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Research Article

Evaluating the germination indices of two bean species (*Phaseolus vulgaris* L. and *Pachyrhizus erosus* (L.) urban) under drought stress and fertilizer treatments

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ABSTRACT- The quality of seeds plays a fundamental role in advancing agriculture and enhancing crop yields. However, drought remains a major constraint, severely limiting crop growth and productivity. To assess how drought stress and different fertilizer treatments influence the germination performance of two bean species, i.e., common bean (*Phaseolus vulgaris* cv. 'Goli') and Jicama bean (*Pachyrhizus erosus* (L.) Urban), a study was conducted at Shahrekord University during the 2022–2023 growing season. The experiment followed a completely randomized design with three replications, incorporating four levels of drought stress (0, -6, -9, and -12 bars) and four fertilizer treatments: (1) no fertilizer (control), (2) biofertilizer at the recommended level, (3) Vinasse organic fertilizer, and (4) a combination of biofertilizer and organic fertilizer at the recommended level. Biochemical markers such as seed proline content, malondialdehyde concentration, and catalase enzyme activity varied significantly across treatments ($P < 0.01$). Jicama bean demonstrated superior germination performance under favorable conditions. When grown without drought stress and treated with the combined fertilizer application, it achieved the highest germination percentage (100%), germination rate (42.48 seeds/day), seedling vigor index (8.55), shoot length (5.66 cm), shoot fresh weight (0.694 g), shoot dry weight (0.125 g), root fresh weight (0.772 g), and root dry weight (0.109 g). Notably, moderate drought stress led to the longest root length (6.333 cm) in this species. Under severe drought conditions, Jicama bean exhibited the highest proline accumulation (0.317 µg/g fresh weight) and catalase activity (0.373 enzyme units/mg protein), indicating enhanced stress tolerance. Severe drought stress without fertilizer caused the highest malondialdehyde content (65.89 nmol/g fresh weight). Fertilizer applications mitigated the negative impacts of drought stress on seed germination. Jicama bean consistently outperformed common bean in germination and early seedling growth, highlighting its resilience and adaptability under varying environmental conditions.

INTRODUCTION

Bean species account for nearly half of the world's legume cultivation area, underscoring their agricultural significance. Rich in protein, fiber, and essential vitamins, beans serve as a vital food source globally. Unlike many other crops, they require no industrial processing and can be consumed directly (Mc Clean et al., 2004). Jicama bean (*Pachyrhizus erosus* (L.) Urban), a member of the Fabaceae family is particularly notable for its high oil and protein content, comprising approximately 20–28% and 23–34% of seed weight, respectively. Native to Mexico and Central America, this species thrives in harsh environments due to its strong adaptability to drought stress and its ability to grow in low-fertility soils, making it suitable for marginal lands (Sorensen, 1996). Red bean (*Phaseolus vulgaris* L.), often referred to simply as "bean," is an annual, heat-loving crop originating from South America. It belongs to a diverse group of Fabaceae species (Boroujerdnia et al., 2016). The Goli cultivar, classified under the Red Mexican commercial class, has been extensively cultivated in Iran due to its strong adaptation to the country's climatic conditions (Najarzadeh et al., 2014). Globally, beans are recognized as one of the most critical sources of protein-rich food, ranking as the second most important crop for human consumption after cereals (Boroujerdnia et al., 2016). Beyond their nutritional value, they play a pivotal role in sustainable agriculture by improving soil health through nitrogen fixation and enhancing its physical, chemical, and biological properties. As a result, legumes are widely incorporated into cereal-based cropping systems to promote agricultural diversification.

In Iran, research on bean varieties has gained importance due to the expansion of cultivated areas, the need to sustain cereal production systems, and their essential role in household food security (Hasani et al., 2022). Seeds serve as reservoirs of essential nutrients and biochemical

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compounds that sustain seedlings from germination through early growth until they reach self-sufficiency. Germination is a highly dynamic process characterized by a series of physiological and biochemical transformations that activate the embryo and initiate seedling development. The period between sowing and full seedling establishment is a critical phase in crop production, directly influencing plant growth, yield potential, and post-harvest seed quality. However, unfavorable soil conditions often hinder germination and early seedling growth. Various biotic and abiotic stress factors, such as extreme temperatures, drought, waterlogging, salinity, soil fertility depletion, pests, and pathogens can significantly impair seed germination and seedling emergence (Hosseinzadeh et al., 2015). Addressing these challenges is essential for improving crop establishment and ensuring sustainable agricultural productivity.

Drought is an inevitable environmental challenge that disrupts agricultural productivity worldwide. Occurring with varying intensity and frequency across different regions, it significantly affects crop production every year (Lukić et al., 2020). To mitigate the adverse effects of drought, biofertilizers have gained prominence as a sustainable solution. These fertilizers contain beneficial microorganisms such as fungi, bacteria, and actinomycetes that enhance plant nutrition and soil health. The increasing adoption of biofertilizers in modern agriculture reflects their potential to improve crop resilience and sustainability (Rasouli-Sadaghiani and Tavakoli, 2011). One widely used biofertilizer is Effective Microorganisms (EM), a specialized blend of 120 aerobic and anaerobic microbial species. These microorganisms coexist harmoniously in a liquid culture, enhancing soil fertility and plant growth. For optimal crop production and quality, it is crucial to establish favorable growth conditions by ensuring adequate nutrient availability and appropriate fertilizer application (Yaseen et al., 2011). Molasses, a by-product derived from *Saccharum officinarum* and *Beta vulgaris*, serves as a key raw material in ethanol production. A major by-product of this process is Vinasse, an organic effluent generated at a ratio of approximately 12 liters per liter of alcohol produced. Rich in organic matter and essential nutrients such as nitrogen, potassium, calcium, magnesium, and phosphorus, Vinasse is widely recognized as a valuable resource in organic farming (Jamili, 2012). DS poses a severe threat to plant establishment, particularly during the early stages of growth. Research has shown that water scarcity can disrupt germination and seedling development (Mamdi et al., 2016). Studies by Sadouqh et al. (2012) indicate that reducing osmotic potential through PEG leads to a decline in germination percentage. Similarly, Bagheri and Hasan Beigi (2018) reported stunted shoot growth under increasing drought stress, a finding further supported by Kafi et al. (2019). However, the application of biological fertilizers has been shown to enhance seed germination and improve plant tolerance to water stress, making them a promising pre-sowing treatment (Kauser Malik and Azam, 2011). The benefits of integrating organic and biological fertilizers have been demonstrated in various plant species. For instance, Tabrizi and Koocheki (2014) found that applying these fertilizers to *Calendula officinalis* significantly improved germination percentage, seedling length, and seed vigor index compared to untreated seeds (Higa and Parr, 1994). Under stress conditions, plants activate antioxidant defense mechanisms to counteract oxidative damage. An increase in reactive oxygen species (ROS) triggers the activation of enzymes such as catalase, which plays a crucial role in detoxifying harmful oxygen radicals. Additionally, MDA, a by-product of lipid peroxidation in cell membranes, serves as a key indicator of oxidative stress. Excessive accumulation of free radicals leads to heightened MDA production, further highlighting the damaging effects of drought stress (Xia et al., 2014). A severe reduction in root growth under drought conditions is a primary factor limiting nutrient absorption from the soil. However, organic fertilizers can alleviate drought stress by increasing proline levels and soluble sugars, as well as enhancing the uptake of essential minerals such as potassium and phosphorus. These mechanisms contribute to improved drought tolerance and higher crop yields (Selim et al., 2019). Despite extensive research on drought stress and fertilizer applications, limited studies have compared the germination responses of Jicama bean and red bean under such conditions. Therefore, this study aims to evaluate the impact of organic and biological fertilizers on the seed germination of these two species under drought stress.

MATERIALS AND METHODS

The experiment followed a factorial design within a completely randomized layout, incorporating three replications, and was conducted in the Physiological Laboratory of Shahrekord University during 2022–2023. The study examined the effects of drought stress (DS) and fertilizer treatments on seed germination. Drought stress (DS) was simulated using a polyethylene glycol (PEG), molecular weight 6000 amu) solution at four osmotic potential levels: 0, -3, -6, and -12 bars. Fertilizer treatments included four levels: (1) control (no fertilizer), (2) EM biofertilizer (applied at the optimal level), (3) Vinasse organic fertilizer (applied at the optimal level), and (4) a combined treatment of EM biofertilizer and Vinasse organic fertilizer (both at their optimal levels). The optimal fertilizer concentrations for seed germination under Petri dish conditions were determined by the fertilizer manufacturers, who recommended a 1:10 dilution with water. The EM biofertilizer was sourced from Emkan Pazir Pars Company, Shiraz, Iran, the exclusive representative of EMRO Japan. Vinasse organic fertilizer was obtained from Khorramshahr Alcohol Company under the order of Tehzih Sanat Baran Company, Tehran, Iran. For each experimental unit, Jicama bean and red bean seeds were tested separately. A total of 25 healthy seeds of each species were placed in sterile Petri dishes, to which 5 mL of the designated fertilizer treatment was added. The Petri dishes were then incubated in a germinator set to 25 °C under dark conditions. Germination counts began 24 hours after incubation, with daily observations continuing for nine days. According to ISTA standards, a seed was considered germinated when its radicle extended at least 2 mm. At the end of the experiment, germination indices and seedling traits were measured following the methodology described by Ahmadpour et al. (2015).

$$Gp = Gn/Tn \times 100 \qquad \text{Eq. (1)}$$

where Gp is the germination percentage, Gn is the number of germinated seeds, and Tn is the total number of seeds.

$$Gr = \sum Gi/Ti \qquad \text{Eq. (2)}$$

where Rs is the germination rate, Gi is the number of seeds germinated each day, and Ti is the number of days.

$$SVi = Ls \times (Gp/100) \qquad \text{Eq. (3)}$$

where SVi is the seedling vigor index, Ls is the average seedling length (sum of average root and shoot length), and Gp is the seed germination percentage.

Finally, radicles and seedlings were collected, and their fresh mass was recorded. Subsequently, seedling roots and shoots were oven-dried at 72°C for 48 hours, after which their dry mass was measured. Proline content was determined using the Bates method (1973), while malondialdehyde (MDA) levels were assessed following the procedure outlined by Heath and Packer (1968). Catalase activity was evaluated according to the method described by Abi (1950). The collected data were analyzed using SAS statistical software (version 9.2). Mean comparisons were conducted using the least significant difference (LSD) test with MSTAT-C software (version 16) at a 5% probability level. Data visualization, including charts and statistical tables, was carried out using Microsoft Word and Excel.

RESULTS AND DISCUSSION

Germination percentage

The analysis of variance revealed that species, DS, and fertilizer treatments had significant effects on the germination percentage of Jicama bean and red bean seeds ($P < 0.01$). Additionally, significant interactions were observed between species and DS, fertilizer and DS, as well as the combined effect of species, DS, and fertilizer. However, the interaction between species and fertilizer had no significant impact on germination percentage (Table 1). The highest germination percentage was recorded in Jicama bean seeds under combined fertilizer (100%), organic fertilizer (68%), and biological fertilizer (68%) treatments when no DS was applied (control drought treatment). Similarly, in red bean seeds, all fertilizer treatments under non-drought-stress conditions resulted in higher germination rates. Conversely, the lowest germination percentage (2.3%) was observed in red bean seeds subjected to both severe DS (-12 bar) and the absence of fertilizer (control treatment) (Fig. 1). The superior germination traits of Jicama bean compared to red bean may be attributed to its greater water absorption capacity under stress conditions. However, in both species, the overall decline in germination percentage under DS was likely due to a reduction in the initial water uptake rate. DS disrupts seed reserve mobilization and directly affects embryo structure by limiting water absorption, ultimately reducing germination success (Dashtaki & AziziNejad, 2019). Tabrizi and Koochaki (2014) reported that the combined application of organic and biological fertilizers significantly enhanced germination percentage, seedling length, and seed vigor index in marigolds compared to the untreated seeds. The observed decline in germination rate under severe DS conditions can be attributed to the reduced cell turgor pressure, which affects cell wall integrity, protein synthesis, and hormone secretion. Furthermore, DS restricts water absorption, thereby impeding the mobilization and transfer of seed reserves (Oveisi, 2017). Consistent with previous findings, the results of this study confirm that DS significantly reduces germination percentage compared to non-stressed conditions.

Germination rate

The findings indicated that seed germination rate in Jicama bean and red bean was significantly influenced by the main effects of treatments, as well as the interaction between species and fertilizer, and the three-way interaction among species, DS, and fertilizer ($P < 0.01$) (Table 1). As shown in Fig. 2, the highest germination rate (42 seeds/day) was recorded under the combined fertilizer treatment in non-drought-stress conditions (control drought treatment). In contrast, the lowest germination rate (2.7 seeds/day) was observed in red bean seeds subjected to severe DS (-12 bar) and the no-fertilizer treatment. Seed germination rate serves as a key indicator of drought tolerance, as higher germination speeds improve the likelihood of successful seedling establishment under water-limited conditions. Some researchers suggest that the reduced germination observed under DS reflects an evolutionary adaptation in plants from arid environments (Armand et al., 2015). For instance, a study on *Lens culinaris* genotypes found that both germination speed index and germination percentage declined under DS (Armand et al., 2015). Similarly, research on *Glycine max* demonstrated that the application of organic fertilizers enhanced the germination rates (Gulser et al., 2010). The decline in both germination rate and percentage under DS can be attributed to the decreased activity of the alpha-amylase enzyme. Limited water availability reduces seed hydration, subsequently inhibiting enzymatic processes essential for germination (Rahbarian et al., 2012). The results of this study align with previous research, further confirming that DS significantly impairs germination dynamics.

Seediling vigor index

The analysis revealed that species, DS, and fertilizer treatments, along with their interactions, had a significant impact on the seedling vigor index of Jicama bean and red bean ($P < 0.01$) (Table 1). The highest seedling vigor index values (8.31 and 8.55) were recorded in Jicama bean under the combined fertilizer treatment, both in non-drought-stress conditions and under mild DS (-6 bar). In contrast, red bean exhibited the lowest seedling vigor index across all fertilizer treatments, except for the combined fertilizer treatment, when subjected to severe DS (-12 bar) (Fig. 3). The decline in seedling vigor under DS, compared to non-stressed conditions, can be attributed to a significant reduction in germination rate and percentage, as well as a decrease in hypocotyl and radicle length at higher drought levels (Ahmadpour et al., 2015).

Table 1. Mean square (MS) from the analysis of variance for germination indices and seedling traits of Jicama bean and red bean, influenced by fertilizer and drought stress.

Source of variation	DF	Seed germination percentage	Germination rate	Seedling vigor index	Hypocotyl length	Fresh weight of hypocotyl	Dry weight of hypocotyl
Species	1	5828.166**	421.011**	110.124**	33.606**	0.166**	0.024**
Drought-stress	3	30508.694**	4409.357**	192.807**	55.620**	0.348**	0.012**
Fertilizer treatment	3	356.194**	148.869**	7.115**	3.071**	0.041**	0.0013**
Species × drought-stress	3	715.361**	28.710**	15.209**	2.646**	0.018**	0.0014**
Species × fertilizer	3	4.083**	2.079ns	0.862**	0.038*	0.0031ns	0.000046ns
Drought-stress × fertilizer	9	24.203**	8.325**	0.402**	0.140**	0.0057*	0.0001ns
Species × drought-stress × fertilizer	9	18.092*	3.110**	0.102**	0.0401**	0.0079**	0.00018*
Error	64	8.531	1.212	0.032	0.012	0.0028	0.000078
C.V. (%)	-	5.35	5.35	4.77	3.61	11.36	11.54

Source of variation	DF	Radicle length	Fresh weight of radicle	Dry weight of radicle	Proline content	Malondialdehyde	Catalase
Species	1	37.375**	0.137**	0.027**	0.651**	1027.826**	0.187**
Drought-stress	3	78.833**	0.342**	0.014**	0.224**	3782.303**	0.322**
Fertilizer treatment	3	1.720**	0.039**	0.00097**	0.008**	199.971**	0.012**
Species × drought-stress	3	5.090**	0.0108*	0.00094**	0.006**	26.511**	0.00083 ns
Species × fertilizer	3	0.065*	0.0038ns	0.00012ns	0.0002**	2.620 ns	0.00057 ns
Drought-stress × fertilizer	9	0.075**	0.0061**	0.00011ns	0.0003**	13.463**	0.00057 ns
Species × drought-stress × fertilizer	9	0.073**	0.0066**	0.0002*	0.0001*	5.445**	0.0021**
Error	64	0.025	0.0016	0.00009	0.00006	1.289	0.00077
C.V. (%)	-	5.36	7.41	15.55	4.92	2.54	2.58

ns: non-significant. * and **: significant at 5% and 1% probability levels, respectively.

Implementing suitable treatments under DS conditions encourages both shoot and root elongation, ultimately strengthening seedling germination vigor and improving structural attributes. Organic and biological fertilizers, rich in plant hormones such as auxins and gibberellins, play a significant role in accelerating seedling growth. These hormones are vital for stimulating shoot and root development, which in turn contributes to the formation of a sturdy plant structure. Consequently, the integration of hormonal compounds and organic fertilizers is essential for fostering vigorous seedling growth and facilitating successful plant establishment. Research on beans and *Cicer arietinum* exposed to DS has shown that applying organic fertilizer extracts enhances shoot and root length, while counteracting the detrimental effects of water deficiency (Ahmadpour et al., 2016). Consistent with these findings, the present study demonstrated that as DS intensity increased, germination percentage, germination speed, germination capacity, and the seed germination index exhibited a remarkable decline.

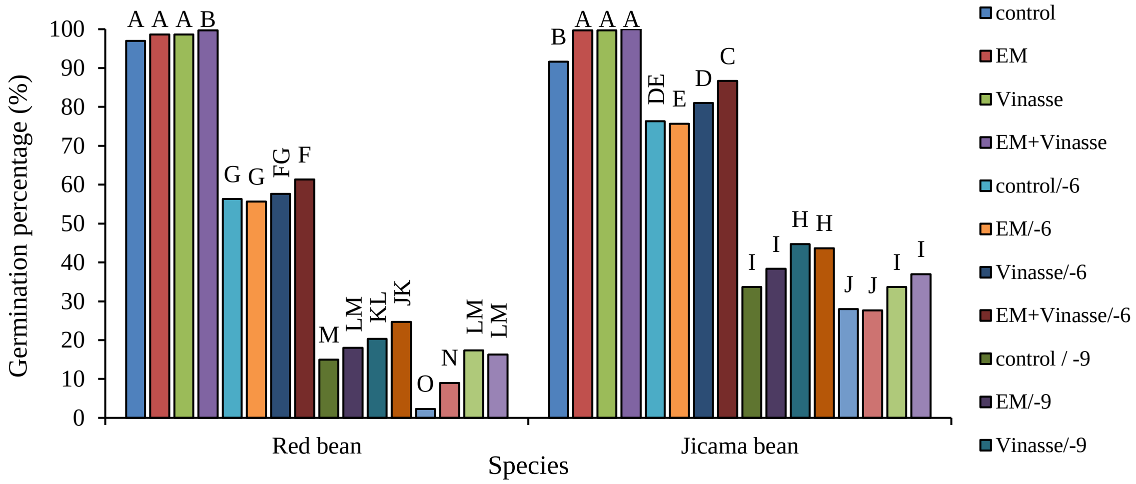

Fig. 1. The effect of species, fertilizer, and drought stress on germination percentage of Jicama bean and red bean seeds (Control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

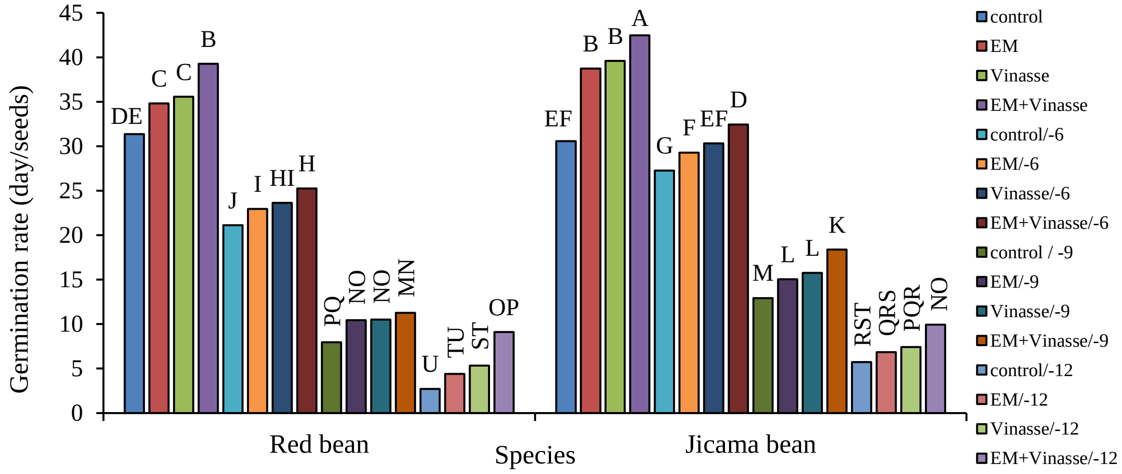

Fig. 2. The effect of species, fertilizer, and drought stress on Germination rate of Jicama bean and red bean seeds (Control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

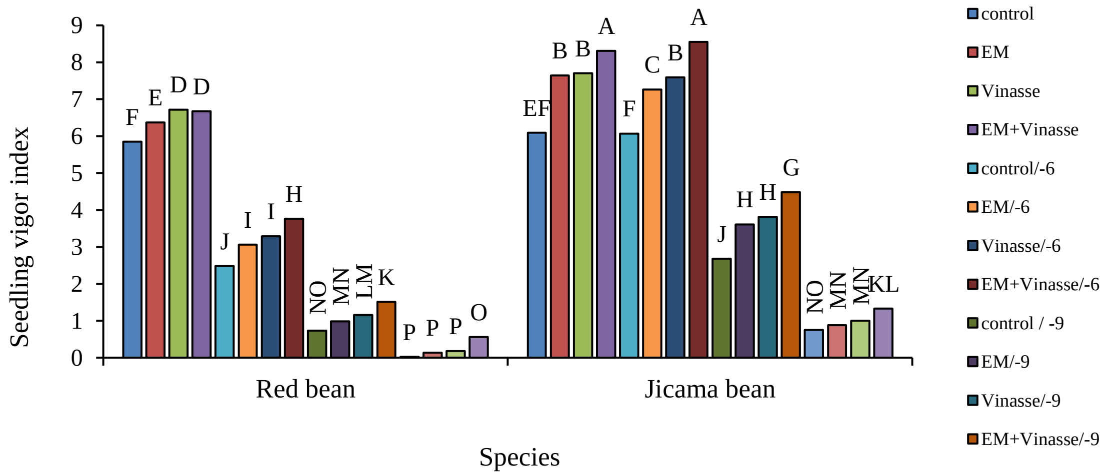

Fig. 3. The effect of species, fertilizer and drought-stress on seedling vigor index of Jicama bean and red bean seeds (Control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

Hypocotyl length

The impact of species, DS, fertilizer application, and their interactions on hypocotyl length was statistically significant ($P < 0.01$) (Table 1). Additionally, the combined influence of species and fertilizer exhibited a notable effect on hypocotyl elongation (LSD 5%). Among the treatments, the Jicama bean species showed the greatest hypocotyl length (5.667 cm) when exposed to a combination of fertilizer application and non-drought-stress conditions (control drought treatment). In contrast, the shortest hypocotyl length (0.366 cm) was observed in the red bean species grown without fertilizer under severe DS (-12 bar) (Fig. 4). Under drought conditions, dry matter accumulation in hypocotyl tissue tends to increase. Genotypes that exhibit a greater ability to elongate their hypocotyls under water-limited environments or experience minimal reductions in hypocotyl length as drought severity escalates are regarded as more drought-tolerant at the seedling stage (Rahimi et al., 2017). The availability of essential nutrients through fertilizer application plays a crucial role in enhancing seed germination parameters, particularly by promoting stem elongation. Research by Sabokdast et al. (2018) revealed that DS significantly suppresses stem and radicle growth in lentils compared to non-stressed conditions, a phenomenon attributed to a decline in physiological and biochemical activities. The regulation of hypocotyl and radicle elongation under DS is largely dependent on fluctuations in turgor pressure within their cells. As water availability decreases, the structural integrity of the cell walls in both the stem and radicle becomes more rigid, thereby restricting their expansion, limiting longitudinal growth, and reducing dry matter accumulation. Findings from Kauser Malik and Azam (2011) further support this, demonstrating that organic fertilizer application enhanced both stem and root growth in *Triticum*. However, root elongation was more significantly influenced by fertilization compared to stem growth, highlighting the differential impact of nutrient availability on plant development under stress conditions.

Fresh weight of hypocotyl

The variance analysis results indicated that hypocotyl fresh weight was significantly influenced by species, DS, and fertilizer application as well as the combined interaction of species, DS, and fertilizer ($P < 0.01$). Additionally, a significant interaction was observed between fertilizer and DS ($P < 0.05$), while the interaction between species and fertilizer did not show a statistically significant effect (Table 1). Among the treatment combinations, the highest hypocotyl fresh weight (0.694 g) was recorded in the Jicama bean species when subjected to fertilizer application under non-drought-stress conditions (control fertilizer treatment). In contrast, the red bean species exhibited the lowest hypocotyl fresh weight (0.073 g) under severe DS (-12 bar) when grown without fertilizer application (control fertilizer treatment) (Fig. 5). Seedlings that demonstrate greater drought tolerance and exhibit enhanced root and shoot development at early growth stages are more likely to withstand DS throughout their lifecycle. Research by Motamednejad et al. (2016) suggests that biological fertilizers play a crucial role on increasing seedling weight. This effect is attributed to the activity of growth-promoting bacteria, which enhance cell proliferation and elongation by producing plant hormones. These processes, in turn, improve water uptake, stimulate overall growth, and contribute to an increase in seedling fresh weight (Motamednejad et al., 2016). Similarly, Armand et al. (2015) reported that organic fertilizers positively influence wheat seedling weight. However, as DS intensifies, the water content available to the plant's initial shoots declines, leading to the reduced seedling growth.

Dry weight of hypocotyl

The analysis revealed that species, DS, and fertilizer as well as the interaction between species and DS had a highly significant effect ($P < 0.01$). Additionally, the combined interaction of species, DS, and fertilizer showed a significant influence ($P < 0.05$) (Table 1). However, no statistically significant effect was observed for the interaction between species, fertilizer, and DS. Among the treatment combinations, the highest hypocotyl dry weight (0.125 g) was recorded in the Jicama bean species when exposed to fertilizer application under non-drought-stress conditions (control drought-stress treatment).
In comparison, the red bean species grown without fertilizer under severe DS (-12 bar) exhibited the lowest hypocotyl dry weight, measuring only 0.014 g (Fig. 6). The significant reduction in seedling stem dry weight under DS conditions can be attributed to the limited nutrient mobility and a decreased transfer of reserves from the cotyledon to the embryonic axis (Rahbarian et al., 2012). Armand et al. (2015) observed that seed treatment with organic fertilizers resulted in a 38% increase in stem dry weight, highlighting the positive impact of organic amendments on seedling growth. Similarly, research by Hosseinzadeh et al. (2015) identified a direct correlation between dry matter accumulation and longitudinal stem growth in drought-resistant plants. This suggests that the observed decline in chickpea seedling stem dry weight under severe DS is closely linked to a reduction in stem elongation at higher stress levels. As DS intensifies, both the length and dry weight of chickpea seedling stems progressively decrease. Deilam et al. (2018) reported a similar trend, demonstrating that seedling stem dry weight diminishes as stress severity increases. This reduction indicates that water scarcity impedes seedling stem elongation by limiting water absorption. It is likely that the decline in dry weight under extreme drought conditions is due to the restricted nutrient transfer from the cotyledons to the embryonic axis. Kaya and Coşkun (2020) further supported this concept, showing that when *Lactuca sativa* and marigold seeds were treated with organic amendments in Petri dishes, their seedling dry and fresh weights significantly increased. This enhancement was attributed to the improved cell elongation and greater water absorption efficiency, reinforcing the beneficial role of organic treatments in mitigating the adverse effects of DS on seedling development.

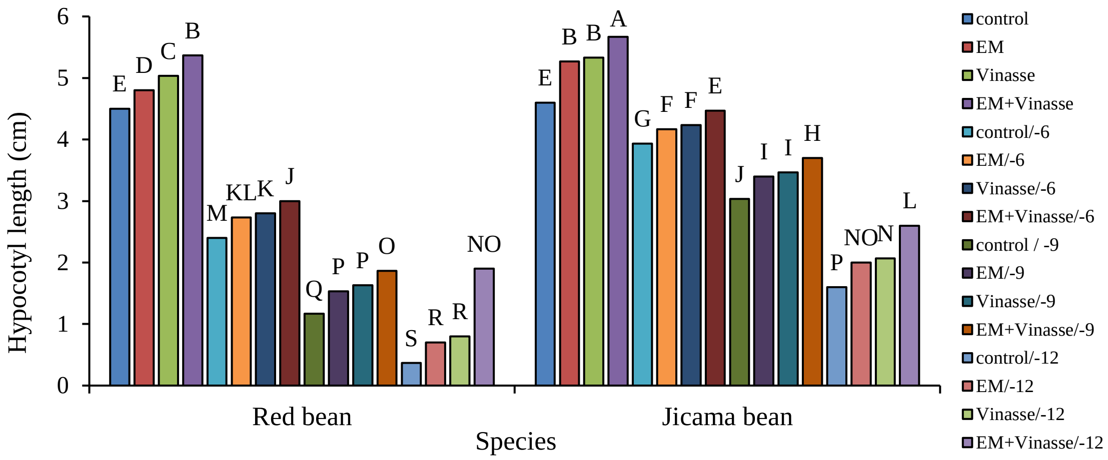

Fig. 4. The effect of species, fertilizer, and drought stress on hypocotyl length of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($P < 0.05$).

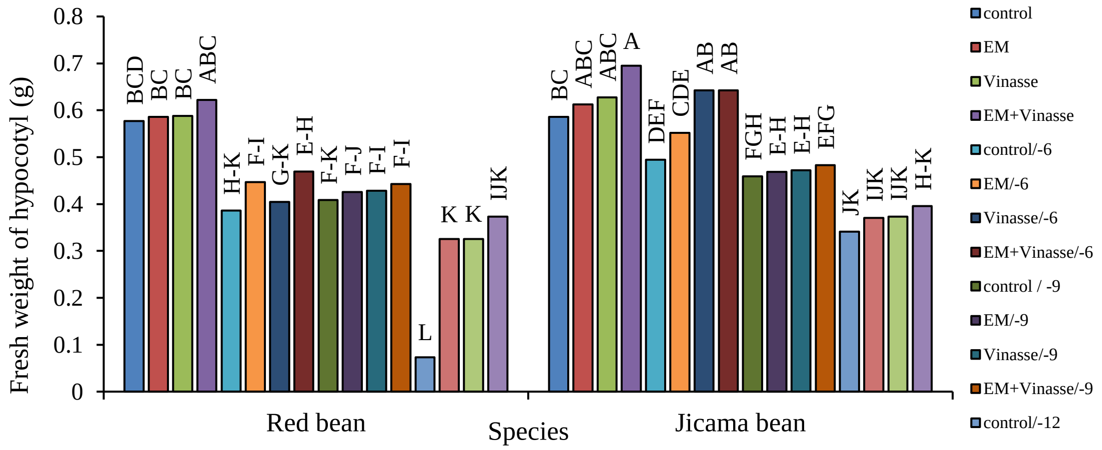

Fig. 5. The effect of species, fertilizer, and drought stress on fresh weight of hypocotyl of Jicama bean and red bean seeds (Control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($P < 0.05$).

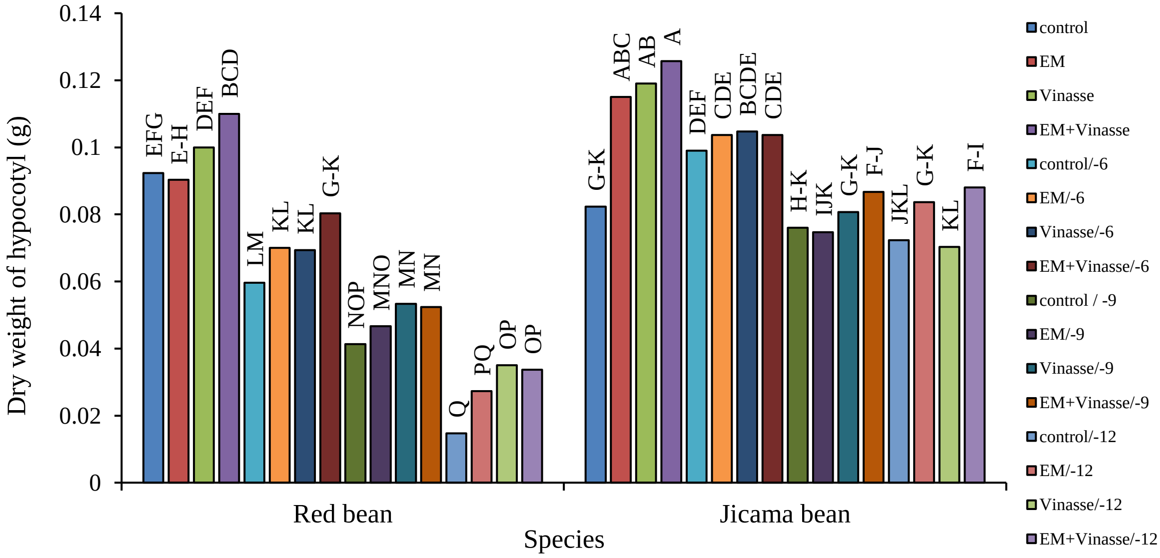

Fig. 6. The effect of species, fertilizer, and drought stress on dry weight of hypocotyl of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($p < 0.05$).

Radicle length

Radicle length was significantly influenced by species, DS, fertilizer application, and their interactions at the 1% probability level (LSD 1%). Additionally, a highly significant difference (P < 0.01) was observed in the interaction between species and fertilizer (Table 1). Among the treatment combinations, the longest radicle (6.333 cm) was recorded in the Jicama bean species under the combined fertilizer application and moderate DS conditions (-9 bar). In contrast, the shortest radicle length (0.533 cm) was observed in the red bean species grown without fertilizer under severe DS (-12 bar) (Fig. 7). The reduction in both seedling stem and root length under mild DS is primarily due to the limited nutrient translocation. However, at higher stress levels, growth suppression is more closely linked to the inadequate transfer of essential growth-promoting substances (Gopal et al., 2010). Furthermore, insufficient water availability reduces hormone secretion and enzyme activity, ultimately hindering root and stem development (Gopal et al., 2010). Taghvaei and Ali Olad (2015) also reported a significant decrease in seedling stem and root length, dry weight of the stem, root, and plant, as well as the seedling vigor index under DS conditions. Meanwhile, research on *Oryza sativa* indicated that growth-promoting bacteria positively influenced the seedling vigor index, root and stem length, and dry weight. The observed decline in root length under various DS levels, compared to the control, may be attributed to the multiple factors, including reduced mitotic activity in the root meristem, diminished enzyme function in germination-related biochemical processes, and impaired water uptake under severe DS conditions (Liga et al., 2003). Organic treatments have been found to enhance germination percentage, germination rate, seedling root, and stem length in wheat under DS, which contributes to the plant establishment, water uptake efficiency, dry matter accumulation, and ultimately, increased yield. The notable improvement in wheat seedling growth following organic treatment is primarily linked to the enhanced water and nutrient absorption. Consequently, the application of these treatments significantly promoted root elongation, whereas the shortest roots were recorded in untreated control seedlings among the measured growth parameters, root length emerged as the most reliable indicator for assessing cultivar responses to DS. This phenomenon is likely a result of increased PEG concentration, which elevates the osmotic potential of the culture medium, thereby restricting water absorption and disrupting normal seedling physiological processes. Similar trends have been observed in lentils, where a decline in water potential led to the reduced germination percentage and rate as well as a decrease in seedling root and stem length (Armand et al., 2015).

Fresh weight of radicle

The analysis of variance revealed that species, DS, fertilizer application, and their interactions had a highly significant impact on radicle fresh weight (P < 0.01) (Table 1). Examination of treatment interactions demonstrated that the Jicama bean species exhibited the highest radicle fresh weight (0.772 g) when subjected to a combination of fertilizer application and non-drought-stress conditions (control drought-stress treatment). In contrast, the lowest fresh weight (0.150 g) was recorded in the red bean species grown without fertilizer (control fertilizer treatment) under severe DS (-12 bar) (Fig. 8). Findings from this study indicated that DS significantly reduced root fresh weight. However, the application of biological and organic fertilizers effectively mitigates this decline, leading to a substantial increase in root biomass even under water-limited conditions. Research by Kaya and Coşkun (2020) further supports this observation, as their study on rapeseed revealed that the highest root fresh weight was obtained when organic fertilizer was applied. Despite this improvement, their study found no statistically significant differences between various fertilizer treatments in terms of root fresh weight (Kaya and Coşkun, 2020).

Dry weight of radicle

The findings indicated that the effects of species, DS, and fertilizer as well as their interactions (specifically species × DS) had a significant impact on the dry weight of the radicle (P < 0.01), while the interaction involving species, DS, and fertilizer was also notable (P < 0.05). However, no significant effect was observed from the interaction between fertilizer, DS, and species with fertilizer (refer to Table 1). The interaction between the various treatments revealed that the Jicama bean species, under conditions with combined and organic fertilizers and without DS (control condition) had the highest dry weight of the radicle. In contrast, the red bean species, without fertilizer under severe DS (-12 bar), exhibited the lowest radicle dry weight, with a significant decrease of 97.55% and 97.56%, respectively (see Fig. 9). When comparing the average dry weight of radicles and stems, both showed a reduction in weight as DS levels increased. However, radicles demonstrated a more pronounced sensitivity to the stress, showing a sharper decline than stems by increasing stress intensity. Importantly, DS was found to significantly reduce the germination rate as well as the length and dry weight of both radicles and stems in marigolds. The results suggested that the reduction in dry weight at high levels of DS could be attributed to a decrease in nutrient transport, specifically from the cotyledons to the embryonic axis. Moreover, at an organic treatment concentration of 400 mg per liter, a significant increase in radicle dry weight was observed. Enzyme activity also rose by 23% to 100%, which contributed to the enhanced root respiration and growth. The use of organic compounds led to the greater radicle growth in comparison with stems, likely due to the improved nutrient access, such as phosphorus (Tabrizi and Koocheki, 2014). In addition, the application of PEG, which limited water availability and induced DS, resulted in a decrease in physiological and metabolic processes during germination, alongside reduced radicle growth and dry weight (Ahmadpour et al., 2015). Consistent with previous research, the present study confirmed that DS led to a significant decline in root-related characteristics.

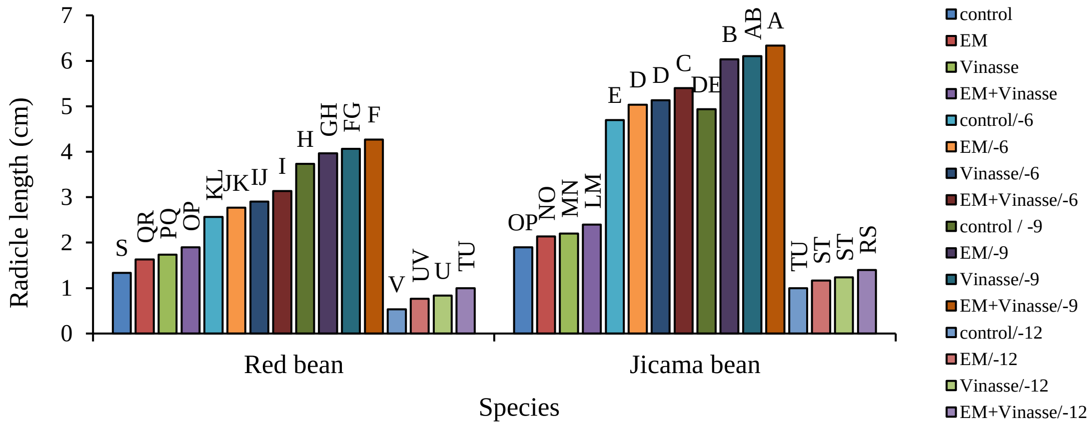

Fig. 7. The effects of species, fertilizer, and drought stress on radicle length of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($P < 0.05$).

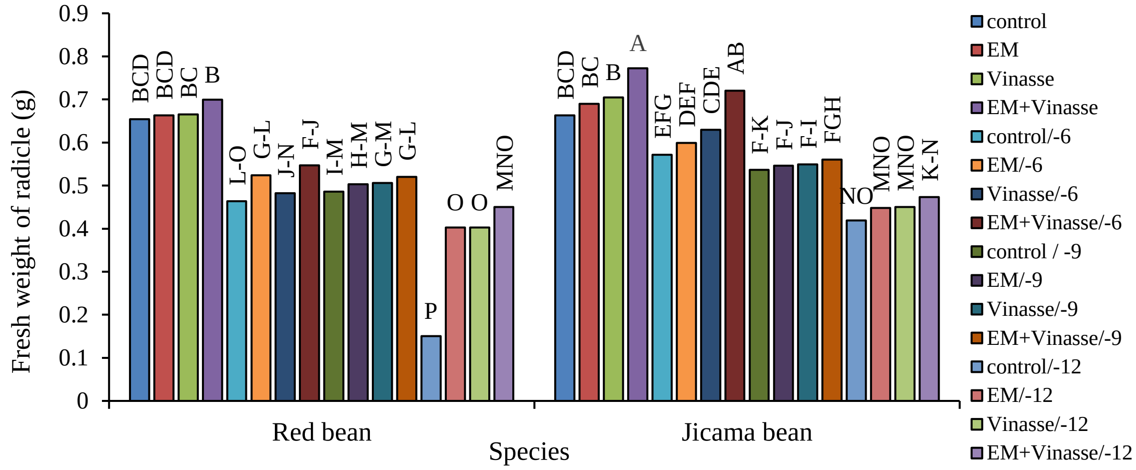

Fig. 8. The effect of species, fertilizer, and drought stress on fresh weight of radicle of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($P < 0.05$).

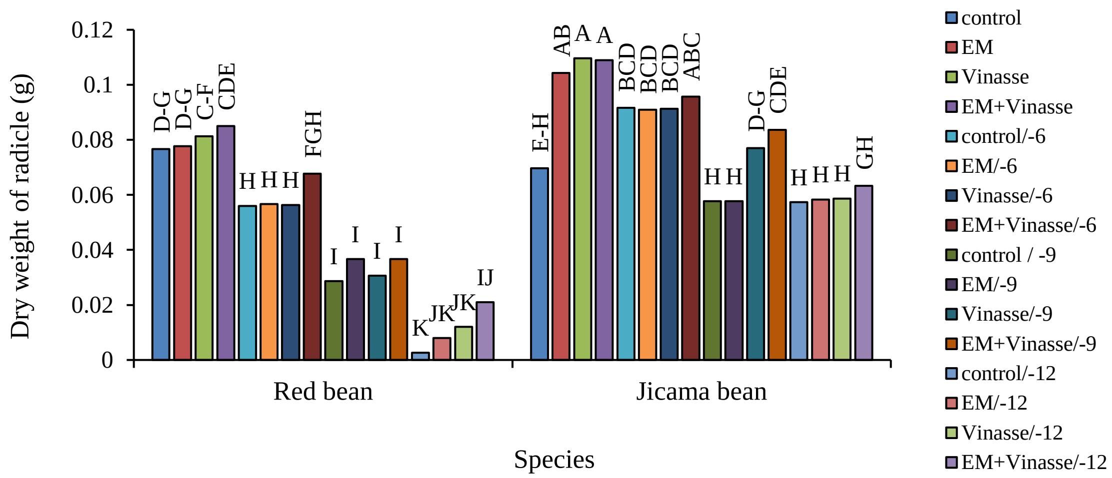

Fig. 9. The effect of species, fertilizer, and drought stress on the dry weight of radicle of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different ($P < 0.05$).

Proline content

The findings revealed that the primary effects of treatments, along with their interactions with proline content were significant (Table 1). As illustrated in Fig. 10, the interaction between treatments demonstrated that Jicama beans exposed to combined fertilizer treatment under severe DS conditions (-12 bar) exhibited the highest proline content (0.317 μg/g fresh weight). Conversely, the lowest proline content (0.019 μg/g fresh weight) was recorded in red bean plants grown without fertilizer and under non-drought-stress conditions. Proline, an amino acid predominantly synthesized in plant leaf tissues during water deficiency, is known to increase as part of an adaptive mechanism for osmotic adjustment under extreme environmental stress (Aziz et al., 2018). The elevated accumulation of proline during severe DS is attributed to its non-oxidation under such conditions. This lack of oxidation allows proline to remain stable and accumulate, aiding plants in maintaining osmotic balance in response to heightened drought-induced osmotic potential (Aziz et al., 2018). The varying responses of plant species to proline accumulation at different stress levels suggest that proline levels are closely linked to a species' drought tolerance. Species with greater resilience to DS can effectively regulate the signaling pathways involved in proline biosynthesis and activate the associated genes (Aziz et al., 2018). According to Selim et al. (2019), the increase in proline levels during DS plays a crucial role in osmotic regulation and in preventing intracellular oxidation. In this study, the use of fertilizer treatments appears to have improved nutrient availability during germination. As a result, the adverse effects of drought-induced osmotic stress were mitigated, subsequently lowering proline accumulation in Jicama bean plants.

Malondialdehyde

The effects of species, DS, and fertilizer treatments, along with their interactions (except for species × fertilizer) as well as the combined influence of these factors on MDA levels, were found to be statistically significant ($P < 0.01$) (Table 1). The data indicated that, under the interaction between treatments, the MDA concentration in Jicama bean plants subjected to combined fertilizer treatment without DS showed a reduction of 62.02% compared to the beans exposed to severe DS (-12 bar) without fertilizer application (Fig. 11). Under stress conditions, plants tend to produce more MDA as part of their response to counteract ROS and minimize potential cellular damage. Stress-induced damage leads to increased cell membrane degradation in leaf tissues, disrupting electron transfer due to the action of free radicals, which in turn causes oxidative damage to membranes and enhances lipid peroxidation. This process prompts the plant to respond by increasing MDA production. Similar patterns of increased lipid peroxidation and elevated MDA levels under stress-induced exhaustion have also been reported in soybeans (Xia et al., 2014). The findings suggest that reduced activity of the catalase enzyme under stress conditions may contribute to the breakdown of cell membranes, which in turn raises the concentration of MDA. This highlights the role of oxidative stress in driving cellular damage and the plant's compensatory response through increased MDA production.

Catalase

Table 1 shows significant effects of species, DS, fertilizer, and their interactions on the catalase enzyme ($P < 0.01$). Their interactions showed no significance, however. The highest catalase level (97.32%) in interaction treatments were obtained by Jicama bean species under combined fertilizer treatments and organic fertilizer under severe DS conditions (-12 bar treatment), and the lowest level (97.16%) was achieved in red bean under no fertilizer application (control fertilizer treatment) without DS (Fig. 12). Under drought and other abiotic stress conditions, catalase activity tends to rise in most plants, although in some instances, it may remain stable or even decrease. Catalase plays a crucial role in breaking down and neutralizing ROS, thereby helping to limit the extent of damage caused by DS (Anjum et al., 2012). Research has shown that enzymes such as catalase and peroxidase are involved in detoxifying ROS, including hydrogen peroxide (H_2O_2) (Anjum et al., 2012). Hydrogen peroxide has a dual role in plant physiology. At moderate levels, it acts as a signaling molecule, contributing to the synthesis of cell wall proteins and regulating various cellular processes. However, when its concentration becomes excessively high, H_2O_2 turns toxic, leading to oxidative stress and cellular damage (Zlatev et al., 2012). In the present study, the use of nutrient-rich fertilizer treatments combined with the beneficial effects of microorganisms present in biofertilizers appears to have alleviated DS in beans. By creating more favorable growth conditions, these treatments likely reduced the plant's need to produce defensive enzymes, leading to a decrease in catalase activity.

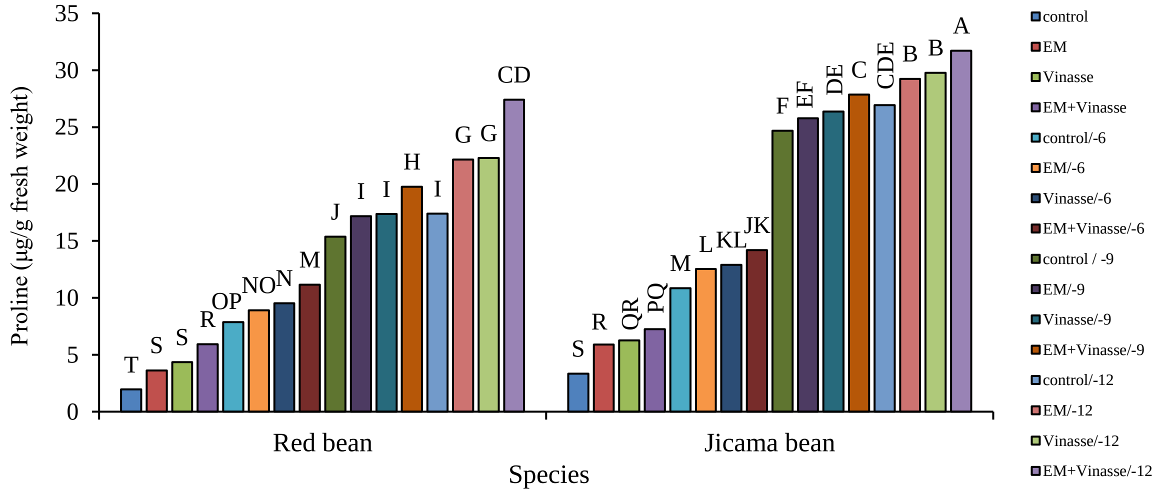

Fig. 10. Comparison of mean values of species, fertilizer, and drought-stress interaction effect on proline in Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

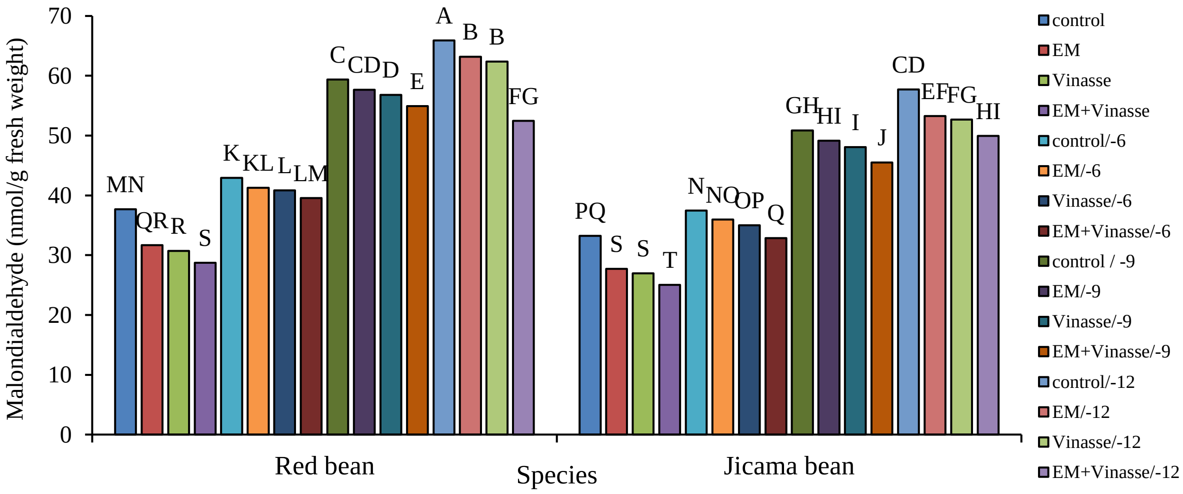

Fig. 11. The effects of species, fertilizer, and drought stress on Malondialdehyde of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

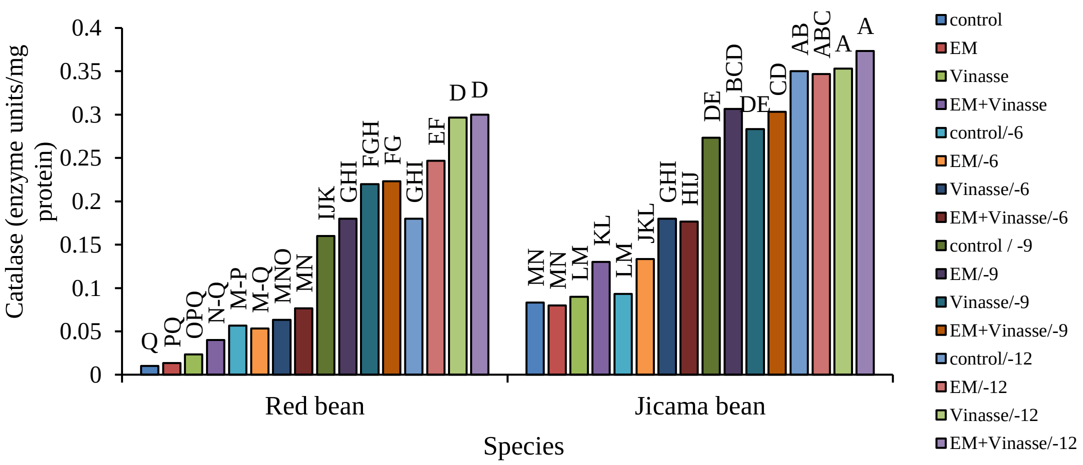

Fig. 12. The effect of species, fertilizer, and drought stress on catalase of Jicama bean and red bean seeds (control: no fertilizer, EM at optimal level, Vinasse at optimal level, and a combination of EM and Vinasse at optimal level. Drought stress at four osmotic potential levels of 0, -3, -6, and -12 bars). Columns with similar letters are not significantly different (*P* < 0.05).

CONCLUSION

The fertilizer treatments used in this study, which contained a rich blend of macro and microelements, organic matter, and beneficial microorganisms, significantly enhanced germination percentage, germination vigor, and seedling vigor index compared to the untreated control. The results demonstrated that both Jicama bean and red bean species exhibited strong germination performance and high vigor. However, the two species responded differently to the DS, with Jicama bean showing greater tolerance than red bean. Under drought conditions, Jicama bean maintained a higher germination percentage, highlighting its superior adaptability to stress. With an increasing interest in expanding bean cultivation and introducing new legume varieties in Iran, the use of EM biofertilizers and Vinasse organic fertilizers could play a crucial role on improving germination rates, enhancing seedling establishment, and strengthening plant growth traits. This strategy would not only increase the plants' ability to withstand stress but also contribute to higher crop yields.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Marzieh Hassani, Mahmoud Reza Tadayon, and Majid Olia; Methodology: Marzieh Hassani and Mahmoud Reza Tadayon; Software: Marzieh Hassani and Mahmoud Reza Tadayon; Validation: Marzieh Hassani; Formal analysis: Marzieh Hassani and Mahmoud Reza Tadayon; Investigation: Marzieh Hassani, Mahmoud Reza Tadayon, and Majid Olia; Resources: Marzieh Hassani; Data curation: Marzieh Hassani and Mahmoud Reza Tadayon; Writing—original draft preparation: Marzieh Hassani and Mahmoud Reza Tadayon; Writing—review and editing: Marzieh Hassani, Mahmoud Reza Tadayon, and Majid Olia; Visualization: Marzieh Hassani and Mahmoud Reza Tadayon; Supervision: Marzieh Hassani, Mahmoud Reza Tadayon, and Majid Olia; Project administration: Marzieh Hassani; Funding acquisition: Marzieh Hassani, Mahmoud Reza Tadayon, and Majid Olia.

DECLARATION OF COMPETING INTEREST

The author discloses no apparent personal relationships or financial ties that could have biased the research results.

ETHICAL STATEMENT

This work is not related to experimental animals or specific human diseases that requires publication and approval of publication ethics.

DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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