

Research Article

Chitosan and humic acid as biostimulant seed coatings enhance germination and seedling growth in two forage pea varieties (*Pisum sativum* var. *arvense* (L.)) under drought stress

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ABSTRACT- Forage pea is an important legume used as livestock feed; however, environmental stresses, particularly drought, significantly affect its germination and seedling growth. Seed coating technology is an effective strategy for enhancing seed germination and seedling growth under drought conditions. This study was conducted to evaluate the potential of seed coating with chitosan (0.1%, 0.15%, 0.2%, and 0.3%) and humic acid (16, 17, 18, and 19 g kg⁻¹ seeds) under drought stress (0, -3, -6, and -9 bar) in two forage pea varieties (Pionir and Arda). Evaluations considered final germination percentage, mean germination rate, and shoot and root length. Overall, the Pionir variety showed higher final germination percentage, mean germination rate, and shoot and root length than the Arda variety under both normal and drought stress conditions. In the Arda variety, significant decreases in the measured traits were observed under -6 bar drought stress. The two cultivars showed significant differences in shoot length, whereas no significant differences were observed in root length. The results for the Pionir variety indicated that seed coating with chitosan (0.2%) improved shoot length by 41.95% and root length by 30.15%, whereas humic acid (19 g kg⁻¹ seeds) increased shoot length by 28.79% and root length by 14.03%. Similarly, in the Arda variety, chitosan (0.2%) increased shoot length by 57.44% and root length by 8.07%, while humic acid (19 g kg⁻¹ seeds) improved shoot length by 32.87% and root length by 30.25%. Thus, these biostimulants can enhance forage pea seed germination and seedling growth.

INTRODUCTION

Forage pea (*Pisum sativum* var. *arvense* (L.)) is one of the oldest cultivated forage legumes in the world and is widely used as fodder and green manure. It is considered a valuable source of protein, fiber, vitamins, minerals, slowly digestible starch, and soluble sugars (Demirkol et al., 2019). Despite its high genetic diversity and numerous agronomic advantages, the global production of this important legume has remained relatively low, according to both research findings and statistical data (Isik et al., 2025). Drought is a major environmental stress that restricts seed germination, seedling growth, and crop productivity in most cultivated plants (Farooq et al., 2021; Izadi et al., 2025). The application of new technologies, such as biostimulant seed coating, has been reported as an effective and practical approach to improve rapid and uniform seed germination, seedling development, and plant growth under unfavorable environmental conditions. Humic acid (HA) is a type of humic substance (HS) that generally exhibits macromolecular characteristics and contains both

carboxylic and phenolic functional groups (Canellas et al., 2015). These functional groups cause HA to carry negative charges in aqueous solutions (Zhang and Bai, 2003). Previous studies have demonstrated the beneficial effects of seed film coating with biostimulants such as humic acid. According to research, seed film coatings enhanced seed germination (Neamatollahi et al., 2024), increased soil phosphorus availability around treated seeds, improved phosphorus uptake by plants, and made greater biomass production (Pilar-Izquierdo et al., 2012). HA also stimulates plant growth and development by increasing root length, root and shoot fresh weight, root-to-shoot ratio, activating antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GPX) (Makhlouf et al., 2022), and improving nutrient uptake by plants (De Avila et al., 2024). Several studies have also confirmed its ability to mitigate abiotic stresses such as drought. For example, (Zhuo et al., 2026) reported that HA application in tomato increased root activity and root length by 105.8% and 530.4%, respectively, while also enhancing physiological performance and antioxidant defense systems. Chitosan (CH) is a natural biopolymer and one of the most abundant

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polymers found in nature. It has the ability to adsorb metals from various aqueous solutions (Katiyar et al., 2015). Biological, biodegradable, and biocompatible properties of CH make it a promising bioactive compound for agricultural applications (Zhang and Bai, 2003). Previous studies have shown that seed coating with CH can enhance plant growth and improve plant performance under stress conditions, such as limited water availability (Katiyar et al., 2015). CH has been reported to increase the activity of antioxidant enzymes, including Superoxide dismutase (SOD) and peroxidase (POX), as well as plant height, stem diameter, root length and number, and chlorophyll content in maize (*Zea mays* L.) (Choudhary et al., 2017). Furthermore, the application of CH as a seed treatment in legumes has been shown to be effective due to its excellent film-forming properties, which help maintain seed moisture and absorb soil moisture, thereby promoting seed germination and seedling growth (Katiyar et al., 2015). In addition, coating seeds with 0.5% CH significantly improved the morphological, physiological, and biochemical responses of sweet corn seedlings under low water supply conditions (Behboud et al., 2024). Despite this potential, only a limited number of studies have examined the effectiveness of seed coating with CH and HA on the germination of forage pea seeds under drought stress. Therefore, the present study was conducted to evaluate the effects of seed coating with different concentrations of CH and HA on final germination percentage (FGP), mean germination rate (MGR), shoot length (SL), and root length (RL) of two forage pea varieties (Pionir and Arda) under different levels of drought stress, with the aim of identifying and recommending the most effective seed coating treatments under both normal and drought stress conditions.

MATERIALS AND METHODS

Plant materials

Seeds of two forage pea varieties (Pionir and Arda), differing in seed germination percentage, were obtained from the Razavi Seed and Plant Company, Mashhad, Iran. The initial germination percentages of the Pionir and Arda varieties were 94% and 82%, respectively. A gibberellic acid dormancy-breaking test was conducted to examine possible seed dormancy in the Arda variety; however, the germination percentage did not change, indicating the absence of dormancy. Seeds of uniform size were used throughout the experiment and were film-coated at the Seed Laboratory of the Razavi Seed and Plant Company, Mashhad, Iran.

Growth conditions

The experiment was conducted at the Crop Physiology Laboratory, School of Agriculture, Shiraz University, Iran, in April 2023. Seeds were germinated under four levels of water potential: 0 (control), -3, -6, and -9 bar. The lowest level of water potential was selected based on preliminary experiments on forage pea seed germination, which showed that no germination occurred at water potentials lower than -9 bar (data not shown). Drought stress was simulated using polyethylene glycol 6000 (PEG-6000). Concentrations of PEG were dissolved in deionized water according to the equation proposed by Michel and Kaufmann (1973). Based on preliminary experiments, nine seed-coating treatments

were evaluated: control (uncoated seeds), humic acid coatings at 16, 17, 18, and 19 g kg⁻¹ seed (HA-coated), and chitosan coatings at 0.1%, 0.15%, 0.2%, and 0.3% (CH-coated). For each treatment, twenty-five seeds were placed in sterile germination boxes (115 × 95 mm) between two layers of sterile filter paper (Whatman® No. 1) moistened with 30 mL of either distilled water (control) or the corresponding PEG solution. CH (molecular weight 190,000–310,000 Da and 75–85% N-deacetylation) was purchased from Sigma-Aldrich (St. Louis, MO), and HA was obtained from GREENHUM, Italy. Seeds were film-coated at pH = 6 (Zhang and Bai, 2003). The germination boxes were placed in an incubator at 20 °C, which is the optimum temperature for forage pea seed germination (ISTA, 2022) and maintained under dark conditions for 7 days. Seed germination was recorded daily until a constant count was reached. Seeds were considered germinated when the primary root reached a length of at least 2 mm.

Measurements

At the end of the experiment, measurements were aimed at FGP and MGR according to Eq. (1) and Eq. (2), respectively.

$$\text{FGP (\%)} = \left(\frac{N}{N_t} \right) \times 100 \quad \text{Eq. (1)}$$

where N is the number of germinated seeds at the final count, and N_t is the total number of seeds in the germination box.

$$\text{MGR} = \frac{(\sum n)}{(\sum nt)} \quad \text{Eq. (2)}$$

where n is the number of newly germinated seeds at time t, and t is the number of days from planting. At the end of the experiment, the seedlings were dissected into roots and shoots, and their length and dry weight were measured separately. Dry weight was measured after drying the fresh plant material in an oven at 70 °C until a constant weight was reached (approximately 48 h).

Statistical analysis

The experiment was conducted as a factorial in a completely randomized design with four replications. Using the Arc sin, the germination percentage, MGR, SL, and RL were transformed and subsequently analyzed by ANOVA using SAS software (version 9.4). The analysis was conducted on the basis of variety, seed coating, and drought levels as fixed factors and when F ratios were significant. The differences between mean values were compared using the LSD test (≤ 0.05). Regarding seeds that did not germinate in response to the -9 bar drought stress, their data were excluded from analyses.

RESULTS AND DISCUSSION

Effects of variety and drought interaction on seed germination and seedling growth of forage pea

Variety and its interaction with drought stress had significant effects on FGP, MGR, SL, and RL (Table 1). In the Pionir variety, the highest FGP (88%) was observed

under -6 bar drought stress. In contrast, in the Arda variety the highest FGP (88%) occurred at -3 bar drought stress, while the lowest FGP (1%) was recorded at -6 bar, which was significantly lower than all other treatments (Table 2). Under -6 bar drought stress, the FGP of the Pionir variety (88%) was significantly higher than that of the Arda variety (1%). Regarding MGR, seeds of both the Pionir (0.44 germinated seeds day⁻¹) and Arda (0.45 germinated seeds day⁻¹) varieties showed the highest values under normal conditions. With increasing drought stress, MGR significantly decreased in both varieties, and the lowest values were recorded at -6 bar. However, under -6 bar drought stress, the Pionir variety maintained a higher MGR (0.24 germinated seeds day⁻¹) compared to the Arda variety (0.03 germinated seeds day⁻¹). In both varieties, increasing drought levels significantly reduced seedling SL and RL compared to normal conditions, except for RL in the Arda variety at -3 bar. Under control conditions (0 bar), the Pionir variety had significantly greater SL and RL (7.34 cm and 7.51 cm, respectively) than the Arda variety (4.22 cm and 6.95 cm). Similarly, under -6 bar drought stress, Pionir maintained significantly greater SL and RL (0.36 cm and 2.64 cm, respectively) than Arda (0.00 cm and 0.30 cm). Poor and non-uniform seed germination and weak seedling establishment under stress conditions, particularly drought, are among the major constraints in forage pea cultivation (Griga and Novak, 1990; Kucukozcu and Avci, 2020). Drought stress has been reported to cause substantial reductions in growth parameters of forage pea (Farooq et al., 2021; Gurkan et al., 2023). Pea plants exposed to drought show reduced photosynthesis, transpiration, glycolate oxidase, glutathione reductase, ascorbate peroxidase, and CAT activity, along with slight reductions in chlorophyll a and carotenoid contents (Moran et al., 1994). Under severe drought stress, increased accumulation of total flavonoids plays an important role in antioxidant activity in pea plants. Additionally, the activity of some antioxidant enzymes, such as GPX, significantly increases, whereas SOD and CAT activities decrease significantly (Farooq et al., 2021).

Effects of variety and seed coating interaction on seed germination and seedling growth of forage pea

The Pionir variety showed a higher FGP (93%) compared to the Arda variety (58%) (Table 3). The results also indicated that seed coating was relatively more effective in the Arda variety than in the Pionir variety under drought stress. In the Pionir variety, most seed coating treatments produced responses similar to the control. All treatments, except 0.1% CH, showed comparable FGP values, whereas 0.1% CH resulted in the lowest FGP (80%) compared to the other treatments and the non-coated seeds. The highest FGP in Pionir was observed in the non-coated seeds. In contrast, in the Arda variety the highest FGP (60%) was obtained with 19 g HA, although it was not significantly different from the other treatments or the control. Regarding MGR, the Pionir variety exhibited a higher MGR (0.33 germinated seeds day⁻¹) than the Arda variety (0.27 germinated seeds day⁻¹), indicating faster germination in Pionir. In the Pionir variety, the highest MGR was recorded with 19 g HA (0.37 germinated seeds day⁻¹), which was significantly higher than most other treatments except 0.15% and 0.2% CH (0.36 and 0.36 germinated seeds day⁻¹, respectively) and 17 g HA (0.36 germinated seeds day⁻¹). In the Arda variety, the

highest MGR was observed with 19 g HA (0.35 germinated seeds day⁻¹), followed by 16 g HA (0.32 germinated seeds day⁻¹), both of which were higher than the control (0.27 germinated seeds day⁻¹). The two varieties differed significantly in SL, while no significant differences were observed in RL. The average SL in the Pionir variety was 3.19 cm, which was significantly higher than that of the Arda variety (1.54 cm). The average RL in Pionir was (4.52 cm), which was not significantly different from that of the Arda variety (4.33 cm). In both varieties, seed coating significantly increased SL and RL. In the Pionir variety, the greatest increase in SL was observed in seeds coated with 0.2% CH (4.55 cm). In the Arda variety, the highest SL was recorded with 0.1% and 0.15% CH (2.88 and 2.52 cm, respectively), both of which were significantly higher than the control. In addition, significantly greater RL in Arda was observed with 19 g and 18 g HA (6.06 and 5.15 cm, respectively). In the Pionir variety, RL increased significantly in most seed coating treatments. The highest RL was recorded with 17 g HA (5.63 cm) compared to the control (4.52 cm). However, no significant differences were observed between 17 g HA and 16 g and 19 g HA (5.13 and 5.22 cm), or between 17 g HA and 0.15%, 0.2%, and 0.3% CH (5.25, 5.55, and 5.50 cm, respectively). (Rasheed et al., 2020) reported that pea seed priming with lower concentrations of CH (less than 0.5%) was more effective than higher concentrations. According to the previous studies, CH can significantly improve plant growth parameters, enhance photosynthetic pigments and secondary metabolite production, reduce oxidative stress damage, and increase nutrient uptake, particularly phosphorus and potassium (Xu et al. 2020).

Effect of seed coating and drought stress on germination characteristics and seedling growth

In the absence of drought stress, seed coating with CH and HA did not significantly affect FGP compared to non-coated seeds, except for 0.1% and 0.2% CH, which recorded the lowest FGP values among all treatments (80% and 81%, respectively) (Fig. 1). Under -3 bar drought stress, the highest FGP values were obtained in non-coated seeds (91%), followed by 0.3% and 0.1% CH (90% and 89%, respectively), 18 g HA (88%), 0.2% CH (87%), and 17 g HA (87%). Under -6 bar drought stress, the highest FGP values were recorded for non-coated seeds and 16 g HA (both 74%), followed by 19 g HA (46%), 0.3% CH (43%), 0.4% CH (43%), and 18 g HA (43%). Several studies demonstrated that increasing drought intensity significantly reduces seed germination and seedling growth. In the present study, the application of HA as a seed-coating agent highly improved seed germination and seedling growth of forage pea under drought stress. Previous studies have also reported that HA enhances seed germination, seedling growth, overall plant development and integrity under various stress conditions (Gursoy et al., 2024) by improving the physiological status of plants and increasing the uptake of beneficial nutrients. These effects also occurred by enhancements in primary and secondary metabolic processes associated with abiotic stress tolerance (Canellas et al., 2015; Lasheen et al., 2024).

Under normal conditions, no significant differences in MGR were observed among all seed-coating treatments compared to the non-coated seeds (Fig. 2). However, under -3 bar drought stress, the application of HA at all tested

concentrations (16, 17, 18, and 19 g) significantly improved MGR (0.38, 0.37, 0.37, and 0.39 germinated seeds day⁻¹, respectively), indicating faster germination compared to the non-coated seeds (0.34 germinated seeds day⁻¹). The lowest MGR values were recorded in non-coated seeds (0.34 germinated seeds day⁻¹), followed by 0.1% and 0.3% CH (0.36 and 0.37 germinated seeds day⁻¹, respectively). Under -6 bar drought stress, 19 g HA (0.24 germinated seeds day⁻¹), followed by 16 g HA (0.17 germinated seeds day⁻¹), resulted in significantly higher MGR and faster germination compared to the control (0.11 germinated seeds day⁻¹). (Guan et al., 2009) reported that treating maize seeds (*Zea mays* L.) with CH had no significant effect on germination percentage but significantly enhanced germination rate, resulting in faster seed germination. Similarly, (Durgadevi et al., 2025) emphasized the potential of CH as a seed-coating agent for promoting faster germination. In addition, (Amirkhani et al., 2019) and (Taylor et al., 2021) demonstrated that seed coating with HA significantly improved germination rate. Regarding seedling SL, the response of forage pea seedlings across all coated seed treatments and non-coated seeds was generally better under normal conditions but increasing drought levels significantly reduced SL (Fig. 3). Across all treatments and

drought levels, the highest average SL was obtained from seeds coated with 0.2% CH (6.86 cm), which was significantly higher than the other treatments and the control. The second-highest SL values were recorded at 0.15% CH, 19 g HA, 16 g HA, 17 g HA, and 0.3% CH (6.27, 6.08, 6.01, 6.00, and 5.95 cm, respectively), all of which were significantly higher than the non-coated seeds (4.98 cm). Under drought stress (-6 bar), CH-coated seeds exhibited better SL compared to other treatments. The highest SL values were recorded for 0.1% CH (3.38 cm), followed by 0.2% CH (3.15 cm) and 0.15% CH (2.99 cm). However, no significant differences in SL were observed among all seed-coating treatments and the control under this stress level. (Jabeen et al., 2012) reported that CH is an effective biostimulator, and seed priming with low concentrations of CH (less than 0.5%) enhanced seedling growth in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), increased germination percentage, and reduced proline and malondialdehyde (MDA) contents as well as POX and CAT activities under stress conditions. Similarly, field cultivation of soybean seeds surface-coated with CH increased seed chitinase activity, germination rate, and yield parameters (Hirano et al., 2000).

Table 1. Variance analysis for the effects of variety and coating treatments on final germination percentage (FGP), mean germination rate (MGR), shoot length (SL), and root length (RL) of forage pea under different levels of drought stress

SOV	df	MS			
		FGP (%)	MGR (germinated seeds day ⁻¹)	SL (cm)	RL (cm)
Variety	1	5632.44**	0.02**	125.82**	1.34 ^{ns}
Drought	2	7029.67**	1.08**	564.91**	585.38**
Coating	8	231.37**	0.01**	2.12**	3.36**
Variety × Drought	2	6020.33**	0.03**	36.74**	71.38**
Variety × Coating	8	115.17*	0.01**	3.16**	1.26**
Drought × Coating	16	102.27**	0.00 ^{ns}	1.34**	1.17**
Variety × drought × Coating	16	130.19**	0.00 ^{ns}	1.36**	1.07**
Error	162	46.56**	0.00	0.25	0.46
R ²		0.97	0.97	0.97	0.95
CV (%)		8.62	6.48	17.42	13.06

* and ** are significant at 1% and 5%, respectively and ns is non-significant different. SOV, source of variation; MS, mean squares; and CV, coefficient of variation.

Table 2. Effects of variety and drought interaction on final germination percentage (FGP), mean germination rate (MGR), shoot length (SL), and root length (RL)

Variety	Drought levels (bar)	FGP (%)	MGR (germinated seeds day ⁻¹)	SL (cm)	RL (cm)
Pionir	Control	85 ^b	0.44 ^a	7.34 ^a	7.51 ^a
	-3	88 ^a	0.37 ^b	3.33 ^c	5.25 ^c
	-6	88 ^a	0.24 ^c	0.36 ^e	2.64 ^d
Arda	Control	84 ^b	0.45 ^a	4.22 ^b	6.95 ^b
	-3	88 ^a	0.37 ^b	2.23 ^b	7.66 ^a
	-6	1 ^c	0.03 ^d	0.0027 ^f	0.3 ^e
Variety		**	**	**	**
Drought		**	**	**	**
Variety × Drought		**	**	**	**
R ²		0.97	0.97	0.97	0.95
CV (%)		8.15	9.27	17.42	13.05

Means in the same column followed with similar letters are not significantly different. ** Significant at 1% probability level. CV, coefficient of variation.

Table 3. Effects of variety and coating interaction on final germination percentage (FGP), mean germination rate (MGR), shoot length (SL) and root length (RL)

Variety	Coating treatments	FGP (%)	MGR (germinated seeds day ⁻¹)	SL (cm)	RL (cm)
Pionir	Control	93 ^a	0.33 ^d	3.19 ^{de}	4.52 ^{fg}
	16 g HA	87 ^b	0.35 ^{bcd}	3.52 ^{cd}	5.13 ^{bcd}
	17 g HA	84 ^{bc}	0.36 ^{ab}	3.99 ^b	5.63 ^{ab}
	18 g HA	85 ^b	0.37 ^{bcd}	3.50 ^{cd}	4.99 ^{cdef}
	19 g HA	84 ^{bc}	0.37 ^a	4.00 ^b	5.22 ^{bcd}
	0.1% CH	80 ^c	0.34 ^{bcd}	2.73 ^{fg}	4.38 ^g
	0.15% CH	84 ^{bc}	0.36 ^{ab}	3.78 ^{bc}	5.25 ^{bcd}
	0.2% CH	87 ^b	0.36 ^{ab}	4.55 ^a	5.55 ^{ab}
	0.3% CH	87 ^b	0.35 ^{bc}	3.84 ^{bc}	5.50 ^{bcd}
	Arda	Control	58 ^e	0.27 ^e	1.54 ^l
16 g HA		59 ^d	0.32 ^d	2.38 ^{ghi}	5.45 ^{bcd}
17 g HA		57 ^e	0.28 ^e	1.94 ^{kl}	5.00 ^{cdef}
18 g HA		57 ^e	0.28 ^e	1.82 ^{kl}	5.15 ^{abce}
19 g HA		60 ^{de}	0.35 ^{bc}	1.93 ^{kl}	6.06 ^a
0.1% CH		57 ^e	0.26 ^e	2.88 ^{ef}	4.54 ^{fg}
0.15% CH		59 ^{de}	0.28 ^e	2.52 ^{fgh}	4.61 ^{efg}
0.2% CH		58 ^d	0.26 ^e	2.31 ^{hij}	4.64 ^{efg}
0.3% CH		57 ^e	0.27 ^e	2.04 ^{ijk}	4.93 ^{def}
Variety			**	**	**
Coating		**	**	**	**
Variety × Coating		**	**	**	**
R ²		0.97	0.97	0.97	0.95
CV (%)		8.15	9.27	17.42	13.05

Means in the same column followed with similar letters are not significantly different. ** Significant at 1% probability level. HA, Humic acid; CH, chitosan; and CV, Coefficient of variation.

With increasing drought intensity, RL significantly decreased in forage pea; however, seed coating with CH and HA significantly improved RL (Fig. 4). Under normal conditions, most seed-coating treatments significantly increased RL compared to the non-coated seeds (6.33 cm), except for 0.1% CH (5.77 cm). The highest RL values were observed in seeds treated with 17, 16, and 19 g HA (7.92, 7.84, and 7.70 cm, respectively), followed by 0.2%, 0.3%, and 0.15% CH (7.57, 7.40, and 7.26 cm, respectively), and 18 g HA (7.92 cm). However, no significant differences were found among these treatments. Under -3 bar drought stress, most HA concentrations, i.e., 18, 19, and 17 g HA (6.82, 6.76, and 6.73 cm, respectively), and 0.3% CH (6.79 cm) produced significantly greater RL compared to the non-coated seeds (5.71 cm). Under -6 bar drought stress, 19 g HA (2.47 cm) resulted in the highest RL compared to all other seed-coating treatments and the non-coated seeds (1.15 cm). The results of the present study indicate that most HA concentrations enhanced RL, with the highest RL observed in seeds coated with 19 g HA. Similarly, (Arslan et al., 2020) reported that the application of HA can improve plant drought tolerance by regulating different metabolic pathways, including the accumulation of abscisic acid (ABA). (Canellas and Olivares, 2014) reported that HA-based seed coating is an effective technology for enhancing seed germination and seedling development under stress conditions. This improvement is mainly attributed to modifications in root architecture, including increased root size, induction of lateral root formation, greater branching, higher root hair density, and changes in transport systems across root

plasma membranes. In maize, HA application stimulated lateral root formation, increased RL, and enhanced root biomass. Additionally, it influenced proteins involved in energy metabolism, protein folding, RNA processing, stress responses, nitrogen assimilation, and protein transport (Nunes et al., 2019).

Effects of variety and seed coating treatments on seedling growth of forage pea under different levels of drought stress

With respect to SL, the highest value was obtained from seeds coated with 0.2% CH (9.17 cm) in the Pionir variety under normal conditions. This value was significantly higher than those of all other seed-coating treatments across all drought levels as well as those observed in the Arda variety (Table 4). In the Arda variety, the highest SL values were recorded for 0.1% CH (5.21 cm), followed by 16 g HA (4.98 cm), 0.15% CH (4.63 cm), and 0.2% CH (4.55 cm); however, these values were not significantly different from each other. Increasing drought stress significantly reduced SL in both forage pea varieties, with the reduction being more pronounced in the Arda variety. Under -3 bar drought stress, most seed-coating treatments produced higher SL values than the control in the Pionir variety (2.82 cm). Among all treatments, the highest SL in Pionir was recorded for 0.2% CH (3.91 cm), which was significantly higher than the control. In the Arda variety, none of the HA seed-coating treatments significantly increased SL compared to the control (1.73 cm) under -3 bar drought stress (Fig. 5).

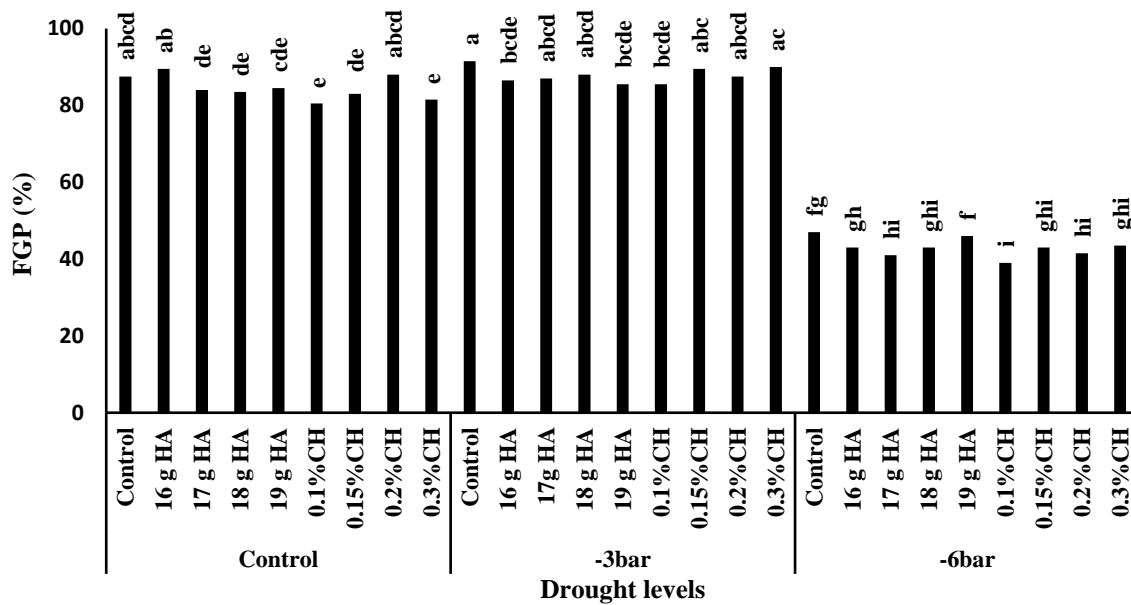


Fig. 1. Effect of seed coating on final germination percentage (FGP) of forage pea under different levels of drought stress. Treatments with different letters represent significant differences at 5% probability level.

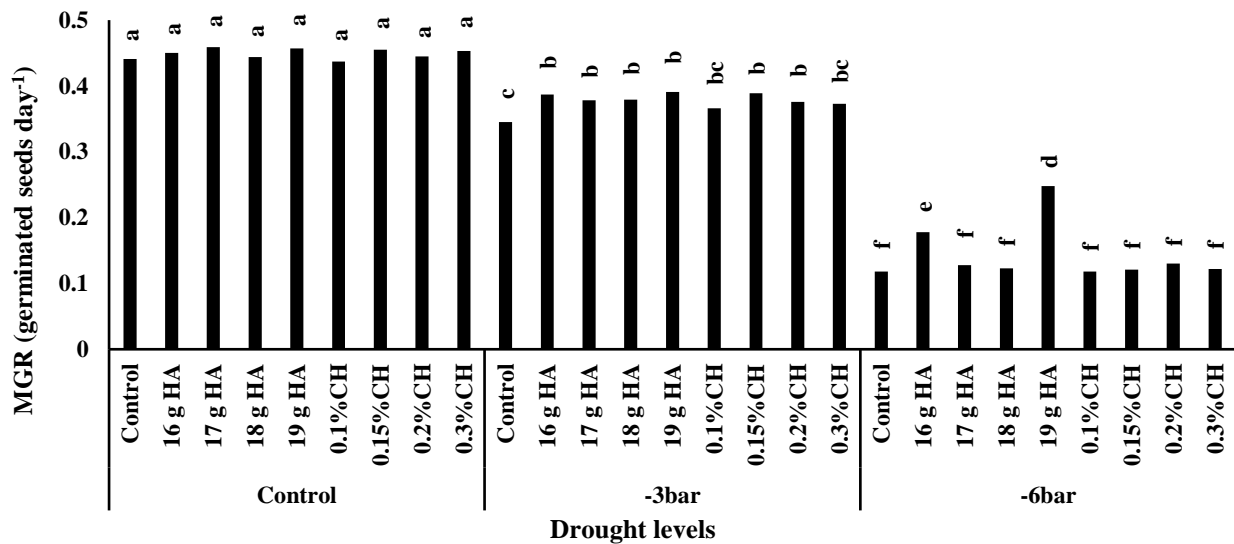


Fig. 2. Effect of seed coating treatments on mean germination rate (MGR) of forage pea under different levels of drought stress. Treatments with different letters represent significant differences at 5% probability level.

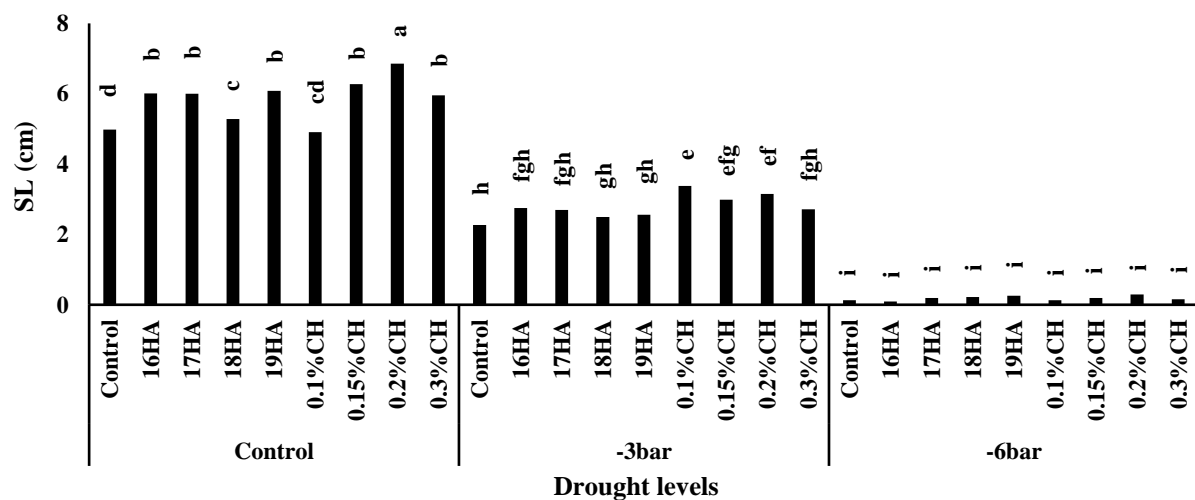


Fig. 3. Effect of seed coating treatments on seedling shoot length (SL) of forage pea under different levels of drought stress. Treatments with different letters represent significant differences at 5% probability level.

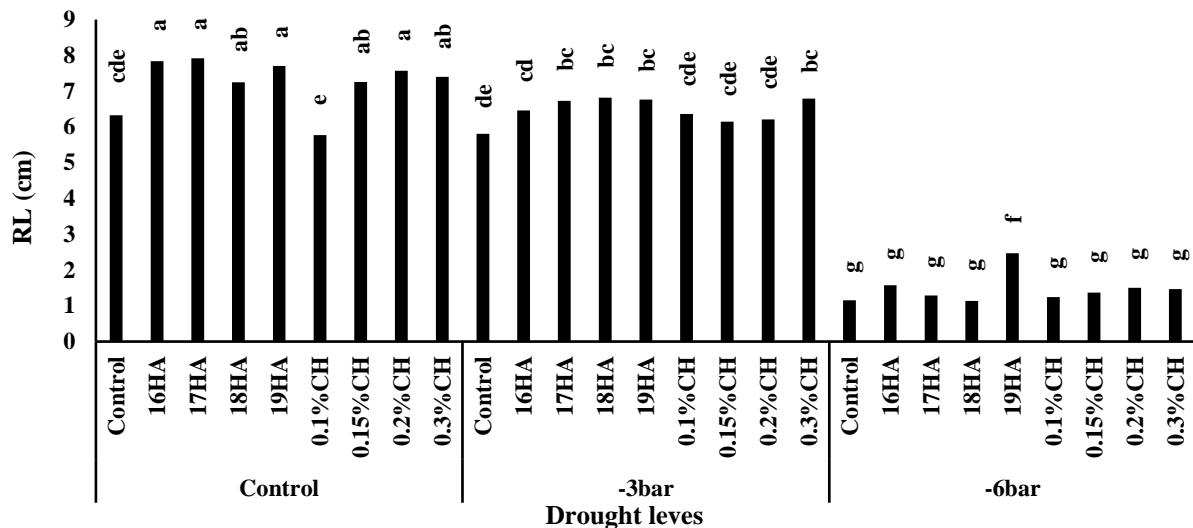


Fig. 4. Effect of seed coating treatments on root length (RL) of forage pea under different levels of drought stress. Treatments with different letters represent significant differences at 5% probability level.

In contrast, the application of CH at rates of 0.1%, 0.15%, and 0.2% (3.43, 2.94, and 2.38 cm, respectively) significantly increased RL compared to the control. Under the -6 bar drought stress, the lowest RL values were recorded for all treatments in both varieties. The highest RL was observed in the Pionir variety with 17 g HA (8.79 cm), which was markedly higher than most other treatments, except for 0.2% CH (8.72 cm), 16 g HA (7.98 cm), and 0.15% CH (7.9 cm). In the Arda variety under -3 bar drought stress, the highest RL values were recorded for HA treatments, particularly 19 g HA (8.45 cm), 16 g HA (7.98 cm), and 17 g HA (7.95 cm). Generally, the Pionir variety exhibited greater RL than the Arda variety under normal conditions. In Pionir, the highest RL under these conditions was recorded for 17 g HA (8.79 cm), which was not significantly different from 0.2% CH (8.72 cm), 16 g HA (7.98 cm), and 0.15% CH (7.90 cm). In the Arda variety, the highest RL was recorded for 16 g HA (7.70 cm). All HA coating treatments (16, 17, 18, and 19 g) increased RL compared to the control (5.95 cm), with RL values of 7.70, 7.05, 7.70, and 7.75 cm, respectively. However, CH-coated seeds did not show increased RL compared to the control. Under the -3 bar drought stress, the highest RL values in the Pionir variety were associated with 18 g HA (5.9 cm), 0.3% CH (5.75 cm), 0.1% CH (5.47 cm), 0.15% CH (5.11 cm), and 17 g HA (5.12 cm), all of which were higher than the non-coated seeds (4.57 cm). In contrast, in the Arda variety, several HA treatments, including 19, 16, and 18 g HA (8.45, 7.98, and 7.95 cm, respectively), increased RL compared to the non-coated seeds (7.05 cm). Similar to the normal conditions, CH application did not improve RL compared to the non-coated seeds. Under the -6 bar drought stress, coating treatments in the Pionir variety did not improve RL compared to non-coated seeds (2.29 cm). However, in the Arda variety, seed coating with 19 g HA (1.98 cm) increased RL

compared to the non-coated seeds (0.00 cm) (Fig. 6). Furthermore, under -6 bar drought stress in the Arda variety, HA seed coating increased RL, and no significant differences were observed between 19 g HA and most other seed coating treatments, except for 0.2% CH (3.00 cm) and the non-coated seeds. The current results indicated that coating seeds with 0.2% CH produced the highest RL across drought levels and among seed coating treatments. These findings are consistent with (Xu et al., 2020), who reported that a CH concentration of 2 g L⁻¹ was optimal for improving seedling shoot biomass and shoot length. Improvements were associated with increased SOD and CAT activities, more nutrient uptake, higher total chlorophyll content, greater photosynthetic capacity, and enhanced soluble protein content, but reduced MDA and proline contents.

CONCLUSION

With increasing drought stress levels, the mean germination rate decreased, and the germination period increased in both varieties. The Pionir variety showed better performance in seed germination and seedling growth compared to the Arda variety under drought stress. Pionir also exhibited greater SL and RL than Arda not only under normal conditions but also under drought stress. However, under -3 bar drought stress, the Arda variety showed the highest RL among all drought levels. Based on the findings of the present study, seed coating with 0.2% CH significantly improved SL, while seed coating with 19 g HA significantly increased RL. These treatments enhanced seed germination and seedling growth under different levels of drought stress and therefore could be considered effective seed coating agents. From a practical perspective, seed coating is recommended as an effective approach to improve seedling growth.

Table 4. Effects of variety and coating treatments on final germination percentage (FGP), mean germination rate (MGR), shoot length (SL) and root length (RL) of forage pea under different levels of drought stress

Variety	Drought (bar)	Coating treatments	FGP	MGR (germinated seeds day ⁻¹)	SL (cm)	RL (cm)	
Pionir	0	Control	93 ^{ab}	0.43 ^{abc}	6.46 ^d	6.70 ^{ghijk}	
		16 g HA	90 ^{abcd}	0.45 ^{ab}	7.13 ^{cd}	7.98 ^{abcd}	
		17 g HA	85 ^{cdefghij}	0.45 ^{ab}	7.94 ^b	8.79 ^a	
		18 g HA	83 ^{efghij}	0.41 ^{bcde}	6.77 ^d	6.80 ^{fghij}	
		19 g HA	85 ^{cdefghij}	0.47 ^a	8.32 ^b	7.64 ^{cdefg}	
		0.1% CH	77 ^j	0.43 ^{abcd}	4.69 ^{efg}	5.19 ^{nop}	
		0.15% CH	81 ^{ghij}	0.45 ^{ab}	7.90 ^b	7.90 ^{abcde}	
		0.2% CH	90 ^{abcde}	0.45 ^{ab}	9.17 ^a	8.72 ^{ab}	
		0.3% CH	80 ^{hij}	0.45 ^{ab}	7.79 ^{bc}	7.81 ^{bcde}	
	-3	Control	92 ^{abc}	0.32 ^h	2.82 ^{lmn}	4.57 ^p	
		16 g HA	87 ^{bcdefgh}	0.37 ^{fg}	3.25 ^{jkl}	4.94 ^{op}	
		17 g HA	85 ^{cdefghij}	0.37 ^{efg}	3.64 ^{ijk}	5.12 ^{lmnop}	
		18 g HA	88 ^{abcdefg}	0.37 ^{efg}	3.32 ^{jkl}	5.90 ^{klmn}	
		19 g HA	85 ^{cdefghij}	0.38 ^{efg}	3.20 ^{jkl}	5.07 ^{nop}	
		0.1% CH	85 ^{cdefghij}	0.37 ^{efg}	3.32 ^{jkl}	5.47 ^{mnp}	
		0.15% CH	86 ^{bcdefghi}	0.38 ^{efg}	3.05 ^{klm}	5.11 ^{nop}	
		0.2% CH	87 ^{bcdefgh}	0.39 ^{efg}	3.91 ^{ghij}	4.94 ^{op}	
		0.3% CH	93 ^{ab}	0.36 ^{fg}	3.41 ^{ijkl}	5.75 ^{klmno}	
	-6	Control	94 ^a	0.23 ^{ij}	0.27 ^p	2.29 ^{qr}	
		16 g HA	84 ^{defghij}	0.23 ^j	0.17 ^p	2.48 ^{qr}	
		17 g HA	82 ^{fghij}	0.25 ^{ij}	0.38 ^p	2.57 ^{qr}	
		18 g HA	86 ^{bcdefgh}	0.24 ^{ij}	0.44 ^p	2.29 ^{qr}	
		19 g HA	83 ^{defghij}	0.27 ⁱ	0.49 ^p	2.96 ^{qr}	
		0.1 % CH	78 ^{ij}	0.23 ^{ij}	0.26 ^p	2.47 ^{qr}	
		0.15 % CH	86 ^{cdefghij}	0.24 ^{ij}	0.39 ^p	2.74 ^{qr}	
		0.2 % CH	83 ^{efghij}	0.26 ^{ij}	0.58 ^p	3.00 ^q	
		0.3 % CH	87 ^{abcdefgh}	0.24 ^{ij}	0.32 ^p	2.94 ^{qr}	
	Arda	0	Control	82 ^{fghij}	0.44 ^{abc}	2.89 ^{lmn}	5.95 ^{klmn}
			16 g HA	89 ^{abcde}	0.45 ^{ab}	4.98 ^e	7.70 ^{cdef}
			17 g HA	83 ^{efghij}	0.46 ^a	4.07 ^{fghi}	7.05 ^{defghi}
			18 g HA	84 ^{defghij}	0.47 ^a	3.79 ^{ij}	7.70 ^{l cdef}
			19 g HA	84 ^{defghij}	0.44 ^{abc}	3.84 ^{hij}	7.75 ^{cde}
			0.1% CH	84 ^{defghij}	0.44 ^{abc}	5.21 ^e	6.35 ^{ijklm}
			0.15% CH	85 ^{cdefghij}	0.45 ^{ab}	4.63 ^{ef}	6.63 ^{hijk}
			0.2% CH	86 ^{bcdefghi}	0.44 ^{abc}	4.55 ^{efgh}	6.43 ^{ijkl}
			0.3% CH	83 ^{efghij}	0.46 ^a	4.10 ^{fghi}	6.75 ^{efghi}
-3		Control	91 ^{abcd}	0.36 ^{fg}	1.73 ^o	7.05 ^{defghi}	
		16 g HA	86 ^{bcdefghij}	0.40 ^{cdef}	2.26 ^{no}	7.98 ^{abcd}	
		17 g HA	89 ^{abcdef}	0.37 ^{efg}	1.75 ^o	7.95 ^{abcd}	
		18 g HA	88 ^{abcdefg}	0.37 ^{efg}	1.68 ^o	7.75 ^{cdef}	
		19 g HA	86 ^{cdefghij}	0.39 ^{defg}	1.92 ^o	8.45 ^{abc}	
		0.1% CH	86 ^{cdefghi}	0.35 ^{hg}	3.43 ^{ijkl}	7.26 ^{defghi}	
		0.15% CH	93 ^{ab}	0.39 ^{defg}	2.94 ^{klmn}	7.19 ^{defghi}	
		0.2% CH	88 ^{abcdefg}	0.36 ^{fgh}	2.38 ^{mno}	7.49 ^{defgh}	
		0.3% CH	87 ^{bcdefgh}	0.37 ^{efg}	2.00 ^o	7.82 ^{bcde}	
-6		Control	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		16 g HA	2.00 ^l	0.12 ^k	0.02 ^p	0.67 ^s	
		17 g HA	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		18 g HA	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		19 g HA	9.00 ^k	0.22 ^j	0.03 ^p	1.98 ^r	
		0.1% CH	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		0.15% CH	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		0.2% CH	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
		0.3% CH	0.00 ^l	0.00 ^l	0.00 ^p	0.00 ^s	
Variety × Drought			**	**	**	**	
Variety × Coating			**	**	**	**	
Drought × Coating			**	**	**	**	
Variety × Drought × Coating			**	**	**	**	
R ²			0.97	0.97	0.97	0.96	
CV (%)			8.15	9.26	17.42	13.53	

Means in the same column followed by similar letters are not significantly different. ** Significant at 1% probability. HA, Humic acid; CH, chitosan; and CV, Coefficient of variation.

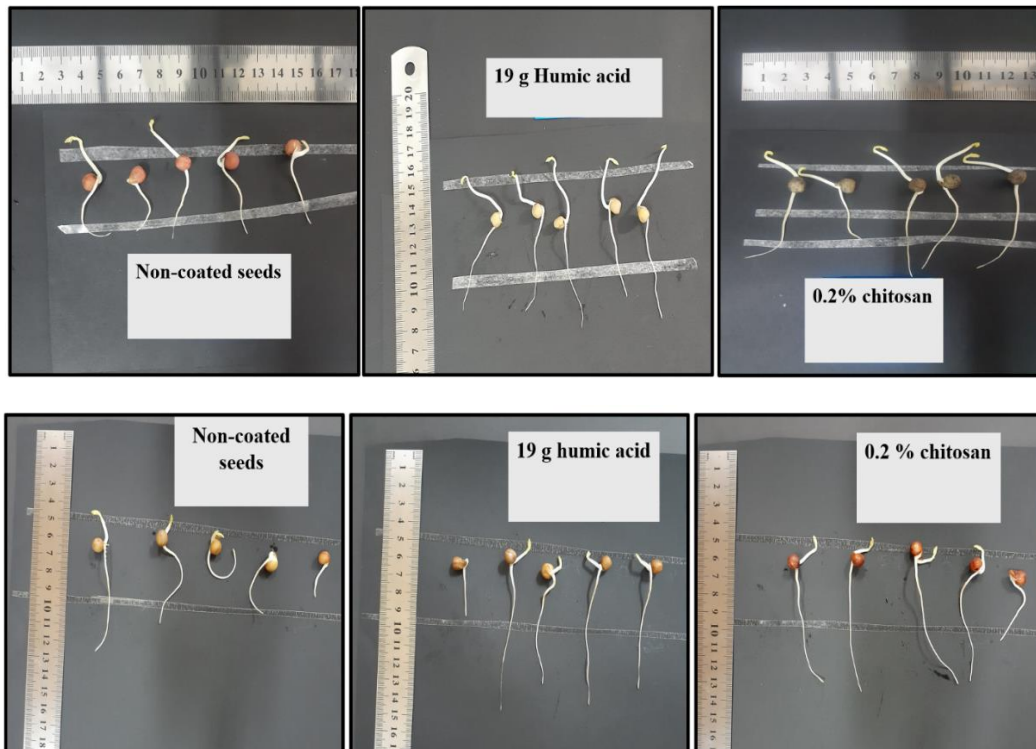


Fig. 5. Effects of CH and HAs seed coating treatments on seed germination and seedling growth of two varieties of forage pea, Pionir and Arda under -3 bar drought stress. **A-C** Pionir variety, **A** = non-coated seeds, **B** = 19g humic acid, **C** = 0.2% CH; **D-F** Arda variety, **D** = non-coated seeds, **E** = 19g humic acid, **F** = 0.2% CH.

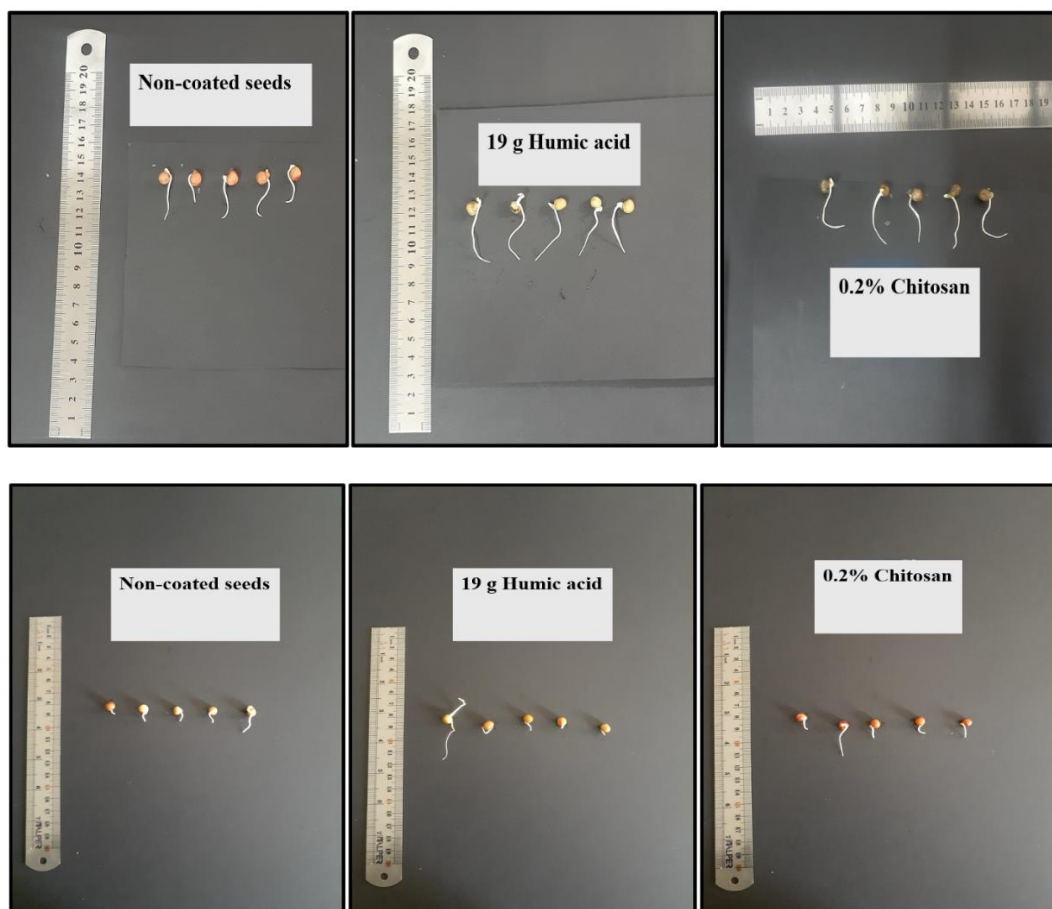


Fig. 6. Effects of CH and HAs seed coating treatments on seed germination and seedling growth of two varieties of forage pea, Pionir and Arda under -6 bar drought stress. **A-C** Pionir variety, **A** = non-coated seeds, **B** = 19g humic acid, **C** = 0.2% CH; **D-F** Arda variety, **D** = non-coated seeds, **E** = 19g humic acid, **F** = 0.2% CH.

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CRedit AUTHORSHIP CONTRIBUTION STATEMENT

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DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

Approval was granted by the Ethics Committee of University Shiraz.

DATA AVAILABILITY

All data analyzed and generated during this study are included in this published article.

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