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Hemp Seed Priming via Different Agents to Alleviate Temperature Stress

Sibel Day[a*], Nilüfer Koçak-Şahin[a], Burak Önol[a]

[a]*Ankara University, Faculty of Agriculture, Department of Field Crops, 06110, Dışkapı, Ankara, TÜRKİYE*

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Corresponding Author: Sibel Day, E-mail: day@ankara.edu.tr
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ABSTRACT
Hemp (*Cannabis sativa* L.) seeds were treated with different priming agents (water, -0.8 MPa PEG 6000, 50 mMol thiamine and 10 mMol mannitol) and subjected to different temperatures (10, 15, 20, 25 and 30 °C). The impacts of low and high temperature on germination and initial growth, along with optimal conditions, were evaluated with priming agents. Results revealed that seed treatment accelerated mean germination time and increased emergence percentage at lower temperatures. The minimum mean germination times of 1.38 and 1.39 days were obtained at 20 °C with hydro priming and mannitol priming. The maximum germination percentages of 89.5 and 90% were observed with mannitol and hydro priming at 20 °C. Retarded seedling emergence was noted at 10 °C. Minimum emergence percentage at 10 °C was 46% in control samples. Seed pre-treatments also promoted shoot length at all temperatures. However, root length promoting effects of seed treatments were more evident at 10, 15 and 20 °C. The minimum root length was 3.04 cm in controls at 30 °C. Seedling fresh and dry weight reached maximum values at 20 °C with water, thiamine and mannitol treatments. Chlorophyll and leaf proline content reached their highest values at 20, 25 and 30 °C. Overcoming temperature stress and promoting germination is important for hemp development. It was concluded that hemp seeds primed with water, thiamine, and mannitol had the highest biomass values for low, optimum, and high temperatures in this study. This indicates that these seed treatments are suitable for hemp plants that could experience low or high temperatures during germination and early growth stages.

Keywords: *Cannabis sativa* L., Hydro priming, Mannitol, Osmo priming, Thiamine

1. Introduction

Cannabis sativa L. (hemp) has been cultivated for more than 5000 years for its fibre, seeds and phytochemicals used in ethno-medicines and pharmacotherapy (Odieka et al. 2022). It is highly resistant against a large number of insect pests, and has a well-developed root system (Visković et al. 2023).

Hemp is cultivated in a wide range of climatic areas in Türkiye and the world with diversity in sowing time and temperatures depending on the longitude (day length), latitude, and altitude (meteorological parameters). The cultivation area in Türkiye is increasing due to increasing demand for hemp fibre. The most important factor in hemp cultivation is temperature. The seeds germinate best when the soil temperature is above 15 °C (Geneve et al. 2022). Most crops exhibit temperature-dependent seed germination. The temperature has an effect on both the percentage of seed germination and the time it takes for the first root to emerge. It is important to study seed germination in hemp at different temperatures. The temperature limits for hemp are important and global warming has motivated scientists to research the impacts of high temperatures. Exposure of seeds to high temperature stress during germination leads to delayed mobilisation of storage reserves, protein degradation, loss of enzyme activity, de novo protein synthesis, and membrane integrity resulting in cellular damage and collapse after prolonged exposure (Essemine et al. 2010). Although, hemp is a multifunctional medicinal plant and an alternative crop like sweet basil (Day 2021), safflower (Day et al. 2017) and black cumin (Day et al. 2022), studies about its germination, emergence and early seedling growth stages are rare.

Therefore, it is desirable to determine the right time and climate to ensure appropriate and good yield. There are a limited number of cultivars and little knowledge about the impact of temperature on seed germination, which is generally genotype dependent. Slow and reduced germination or late sowing could lead to low yield (Day 2022). The uncertain Central Anatolian climatic conditions, with low moisture, low temperature and frequently changing meteorological conditions, leave farmers with many problems during seed sowing and emergence.

Several strategies are employed by farmers in soil to improve germination and emergence. Seed treatments before sowing are widely used as an easy way to biofortify seeds with different chemicals or nutrient elements to stimulate their germination and emergence and inhibit the effects of external stresses, such as temperature or water deficit (Day & Koçak-Şahin 2023a).

Seed priming is a technique inducing pre-germinative metabolism via controlled rehydration (Paparella et al. 2015). The advantages of seed priming were revealed in both optimal and unfavourable conditions for many oil seed crops such as corn, sunflower and canola (Shrestha et al. 2019; Bourioug et al. 2020; Day 2022). This technique could also be used in industrial hemp to improve germination performance. Hemp seeds were primed with GA3 to alleviate drought stress and enhancing impacts were reported with 400 and 600 mg L^{-1} GA3 doses (Du et al. 2022).

The detrimental impact of hydro priming could be reduced by inducing high osmotic pressure, so polyethylene glycol (PEG 6000) is one of the solutes frequently used for seed priming (Lemmens et al. 2019). Thiamine (vitamin B1) is produced by microbes and plants (Suohui et al. 2022) and it is well known that both thiamine and its phosphate esters are modulated by stress factors, which end up increasing total contents in many plant species (Rapala-Kozik et al. 2008; Tunc-Ozdemir et al. 2009). Many studies reported that the continuous synthesis of thiamine or enhanced endogenous levels could to improve the health of plants and their tolerance against harsh stressful conditions (Jabeen et al. 2022).

In plants, mannitol provides resistance under abiotic stress conditions (Hema et al. 2014). Increased concentrations improve resistance against salinity or drought stress conditions, as evident in peach (Lo Bianco et al. 2000), transgenic wheat, sugarcane, rice and sorghum (Cha-um et al. 2009). Improving effects of mannitol were also observed on *Zea mays* L. under saline conditions and Cr toxicity with exogenous application (Kaya et al. 2013; Habiba et al. 2019).

Overall, the purpose of the experiment was to evaluate the effect of several priming agents at different temperatures on the germination of industrial hemp seeds and to reduce the germination time. Furthermore, the study evaluated the use of substantially different agents to improve hemp seed germination and seedling growth at different temperatures.

2. Material and Methods

The hemp seeds used in this study belonged to the Narlısaray population procured from the Directorate of Agricultural Management at Gökhöyük, Amasya, Türkiye. The hemp seeds were primed using following treatments:

I. Control: The seeds were germinated without any pre-treatment.
II. Hydro priming: The seeds were soaked in distilled water at 20 °C for 8 h.
III. Polyethylene glycol: The seeds were soaked in -0.8 MPa PEG 6000 solution at 20 °C for 8 h. Osmotic potential of PEG 6000 was adjusted according to Michel & Kaufmann (1973).
IV. Thiamine: The seeds were soaked in 50 mMol thiamine solution for 8 h.
V. Mannitol: The seeds were soaked in 10 mMol mannitol solution for 8 h.

After priming with PEG, thiamine and mannitol, the seeds were rinsed with distilled water to remove any traces of the respective chemicals from the surface of the seeds, followed by drying to initial weight.

2.1 Germination test

The experiment was conducted with four replications using 50 seeds in each replicate. Seeds from each treatment were placed into three layers of filter paper moistened with 21 mL of deionized water to check their seed viability. The rolled papers were put into sealed plastic bags and placed in an incubator in the dark. The seeds with a radicle length of 2 mm were counted as germinated. The germinated seeds were recorded daily for 14 days to calculate the mean germination time (MGT), and the germination percentage (GP). Germination and early seedling growth of control and primed seeds were evaluated at low temperatures (10±1 and 15±1 °C), room temperatures (20±1 and 25±1 °C) and high temperature (30 °C).

Germination speed was evaluated according to mean germination time (MGT) using the formula given below (ISTA 2017):

$$MGT = \frac{\Sigma(n \times t)}{N}$$

Where; n is the number of germinated seeds on each day, t is the days from planting and N is the total number of germinated seeds.

The germination percentage was calculated with the following equation (Day and Koçak-Şahin 2023b):

$$GP = \frac{NG}{NT} \times 100$$

Where; *GP* is the emergence percentage, *NG* is the number of germinated seeds and *NT* is the total number of seeds.

Germination index (GI) calculation was done according to the equation given below (Abdul-Baki & Anderson (1973):

$$GI = Number\ of\ germinated\ seeds/day\ of\ first\ count + \cdots + Number\ of\ germinated\ seeds/day\ of\ final\ count.$$

2.2 Emergence tests

Four replicates of 50 seeds (50 × 4= 200 seeds) were sown at a depth of 2 cm in plastic trays (30 × 21 × 9 cm) containing peat and placed in a growth chamber (Sanyo versatile growth chamber, Japan) at 10±1, 15±1, 20± 1, 25±1, and 30±1°C under 45 µM photons m^{-2} s^{-1} light intensity for 16 h with 45% humidity. The peat used in the study had pH of 6.5 and EC of 40 mS m^{-1}, with porosity of around 69% (v w^{-1}).

The number of emerged seedlings (unfolding cotyledons on the surface) was counted daily up to 25 days, along with the emergence percentages of the respective seedlings. The plants were irrigated with 50 ml water 5 times during the 26 days of the experiment. The mean emergence time (MET, days), emergence percentage and emergence index were calculated according to the formulas given in the germination test.

Chlorophyll contents were measured on the 25th day. Shoot length, root length, seedling fresh and dry weight were measured for all seedlings from each replicate after the 26th day. Fresh weights of seedlings were measured soon after harvest to avoid weight loss (Day 2016). The dry weight of the seedlings was measured after drying the samples in an oven at 70 °C for 48 h (Yildiz et al. 2010).

2.3 Chlorophyll index and proline content determination

Chlorophyll measurements were done with SPAD-502 Plus (Konica Minolta) using five leaves per seedling. Ten seedlings from each replicate were used for sampling.

Free proline quantification in the shoots was determined following Bates et al. (1973). Proline was extracted from 100 mg of leaf samples by using ninhydrin reagent in 3% (w/v) aqueous sulfosalicylic acid. The organic toluene phase was separated and the absorbance of the red colour was read at 520 nm. Concentration of proline (µmol g^{-1} FW) was determined using a calibration curve prepared with L-proline.

2.4 Experimental design and statistical analysis

The design of the study was based on two factorials (5 × 5) arranged in a randomized complete block design with four replicates. The main factor was temperature and the sub-factor was seed priming treatments. Data for the emergence percentage were subjected to arcsine transformation before ANOVA. The differences between the means were compared using Duncan's multiple range test ($P<0.01$).

3. Results

3.1 Germination and emergence test

Significant differences between priming agents were determined particularly at 10, 15 and 20 °C for mean germination time and germination percentage (Table 1). Decreased temperature led to an increase in MGT. The germination percentage and mean germination time were closely affected by temperature. PEG increased the mean germination time (6 days) compared to other treatments at 10 °C. However mean germination time of control treatments at other temperatures was longer than with the priming treatments. The lowest mean germination time for control and other treated seeds was observed at 30 °C with non-significant numerical differences of 1.67-1.82 d to germinate. Overall, the minimum mean germination time was observed with hydro priming (1.38 day) and mannitol priming (1.39 day) at 20 °C.

The maximum germination percentage values (89.5 and 90%) were recorded for mannitol based osmo priming and hydro priming at 20 °C (Table 1). Mannitol based osmo primed seeds failed to induce similar germination behaviour at 10-15 °C when compared with their performance at 20-25 °C.

Table 1- Impact of priming on MGT and germination percentages for hemp at different temperatures

Priming	*Mean germination time (day)*					*Germination percentage (%)*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30 °C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	5.21^{b}	4.10^{c}	2.87^{ef}	2.92^{ef}	1,80^{g}	80.0^{bcd}	77.5^{bcd}	85.5^{abc}	83.0^{abc}	84.5^{abc}
Water	5.29^{b}	3.04^{e}	1.38^{g}	2.56^{ef}	1,67^{g}	83.5^{abc}	86.0^{ab}	90.0^{a}	85.5^{abc}	86.5^{ab}
PEG	6.00^{a}	3.02^{e}	2.70^{ef}	2.57^{ef}	1,78^{g}	77.5^{bcd}	86.0^{ab}	81.0^{bcd}	83.5^{abc}	72.0^{d}
Thiamine	5.26^{b}	2.90^{ef}	2.45^{f}	2.66^{ef}	1,82^{g}	80.5^{bcd}	84.5^{abc}	80.0^{bcd}	84.0^{abc}	77.5^{bcd}
Mannitol	4.89^{b}	3.50^{d}	1.39^{g}	2.53^{ef}	1,74^{g}	78.0^{bcd}	76.5^{cd}	89.5^{a}	85.0^{abc}	77.5^{bcd}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

Mean emergence time under different temperatures showed diversity (Table 2). Retarded seedling emergence was noted at 10 °C among the temperatures. All priming treatments at 10 °C had insignificant differences. At 15 °C, control seeds emerged earlier than treated seeds. Increased temperature shortened the emergence days of hemp with all treatments. At 25 °C, particularly PEG 6000 osmo primed seeds showed early emergence compared to controls, whereas at 30 °C PEG 6000, thiamine and mannitol-based priming treatments had earlier seedling emergence compared to the control and hydro priming treatments. The results clearly demonstrate that all types of priming treatments could not reduce the emergence time below 7.41 days at 10, 15 and 20 °C.

Table 2- Impact of priming on MET and emergence percentage of hemp at different growing temperatures

Priming	*Mean emergence time (day)*					*Emergence percentage (%)*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30°C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	13.03^{a}	8.00^{def}	7.69^{ef}	7.78^{ef}	8.08^{c-f}	46.00^{d}	47.50^{cd}	83.00^{a}	63.50^{b}	27.00^{e}
Water	12.99^{a}	9.32^{bcd}	8.06^{c-f}	6.84^{fg}	8.24^{b-f}	51.00^{cd}	53.50^{bcd}	76.00^{a}	56.00^{bcd}	29.00^{e}
PEG	13.45^{a}	9.52^{bc}	9.16^{b-e}	6.01^{g}	6.82^{fg}	52.50^{cd}	48.00^{cd}	75.00^{a}	48.00^{cd}	31.50^{e}
Thiamine	13.01^{a}	9.65^{b}	7.92^{def}	7.29^{fg}	6.74^{fg}	58.00^{bc}	49.00^{cd}	82.50^{a}	52.00^{cd}	32.50^{e}
Mannitol	13.97^{a}	9.07^{b-e}	7.41^{fg}	7.00^{fg}	6.77^{fg}	50.00^{cd}	56.00^{bcd}	79.00^{a}	54.50^{bcd}	32.00^{e}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

Seed treatments provided advantages for emergence percentage at 10 and 15 °C compared to controls but insignificant differences were observed (Table 2). At 10 °C, the minimum emergence percentage (46%) was obtained for control seeds. Emergence percentage had the highest results with all priming treatments at 20 °C. The minimum emergence percentages for all priming treatments were observed at 30 °C.

The germination index is a measure of the vigorousness of seeds, and a higher germination index indicates more vigorous seeds. At 10 °C, the best results were obtained from hydro priming with 36.56 and the hydro primed seeds also showed more vigour at 20, 25 and 30 °C. The maximum germination vigour was observed in mannitol-primed seeds at 20 °C (Table 3).

The emergence index values for all treatments reached their highest level at 20 °C and seeds osmo primed with PEG showed difference compared to other treatments (Table 3).

Table 3- Impact of priming on germination index and emergence index of hemp at different growing temperatures

Priming	*Germination index*					*Emergence index*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30°C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	20.44^{i}	29.77^{ghi}	38.97^{fg}	30.68^{ghi}	56.90^{bc}	7.86^{fg}	11.65^{efg}	30.71^{a}	14.56^{cde}	7.86^{fg}
Water	36.56^{fgh}	39.57^{fg}	80.58^{a}	46.16^{c-f}	65.65^{b}	7.96^{fg}	12.48^{def}	28.83^{a}	18.56^{bc}	8.22^{fg}
PEG	27.33^{hi}	40.76^{efg}	43.27^{def}	43.53^{def}	51.87^{cde}	8.45^{fg}	11.25^{efg}	21.50^{b}	17.61^{bcd}	11.34^{efg}
Thiamine	25.77^{i}	52.28^{cd}	46.21^{c-f}	41.79^{def}	53.10^{cd}	10.22^{efg}	9.73^{efg}	30.84^{a}	14.59^{cde}	11.21^{efg}
Mannitol	22.92^{i}	37.31^{fgh}	82.21^{a}	47.19^{c-f}	56.19^{bc}	6.04^{g}	13.00^{def}	27.40^{a}	17.49^{bcd}	12.71^{def}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

3.2 Seedling growth parameters

Smaller shoot length was noted in control treatments regardless of temperature (Table 4). The shoot length in all priming treatments had significant differences compared to control at 10, 15 and 20 °C. The largest shoots were noted at 20 °C with all priming treatments, including the mannitol-based priming, without sharp differences among them. The minimum shoot length with all priming treatments was observed at 30 °C. It is clear and established that increasing temperatures (25 - 30 °C) encouraged reductions in shoot length. Improved shoot lengths were noted for hydro primed, thiamine, and mannitol-based osmo primed seedlings at all temperatures.

Table 4- Impact of priming on shoot length and root length of hemp seedlings at different growing temperatures

Priming	*Shoot length (cm)*					*Root length (cm)*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30°C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	9.19^{g-j}	9.84^{e-h}	12.60^{bc}	8.59^{h-k}	7.27^{k}	3.78^{f-i}	3.99^{e-h}	5.06^{a-d}	4.34^{d-g}	3.04^{i}
Water	11.13^{cde}	11.00^{def}	14.20^{a}	9.99^{d-h}	7.94^{jk}	4.56^{c-f}	5.11^{a-d}	5.54^{ab}	3.50^{ghi}	3.67^{ghi}
PEG 6000	10.34^{d-g}	10.44^{d-g}	13.47^{ab}	8.73^{h-k}	7.70^{jk}	5.54^{ab}	4.62^{c-f}	4.68^{b-e}	3.08^{i}	3.38^{hi}
Thiamine	11.48^{cd}	11.34^{cde}	13.66^{ab}	9.51^{f-i}	7.85^{jk}	5.12^{a-d}	5.34^{abc}	5.22^{a-d}	3.41^{hi}	3.28^{hi}
Mannitol	10.80^{def}	11.42^{cd}	14.36^{a}	10.82^{def}	8.15^{ijk}	5.21^{a-d}	5.69^{a}	5.85^{a}	4.55^{c-f}	3.23^{hi}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

The longest roots with mannitol priming for all temperature levels are shown in Table 4. Hydro priming, PEG 6000 and thiamine-based priming also clearly enhanced root length at 10 and 15 °C. Significant drops in root length were observed for all priming treatments at 25 and 30 °C. The minimum root length (3.04 cm) was obtained in control at 30 °C.

Seedling fresh weight had maximum values at 20 °C for all priming treatments (Table 5). The heaviest seedling fresh weight (582.5 mg plant^{-1}) was recorded after mannitol priming at 20 °C.

Table 5- Impact of priming on fresh and dry weights of hemp seedlings at different growing temperatures

Priming	*Fresh weight (mg plant^{-1})*					*Dry weight (mg plant^{-1})*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30°C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	205.0^{ghi}	292.5^{c-g}	395.0^{bc}	220.0^{f-i}	160.0^{hi}	14.50^{j}	24.50^{f-j}	35.75^{cde}	34.00^{d-g}	17.25^{ij}
Water	230.0^{e-h}	297.5^{c-g}	487.5^{b}	322.5^{c-f}	197.5^{ghi}	21.50^{hij}	28.25^{e-h}	45.00^{abc}	41.50^{a-d}	22.25^{hij}
PEG6000	257.5^{e-h}	247.5^{e-h}	285.0^{d-g}	207.5^{ghi}	122.5^{i}	16.75^{ij}	24.00^{g-j}	39.00^{bcd}	36.50^{b-e}	17.50^{ij}
Thiamine	240.0^{e-h}	237.5^{e-h}	380.0^{cd}	272.5^{d-g}	300.0^{c-g}	17.75^{hij}	26.75^{e-i}	46.00^{ab}	46.25^{ab}	24.75^{f-j}
Mannitol	330.0^{c-f}	335.0^{cde}	582.5^{a}	250.0^{e-h}	295.0^{c-g}	17.00^{ij}	34.50^{def}	51.00^{a}	34.00^{d-g}	18.50^{hij}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

Dry weight gains at low and high temperatures (10 and 30 °C) were reduced in all priming treatments compared to their weight at other temperatures. Seedling dry weights with all priming treatments reached their highest values at 20 and 25 °C. The maximum dry weight (51.00 mg plant^{-1}) was obtained at 20 °C after mannitol priming treatment.

3.3 Leaf chlorophyll index and proline content

The minimum chlorophyll index values were observed for all treatments at 10 °C (Table 6). Only seedlings of PEG 6000 primed seeds with 34.83 SPAD value had significant differences compared to control and other osmo priming treatments. Chlorophyll index reached its highest value at 25 °C and the maximum value was obtained for thiamine priming with 56.03 SPAD value. Especially seedlings obtained from thiamine priming at 25 °C had the highest chlorophyll index. Thiamine and mannitol priming especially at 25 and 30 °C resulted in better chlorophyll index compared to controls.

Table 6- Impact of priming on chlorophyll and proline content in seedling leaves of hemp at different growing temperatures

Priming	*Chlorophyll index (SPAD)*					*Leaf proline content (µmol g^{-1})*				
	10 °C	*15 °C*	*20 °C*	*25 °C*	*30°C*	*10 °C*	*15 °C*	*20 °C*	*25 °C*	*30 °C*
Control	29.64^{i}	35.81^{g}	49.19^{b-e}	46.59^{cde}	46.21^{de}	0,96^{ij}	1.49^{ghi}	1.94^{d-h}	2.43^{bcd}	1.39^{hi}
Water	31.49^{hi}	34.99^{gh}	48.22^{b-e}	50.05^{bcd}	49.77^{bcd}	0,55^{j}	1,34^{hi}	1.76^{e-h}	2.79^{b}	1.48^{ghi}
PEG6000	34.83^{gh}	35.94^{g}	45.31^{e}	48.87^{b-e}	48.90^{b-e}	0,97^{ij}	1.49^{ghi}	2.38^{bcd}	2.09^{c-f}	1.67^{fgh}
Thiamine	30.79^{i}	36.40^{g}	50.00^{bcd}	56.03^{a}	50.87^{bc}	0.59^{j}	1.40^{ghi}	2.27^{b-e}	2.48^{bcd}	1.93^{d-h}
Mannitol	30.38^{i}	41.17^{f}	50.37^{bcd}	51.95^{b}	51.30^{b}	0.71^{j}	1.99^{d-g}	2.37^{bcd}	3.94^{a}	2.59^{bc}

**, All values shown in a block with different letters are dissimilar according to DMRT at P<0.01 level of significance

Proline level increased at room temperatures of 20 and 25 °C and decreased at 30 °C. The minimum proline levels were noted in all treatments at 10 °C. The maximum proline level 3.94 µmol g^{-1} was obtained in seedlings from mannitol-primed seedlings at 20 °C. The seed treatments did not improve leaf proline contents at 10 and 15 °C compared to the control treatment.

4. Discussion

4.1 Germination and Emergence tests

The study's results revealed that increasing temperatures shortened the mean germination time. Seed treatments including hydro priming, -0.8 Mpa PEG 6000, thiamine, and mannitol priming at 10, 15, 20, and 30 °C had positive impacts on the acceleration

of germination, and mobilized responses for the early growth of hemp seedlings under low temperatures. Decreasing time of germination using hydro priming under the low and room temperature conditions is very encouraging and similar enhancing results were reported for canola (Day 2022). The impact of temperature on germination was similar to results by Geneve et al. (2022) who reported that seed germination of hemp was highest at 19 °C.

Seedling emergence is directly influenced by vigour because this shows the ability of seeds to emerge under optimal or adverse field conditions (Kandasamy et al. 2020). All treatments were effective in enhancing germination and emergence indexes. Emergence index values were found to be statistically significant, with values at 20 °C superior to other temperatures. Osmo priming with PEG 6000 decreased the emergence index and this treatment led to retarded emergence at 20 °C compared to control and other treatments.

It was noteworthy that increasing temperatures shortened the time to emergence of the hemp seeds. However, the results suggest taking care in the selection of chemicals for priming treatments because dissimilar chemical agents were not equally effective to shorten the seedling emergence time at different temperatures.

Although hydro primed seeds at 10 °C had a reduced time of 12.99 d for emergence, the control treatment and emergence due to other priming treatments and control treatments had insignificantly different emergence times. It is well known that low temperatures may have full or partial negative delaying effects on seed germination and seedling emergence at low temperatures. In this study, the accelerating impact of seed pre-treatments on seedling emergence, especially for low temperatures, was not seen.

Emergence percentage was enhanced by seed treatments at 10 °C, it increased from 46% in control to 58% with thiamine treatment. All the treatments were effective to improve emergence at 10 °C but there was no significant difference in priming treatments. At all other temperatures, seed pre-treatments did not show significant differences compared to control. Particularly at 20 °C higher germination percentage values were observed compared to lower or higher temperatures. The promoting effects seen with thiamine (incipient thiol) and mannitol priming could be explained by the increased activity of thiol-dependent physiological reactions during germination and seedling growth. In plants, mannitol is an osmolyte and protects thiol-regulated enzymes (e.g., phosphoribulokinase) against hydroxyl radicals, which are abundant during the oxidative stress process and remobilization of stored nutrients during germination (Neumann et al. 1999; Shen et al. 1997).

4.2 Seedling growth parameters

Seed priming promoted seedling growth of hemp at all temperatures. Particularly while mannitol priming at low temperatures and 20 °C helped seedlings to gain more weight, thiamine and hydro priming supported weight gain at 25 and 30 °C. Thiamine efficiency increasing at 25 and 30 °C due to thiamine priming is clearly demonstrated in this study. Thiamine biosynthesis is light dependent and photosynthetic tissues are the main process area (Mozafar & Oertli 1993). The thiamine reserves are stored in seed embryos (Sherwood et al. 1941; Belanger et al. 1995) and influence their elongation and growth, facilitating early and prolonged growth of crop plants and improving chlorophyll pigmentation along with photosynthesis.

Mannitol enrichment of seeds via priming in the present study significantly increased seedling growth parameters and biomass, especially at low temperatures. This enhancement could be attributed to the role of mannitol in cellular osmotic adjustments and its role in scavenging hydroxyl radicals (•OH) (Srivastava et al. 2010; Kaya et al. 2013). During seed priming or seed germination in field conditions, seeds face stress factors related to the seed itself or the environment. Mannitol was shown to have a supporting impact on hemp seed germination and early growth at low temperatures in this study.

4.3 Leaf chlorophyll index and proline content

Chlorophyll pigments are the main constituents of chloroplasts, which regulate the rate and the process of photosynthesis. It is reported that the SPAD value used in chlorophyll measurement is mainly related with nitrogen in plants and is sensitive to environmental stress. Thiamine priming of hemp, especially at 25 and 30 °C, significantly influenced the relative greenness (SPAD value) due to thiamine's role in carbon assimilation (photosynthesis) and respiration. Furthermore, thiamine-based priming led to increased thiamine reserves in hemp seeds and less energy was used for thiamine biosynthesis in chloroplasts. Previous studies also reported a positive impact of exogenous thiamine and thiamine priming on photosynthetic pigments (Bahuguna et al. 2012). Apart from thiamine accumulation in plants during abiotic stress, its exogenous application could provide resistance against abiotic stresses (Kaya et al. 2015; Ghaffar et al. 2019).

Chloroplasts and mitochondria are the main places where proline catabolism occurs (Raza et al. 2023). During the development of chloroplasts, the number, size and composition of plastids change depending on the environmental factors. Particularly, low temperature is an adverse environmental signal that alters processes during development of chloroplasts and influences other issues such as chlorophyll biosynthesis, light energy absorption, and photosynthetic electron transport occurring in chloroplasts (Liu et al. 2018). The low level of chlorophyll index and proline contents at low temperatures could be attributed to retarded and decreased chloroplast development during early seedling growth of hemp.

5. Conclusions

Germination and early seedling growth stages are the most important stages in all crops. Hemp is globally important due to its fibre and chemical constituents, which makes its cultivation important. Its cultivation area is expanding due to changing climate conditions and increasing demand. With increasing cultivation area, hemp could experience different temperature stresses during germination and early seedling growth. The plant is vulnerable to temperature stress in these stages. Overcoming temperature stress is important for hemp development. Hemp seeds primed with water, thiamine, and mannitol had the highest biomass values at low, optimum, and high temperatures in this research, which makes these seed treatments suitable for hemp plants that could experience low or high temperatures during germination and early growth stages.

In conclusion, the following results were observed; accelerated mean germination time and increases in emergence percentage at lower temperatures. Root length promoting effects of seed pre-treatments were more evident at 10, 15, and 20 °C. Seedling fresh and dry weight reached maximum values at 20 °C with water, thiamine and mannitol treatments. Chlorophyll and leaf proline content reached their highest values at 20, 25 and 30 °C.

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