Article

Productivity and Quality of Garlic Produced Using Below-Zero Temperatures When Treating Seed Cloves

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Abstract: Garlic cultivation has increased in Brazil in recent years primarily due to the adoption of appropriate technologies, such as the use of low temperatures during the maintenance of garlic seeds to overcome dormancy. However, there is no information on the effects of below-zero temperatures when treating seed cloves on garlic development. Therefore, this study's objective was to evaluate the effects of below-zero temperatures and different visual indices of overcoming dormancy (VIDs) on garlic performance in Cristalina County, Goiás State, Brazil. The experiment was conducted in a randomized block design with four replicates in a 2 × 3 factorial scheme: with two VIDs (40% and 60%), and three temperature ranges (−1 to −3 °C, 1 to 3 °C, and 2 to 4 °C). Vegetative characteristics, bulbar ratios, and commercial bulb yields were evaluated. The results showed that below-zero temperatures resulted in better vegetative characteristics. The yield increased after using below-zero temperatures to treat seed cloves with a VID of 60%. The garlic produced had a higher market value. We concluded that there is an enormous potential for using below-zero temperatures to improve the performance of the “Ito” garlic variety, and more studies should be conducted with other varieties of economic importance to enhance Brazilian garlic production.

Keywords: *Allium sativum* L.; below-zero temperature; visual index of overcoming dormancy; noble variety

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1. Introduction

Garlic crops (*Allium sativum* L.) are economically important in Brazil, with a cultivation area of 16,000 hectares and production of approximately 290 thousand tons [1].

The nationwide production of garlic only accounts for 45% of consumption, with the other 55% being imported from China (46%), Argentina (39%), Spain (12%), and other countries (3%) [2].

Therefore, to compete with imported garlic, Brazilian growers should achieve greater productivity through the implementation of proper management and innovative technologies, such as the use of improved cultivars, and the adoption of virus-free clove seeds [3].

Because of its origin (Central Asia—Afghanistan), garlic requires low temperatures and photoperiods (13–14 h) to induce bulbil differentiation. However, after undergoing mutation, the noble garlic planted in Brazil's southern regions does not demand low temperatures, and the region's prevailing temperatures are sufficient for bulbil differentiation [4].

The national garlic yield has gradually increased because of new technologies and more productive garlic varieties, allowing crop expansion to new planting areas in some regions [5]. Recently, the adoption of low temperature practices has enabled the planting of noble garlic cultivars from Argentina and southern Brazil in regions where the plant's thermoperiodic requirements are not met [6]. There is a possibility for growers to produce commercial crops in the off-season or to supply fresh-cut flowers year-round by controlling the growth temperature or plant age [7].

The most striking effect of low temperature treatment is the increase in earliness, especially in cultivars with stricter low-temperature requirements for plant development. Yet, researchers have conflicting opinions regarding the effects of cold conditioning on garlic yield. While some studies have observed increases, others have reported inconsiderable differences and depressed yields [8].

The storage of bulbs at temperatures from 0 to 10 °C for 2 months before planting accelerates the plant cycle and possibly supplies the initial climatic requirements of garlic. The exposure of bulbs to low temperatures changes their hormonal balance and promotes early plant development [8].

It is necessary to provide reserve leaf (differentiation management), enabling the crop to reach a cycle capable of good productivity and bulb quality for each location, planting time, and genetic material cultivated.

Several studies concluded that even before current practices (such as low temperature treating garlic seeds), it was necessary for the garlic to reach a visual index of overcoming dormancy (VID) of at least 25%; thus, by the time of planting, the index should be equal to or greater than 70%. Therefore, dormancy is not a problem because plants demand a low photoperiod for bulbification.

In Brazil, the practice of using low temperatures to treat garlic clove seeds is related to the induction of bulbification and is called "vernalization". Under Brazilian conditions, flowering is not normally expected, however, the crop produces the commercial bulb. In some regions with lower temperatures, flowering can occur, when the plants produce a small floral structure (called "Pito" in Brazil), but this does not influence the bulbification process. Therefore, under Brazilian conditions the use of low temperatures during garlic seed treatment is more important in inducing garlic bulbification than in flowering.

Meanwhile, with some variations, Brazilian garlic growers have been working with available data for each garlic variety and planting location, considering the duration, temperature, and relative humidity for low temperatures to treat seed cloves. In this context, when reducing the temperature range, the treatment duration must also be reduced, for the same objective of inducing bulbification.

Considering the diverse information on low temperatures practices since 2015, growers and technicians from São Gotardo County have been conducting trials with below-zero temperatures for the treatment of garlic bulbs without a scientific basis.

Further trials conducted in 2017 produced better yields and bulb quality, revealing that below-zero temperatures were a viable option.

However, there is a lack of technical and scientific information regarding the application of below-zero temperatures, which is a relatively new technique. Therefore, this study aimed to evaluate the effects of below-zero temperatures when treating seed cloves, combined with different VIDs, on garlic productivity and bulb quality.

2. Materials and Methods

2.1. Experimental Area

The experiment was conducted at Agricola Wehrmann, located in the rural area of Cristalina County, State of Goiás, Brazil (17°02'45'' S, 47°45'24'' W), at an altitude of 980 m.

The soil is classified as a red-yellow latosol of medium texture, with a nonuniform relief along the plane. According to the Koppen classification, the area is characterized as Aw (tropical, hot, and humid areas with cold and dry winters). The agricultural region is in

the Central Plateau mesoregion of eastern Goiás State, with a precipitation of 1500 mm and an annual average temperature of 20.9 °C.

2.2. Experimental Design

The experiment was performed in 2018, in a randomized block design (DBC) using a 3×2 factorial scheme with four replicates: three temperature ranges to treat garlic seed cloves (−1 to −3 °C, 1 to 3 °C, and 2 to 4 °C) and two levels of VID, 40% and 60%. The experiment was carried out in triplicate at three different planting times (26 March, 13 April, and 4 May). During the experimental period, meteorological conditions were obtained for each planting period. For the first planting, the maximum temperature ranged from 21 to 26 °C, the minimum temperature ranged from 8 to 20 °C, 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 a median value of 11 h and 10 min during bulb enlargement. For the second planting, the maximum temperature ranged from 19 to 30 °C, the minimum temperature ranged from 8 to 19 °C, the accumulated precipitation was 79 mm, and the photoperiod ranged from 11 h and 4 min to 11 h and 28 min during plant growth, with a median value of 11 h and 14 min during bulb enlargement. For the third planting, the maximum temperature ranged from 18 to 30 °C, the minimum temperature ranged from 10 to 18 °C, the accumulated precipitation was 32.4 mm, and the photoperiod ranged from 11 h and 27 min to 11 h and 50 min during the plant growth, with an average of 11 h and 27 min during bulb enlargement. These conditions are typical for the autumn–winter season in Brazil.

Each experimental plot was 1.2 m wide and 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 garlic seeds used belong to the “Ito” variety, a noble garlic variety characterized as round, carrying uniform and vigorous bulbs, with a white outer tunic containing seven–ten bulbils per bulb with a purple film [9]. This variety originated in southern Brazil and is the result of the selection of cultivars made in the state of Santa Catarina, from the Chonan variety, maintained by a Japanese immigrant agricultural technician, Takashi Chonan. This variety eventually spread to all producing regions.

The bulbs used had a commercial classification of 6, were 51–55 mm in diameter, and were from the 3rd generation of commercial crops. The garlic for planting was stored in a cold chamber for 15 and 25 days to reach 40% and 60% VID, respectively. The relative humidity in the chamber was between 60% and 70%. Subsequently, the bulbs were subjected to 50 days of treatment with different temperatures.

The VID was obtained from the longitudinal length of the sprouting leaf (SL) and the reserve leaf (RL) in a longitudinal section along its convex face, expressed as a percentage ($\text{VID} = (\text{SL}/\text{RL}) \times 100$) [10].

2.3. Temperature Treatments

The garlic seed cloves’ cold treatments were applied in three cold chambers, adjusted to different temperature ranges: −1 to −3 °C, 1 to 3 °C, and 2 to 4 °C. The dimensions of the cold chambers were $2 \times 2 \times 2$ m, built with insulating material in a metallic structure.

An air-cooling refrigeration set was installed in each chamber with a single-phase compressor and fan. The cold chambers had forced-air fans, an electric control panel, an electronic temperature gauge, and a defrost controller. There were also refrigerant gas, high- and low-pressure switches, copper pipes and connectors, a thermostatic expansion valve, accessories for thermal and pipe insulation, a revolving refrigerator door made of polyurethane, measuring 1.80×0.80 m, which was covered in a “pre-painted” and treated steel plate, a curtain flexible door with stainless steel support, and a mini exhaust fan for air renewal and control. Next to each chamber, a portable dehumidifier was installed to maintain a relative humidity of approximately 65%, to prevent the dehydration of the

bulbs (in case of very low humidity) or the appearance of pathogenic fungi (in case of high humidity). During this process, the bulbs were kept in the dark.

2.4. Vegetative Assessments

The relative index of chlorophyll (RIC) was determined using a SPAD-502 chlorophyll meter (Spectrum Technologies, Aurora, IL, USA) to sample the central part of fully expanded and physiologically mature leaves. The length of the largest leaf was measured from the ground level to its upper end, 38 and 77 days after planting (DAP).

The nutrient content of the garlic leaves was evaluated according to previously described methods [11]. Consequently, the youngest fully developed leaf was collected, which corresponded to the fourth or fifth leaves. Twenty leaves were collected from each plot.

Bulbar ratios were measured at 73 and 94 DAP. This variable expresses the degree of bulb development [12]. It was determined by measuring the diameter of the pseudostem 5 cm above the ground and dividing it by the diameter of the median part of the bulb. Finally, the number of fully developed leaves was determined by counting the leaves at 75 DAP.

2.5. Harvesting and Classification

Harvesting was conducted during the garlic crop senescence phase, with approximately 4 remaining fresh leaves (Table 1). The leaves and bulbs of plants in the central double row (useful plot) were harvested and stored in a protected environment for 30–45 days to remove excessive humidity (“curing” process). The garlic bulbs were cleaned, separated from the pseudostem, and the roots and dirty films were removed.

Table 1. Cycle of garlic crop (days) under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	VID (%)	Harvest (Days after Planting—DAP)		
		Temperature Ranges		
		2 to 4 °C	1 to 3 °C	−1 to −3 °C
March 26	40	106	106	115
	60	102	104	115
April 13	40	113	113	122
	60	113	113	122
May 4	40	118	118	130
	60	118	118	130

The bulbs were weighed, counted, and classified commercially. The classification range used was from 2 to 8, 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. This is the commercial classification used for postharvest Brazilian garlic.

Based on the bulbs’ weights, the crop productivity (t ha^{-1}) was estimated by sampling ten bulbs in classes 6 to 8 per plot.

2.6. Data Analysis

The data were tested for normality and homogeneity of variance using the Shapiro–Wilk and Levene’s tests, respectively. The level of significance was set at $p < 0.01$. An analysis of variance (ANOVA) was performed using the F test to determine significance levels at $p < 0.05$ and $p < 0.01$. The means were compared using Tukey’s test (5% probability level). To assess the productivity, a joint analysis was performed for different planting seasons.

3. Results

3.1. Relative Index of Chlorophyll (RIC)

Assessments at 38 and 77 DAP for the RIC showed no significant interaction between temperatures ranges and VID for the three planting times (Table 2). The highest values were obtained for the first and second planting times at 77 DAP using a VID of 60%. For the second planting time, the lower temperature range showed a significant difference from other treatments, with higher values at 38 DAP.

Table 2. Relative index of chlorophyll (RIC) in garlic plants of the Ito variety at 38 and 77 days after planting (DAP), under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	38 DAP				77 DAP			
	VID (%)	Temperature Ranges						
		2 to 4 °C	1 to 3 °C	−1 to −3 °C	2 to 4 °C	1 to 3 °C	−1 to −3 °C	Means
26 March	40	63.57	65.37	64.54	83.29	85.87	86.62	85.26 B
	60	64.66	63.71	63.15	130.66	130.90	125.25	128.93 A
13 April	40	65.86	69.73	69.54	79.75	92.42	93.81	88.66 B
	60	60.70	65.61	76.59	88.58	97.52	100.31	95.47 A
	Means	63.28 b	67.49 ab	73.06 a	84.17	94.73	97.03	
4 May	40	72.86	63.46	75.82	64.67	62.69	65.64	64.13 A
	60	74.86	74.12	83.95	62.69	63.04	65.48	63.73 A
CV (%) time 1			5.92				14.52	
CV (%) time 2			8.43				12.14	
CV (%) time 3			14.62				4.20	
Fc time 1			0.7343 ^{ns}				0.0097 *	
Fc time 2			0.0130 *				0.9498 ^{ns}	
Fc time 3			0.1244 ^{ns}				0.6489 ^{ns}	

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation; Fc: calculated F value; *: significant; ^{ns}: not significant.

All plants had ideal nutrient levels (Table 3).

Table 3. Levels of foliar nutrients in garlic plants, under different temperatures ranges and visual index of overcoming dormancy (VID).

26 March												
VID (%)	Temperature Ranges (°C)	Macronutrients (g kg ^{−1})						Micronutrients (mg kg ^{−1})				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
40	−1 to −3 °C	39	5	34	13	4	15	42	61	201	28	31
	1 to 3 °C	46	5	34	12	5	13	60	66	234	34	38
	2 to 4 °C	41	5	35	13	5	15	61	63	341	30	40
60	−1 to −3 °C	46	6	35	12	4	15	64	48	208	27	39
	1 to 3 °C	34	4	31	12	5	12	42	81	326	42	42
	2 to 4 °C	42	5	34	13	5	15	50	86	216	38	50
13 April												
40	−1 to −3 °C	38	5	20	6	4	4	48	160	184	162	98
	1 to 3 °C	39	5	24	6	4	5	38	244	206	181	122
	2 to 4 °C	38	4	25	7	5	4	39	207	151	143	104
60	−1 to −3 °C	38	5	24	5	4	4	42	168	175	142	99
	1 to 3 °C	37	5	25	5	4	4	34	208	187	160	114
	2 to 4 °C	37	6	25	8	5	6	43	221	680	166	118

Table 3. Cont.

26 March												
VID (%)	Temperature Ranges (°C)	Macronutrients (g kg ^{−1})						Micronutrients (mg kg ^{−1})				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
4 May												
40	−1 to −3 °C	50	5	24	5	2	3	35	84	142	31	64
	1 to 3 °C	32	3	26	5	2	2	43	141	148	21	45
	2 to 4 °C	40	5	28	5	3	2	33	151	117	15	50
60	−1 to −3 °C	37	4	29	7	3	2	36	151	110	22	58
	1 to 3	35	4	27	7	3	2	44	151	122	52	53
	2 to 4	35	4	29	6	3	2	44	189	101	71	68

3.2. Plant Height

A significant interaction between temperatures ranges, and VID was observed for plant height at only two time points (Table 4). At 38 days after the second planting, the highest values occurred at a VID of 40% at the highest temperature. When the culture indicated an advanced cycle 77 days after the first planting, the greatest heights occurred with a VID of 60%, combined with a treatment at below-zero temperatures.

Table 4. Height of garlic plants of the Ito variety, at 38 and 77 days after planting (DAP), under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	38 DAP				77 DAP		
	VID (%)	Temperature Ranges					
		2 to 4 °C	1 to 3 °C	−1 to −3 °C	2 to 4 °C	1 to 3 °C	−1 to −3 °C
26 March	40	56.25	57.25	59.00	62.16 bB	61.5 bB	66.45 aB
	60	57.25	58.00	60.25	66.16 aA	67.71 aA	70.125 aA
	Means	56.75 b	57.62 ab	59.62 a	72.83	72.79	70.04
13 April	40	70.50 aA	70.60 aA	64.02 bB	73.24	74.82	76.00
	60	65.45 bB	69.25 aA	68.97 aA	73.04	72.89	73.81
4 May	40	56.12	57.50	69.00	57.90	58.67	70.48
	60%	59.09	60.22	65.91	61.95	62.33	67.62
	Means	57.58 b	59.06 b	67.50 a	60.11 b	60.31 b	69.05 a
CV (%) _{time 1}		3.50				3.38	
CV (%) _{time 2}		2.73				4.26	
CV (%) _{time 3}		7.10				6.97	
Fc _{time 1}		0.035 *				0.0012 *	
Fc _{time 2}		0.003 *				0.2530 ^{ns}	
Fc _{time 3}		0.008 *				0.0013 *	

Means followed by distinct letters, lowercase in rows, uppercase in columns, significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant; ^{ns}: not significant.

The treatment at below-zero temperatures resulted in taller plants, with significant differences at the third planting time, in both evaluations. The highest average heights were 71 and 76 cm at 38 and 77 DAP, respectively, 14 cm higher than the plants submitted at the commonly used temperature (4 °C).

3.3. Bulbar Ratio

A significant interaction between VID and temperature ranges was observed 73 days after the first planting (Table 5), with the highest ratios obtained with the treatment at below-zero temperatures. For other periods, in both evaluations, below-zero temperatures also resulted in relatively high bulbar ratios.

Table 5. Bulbar ratios of garlic plants at 73 and 94 days after planting (DAP), under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	73 DAP				94 DAP		
	VID (%)	Temperature Ranges					
		2 to 4 °C	1 to 3 °C	−1 to −3 °C	2 to 4 °C	1 to 3 °C	−1 to −3 °C
26 March	40	0.18 bA	0.14 cA	0.21 aA	0.24	0.23	0.29
	60	0.18 aA	0.16 aA	0.18 aB	0.26	0.23	0.29
	Means	0.18	0.15	0.20	0.25	0.23	0.29
13 April	40	0.33	0.34	0.40	0.19	0.23	0.31
	60	0.33	0.32	0.42	0.19	0.21	0.29
	Means	0.33 b	0.33 b	0.41 a	0.19 b	0.22 b	0.30 a
4 May	40	0.37	0.39	0.53	0.38	0.32	0.50
	60	0.37	0.37	0.50	0.34	0.34	0.49
	Means	0.37 b	0.38 b	0.52 a	0.36 b	0.33 b	0.50 a
CV (%) _{time 1}		8.69				18.28	
CV (%) _{time 2}		6.84				11.98	
CV (%) _{time 3}		11.88				13.11	
Fc _{time 1}		0.0173 *				0.9246 ^{ns}	
Fc _{time 2}		0.000 *				0.000 *	
Fc _{time 3}		0.000 *				0.000 *	

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant; ^{ns}: not significant.

3.4. Leaves

The highest number of leaves per plant was obtained at the third planting time (Table 6), but significant interactions between the variables were observed only for the second planting time. Here, the highest number of leaves was observed in plants treated with below-zero temperatures combined with a VID of 60%, which demonstrated comparatively greater plant vigor and performance. There was no incidence of secondary growth in this experiment.

Table 6. Average number of leaves of garlic plants, Ito variety, at 75 days after planting (DAP), under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	VID (%)	Temperature Ranges		
		2 to 4 °C	1 to 3 °C	−1 to −3 °C
26 March	40	8.29	8.79	8.79
	60	8.04	8.25	8.33
13 April	40	8.58	8.83	9.66
	60	8.91	8.59	9.37
	Means	8.75 b	8.71 b	9.52 a
13 May	40	8.75	8.91	8.91
	60	9.25 aB	9.33 aB	9.75 aA
CV (%) time 1		7.32		
CV (%) time 2		4.69		
CV (%) time 3		6.04		
Fc time 1		0.8884 ^{ns}		
Fc time 2		0.0023 *		
Fc time 3		0.0085 *		

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant; ^{ns}: not significant.

3.5. Productivity

Bulb productivity values confirmed the results previously obtained with other variables, and it was possible to observe significantly high productivity in treatments with below-zero temperatures at all planting times (Table 7).

Table 7. Productivity of garlic (t ha^{-1}) plants of the Ito variety, under different temperatures ranges and visual index of overcoming dormancy (VID).

Planting Time	VID (%)	Temperature Ranges		
		2 to 4 °C	1 to 3 °C	−1 to −3 °C
26 March	40	12.81 bB	11.36 cB	16.75 aB
	60	14.35 bA	15.01 bA	18.08 aA
13 April	40	16.93 aA	16.20 aB	16.73 aB
	60	16.45 bA	17.83 aA	18.76 aA
4 May	40	10.87	11.62	15.56
	60	11.42	11.12	16.55
	Means	11.14 b	11.37 b	16.05 a
	CV (%) time 1			3.81
	CV (%) time 2			4.01
	CV (%) time 3			5.75
	Fc time 1			0.001 *
	Fc time 2			0.005 *
	Fc time 3			0.000 *

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant.

A significant interaction between the factors was observed for the first and second planting times, with the highest productivity obtained with the below-zero temperature range and the highest VID.

In Table 8, a significant interaction can be observed by comparing planting times and temperature ranges. The highest productivity was obtained at the second planting time, which was also associated with the lowest temperature treatment.

Table 8. Total productivity (t ha^{-1}) of garlic plants, Ito variety, under different temperatures ranges and planting times.

Temperature Ranges	Planting Time		
	26 March	13 April	4 May
2 to 4 °C	13.62 bB	16.62 aB	11.12 cB
1 to 3 °C	13.12 bB	17.00 aA	11.37 bB
−1 to −3 °C	17.30 aA	17.75 aA	16.12 bA
Fc temperature × time		0.0292 *	
CV (%)		4.01	

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant.

3.6. Classification

The results for bulb classification showed significant differences among treatments. The largest quantity of garlic bulbs of smaller size and inferior commercial classification (2–5) were obtained at higher temperature range during the first and second planting times (Table 9). For the third planting time, the values were similar regardless of the interaction between factors.

Table 9. Bulb productivity (t ha^{-1}) according to classifications 2 to 5 and 6 to 8, temperatures ranges, and visual index of overcoming dormancy (VID).

Planting Time	VID (%)	Classes 2 to 5 (t ha^{-1})		
		Temperature Ranges		
		2 to 4 °C	1 to 3 °C	−1 to −3 °C
26 March	40	10.47	11.19	6.52
	60	12.26	12.39	7.42
	Means	11.36 a	11.79 a	6.97 b
13 April	40	11.65	11.91	10.88
	60	11.71 a	10.11 b	9.45 c
4 May	40	10.15 bB	11.09 aA	10.92 abA
	60	11.11 aA	10.44 aA	10.49 aA
Classes 6 to 8 (t ha^{-1})				
26 March	40	2.33	0.17	10.23
	60	2.09	2.62	10.65
	Means	2.21 b	1.39 b	10.44 a
13 April	40	5.28 aA	4.29 aB	5.84 aB
	60	4.74 bA	7.72 aA	9.30 aA
4 May	40	0.72	0.52	5.63
	60	0.31	0.68	5.06
	Means	0.51 b	0.60 b	5.35 a
Bulbs of classes 2 to 5: CV (%) time 1 : 6.53; CV (%) time 2 : 10.83; CV (%) time 3 : 4.11; Fc time 1 : 0.4062 ^{ns} ; Fc time 2 : 0.0670 ^{ns} ; Fc time 3 : 0.0045 * Bulbs of classes 6 to 8: CV (%) time 1 : 22.27; CV (%) time 2 : 25.44; CV (%) time 3 : 39.52; Fc time 1 : 0.000 *, Fc time 2 : 0.0345 *, Fc time 3 : 0.000 *				

Means followed by distinct letters, lowercase in rows, uppercase in columns significantly differ for each planting time; CV (%): coefficient of variation value; Fc: calculated F value; *: significant; ^{ns}: not significant.

The treatment at below-zero temperatures provided the highest yields of higher-quality bulbs (classification 6 to 8) in the first and third planting times.

4. Discussion

The garlic plants showed adequate leaf nutrient levels that were higher than the values obtained by Boutasknit et al. [13], who evaluated different sources of nutrients for the cultivation of garlic with good productivity. The leaf nutrient levels also presented appropriate values, according to Resende and Filho [14], and as shown by the RIC values.

The assessment of chlorophyll levels revealed that garlic plants presented acceptable physiological parameters without cell damage in the treatments.

The results obtained for plant height were superior to those reported by Lopes et al. [15], who also evaluated different temperature treatments periods, with a significant increase only observed for the third planting (23 June), with a maximum height of 49.14 cm when garlic was treated for 64 days at a temperature of 4 °C.

Resende et al. [16] reported greater plant heights in the first growing season (23 March) than those in April and May, with the Roxo Pérola de Caçador and Quitéria varieties. The differences in the means observed compared with other studies may be because of different evaluation times, as in the present study, where evaluations were performed at different times. Additionally, the observed differences could be due to different physiological responses of the cultivars and edaphoclimatic conditions in the evaluated regions.

Some varieties may have peculiar characteristics regarding the need for low temperatures for optimal productivity. The same variety can also behave differently depending on the region of growth in the country. This indicates that the storage period's length and the low temperature have different effects on the responses of garlic plants. In addi-

tion to their immediate effects, environmental factors also have long-term effects at each developmental stage [8].

Regarding the bulbar ratio, the higher values obtained by the treatment at below-zero temperatures showed that the bulbs could still attain a larger size compared with treatment at above-zero temperatures. The treatments with the lowest bulbar ratio of 94 DAP did not necessarily lead to the most productive harvest, implying that there was greater bulb development in treatments with below-zero temperatures from the development of the crop to its harvesting stage.

Preliminary tests have shown a slower initial development of garlic conditioned at below-zero temperatures compared with traditional practice. However, in the final stage of bulb development, there was an acceleration in the crop cycle (see Table 1), leading to greater bulb development, as confirmed by the productivity data (see Table 7).

Our findings for the number of leaves were similar to those obtained by Soares et al. [17], who evaluated the planting times and production of different garlic varieties in Brazil's northern region.

The average productivity with below-zero temperature was higher than the average values for Brazil (18 t ha^{-1}) and Minas Gerais State (15.8 t ha^{-1}) [18]. Similar results were found by Lopes et al. [15], who evaluated the effects of planting times and periods of low temperature.

Therefore, it was confirmed that there was a significant increase in productivity by more than 4 t ha^{-1} compared with higher temperatures treatment, indicating the potential of below-zero temperature for providing higher yields of garlic crop in the study region.

Brazil can reduce its garlic imports by increasing production and adopting below-zero temperature treatment, allowing producers to extend planting areas and consequently increase production. The use of below-zero temperatures is a pioneering technique that opens prospects for other countries with edaphoclimatic conditions similar to the Brazilian savanna to become garlic producers.

When compared with Brazilian varieties, garlic cultivars of Asian origin may not demonstrate the same results using low temperatures. Wu et al. [7] studied cv G064 (Chinese garlic, bolting type, 250 days, 13 h photoperiod, 20°C to bulbification) and found that subjecting garlic cloves to low temperatures (5°C) for 60 days significantly increased the bolting rate and decreased the number of days until harvest (212 days), compared with a control (22°C , 20 days of treatment and 234 days to harvest). In addition, cold treatment reduced the plant growth period, mean bulb weight, and bulb yield compared to plants grown in the open field under natural conditions. One possible reason for the decreased yield could be early ripening in longer cold treatments that shortens the growth period compared with shorter cold treatments.

Dufoo-Hurtado et al. [8] reported that the low-temperature conditioning of "seed" cloves for 5 weeks at 5°C affected various metabolic pathways and physiological processes. The affected physiological processes included cellular growth, antioxidative/oxidative state, macromolecule transport, protein folding, and transcription regulation. The affected metabolic pathways included protein biosynthesis and quality control systems, photosynthesis, photorespiration, energy production, and carbohydrate and nucleotide metabolism.

Low temperature treatment significantly increased the bolting rate in plants at all tested ages, and the youngest plants produced the highest bolting rates at $10/5^\circ\text{C}$ (day/night). Low temperature treatment reduced vegetative growth in garlic. Peroxidase and superoxide dismutase activities also increased, with the greatest activities observed in plants treated at the lowest temperatures [7].

Therefore, below-zero temperature improves bulb yield in Brazil as it enables the garlic crop to meet its requirements in Brazil's particular edaphoclimatic conditions.

Concerning garlic yield and classification, the results revealed that below-zero temperatures resulted in the larger bulbs preferred by consumers. Fewer cloves are also preferable. Both bulb size and clove number are essential considerations during the garlic commercialization process as bigger bulbs fetch higher prices [15].

For the Ito variety, below-zero temperatures have proven to be advantageous, with positive effects on development characteristics and bulb productivity. The cold temperatures associated with below-zero temperatures provide the physiological stimuli necessary for garlic to reach their production potential.

Additionally, garlic seeds conditioned at below-zero temperatures present root differentiation not evident in those conditioned at above-zero temperatures. Significant root development makes plants susceptible to damage during manual planting.

Furthermore, garlic conditioned at below-zero temperatures exhibits a slower initial development, most likely due to less developed roots at the time. However, at 38 DAP, the plants had equal or even greater height than plants conditioned at above-zero temperatures (Table 4).

It is also plausible that garlic seeds treated with below-zero temperatures could have a lower active viral load than garlic seeds subjected to above-zero temperatures, leading to better plant development and productivity.

The increase in the cycle probably occurred owing to the reasons already discussed, and the longer cycle allowed a substantial accumulation of photoassimilates, providing greater productivity.

The explanations given here are based on observations not quantified in the current work; therefore, they should be the subject of future research addressing the inferences made. It is also important to conduct similar studies with other varieties of economic importance to improve the productivity of other noble garlic varieties in Brazil.

5. Conclusions

Below-zero temperatures during the treatment of seed cloves favor the development of garlic of the Ito variety, providing greater plant height, bulbar ratio, and number of leaves.

A higher productivity and quality of the bulbs can be achieved by combining below-zero temperatures with a VID of 60%.

The results demonstrated the potential for increasing noble garlic production using below-zero temperatures when treating seed cloves.

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