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Original Research

Counteracting Heat, Salinity, and Osmotic Stresses by Reconciling Seed Size and Sowing Depth for Bolstering Germination and Seedling Growth of Cluster Bean (*Cyamposis tetragonoloba* L. Taub.)

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Abstract

Under abiotic stresses (AS, heat, salinity, and osmotic stresses), seed germination and seedling growth of legumes like cluster beans are critical phases of the crop growth cycle that determine their productivity. Research gaps exist regarding the impacts of seed size, sowing depth, and soil pH on the germination, root, and shoot growth attributes of cluster beans when exposed to AS. Five different trials were executed to assess the comparative performance of cluster bean seed sizes (small, medium, and large) in response to different regimes of temperature (T, 10, 20, and 30°C), salinity (SS, 50, 100, 150, and 200 mM), osmotic stress (OS, 0.2, 0.4, 0.6, 0.8, and 1.0 Mpa), soil pH (5, 6, 7, 8, 9, and 10), and sowing depths (SD, 0, 2, 4, 6, and 8 cm). The response variables included germination (GP), germination index (GI), and time taken to 50% germination (TG), along with length, fresh, and dry weights of the shoot and root of the cluster bean. The results exhibited that large-size seeds sown at 4 cm depth remained unmatched by recording the maximum GP and GI up to 20-30°C temperature, 0-50 mM salinity level, 0-0.4 Mpa osmotic stress, and 6-7 pH. The same range of employed treatments also remained effective in a pronounced reduction of time taken to the TG. Moreover, cluster bean large-size seeds sown in 4 cm depth depicted the maximum root and shoot attributes, whereas smaller seed sizes sown in 0, 6, and 8 depths when exposed to 10°C temperature, 100-200 mM SS levels, soil pH (5, 9, and 10), and 0.6, 0.8, and 1.0 Mpa of OS recorded significantly reduced shoot and root traits. Thus,

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these findings reveal the feasibility of alleviating moderate levels of AS for cluster beans with large seed sizes and sowing depth reconciliation under semi-arid conditions.

Keywords: guar, germination index, legumes, environmental stresses, drought

Introduction

Cluster bean, commonly known as guar (*Cyamposis tetragonoloba* L. Taub.), is an annual summer leguminous crop belonging to the family of Leguminosae (Fabaceae) [1]. It is believed to have originated in the Indo-Pakistan sub-continent region [2] and is mostly grown in arid and semi-arid zones of India, Pakistan, and tropical and sub-tropical regions of Africa and North America [3]. Major exporters of guar are India (80%) and Pakistan (15%), followed by Sudan and the United States. Recently, its demand has persistently increased in developed countries due to its industrial uses [4], such as in gum preparation (utilized as a stabilizer, emulsifier, and thickener in food and feed products) [5]. Additionally, residues that remain after the extraction of gum are known as guar meal, which serves as protein-rich (33-60%) feed for dairy animals [6]. Moreover, guar is being used as a vegetable and grain for human consumption, along with fodder for animals and a green manure crop for boosting soil fertility status sustainably [7].

However, guar yield has remained below par in the Indo-Pakistan sub-continent owing to poor germination, sub-optimal seedling growth, and frequent incidences of abiotic stresses, which has necessitated finding biologically feasible strategies to bolster its germination and seedling growth. Previously, larger seeds (LS) and sowing depth adjustment (1 cm) performed better than smaller ones and produced significantly higher yields by boosting germination rate, root weight, and seedling growth in *Brassica napus* [8]. Additionally, the LS recorded better crop establishment and a higher seedling survival rate under stressful environmental conditions [9]. Likewise, the LS sown in 1 cm depth produced better crop stand and more vigorous seedlings, while seed vigor was directly associated with seed size in rice [10]. Moreover, LS produced healthy seedlings because of immense storage potential and high food reserves, as seed germination, seedling length, and biomass were pronouncedly increased when sowing depths of 4 cm and 2 cm were maintained for *B. retusa* and *B. variegata*, respectively [11]. However, research findings are scarce pertaining to seed size association with germination and seedling growth of cluster beans under semi-arid conditions.

Globally, environmental stresses, particularly elevated temperature, have led to a frequent incidence of heat stress (HS), which directly affects the vital metabolic processes by preventing gene expression leading to oxidative stress in crop plants [12]. Moreover, the HS disrupted many vital metabolic processes and enzymatic activities associated with the regulation of seed germination, which resulted in poor crop establishment and significantly reduced crop yield [13]. Besides HS, salinity stress (SS) has recently emerged as one of the most daunting constraints, which resulted in osmotic pressure buildup and drastically reduced water uptake by seeds, resultantly, the seed imbibition process was delayed, leading to a significant reduction in seed germination and seedling growth [14, 15]. In addition, the SS, by virtue of excessive sodium and chloride ions, imparted toxic effects and disrupted morphological, biochemical, and physiological processes (photosynthesis, glycolysis, partitioning of assimilates, etc.) of crop plants [16]. Along with HS and SS, global warming has also resulted in the emergence of drought or osmotic stress (OS) that has pronouncedly deteriorated soil fertility status and imparted deleterious effects, particularly at the early growth stages of crop plants [17, 18]. Moreover, OS also reduced seedling establishment and caused a significant decline in the economic yield of crops owing to unsynchronized seedling growth [19-21].

The frequent incidence of abiotic stresses, rapidly increasing human population, and decreasing land area owing to uncontrolled urbanization have necessitated finding biologically viable, pro-environment, and farmer-friendly agronomic strategies. Adjustments in sowing depth and seed size might be developed as potent strategies to cope with the deleterious effects of abiotic stresses. Previously, it was affirmed that deep sowing caused a pronounced reduction in seed germination and seedling emergence [22]. Contrastingly, it was also inferred that, owing to moisture deficiency in the upper soil layer, shallow seed sowing also resulted in sub-optimal seed germination and restricted seedling growth. Deep planting delayed seedling's emergence, and young seedlings were exposed to pathogen attack [23], whereas Chachalis and Reddy [24] also demonstrated that seedling emergence was decreased with an increase in seeding depth. Furthermore, soil pH has also been reported to influence seed germination and seedling growth processes like nutrient availability, solubility, and microorganism activity. Moreover, the depth of seed sowing influenced the availability of oxygen, moisture content, and microbial activity, which imparted significant impacts on germination, root development, and seedling growth [25]. Although several studies have studied the impact of seed depth or seed size on germination and seedling growth of crop plants, pronounced research gaps have existed pertaining to the mitigation of abiotic stresses with adjustments of sowing depth, seed size, and soil pH for cluster beans.

Therefore, to bridge the research gaps, a comprehensive study entailing five trials was designed with a research hypothesis that seed size, sowing depth, and soil pH could significantly influence the germination rate and seedling growth traits of cluster beans by neutralizing the deleterious effects of abiotic stresses. Thus, the prime aims of the study were to delve into the impacts of heat, osmotic stress, soil pH, and saline environment on seed germination and seedling growth traits of cluster beans.

Experimental

Experimental Description

Five trials were executed at the Microbiology Lab of the Agronomy Department, University of Agriculture Faisalabad, Pakistan, during 2019-2020. Seeds of guar (cv. BR-17) were placed in each Petri plate (9 cm diameter) having three layers of Whatman No. 1 filter paper and replicated thrice. Before each trial, seeds were soaked for 5 minutes in 0.5% NaClO (sodium hypochlorite) solution and then washed with distilled water thoroughly (by repeating the procedure thrice to minimize the residual chlorine) to avoid fungal attack and allowed to dry at room temperature for 5 hrs, before placing those at the filter papers. According to the requirement, the filter papers were moistened regularly with deionized water using a pipette. For sowing depth treatments, plastic pots were filled with soil, and germination was noted for three weeks. The humidity (40%) and constant fluorescent light (400-700 nm and 100 μmol m^{-2} s^{-1}) conditions were kept similar for all treatments in five trials of cluster bean.

Imposition of Abiotic Stresses and pH Level Adjustments

For imposing HS, different levels of constant temperature (T, 10, 20, and 30°C) were maintained to examine its effect on seed germination in a growth chamber (ICO105, Memmert GmbH+ Co. KG, Schwabach, Germany). Data from each trial were recorded daily for two weeks except for sowing depth treatments. Seeds were considered to have germinated when radical protrusion reached up to 2 mm. For seed size categorization, guar seeds were classified by visual observation and then based on 1000-grain weight. In each Petri plate (9 cm), 9 guar seeds were placed according to their sizes (small, medium, and large) with three replicates and incubated in a growth chamber at all the above levels separately.

For imposing salinity stress treatments, salt solutions were prepared by adding 1 molar solution of NaCl (58.5 g molecular mass of NaCl) in 1000 ml of distilled water in accordance with [26].

$$\text{Salinity level} = \frac{\text{Molecular mass}}{1000} \times 100$$

So, in this way, 2.92, 5.85, 8.77, and 11.7 grams of NaCl were dissolved in 1000 ml of distilled water to obtain 50, 100, 150, and 200 mM salt solutions. Different-sized seeds of guar (9 per Petri plate) were treated with all these above solutions. Initially, Petri plates were treated with 5 ml of each solution (50, 100, 150, and 200 mM) separately, but further concentrations of these solutions were applied according to requirement. Petri plates were kept in the incubator at 27 °C temperature.

For imposing the osmotic stress treatments, different osmotic solutions were prepared as suggested by [27].

$$\text{Water potential} = -(1.18\times10^{-2})\times C - (1.18\times10^{-4})\times C + (2.67\times10^{-4})\times C\times T + (8.39\times10^{-7})\times C^2T$$

Where C = concentration of PEG in g/kg of distilled water and T = temperature (°C)

These solutions were prepared by using PEG 6000, and the experiment was conducted in a growth chamber. In the control treatment, distilled water was used, whereas osmotic stress was imposed using different concentrations of PEG (0.028, 0.045, 0.058, 0.069, and 0.079 g dissolved in 1000 ml of water) for imposing stress levels of -0.2, -0.4, -0.6, -0.8, and -1.0 Mpa, respectively.

To assess the impact of different pH levels, these were adjusted by using different solutions, such as pH_5 and pH_6 solutions of 2 mM MES [2-(N- morpholino) ethanesulfonic acid], which was adjusted by using a 0.1 N solution of sodium hydroxide. For making pH_7 and pH_8 solutions, HEPES [N-(2-hydroxymethyl) piperazine–N–(2-ethane sulfonic acid) solution was adjusted by a 0.1 N of sodium hydroxide solution. Tricine was adjusted by 0.1N sodium hydroxide solution for pH levels of 9 and 10 [24].

Sowing Depth Treatments

Sowing depth treatments were comprised of 0, 2, 4, 6, and 8 cm depth, wherein control treatment (0 cm) was implied by placing seeds on the soil surface. Twenty guar seeds were placed in plastic pots (15 cm), which were filled with soil (40% sand, 30% silt, and 30% clay), and seeds were sown according to the treatments with the help of a wooden scale. Distilled water was provided (by maintaining sufficient soil moisture level at different depths by pulverizing the soil with an iron rod and ensuring water application until it started to leak out from the pot's bottom hole) to pots according to their requirements and seeds were considered to have germinated by the visibility of plumule (2 mm).

Response Variable Recordings

Germination (G, %) was assessed by using the formula described by Iqbal [28].

$$GP = \frac{\text{Germinated seeds} \times 100}{\text{Total seeds}}$$

Seed germination was recorded daily to determine the germination index (GI) as suggested by Iqbal [7].

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds}}{\text{Days of second count}} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Mean germination time was also estimated by following the equation reported by Ali et al. [29].

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

Where D=number of days from the start of germination/emergence and n=number of seeds germinated/ emerged on day D.

Moreover, T_{50} was calculated according to the formula given by Farooq et al. [30].

$$T_{50} = t_i + \frac{(N/2 - n_i)(t_j - t_i)}{(n_j - n_i)}$$

t_j and t_i represent the adjacent times. N denotes the final germinated seed, while n_i and n_j represent the cumulative number of germinated seeds by contiguous counts at time t_j and ti, where $n_i < N/2 < n_j$.

Statistical Analyses

The collected data of all response variables of guar were arranged and subjected to a one-way Fisher's analysis of variance (ANOVA) technique for estimating the overall significance of employed treatments with the help of the computer-run statistical package Statistix (8.1 version). Thereafter, the least significant difference (LSD) test at the 5% probability level was employed to sort out the significance among the treatment means [31].

Results and Discussion

Temperature and Seed Sizes Interactive Effects

The recorded findings revealed that different sizes of cluster bean seeds and different temperature regimes interacted significantly to influence germination and seedling growth (Table 1). The maximum germination (100%) was recorded at 20-30°C for all sized seeds, but at 10°C, small-sized seeds showed a significant reduction in final germination (70%). Likewise, the germination index and time to 50% germination were also significantly affected under different temperatures and with seed sizes, but at 20°C, the final germination and germination index were increased. Thus, these findings showed that any increase and decrease in temperature from 20°C and decrease in seed size led to a significant increase in the germination time and time taken to 50% germination. For all seedling parameters under investigation, the maximum root and shoot length (4.7 and 7.3 cm, respectively) were recorded by the large seeds when incubated at 20°C, and the minimum (1.2, 2.3 cm) corresponding values were demonstrated by small-sized seeds at 10°C. Moreover, the maximum root, shoot fresh, and dry weights were also observed for large seeds incubated at 20°C. These findings were also correlated with previous studies whereby a significant influence of temperature was noted on seed germination and seedling growth [32, 33]. Gresta et al. [34] concluded the same results, as the mean germination time was pronouncedly increased with fluctuations in the optimum temperature. Likewise, these results were in accordance with EL-Abady [35], who noted the significant impact of temperature alterations on the seedling growth traits of maize. Chaturvedi et al. [13] observed that temperature regulated the germination process by influencing many metabolic processes (enzymatic activity). Moreover, it was also demonstrated that many catabolic activities were inhibited by chilling stress, whereas elevated temperature led to the inactivation of many enzymes and the denaturation of many vital proteins [36]. Similar to our findings, Al Khteeb [26] inferred that germination rate and seedling parameters of *panicum turgidum* were significantly enhanced at the optimum temperature of 20-30°C, whereas higher temperatures caused a significant reduction in germination indices and seedling growth traits.

Salinity Levels and Seed Sizes Interactive Effects

Germination and seedling growth of different-sized seeds of guar were evaluated under various salinity levels (SS, control, 50, 100, 150, and 200 mM), and the results exhibited that an increase in SS and a decrease in seed size led to a pronounced decrease in the germination of guar (Table 2). Small seeds showed tolerance to SS of 50 mM. Mean germination time and time to 50% germination were increased with the increase in SS coupled with a decrease in seed size. Small-sized seeds showed the lowest germination index when exposed to the 200 mM salt solution, and the highest germination index was observed in large-sized seeds when treated with distilled water (control) up to 100 mM salt solution. In terms of seedling growth traits under investigation, large-sized seeds performed better even when exposed to the 100 mM salt solution. Likewise, the large-sized seeds depicted the highest root

Table 1. The interactive impact of different seed sizes and temperature regimes on germination and seedling growth traits of cluster bean.

Temperature regimes × seed size	Germination (%)	Germination index	Time to 50% germination (days)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Small×10°C	70.00 d	3.43 g	4.44 a	2.31 h	0.75 e	0.14 c	1.27 f	0.74 d	0.14 d
Small×20°C	75.00 e	12.03 d	4.18 b	4.32 e	1.77 cd	0.15 c	2.25 c	0.85 cd	0.17 cd
Small×30°C	78.00 f	5.93 f	4.29 b	3.17 g	1.36 d	0.14 c	1.68 e	0.87 cd	0.38 cd
Medium×10°C	85.00 c	8.42 e	3.81 c	3.53 f	0.78 e	0.15 c	1.78 d	0.93 c	0.08 d
Medium×20°C	86.67 bc	17.51 b	3.48 d	6.17 c	2.13 c	1.06 b	3.81 b	1.16 b	1.02 b
Medium×30°C	88.33 b	11.82 d	3.59 d	5.36 d	1.65 cd	0.46 c	2.16 cd	1.19 b	1.14 b
Large ×10°C	86.00 bc	14.05 c	2.88 e	5.17 d	1.36 d	0.41 c	2.30 c	1.25 bc	0.74 bc
Large ×20°C	93.00 a	19.27 a	2.31 f	7.32 a	3.88 a	2.15 a	4.75 a	3.04 a	2.06 a
Large ×30°C	95.00 a	18.83 a	2.41 f	6.56 b	3.21 b	2.07 a	3.76 b	3.01 a	1.79 a
LSD at 5%	2.83	1.15	0.13	0.23	0.56	0.43	0.49	0.69	0.57

Note: Values having different letters within the same column vary significantly at 5% probability level.

and shoot length (8.9, 7.6 cm) as compared with small and medium-sized seeds. Under high SS levels, seedling fresh and dry weights were greatly reduced, particularly in small-sized seeds. These results were contradictory to Kaya et al. [37], who reported that larger seeds of chickpea acquired the highest mean germination time under all SS levels; however, Teolis et al. [38] noted that guar seeds showed variations in germination under various SS levels. Chauhan and Johnson [39] also recorded similar results in African mustard and inferred that the reduction in germination under high SS levels might be due to the toxic effects of accumulated salts, which reduced the uptake of water and nutrients. Like our findings, higher levels of salt ions caused a reduction in the germination of okra and suppressed cell division, causing inhibition in growth [40]. The same findings were reported by [41] in shallot, whereby a saline environment restricted moisture and nutrient uptake, and growth was pronouncedly restricted. Moreover, SS seriously reduced plant traits, while hydrolyzed gellan gum reduced the deleterious effects of a saline environment [42].

Osmotic Stress and Seed Sizes Interactive Effects

Germination and seedling growth of different-sized seeds of guar were evaluated under different osmotic stresses (OS, control, -0.2, -0.4, 0.6, -0.8, and -1.0 Mpa), and the results exhibited that large-sized seeds performed much better under all osmotic stresses as compared to medium and small-sized seeds (Table 3). The maximum germination was recorded in all-sized seeds in the control treatment that supplied the distilled water, while the lowest germination (25%) was recorded by small seeds when exposed to the -1.0 Mpa solution. Likewise, the GI was also reduced in small-sized seeds exposed to a higher osmotic stress level (-1.0 Mpa). Likewise, the mean germination time and time taken to 50% germination were significantly increased with higher levels of OS, whereas these parameters were also reduced with a decrease in seed size (large to small). Seedling parameters were also reduced under high OS, particularly in small-sized seeds. The maximum root and shoot length were recorded in large-sized seeds in all osmotic stress levels as compared with medium and small-sized seeds. Moreover, seedling fresh and dry weights recorded a significant reduction with the increase in OS, especially the small-sized seeds that were not able to develop their seedling at -1.0 Mpa solution. These findings agreed with those of Mut and Akay [43], who reported that large-size seeds effectively offset the deleterious effects of osmotic stress owing to being more vigorous in nature and resulting in a pronouncedly greater germination percentage. Likewise, in line with our findings, previous results have been reported by Pratiwi et al. [44] in shallot (*Allium ascalonicum* L.) and triticale [45]. Muscolo et al. [46] described that root length and germination percentage recorded a pronounced reduction under

Table 2. The interactive impact of different seed sizes and salinity levels on germination and seedling growth traits of cluster bean.

Salinity levels × seed size	Germination (%)	Germination index	Time taken to 50% germination (days)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Small× Control	89.3 b	9.26 c	5.92 e	5.64 g	3.70 b	1.84 c	4.27 c	2.83 c	0.84 c
Small×50 mM	77.0 de	7.04 d	6.33 d	5.38 gh	2.45 de	1.82 c	4.08 c	2.72 d	0.81 cd
Small×100 mM	74.6 e	5.80 e	6.81 c	5.22 gh	2.35 de	1.58 d	3.95 c	2.69 d	0.77 d
Small×150 mM	70.1 f	4.41 f	7.11 b	5.11 h	2.19 f	1.49 e	2.75 d	2.43 e	0.69 e
Small×200 mM	63.9 g	3.65 f	7.69 a	4.42 i	2.01 f	1.23 f	1.93 e	2.26 f	0.67 e
Medium × Control	91.0 b	11.87 b	5.87 e	7.59 c	3.69 b	1.98 b	6.21 b	2.84 c	0.93 b
Medium ×50 mM	88.6 c	11.40 b	5.92 e	7.35 cd	2.69 d	1.84 c	6.09 b	2.82 c	0.91 bc
Medium×100 mM	83.3 d	11.50 b	6.00 e	7.25 cde	2.14 f	1.76 cd	5.87 b	2.65 d	0.86 c
Medium×150 mM	80.1 de	11.09 b	7.09 b	6.77 ef	2.10 f	1.59 d	5.71 b	2.49 e	0.84 c
Medium ×200 mM	78.4 e	7.69 d	6.29 d	7.01 def	2.17 f	1.54 de	5.48 b	2.44 e	0.80 d
Large × Control	94 a	13.58 a	4.44 h	8.94 a	3.98 a	2.09 a	7.66 a	2.98 a	1.13 a
Large ×50 mM	93.3 a	13.14 a	4.69 h	8.74 a	3.70 b	2.06 ab	7.53 a	2.95 b	1.09 a
Large ×100 mM	90.9 b	13.07 a	5.13 f	8.63 ab	3.15 c	1.96 b	7.40 a	2.86 c	1.03 ab
Large ×150 mM	87.3 c	11.88 b	5.82 g	8.17 b	2.73 d	1.87 c	6.20 b	2.73 d	0.96 b
Large ×200 mM	84.0 d	7.70 d	5.98 g	6.71 f	1.87 e	1.61 d	6.06 b	2.69 d	0.91 bc
LSD at 5%	2.86	1.02	0.48	0.52	0.39	0.24	0.79	0.13	0.22

Note: Values having different letters within the same column vary significantly at 5% probability level.

Table 3. The interactive impact of different seed sizes and osmatic stress levels on germination and seedling growth traits of cluster bean.

Osmatic stress levels × seed size	Germination (%)	Germination index	Time to 50% germination (days)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Small× Control	100.00 a	10.36 g	5.05 de	7.40 b	2.79 cd	1.71 bcde	3.91 f	1.80 efg	0.92efg
Small×-0.2 Mpa	95.00 abc	6.35 h	5.36 cd	7.38 b	2.61 de	1.24 ef	3.70 f	1.55 fg	0.77 fg
Small×-0.4 Mpa	83.33 abcd	5.16 i	5.77 c	6.75 e	2.35 def	0.48 ghij	2.58 g	1.25 gh	0.67 g
Small×-0.6 Mpa	78.33 cde	4.74 i	6.38 b	6.71 e	1.19 gh	0.58 ghi	2.07 gh	0.67 hi	0.15 h
Small×-0.8 Mpa	68.33 de	2.72 k	6.69 b	5.98 f	0.75 h	0.42 hij	1.57 h	0.06 i	0.03 h
Small×-1.0 Mpa	25.00 f	1.45 l	7.48 a	5.11 g	0.00 i	0.00 j	0.33 i	0.00 i	0.00 h
Medium× Control	100.00 a	12.55 cd	4.15 g	7.48 b	2.76 de	1.87 abc	5.78 bcd	2.52 cde	1.68 ab
Medium×-0.2 Mpa	93.33 abc	12.00 de	4.48 fg	7.45 b	2.59 de	1.77 bcde	5.57 cd	2.31 cdef	1.26 cde
Medium×-0.4 Mpa	90.00 abc	11.41 ef	4.81 ef	7.31 c	2.31 ef	1.29 def	5.03 de	1.88 defg	1.15 def
Medium× -0.6 Mpa	81.67 bcd	11.07 fg	4.97 def	7.16 d	2.05 f	2.05 f	4.09 f	1.70 fg	1.02 efg
Medium×-0.8 Mpa	81.67 bcd	3.72 j	5.05 de	6.83 e	1.35 g	0.96 fgh	4.39 ef	1.87 efg	0.94 efg
Medium×-1.0 Mpa	63.33 e	2.03 kl	5.48 cd	6.14 e	0.12 i	0.06 ij	2.03 gh	0.42 i	0.04 h
Large× control	100.00 a	13.77 a	2.15 j	7.65 a	3.73 a	2.36 a	7.57 a	3.93 a	2.05 a
Large×-0.2 Mpa	98.33 ab	13.41 ab	2.70 i	7.60 a	3.41 ab	1.85 abcd	7.42 a	3.73 ab	1.98 a
Large×-0.4 Mpa	96.67 ab	13.00 bc	2.99 hi	7.51 b	3.24 bc	2.11 ab	6.15 bc	3.49ab	1.57 bc
Large×-0.6 Mpa	88.33 abc	2.72 k	3.31 h	7.38 c	2.67 de	1.25 ef	6.50 b	3.02 bc	1.42 bcd
Large×-0.8 Mpa	90.00 abc	12.25 cd	3.02 hi	7.36 c	2.64 de	1.04 fg	6.00 bc	2.64 cd	1.30 bcde
Large×-1.0 Mpa	66.67 de	2.72 k	3.37 h	7.21 d	1.45 g	0.22 ij	2.44 g	0.56 hi	0.24 h
LSD at 5%	2.955	1.39	0.55	0.43	0.47	0.56	0.77	0.75	0.39

Note: Values having different letters within the same column vary significantly at 5% probability level.

Table 4. The interactive impact of different seed sizes and pH levels on germination and seedling growth traits of cluster bean.

pH levels × seed size	Germination (%)	Germination index	Time to 50% germination (days)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Small× 5	60.00 f	5.22ij	4.93 f	5.18 g	1.52 g	0.82 ef	2.33 jk	0.93 ef	0.59 f
Small× 6	73.33 e	5.55 i	4.74 e	5.56 f	2.35 ef	1.24 cdef	3.30 hi	0.99 def	0.57 fg
Small× 7	98.33 a	10.50g	3.69 b	7.28 b	2.70 de	1.40 cde	4.32 fg	1.77 c	1.39 cde
Small× 8	93.30 abc	6.47 h	3. 71 b	7.25 b	2.59 de	0.90 def	3.78 ghi	1.59 cd	1.05 e
Small× 9	51.66 fg	2.84l	3.98 c	6.76 d	1.23 g	0.74 f	2.22 jk	0.45 fgh	0.22 gh
Small× 10	20.00 h	0.97 m	4.09 c	6.17 e	0.00 i	0.00g	0.00	0.00 h	0.00 h
Medium×5	81.66 cde	11.55f	4.60 d	6.23 e	1.45 g	0.82 ef	3.89 fgh	0.98 def	0.65 f
Medium×6	88.33 abcd	12.22 def	4.71 e	6.78 d	2.94 d	0.92def	5.49 de	1.54 cde	1.25 de
Medium×7	98.33 a	12.09 ef	3.84 a	7.26 b	3.74 bc	1.87 abc	6.52 bc	2.85 b	1.45 bcd
Medium×8	95.00 ab	12.86bcd	4.00 c	7.19 b	3.56 c	1.51 abcd	5.97 cd	2.63 b	1.32 cde
Medium×9	73.33 e	3.76 k	4.61 d	7.04 c	1.23 g	0.95def	3.01 ij	0.78 fg	0.30 fgh
Medium×10	46.66 g	3.27kl	4.78 e	6.83 d	0.12 hi	0.08g	1.68 k	0.08 h	0.06 h
Large×5	85.00 bcde	12.94 bc	4.00 c	6.91 d	2.94 d	1.48 bcd	5.23 de	1.78 c	0.63 f
Large×6	91.66 abc	12.55 cde	3.64 bc	7.09 c	3.24 bc	2.14 a	6.87 ab	2.81 b	1.67 abc
Large×7	98.33 a	13.83 a	3.88 a	7.58 a	3.89 a	2.10 ab	7.54 a	3.73 a	1.82 a
Large×8	96.66 ab	13.44 ab	3.71 b	7.51 a	3.81 a	0.88def	7.39 a	2.72 b	1.78 ab
Large×9	78.33 de	4.72 j	3.67bc	7.33 b	2.01 f	0.62 fg	4.68 ef	1.67 c	0.58 f
Large×10	76.66 de	3.65 k	4.05 c	7.12 c	0.45 h	0.62fg	1.75 k	0.23 gh	0.16 h
LSD at 5%	2.93	0.71	0.23	0.37	0.40	0.64	0.84	0.64	0.35

Note: Values having different letters within the same column vary significantly at 5% probability level.

OS in all lentil genotypes because the germination process involved several enzymes, while osmotic stress disrupted the activities of enzymes like glucosidase and amylase. Contrastingly, the OS inhibited germination by restricting the seed imbibition process due to a significant reduction in water uptake [18]. Ghorbani et al. [47] also observed that different morphological traits of guar were reduced under osmotic stress. Mujtaba et al. [48] and Khayatnezhad et al. [49] inferred that no wheat seedling showed survival at the OS level of -1.0 Mpa, and it also caused a significant reduction in seedling growth of maize. In agreement with our findings, several other studies have also reported the deleterious effects of OS on germination and seedling growth in many field crops like maize, rice, and camelina [50-52]. The underlying reason was the osmotic stress-driven injury in mitochondria and chloroplast that resulted in the overproduction of reactive oxygen species (ROS), and growth was hampered in *Melilotus albus* [53]. Moreover, PEG-induced osmotic stress pronouncedly reduced germination and seedling growth by triggering the biosynthesis of ROS in *Sophora viciifolia*, whereas exogenous abscisic acid mitigated the adverse effects of OS [54].

pH Solutions and Seed Sizes Interactive Effects

The results pertaining to the different-sized seeds of guar sown in a wide range of pH solutions (5, 6, 7, 8, 9, and 10) revealed that the maximum germination percentage was recorded at 7-8 pH (Table 4). Large-sized seeds performed superiorly under all pH levels as compared to medium and small-sized seeds. The highest germination (98%) was recorded by large seeds at 7 pH, whereas germination was reduced significantly with increasing pH levels, particularly for small-size seeds (20%) when exposed to 10 pH. Following the trend, the highest mean germination time (8.6 days) was exhibited by small seeds at 10 pH. Interestingly, it was also observed that the time to 50% germination was also increased with an increase or decrease of optimum pH (7), especially in small-sized seeds. Moreover, the large seeds demonstrated the highest germination index (GI) at 7 pH (13.8), whereas the lowest corresponding value was recorded by small seeds at 10 pH. Furthermore, seedling growth was significantly affected by the pH levels and seed sizes (Table 4). The minimum development in root and shoot traits (length, fresh and dry weights) was recorded by small seeds at pH 5 and 10 levels. Small-sized seeds even did not develop their seedling when exposed to a 10-pH solution. Like these results, Dorner et al. [55] demonstrated that any increase or decrease in optimum pH caused a reduction in canary grass, and it was attributed to a disruption in many enzyme (lipase, protease, and diastase) activities. Contrastingly, it was inferred that soil pH influenced the availability of essential plant nutrients and altered the correlations between the uptake of macro and micronutrients [56-58]. Moreover, biochar-induced increment in pH caused osmotic stress along with nutrient imbalance owing to the insolubilization of minerals, and thus reduced availability of vital nutrients suppressed the seedling growth in tomatoes. Moreover, Yang et al. [59] stated that high alkalinity caused an ionic imbalance, and inhibition of ion uptakes led to the reduction of seedling growth. However, these findings were in contradiction with those of Gentili et al. [60], who recorded minimum growth of *Ambrosia artemisiifolia* at neutral pH, which might be interpreted in terms of different species and plant materials used in the study.

Sowing Depth and Seed Sizes Interactive Effects

The recorded findings regarding the emergence and various seedling parameters of different-sized seeds of guar sown under various sowing depths (SD, 0, 2, 4, 6, and 8 cm) revealed that the maximum emergence (80%) was recorded by large seeds, and it was significantly decreased with an increase in the SD (Table 5). The highest mean emergence (9.8 days) and time to 50% emergence (7.5 days) were recorded in small seeds at 8 cm depth. The maximum emergence index was recorded in large-sized seeds at 4 cm and the minimum emergence index (1.96 seed day^{-1}). In terms of all seedling parameters, large-size seeds performed much better under all sowing depths as compared to medium and small-sized seeds. Large seeds had better root length (7.6 cm) and shoot length (8.6 cm) under 2 cm depth. Seedling fresh and dry weights were highly reduced in small-sized seeds when these seeds were sown at 8 cm SD. It might be interpreted that seed germination tends to depend on various ecological conditions, particularly moisture, light, and temperature, and these conditions vary at different soil depths, which led to different germination indices in this study. In previous studies, it was inferred that seeds showed the minimum emergence when buried too shallow due to insufficient moisture at the uppermost layer of soil [61, 62]. Likewise, Heckman et al. [63] reported that seeds sown at 2 cm depth recorded greater germination and developed a better root system to uptake water and nutrient contents, which resulted in better seedling vigor. The same conclusions have been given by Emenky and Khalaf [64] in chickpeas. Deep planting depth caused a negative effect on seedling emergence, as reported by Nabi et al. [65] in cotton. Boyd and Van Acker [61] reported that seed emergence was reduced by the increase in planting depth in some annual and perennial weed species. Similar results were reported for *Galium tricornutum* seeds, and seeds sown at 8 cm of depth showed no germination, while seeds sown at depths of 0.5-2 cm recorded the highest seedling emergence [62]. Moreover, it was revealed that seeds buried in different soil depths tend to experience atypical environmental conditions, particularly; oxygen availability, CO_2 exchange between soil layers, temperature, moisture, and nutrient deficiency, which affect seed germination, time taken to 100% germination, and seedling growth traits (root

Table 5. The interactive impact of different seed sizes and sowing depths on germination and seedling growth traits of cluster bean.

Sowing depths × seed size	Germination (%)	Germination index	Time taken to 50% germination (days)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Small× 0 cm	9.33 b	3.60 g	7.40 a	2.71 l	1.80 d	0.58 f	4.27 c	1.60 bc	0.27 g
Small× 2 cm	7.44 e	7.13 d	5.06 cd	4.76 i	2.39 c	1.50 cd	4.08 c	1.15 e	0.54 ef
Small× 4 cm	8.31 d	5.91 e	5.94 b	3.36 k	1.71 d	1.40 d	3.95 c	1.09 e	0.43 fg
Small× 6 cm	9.35 b	3.05 g	7.06 a	2.41 m	1.19 e	0.59 f	2.75 d	1.04 e	0.24g
Small× 8 cm	9.80 a	1.960 j	7.52 a	1.43 n	0.79 f	0.05 g	1.93 e	0.05 f	0.00 h
Medium×0 cm	8.39 d	4.94 f	4.59 d	5.61 h	2.69 c	1.44 cd	6.21 b	1.60 bc	0.71 de
Medium×2 cm	6.48 g	13.50 a	3.55 e	6.90 e	2.69 c	1.59 c	6.09 b	2.91 a	1.19 b
Medium×4 cm	7.73 e	11.46 c	3.95 e	5.96 g	2.45 c	1.45 cd	5.87 b	1.77 b	0.78 cd
Medium×6 cm	7.58 e	3.49 g	4.93 cd	4.91 i	1.79 d	0.91 e	5.71 b	1.44 d	0.52 ef
Medium×8 cm	8.92 c	2.40 ij	5.34 c	4.07 j	1.39 e	0.67 f	5.48 b	1.14 e	0.25 g
Large×0 cm	7.49 e	11.053c	3.69 e	7.73 c	3.52 ab	1.81b	7.66 a	1.77 b	0.87 c
Large×2 cm	6.31 g	13.52 a	2.53 f	8.69 a	3.82 a	2.00 a	7.53 a	2.91 a	1.83 a
Large×4 cm	9.81 a	13.86 a	2.95 f	8.39 b	3.72 a	1.85 ab	7.40 a	1.67 bc	0.85 c
Large×6 cm	8.48 d	4.46 f	3.97 e	7.16 d	3.25b	1.52 cd	7.20 a	1.72 bc	0.56 de
Large×8 cm	8.87 c	2.81 hi	4.84 d	6.53 f	2.41 c	0.99 e	7.06 a	1.19 e	0.41 fg
LSD at 5%	0.31	0.61	0.48	0.22	0.29	0.39	0.87	0.23	0.23

Values having different letters within the same column vary significantly at 5% probability level.

and shoot length and their fresh and dry weights). Interestingly, deeply buried seeds need to consume extra food reserves in pushing their shoot apices at the soil surface in comparison to the seeds sown at shallower depths. Contrastingly, seeds present on the soil surface were exposed to air and litter, which reduced their viability owing to rapid loss of moisture [25]. Thus, the findings of our trial put forward the concept of optimization of seed size and SD reconciliation for ensuring maximum emergence and seedling growth under normal and suboptimal growth conditions.

Conclusions

Based on recorded findings, it might be inferred that research results were in concurrence with the research hypothesis because the deleterious effects of abiotic stresses vary depending on the seed sizes and sowing depths of cluster beans. Overall, it was revealed that in comparison to small and medium-sized seeds, large seeds sown at 4 cm depth effectively offset the adverse effects of imposed abiotic stresses by recording the maximum germination percentage and germination index along with shoot and root length and fresh and dry weights. However, this treatment combination could not perform at par when exposed to 10°C temperature, 100-200 mM salinity levels, soil pH (5, 9, and 10), and 0.6, 0.8, and 1.0 Mpa of osmotic stress. Thus, based on recorded findings, larger seeds sown at 4 cm depth could be recommended to cluster bean growers; however, these findings are limited in scope, and future studies need to ascertain the underlying mechanisms that promote seed germination and seedling growth under different environmental stresses in varying soil and agro-climatic conditions. Moreover, future studies might investigate short-term soil pH modifications during the germination phase of cluster beans using lime (calcium carbonate) and wood ash for increasing soil pH or acidifying substances (acidic mulches, elemental sulfur, iron or aluminum sulfate, acidifying fertilizers like ammonium sulfate) to enhance germination rate, reduce time taken to germination, and improve the seedling establishment under abiotic stresses.

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Conflict of Interest

The authors declare no conflict of interest.

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