RL), fresh seedling weight (FSW), and dry seedling weight (DSW) of garden pea cultivars under optimal conditions (a) and heat stress (b).

Treatments	Traits							
	SL (mm)		RL (mm)		FSW (g)		DSW (g)	
	cv. 1	cv. 2	cv. 1	cv. 2	cv. 1	cv. 2	cv. 1	cv. 2
				Optimal Conditions (a)				
Control	23.8 e	26.2 d	65.9 e	82.4 d	1.91 f	2.83 d	0.20 g	0.27 d
Hydropriming (H_2O)	24.3 e	30.3 c	67.5 e	112 c	2.49 e	3.48 c	0.24 e	0.32 c
Osmopriming ($CaCl_2$)	24.6 e	35.4 a	67.2 e	129 a	2.46 e	4.37 a	0.23 f	0.38 a
Hormopriming (SA)	24.2 e	34.1 b	67.7 e	122 b	2.46 e	3.77 b	0.23 ef	0.37 b
Average	24.0 B	31.5 A	66.6 B	111 A	2.33 B	3.61 A	0.22 B	0.33 A
				Heat Stress (b)				
Control	10.6 e	13.6 d	41.2 d	62.6 c	1.13 f	1.62 d	0.13 g	0.20 d
Hydropriming (H_2O)	12.4 d	20.8 c	42.4 d	97.7 b	1.22 e	2.25 c	0.14 fg	0.24 c
Osmopriming ($CaCl_2$)	13.5 d	26.5 a	45.0 d	110 a	1.23 e	2.45 b	0.15 e	0.30 a
Hormopriming (SA)	14.1 d	22.5 b	43.3 d	101 b	1.26 e	3.00 a	0.14 f	0.28 b
Average	12.6 B	20.9 A	43.0 B	92.9 A	1.21 B	2.33 A	0.14 B	0.26 A

Data are represented as mean (n = 3). Differences between treatments were analyzed using Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different.

Moreover, a significant effect of priming treatments, in relation to the control, was observed in fresh and dry seedling weight of cv. 1 under optimal conditions; shoot length, fresh seedling weight, and dry seedling weight (except hydropriming) of cv. 1 under heat stress; shoot and root length, fresh and dry seedling weight of cv. 2, under both conditions (Table 3a,b). Additionally, significant differences between individual treatments were noted, except for shoot length, root length, and fresh seedling weight (H_2O vs. $CaCl_2$ vs. SA)

of cv. 1 under both conditions; dry seedling weight of cv. 1 under optimal conditions (H_2O vs. SA and $CaCl_2$ vs. SA) and heat stress (H_2O vs. SA) (Table 3a,b). Under optimal conditions, the highest increase in shoot length of cv. 1 and cv. 2 (3.4% and 35.2%), as well as root length, fresh seedling weight, and dry seedling weight of cv. 2 (56.8%, 54.4%, and 40.9%), as compared to control, was observed after seed priming with $CaCl_2$ (Table 3a). On the contrary, other treatments exhibited a higher effect on root length (SA), and fresh and dry seedling weight (H_2O) of cv. 1 in the same conditions, leading to an increase of 2.7%, 30.4%, and 20.9%, respectively (Table 3a). Priming treatments had a relatively similar pattern and stronger influence under heat stress, where root length and dry seedling weight of cv. 1 (9.2% and 13.5%) and cv. 2 (76.5% and 45.3%), as well as shoot length of cv. 1 (32.7%) were greatly improved after osmopriming (Table 3b). Moreover, the highest increase in shoot length of cv. 1 (32.7%), and fresh seedling weight of cv. 1 (11.5%) and cv. 2 (85.2%) over control were recorded after seed priming with SA (Table 3b).

Table 4. Effect of seed priming treatments on shoot elongation rate (SER), root elongation rate (RER), relative water content (RWC), chlorophyll content (Chl), and membrane stability index (MSI) of garden pea cultivars under optimal conditions (a) and heat stress (b).

Treatments	Traits									
	SER		RER		RWC (%)		Chl (mg g^{-1} FW)		MSI	
	cv.1	cv.2	cv.1	cv.2	cv.1	cv.2	cv.1	cv.2	cv.1	cv.2
Optimal Conditions (a)										
Control	5.60 d	6.27 c	10.3 f	15.7 d	79.5 e	81.6 cd	0.84 d	1.04 c	78.4 d	78.8 cd
Hydropriming (H_2O)	5.72 d	6.74 b	11.1 f	18.7 c	80.6 d	83.2 b	1.16 abc	1.10 bc	79.4 bcd	80.1 bc
Osmopriming ($CaCl_2$)	6.27 c	7.35 a	14.2 e	21.3 a	81.8 c	84.5 a	1.19 abc	1.13 abc	80.2 bcd	81.1 ab
Hormopriming (SA)	6.99 ab	7.01 ab	16.2 d	19.9 b	82.4 bc	83.4 b	1.26 a	1.24 ab	80.5 ab	82.0 a
Average	6.14 B	6.84 A	13.0 B	18.9 A	81.1 B	83.2 A	1.11 A	1.13 A	79.6 B	80.5 A
Heat Stress (b)										
Control	2.79 e	2.79 e	6.89 g	11.6 d	68.5 d	63.4 e	0.74 d	0.83 c	70.6 de	69.1 f
Hydropriming (H_2O)	3.39 d	3.79 c	6.57 fg	15.6 c	71.3 c	74.8 b	0.84 c	0.93 b	70.4 def	69.6 ef
Osmopriming ($CaCl_2$)	3.53 cd	5.37 a	8.14 e	17.5 a	71.4 c	74.3 b	0.97 b	0.97 b	71.3 cd	74.7 a
Hormopriming (SA)	3.71 cd	4.58 b	7.56 ef	16.6 b	75.2 b	76.5 a	1.11 a	1.05 a	72.5 bc	73.3 b
Average	3.36 B	4.13 A	7.29 B	15.3 A	71.6 B	72.3 A	0.91 A	0.94 A	71.2 A	71.7 A

Data are represented as mean (n = 3). Differences between treatments were analyzed using Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different.

Furthermore, priming treatments had significant and positive effects, compared to the control, except hydropriming in case of shoot and root elongation rate (cv. 1), chlorophyll content (cv. 2), membrane stability index (both cultivars), as well as osmopriming in case of chlorophyll content (cv. 2) and membrane stability index (cv. 1) under optimal conditions (Table 4a); hydropriming in case of root elongation rate (cv. 1), membrane stability index (both cultivars), as well as osmopriming in case of membrane stability index (cv. 1) under stress conditions (Table 4b). In addition, significant differences between treatments were recorded as follows: $CaCl_2$ vs. SA for shoot elongation rate (cv. 1), root elongation rate (both cultivars), and relative water content (cv. 2) under optimal conditions (Table 4a), as well as shoot and root elongation rate (cv. 2), relative water content (both cultivars) chlorophyll content (both cultivars), and membrane stability index (cv. 2) in heat stress (Table 4b); $CaCl_2$ vs. H_2O in all parameters (both cultivars), except chlorophyll content and membrane stability index under optimal conditions (Table 4a), shoot elongation rate (cv. 2), root elongation rate (both cultivars), chlorophyll content (cv. 1), and membrane stability index (cv. 2) in heat stress (Table 4b); and SA vs. H_2O in shoot elongation rate (cv. 1), root elongation rate (both cultivars), relative water content (cv. 1), and membrane stability index (cv. 2) under optimal conditions (Table 4a), shoot and root elongation rate (cv. 2), relative

water content, chlorophyll content, and membrane stability index (both cultivars) under stress conditions (Table 4b).

Under optimal conditions, priming with SA led to the highest increase in comparison to the control in shoot elongation rate (24.8%), root elongation rate (57.3%), relative water content (3.6%), chlorophyll content (49.6%), and membrane stability index (2.7%) of cv. 1, as well as chlorophyll content (18.5%) and membrane stability index (4.1%) of cv. 2 (Table 4a). Additionally, the shoot elongation rate (17.2%), root elongation rate (36%), and relative water content (3.6%) of cv. 2 after priming with $CaCl_2$ showed the highest enhancement over the control in the same conditions (Table 4a). Similarly, the greatest improvements in shoot elongation rate (33%), relative water content (9.8%), chlorophyll content (50.2%), and membrane stability index (2.7%) of cv. 1, as well as relative water content (20.7%) and chlorophyll content (27.4%) of cv. 2 in heat stress, compared to the control, were observed after hormopriming (Table 4b). Moreover, osmopriming was the best treatment in the case of root elongation rate (18.1%) of cv. 1, as well as shoot elongation rate (92.5%), root elongation rate (50.5%), and membrane stability index (8.1%) of cv. 2 under stress conditions, as compared to control (Table 4b).

Correlation analysis confirmed the beneficial effects of seed priming treatments in both optimal and heat stress conditions. A positive relationship was established between examined parameters, with the exception of abnormal seedlings (Figure 1a,b). All parameters of normal seeds were significantly interrelated, except chlorophyll content, which was significantly correlated only with relative water content, membrane stability index, shoot elongation rate, and root elongation rate (Figure 1a). Similarly, a significant dependence was observed between chlorophyll content and germination energy, final germination, shoot length, relative water content, membrane stability index, and shoot elongation rate of heat-treated seeds (Figure 1b). Furthermore, relative water content and membrane stability index were significantly interrelated, as well as with shoot length, root length, seedling vigor index, fresh seedling weight, and dry seedling weight, while other examined parameters of seeds under stress were also strongly related (Figure 1b).

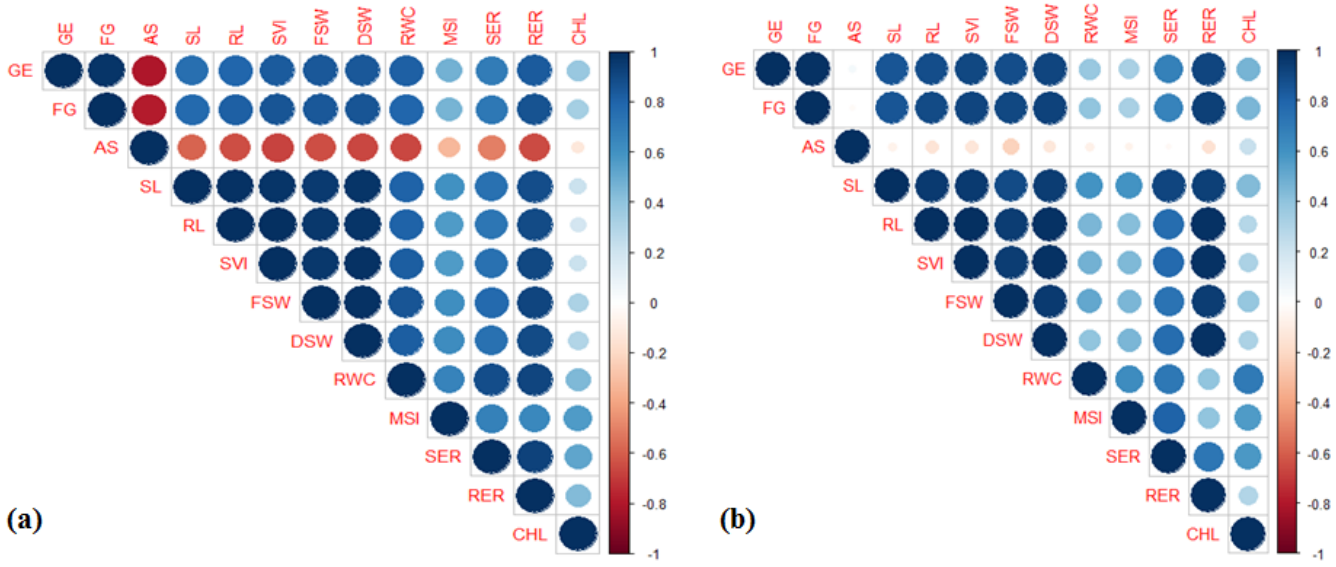

Figure 1. Correlation heat map of germination, initial seedling development and physiological traits of a garden pea. (**a**) Optimal Conditions; (**b**) Heat stress. Blue colors indicate positive relationships, and red colors indicate negative relationships. Note: (GE) germination energy; (FG) final germination; (AS) abnormal seedlings; (SL) shoot length; (RL) root length; (SVI) seedling vigor index; (FSW) fresh seedling weight; (DSW) dry seedling weight; (RWC) relative water content; (MSI) membrane stability index; (SER) shoot elongation rate; (RER) root elongation rate; (CHL) chlorophyll content.

4. Discussion

In this study, heat stress had an evident negative effect on seed germination and germination-related parameters as well as on physiological parameters such as relative water content, membrane stability index, and chlorophyll content, while both garden pea cultivars showed a significant reduction in the above-mentioned parameters following heat stress treatment. The detrimental impact of heat stress can be attributed to physiological and biochemical changes such as disruption of biomembranes, misbalance of phytohormones, deterioration of main enzymes, accumulation of reactive oxygen species (ROS), damage of protein and nucleic acid structures, reduction in photosynthesis, etc. [2,25,42,43]. It has been reported that heat stress causes a reduction in shoot growth, root growth, plant height, biomass, relative water content, and chlorophyll content of faba bean, which is consistent with our results [44]. Similar results have also been obtained in *Brassica oleracea* [45] and alfalfa [46].

Numerous studies described seed priming as a common technique for promoting germination, improving morphological characteristics, and enhancing plant development under both non-stress and stress conditions [28,47]. Seed priming is known to improve germination performance through early emergence and uniform crop establishment [22]. Priming induces a variety of metabolic changes in the seed during germination and increases seed vitality, resulting in quick and homogenous emergence, as well as strong stand establishment [34,48]. In this regard, our results revealed the beneficial effects of hydropriming, halopriming, and hormopriming on seed germination and germination-related parameters of garden pea cultivars under both optimal and heat stress conditions. Hydropriming led to a significant increase in the above-mentioned parameters under both non-stress and stress conditions due to its effects on partial hydration of seeds and initiation of early activities and metabolic processes [25], but to a lesser extent than other tested priming treatments. The greatest improvement in seed germination was observed with priming with SA, followed by priming with $CaCl_2$ in both conditions. Seed priming techniques such as osmopriming and hormopriming have been suitable for overcoming the adverse effects of salinity and drought [26,49,50], as well as heat stress [22]. Seed priming triggers a series of biochemical changes, such as enzyme activation, hydrolysis [22,51], metabolic reparation [52], and build-up of germination-enhancing metabolites [53], which leads to accelerated seed germination, as well as vigorous plant growth. Improvement of seed quality and seedling growth due to seed priming subsequently leads to a higher seedling vigor index. In our study, all priming treatments improved the seedling vigor index compared to the control. The highest increase was observed in osmopriming, followed by hormopriming both under optimal and high-temperature conditions (Table 1). Previous studies have also confirmed that osmopriming with $CaCl_2$ improved seed germination, seedling length, and seedling vigor index under stress conditions such as low temperature [54].

According to the cultivar average, different effects of priming treatments on most parameters were recorded under both optimal and high-temperature conditions. A different response to priming treatments of the examined cultivars, especially in growth, biomass, and physiological parameters, indicates the possibility of improving the performance and heat stress tolerance of garden pea by selecting the proper priming treatment for specific cultivars.

An increase in seedling growth and seedling weight by seed priming with SA, followed by priming with $CaCl_2$, has also been reported in maize. This is due to a higher rate of cell division within apical meristem and regulated plant growth through enhanced cell enlargement and cell division in seedlings [22,55]. Furthermore, our study observed that pea cultivars face heat stress in the early stages of development, causing a decrease in shoot and root elongation rate, relative water content, chlorophyll content, and membrane stability index (Table 4b). The greatest increase in shoot elongation rate and root elongation rate was observed after $CaCl_2$ priming, followed by SA, but the opposite results were obtained for relative water content. According to Iqbal et al. [22], primed seeds emerged earlier and stimulated seedling vigor, ultimately resulting in greater seedling

length and accumulation of biomass under heat stress, which is in agreement with our results. The decrease in relative water content, as a suitable indicator of heat stress sensitivity, could be associated with slower root growth and might affect plant metabolism [46]. Iqbal et al. [22] stated that decreased relative water content due to heat stress might be the result of lower metabolites and osmotic concentration within tissue to hold water. The improvement of relative water content due to seed priming with $CaCl_2$ and SA under various temperature conditions was observed in maize [56], which is in agreement with our results. Ranty et al. [57] stated that calcium acts as a secondary messenger that regulates stress mechanisms that help plants adapt to adverse conditions, as well as regulates plant cell metabolism and thus increases plant stress tolerance. On the other hand, priming with SA increased the relative water content of pea cultivars under optimal and stress conditions, which might be due to the enhanced plant tolerance to stress by modifying the antioxidant activity system [22].

Hydropriming also significantly improved pea parameters under stress conditions but to a lesser extent. Similarly, despite the decrease in chlorophyll content and membrane stability index in the control due to heat stress, seed priming treatments improved these parameters, except for the effect of hydropriming on the membrane stability index (Table 4b). Ahmad et al. reported similar results in maize [58]. The cultivar average revealed that priming with SA had the highest increase in chlorophyll content, while priming with $CaCl_2$, followed by priming with SA had the highest increase in membrane stability index compared to the control. SA proved to be an efficient treatment for improving chlorophyll content due to an enhancement in antioxidants, which might mitigate the deleterious effects of heat stress and prevent the degradation of chlorophyll [22,58]. Additionally, according to Iqbal et al. [22], both priming with $CaCl_2$ and SA also increased the membrane stability index in maize under heat stress. However, they stated that hormopriming, followed by osmopriming, was ranked the highest, which is contrary to our results.

Overall, the examined cultivars positively responded to all priming treatments, both under optimal and stress conditions. In both conditions, hormopriming had the predominant effect on germination and germination-related parameters of cultivars, whereas only the seedling vigor index of cv. 2 had the highest value after osmopriming. Furthermore, the results of this study showed that cultivars reacted differently to the priming treatment for certain parameters. Namely, seed priming with $CaCl_2$ had a prevailing effect on seedling growth, biomass accumulation, and growth-related parameters, leading to the largest improvements in shoot length of cv. 1 and cv. 2, as well as root length, fresh and dry seedling weight, shoot and root elongation rate of cv. 2, under optimal conditions; root length, dry seedling weight, and root elongation rate of cv. 1, as well as shoot length, root length, dry seedling weight, shoot and root elongation rate of cv. 2, under heat stress. In the case of physiological treatments, hormopriming had the highest effect on relative water content, membrane stability index, and chlorophyll content of cv. 1 in both conditions; membrane stability index and chlorophyll content of cv. 2 under optimal conditions, as well as relative water content, and chlorophyll content of cv. 2 in heat stress. Additionally, osmopriming had the strongest influence only on relative water content and membrane stability index of cv. 2, under optimal and stress conditions, respectively.

The different effects of the applied treatments on certain parameters in the examined cultivars can be attributed to the cultivar characteristics and the quality of a seed lot. The selected garden pea cultivars differ in the length of vegetation, plant height, grain weight, the shape of the pods, the number of pods per plant, and grain yield. Cultivar cv. 1 has a shorter growing season (very early variety, 55–60 days), while cv. 2 has a slightly longer growing season (early variety, 58–63 days). Moreover, pea cultivars cv. 1 and cv. 2 also differ in grain yield (5.6 t ha^{-1} and 6.6 t ha^{-1}, respectively). Garden pea cultivar cv. 1 enters the flowering phase in optimal climatic conditions, blooms, and pollinates earlier, which gives it more reliable opportunities to form grain yield than cv. 2, due to the depressing effect of high temperature during flowering. Pea cultivar cv. 1 is created for the needs of the green market as fresh grain, as well as the needs of the industry for

freezing young green grain, while cv. 2 forms larger grains, has a high yield potential, and is created for the needs of industrial production. Climatic conditions in the Vojvodina Province (Serbia) are continental, with frequent drought in winter and high-temperature periods in summer. The critical period for garden pea yield formation is considered to be the emergence in February and March and the period during and after flowering in April and May when high temperatures occur in most years. In drought conditions, the pods of both cultivars lag behind in growth and form fewer grains. Therefore, we hypothesized that heat stress could have a predominant effect on garden pea production through the effect on seed germination and the initial seedling growth and development, which was confirmed in this study under different laboratory conditions. Further research on effective seed priming treatments through field trials will be necessary in order to establish their efficiency in different environmental conditions. Implementation of this strategy in the current agronomic practice could contribute to the prevention of crop losses and improve garden pea production under changing climate conditions.

5. Conclusions

This study confirmed that heat stress significantly decreased seed germination and hindered stand establishment, causing increased membrane leakage and reduced relative water and chlorophyll contents of examined garden pea cultivars. Different priming techniques used in the study improved germination performance, seedling development, and physiological characteristics of garden pea cultivars under both optimal and adverse conditions, mitigating the deleterious effect of heat stress. Overall, osmopriming and hormopriming had a significant effect compared to the control and better results compared to hydropriming. Both priming techniques could be recommended as important heat stress-resistant strategies for the improvement of garden pea production, especially in future climate change scenarios. To the best of our knowledge, this is the first study on the effects of seed priming treatments for improving seed quality and initial plant development of garden pea under heat stress. Future research should focus on testing the various concentrations and priming conditions of effective treatments in different cultivars and environmental conditions.

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