

## Agronomic performance, flowering, physicochemical characteristics and genetic divergence in garlic accessions from Brazil

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### Abstract

The evaluation of garlic accessions is important in the search for superior, adapted and flowering cultivars for botanical seed production. The objective of this work was to assess the agronomic performance, flowering capacity and genetic divergence of 13 experimental garlic accessions bred in Brazil. The experiment was laid out as a completely randomized block design with four replications at Lavras, Minas Gerais, Brazil from May to October 2018. A total of 11 accessions were experimental and two were control varieties, Quitéria and Caçador. The control varieties are commercially produced in Brazil and the 11 experimental accessions are elite. The emergence, emergence speed index, plant height, percentage of overcrowding, average weight of commercial bulb, number of bulbs per bulb, total yield, commercial yield, flowering percentage, length and diameter, soluble solids, titratable acidity, pH and ratio soluble solids and titratable acidity of the exhaust floral were measured. The data obtained were submitted to analysis of variance ( $p \leq 0.05$ ), and when significant the effects of genotypes, their means grouped by the Scott-Knott test. We also proceeded to study genetic divergence through multivariate analysis, adopting the canonical variables technique. Variance analysis indicated significant differences between genotypes for emergence speed index, flowering percentage, floral scape diameter, floral scape length and commercial yield. The highest commercial yield and flowering percentage were observed in RAL (8.82 t ha<sup>-1</sup>) and DDR 6024 (80.92%) access, respectively, which differed only from commercial cultivars (Quitéria and Caçador). The greatest genetic divergence was verified between experimental accessions and commercial cultivars. The experimental accessions presented flowering potential and botanical seed production in the studied region.

**Keywords:** *Allium sativum* L.; Flowering; Productivity; Seeds.

**Abbreviations:** UFLA\_Federal University of Lavras; EMBRAPA\_Brazilian Agricultural Research Corporation; MAPA\_Ministry of Agriculture, Livestock and Supply; MCTESTP\_Ministry of Science Technology, Higher Education and Professional Technical of Mozambique; CNPq\_National Council of Development and Technological; CAPES\_Higher Education Personnel Improvement Coordination Brazil; ESI\_emergence speed index; PHG\_plant height; PO\_percentage of overcrowding; AWCB\_average weight of commercial bulbs; NB\_number of bulbils per bulb; TY\_total yield; CY\_commercial yield; FP\_flowering percentage; FSL\_floral scape length; DFS\_floral scape diameter; pH\_hydrogenionic potential; SS\_soluble solids; TA\_titratable acidity; SS/TA\_ratio of soluble solids and titratable acidity NPK\_nitrogen, phosphor and potassium; ANOVA\_analysis of variance; KPa\_kilopascal; MAX. T.\_maximum temperature; MIN. T.\_minimum temperature; AVER. T.\_average temperature; RH\_relative humidity; CV\_1\_canonical variable 1; CV\_2\_canonical variable 2.

### Introduction

Garlic (*Allium sativum* L.) is a compulsory exclusively apomitic species, being propagated only vegetatively because of its unviable sexual propagation (Simon and Jenderek, 2003; Mayer and Goldstein, 2019). For commercial production underground bulb bulbs are used, or less frequently, mini bulbs, present in the floral leaks or inflorescences (Novak, 1972; Mayer et al., 2015). However, asexual multiplication using so-called seed bulbs, originating from previous production and without prior disinfection,

generates the problem of viral accumulation across generations. This is called degeneration, which has significantly contributed to the low productivity of garlic (Simon and Jenderek, 2003; Oliveira, 2010).

The cause of the unfeasibility of botanical garlic seed production is attributed to several possible mechanisms: development of garlic flower buds that are not able to compete with aerial bulbs; the pollen sac degenerates before pollen mitosis; degenerative diseases or even

morphological abnormalities already observed in garlic flowers, which may interfere with sexual reproduction and contribute to the sterility of the species (Etoh, 1985; Tchórzewska et al., 2018; Mayer and Goldstein, 2019).

In addition, during the domestication process, garlic was propagated asexually for many generations. This may have resulted in accumulation of chromosomal aberrations, such as aneuploidy, and translocations and / or inversions, which may have contributed to reducing the incidence of fertile gametes (Kamenetsky et al., 2005). Therefore, species breeding for new cultivars is only possible through the selection of variants resulting from natural or tissue culture induced mutations (Konvicka et al., 1978; Simon and Jenderek, 2003). However, sexual reproduction, whether natural or human-facilitated, is the cheapest and easiest way to develop garlic genetic breeding programs. Therefore, identifying productive and better flowering garlic genotypes is an important strategy to obtain genetic variability, allowing to find genotypes with higher potential for seed production.

Garlic flowering for later botanical seed production is a viable solution to generate greater genetic variability. This can further ensure virus-free plants and could somehow contribute to increased yield (Zhang et al., 2018). However, commercially available commercial cultivars lost their flowering capacity during the selection process aiming at better bulb yield and the elimination of the floral stem, thus reducing the nutrient dispute between inflorescence and bulbs (Mayer and Goldstein, 2019). This phenomenon had a very significant contribution to the occurrence of several diseases, especially viruses, favored by the continuous use of the same genetic materials, which contributed to reductions in garlic productivity (Oliveira, 2010).

Currently, one way to reduce or even eliminate viral load for increased yield has been the use of tissue culture technology. However, in addition to being costly and laborious, the technique requires specialized labor, which is often not feasible for small and medium garlic producers (Macêdo et al., 2009). Studying the genetic divergence in garlic allows us to identify the genetic variability of genotypes in relation to the various characteristics to be studied (Viana et al., 2016). In plants with normal flowering and seed production, information on genetic divergence allows the establishment of recombination through crosses that enhance the increase of genetic diversity in subsequent generations.

The pursuit of botanical seed production is the most viable and affordable way to establish a solid breeding program with garlic. This facilitates the production of virus-free plants and therefore more productive. Thus, the objective of this study was to evaluate the agronomic performance, flowering capacity and genetic divergence of garlic accessions.

## Results and discussion

The analysis of variance (Table 1) showed a significant effect of genotypes on emergence speed index (ESI), commercial yield (CY), flowering percentage (FP), floral scape length (FSL), floral scape diameter (DFS), soluble solids (SS), pH, titratable acidity (TA) and ratio of soluble solids and titratable acidity (SS/TA). There was no significant effect of

treatments for emergence, plant height (PHG), percentage of overcrowding (PO), average weight of commercial bulbs (AWCB), number of bulbils per bulb (NB) and total yield (TY). The variables presented coefficients of variation between 0.43 and 27.26%, which indicates an acceptable precision of the assay, according to the adopted design.

### *Agronomic characteristics and flowering*

The highest values of emergence speed index were observed for cultivar Caçador, with an average of 35.28 (Table 2). This treatment differed statistically only from the RAL 27, OU 73, RE PSK and RE 6820 accessions, which presented the lowest values (Table 2). Importantly, the cultivars Caçador and Quitéria are the earliest genotypes, with cycles ranging from 120 to 135 days, while for other accessions, the cycle needs further studies (Resende et al., 2016). Experimental accessions with emergence speed index similar to commercial cultivars are favorable features, as these may indicate greater precocity of these materials.

Trenhago et al. (2011) reported that factors such as region, climate, temperature and vernalization directly influence the acceleration of garlic emergence. When there is a positive response to these factors, there is slight acceleration in the emergency. The difference found between genotypes may have been influenced by one or more of these factors. Such a difference can be attributed to genetic variability, or the differentiated response of genotypes to the environment, or even to the quality of seedlings (Tchórzewska et al., 2018). This relationship between emergence and seedling quality is related to the possibility that they may be in a state of differentiated physiological maturation. The ripening is mainly influenced by factors such as photoperiod, temperature, high altitude, soil fertility and irrigation management, which can interfere with cultivar development (Oliveira, 2010).

Experimental accessions presented higher commercial yield in relation to commercial cultivars (Table 3), which is related to the higher amount of disposal found in commercial cultivars. This was due to the higher production of small bulbs in these treatments, considerably influencing the results. When considering total yield, there was no significant difference between genotypes.

Although commercial yields of experimental accessions were higher than those of commercial cultivars, these values are lower than the average garlic yield in Minas Gerais State, Brazil, which has an average of 14.9 t ha<sup>-1</sup> (Ibge, 2018; Conab, 2018; Anapa, 2019). In addition to genetic factors, the low yield found in this study may also be associated with an accumulated viral load, or even with the genetic performance of accessions in the studied environment (Macêdo et al., 2009; Fernandes et al., 2013). The early age of commercial cultivars may also have contributed to the low yield, as late cultivars tend to be more productive than early-growing ones.

The highest flowering percentage was verified in DDR 6024 access, with an average of 80.92%. However, it differed significantly only from the commercial varieties Caçador and Quitéria, which presented lower flowering percentage, with average values of 40.05 and 20.39%, respectively (Table 4). The highest values of length and diameter of floral scape were observed in cultivar Caçador, with an average of 61.16 cm and 17.99 mm in length and diameter, respectively

(Table 4). In general, the other genotypes did not differ in these two characteristics, with average values ranging from 44.32 to 51.68 cm for the length, and from 10.52 to 15.11 mm for the diameter.

The most widely cultivated commercial garlic cultivars in Brazil have low flowering capacity, or even almost nonexistent, especially in the Southeast, Midwest and Northeast regions of the country, even under vernalization conditions, due to their genetic conditions, as reported by authors (Etoh and Simon, 2002; Tchórzewska et al., 2018; Mayer and Goldstein 2019) in their studies of flowering under similar conditions. This is in agreement with the findings of this paper. The other accessions tested showed high flowering aptitude.

Based on Table 4, there is a flowering potential of experimental accessions in relation to commercial cultivars. This high percentage of flowering of accessions is a strong indication of environmental adaptation to flowering (Mueller et al., 1998; Chaturvedi et al., 2018). However, it is important to mention that it was not possible to obtain botanical seeds in this trial, which may be justified by the occurrence of rainfall at the end of the cycle (beginning of flowering), as observed in Fig. 1, besides the genetic limitations of the crop.

This may have affected the development and maturation of the inflorescences, as well as favoring abortion and not allowing the production of true seeds. A similar study was developed by Jenderek and Hannan (2004), who evaluated 45 accessions of garlic and found that all of them presented flowering, but with different intensities. However, of the 45 accessions, only 19 produced true seeds. Rotem et al. (2007), also developed a similar study with 2 accessions of garlic, both of which flowered, but only one of them produced botanical seeds and the other aborted in the early phase of inflorescence development. This reinforces the difficulty in obtaining botanical seeds in garlic, even under experimental conditions.

Floral induction and development is a complex process and not only depends on environmental factors, but also involves the genetic factor (Pooler and Simon, 1993). Thus, the genetic characteristic of genotypes influences the capacity of botanical seed production. This indicates the need for more detailed studies at different growing seasons to evaluate the effects of genotype x environment interaction (G x E) and to identify potential seed producing genotypes.

Other studies have indicated that there is no correlation between these characteristics of floral scape length and diameter and the ability of the genotype to produce botanical seeds (Etoh and Simon 2002; Tchórzewska et al., 2018; Mayer and Goldstein 2019). However, the emission of the floral stem means that the garlic cultivar is adapted to flowering in a given region (Hong and Etoh, 1996; Lopez-Bellido et al., 2016). Thus, based on this information, the emission of floral escapes indicates that genotypes have adaptability to flowering in the region, but with different intensities.

This floral induction, in addition to the genetic factor, is influenced by the photoperiod, growth temperature, and bulb shelf life and storage temperature (Brewster 1982a; Brewster, 1982b; Brewster 1983; Bertaud, 1988; Chaturvedi et al., 2018). Increasing the length and diameter of the

exhaust, in addition to climatic factors, can often be associated with greater efficiency in nutrient utilization, especially nitrogen, which tends to be transferred to these regions because they are preferential drains. This fact generally implies a reduction in the size of the bulb, and consequently of the bulbils (Zhang et al., 2018; Liu et al., 2019).

The flowering capacity observed in the evaluated experimental accessions is a positive and extremely important result from the point of view of garlic botanical seed production and the implementation of a genetic improvement program with the crop conducting new research seeking flowering and viable botanical seed production in garlic genotypes.

#### **Biochemical characteristics**

The highest percentage of soluble solids (SS) was presented by access RE 493099 (33.50%), the lowest obtained by access RAL 159 (25.00%) (Table 5). The importance of knowing the soluble solids content is in choosing the best time for harvest planning. The results found are close to those obtained by Oliveira (1999), who verified a variation of 28.83 to 38.45% of soluble. This author also states that the increase in storage days influences the increase in the percentage of soluble solids.

The pH varied from 6.27 to 6.43, with the lowest value observed for RE PSK access and the highest value for RAL 159 access, respectively (Table 5). The results obtained are close to those found by Bessa et al. (2017), who found a variation from 5.88 to 6.7, and were different from those found by Mota et al. (2003), in the evaluations made in the municipality of Lavras, whose pH varied from 6.60 to 7.06.

The highest levels of titratable acidity (TA) were observed in the cultivar Quitéria, with 1.29%, which differed from the other genotypes (Table 5). The lowest values were observed in the Caçador and RE 6820 genotypes, with 0.38% TA. These results are close to those found by Mota et al. (2003), evaluating garlic genotypes in the Lavras municipality, where they found values between 0.83 to 1.29% of TA.

The highest percentage of the ratio of soluble solids / titratable acidity was presented by cultivar Caçador with 71.92%, and the lowest, by cultivar Quitéria with 25.16%. The SS / TA ratio is one of the best ways of assessing the taste of fruits and vegetables, being more representative than the isolated measurement of sugars or acidity, as it reflects the balance between sugars and acids (Chitarra and Chitarra, 2005). The values of the SS / AT ratio are close to that found by Soares (2013), when evaluating different garlic cultivars, values between 39.6 and 61.6% were obtained in the SS / AT ratio.

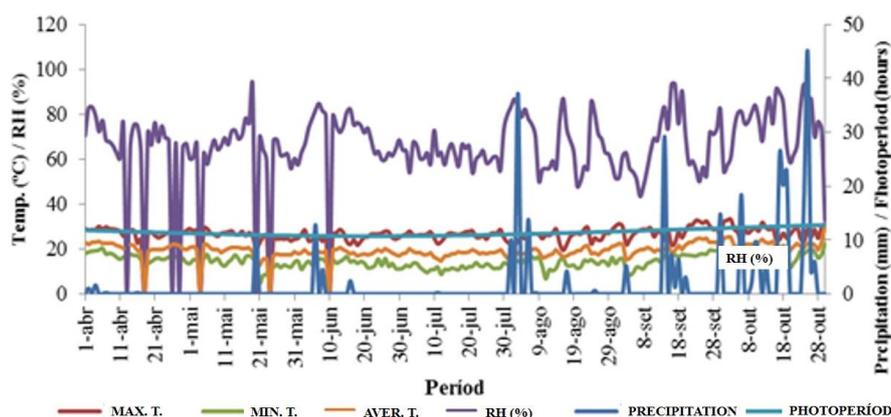
Physico-chemical analyzes of pH, TA and SS are normally performed for most plant products, as these are directly associated with the flavor presented. Their concentrations can influence their acceptance with the consumer. The information generated through the physicochemical analysis of garlic indicates whether the product is more suitable for fresh consumption or for industry, greater post-harvest storage capacity and also, according to Oliveira (1999), the best time for harvesting.

The values of titratable acidity, pH and soluble solids found in the present work are considered ideal to those accepted by the consumer market, when compared to existing

**Table 1.** Summary analysis of variance for emergence, emergence speed index (ESI), plant height (PHG), percentage of overcrowding (PO), average weight of commercial bulbs (AWCB), number of bulbils per bulb (NB), total yield (TY), commercial yield (CY), flowering percentage (FP), floral scape length (FSL), floral scape diameter (DFS), soluble solids (SS), Hydrogenionic potential (pH), titratable acidity (TA) and ratio of soluble solids and titratable acidity (SS/TA) in experimental accessions and commercial garlic cultivars. UFLA, Lavras-MG, Brazil, 2018.

CF	ddf	Mean squares					
		Germination and morphology					
		Emergence	ESI	PHG	PO	AWCB	NB
Block	3	0.121	121.630	66.939	84.536	19.273	1.641
Genotypes	12	0.017 <sup>ns</sup>	32.439*	33.484 <sup>ns</sup>	0.927 <sup>ns</sup>	19.141 <sup>ns</sup>	1.898 <sup>ns</sup>
Residue	36	0.011	6.288	32.642	0.804	25.110	0.779
CV (%)		7.57	7.93	10.84	27.26	16.12	8.36
CF	df	Mean squares					
		Germination and morphology					
		TY	CY	FP	FSL	DFS	
Block	3	13.584	13.702	190.910	320.650	194.073	
Genotypes	12	1.246 <sup>ns</sup>	18.086*	921.790*	119.720*	16.012*	
Residue	36	12.170	15.039	83.220	60.870	35.255	
CV (%)		12.92	16.65	14.5	16.47	19.46	
FV	GL	Mean Square					
Physico-Chemical Characteristics							
		SS (%)	pH	TA (%)	SS/TA (Ratio)		
Block	3	0.632	0.001	0.002	25.249		
Genotypes	12	41.794*	0.016*	0.209*	385.26*		
Residue	36	2.109	0.001	0.002	28.88		
CV (%)		5.05	0.43	8.21	9.88		

Significant (\*) and not significant (<sup>ns</sup>) by the F test ( $p \leq 0.05$ ).

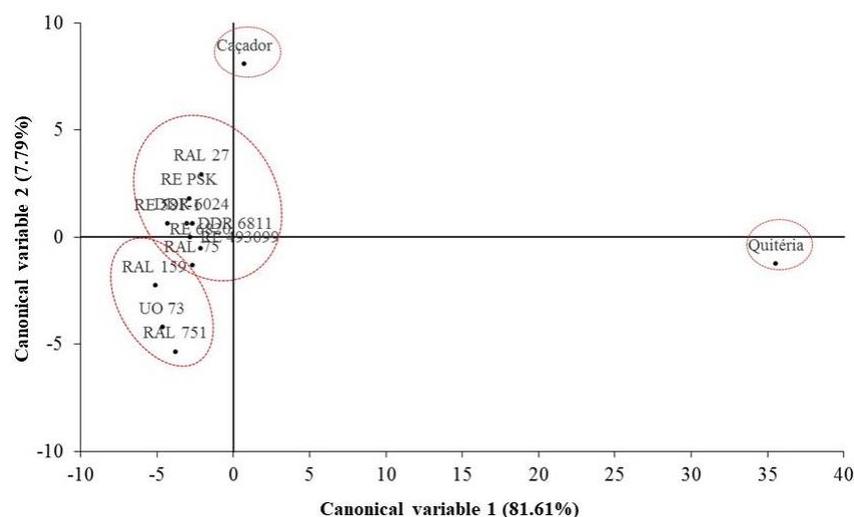


**Figure 1.** Maximum (MAX. T.), minimum (MIN. T.) and average (AVER. T.) temperature, relative humidity (RH), precipitation and photoperiod in the municipality of Lavras, MG, Brazil, from April to October 2018.

**Table 2.** Emergence, emergence speed index (ESI), plant height (PHG) and percentage of overcrowding (PO) in experimental accessions and commercial garlic cultivars. UFLA, Lavras-MG, Brazil, 2018.

Genotypes	Emergence (%)	ESI	PHG (cm)	PO (%)
Caçador	97.92 a	35.28 a	57.91 a	59.38 a
DDR 6024	97.92 a	34.28 a	52.47 a	54.51 a
DDR 6811	97.22 a	32.93 a	55.18 a	59.38 a
Quitéria	95.83 a	32.64 a	52.12 a	42.71 a
RAL 159	96.53 a	31.04 a	59.16 a	44.10 a
RAL 27	93.06 a	30.15 b	50.55 a	38.19 a
RAL 75	97.92 a	31.91 a	51.55 a	44.45 a
RAL 751	94.79 a	33.26 a	48.09 a	53.82 a
RE 493099	94.45 a	32.13 a	51.61 a	54.86 a
RE 518-1	97.92 a	34.67 a	54.53 a	46.88 a
RE 6820	87.50 a	26.67 b	50.63 a	56.94 a
RE PSK	89.24 a	27.93 b	54.38 a	39.58 a
UO 73	92.01 a	27.49 b	56.63 a	54.17 a

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ( $p \leq 0.05$ ).



**Figure 1.** Multivariate analysis performed with 15 significant variables from those evaluated in experimental accessions and commercial garlic cultivars. UFLA, Lavras - MG, Brazil, 2018.

**Table 3.** Average weight of commercial bulb (AWCB), number of bulb per bulb (NB), total yield (TY) and commercial yield (CY) in experimental accessions and commercial garlic cultivars. UFLA, Lavras-MG, Brazil, 2018.

Genotypes	AWCB (g bulb <sup>-1</sup> )	NB (bulbs bulb <sup>-1</sup> )	TY (t ha <sup>-1</sup> )	CY (t ha <sup>-1</sup> )
Caçador	33.42 a	11.50 a	8.91 a	2.89 b
DDR 6024	28.88 a	10.50 a	8.19 a	8.12 a
DDR 6811	32.98 a	10.84 a	9.10 a	8.10 a
Quitéria	34.70 a	9.17 a	7.60 a	2.48 b
RAL 159	31.00 a	11.33 a	8.81 a	8.54 a
RAL 27	31.30 a	9.92 a	8.94 a	8.82 a
RAL 75	31.33 a	10.00 a	8.86 a	8.70 a
RAL 751	28.15 a	10.42 a	7.99 a	7.94 a
RE 493099	31.34 a	10.75 a	8.87 a	8.39 a
RE 518-1	31.31 a	10.00 a	8.83 a	8.57 a
RE 6820	28.84 a	10.50 a	8.04 a	7.60 a
RE PSK	27.66 a	10.75 a	7.67 a	7.23 a
UO 73	33.28 a	11.58 a	9.21 a	8.39 a

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ( $p \leq 0.05$ ).

**Table 4.** Flowering percentage, length and diameter of floral scape in experimental accessions and commercial garlic cultivars. UFLA, Lavras-MG, Brazil, 2018.

Genotype	Flowering (%)	Floral Scape	
		Length (cm)	Diameter (mm)
Caçador	46.06 b	61.16 a	17.99 a
DDR 6024	80.92 a	51.68 b	11.97 c
DDR 6811	66.78 a	43.61 b	11.03 c
Quitéria	20.39 c	45.49 b	10.54 c
RAL 159	65.79 a	44.56 b	12.32 c
RAL 27	67.43 a	50.20 b	13.21 c
RAL 75	65.46 a	46.55 b	13.55 c
RAL 751	72.04 a	46.99 b	15.11 b
RE 493099	63.82 a	46.02 b	10.69 c
RE 518-1	71.38 a	48.31 b	11.98 c
RE 6820	62.17 a	44.32 b	12.05 c
RE PSK	60.86 a	44.60 b	10.52 c
UO 73	74.67 a	50.04 b	12.99 c

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ( $p \leq 0.05$ ).

**Table 5.** Percentage of soluble solids, pH and titratable acidity in experimental accessions and commercial garlic cultivars. UFLA, Lavras – MG, Brazil, 2018.

Genotype	Soluble solids (%)	pH	Titratable acidity (%)	SS/TA (Ratio)
Caçador	27.33 b	6.34 c	0.38 d	71.92 a
DDR 6024	25.50 c	6.32 c	0.56 b	42.18 c
DDR 6811	27.50 b	6.34 c	0.47 c	59.05 b
Quitéria	32.50 a	6.31 c	1.29 a	25.16 d
RAL 159	25.00 c	6.43 a	0.47 c	53.69 b
RAL 27	26.83 c	6.21 e	0.56 b	48.22 b
RAL 75	29.17 a	6.33 c	0.59 b	49.89 b
RAL 751	33.00 a	6.42 a	0.62 b	54.54 b
RE 493099	33.50 a	6.33 c	0.53 b	63.40 a
RE 581-1	25.00 c	6.36 b	0.47 c	53.24 b
RE 6820	26.50 c	6.42 a	0.38 d	69.84 a
RE PSK	28.83 b	6.27 d	0.53 b	54.57 b
UO 73	33.17 a	6.40 a	0.59 b	56.87 b

The means followed by the same letter do not differ from each other by the Scott-Knott test ( $p \leq 0.05$ ).

**Table 6.** Correlations established between the first two canonical variables and nine characteristics evaluated in experimental accessions and commercial garlic cultivars. UFLA, Lavras – MG, Brazil, 2018.

Characteristics	Canonical variable 1	Canonical variable 2
Emergency speed index (ESI)	9.00%	26.00%
Flowering percentage (FP)	-77.00%	-22.00%
Floral scape length (FSL)	-5.00%	47.00%
Diameter of floral scape (DFS)	3.00%	27.00%
commercial yield (CY)	-67.00%	-40.00%
Soluble solids (SS)	31.00%	-43.00%
Titratable acidity (TA)	90.00%	-29.00%
Hydrogen potential (pH)	-21.00%	-51.00%
Soluble solids / titratable acidity (SS/TA)	-66.00%	29.00%

commercial cultivars. Regarding the physicochemical quality of garlic, the values accepted by the market are above 0.30% of titratable acidity, 30% of soluble solids and in relation to pH, below 7 (Bessa et al., 2017).

#### Genetical diversity

There was a negative correlation between flowering (-77.00%), commercial yield (-67%) and SS / TA ratio (-66.00%) with the canonical variable 1 (CV1), in addition to a strong positive correlation with titratable acidity (90%) (Table 6). Thus, it is possible to infer that commercial cultivars were more dispersed (divergent) in relation to experimental accessions, mainly because they presented less flowering and less commercial production (Figure 2). In addition, it appears that the cultivar Quitéria diverged strongly from the other genotypes mainly due to its higher content of titratable acidity.

The pH was the variable that showed the greatest negative correlation with the canonical variable 2, whose value was -51% (Table 6). Based on the dispersion of the genotypes (Figure 2), it is observed that the experimental accessions RAL 751, UO 73 and RAL 751 are among those with the highest pH. In canonical variable 1, the divergence of the cultivar Quitéria was influenced by the variables, commercial yield, percentage of flowering and titratable acidity, while the cultivar hunter was due to pH. This analysis further strengthens the potential of experimental accesses, in general, in relation to commercial cultivars, being ideal for carrying out further studies.

#### Materials and methods

##### Study location

The work was conducted in the experimental area of the Olericulture Sector of the Department of Agriculture of the Federal University of Lavras (UFLA), located in Lavras (21 ° 13'20.54" south latitude, 44 ° 58'7.99" west longitude and 910 m altitude), southern region of the state of Minas Gerais. This work is part of the master's thesis of the first author, held at UFLA (Taula, 2011). The local climate, according to Köppen classification, is Cwa type, subtropical (mesothermal) temperate with dry winter and rainy summer, characterized by a dry season from April to September and a rainy season from October to March (Köpen, 1948). The soil of the experimental area is classified as Typical Distroferric Red Latosol, with clay texture, with 33% of sand, 18% of silt and 49% of clay (Curi et al., 2017). The climatic data of the region are presented in Fig. 1:

##### Study materials

As treatments were evaluated 11 experimental accessions from Embrapa Vegetables Garlic Germplasm Bank (DDR 6024, RAL 159, RAL 75, RAL 751, RAL 27, RE 518-1, DDR 6811, RE 6820, RE 493099, RE PSK e OU 73) and two commercial cultivars (Caçador and Quitéria), used as treatments control.

The experimental accessions used in the study came from the University of Wisconsin / USA, indicated with aptitude for botanical seed production.

The seed garlic bulbs were subjected to a vernalization process for 50 days in a cold room at  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 70% relative humidity. The bulbs were removed from the cold chamber one week before planting for threshing. After threshing, the bulbils were classified according to size (Regina and Rodrigues, 1970), and those retained in sieves 1 and 2 were used for planting the experiment.

#### **Experimental design**

The experiment was conducted between May and October of 2018, in a randomized complete block design with 13 treatments and 4 replications. The tillage consisted of plowing and harrowing, followed by raising the beds. The plots consisted of beds of 0.2 m in height, 1.0 m in width and 2.0 m in length, totaling  $2\text{m}^2 \text{plot}^{-1}$ . In the planting were used 4 simple lines, with 19 plants per line, totaling 76 plants per plot. The bulbs were planted at a depth of 0.05 m, with a spacing of 0.20 m between rows and 0.10 m between bulbs. Planting fertilization was made with formulated NPK (nitrogen, phosphor and potassium) (4:14:8),  $150 \text{g m}^{-2}$ . In addition, an organic compound fertilizer in the amount of 20 tons  $\text{ha}^{-1}$  was added. The top dressing was applied in two applications, one at 30 days and one at 60 days after planting, with ammonium sulfate, in the proportions of  $10 \text{g m}^{-2}$ , corresponding to  $400 \text{kg ha}^{-1}$ .

The micro sprinkler irrigation system was used, with a flow rate of  $27 \text{l h}^{-1}$  by micro sprinkler, pressure of 200 KPa, and the estimated amount of water applied according to the crop evapotranspiration. Weeding was carried out manually as needed to keep the crop clean. Insecticides and fungicides were also sprayed for disease prevention and control.

#### **Variables collected**

The emergence speed index was evaluated by daily counting of emerged plants in all plots for seven days after the emergence of the first seedlings, calculated according to Maguire (1962); emergence (%) by counting plants that emerged at 25 days after planting; plant height (cm) assessed at 115 days after planting, as measured by the distance from ground level to the longest leaf edge; the flowering percentage (%) observed and accounted for the plants that emitted the floral stem per plot; the length (cm) and diameter (mm) of floral scape, where 20 plants were selected per plot with floral tassel and measured with the aid of a tape measure for vertical length and a caliper for diameter.

Bulbs were harvested at 140 days after yellowing and dryness of 2/3 of the aerial part of the plants. During harvest, 5 plants with floral scape were left in each row for the production of botanical seeds. The harvested plants were submitted to the pre-cure process, remaining for three days in the open, protected from direct sunlight. Then, the plants were stored for 30 days in the shed (dry and ventilated environment), and later evaluated for production and quality of bulbs.

The average weight of the commercial bulb ( $\text{g bulb}^{-1}$ ) was estimated based on the weight of the bulbs classified as commercial (in grams) divided by their number of bulbs, the adjusted values being expressed in  $\text{g bulb}^{-1}$ ; for the number of bulbs per bulb, 20 bulbs per plot were randomly selected and threshed, then the bulbs were accounted for grouped by determining the mean expressed in  $\text{bulbs bulb}^{-1}$ ; the total

yield was evaluated from the weighing of the whole production of the plot, and the commercial from the bulbs that presented good phytosanitary and physiological conditions with commercial value according to the classification established by Ministry of Agriculture, Livestock and Supply (MAPA), whose results were expressed in  $\text{t ha}^{-1}$ .

Physical-chemical analyzes of titratable acidity, pH and soluble solids were also performed. The determination of titratable acidity was carried out by titration with 0.1N sodium hydroxide solution (NaOH), using phenolphthalein as an indicator, according to the Instituto Adolfo Lutz (2005). 5 g of garlic paste, diluted in distilled water to a volume of 20 ml, was used. Two drops of 1% alcoholic phenolphthalein were added. The results were expressed in g of pyruvic acid (predominant acid) per 100g. The pH was determined using a pH meter Schott Handylab, according to the AOAC technique (2012). The total soluble solids were determined by refractometry, and the digital refractometer ATAGO PR-100 with automatic temperature compensation at  $25^{\circ}\text{C}$  was used, according to Instituto Adolfo Lutz (2005) and the results expressed as a percentage. In addition, the soluble solids / titratable acidity ratio (Ratio) was obtained, obtained by dividing the soluble solids content by the titratable acidity.

#### **Statistical analysis**

The data were submitted to analysis of variance (ANOVA) by the F test ( $p \leq 0.05$ ), and when significant the effects of the genotypes, the means of the treatments were grouped using the Scott-Knott test, adopting the 5% significance level. Multivariate data analysis was also performed, considering the characteristics with significant effect ( $p \leq 0.05$ ) by ANOVA. The purpose of this analysis was to verify the degree of similarity and dissimilarity between the treatments in the multivariate aspect, which was studied through canonical variables. Data analyzes were performed using the R statistical software (R Core Team, 2018).

#### **Conclusions**

The experimental accessions presented the highest values of bulb commercial yield and flowering percentage, in Lavras, MG. The experimental accessions presented flowering potential and botanical seed production in the region of Lavras, MG. Genetic divergence between experimental accessions and commercial cultivars was verified, mainly in relation to flowering capacity and commercial yield.

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## References

- ANAPA - National Association of Garlic Producers. Crop 2019. Available in: <<https://anapa.com.br/>>. Access on January, 12, 2019.
- AOAC - Association of Official Analytical Chemists (2012) Official methods of analysis. AOAC International, 18th edn. Gaithersburg, USA.
- Bertaud DS (1988) Effects of chilling duration, photoperiod, and temperature on floral initiation and development in sprouted and unsprouted onion bulbs. Symposium on Allium: EUCARPIA - Section Vegetables, 4th Coventry Sep 6-9, United Kingdom GB.
- Bessa AT, Lopes WAR, Silva OMP, Lima MFP, Oliveira PRH, Souza HC, Aguiar AF, Negreiros MZ (2017) Caracterização físico-química de alho 'BRS Hozan' e 'Roxo Pérola de Caçador' em função do tempo de armazenamento. Rev Colomb Cienc Hortic. 11: 368-377.
- Brewster JL (1982a) Flowering and seed production in overwintered cultivars of bulb onions. I. Effects of different raising environments, temperatures, and daylengths. J Hortic Sci. 57: 93-101.
- Brewster JL (1982b) Flowering and seed production in overwintered cultivars of bulb onions. II. Quantitative relationships between mean temperatures and daylengths and the rate of inflorescence development. J Hortic Sci. 57: 103-108.
- Brewster JL (1983) Effects of photoperiod, nitrogen nutrition, and temperature on inflorescence initiation and development in onion (*Allium cepa* L.). Ann Bot. 51: 429-440.
- Chaturvedi AK, Shalom SR, Faigenboim-doron A, Teperbamnolker P, Salam BB, Daus A, Kamenetsky R, Eshel D (2018) Differential carbohydrate gene expression during preplanting temperature treatments controls meristem termination and bulbing in garlic. Environ Exp Bot. 150: 280-291.
- Chitarra MIF, Chitarra AB (2005) Pós-colheita e frutos e hortaliças: fisiologia e manuseio. 2 ed. Editora UFLA: ESAL/FAEPE, Lavras, MG.
- CONAB - National Supply Company (2018) National garlic supply company. Available in: [http://www.conab.gov.br/olalaCMS/uploads/file/11\\_03\\_11\\_17\\_12\\_15\\_alhojaneiro2018](http://www.conab.gov.br/olalaCMS/uploads/file/11_03_11_17_12_15_alhojaneiro2018). Access on July, 09, 2019.
- Curi N, Silva SHG, Poggere GC, Menezes MD (2017) Mapeamento de Solos e magnetismo no Campus da UFLA como traçadores ambientais. 1ed. Editora UFLA, Lavras, MG.
- Etoh T (1985) Studies on sterility in garlic (*Allium sativum* L.). Mem Fac Agri Kagoshima Univ. 21: 77-132.
- Etoh T, Simon PW (2002) Diversity, fertility and seed production of garlic. In: H.D. Rabinowitch and L. Currah (eds.) Allium Crop Science – Recent Advances, 1rd edn. Wallingford, UK. 101-117.
- Fernandes RF, Dusi AN, Resende FV (2013) Viroses do alho no Brasil: importância e principais medidas de controle. Brasília, BR: Embrapa Hortaliças, Circular Técnica, 1: 8p.
- Hong CJ, Etoh T (1996) Fertile clones of garlic (*Allium sativum* L.) abundant around the Tien Shan Mountains. Breeding Sci. 46: 349-353.
- IBGE - Brazilian Institute of Geography and Statistics (2018) Available in: <http://www.sidra.ibge.gov.br/>. Access on June, 17, 2018.
- Instituto Adolfo Lutz (2005) Métodos físico-químicos para análise de alimentos. 4 ed. São Paulo, SP.
- Jenderek M, Hannan RM (2004) Variation in reproductive characteristics and seed production in the USDA garlic germplasm collection. Hortscience. 39: 485-488.
- Kamenetsky R, Shafir IL, Khassanov F, Kik C, Van Heusden AW, Ginkel MV, Burger-Meijer K, Auger J, Arnault I, Rabinowitch HD (2005) Diversity in fertility potential and organo-sulphur compounds among garlics from Central Asia. Biodivers Conserv. 14: 281-295.
- Konvicka O, Nienhaus T, Fischbeck G (1978) Investigations into the causes of pollen sterility in *Allium sativum* L. Pflanzenzucht. 80: 265-276.
- Köppen W (1948) Climatologia: com um estudio de los climas de La tierra. Fondo de Cultura Económica, México.
- Liu H, Deng R, Huang C, Cheng Z, Meng H (2019) Exogenous gibberellins alter morphology and nutritional traits of garlic (*Allium sativum* L.) bulb. Sci Hortic. 246: 298-306.
- Lopez-bellido FJ, Lopez-bellido RJ, Muñoz-romero V, Fernandez-garcia P, Lopez-bellido L (2016) New phenological growth stages of garlic (*Allium sativum*). Ann Appl Biol. 169: 423-439.
- Macêdo FS, Souza RJ, Oak JG, Santos BR, Milk LVR (2009) Productivity of vernalized garlic as a function of nitrogen and molybdenum doses. Bragantia 68: 657-663.
- Maguire JD (1962) Speed germination: AID in selection and evaluation for seedling emergence and vigour. Crop Sci. 2: 176-177.
- Mayer ES, Bem-Michael T, Kimhi S, Forer I, Rabinowitch HD, Kamenetsky (2015) Effects of different temperature regimes on flower development, microsporogenesis and fertility in bolting garlic (*Allium sativum*). Funct Plant Biol. 42: 514-526.
- Mayer ES, Goldstein RK (2019) Recent Advances in Sexual Propagation and Breeding of Garlic. Hortic Rev. 46: 1-38.
- Mota JH, Yuri JE, Souza RJ, Resende GM, Freitas SAC, Rodrigues Júnior JC (2003) Características físico-químicas de cultivares de alho (*Allium sativum* L.) do grupo semi-nobre, nas condições de Lavras, MG. Hortic Bras. 21: 1-4.
- Mueller S, Kreuz CL, Mondardo M (1998) Produtividade, qualidade e lucro em função de espaçamentos de plantio e pesos de bulbilhos-sementes de alho. RAC. 11: 52-55.
- Novak FJ (1972) Tapetal development in the anthers of *Allium sativum* L. and *Allium longicuspis* Regal. Experientia. 28: 1380-1381.
- Oliveira FLD, Doria H, Teodoro RB, Resende FV (2010) Características agronômicas de cultivares de alho em Diamantina. Hortic Bras. 28: 355-359.
- Oliveira CMD (1999) Determinação do ponto de colheita em cultivares de alho. Masters dissertation. Universidade Federal de Lavras, Lavras, Brazil.
- Pooler MR, Simon PW (1993) Garlic flowering in response to clone, photoperiod, growth temperature, and cold storage. Hortscience. 28: 1085-1086.
- R Core Team (2018) R: A Language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Regina SM, Rodrigues JV (1970) Peneiras já classificam o alho-planta. Belo Horizonte:ACAR, 1970. 6 p. (Comunicado técnico).

- Resende FV, Haber LL, Pinheiro JB (2016) Sistema de produção de alho. Brasília: Embrapa Hortaliças. Available in: <https://www.embrapa.br/documents/1355126/9124396/Sistema+de+Produ%C3%A7%C3%A3o+de+Alho/64258d94-6bb8-4826-a0e9-ece47aa434ff> Acess on April, 09, 2019.
- Rotem N, Shemesh E, Peretz Y, Akad F, Edelbaum O, Rabinowitch HD, Sela I, Kamenetsky R (2007) Reproductive development and phenotypic differences in garlic are associated with expression and splicing of LEAFY homologue gaLFY. *J Exp Bot.* 58: 1133-1141.
- Simon PW, Jenderek MM (2003) Flowering, seed production and the genesis of garlic breeding. *Plant Breeding Rev.* 23:211-214.
- Soares AM (2013) Avaliação de cultivares de alho no município de Governador Dix-sept Rosado-RN. Masters dissertation. Universidade Federal Rural do Semi-árido, Mossoró, Brazil.
- Taula AJV (2013) caracterização de acessos de alho quanto ao desempenho agrônomo e capacidade de florescimento. Masters dissertation. Universidade Federal De Lavras, Lavras, Brazil.
- Tchórzewska D, Luchowski R, Gruszecki WI, Winiarczyk K (2018) Comparative studies of live tapetum cells in sterile garlic (*Allium sativum*) and fertile leek (*Allium ampeloprasum*) using the fluorescence lifetime imaging analytical method. *S Afr J Bot.* 117: 222-231.
- Trenhago R, Gottems A, Roggia C, Alves F, Guimarães A, Kofender J (2011) Avaliação do IVG no alho plantado em diferentes formas no solo. In: XVI Seminário Institucional de Ensino Pesquisa e Extensão 1:3.
- Viana JPG, Pires CJ, Pinheiro JB, Valente SEDS, Lopes ÂCA, Gomes RLF (2016) Divergência genética em germoplasma de alho. *Cienc Rural* 46: 203-209.
- Zhang YJ, Gao H, Cheng HY, WANG YW (2018) Effects of different nitrogen levels on photosynthetic characteristics, chlorophyll fluorescence and yield of *Allium sativum* cv 'Minlezipi'. *Res J Biotechnol.*13: 27-31.