

# Vernalization temperature and maturation point of seed cloves on garlic production and quality

## Temperatura de vernalização e ponto de maturação de bulbilhos-sementes na produtividade e qualidade do alho

José Magno Queiroz Luz<sup>1</sup>, Carlos Inácio Garcia de Oliveira<sup>2</sup>, Sérgio Macedo Silva<sup>3</sup>, Túlio Garcia Oliveira<sup>4</sup>, Renata Castoldi<sup>5\*</sup>

<sup>1</sup>Universidade Federal de Uberlândia/UFU, Instituto de Ciências Agrárias/ICIAG, Campus Glória, Uberlândia, MG, Brasil

<sup>2</sup>Supere Planejamento, Consultoria e Assistência Agrônômica, Uberlândia, MG, Brasil

<sup>3</sup>Universidade Federal dos Vales do Jequitinhonha e Mucuri, Unai, MG, Brasil

<sup>4</sup>Grupo Seletto, Uberlândia, MG, Brasil

<sup>5</sup>Universidade Federal de Uberlândia/UFU, Instituto de Ciências Agrárias/ICIAG, Monte Carmelo, MG, Brasil

\*Corresponding authors: [rcastoldi@ufu.br](mailto:rcastoldi@ufu.br)

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### ABSTRACT

Brazilian producers have been seeking to improve garlic productivity and quality via vernalization at negative temperatures. However, more concrete information about the application and effects of this technique on the productivity and quality of noble garlic varieties is lacking. Moreover, there are also questions about the use and influence of seed cloves at different maturation points on this crop's yield. Therefore, the objective of this work was to evaluate the effects of vernalization temperatures and different maturation points of seed cloves on the yield and quality of noble garlic cultivars. Three experiments were conducted using the Ito cultivar over three garlic planting seasons in Cristalina, GO, Brazil. The experimental design followed a 3 x 3 factorial scheme: three vernalization temperature ranges (-1 to -3 °C; 1 to 3 °C; and 2 to 4 °C) and three seed clove maturation points (normal, early and late). Plant height, aboveground fresh mass, bulbar ratio, and bulb yield and quality were evaluated after harvesting. The negative vernalization temperature had significant results with the highest garlic yields occurring in all three planting seasons, with a considerable increase in the quantity of bulbs with better commercial value ('class 6') and a decrease in the quantity of 'discard' bulbs. In comparison to the other temperatures, negative vernalization temperatures also yielded higher fresh plants in all evaluated seasons. The normal maturation point resulted in gains in total bulb yield. The use of below-zero vernalization temperatures increased the productivity of the garlic cultivar Ito under the conditions found in Brazil.

**Index terms:** Noble garlic; Ito cultivar; below-zero temperature; bulb productivity; class 6.

### RESUMO

Os produtores brasileiros têm buscado melhorar a produtividade e a qualidade do alho, aplicando a técnica de vernalização com temperaturas negativas. No entanto, faltam informações mais concretas sobre os efeitos e aplicação desta técnica na produtividade e qualidade do alho nobre. Existem dúvidas também sobre o uso de bulbilhos-sementes com diferentes pontos de maturação e sua influência na produção desta cultura. Portanto, o objetivo do trabalho foi avaliar os efeitos de temperaturas de vernalização, incluindo temperaturas negativas e diferentes pontos de maturação do bulbilho-semente, sobre a produtividade e a qualidade do alho-nobre. Foram conduzidos 3 experimentos, em três épocas de plantio de alho, com a variedade Ito, nas condições de Cristalina, GO, Brasil. O delineamento experimental foi em esquema fatorial 3 x 3, sendo três faixas de temperatura de vernalização (-1 a -3 °C; 1 a 3 °C e 2 a 4 °C) e três pontos de maturação de bulbilho-semente (precoce, normal e tardio). Foram avaliadas altura de plantas, massa fresca da parte aérea e razão bulbar, além de produtividade e qualidade de bulbos, após a colheita. Nas três épocas de plantio, a temperatura negativa de vernalização proporcionou, significativamente, as maiores produtividades de alho, elevando consideravelmente a quantidade de bulbos de melhor valor comercial ('classe 6'), e reduzindo a quantidade de bulbos do tipo 'descarte'. As temperaturas negativas de vernalização também proporcionaram maior massa de plantas, em todas as épocas avaliadas. O ponto de maturação normal proporcionou ganhos de produtividade total de bulbos. O uso de temperaturas negativas na vernalização proporciona incrementos na produtividade de alho cultivar Ito, nas condições brasileiras.

**Termos para indexação:** Alho nobre; cultivar Ito; temperatura negativa; produtividade de bulbos; classe 6.

## INTRODUCTION

Garlic (*Allium sativum* L.) is considered the second main species of the *Allium* genus and is the most widely consumed garlic species in the world (Food and Agricultural Organization of the United Nations - FAO, 2020). Brazil produces the fourth most garlic globally, with ca. 156 thousand tons of bulbs produced over approximately 12 thousand hectares, within the states of Minas Gerais, Goiás, Santa Catarina, and Rio Grande do Sul, accounting for 92% of national production (Associação Nacional dos Produtores de alho - ANAPA, 2022).

Despite its high level of garlic production (approximately 167 thousand tons), Brazilian production can only provide 44% of the domestic market needs, requiring imports mainly from China, Argentina, and Spain (Resende et al., 2018). Environmental conditions, such as air temperature, photoperiod (Mathew et al., 2011), solar radiation (Rizzalli; Villa Lobos; Orgaz, 2002), soil temperature (Rahim; Fordham, 1990), water availability (Cortés; Olalla; Urrea, 2003), storage period and temperature of seed cloves (Atashi et al., 2011), constitute one of the major hurdles to producing noble garlic varieties in Brazil, as these factors interfere with plant growth, development, and productivity.

In Brazil, garlic seed cloves are usually stored in cold chambers prior to planting, a technique known as vernalization. This technique is applied worldwide as a physiological process to induce flowering, wherein plants of some species are exposed to low, nonfreezing temperatures (Taiz; Zeiger, 2017).

Several agricultural species require vernalization for production; however, this process usually occurs in plants that propagate through vegetative propagules (bulbs, shoots, tubers, or rhizomes), such as onions when propagated vegetatively (D'Aangelo; Goldman, 2018), strawberries (Buffon et al., 2021), amaryllis (Andini; Setiadi; Siregar, 2019), and gladiolus (Tomiozzo et al., 2019). However, in Brazil, the vernalization process for garlic consists of applying low temperatures to seed cloves to induce bulbification (Luz et al., 2022).

The storage of bulbs at temperatures of 3-5 °C for a period of 40-60 days before planting at a humidity of 70-80% (Resende et al., 2011) stimulates the accumulation of morphological change-inducing hormones, such as free gibberellins and cytokinins (Lucena et al., 2016). This period is necessary to provide the differentiation in the 'reserve' leaf and allow the crop to have a cycle capable of ensuring good productivity and quality for each location, planting time, and genetic material cultivated.

The period, as well as the permanence time in the cold chambers, reflects the performance of the plant in the field. Since the use and maintenance of cold chambers are expensive, studies with different vernalization times are necessary to determine the minimum submission period of seed cloves to low temperatures. In addition to the high cost of cold chambers, there is also the high cost of obtaining seed bulbs (Coimbra et al., 2007).

According to Smaniotto et al. (2014), seed quality is an extremely important factor in attaining the expected productivity. Some of the factors that can help maintain this physiological quality include storage, maturation point, temperature, and storage time. Oliveira, Souza and Mota (2003) state that the ideal maturation point for harvesting garlic – allowing for variations according to the cultivar – is when the bulb is mature.

Garlic producers have been using vernalization time, temperature, and seed clove maturation point data for each cultivar and location. However, there is still a lack of information on the vernalization of seed cloves at temperatures below zero, their planting at different maturation points, and their possible influences on garlic productivity and quality for consumption. Thus, this work aimed to evaluate the effect of three vernalization temperatures, including below-zero temperatures, and three seed clove maturation points on the yield and quality of the garlic cultivar Ito.

## MATERIAL AND METHODS

### Experimental area

The experiments were conducted in three distinct seasons: from March to June 2019 (Season I) from April to July 2019 (Season II), and from May to August 2019 (Season III) in the experimental area of Grupo Agrícola Wehrmann, located in Cristalina-GO, Brazil (17°02'45"S, 47°45'24"W, altitude of 980 m).

The soil is classified as a Red-Yellow Latosol of medium texture, with a nonuniform relief from gently undulating to flat. The climate of the region, according to the Köppen climate classification, is temperate humid, characterized by hot summers and dry winters, with annual rainfall and average temperature values of 1500 mm and 20.9 °C, respectively.

During the experimental period, meteorological data were obtained for each planting period. In Season I, the maximum and minimum temperatures ranged between 21 and 26 °C and 8 and 20 °C, respectively; the accumulated precipitation was 215 mm, and the

photoperiod ranged from 12 h and 3 min to 11 h and 17 min during plant growth, with an average value of 11 h and 10 min during bulb enlargement. In Season II, the maximum and minimum temperatures ranged between 19 and 30 °C and 8 and 19 °C, respectively; the accumulated precipitation was 79 mm, and the photoperiod varied from 11 h and 4 min to 11 h and 28 min during plant growth, with an average value of 11 h and 14 min during bulb enlargement. In Season III, the maximum and minimum temperatures ranged between 18 and 29 °C and 9 and 18 °C, respectively; the accumulated precipitation was 51 mm, and the photoperiod varied from 11 h and 2 min to 11 h and 36 min during plant growth, with an average value of 11 h and 14 min during bulb enlargement.

### Design and treatments

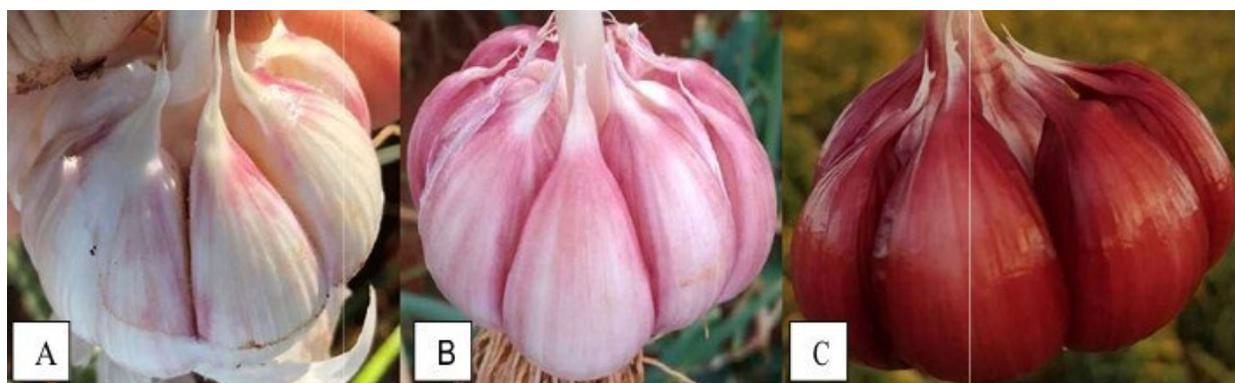
The experiment was performed in a randomized block design using a 3 x 3 factorial scheme, totalling nine treatments, with four replicates per treatment. The treatments consisted of three vernalization temperature ranges (-1 to -3 °C; 1 to 3 °C; and 2 to 4 °C) and three seed clove maturation points (normal, early and late). The maturation point of a seed clove referred to the time when the bulb was harvested, which was based on the colour of the seed clove protective leaf (Figure 1). Thus, when the bulb harvest was anticipated, the protective leaf of the seed clove had a slight purple colour, and this maturation was called early (Figure 1A); when the bulb was harvested when the colour of the protective leaf was normal purple, it was called normal maturation (Figure 1B); and when the bulb was harvested when the protective leaf was intensely purple, it was called late maturation (Figure 1C).

Each experimental plot was 1.2 m wide × 6 m long with a double-row planting system: 0.1 m spacing between

plants and 0.4 m between double rows. A total of 360 plants were planted per plot.

The seed cloves were from the Ito cultivar, a noble garlic variety, and this cultivar contains round, uniform, and vigorous bulbs, with a white outer tunic; each bulb contains seven to ten seed cloves covered by a purple film (Resende et al., 2013). The bulbs belonged to commercial class 6, i.e., 51-55 mm in diameter, and were from the third generation of commercial crops. Before planting, the bulbs were stored in a cold chamber with a relative humidity of 60-70% for 25 days to reach 70% of the Visual Index of Overcoming Dormancy (VID). This index was calculated according to Reghin and Kimoto (1998) using the formula  $VID = [(CLB/CLR) \times 100]$ , where VID = visual index of overcoming dormancy, CLB = longitudinal length of the sprouting leaf, and CLR = longitudinal length of the reserve leaf.

Bulbs were subsequently subjected to 50 days at different temperatures (vernalization), according to each treatment. The vernalization process of the seed cloves was conducted in three different cold chambers, adjusted to the following temperature ranges: -1 to -3 °C; 1 to 3 °C; and 2 to 4 °C. Each cold chamber was fitted with an air-condensing refrigeration set, with a single-phase compressor and fan that had forced-air fans, an electrical control panel, electronic temperature and defrosting controllers, refrigerant gas, high- and low-pressure switches, copper pipes and connectors, a thermostatic expansion valve, accessories for thermal and pipe insulation, a revolving refrigerated door of polyurethane measuring 1.80 × 0.80 m covered in a treated and prepainted steel plate, a flexible curtain door with stainless steel support, and a mini exhaust fan for air renewal and control. A portable dehumidifier was installed next to each chamber.



**Figure 1:** Beginning of purple colouration (A), normal purple colouration staining (B), intense purple colouration (C).

Before planting, soil analysis was performed to define fertilization management. Soil preparation was performed by heavy harrowing, mouldboard ploughing, light harrowing, subsoiling, and then bed building (0.20 m height  $\times$  1.20 m width). After preparing the beds, garlic bulbs were planted manually in a double row system, totalling 3 double rows per bed.

The crop was drip irrigated throughout the crop cycle, starting from the induction of axillary sprouts to tissue differentiation. Irrigation was controlled to induce the necessary stress but to prevent oversprouting and promote the adequate formation of bulbs and bulbils. Other cultural and phytosanitary treatments were those normally employed in the culture.

### Evaluation

At 48 days after planting (DAP) for Season I and at 49 DAP for Seasons II and III, four plants from the centre of the double-row planting system of each experimental plot (useful plot) were evaluated for plant height, measured from ground level to the tip of the largest leaf, expressed in cm. At 70 DAP for Season I and at 98 DAP for Seasons II and III, plant samples from each plot were collected for evaluation of the fresh shoot mass. The bulbar ratio – a variable proposed by Mann (1952), which expresses the degree of bulb development through the relationship between the diameter of the pseudostem at the neck and the diameter of the median part of the bulb – was also measured.

Harvesting was conducted during the crop senescence phase, with approximately four remaining fresh leaves, i.e., at 92 DAP (Season I), 99 DAP (Season II), and 110 DAP (Season III). After harvesting, the plants were stored and hung in an open shed for 40 days to undergo the “curing” process. Bulbs were cleaned by separating the pseudostem 2 cm above the bulb and removing roots and dirty outer films.

Subsequently, the bulbs were weighed, counted and classified according to the commercial bulb classification model by applying sieves in the 2–8 range based on bulb diameter, as follows: class 2, <35 mm; class 3, 36–40 mm; class 4, 41–45 mm; class 5, 46–50 mm; class 6, 51–55 mm; class 7, 56–60 mm; and class 8, >60 mm. Within each class, the bulbs of the extra and industry types were separated, as well as the ‘Discards’ (noncommercial material). Based on the weight, the total productivity of the culture was estimated ( $\text{t ha}^{-1}$ ).

### Data analysis

The data were tested to determine whether they met the analysis of variance (ANOVA) assumptions of residual

normality, homogeneity of variance and block additivity at 5% probability by Shapiro Wilks, Levene’s, and Tukey’s tests, respectively. Once the assumptions were met, the means were subjected to ANOVA, and the variables that showed significant differences by the  $F$  test ( $\leq 0.05$ ) were compared by Tukey’s test ( $p \leq 0.05$ ) using the statistical software R Core Team (2019).

## RESULTS AND DISCUSSION

### Season I

Regarding the height and fresh mass variables, there was no significant interaction between maturation points and vernalization temperatures (Table 1). On average, the plants reached >75 cm in height and >350 g in fresh mass.

**Table 1:** Mean height, fresh mass, and bulbar ratio values of noble garlic plants, cultivar Ito, in Season I, at different seed clove maturation point and vernalization temperature and the conditions of Cristalina-GO, 2019.

Evaluated factors	Plant height (cm)	Fresh mass (g)	Bulbar ratio
Maturation Point (MP)			
Normal	77.84 <sup>a</sup>	372.82 <sup>a</sup>	0.36 <sup>a</sup>
Early	75.59 <sup>a</sup>	374.47 <sup>a</sup>	0.37 <sup>a</sup>
Late	76.63 <sup>a</sup>	362.09 <sup>a</sup>	0.36 <sup>a</sup>
$F_{MP}$	3.09 <sup>ns</sup>	0.26 <sup>ns</sup>	0.34 <sup>ns</sup>
Vernalization Temperature (VT)			
-1 to -3 °C	77.53 <sup>a</sup>	379.32 <sup>a</sup>	0.39 <sup>a</sup>
1 to 3 °C	75.94 <sup>a</sup>	372.24 <sup>a</sup>	0.35 <sup>b</sup>
2 to 4 °C	76.60 <sup>a</sup>	357.82 <sup>a</sup>	0.35 <sup>b</sup>
$F_{VT}$	1.56 <sup>ns</sup>	0.70 <sup>ns</sup>	6.96 <sup>*</sup>
$F_{MP \times VT}$	2.28 <sup>ns</sup>	2.26 <sup>ns</sup>	4.18 <sup>*</sup>
C.V. (%)	4.09	17.39	10.98

Means followed by distinct lowercase letters in the column differ by Tukey’s test at the 0.05 significance level; \* = significant at the 5% probability level; ns = not significant;  $F_{MP}$  =  $F$  calculated for maturation point;  $F_{VT}$  =  $F$  calculated for vernalization temperature;  $F_{MP \times VT}$  =  $F$  calculated for the interaction maturation point  $\times$  vernalization temperature; C.V. = coefficient of variation.

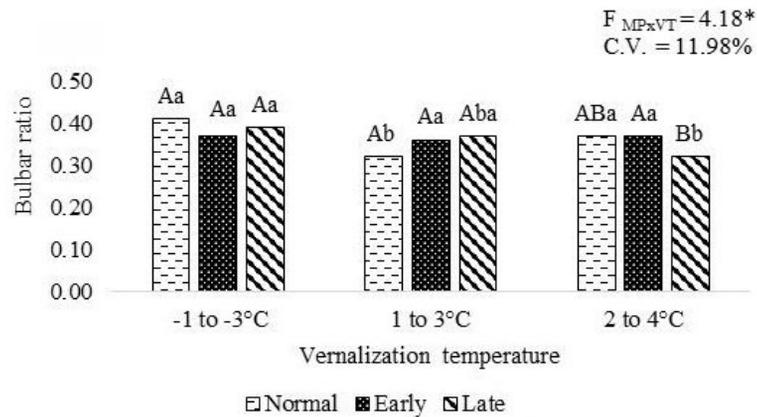
For the bulbar ratio, there was an interaction between maturation points and vernalization temperatures (Table 1). In general, of the temperatures,

the below-zero vernalization temperature resulted in the highest bulbar ratios (Figure 2). This finding may stem from the fact that bulbs subjected to this treatment were still developing in this period compared to those in the other treatments.

The highest total garlic bulb yields were obtained when below-zero vernalization temperatures were applied,

which provided a bulb quantity increase of at least 2.3 t ha<sup>-1</sup>. The normal and early maturation points also provided the highest total productivity values (Table 2).

Similar to the total productivity index, the bulb quantity of classes 2 to 5 increased with the below-zero vernalization temperature by 2.03 t ha<sup>-1</sup> compared to with the higher temperature other treatments.



**Figure 2:** Interaction analysis of bulbar ratio of noble garlic plants, cultivar Ito, in Season I, at the different bulb-seed maturation point (normal, early, and late) and vernalization temperature (-1 to -3 °C; 1 to 3 °C; and 2 to 4 °C), under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.

**Table 2:** Mean values of total bulb productivity (TBP) (t ha<sup>-1</sup>): bulb productivity of class 2 to 5 (BP2-5), bulb productivity of class 6 (BP6), and bulb productivity of 'discard' class (BPD) of noble garlic, cultivar Ito, in Season I, at the different seed clove maturation point and vernalization temperature, under the conditions of Cristalina-GO, 2019.

Evaluated factor	TBP	BP2-5 (<35-50 mm)	BP6 (51-56 mm)	BPD
Maturation Point (MP)				
Normal	14.03 <sup>a</sup>	12.62 <sup>a</sup>	0.24 <sup>ab</sup>	1.16 <sup>a</sup>
Early	14.04 <sup>a</sup>	12.53 <sup>a</sup>	0.52 <sup>a</sup>	0.98 <sup>a</sup>
Late	12.57 <sup>b</sup>	11.54 <sup>b</sup>	0.12 <sup>b</sup>	0.90 <sup>a</sup>
F <sub>MP</sub>	20.56*	7.25*	3.35*	0.91 <sup>ns</sup>
Vernalization Temperature (VT)				
-1 to -3 °C	15.18 <sup>a</sup>	13.62 <sup>a</sup>	0.68 <sup>a</sup>	0.87 <sup>a</sup>
1 to 3 °C	12.87 <sup>b</sup>	11.59 <sup>b</sup>	0.10 <sup>b</sup>	1.17 <sup>a</sup>
2 to 4 °C	12.59 <sup>b</sup>	11.48 <sup>b</sup>	0.10 <sup>b</sup>	1.00 <sup>a</sup>
F <sub>VT</sub>	58.40*	29.51*	8.80*	1.20 <sup>ns</sup>
F <sub>MP×VT</sub>	1.43 <sup>ns</sup>	0.31 <sup>ns</sup>	5.07*	1.39 <sup>ns</sup>
C.V. (%)	6.72	8.87	185.24	66.52

Means followed by distinct lowercase letters in the column differ by Tukey's test at the 0.05 significance level; \* = significant at the 5% probability level; <sup>ns</sup> = not significant; F<sub>MP</sub> = F calculated for maturation point; F<sub>VT</sub> = F calculated for vernalization temperature; F<sub>MP×VT</sub> = F calculated for the interaction maturation point x vernalization temperature; C.V. = coefficient of variation.

A significant interaction among factors was observed only for the productivity of class 6 bulbs, with the highest values obtained with the below-zero vernalization temperature associated with early maturation of garlic seeds (Figure 3). No significant differences were found among treatments for the productivity of garlic classified as 'discard' (Table 2).

### Season II

In Season II, there was a significant interaction between maturation point and vernalization temperature on plant height, fresh mass, and bulbar ratio (Table 3). The highest plant heights were obtained when using seed cloves at the late-maturation point and below-zero vernalization temperature (Figure 4). Cloves at the normal and late-maturation stages subjected to temperatures ranging from -1 to -3 °C and from 1 to 3 °C yielded heavier bulbs than those subjected to the 2 to 4 °C temperature range (Figure 5). The highest bulbar ratio values were found for seed cloves at the normal and late-maturation stages subjected to below-zero temperatures (Figure 6).

In comparison to the other treatments, the below-zero vernalization temperature associated with the normal maturation stage resulted in slower bulb development, further indicating greater bulb growth potential compared to that in the other treatments, possibly resulting in greater size and weight gain at harvesting.

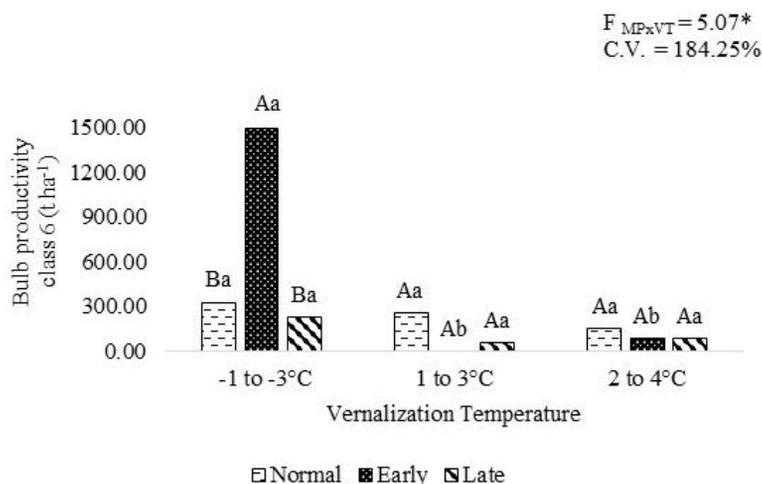
The total bulb yield was higher when using late-maturation seed cloves subjected to low temperatures, with increments of ca. 1.5 t ha<sup>-1</sup> relative to the highest vernalization temperature (Figure 7).

The other classes showed no significant interaction between the factors; however, the lower vernalization temperature range ensured the best quality of class 6 bulbs, with gains of 4.3 t ha<sup>-1</sup> compared to those at the intermediate temperature range (Table 4). For the bulbs classified as 'discard', the highest yield values were obtained with the highest vernalization temperatures, which demonstrates the great advantage of using negative temperatures.

### Season III

In Season III, there was no significant interaction between the factors for any of the evaluated variables. Garlic plants reached an average height of 78.42 cm and a fresh mass of 351.19 g, with no significant differences among treatments (Table 5). The bulbar ratio was again significantly higher at the below-zero vernalization temperatures compared to in the other treatments, regardless of the maturation stage of seed cloves.

For total bulb yield, the highest value was obtained using seed cloves at the normal maturation stage subjected to below-zero vernalization temperatures (Table 6); increases of approximately 3 t ha<sup>-1</sup> were obtained compared to those at the 2 to 4 °C range. For the other classes, significant interactions between treatments were observed (Table 6).

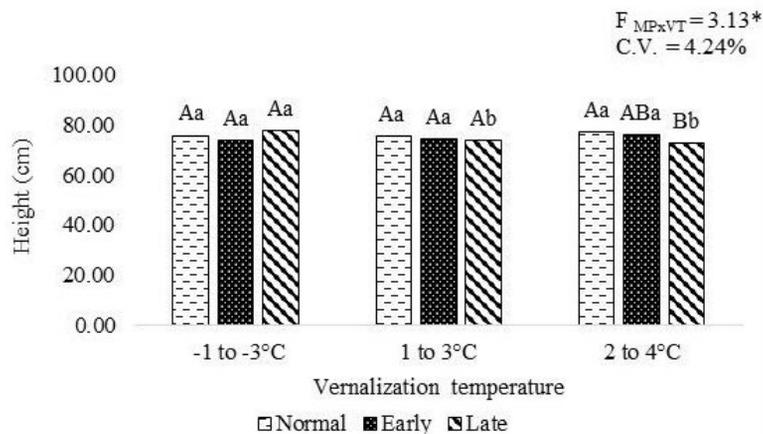


**Figure 3:** Interaction analysis of class 6 bulb productivity (t ha<sup>-1</sup>) of noble garlic plants, cultivar Ito, in Season I, at different maturation points of the seed bulbs (normal, early, and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C; and 2 to 4 °C), under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.

**Table 3:** Mean values of height, fresh mass, and bulbar ratio of noble garlic plants, cultivar Ito, in Season II, at different seed clove maturation stages for crop and vernalization temperatures under the conditions of Cristalina-GO, 2019.

Evaluated factor	Plant height (cm)	Fresh mass (g)	Bulbar ratio
Normal	76.10 <sup>a</sup>	575.39 <sup>a</sup>	0.36 <sup>a</sup>
Early	74.72 <sup>a</sup>	520.13 <sup>b</sup>	0.33 <sup>b</sup>
Late	74.49 <sup>a</sup>	546.31 <sup>b</sup>	0.32 <sup>b</sup>
F <sub>MP</sub>	1.80 <sup>ns</sup>	5.35 <sup>**</sup>	3.92 <sup>**</sup>
-1 to -3 °C	75.70 <sup>a</sup>	569.99 <sup>a</sup>	0.38 <sup>a</sup>
1 to 3 °C	74.45 <sup>a</sup>	553.22 <sup>ab</sup>	0.31 <sup>b</sup>
2 to 4 °C	75.17 <sup>a</sup>	518.62 <sup>b</sup>	0.33 <sup>b</sup>
F <sub>VT</sub>	0.93 <sup>ns</sup>	4.81 <sup>*</sup>	14.88 <sup>*</sup>
F <sub>MPxVT</sub>	3.13 <sup>*</sup>	2.60 <sup>*</sup>	3.69 <sup>*</sup>
C.V. (%)	4.24	10.70	13.73

Means followed by distinct lowercase letters in the column differ by Tukey's test at the 0.05 significance level; \* = significant at 5% probability; \*\* = significant at 1% probability, <sup>ns</sup> = not significant; F<sub>MP</sub> = F calculated for maturation point; F<sub>VT</sub> = F calculated for vernalization temperature; F<sub>MPxVT</sub> = F calculated for the interaction maturation point x vernalization temperature; C.V. = coefficient of variation.



**Figure 4:** Interaction analysis of the height (cm) of noble garlic plants, cultivar Ito, in Season II, at different seed clove maturation stages (normal, early, and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C and 2 to 4 °C), under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.

In class 2 to 5 bulbs, the highest yields were obtained with late-maturation stage seeds associated with lower vernalization temperatures (-1 to -3 °C, and 1 to 3 °C - Figure 8A). The below-zero temperature, regardless of the maturation stage, provided gains in the productivity of larger (class 6) bulbs, i.e., those with higher commercial value (Figure 8B). Regarding the 'discard' class, the highest

value was obtained when applying the 2 to 4 °C temperature range using late-maturation stage seed cloves (Figure 8C).

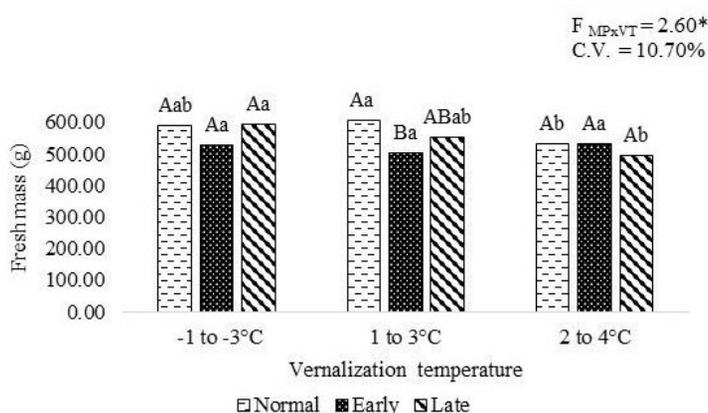
Garlic plants were vigorous and had well-developed leaves throughout the cycle, as indicated by the high plant heights in all three seasons.

The results obtained in this study are similar to those in Wu et al. (2015), who evaluated different garlic

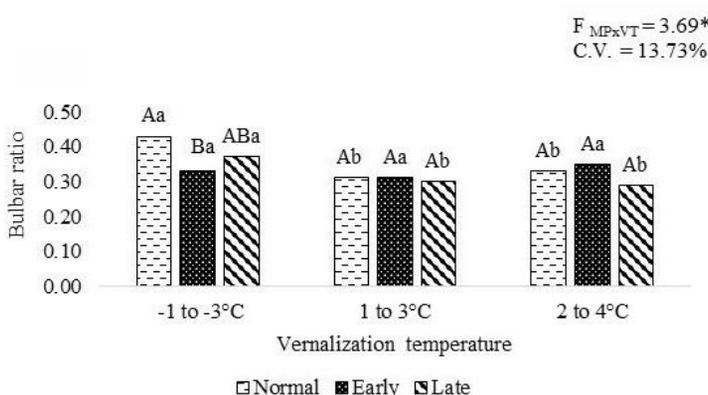
vernalization temperatures (5 °C, 10 °C, and 15 °C) and obtained plants over 70 cm high from 136 DAP; in comparison to those subjected to the other temperatures, the plants subjected to the temperature of 5 °C were significantly larger. The results of the present study also corroborate those of Luz et al. (2022), who evaluated the cultivar Ito under similar experimental conditions.

Seed cloves treated with low temperatures can affect plant growth promoters (growth regulators), which can trigger early plant emergence and subsequently rapid growth (Lucidos et al., 2014), leading to optimal development and vigour levels.

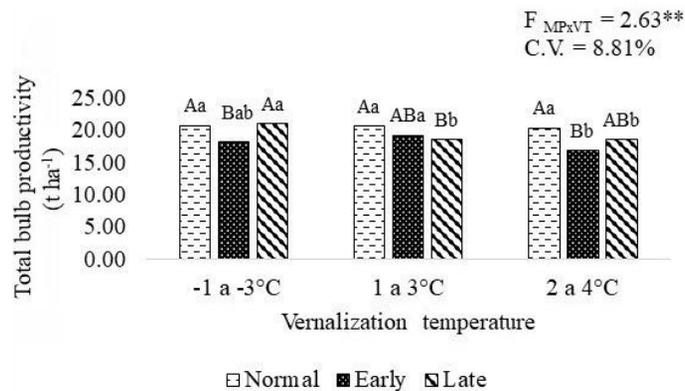
On the other hand, Lopes et al. (2016) evaluated the noble garlic cultivar “Roxo Pérola de Caçador”, whose seed cloves were subjected to a vernalization temperature of 4 °C in three planting seasons in the semiarid region of northeastern Brazil, and obtained a maximum plant height of 55 cm with the longest vernalization time. Thus, each genotype requires specific treatments and can generate physiologically different responses according to location or production environment, day length, temperature, and other environmental factors that benefit bulb production (Michael et al., 2018).



**Figure 5:** Interaction analysis of fresh mass (g) of noble garlic plants, cultivar Ito, in Season II, at different seed clove maturation stages (normal, early, and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C and 2 to 4 °C), under Cristalina GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.



**Figure 6:** Interaction analysis of the bulbar ratio of noble garlic plants, cultivar Ito, in Season II, at different seed clove maturation stages (normal, early, and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C and 2 to 4 °C), under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.



**Figure 7:** Interaction analysis of the productivity of total bulbs of noble garlic plants, cultivar Ito, in Season II, at different seed clove maturation stages (normal, early, and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C and 2 to 4 °C), under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.

**Table 4:** Mean values of total bulb productivity (TBP) (t ha<sup>-1</sup>), class 2 to class 5 bulb productivity (BP2-5), class 6 bulb productivity (BP6), and 'discard' class bulb productivity (BPD) of noble garlic, variety Ito, in Season II at different seed clove maturation stages and vernalization temperatures under the conditions of Cristalina-GO, 2019.

Evaluated factor	TBP	BP2-5 (<35 to 50 mm)	BP6 (51 to 56 mm)	PBD
Maturation Points (MP)				
Normal	20.57 <sup>a</sup>	5.71 <sup>a</sup>	6.06 <sup>a</sup>	7.88 <sup>a</sup>
Early	18.09 <sup>c</sup>	6.30 <sup>a</sup>	5.45 <sup>a</sup>	6.33 <sup>a</sup>
Late	19.33 <sup>b</sup>	6.58 <sup>a</sup>	6.06 <sup>a</sup>	6.70 <sup>a</sup>
F <sub>MP</sub>	12.74*	0.83 <sup>ns</sup>	1.95 <sup>ns</sup>	1.59 <sup>ns</sup>
Vernalization Temperatures (VT)				
-1 to -3 °C	19.98 <sup>a</sup>	5.51 <sup>a</sup>	9.35 <sup>a</sup>	5.11 <sup>b</sup>
1 to 3 °C	19.47 <sup>ab</sup>	6.01 <sup>a</sup>	5.13 <sup>b</sup>	8.33 <sup>a</sup>
2 to 4 °C	18.53 <sup>b</sup>	7.07 <sup>a</sup>	4.00 <sup>b</sup>	7.46 <sup>a</sup>
F <sub>VT</sub>	4.42*	2.67 <sup>ns</sup>	26.22*	6.73*
F <sub>MPxVT</sub>	2.63*	1.41 <sup>ns</sup>	1.83 <sup>ns</sup>	0.89 <sup>ns</sup>
C.V. (%)	8.81	38.49	43.78	45.06

Means followed by distinct lowercase letters in the column differ by Tukey's test at the 0.05 significance level; \* = significant at the 5% probability level; <sup>ns</sup> = not significant; F<sub>RP</sub> = F calculated for maturation point; F<sub>VT</sub> = F calculated for vernalization temperature; F<sub>MPxVT</sub> = F calculated for the interaction maturation point x vernalization temperature; C.V. = coefficient of variation.

Except for Season II, which involved more vigorous growth, the use of lower temperatures during vernalization yielded plants with high biomass; in the other seasons, the values for plant biomass were very similar and did not differ between treatments.

For Dufoo-Hurtado et al. (2015), conditioning garlic seed cloves at low temperatures (5 °C) for five weeks affected different metabolic pathways and

physiological processes, and the latter included cell growth, antioxidant/oxidative status, macromolecular transport, protein folding, and transcription regulation. In turn, these factors triggered fundamental metabolic pathways, which included protein biosynthesis and quality control systems, photosynthesis, photorespiration, energy production, and carbohydrate and nucleotide metabolism.

**Table 5:** Mean plant height, fresh mass and bulbar ratio values of noble garlic plants, cultivar Ito, in Season III, at different seed clove maturation stages and vernalization temperatures under the conditions of Cristalina-GO, 2019.

Evaluated factors	Height (cm)	Fresh mass (g)	Bulbar ratio
Maturation Points (MP)			
Normal	79.01 <sup>a</sup>	356.51 <sup>a</sup>	0.23 <sup>a</sup>
Early	77.09 <sup>a</sup>	352.16 <sup>a</sup>	0.23 <sup>a</sup>
Late	79.17 <sup>a</sup>	348.90 <sup>a</sup>	0.23 <sup>a</sup>
F <sub>MP</sub>	3.20 <sup>ns</sup>	0.14 <sup>ns</sup>	0.19 <sup>ns</sup>
Vernalization Temperatures (VT)			
-1 to -3 °C	77.88 <sup>a</sup>	363.90 <sup>a</sup>	0.26 <sup>a</sup>
1 to 3 °C	77.67 <sup>a</sup>	341.09 <sup>a</sup>	0.22 <sup>b</sup>
2 to 4 °C	79.73 <sup>a</sup>	352.57 <sup>a</sup>	0.21 <sup>b</sup>
F <sub>VT</sub>	3.07 <sup>ns</sup>	1.27 <sup>ns</sup>	26.94 <sup>*</sup>
F <sub>MPxVT</sub>	1.89 <sup>ns</sup>	1.78 <sup>ns</sup>	1.26 <sup>ns</sup>
C.V. (%)			

Means followed by distinct lowercase letters in the column differ by Tukey's test at the 0.05 significance level; \* = significant at the 5% probability level; <sup>ns</sup> = not significant; F<sub>MP</sub> = F calculated for maturation point; F<sub>VT</sub> = F calculated for vernalization temperature; F<sub>MPxVT</sub> = F calculated for the interaction maturation point x vernalization temperature; C.V. = coefficient of variation.

As observed, higher bulbar ratios were attained in plants submitted to below-zero vernalization temperatures over the evaluation period, mainly associated with the normal maturation point – hence demonstrating that the bulbs could still develop and reach larger diameters until harvesting compared to plants submitted to above-zero temperature ranges.

Similarly, the treatments that yielded the lowest bulbar ratios during the evaluation period were not associated with the highest yields and garlic qualities in the harvesting period. Therefore, there was greater vigour and growth increments; i.e., greater bulb development and growth occurred when subjected to below-zero temperatures, from crop development to harvest.

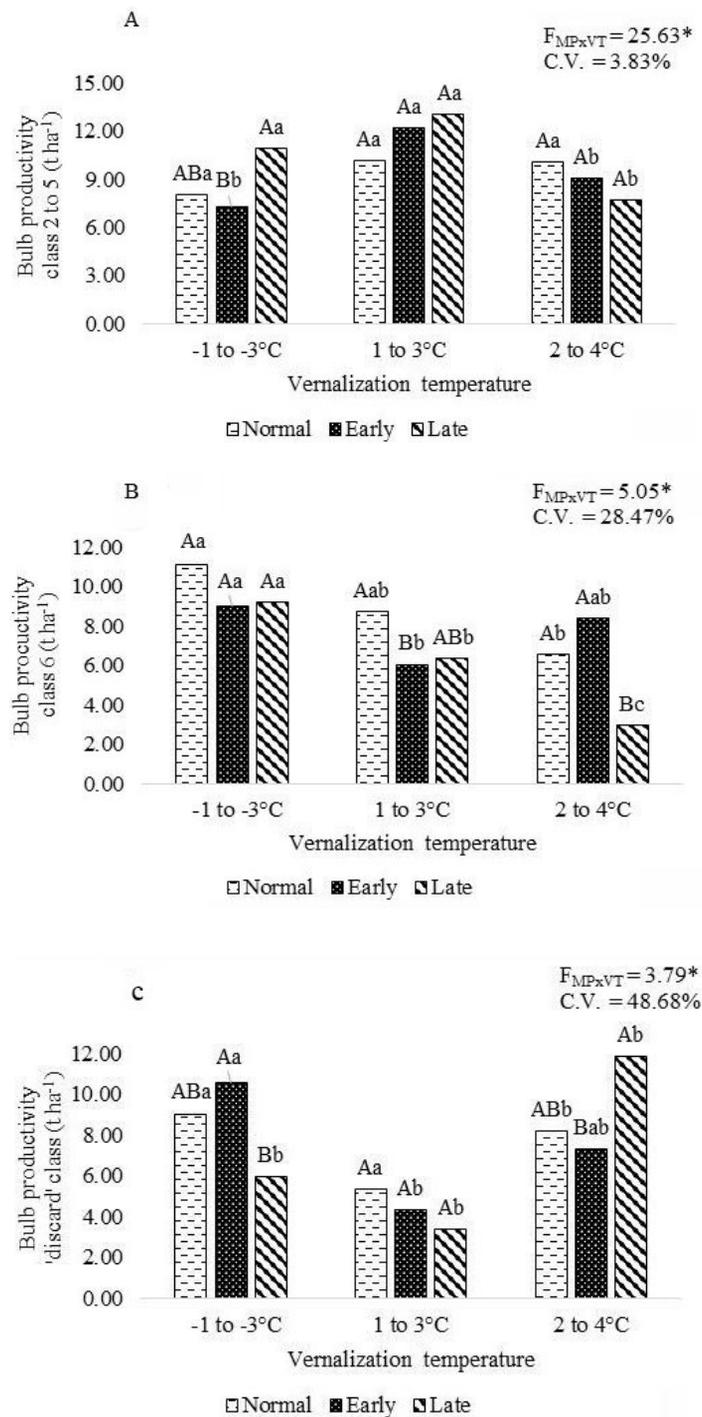
These findings allowed the inference that there was a slower initial development of garlic vernalized at below-zero temperatures compared to the garlic in the other treatments. However, in the final phase of bulb development, the crop cycle accelerated, leading to enhanced bulb development, which was confirmed by the productivity data and corroborated by Luz et al. (2022).

In our study, the average bulb yield was positively influenced by the below-zero vernalization temperature. The averages of Seasons II and III were much higher

**Table 6:** Mean values of total bulb productivity (TBP) (t ha<sup>-1</sup>), class 2 to class 5 bulb productivity (BP2-5), class 6 bulb productivity (BP6), and 'discard' class bulb productivity (DBP) of noble garlic, variety Ito, in Season III at the different seed clove maturation stages and vernalization temperatures under the conditions of Cristalina-GO, 2019.

Evaluated factors	TBP	BP2-5 (<35 to 50 mm)	BP6 (51 to 56 mm)	DBP
Maturation Points (MP)				
Normal	25.76 <sup>a</sup>	9.43 <sup>a</sup>	8.81 <sup>a</sup>	7.51 <sup>a</sup>
Early	24.74 <sup>ab</sup>	9.54 <sup>a</sup>	7.81 <sup>a</sup>	7.39 <sup>a</sup>
Late	23.85 <sup>b</sup>	10.60 <sup>a</sup>	6.18 <sup>b</sup>	7.06 <sup>a</sup>
F <sub>MP</sub>	7.08 <sup>*</sup>	1.59 <sup>ns</sup>	9.01 <sup>*</sup>	0.10 <sup>ns</sup>
Vernalization Temperatures (VT)				
-1 to -3 °C	27.05 <sup>a</sup>	8.78 <sup>b</sup>	9.77 <sup>a</sup>	8.51 <sup>a</sup>
1 to 3 °C	23.24 <sup>b</sup>	11.82 <sup>a</sup>	7.07 <sup>b</sup>	4.34 <sup>b</sup>
2 to 4 °C	24.06 <sup>b</sup>	8.78 <sup>a</sup>	5.97 <sup>b</sup>	9.13 <sup>a</sup>
F <sub>VT</sub>	31.07 <sup>*</sup>	10.95 <sup>*</sup>	19.59 <sup>*</sup>	12.79 <sup>*</sup>
F <sub>MPxVT</sub>	1.09 <sup>ns</sup>	3.83 <sup>*</sup>	5.05 <sup>*</sup>	3.79 <sup>*</sup>
C.V. (%)	7.11	25.63	28.47	48.68

Means followed by distinct lowercase letters in the column differ by Tukey's test at the 0.05 significance level; \* = significant at the 5% probability level; <sup>ns</sup> = not significant; F<sub>MP</sub> = F calculated for maturation point; F<sub>VT</sub> = F calculated for vernalization temperature; F<sub>MPxVT</sub> = F calculated for the interaction maturation point x vernalization temperature; C.V. = coefficient of variation.



**Figure 8:** Interaction analysis of the productivity of bulbs of class 2 to 5 (A), class 6 (B) and 'Discard' class (C) of noble garlic plants, cultivar Ito, in Season III at different seed clove maturation stages (normal, early and late) and vernalization temperatures (-1 to -3 °C; 1 to 3 °C and 2 to 4 °C) under Cristalina-GO conditions, 2019. Lowercase letters on the bars compare vernalization temperatures at each maturation point, and uppercase letters compare maturation points at each vernalization temperature.

than the national average productivity, which is ca. 13 t ha<sup>-1</sup> (ANAPA, 2022), mainly using the below-zero vernalization temperature. Luz et al. (2022) obtained high productivity values with the cultivar Ito in 2018, also applying vernalization with below-zero temperatures.

Despite the significant features of this technique, the total bulb productivity and, particularly, the class 6 bulb productivity showed a gradual and significant increase in each planting season: on average, there was a 5 t ha<sup>-1</sup> increase from Season I to Season II and more than 10 t ha<sup>-1</sup> from Season I to Season III. It is worth noting that the increase in total bulb yield, unfortunately, was also accompanied by a considerable increase in 'discard' class bulbs in Seasons II and III. Considering that the same treatments and crop management techniques were performed and that the same cultivar was used in all experimental seasons, this result was due to the influence of climatic factors during the cultivation of garlic in the field.

Thus, considering the Asian origin of the garlic species, noble garlic varieties respond well to low environmental temperatures. Even the Ito cultivar, which is characterized as mature compared to the other noble-type garlic, was able to respond positively in terms of productivity gains and bulb quality upon the decrease in average temperature and precipitation during the winter months.

Some varieties may present unusual characteristics regarding the low temperature requirement for optimum productivity. The same variety may also behave differently depending on the region of the country, indicating that the length of the storage period and the vernalization temperature may affect the responses of the garlic plant differently. In addition to their immediate effects, environmental factors also pose long-term effects at each stage of development (Dufoo-Hurtado et al., 2015).

As observed, the treatment with below-zero vernalization temperatures promoted productivity gains on the order of 3 t ha<sup>-1</sup> compared to those of the other treatments in all experimental seasons, confirming the potential of this technique for raising garlic crop yields. For Lopes et al. (2016), below-zero vernalization temperatures also resulted in larger bulbs, which better met consumer demands. Thus, both bulb size and number are essential considerations in garlic marketing, as larger bulbs reach higher prices.

Unlike in the present study, vernalization of garlic plants with low temperatures may not result in significant crop gains. For instance, Wu et al. (2015) obtained cycle maturity of cultivar G064, reducing the cycle from 250 days to 212 days to harvest, followed by an increase in

the bolting rate compared to the control treatment (no vernalization), after subjecting garlic plants to 5 °C for 60 days. This was followed by lower bulb weights and yields.

The vernalization treatment significantly increased the bolting rate in plants at all ages evaluated, and the youngest plants produced the highest bolting rates at 10 °C/5 °C (day/night). Vernalization also reduced vegetative growth in garlic (Wu et al., 2016).

Finally, for the garlic cultivar Ito, the application of below-zero vernalization temperatures to seed cloves was very advantageous, with positive effects on development characteristics and bulb productivity at the end of the cycle. Low vernalization temperatures provided the necessary physiological stimuli for garlic to reach its productive potential under unfavourable growing conditions (Luz et al., 2022).

## CONCLUSIONS

Below-zero vernalization temperatures provide significant increases in bulb yield and quality of the Ito cultivar under Cerrado conditions; the normal maturation point promotes significant increases in total bulb yield; additional studies with below-zero vernalization temperatures should be conducted in other regions of the country to confirm the potential of its application and recommendation for other noble garlic varieties, of great economic importance.

## AUTHOR CONTRIBUTIONS

Conceptual idea: Luz, J.M.Q., Oliveira, C.I.G.; Methodology design Luz, J.M.Q., Oliveira, C.I.G., Silva, S.M.; Data collection: Oliveira, T.G.; Data analysis and interpretation: Luz, J.M.Q., Oliveira, C.I.G., Silva, S.M., Castoldi, R.; and Writing and editing: Luz, J.M.Q., Silva, S.M., Castoldi, R.

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