horticultura brasileira

Research

MARODIN, JC; RESENDE, FV; RESENDE, JTV; GABRIEL, A; ZEIST, AR; CONSTANTINO, LV; SANZOVO, AWS. 2020. Virus-free garlic: yield and commercial classification as a function of plant spacing and seed size. *Horticultura Brasileira* 38: 295-300. http://dx.doi.org/10.1590/S0102-053620200309

Virus-free garlic: yield and commercial classification as a function of plant spacing and seed size

Josué C Marodin [1]; Francisco V Resende [2]; Juliano TV de Resende [3]; André Gabriel [4]; André R Zeist [5]; Leonel V Constantino [3]; Alisson WS Sanzovo [3]

[1]Universidade Federal de Lavras (UFLA), Lavras-MG, Brasil; josuemarodin@gmail.com; [2]Embrapa Hortaliças, Brasília-DF, Brasil; francisco.resende@embrapa.br; [3]Universidade Estadual de Londrina (UEL), Londrina-PR, Brasil; jvresende@uel.br; leonel@uel.br; allissonuenp@gmail.com; [4]Universidade Estadual do Centro-Oeste (Unicentro), Guarapuava-PR, Brasil; andre.gb85@hotmail.com; [5]Universidade do Oeste Paulista (UNOESTE), Presidente Prudente-SP, Brasil. andrezeist@unoeste.br

ABSTRACT

Studies on the interaction between garlic plant density and virus-free seed size are scarce in Brazil. Thus, this study was installed to evaluate the effect of plant spacing and seed size on garlic traits and yield for infected and virus-free bulbs. Treatments were arranged in a randomized block design and 2x5x3 factorial combination [infected and virus-free bulbs, five plant spacings (210, 260, 300, 360, and 390 cm^2 per plant), and three bulbous seed sizes (sieve one, two, and three)]. The highest bulb yield was observed for virus-free seeds at a plant spacing of 390 cm^2 $plant^{-1}$, while the highest commercial yield was verified for the spacing of 210 cm^2 $plant^{-1}$. The combination of virus-free seeds, larger bulbs, and spacing of 332 cm^2 $plant^{-1}$ promoted the highest leaf area index. For virus-free garlic, lower plant densities resulted in higher yields and garlic bulb quality. The best option for higher yields and improved commercial quality bulbs was the use of medium-sized virus-free bulbils at a plant density of about 300 thousand plants ha^{-1}.

Keywords: *Allium sativum*, degeneration, plant density, vegetative propagation.

RESUMO

Alho livre de vírus: produtividade e classificação comercial em função do espaçamento e tamanho do bulbilho semente

Estudos que relacionam a interação entre densidade de plantio e tamanho de alho com sementes livre de vírus são escassos no Brasil. Assim, avaliou-se o efeito do espaçamento de plantio e tamanho do alho-semente sobre as características da planta e produtividade de alho infectado e livre de vírus. Os tratamentos foram dispostos em blocos ao acaso em esquema fatorial 2x5x3, sendo bulbilhos infectados e livres de vírus, cinco espaçamentos entre plantas (210, 260, 300, 360 e 390 cm^2) e três tamanhos de bulbilhos (peneiras um, dois e três). A maior produtividade de bulbos foi observada para sementes livre de vírus no espaçamento 390 cm^2 $planta^{-1}$, enquanto a maior produtividade comercial foi verificada para o espaçamento de 210 cm^2 $planta^{-1}$. Alho-semente livre de vírus e bulbilho de maior tamanho no espaçamento de 332 cm^2 $planta^{-1}$ proporcionaram o maior índice de área foliar. Para os bulbilhos livre de vírus, as menores densidades de plantio resultaram nas maiores produtividades e maior qualidade dos bulbos de alho. O uso de bulbilhos semente livre de vírus com tamanho médio em cultivos com densidade de plantio em torno de 300 mil plantas ha^{-1}, consiste na melhor opção para obtenção de maiores produtividades e bulbos de melhor padrão comercial.

Palavras-chave: *Allium sativum*, degenerescência, densidade de plantio, propagação vegetativa.

Received on September 3, 2019; accepted on May 20, 2020

Garlic (*Allium sativum*) is propagated exclusively through bulbs and bulbils (clones); however, these may increase the incidence and spread pathogens from one crop cycle to another (Marodin *et al*., 2019). Viral complexes heavily jeopardize crop growth and yield (Oliveira *et al*., 2014; Marodin *et al*., 2019). As a result, seed bulbs from virus-free stock plants have been recommended, as stocks are produced using apical stems from *in vitro* culture associated with thermotherapy (Oliveira *et al*., 2014; Vieira *et al*., 2015). In this sense, virus-free seed bulbs can increase yield, commercial quality of bulbs, and sustainability of garlic production systems (Gimenez *et al*., 2016; Marodin *et al*., 2019).

Bulbil plant density is a major factor for garlic production for being able to increase yields of such economically-important vegetable (Rathinakumari *et al*., 2015). In terms of the final product, small bulbs have less commercial value if compared to larger ones, thus decreasing farmer profits. Moreover, higher garlic yields are observed in systems using high plant densities and larger garlic seeds, especially when combined with high technification (Asgharipour & Arshadi, 2012; Lima *et al*., 2019).

High planting densities increase crop yield given the larger number of bulbs produced per unit area (Doro, 2012; Muneer *et al*., 2017), despite increasing competition between plants (Moravčević *et al*., 2011, Rostami & Mohammadi, 2018). However, bulbs of better commercial classes are mostly produced in crops with lower plant densities, as they promote larger bulb sizes and average weights (Castellanos *et al*., 2004; Alam *et al*., 2010).

Planting of larger bulbils can also provide higher yields (Marodin *et al*., 2019) as they increase vegetative growth for having a surplus of nutritional reserves (Stahlschmidt *et al*., 1997). Gedamu *et al*. (2008) observed an interaction between garlic seed size and plant spacing, wherein small bulbils promoted higher yields in denser spacings. On the contrary, less dense spacings only produce larger average weights of bulbs when using larger garlic seeds. Moreover, larger garlic seeds provide greater vegetative growth mainly when associated with increased plant spacing, as it increases light uptake and production of larger bulbs at harvest time (Moravčević *et al*., 2011).

Despite the above reports, there is a lack of studies on the potential interaction between planting density and size of garlic seeds. Given the non-degeneration, clones free of viral complexes, such as those composed of *Allexivirus*, *Carlavirus*, and *Potyvirus* genera (Fayad-André *et al*., 2011; Oliveira *et al*., 2014), provide more vigorous vegetative growth than virus-infected plants (Gimenez *et al*., 2016). Therefore, plant spacings other than those in systems using virus-infected garlic seeds may be suitable for increased yields.

Given the above, in our study we aimed to evaluate the garlic traits and yield for garlic cropping using virus-infected and -free seeds at different plant densities and seed sizes.

MATERIAL AND METHODS

An experiment was carried out at Embrapa Hortaliças, located in Brasília-DF, Brazil (15°56'S, 48°9'W, 1150 m altitude). According to Köppen's classification, the local climate is classified as *Aw* type, which stands for a tropical climate with dry winters and rainy summers. The experiment was conducted from May to October 2013, when temperature and relative humidity averages were 21.3°C and 59%, respectively. The local soil is classified as eutrophic Yellow Latosol (Embrapa, 2006), i.e., Oxisol, with a sandy-silty-clayey texture.

The experiment was carried out in a randomized block design, with four replications and split plots. Treatments comprised virus-infected and -free garlic seeds in plots, and plant spacings and seed sizes in subplots. The cultivar Chonan was used as seeds source. Some of these seeds were infected by virus and some were virus-free. Seeds were divided into three size groups (from sieve 1: 15x25 mm; sieve 2: 10x20 mm; and sieve 3: 8x17 mm). Seeds were planted at five plant spacings (210, 260, 300, 360, and 390 cm^2 per plant).

Virus-infected seeds came from the garlic germplasm bank of Embrapa and were maintained under field conditions. Yet, virus-free seeds were obtained using apical stems from *in vitro* culture, maintained under a controlled environment. Dot-Elisa tests confirmed viral complex presence and absence (*Allexivirus*, *Carlavirus* and *Potyvirus* genera) in the propagation materials. These belong to Embrapa Hortaliças. The cultivation of virus-free materials for a single generation has been proven to not compromise plant development, even if grown close to virus-infected plants (Marodin *et al*., 2019). The cultivar Chonan is of the noble group and has an average of twelve bulbils per bulb but is susceptible to overgrowth.

Bulbils were planted in beds, and experimental plots were composed of four double rows, totalling 232 plants per plot. The useful area comprised 100 plants in the two central rows, discarding two plants at the end of rows in all plots. Plant spacing varied between single and double rows, as shown in Table 1.

Prior to planting, garlic bulb-seeds were vernalized for 57 days at 3-5°C, in a cold chamber. The average weight of virus-free bulbils from sieves one, two, and three corresponded to 3.75, 2.80, and 1.90 g $bulbil^{-1}$, respectively. Yet, the average weight of virus-infected bulbils from sieves one, two, and three corresponded to 3.25, 2.37, and 1.72 g $bulbil^{-1}$, respectively.

The doses of time-of-planting fertilization were estimated according to the following soil chemical analysis: pH in water = 6.3; organic matter = 31.1 g dm^{-3}; P and K = 22 and 176 mg dm^{-3}, respectively; Ca, Mg, Al, and H+Al= 5.8, 2.4, 0.0, 2.4 $cmol_c$ dm^{-3}, respectively. Based on this, an amount of 10 t ha^{-1} organic compound, 1000 kg ha^{-1} simple superphosphate, 300 kg ha^{-1} Yoorin Master®, 100 kg ha^{-1} potassium chloride, 15 kg ha^{-1} borax, and 10 kg ha^{-1} zinc sulphate were applied at planting.

At 55 days after planting (DAP), water stress was imposed to reduce bulbil sprouting. The plants were kept for 12 days under low water supply. After water stress, two topdressing fertilizations were performed (at 70 and 90 DAP), applying a total of 80 kg N and 80 kg K_2O ha^{-1}, using urea and potassium chloride as sources.

Spontaneous plants were controlled by oxadiazon herbicide and manual weeding. Phytosanitary control was performed using iprodione (fungicide) and deltamethrin (insecticide) against *Alternaria porri* and *Thrips tabaci*, respectively. Irrigation was performed via conventional sprinkling, as a function of crop water requirements at each garlic cultivation stage, applying 5 mm daily, three times a week.

At 90 DAP, four plants were sampled per plot in the morning. The sampled material was divided into bulbs, pseudo-stems, and leaves. The area of green leaves was obtained through a bench-top leaf area meter (Area Meter) LI-COR®, model LI 3100C, and expressed in cm^2 $plant^{-1}$. The total fresh weights of bulbs, pseudo-stems, and leaves were measured on a 0.001-g precision scale.

Bulbs were harvested after maturity, during plant senescence, at 130 DAP. Bulb-curing process was performed under shade for 25 days after harvest (Gabriel *et al*., 2020). The bulbs were cleaned by removing roots and leaves with pruning shears. Then, they were classified into the commercial classes established in Ordinance #242 of 17 September 1992, by the Brazilian

Ministry of Agriculture, Livestock and Food Supply (MAPA, 1992). Such classification rates bulbs as a function of diameter, as follows: refuse (<32 mm), class 3 (32 to 37 mm), class 4 (37 to 42 mm), class 5 (42 to 47 mm), class 6 (47 to 56 mm), and class 7 (>56 mm). Since class-3 bulbs had low occurrence, class-3 and -4 were grouped into one category (class 3+4). After classification, the total weight and number of bulbs were then estimated for each class.

Commercial bulb production (CBP) was evaluated according to average bulb mass (ABM) and percentages of class-7 (PB7), class-6 (PB6), class-5 (PB5), and class 4+3 (PB4+3) among the bulbs harvested in each plot. Given the absence of 'refuse' bulbs, production was estimated by weighing bulbs from all classes and expressed in t ha^{-1}. The average weight of produced bulbs was determined by the ratio of total weight to the number of bulbs, with values expressed in g bulb^{-1}. The rates (%) of class 7, 6, 5, and 4+3 bulbs were determined by the ratio of the number of bulbs in each respective class to the total number of bulbs.

Data were tested for normality and homogeneity of residual variances by the Lilliefors and Bartlett tests, respectively. When the assumptions were met, the data underwent the F-test for analysis of variance, using a joint analysis to compare the effects of using virus-infected and -free seeds. The means of bulb sizes were compared by the Tukey's test ($p \leq 0.05$), while the ones of plant spacing were submitted to regression analysis, with derivation for maximum or minimum values. These statistical analyses were performed using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

The use of virus-free garlic seeds increased all studied parameters but rates of class 5 (PB5) and class 4+3 (PB4+3) bulbs, regardless of seed size and plant spacing (Tables 2 and 3; Figures 1, 2, and 3).

The planting of sieve-1 virus-free garlic seeds showed a quadratic response for leaf area as plant spacing increased. In this treatment, a spacing of 332 cm^2 plant^{-1} reached the largest leaf area (617 cm^2 plant^{-1}). Yet, for infected clones, the maximum leaf area (451 cm^2 plant^{-1}) was achieved using sieve-1 seeds and a plant spacing of 315 cm^2 plant^{-1} (Figure 1).

Over the years, virus degeneration can limit vegetative growth of infected plants, mainly when closer spacing and smaller seeds are used (Lima *et al.*, 2019). The larger leaf areas in virus-free materials (Table 2) may have been due to the greater plant vegetative vigour of plants since they need larger spacings

Table 1. Population of garlic plants (POP) in response to spacing, distance between double rows (DR), single rows (SR) and between plants in the line (PL). Brasília, Embrapa Hortaliças, 2013.

Spacing (cm^2 plant^{-1})	DR (m)	SR (m)	PL (m)	POP (thousand plants/ha)
210	0.30	0.12	0.10	476
260	0.40	0.12	0.10	385
300	0.45	0.15	0.10	333
360	0.45	0.15	0.12	278
390	0.50	0.15	0.12	256

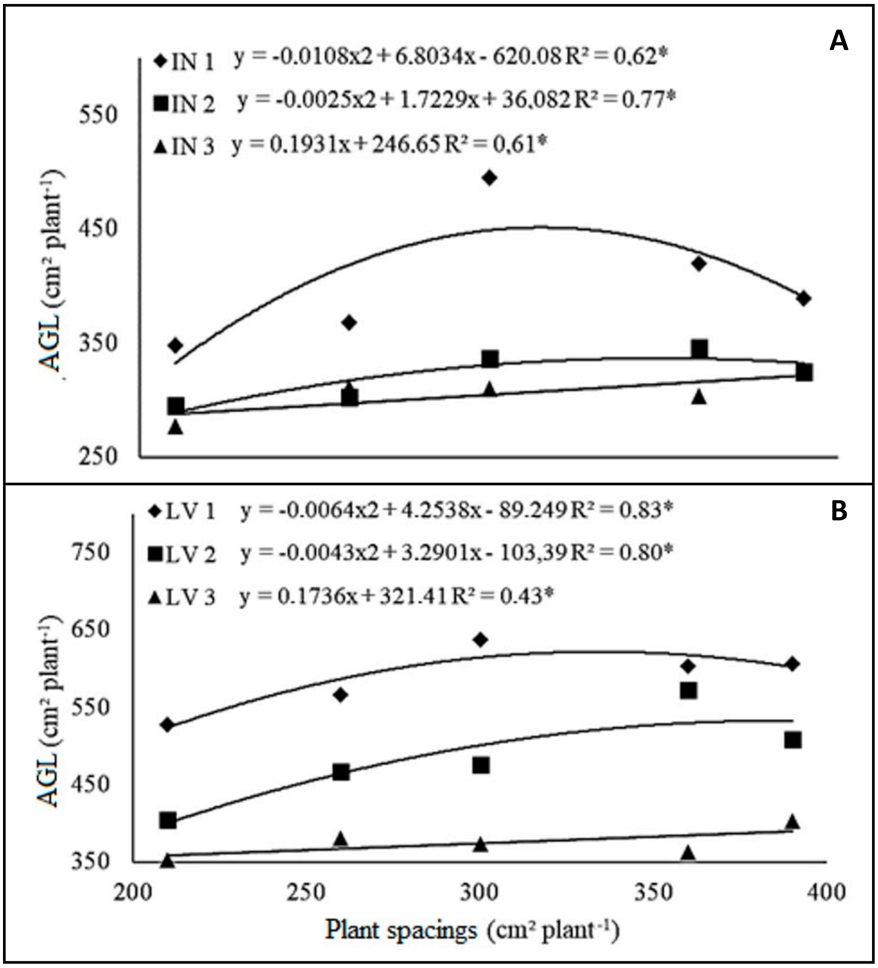

Figure 1. A= area of green leaves (AGL) of garlic, cv. Chonan, virus infected (IN) and B= virus-free (LV) in response to three bulbil sizes [sieves 1 (15x25 mm), 2 (10x20 mm) and 3 (8x17 mm) and five plant spacings (210, 260, 300, 360 and 390 cm²), at 90 days post-planting date]. Brasília, Embrapa Hortaliças, 2013.

to express their maximum growth potential.

For virus-infected garlic, the use of larger seeds may increase vegetative growth due to major nutritional reserve in seed bulbs (Pramesh & Baranwal, 2015). This ensures plants a greater initial growth, higher leaf number and length, which may have resulted in the largest leaf areas (Castellanos *et al.*, 2004). Still, fresh plant weight, average bulb weight, and yield varied with seed size (Table 2), and only sieve-1 bulbs provided higher values for these traits. Bulbs with higher carbohydrate reserves tend to favour plant establishment in the field and hence improve agronomic performance (Lencha & Buke, 2017).

Conversely, for virus-free clones, garlic yield and average bulb weight showed no differences between seed sizes from sieve 1 and sieve 2 (Table 2). This might have occurred due to a lesser virus degeneration of plants (Marodin *et al.*, 2019). Yet, medium-sized seeds provided vegetative growth potential and bulb yield similar to those of treatments using larger seeds (Table 2).

The best vegetative growth in virus-free plants was due to better photosynthetic capacity (Moravčević *et al.*, 2011) and nutrient accumulation in these plants (Fan *et al.*, 2017), increasing vegetative vigour, leaf area, and fresh weight compared to the infected clones (Table 2). This corroborates Resende *et al.* (1999) who observed, in garlic from meristem culture, an increase of 80.3% in shoot dry weight accumulation compared to virus-infected plants. A greater shoot vegetative growth

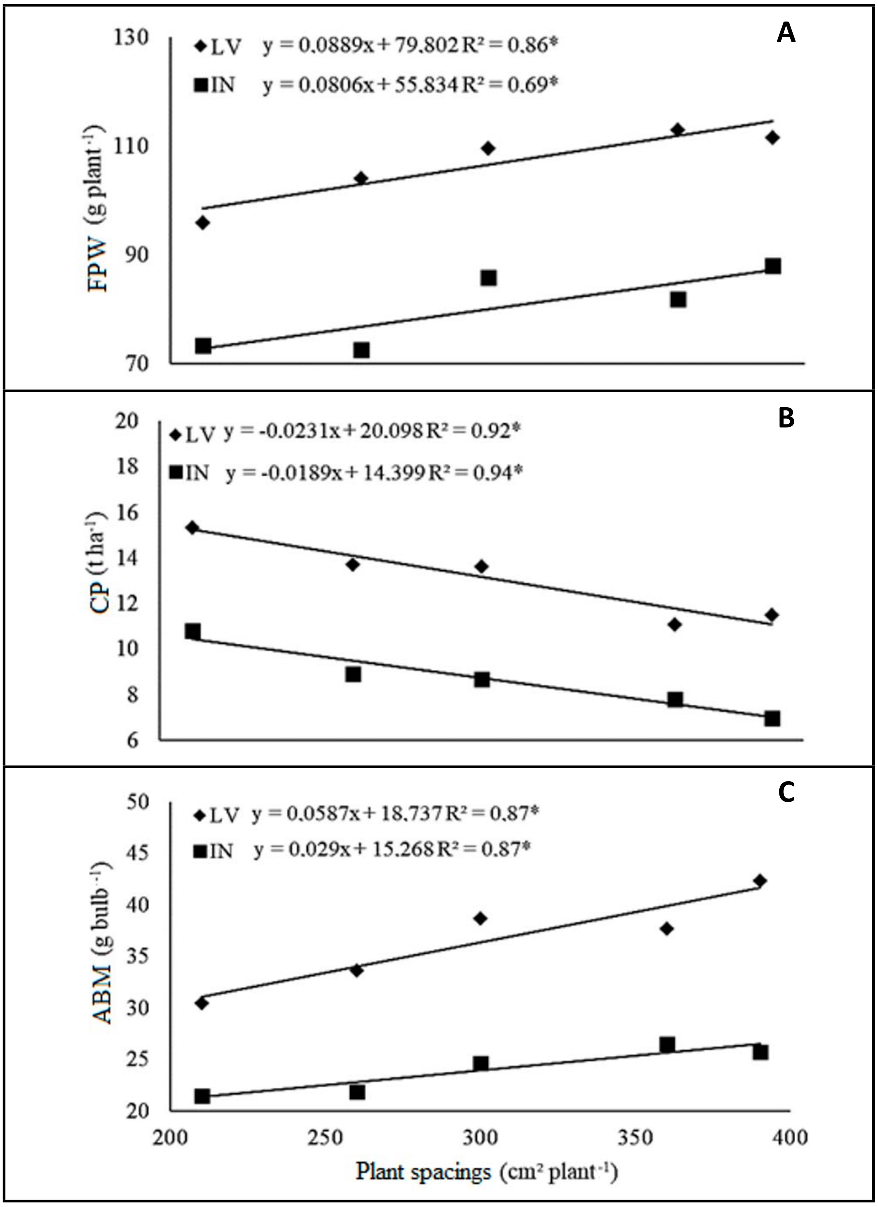

Figure 2. A= fresh plant weight (FPW), B= commercial bulb production (CP) and C= average bulb mass (ABM) of garlic, cv. Chonan virus-free (LV) and infected (IN), in response to three bulbil sizes [sieves 1 15x25 mm), 2 (10x20 mm); and 3 (8x17 mm)]. Brasília, Embrapa Hortaliças, 2013.

Table 2. Area of green leaves (AGL), fresh plant weight (FPW), commercial bulb production (CP) and average bulb mass (ABM) of garlic, cv. Chonan, virus-free (LV) and infected (IN) in response to three bulbil sizes [sieves 1 (15x25 mm), 2 (10x20 mm) and 3 (8x17 mm)]. Brasília, Embrapa Hortaliças, 2013.

FV		AGL (cm^2 $plant^{-1}$)		FPW (g $plant^{-1}$)		CP (t ha^{-1})		ABM (g $plant^{-1}$)	
		LV	IN	LV	IN	LV	IN	LV	IN
Sieves	1	588.6 a	404.0 a	124 a	92.1 a	13.8 a	9.7 a	38.9 a	26.8 a
	2	485.3 b	321.0 b	105 b	78.7 b	13.4 a	8.5 b	37.5 a	23.5 b
	3	374.2 b	305.3 b	91.4 c	70.1 b	12.0 b	7.8 c	33.4 b	21.8 c
Average		482.7 A	343.4 B	13.1 A	80.3 B	13.1 A	8.7 B	36.6 A	24.1 B
CV (%)		14.1		13.7		7.9		7.8	

Means followed by the same lowercase letter in the column do not differ by Tukey test ($p \leq 0.05$), and means followed by the same capital letter in the row do not differ by F test ($p \leq 0.05$). AGL and FPW were evaluated 90 days post-planting date; CP and ABM were evaluated 130 days post-planting date.

increase photo-assimilates that will be directed to bulbs (Marodin *et al.*, 2019). By contrast, infected plants tend to have reduced vegetative growth, early bulbification, and premature senescence (Fayad-André *et al.*, 2011). The presence of viruses does not always lead to garlic death, but makes plants more susceptible to other biotic stresses and adverse environmental conditions (Marodin *et al.*, 2019).

When virus-free seeds were used, larger plant spacings provided increased plant fresh and average bulb weights. Moreover, if compared to virus-infected clones, plant potential expression was enhanced in virus-free ones (Figure 2). As plant spacing increased, competition for light, nutrients, and water decreased, while photosynthetic efficiency and dry matter accumulation increased. Thus, the vegetative growth was improved, and larger garlic bulbs were produced (Ahmed *et al.*, 2017).

Larger plant spacings reduce density per area, decreasing number of produced bulbs. Even with the increase in bulb weight, lower plant densities decreased garlic yield both for virus-free and -infected clones. These traits are, therefore, inversely proportional to plant spacing (Figure 2). Furthermore, Lima *et al.* (2019) found that soluble solids, total soluble sugars, and soluble solids/titratable acidity ratio in bulbs increase as plant spacing decreases, regardless of bulb-seed size and viral infection.

From a qualitative point of view, bulbs of higher commercial classes have a better market share (Marodin *et al.*, 2019). Virus-free clones produced more class-7 and -6 and less class-4 and -3 bulbs. Conversely, virus-infected clones produced less class-7 bulbs. The use of virus-free seeds increased by 393% compared to the infected material. Moreover, for virus-free material, large- and medium-sized seeds (from sieve 1 and 2) increased by up to 36% compared to sieve-3 ones (Table 3).

For virus-free garlic, larger spacings promoted a linear increase in class-7 bulbs, reaching a maximum of 36.4% at the largest spacing (390 cm^2 plant^{-1}). However, when closer spacings were used, the percentage of class-6 bulbs increased. The excessive increase in plant density reduced the quality and sizes of produced bulbs, mainly for infected seeds, with a significant increase of class 4+3 bulbs (Figure 3).

Table 3. Garlic bulbs (%) classes 7, 6, 5 and 4+3, cv. Chonan virus-free (LV) and infected (IN), in response to three bulbil sizes [sieves 1 (15x25 mm), 2 (10x20 mm); and 3 (8x17 mm)], at 130 days post-planting date. Brasília, Embrapa Hortaliças, 2013.

FV		Class-7 (%)		Class-6 (%)		Class-5 (%)		Class-4+3 (%)	
		LV	IN	**LV**	IN	**LV**	IN	**LV**	IN
	1	28.1 a	10.9 a	56.9 ab	51.6 a	11.6 a	18.3 b	3,5 b	19.2 b
Sieves	2	27.9 a	2.42 b	54.9 b	46.6 ab	12.6 a	28.6 a	4,7 b	22.5 b
	3	20.7 b	2.19 b	59.4 a	41.3 b	13.5 a	31.6 a	6,4 a	25.0 a
Average		25.5 A	5.17 B	57.0 A	46.5 B	12.6 B	26.1 A	4.9 B	22.2 A
CV%		23.8		11.2		25.1		26.8	

Means followed by the same lowercase letter in the column do not differ by Tukey test (p≤0.05), and means followed by the same capital letter in the row do not differ by F test (p≤0.05).

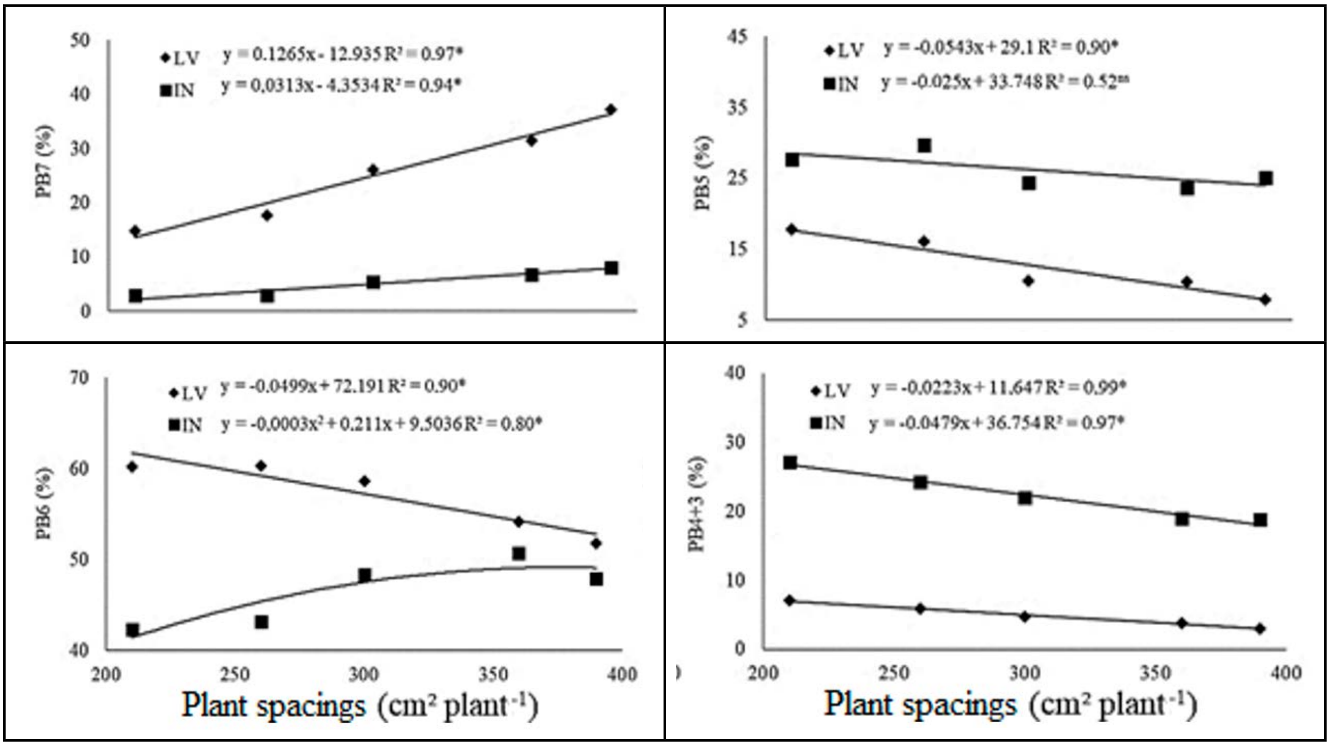

Figure 3. Garlic bulbs (%) infected (IN) and virus-free (LV) classes 7 (PB7), 6 (PB6), 5 (PB5) and 4+3 (PB4+3) in response to five spacings between plants (210, 260, 300, 360 and 390 cm^2), at 150 days after planting date. Brasília, Embrapa Hortaliças, 2013.

Since seeds have a high cost and represent a large part of garlic farming investments, the use of medium-sized garlic seeds can decrease planting costs. Moreover, virus-free clones have satisfactory results at larger plant spacings, improving vegetative growth and bulb commercial classification, as well as reducing expenses per area. As a result, using medium-sized virus-free garlic bulbs (sieve 2) as seed material, at a density of 300,000 plants ha^{-1}, may improve garlic yields and commercial classification of produced bulbs.

REFERENCES

ALAM, MS; RAHIM, MA; BHUYAN, MAH; SIMONAND, PW; MALEK, MA. 2010. Effect of spacing on growth and yield of two lines of garlic under dry land condition. *Journal of Agroforestry and Environment* 4: 151-154.

AHMED, I; KHAN, MA; KHAN, N; AHMED, N; WAHEED, A; SALEEM, FY; KHAN, S; ASLAM, S. 2017. Impact of plant spacing on garlic rust (*Puccinia allii*), bulb yield and yield component of garlic (*Allium sativum*). *Pakistan Journal of Agricultural Research* 30: 380-384.

ASGHARIPOUR, MR; ARSHADI, MJ. 2012. Effect of planting date and plant density on yield and yield components of garlic Infariman. *Advances in Environmental Biology* 6: 583-586.

BRASIL.1992. *Portaria nº 242, de 17 de setembro de 1992.* Available <http://www.codapar.pr.gov.br/arquivos/File/pdf/alho242_92.pdf>. Accessed March 10, 2019.

CASTELLANOS, JZ; VARGAS-TAPIA, P; OJODEAGUA, JL; HOYOS, G; ALCANTAR-GONZALEZ, G; MÉNDEZ, FS; ALVAREZ-SANCHEZ, E; GARDEA, AA. 2004. Garlic productivity and profitability as affected by seed clove size. *Hortscience* 39: 1272-1277.

DORO, AK. 2012. Response of garlic (*Allium sativum* L.) to intra-row spacing at Ajiwa irrigation site of Katsina State. *Journal of Research in National Development* 10: 103-107.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. 2006. *Sistema brasileiro de classificação de solos*. Brasília: EMBRAPA-SPI. 306p.

FAN, B; HE, R; SHANG, Y; LIWEI, X; NINGNING, W; HUI, G; XIAOYING, L; ZHENYING, W. 2017. System construction of virus-free and rapid-propagation technology of Baodi garlic (Allium sativum L.). Scientia Horticulturae 225: 498-504.

FAYAD-ANDRE, MS; DUSI, AN; RESENDE, RO. 2011. Spread of viruses in garlic fields cultivated under different agricultural production systems in Brazil. *Tropical Plant Pathology* 36: 341-349.

FERREIRA, DF. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.

GABRIEL, A; RESENDE, JTV; MARODIN, JC; MATOS, R; ZEIST, AR; MACIEL, CDG. 2020. Chemical stress reduces the lateral shoot growth in vernalized garlic. *Horticultura Brasileira* 38: 41-46.

GEDAMU, F; WITSADIK, K; DECHASSA, N; TIWARI, A. 2008. Effects of clove size and plant density on the bulb yield and yield components of Ethiopian garlic (*Allium sativum* L.). *Pantnagar Journal of Research* 6: 234-238.

GIMENEZ, MD; YAÑEZ-SANTOS, AM; PAZ, RC. 2016. Assessment of genetic and epigenetic changes in virus-free garlic (*Allium sativum* L.) plants obtained by meristem culture followed by *in vitro* propagation. *Plant Cell Reports* 35: 129-141.

LENCHA, B; BUKE, T. 2017. Effects of clove size and plant density on the bulb yield and yield components of garlic (*Allium sativum* L.) in Sodo Zuria Woreda, southern wolaita zone. *Journal of Natural Sciences Research* 7: 1-7.

LIMA, MFP; LOPES, WAR; NEGREIROS, MZ; GRANGEIRO, LC; SOUSA, HC; SILVA, OMP. 2019. Garlic quality as a function of seed clove health and size and spacing between plants. *Revista Caatinga* 32: 966-975.

MARODIN, JC; RESENDE, FV; GABRIEL, A; SOUZA, RJ; RESENDE, JTV; CAMARGO, CK; ZEIST, AR. 2019. Agronomic performance of both virus-infected and virus-free garlic with different seed bulbs and clove sizes. *Pesquisa Agropecuária Brasileira* 54: 01-08.

MORAVČEVIĆ, D; BJELIĆ, V; SAVIĆ, D; VARGA, JG; BEATOVIĆ, D; JELAČIĆ, S; ZARIĆ, V. 2011. Effect of plant density on the characteristics of photosynthetic apparatus of garlic (*Allium sativum* L.). *African Journal of Biotechnology* 10: 15861-15868.

MUNEER, N; HUSSAIN, M; AHMAD, MJ. 2017. Effect of planting density on growth, yield and quality of garlic at Rawalakot, Azad Kashmir. *International Journal of Agronomy and Agricultural Research* 10: 42-51.

OLIVEIRA, ML; NARDINI, JPC; MARCHI, BR; MITUTI, T; BAMPI, D; PAVAN, MA; KRAUSE-SAKATE, R. 2014. Análise da presença de vírus em alho semente da segunda e quarta gerações, produzidos por termoterapia e cultura de tecido. *Summa Phytopathologica* 40: 75-77.

PRAMESH, D; BARANWAL, VK. 2015. Production of virus-free garlic (*Allium sativum* L.) through meristem tip culture after solar or hot air treatment of cloves. *Journal of Horticultural Science and Biotechnology* 90: 180-186.

RATHINAKUMARI, AC; CHANNABASAMMA, BB; KUMARAN, GS. 2015. Physical and mechanical properties of garlic bulbs and cloves (*Allium sativum* L.) relevant to development of garlic bulb breaker. *International Journal of Tropical Agriculture* 33: 1881-1887.

RESENDE, FV; SOUZA, RJ; FAQUIN, V; RESENDE, JT. 1999. Comparação do crescimento e produção entre alho proveniente de cultura de tecidos e de multiplicação convencional. *Horticultura Brasileira* 17: 118-124.

ROSTAMI, M; MOHAMMADI, H. 2018. Effect of plant density and temperature on yield and morpho-physiological traits of garlic (*Allium sativum* L.). *Iranian Journal of Medicinal and Aromatic Plants* 34: 766-780.

STAHLSCHMIDT, O; CAVAGNARO, JB; BORGO, R. 1997. Influence of planting date and seed cloves size on leaf area and yield of two garlic cultivars (*Allium sativum* L.). *Acta Horticulturae* 433: 519-526.

VIEIRA, RL; SILVA, AL; ZAFFARI, GR; STEINMACHER, DA; FRAGA, HPF; GUERRA, MP. 2015. Efficient elimination of virus complex from garlic (*Allium sativum* L.) by cryotherapy of shoot tips. *Acta Physiologiae Plantarum* 37: 1733, 2015.