Effect of osmopriming with PEG 6000 on seed germination performance and early seedling establishment in okra (Abelmoschus esculentus (L.) Moench)

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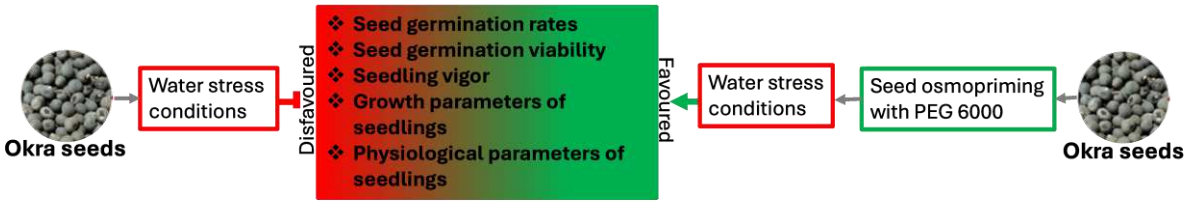
Abstract

Enhancing plant production requires advancing seed germination processes despite environmental constraints. Dry soils often reduce okra yield by hindering seed germination and plant growth. Seed pre-treatment could alleviate water shortage problems. The main objective of this study was to identify water pretreatment conditions conducive to seedling development in okra (*Abelmoschus esculentus* (L.) Moench). We used PEG 6000 solutions with increasing concentrations (*C: 0 MPa; T1: -0.3 MPa; T2: -0.6 MPa; T3: -0.9 MPa; and T4: -1.2 MPa*) to treat okra seeds for 1, 3, 7, and 9 hours. The experiment was conducted using a fully randomized setup with three simultaneous replications. The main findings of the analysis are that water stress has a repressive impact on germination and seedling development in both okra varieties (*Clemson spineless* and *Hire*). Osmotic pretreatments consistently enhanced germination parameters and seedling development, irrespective of the priming duration, particularly showing an improved response in the *Clemson spineless* variety. In comparison to the control group, seeds of the *Clemson spineless* variety that were pretreated exhibited the highest germination rate (95.83%), the highest seedling viability (93.94%), and the greatest seedling vigor (10.61). The highest seedling height (11.55 cm) was achieved in PEG 6000 at a pressure of -0.6 MPa for 9 hours. Under priming conditions, the seedlings of the *Clemson Spineless* variety exhibit an increase in various physiological parameters. The highest values are observed using seeds treated with PEG 6000 at -0.6 MPa for 9 hours (2.77 mg/g for chlorophyll *a*; 1.62 mg/g for chlorophyll *b*; 4.40 mg/g for total chlorophyll, and 6.09 mg/g for carotenoids) compared to the control. Despite the depressive impact of PEG 6000-induced water stress on germination and seedling development, the use of PEG 6000 as a priming promotes germination and seedling development in okra. To achieve optimal okra production in arid or semi-arid environments, it is recommended to utilize seeds of the *Clemson Spineless* variety that have been osmoprimed with PEG 6000.



KeyWords: *Abelmoschus esculentus*, Germination, Osmopriming with PEG 6000, Seedlings, Water stress.

1. Introduction

Increased environmental stressors like salinity, high temperature swings, and water stress are brought on by global change and have a substantial impact on the quantity and quality of plant production [1]. Climate change and global population growth pose significant challenges to agriculture, which uses over 70% of water withdrawals worldwide [2]. A quarter of all farmed area worldwide is used for irrigated agriculture, which produces 40% of the world's food, rainfed agriculture yields less than half as much per unit of land as irrigated agriculture on average [2]. Plant growth, development, and yield are mostly influenced by drought [1]. The amount of drought stress is contingent upon a number of environmental conditions, including the amount and distribution of solar radiation, the requirement for evapotranspiration, and the soil's capacity to hold onto moisture [3]. Thus, in order to reduce the harm that drought stress does to vegetable crops, sustainable breeding technologies and agricultural management techniques should be created to monitor it [4].

Vegetable quality is similarly impacted by drought, and most vegetables become vulnerable to it at volumetric water contents of less than 20% [5]-[6]. One of the primary issues facing modern agriculture is maintaining crop yield and development in the face of unfavorable environmental stressors, including a water deficit [7]. When a plant's water demands are not entirely satisfied, it might hinder regular operations, this is known as a water deficit. This deficit arises when the rate of transpiration from the surface exceeds the rate at which the roots absorb water, resulting in lower soil water potential [8]. Osmotic, ionic, and oxidative stressors brought on by drought cause plants to close their stomata, which reduce their size over time [9]. Reduced CO_2 intake via stomata closure limits carboxylation and lowers internal CO_2 levels, which increases photorespiration [9]. The depletion of water resources, rising food prices, and climate change all exacerbate the effects of drought on agriculture [11]. The quantity of carbon assimilated as biomass or grain produced per unit of water utilized by the crop is known as water usage efficiency [12]. It establishes if a plant can withstand a mild or severe soil water deficiency being a significant element in plant survival in drought conditions [13].

One main objective of breeding and genetic engineering activities is to increase the water use efficiency of crops. Acosta-Motos et *al.* [14] stated that plants are essential to human nutrition and health because they provide vitamins, folic acid, mineral salts, dietary fibers, and therapeutic values. Several plants, including lettuce, carrots, tomatoes, potatoes, and okra, are susceptible to salt and water stresses

[4]. Of these, okra (*Abelmoschus esculentus* (L.) Moench), an industrial crop with high economic value, is native to Africa and is cultivated in southern Europe, Asia, and America [15]-[16]. Because all of its parts roots, stems, leaves, fruits, and seeds have therapeutic, artisanal, culinary, and even industrial uses, okra is a remarkable and adaptable plant [17]. Green okra fruit decoction is used to cure constipation and ease ulcers. The "oily" pulp of fresh fruits is used as a poultice for irritated skin, wounds that are infected, and zits [17];[18]. These elements in okra fruits have the ability to lower blood sugar levels and help diabetes people avoid insulin injections [19]-[20]. Bark fibers can be utilized locally to make cardboard, paper, and cord [21]. Low in cholesterol, calcium, iron, proteins, vitamins A and C, flavonoids, and polysaccharides, okra is a nutrient-dense dietary supplement [18];[20].

Priming is an essential method for seed germination that addresses heterogeneity by controlling metabolic processes prior to radicle emergence to encourage growth, early flowering, and enhanced stress tolerance [22]. According to Hossain et *al*. [23], priming is increasingly being used as a strategy to strengthen plant defense against biotic and abiotic stressors. Through a variety of processes, such as the production of osmoprotective regulators and antioxidation mediated defense system modification, priming improves cellular response [24]. In this procedure, seeds are carefully hydrated using osmotic agents, which are safe for the environment and people's health [25] and include polyethylene glycol (PEG), salts (NaCl; KCl), or polyols [26]. Seed germination is a crucial process that involves various physiological, biochemicals, molecular, and genetic modifications, resulting in enhanced growth, improved yield, and upregulation of specific tolerance genes for abiotic and biotic stresses [27]. Presoaking seeds in water or in solution usually polyethylene glycol has been demonstrated to improve the germination and the vigor of numerous species including vegetables [28]-[29], flowers [30]-[31], wild plants [32], and others [31];[33]-[34]. Priming treatment enhances grain production by promoting germination, stimulating growth, shortening germination time, enhancing stand establishment, emergence, repairing damaged seeds, enhancing plant vigor, osmotic adjustment, fast flowering, and harvesting [35]. It also improves affective oxidative metabolism, which includes antioxidant enzyme activities like those of superoxide dismutase (SOD) and peroxidase (POD), glutathione reductase in aged seeds, enhancing α-amylase, ATPase, as well as acid phosphatase and RNA synthesis [36]. Conducting studies in this area is crucial as it helps in understanding the effects of osmotic pretreatments, which contribute to the uniformity of germination and predisposition to resistance against abiotic stress.

Carrying out this study is important because it enables us to understand the effect of osmotic pretreatments on the uniformity of germination and the ability of seedlings to withstand abiotic stress. It aids in predicting seed behavior during germination and post-stress growth. The main goal is to develop a technical osmotic pretreatment method that maximizes okra development in drought stress conditions.

2. Material and Methods

2.1. Plant material

Two enhanced okra cultivars, *Clemson Spineless* and *Hire*, which were acquired from SEMAGRI SARL, provided the plant material for the trial (Table 1). These kinds were chosen according to their vegetative cycle and grower's preference.

Table 1: Characteristics of the two varieties of okra

Characteristics	Varieties	
	Clemson spineless	*Hire*
Sowing period	All year round	All year round
Precocity (*DAS*)	55-65	60-65
Productivity	Average	High
Fruit	Smooth, oval section	Slightly hairy

DAS: Days After Sowing

2.2. Seed germination

The chosen seeds underwent a sorting procedure before being cleaned for three to five minutes with a 5% bleach solution. After that, the seeds were rinsed three times with distilled water to get rid of any leftover chlorine. The seeds were then allowed to dry on sterile filter paper. Thirty healthy seedlings were held in three separate petri dishes measuring 9 cm in diameter and covered with three layers of sterile filter paper. Following that, 10 ml of each solution distilled water for the control group were used to moisten the seeds, and previously prepared osmopriming solutions (*T1: -0.3 MPa, T2: -0.6 MPa, T3: -0.9 MPa, and T4: - 1.2 MPa*). The osmotic pretreatment solutions were made using the PEG 6000 concentrations that were previously mentioned. These various solutions were used to soak the seeds for sets of time as, one hour, three hours, seven and nine hours. After each soaking duration, the seeds were sun-dried for 48 hours to guarantee total dehydration [37].

A triple repetition system was used for every treatment. The seedlings were incubated in petri plates kept at room temperature in a dark, controlled atmosphere (25 ± 2 °C) in the laboratory. Counting and watering with the appropriate solutions were done every day until the seventh day following seed germination. The initial stage of plant growth is seed germination, which starts with imbibition and concludes with radicle emergence. A minimum of 2 mm in length, the radicle that emerges from the seed coverings indicates germination [38].

2.3. Parameters investigated.

The following were the parameters this study looked into:

The formula is used to compute the Germination Rate (GR), which is the physiological limit of seed germination.

$$GR = \frac{\text{number of seeds germinated}}{\text{total number of seeds sown}} * 100 \; [39];$$

The above equation is used to compute the Germination Viability (GV), which is a measurement of the percentage of fully developed seedlings at the end of the process of seed germination.

$$GV = \frac{\text{number of seedlings formed}}{\text{number of germinated seeds}} * 100 \; [39];$$

The Mean Daily Germination (MDG) is calculated using the formula:

$$MDG = \frac{\text{Germination rate}}{\text{number of day on final germination}} \; [40];$$

The rate at which the population of seeds germinated is shown by the Mean Germination Time (MGT). The population that germinated increased with a decrease in the mean germination time rate. The method by which MGT is computed:

$$MGT = \frac{\sum fx}{\sum f} \; [41];$$

Where "f" refers to number of seeds germinated on day x.

The Germination index (GI) has been reported according to the formula as described by Kader [41].

GI= (7*n1) + (6*n2) + (5*n3) + … + (1*n7)

Therefore, the numbers n1, n2, ... n7 indicated the number of seeds that germinated on the first, second, and up to the seventh day, whereas the numbers 7, 6, ... and 1 corresponded to the number of seeds that germinated on the first, second, and up to the seventh day. The Seedling Vigor Index (SVI) is calculated using the formula:

$$SVI = \frac{\text{Average germination rate} * \text{average seedling length}}{100} \; [42];$$

A ruler with a centimeter grade is used to measure the height of the plant, the length of the stems, and the roots.

The process of extracting photosynthetic pigments involves the use of 80% acetone, as outlined by Arnon [43]. The following formulas [44] are then used to determine the pigment contents of the leaves:

Chlorophyll a (µg/ml) = (12.7 * DO663) – (2.69*DO647);

Chlorophyll b (µg/ml) = (22.9 * DO647) – (4.68*DO663);

Total chlorophyll (µg/ml) = (20.2 * DO645) – (8.602*DO663);

Carotenoids (µg/ml) = (5 * DO 470) + (2.846 * DO 663) – (14.876 * DO647).

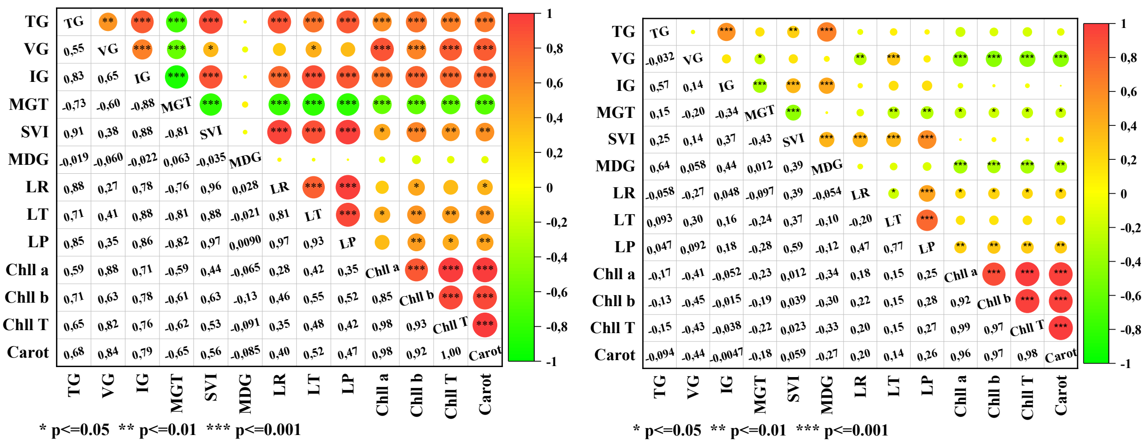
2.4. Statistical analysis

In order to analyze variation between the various means, the data was processed using SPSS version 20.0 software. When a significant difference was found, the means were separated using the Duncan statistical test at the 5% level. Graph Pad version 10.2.3 was used to plot the curves and violin. The software Origin Pro 9.9.0.225 was used to perform the correlations.

3. Results

3.1. Correlation on the germination parameters

The analysis of the correlations between the different parameters (Fig. 1) shows a beneficial effect of priming on the germination parameters of okra. A positive correlation was observed between all the studied parameters except for the germination parameters such as the mean germination time (MGT), the mean germination day (MGD), and germinative viability (Fig. 1a, b). All parameters measured under optimal conditions were significantly correlated, except for the mean germination time (MGT) and the mean germination per day (MGD), where only negative correlations were observed (Fig. 1a). Under osmotic stress conditions induced by PEG 6000, a positive correlation is observed between the parameters except for germinative viability and average daily germination, which only showed a correlation with the germination percentage, seed vigor index, and germination index (Fig. 1b). The best correlations under optimal conditions (Fig. 1a) are obtained between the germination rate (r=0.91), the seedling length (r=0.97), the radicle length (r=0.96), and the seedling vigor. Stressful conditions (Fig. 1b) have strong negative correlations on the expression of various germinative and seedling parameters compared to optimal conditions (Fig. 1a), and germinative viability (GV) is strongly affected by stressful conditions (Fig. 1b).



(a) (b)

Fig. 1 Correlation between the different parameters assessed during water stress

(a) Optimal Conditions; (b) Osmotic stress. Note: (GR) Germination Rate; (GV) Germination Viability; (GI) Germination Index; (MGT): Mean Germination Time; (SVI) Seedling Vigor Index; (MDG): Mean Daily Germination; (RL) Root Length; (SL) Shoot Length; (LP): Length of Plant; (Chll) chlorophyll content; (Carot): Carotenoids.

3.1. Effect of pretreatments on germination parameters

The assessment of water stress on the germination parameters (*germination rate, germinative viability, and seedling vigor index*) of okra is expressed below and shows that the increase in water stress significantly influences (P<0.05) the germination parameters of okra seeds. The overall observation made on the results shows that regardless of the duration of osmotic pretreatment (1h, 3h, 7h and 9h) observed and the variety, a positive improvement in germination parameters (GR, GV, and SVI) is noted (Fig. 2).

3.1.1. Effect of pretreatments on germination rate

The germination rate parameter (Fig. 2, A1 and A2) explains the influence of external conditions on the degradation of embryonic reserves during seed germination. The germination rate of okra seeds decreases within the *Clemson spineless* varieties (0.0 MPa: 94.45% to -1.2MPa: 45.83%) and *Hire* (0.0 MPa: 91.78% to -1.2MPa: 47.92%) depending on the intensity of the water stress. Using these same previous concentrations in pretreatment, an optimization of the germination rate is observed in both varieties, with the highest averages obtained at 1 hour of priming (-0.6MPa: 97.22%) for *Clemson spineless* (Fig. 2, B1) and at 7 hours of priming (-1.2MPa: 99.33%) for *Hire* (Fig. 2, D2) compared to the control.

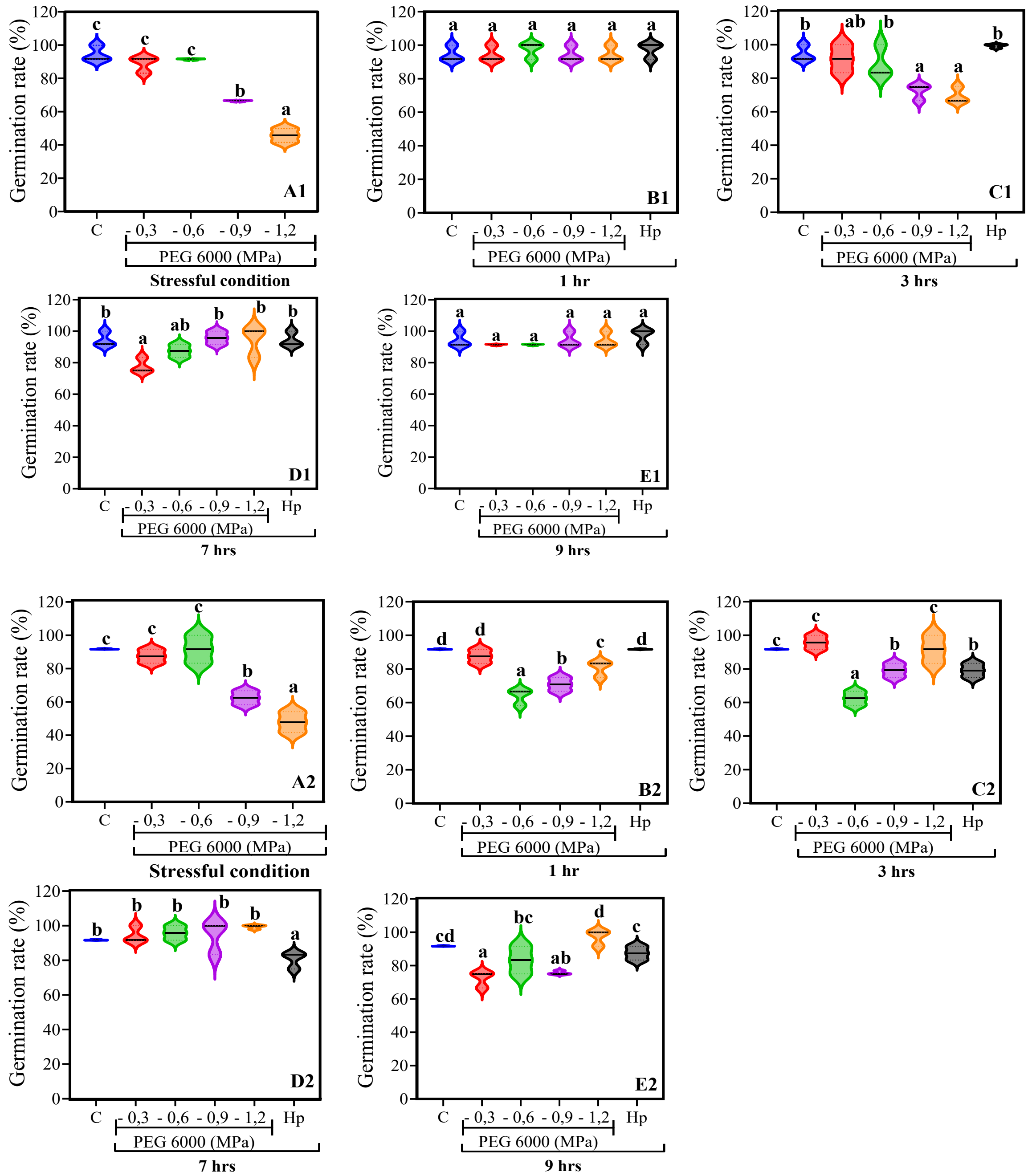


Fig. 2 Effect of osmotic pretreatments on the germination rate of two varieties of okra
*All the violin followed by the same alphabetical letter within each treatment are not significantly different at the 5% threshold, as determined by the Duncan test. C: Control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.1.2. Effect of the priming on the germinative viability

The results on germinative viability (Fig. 3) show that despite high germination rates, the ability of seedlings to follow up is closely influenced by external environmental constraints. Stressful conditions show decreasing seedling viability rates in *Clemson spineless* (-0.3 MPa: 25.46% to -1.2 MPa: 0.00%) and in *Hire* (-0.3 MPa: 93.64% to -1.2 MPa: 0.00%) following the increase in PEG 6000 concentration. During the application of these same treatments in osmotic priming, an increase in the seed viability rate parameter is observed in both studied varieties. The highest germination viability of the seedling is noted in the osmotic pretreatment with PEG 6000 at -0.3 MPa (1 hour and 3 hours: 97.28%) for *Clemson spineless* (Fig. 3, B1) and at -0.6 MPa (1hour: 99.33%) and -0.3 MPa (3 hours: 99.33%) for *Hire* (Fig. 3, B2 and C2).

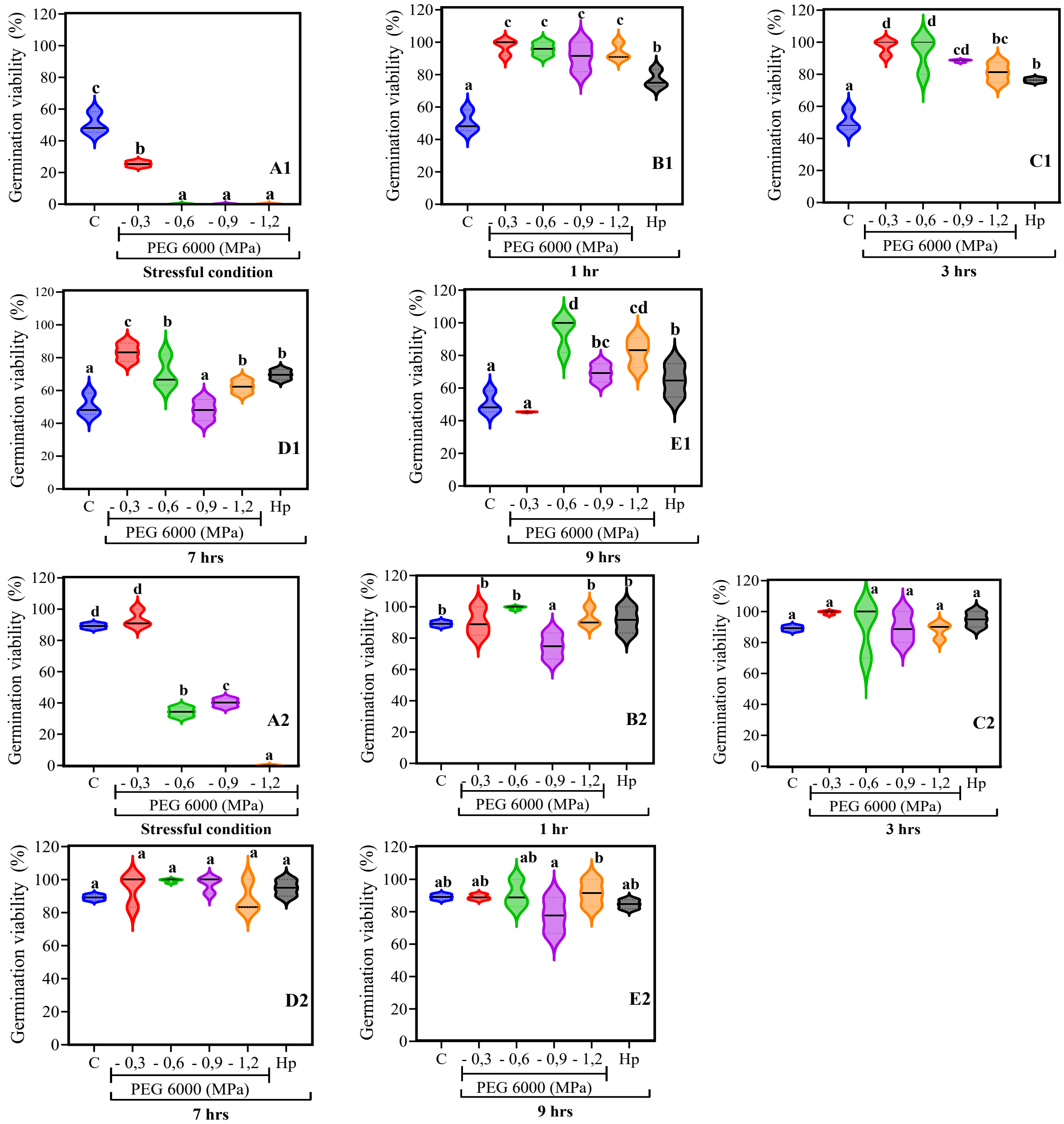


Fig. 3 Effect of osmotic pretreatments on the germination viability of two varieties of okra

*All the violin followed by the same alphabetical letter within each treatment are not significantly different at the 5% threshold, as determined by the Duncan test. C: Control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.1.3. Effect of the priming on the vigor of the seedlings

Under stressful conditions and regardless of the variety, the increase in PEG 6000 concentrations negatively affects the vigor of the seedlings, unlike osmotic pretreatments which optimize their development (Fig. 4, A1 and A2). The vigor of the seedlings decreases and is very low under water stress in *Clemson spineless* (-0.3 MPa: 6.85 to -1.2 MPa: 0.61) and in *Hire* (-0.3 MPa: 4.55 to -1.2 MPa: 0.75) following the increase in PEG 6000 concentration in the medium. The 9-hour pretreatment shows the best seedling vigor in *Clemson spineless* (-0.6 MPa: 10.61) and in *Hire* (-1.2 MPa: 9.67) compared to the control in *Clemson spineless* (C:

4.46) and in *Hire* (C: 5.06). It is important to note that the positive control (hydropriming) shows greater seedling vigor respectively for the variety (*Clemson spineless* and *Hire*) and the pretreatment time (3 hours: 9.03 and 7 hours: 8.35) (Fig. 4: C1, C2, D1 and D2).

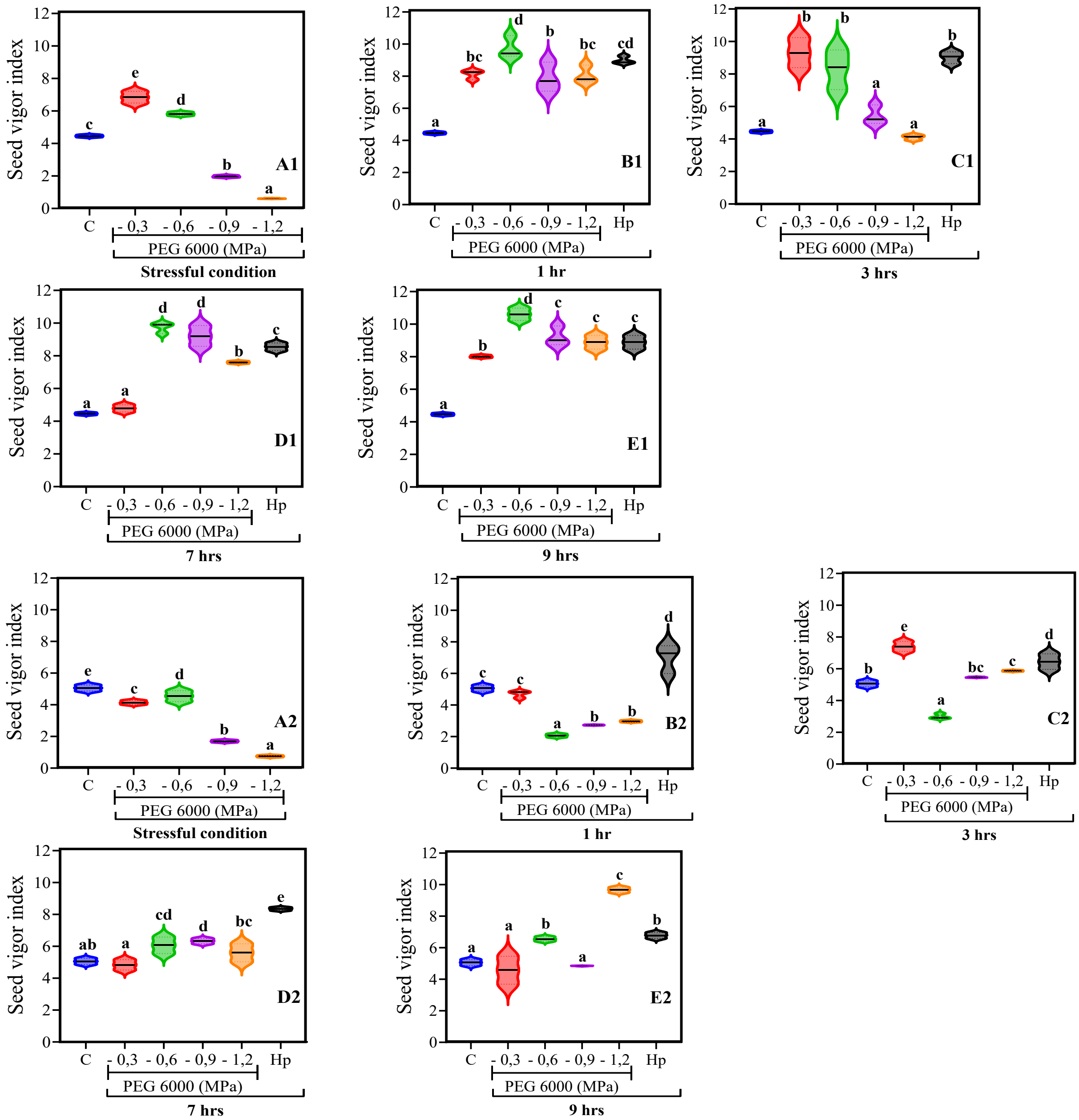


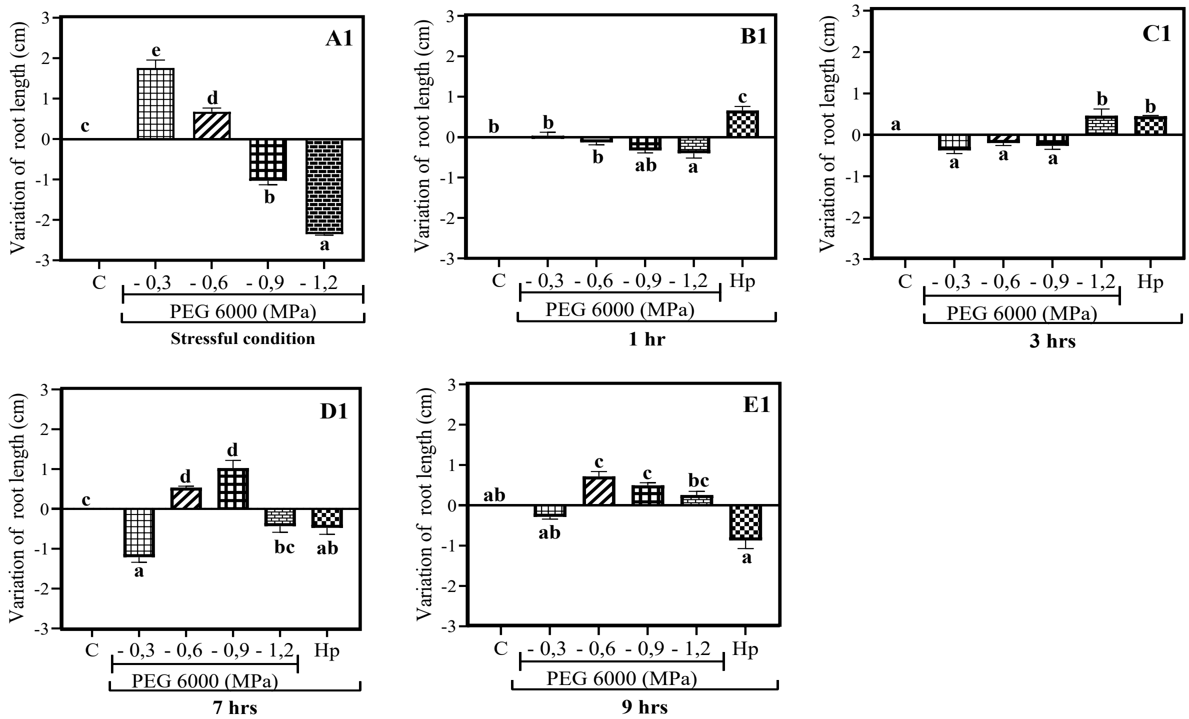
Fig. 4 Effect of osmotic pretreatments on the seedling vigor index of two varieties of okra
*All the violin followed by the same alphabetical letter within each treatment is not significantly different at the 5% threshold, as determined by the Duncan test. C: control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.2. Effect of pretreatments on sowing parameters

Fig. 5 shows the effects of osmotic priming on the seedling parameters (root length, stem length, and plant length) of seedlings from two varieties of okra. The different osmotic pretreatments significantly influence (P<0.05) the seedling parameters depending on the duration of priming and the variety. A global analysis within the two varieties (*Clemson spineless* and *Hire*) and independently of the root parameters shows that water stress significantly influences seedling development and that increasing PEG 6000 concentrations negatively affect seedling parameters. Similarly, it is important to note that osmotic pretreatments improve the seedling parameters compared to the negative control.

3.2.1. Effect of pretreatments on root length of seedlings

The results of the effect of osmotic priming on the seedling parameters of okra in the *Clemson spineless* variety are recorded in Fig. 5 (A1-E1). The evolution of root length under stressful conditions (Fig. 5: A1) shows that low concentrations of PEG 6000 are favorable (-0.3 MPa: 1.76 cm and -0.6 MPa: 0.67 cm) unlike high concentrations (-0.9 MPa: -1.03 cm and -1.2 MPa: -2.35 cm) compared to the control. The root length at the level of osmotic pretreatments enhances the best responses at the level of osmotic treatments at -0.9 MPa (7 hours: 1.03 cm) (Fig. 5: D1) and -0.6 MPa (9 hours: 0.72 cm) (Fig. 5: E1) compared to the control. The results of the effect of osmotic priming on the parameters of okra seedling growth in the *Hire* variety are recorded in Fig. 5 (A2-E2). The evolution of root length under stressful conditions (Fig. 5: A2) shows that low concentrations of PEG 6000 are favorable (-0.3 MPa: 0.15 cm and -0.6 MPa: 0.62 cm) unlike high concentrations (-0.9 MPa: -1.45 cm and -1.2 MPa: -2.35 cm) compared to the control. The best responses expressing root length is at the level of osmotic pretreatment with PEG 6000 at -1.2 MPa (3 hours: 1.04 cm) (Fig. 5: C2) compared to the other treatments.



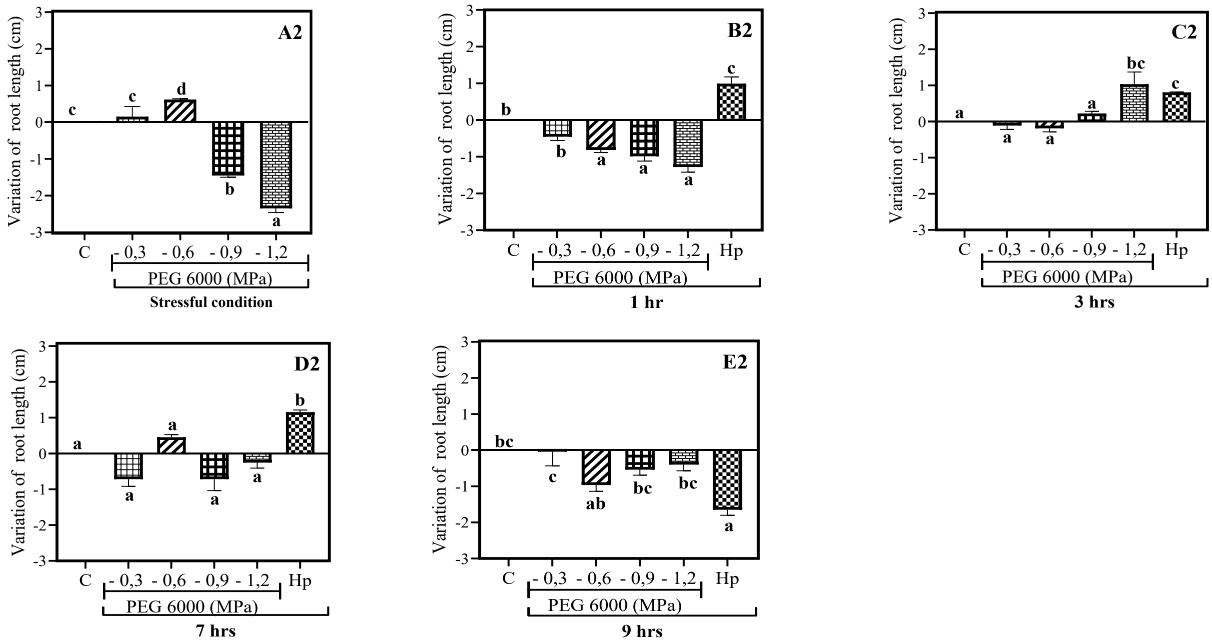


Fig. 5 Effect of osmotic pre-treatments on the root length of okra seedlings
*All histogram bars followed by the same alphabetical letter within each root parameter are not significantly different at the 5% level according to the Duncan test. C: control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.2.2. Effect of pretreatments on seedling stem length

The stem length (Fig. 6: A1) in the *Clemson spineless* variety is also influenced by increasing concentrations of PEG 6000 in the medium compared to the control. The low concentrations of PEG 6000 (-0.3 MPa: 1.68 cm and -0.6 MPa: 0.79 cm) compared to the high concentrations (-0.9 MPa: -0.88 cm and -1.2 MPa: -1.16 cm) differently influence the evolution of root length compared to the control. The best stem lengths are obtained in the osmotic pretreatment with PEG 6000 at -0.6 MPa during the priming times of 1 hour (5.42 cm); 3 hours (4.72 cm); 7hours (5.48 cm) and 9hours (5.62 cm) compared to the control. The stem length in the *Hire* variety (Fig. 6: A2) is influenced by increasing concentrations of PEG 6000 in the medium compared to the controls. Low concentrations of PEG 6000 (-0.3 MPa: 0.24 cm and -0.6 MPa: -0.52 cm) negatively influence the stem length parameter compared to high concentrations (-0.9 MPa: -0.73 cm and -1.2 MPa: -1.04 cm). The best stem lengths are obtained in the osmotic pretreatments with PEG 6000 at -0.3 MPa (3 hours: 2.64 cm); -0.6 MPa (7 hours: 3.27 cm) and -1.2 MPa (9 hours: 4.79 cm).

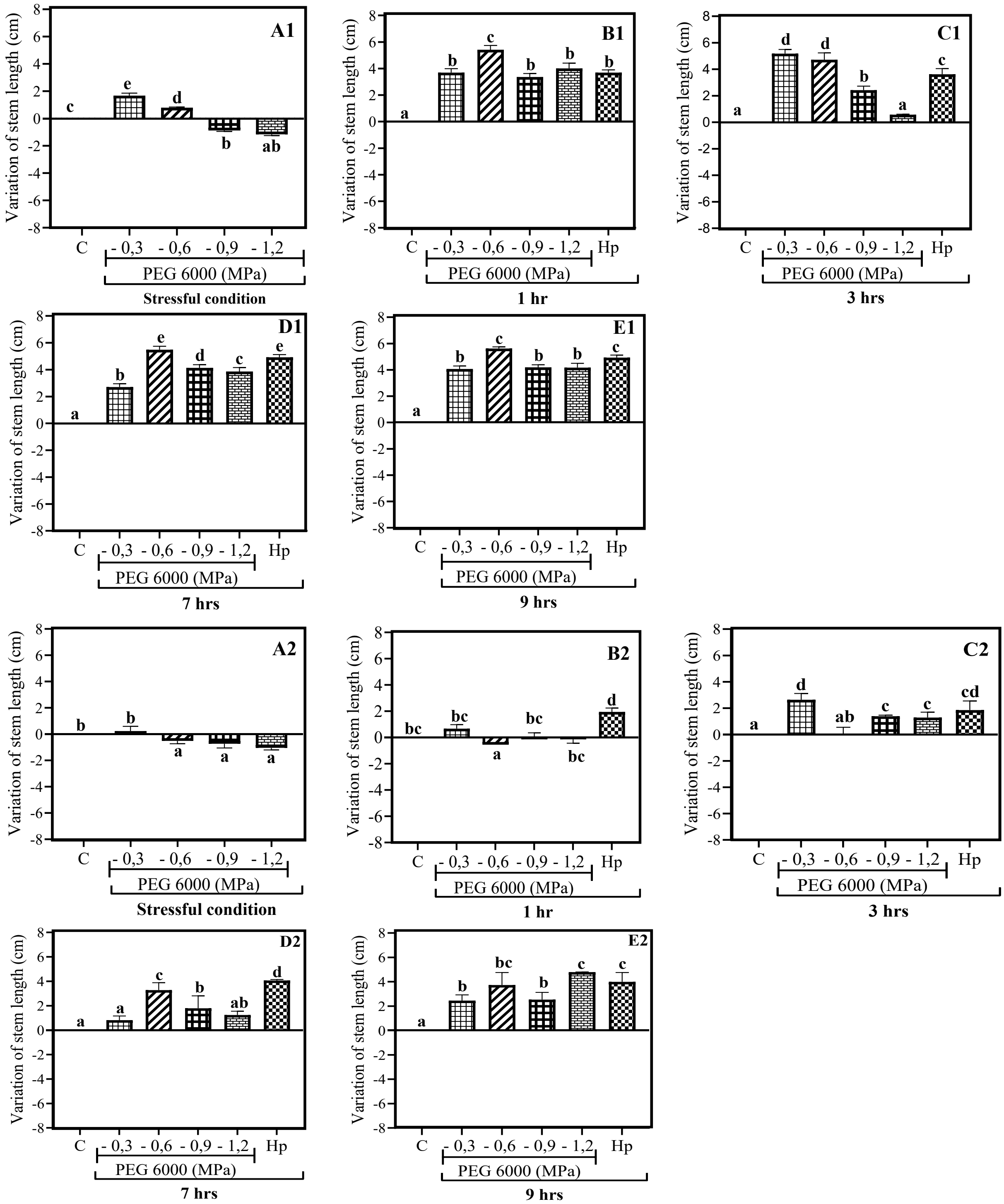


Fig. 6 Effect of osmotic pretreatments on the stem length of okra seedlings

*All histogram bars followed by the same alphabetical letter within each root parameter are not significantly different at the 5% level according to the Duncan test. C: control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.2.3. Effect of the treatment on the expression of plant length

The expression of plant length (Fig. 7: A1-E1) in the *Clemson spineless* variety is significantly reduced by the increase in water stress (-0.9 MPa: -1.9 cm and -1.2 MPa: -3.52 cm) compared to the negative control. The pretreatments provide a more favorable response on the expression of the size of okra seedlings, and the best results are obtained with the osmotic pretreatment PEG 6000 at -0.6 MPa (1hour: 5.1 cm; 3hours: 4.4 cm; 7hours: 6.19 cm; and 9hours: 6.56 cm) compared to the negative control.

The expression of seedling length (Fig. 7: A2-E2) under stressful conditions in the *Hire* variety is significantly reduced by the increase in water stress (-0.9 MPa: -2.92 cm and -1.2 MPa: -3.64 cm). The pretreatments promote an optimization of the expression of okra seedling size, and the best results are obtained with osmotic pretreatments of -0.6 MPa (7hours: 2.97 cm) and -1.2 MPa (9hours: 4.4 cm) compared to the negative control.

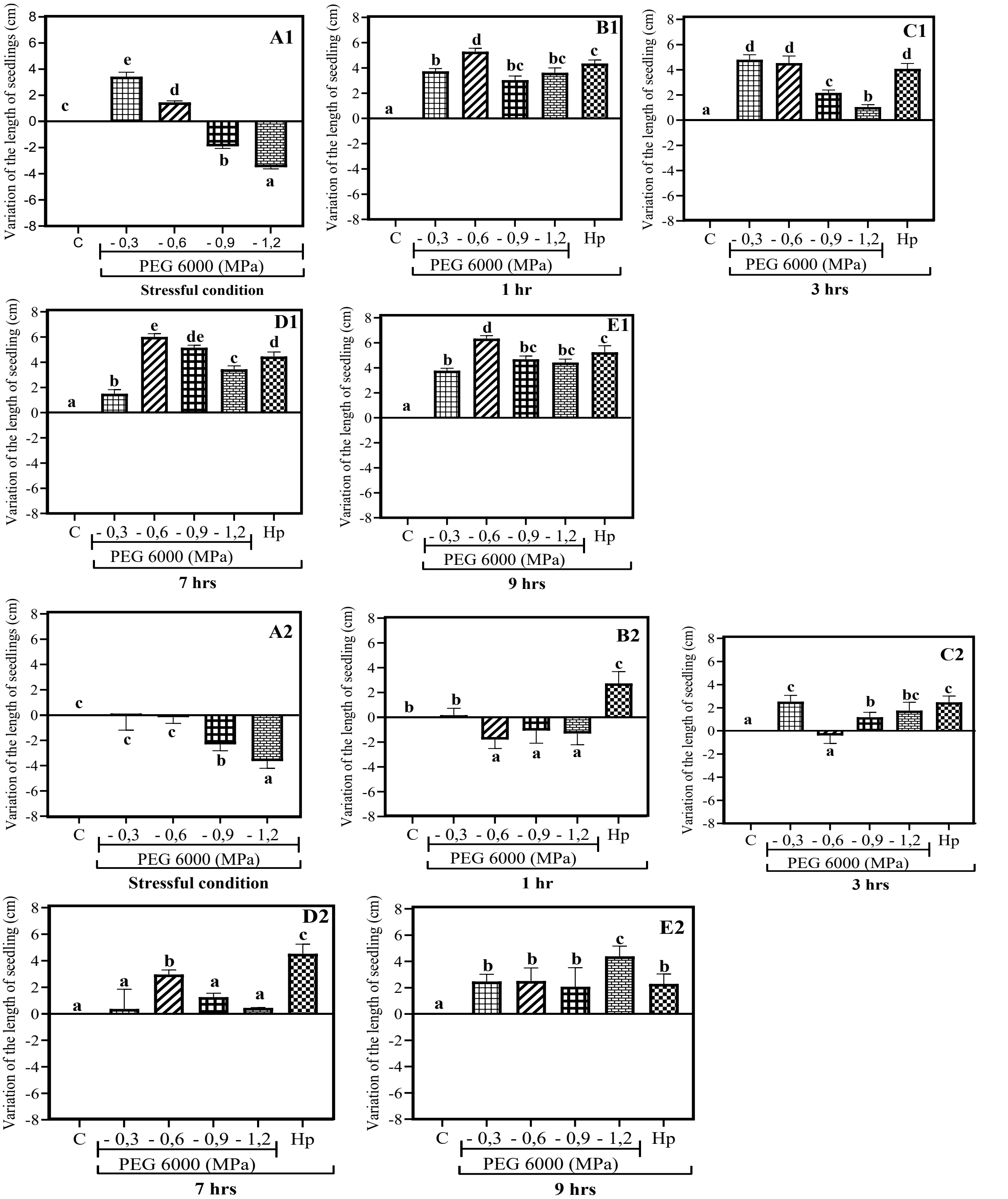


Fig. 7 Effect of osmotic pretreatments on the plant length of okra seedlings

*All histogram bars followed by the same alphabetical letter within each root parameter are not significantly different at the 5% level according to the Duncan test. C: control; B, C, D and E (1hr; 3hrs; 7hrs and 9hrs are respectively hour(s) duration of priming); Hp: hydropriming; *Clemson spineless*: A1-E1; *Hire*: A2-E2.

3.3. Effects of osmopriming on the physiological parameters

Fig. 8 and Fig. 9 below represent the effect of priming on the physiological parameters of okra seedlings (*chlorophyll a content, chlorophyll b content, total chlorophyll content, and carotenoid content*). Regardless of the variety, a progressive decrease in these parameters is observed under stressful conditions following the increase in PEG 6000 concentration (Fig. 8A, Fig. 9A). The responses obtained with osmotic pretreatments using PEG 6000 are satisfactory compared to the control within the *Clemson Spineless* variety (Fig. 8: B, C, D, E, and F).

3.3.1. Effects of osmopriming on the physiological parameters of Clemson Spineless

The levels of photosynthetic pigments under stress conditions (Fig. 8: A) gradually decrease as the concentration of PEG 6000 increases; these values are 2.22 mg/g (Carotenoids); 1.79 mg/g (Total Chlorophyll); 1.10 mg/g (Chlorophyll 'a') and 0.70 mg/g (Chlorophyll 'b') for the concentration of 0 MPa of PEG 6000, and they regress to a value of 0 mg/g for the different levels of photosynthetic pigments for the concentration of 200 MPa of PEG 6000. Under priming conditions, the *Clemson Spineless* variety (Fig. 8) shows a significant increase ($P<0.05$) in various physiological parameters, with the highest values obtained at 50MPa (9hours: 2.77 mg/g for chlorophyll ‘a’ ; 1.62 mg/g for chlorophyll ‘b’ ; 4.40 mg/g for total chlorophyll and 6.09 mg/g for carotenoids) and 100MPa (9h: 2.53 mg/g for chlorophyll ‘a’, 1.49 mg/g for chlorophyll ‘b’, 4.02 mg/g for total chlorophyll, and 5.71 mg/g for carotenoids) compared to the control and hydropriming (Fig. 8: B,C, F).

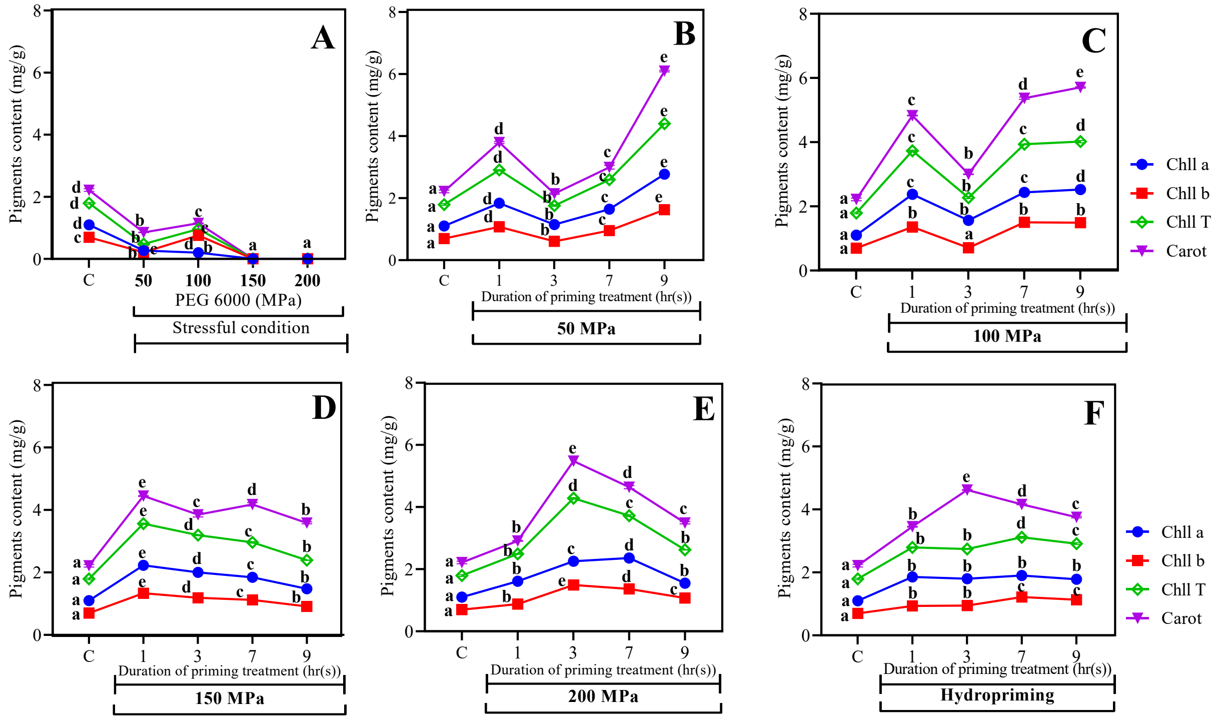


Fig. 8 Effect of priming with PEG 6000 on the pigment content of *Clemson spineless* seedlings
*All curves followed by the same alphabetical letter within each photosynthetic pigment are not significantly different at the 5% level according to the Duncan test. C: Control; Hp: hydropriming.

3.3.2. Effects of osmopriming on the physiological parameters of *__Hire__*

Under stress conditions (Fig. 9: A), the levels of photosynthetic pigments gradually decrease as the concentration of PEG 6000 increases; these average levels are 4.27 mg/g (Carotenoids); 3.47 mg/g (Total Chlorophyll); 2.32 mg/g (Chlorophyll 'a') and 1.15 mg/g (Chlorophyll 'b') for the concentration of 0 MPa of PEG 6000, and they regress to a value of 0 mg/g for the different levels of photosynthetic pigments for the concentration of 200 MPa of PEG 6000. Under priming conditions, the *Hire* variety (Fig. 9) shows an inhibition of the synthesis of various physiological parameters with the lowest values obtained at 200 MPa (1h: 0.88 mg/g for chlorophyll ‘a’; 0.48 mg/g for chlorophyll ‘b’; 1.31 mg/g for total chlorophyll and 1.19 mg/g for carotenoids; and 7h: 0.71 mg/g for chlorophyll ‘a’; 0.40 mg/g for chlorophyll ‘b’; 1.11 mg/g for total chlorophyll and 1.14 mg/g for carotenoids) compared to the control and hydropriming (Fig. 9, B, C, F).

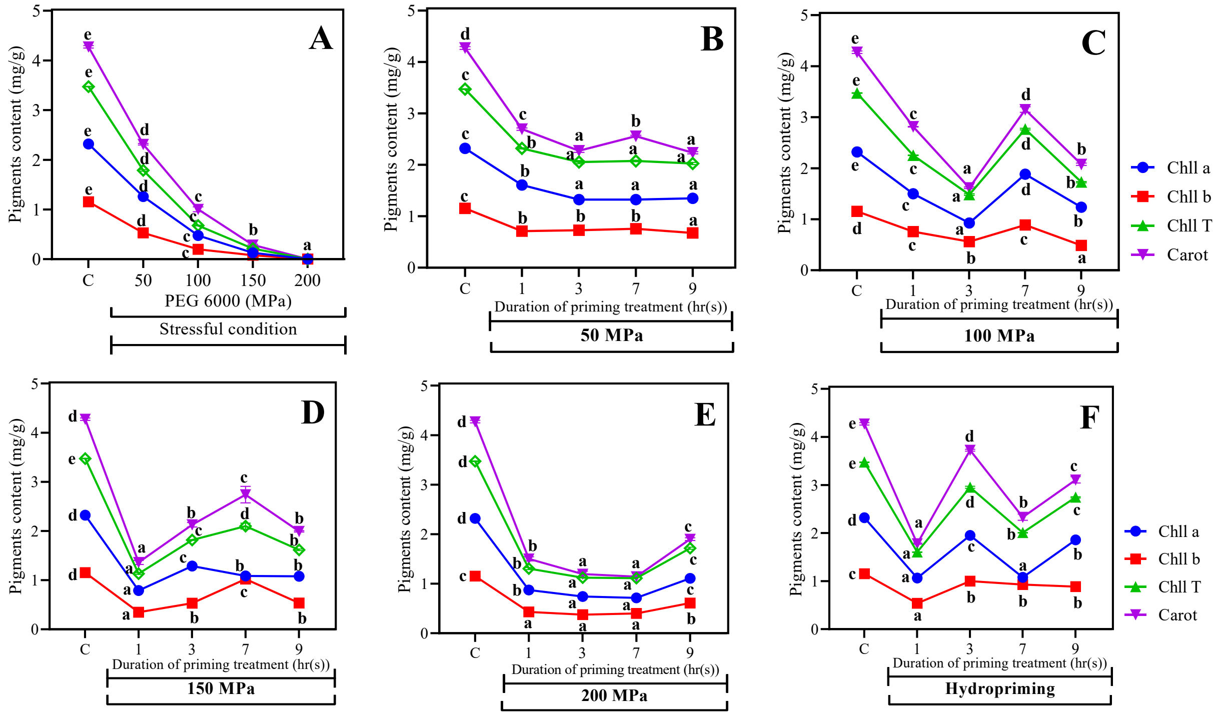


Fig. 9 Effect of priming with PEG 6000 on the pigment content of *Hire* seedlings
*All curves followed by the same alphabetical letter within each photosynthetic pigment are not significantly different at the 5% level according to the Duncan test. C: Control; Hp: hydropriming.

4. Discussion

4.1. Effect of pretreatments on the germination parameters of two okra varieties

For the two varieties studied, the concentration of PEG 6000 as well as the duration of priming significantly influence germination parameters such as the germination rate, the mean germination time, and the seedling vigor index. Indeed, these elements induce an increase in the osmotic potential in the environment surrounding the plant, thereby preventing it from absorbing the water necessary for its growth. The use of PEG 6000 in priming contributed to the increase in the germination percentage and the average germination time. This result would translate into an improvement in protein synthesis in the seeds through osmopriming and the activation of antioxidant enzymes such as catalase and peroxidase dismutase, which reduce in the seeds, and the activation of desoxyribonucleic acid synthesis [45]-[46]. Also, priming improves the synthesis of α-amylases and proteases responsible for the hydrolysis and mobilization of reserves, which are the main source of energy for respiration during germination [28];[47]. Similar results were obtained by Kadir et *al.* [48] on rice germination under water stress conditions.

Moreover, the improvement in seedling vigor could be explained by the increase in water content in the seedlings, which would facilitate metabolic reactions in the plant cells. Indeed, priming allows for the control of water absorption by the seeds at the time of germination, thus providing them with the precise amount of water necessary for this phase of their growth. It also leads to an increase in the regulation of the expression of genes responsible for the synthesis of ABA and reactive oxygen species [49]. Moreover, the dehydration phase induces low-amplitude stress in the seeds, which would lead to better adaptation during a subsequent exposure to water stress. This result is in contrary to that of Kadir et *al*. [48] who showed that osmopriming has no significant effect on seed vigor.

With the increase in priming duration and salt concentration, there is a progressive decrease in germination parameters. This decrease could be attributed to oxidative stress caused by the negative osmotic potential of the solutions [50]. This result is in contradiction with the work of Varshini et *al*. [51] on chickpeas, who observed an increase in germination with the increase in priming duration up to a threshold of 6 hours. However, for the majority of the recorded parameters, quite good results are observed at 9 hours of priming. The reaction to the duration of primings would therefore depend on the plant species studied.

4.2. Effect of osmotic pretreatments on the sowing parameters of two okra varieties

The germination and seedling establishment phases are the stages of plant growth that show the most vulnerability to abiotic stresses [46]. The results obtained show an improvement in seedling parameters, particularly the elongation of the epicotyl and radicle in okra. This result could be explained by the improvement of metabolic activities in the seeds during priming, which would promote multiple cell divisions as well as cell elongation [58]. This result is in agreement with the work of Saini et *al*. [52]on squash.

The increase in the length of the radicle could be explained by an enhancement of cell divisions and elongation, as well as a preparation of the seedlings for potential exposure to abiotic stresses. Indeed, the drying phase included in priming induces a low-intensity stress in the seeds [50] that activates the plant's defense mechanism against stress, thereby improving seedling vigor; similar results were obtained by Varshini et *al*. [51] on chickpeas. The improvement in stem size could be explained by the mobilization of cotyledonary reserves (starch), the activation of enzymes such as α-amylase responsible for the degradation of these reserves, and the activation of metabolic reactions fundamental to plant development. Similar results were obtained by Kadir et *al*. [46] on the growth of rice under water stress conditions.

The overall size of the seedlings was obtained by adding the lengths of the radicle and epicotyl. The largest values of seedling sizes (radicle + epicotyl) were recorded at -0.6 MPa of PEG 6000 for 9 hours of priming, a result in disagreement with the results of Varshini et *al*. [51], who showed that the ideal duration for a significant improvement in seedling size was 6 hours on durum wheat. This difference could be explained by a specificity of the effectiveness of osmopriming to different plant species.

4.3. Effect of pretreatments on the physiological parameters of two varieties of okra

The osmotic stress induced by PEG 6000 led to a decrease in the chlorophyll content (a, b, and total) and carotenoids in the leaves of the okra seedlings in both varieties used. Indeed, as the severity of water stress increases, there is an alteration in membrane and enzymatic activities fundamental to biological processes. For example, a decrease in stomatal diameter is observed, which complicates the entry of CO_2 into leaf cells, leading to a slowdown in photosynthetic activity [54]. Similar results were obtained by Kakar et *al*. [46] regarding the stress induced by PEG 6000, contrary to those of Tsoata et *al*. [55], who showed that water stress has no significant effect on the variation of chlorophyll content in young Fabaceae seedlings.

The use of PEG 6000 solutions for priming improved the chlorophyll and carotenoid content in okra leaves. The improvement in chlorophyll content could be explained by the increased water absorption by the roots, the water content in the seedlings, which would promote better conductance in the xylem vessels, protein synthesis, and the accumulation of sugars necessary for photosynthesis. It could also be due to the improvement in Rubisco synthesis, which is fundamental to carbon metabolism in the Calvin cycle [56]. This result is in agreement with that of Kakar et *al*. [46], who showed an increase in chlorophyll an and total content in the leaves of maize subjected to chitosan osmopriming, and those of Tamindzic et *al*. [57], who demonstrated that priming improves chlorophyll content in pea leaves.

The improvement in carotenoid content can be explained by the induction of osmotic stress during the drying phase, which contributed to the enhancement of antioxidant enzyme synthesis [46] and non-enzymatic and antioxidant compounds such as carotenoids responsible for regulating reactive oxygen species that are signaling agents in response to osmotic stress [58]. This result is in agreement with that of Kakar et *al*. [46], who showed an increase in the content of chlorophyll an and total chlorophyll in maize leaves that underwent osmopriming with chitosan.

5. Conclusion

The results of this study show that water stress induced by PEG 6000 has a repressive effect on the germination parameters and the development of okra seedlings. Apart from this inhibitory effect, we also note from our study that the use of these priming treatments improves germination and seedling development compared to the positive control. These positive results of the effect of osmopriming with PEG 6000 on the germination and development parameters of seedlings could contribute to the popularization and improvement of okra cultivation in areas highly subjected to water stress (*condition of water scarcity*). The positive responses obtained could be explained by better water absorption and improved photosynthesis, which are key phenomena in seed germination and plant growth. For a better understanding of the importance of priming and its mechanism, additional studies, particularly on the molecular, biochemical, and genetic mechanisms, can be conducted.

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