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Effect of alternating temperature regimes on seed germination of guar [*Cyamopsis tetragonoloba* (L.) Taub.]

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Abstract: Guar [*Cyamopsis tetragonoloba* (L.) Taub.] 2*n*=14, is an annual crop that belongs to the Fabaceae family and has a spring–summer growth cycle. This species has been assuming increasing importance among agro-industrial crops due to the high galactomannan content of its seeds for which a multitude of food and non-food applications have been developed. Guar is a viable rotation crop for growers in Mediterranean areas and can result in diversification in crop production systems. The effects of four alternating day/night temperature regimens on *C. tetragonoloba* seed germination were tested: (T1) 23/15 ℃, (T2) 21/13 ℃, (T3) 19/ 11 ℃, and (T4) 17/09 ℃ with a 16/08 h light/dark photoperiod, respectively. Seeds from two guar cultivars (RGC-0936 and RGC-1066) were used. Treatments consisted of four replicates of 50 seeds each, arranged in a split-plot design with temperatures as the main plot and cultivars as the subplot. The results showed that lower temperatures reduced guar seed germination. The highest germination percentages were obtained at the 21/13 ℃ and 23/15 ℃ temperature regimens, while a significant drop in germination percentage occurred for the 19/11 ℃ treatment, and the lowest germination percentages were observed at 17/09 ℃. No statistically significant differences were found between the two cultivars studied for seed germination percentages. The interaction effects among the different alternative temperature regimes on seed germination and guar cultivars were not statistically significantly different. The values obtained at the 21/13 ℃ temperature treatment allow us to conclude that this regimen is the minimum acceptable combination of temperatures to program the sowing date.

Keywords: cluster bean; guar sowing; guar cultivars; guar cycle; Mediterranean.
Abbreviations: ANOVA_analysis of variance, NaOCl_sodium hypochlorite.

Introduction

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] 2n=14, also referred to as cluster bean, belongs to the Fabaceae family and is an annual crop with a spring–summer growth cycle. Guar, generally consisting of deep-rooted 50–150 cm tall and plant, is a drought-tolerant crop that is typically grown on sandy loam soils under hot and dry conditions (Undersander et al., 1991). It has tolerance to salinity (Sandhu et al., 2021) and requires an annual rainfall ranging from 200 to 375 mm, coupled with ample sunshine (NIAM, 2014). Due to the relatively high tolerance of guar to drought and salinity, it can be a valuable alternative crop for utilisation in semi-arid areas (Mahdipour-Afra et al., 2021).
Guar is a multipurpose crop. Its green pods are used as vegetables, grain pulses, plants for fodder, and green manure (Tran, 2015); nevertheless, growing the crop for grain production for industrial use is increasing worldwide. Guar is cultivated predominantly in India, the United States, Pakistan, Australia, and several southern European countries (Vishnyakova et al., 2023). This species produces a seed that contains a galactomannan gum for which a multitude of food and non-food applications have been developed (Pathak, 2015; Soltani et al., 2021). Galactomannan gum is a polysaccharide used as a thickener and stabilizer in food industries, in pharmaceutical industries, and for cosmetic products (Tahmouzi et al., 2023). Furthermore, guar gum is used in the oil and gas industry as a gelling agent; added to water it creates a high-viscosity fluid that fractures the subsurface shale layers for the liberation of oil and natural gas (Gresta et al., 2013; Mudgil et al., 2014). Due to its strategic significance, guar is recognized as one of the most sought-after and promising plant crops worldwide (Vishnyakova et al., 2023).
Guar growing could be economically sustainable and provide an important opportunity for diversification or integration of incomes in Mediterranean areas (Gresta et al., 2014). Guar is a viable rotation crop for growers in Mediterranean areas and can help diversify crop production systems. Particularly, guar may offer the potential to serve as an alternative to cash crops in certain inland areas of Portugal due to its reduced water needs and resilience to drought (Reis et al., 2021). Under these conditions, it is important to have information to help determine the best sowing time.
Guar germination is dependent on different factors, namely seed viability, seed size, seed vigour, soil type, soil moisture, soil temperature, and relative humidity. Depending on the variety, soil, and climatic conditions, crop harvesting is started 90–110 days from the starting date of sowing when 90% of the pods are matured (Pathak, 2015; Singh et al., 2022).

Temperature has a strong effect not only on germination capability but also on germination speed, which is an important parameter for field sowing. Guar is a photosensitive crop and grows well in specific climate conditions, which ensure a soil temperature of approximately 21–25 ℃ for proper germination (Hymowitz and Matlock, 1963). Temperatures should fluctuate between 21 ℃ and 30 ℃ at planting time (Tyagi et al., 1982), and they should never be below 15 ℃ during the growing season. According to Undersander et al. (1991), guar needs high soil temperatures of at least 20-21 ℃ for adequate seed germination, while the optimal temperature is considered 30 ℃. Pathak (2015) also stated that at planting time, soil temperatures should be above 21 ℃ for rapid crop establishment. Hu et al. (2002) suggested that the temperature at which guar seed germinates most quickly is 27 ℃. Singh et al. (2021) concluded that maximum germination can be obtained in the temperature range between 22 ℃ and 28 ℃. Gresta et al. (2018) studied the germination of different genotypes and verified that some of the guar cultivars germinated at 15 ºC fixed temperature, whereas no germination occurred at 5 ℃ and 10 ℃. Guang-hua et al. (1980) did not observe any guar seed germination at temperatures lower than or equal to 10 ºC. Singh et al. (2021) found that some genotypes performed well under low temperatures and germination percentages of 98.5% were reported for cv. Kinman at a fixed temperature of 16 ℃.

When compared to soybean and other warm-season legumes, guar exhibited a higher germination temperature and showed the best germination at high day-time temperatures which are characteristic of tropical and subtropical regions. Cultivated guar originated in a sub-tropical environment, and it has adapted to higher soil temperatures for better seed germination (Singh et al., 2021). One of the aspects of adapting the guar crop to the Mediterranean climate is reconciling the sowing date with the requirements for seed germination. On the other hand, it is important that the crop can complete the cycle without a reduction in the productivity and quality of the seed. There are few studies on the effect of alternating temperatures on guar germination. This study was conducted with the objective of assessing the effects of different day/night alternating temperatures on the germination of two guar cultivars and determining the optimal soil temperature for guar seed germination.

Results and discussion

ANOVA and interaction effects

Statistically significant differences were found among alternative temperature regimes for seed germination percentage $F(3, 24) = 48.17$, $p < 0.0005$. On the other hand, no significant differences were found between the two cultivars studied (RGC-936 and RGC-1066), and their germination behaviours are similar, $F(1, 24) = 0.022$, $p = 0.884$. The interaction effects among the different alternative temperature regimes on seed germination and guar cultivars were not statistically significant, $F(3, 24) = 0.222$; $p = 0.880$ (Table 1). Nevertheless, different guar genotypes may differ in seed germination percentages under different temperature conditions (Gresta et al., 2018; Singh et al., 2021). Singh et al. (2021) investigated six cultivars of guar across six germination temperatures (13, 16, 19, 22, 25, and 28 ℃). Significant variations were observed among guar cultivars for germination percentage. In a study by Gresta et al. (2018), nine worldwide guar genotypes were examined for germination capability and speed in response to seven constant temperature increments (ranging from 5 ℃ to 35 ℃), as well as two alternating temperatures of 15/10 ℃ and 20/15 ℃. Differences were found among cultivars, and at 20/15 ℃, three exhibited germination percentages over 60%.

Germination at four alternating temperature regimes

Results of the effect of four day/night alternating temperature regimes on seed germination (%) from two guar cultivars on days 5 and 10 after assay installation are presented in Table 2. Results showed that alternating temperatures significantly affected guar seed germination, with lower temperatures resulting in reduced seed germination The highest germination percentages were obtained for treatments 21/13 ℃ and 23/15 ℃. Values of 96.5% and 86.0% (RGC-936) and 97.0% and 89.5% (RGC-1066) were recorded, respectively, for treatments 21/13 % and 23/15℃, on day 10 after the assay installation. No statistically significant differences were found between the temperature treatments 21/13 ℃ and 23/15 ℃, according to the Bonferroni post-hoc test. Higher germination percentage values would be expected for the 23/15 ºC treatment. The values obtained are probably due to partial dehydration of the Petri dishes in this treatment during the initial phase of the assay. A significant reduction in the germination percentage occurred for the 19/11 ℃ treatment, with 78% for both cultivars studied, on day 10 after the assay installation. The lowest germination percentages were observed for the 17/09 ℃ treatment, with 40.5% and 34.0%, respectively, in cultivars RGC-936 and RGC 1066 on day 10 after the assay installation. Gresta et al. (2018) stated that there is no germination of guar seeds at 5 ℃ and 10 ℃; moreover, at 15 ℃, germination is negligible. According to the same authors, the maximum germination percentages occur between 25℃ and 30 ℃.

Choosing a sowing date is important, as it is necessary to ensure that the soil has reached the appropriate temperature for maximum germination. On the other hand, it is also necessary to allow time for the plant to complete its cycle and for seed maturation to occur. In Mediterranean areas, such temperatures impose a sowing time no earlier than May or early June (Gresta et al., 2013), which in the case of long-cycle gaur varieties may involve late harvesting (end of October/beginning of November) at a time when rainfall may result in lower seed quality. The harvest index decreases with a delay in the sowing date. This trend might be attributed to the shortening of the growing season, leading to a reduction in the reproductive stage which decreases the number of flowers and pods per plant and, ultimately, the harvest index (Mahdipour-Afra et al., 2021). A possible way to overcome the above-mentioned problems could be to select genotypes with lower temperature requirements for seed germination (Gresta et al., 2018; Singh et al. 2021) or alternatively, obtain genotypes with a cycle 10 to 15 days shorter compared to existing cultivars (Singh et al., 2022).

In our study, the values obtained after using the 21/13 ℃ temperature treatment allow us to conclude that this regimen is the minimum acceptable combination of temperatures to program the sowing date. Although it may be possible to sow at lower temperatures (19/13 ℃, germination 78%), this change implies an increase in costs as more seed is needed. Our data agree with those of Undersander et al. (1991) who reported a threshold soil temperature for seed germination equal to 21 ℃. The results we obtained also agree with those reported by Singh et al. (2021) who stated that lowering the temperature did not change the seed germination percentage significantly until temperatures were below 22 ℃, at which point a significant reduction in germination occurred. According to the same authors, the maximum germination can be achieved with a temperature of 22 ºC, although the speed of germination and seed vigor indices, which are indicators of quick and uniform establishment of guar in the field, continued to increase until 28 ºC.

Table 1. ANOVA Summary Table.

Source size	SS	*df*	MS	*F*	*p*	Effect
Temp. treatments	2.495	3	0.832	48.170	0.000	0.858
Cultivars	0.000	1	0.000	0.022	0.884	0.001
Treatments x cultivars	0.012	3	0.004	0.222	0.880	0.027
Within groups	0.414	24	0.017			
Total	41.524	32				

ANOVA – analysis of variance; SS – Sum-of-squares; MS = Mean squares; effect size = partial $\eta 2$.

Table 2. Effect of four alternating temperature regimes, with a 16/8 h light/dark (L/D) photoperiod, on seed germination (%) from two guar cultivars (RGC-936 and RGC-1066) on days 5 and 10 after assay installation.

Temperature treatments	Photoperiod (L/D)	RGC-936		RGC-1066	
		Day 5	Day 10	Day 5	Day 10
23/15 °C	16/8h	$65.0^{b} \pm 25.6$	$86.0^{ab} \pm 16.9$	$63.0^{a} \pm 34.0$	$89.5^{ab} \pm 4.1$
21/13 °C	16/8h	$93.0^{a} \pm 3.8$	$96.5^{a} \pm 1.9$	$70.0^{a} \pm 37.6$	$97.0^{a} \pm 2.6$
19/ 11 °C	16/8h	$55.5^{b} \pm 14.7$	$78.0^{b} \pm 10.2$	$52.5^{a} \pm 9.6$	$78.0^{b} \pm 11.2$
17/ 09 °C	16/8h	$7.0^{c} \pm 6.8$	$40.5^{c} \pm 10.9$	$11.0^{b} \pm 6.2$	$34.0^{c} \pm 9.5$

Means with different alphabetic superscripts in the same line differ significantly (P < 0.05) according to the Bonferroni post-hoc test.

Our data are in partial agreement with those of Gresta et al. (2018) who reported germination percentages ranging from 66% to 80%, depending on the genotype, at 20/15 ℃ alternating temperatures. In the same study, negligible germination occurred at 15/10 ℃ alternating temperatures. These authors concluded that it is possible to obtain adequate germination and crop establishment for some cultivars by sowing early in the spring if the temperature reaches 20 ℃ for 6 hours daily. In our study, germination percentages of 78% were obtained for both cultivars (RGC-936 and RGC-1066) with the alternating temperatures treatment of 19/11 ℃ 10 days after assay installation. Our data show that sowing at a soil temperature of 19 °C is possible when the seeding density is increased to compensate for germination losses. However, a higher sowing density increases production costs and results in a lower germination speed at this temperature.

The temperature not only influences the germination capacity but also plays a crucial role in determining the speed of germination, which is a critical factor for field sowing. In our study, more than 50% of seed germination occurred within the first five days after assay installation in treatments 23/15 °C, 21/13 °C and 19/11 °C. In the case of the 17/09 °C treatment, the mean germination for both cultivars were 9.0% and 37.3%, respectively, on days 5 and 10.

Guar growing could offer a significant opportunity for diversification and integration of incomes in Mediterranean areas. Particularly, guar might have the potential to be an alternative to cash crops in some inland areas of Portugal due to its lower water requirements and drought resilience. Attaining a high grain yield of guar depends on selecting the appropriate sowing date and density. Accordingly, early sowing (May) is considered suitable for guar cultivation in a Mediterranean climate, provided the soil temperature reaches 21 ℃.

Materials and methods

Plant materials and germination experiments

The effects of four day/night alternating temperature regimes on *C. tetragonoloba* seed germination were studied. Seeds of two guar cultivars (RGC-0936 and RGC-1066) were used. Seeds were sterilized in a 1% NaOCl solution for 10 min and then rinsed three times in distilled water. Treated seeds were placed in Petri dishes on filter paper and moistened with sterilized water. Treatments consisted of four replicates of 50 seeds each, which were arranged in a split-plot design with temperatures as the main plot and cultivars as the subplot. The experiment was conducted in a growth chamber with controlled light and temperature. Four day/night alternating temperature regimes were tested: (T1) 23/15 ℃, (T2) 21/13 ℃, (T3) 19/ 11 ℃, and (T4) 17/09 ℃ with a 16/08 h light/dark photoperiod, respectively. These temperatures were chosen to simulate the conditions that occur at the end of spring when sowing must be done in a Mediterranean environment. This alternating temperature regime is typical in soil during the spring. Petri dishes were moistened with 5 ml of distilled water every two to three days according to temperature regimes. The number of germinated seeds was counted on the fifth and tenth days after the start of the assay. The seed was considered to have germinated when the radicle emerged from the testa (> 1 mm) as described by Bewley and Black (1994).

Data analysis

The germinated percentage for each Petri dish was calculated from the total number of seeds germinated divided by the total number of viable seeds tested and then multiplied by 100. The arcsine and square root transformations of percentage data were conducted to correct situations of non-normality, heterogeneity of variance, and no additivity (Ahrens et al., 1990). Data were subjected to analysis of variance (ANOVA) using the General Linear Model available in IBM SPSS Statistics (version 26) after verifying that the ANOVA assumptions were met (namely, homogeneity of variance and normal distribution as verified by Levene's and the Shapiro–Wilk tests, respectively). No outliers were found, residuals were normally distributed ($p > 0.05$), and homogeneity of variances ($p = 0.126$) was detected. The Bonferroni post-hoc test was used to detect significant differences (P < 0.05) among treatment means.

Conclusions

No statistically significant differences were found between the two studied cultivars (RGC-936 and RGC-1066) for seed germination percentages. The resulting values allow us to conclude that the 21/13 ℃ temperature treatment is the minimum acceptable combination of temperatures to program the guar sowing date. At soil temperatures below 21 ℃, a significant reduction in seed germination percentage may occur. It would be of interest to extend this type of study to a greater number of cultivars and verify whether an interaction between cultivars and alternative temperature regimes for germination percentage does occur. It is important to develop cultivars with lower temperature requirements for seed germination to allow earlier sowing times.

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