Evaluation of arugula cultivars and seed production in the organic system

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ABSTRACT – The production of arugula (Eruca sativa Mill.) in Brazil uses seeds produced in the conventional system, without availability of organic seeds. The aim of this research was to indicate the cultivars of arugula with best performance for commercial production and to evaluate seed production viability, as well as seed quality of these cultivars in the organic production system. Eight cultivars were evaluated in a protected environment for agronomic characteristics in two periods and for seed production. The parameters evaluated were plant height, number of leaves, shoot fresh and dry weight, yield, iron and vitamin C contents. For seed production, time for flowering, number of branches per plant, silique length, number of siliquae per plant, number of seeds per silique, yield, one thousand seed weight, first count of germination, germination and seed health. After storage, first germination count, germination, seedling emergence, emergence speed index and seed health were evaluated. “Astro” and “Giovana” performed well in the organic system. Organic arugula seeds can be produced with high yield and good seed quality in a protected environment, and the cultivars Astro and LG Maia are suitable for this purpose.

Index terms: Eruca sativa Mill., growing seasons, agronomic performance, seed yield, storage.

Introduction

Arugula, which belongs to the family Brassicaceae and is native to the Mediterranean, is a much-appreciated leafy vegetable, consumed in the form of salad, rich in vitamin C, potassium, sulfur and iron, and grown in various regions of Brazil (Filgueira, 2008).

There are 2,756 arugula growers in the organic system registered in Brazil (MAPA, 2018); however, the seeds used by these growers are produced in the conventional system,
not in the organic system. Organic seeds increase production costs because they are more expensive, since the options are either imported seeds - whose price in Brazil is 20% higher than that of conventional seeds (Nascimento, 2013) - or seeds produced in Brazil, whose yield is insufficient to meet the demands of producers.

Seed companies in Brazil do not invest in organic seed production because the use of organic seeds is non-mandatory and there is a low demand for this sector, as a result of the higher cost of seeds. Moreover, there is little research on seed production in this cultivation system.

Producing organic seeds needs further development of technologies adapted to the conditions of Brazil, especially to ensure germination and health, in addition to identifying the most appropriate germplasm, with good commercial characteristics and resistance to pests and diseases (Nascimento, 2013).

Despite the low supply of organic seeds on the market, the cultivation of organic vegetables is on the rise, demonstrating market potential for this type of seed. Vegetable breeding programs for organic cultivation are scarce in Brazil, and conventional cultivars are the only options available to farmers. Therefore, evaluating the performance of cultivars in the organic system is important to check for their agronomic characteristics, as well as their potential for seed production. The aim of this work was to indicate the best performing arugula cultivars during the commercial production phase and to evaluate the viability of producing seeds with high germination rates, vigor and health in an organic system.

**Material and Methods**

Two assays were carried out: the first one evaluated the agronomic performance of eight arugula cultivars in the organic system in two growing periods (summer and fall) and the second assay evaluated the same cultivars for seed production in the organic system in the fall. Both tests were performed in the experimental field of the Mokiti Okada Research Center in a protected environment (without closing the sides). The farm is located in Ipeúna, SP, with latitude 22º26’09” south and longitude 47º43’08” west and altitude of 635 m.

The soil was classified as a red yellow latosol (EMBRAPA, 2006), with the following chemical characteristics (0-0.2 m); first test: P (mg.dm⁻³) 142; O.M. (g.dm⁻³) 37; pH (CaCl₂) 5.3; K (mmolec.dm⁻³) 29.7; Ca (mmolec.dm⁻³) 56; Mg (mmolec.dm⁻³) 31; S (mmolec.dm⁻³) 14; H + Al (mmolec.dm⁻³) 18; Al (mmolec.dm⁻³) 0.2; SB (mmolec.dm⁻³) 117; CEC (mmolec.dm⁻³) 135; V (%) 87; B (mg.dm⁻³) 0.4; Cu (mg.dm⁻³) 13.0; Fe (mg.dm⁻³) 11.0; Mn (mg.dm⁻³): 19.6; Zn (mg.dm⁻³) 3.0; and second test: P (mg.dm⁻³) 204; O.M. (g.dm⁻³) 29; pH (CaCl₂) 5.2; K (mmolec.dm⁻³) 28.7; Ca (mmolec.dm⁻³) 43; Mg (mmolec.dm⁻³) 5; S (mmolec.dm⁻³) 9; H + Al (mmolec.dm⁻³) 12; Al (mmolec.dm⁻³) 0.2; SB (mmolec.dm⁻³) 77.1; CEC (mmolec.dm⁻³) 90.1; V (%) 86; B (mg.dm⁻³) 0.19; Cu (mg.dm⁻³) 9.0; Fe (mg.dm⁻³) 60.0; Mn (mg.dm⁻³): 16.4 and Zn (mg.dm⁻³) 2.7.

**First assay:** The experiment used a randomized block design with eight cultivars (Ágatha, Astro, Apreciatta Folha Larga, Giovana, Folha Larga, Cultivada, Rococó and LG Maia) and eight replications. The soil was prepared with the aid of a tiller and the incorporation of Bokashi fertilizer (100 gm⁻²). Sowing was carried out on January 25th, 2017 (summer) and April 26th, 2017 (fall), in four cultivation rows (0.30 m between them), keeping one plant every 0.15 m. Harvesting and evaluations were performed at 37 days after sowing (DAS) in the summer and at 43 DAS in the fall. Ten plants were evaluated in the two central rows per plot and the other rows were considered as borders. The parameters evaluated were plant height (cm): determined by measuring from the root collar to the end of the largest leaf of each plant; number of leaves per plant: the number of leaves (greater than 0.05 m) was counted; shoot fresh weight (g.plant⁻¹): weighing the set of five plants; shoot dry weight (g.plant⁻¹): determined after drying the shoot in a forced air circulation oven (70 °C) to constant weight; yield (kg.m⁻²): determined by the fresh weight of the evaluated shoot divided by the occupied area of each plant; iron content: 100 g of leaves from four plots were collected and the methodology of Nogueira (2005) was used; vitamin C content: 100 g of leaves from four plots were collected and analyzed according to the methodology of Carvalho et al. (1990).

**Second assay:** The experimental design, cultivars and tillage were the same adopted in the first test, and planting was performed in the fall. Spacing was 0.2 m (plant) and 0.4 m (rows). Number of days for flowering, determined from sowing until approximately 50% of the plants were in the flowering stage and expressed in DAS. The seeds were harvested from the ten central plants of each plot, when more than 50% of the silique of the plant were light brown; after that, the number of branches per plant was determined from shoots originating from the main stem; silique length (cm): six randomized siliquae of each plant were analyzed; total number of siliquae per plant: determined by counting all the siliquae in the plant; number of seeds per siliqua: performed by counting the seeds of six siliquae of each plant of the plot; seed yield in t.ha⁻¹; one thousand seed weight: determined according to Brasil (2009); first germination count and
germination test: performed according to Brasil (2009), using four replications of a hundred seeds with the results expressed as percentage of normal seedlings per cultivar; health test (fungal incidence): performed by the filter paper method with frozen samples, with four replications of fifty seeds left in incubation chamber for 24 hours at 20 ± 2 °C and 12 h photoperiod. After this period, the seeds were kept at -20 °C (freezer) for 24 hours and then transferred to the incubation chamber for a further seven days. The evaluation was performed at the end of this incubation period (Brasil, 2009). The results were expressed as percentage of seeds with incidence of different potentially pathogenic fungi.

Water content was also determined for standardization by the oven drying method (105 ± 3 °C for 24 hours) (Brasil, 2009) and the results were expressed as a percentage.

The remaining seeds were placed in paper bags and stored in a cold chamber at 10 °C and 40% humidity for one year. After this period, the evaluations of germination, first germination count and seed health (fungal incidence) were repeated. The following tests were also performed: seedling emergence and emergence speed index (ESI).

The emergence test was performed with four replications of fifty seeds in plastic trays with the Plant Bokashi substrate, in a protected environment, with intermittent and automatic micro-sprinkler irrigation. The number of normal seedlings emerged daily until emergence stabilization - which occurred ten days after sowing - was calculated, and ESI was calculated using the formula proposed by Maguire (1962). At the end, the total number of emerged normal seedlings was counted, and the results were expressed as a percentage.

The data were submitted to analysis of variance, and the means were compared by the Scott Knott test at 5% probability.

### Results and Discussion

In the first assay, in the summer, there was a difference in the number of leaves (NL) and shoot dry weight (SDW) among the cultivars, with Agatha, Astro, Folha Larga, Giovana and LG Maia having the highest values, which did not differ from one another (Table 1). Cultivars with more leaves per plant are more suitable because they are more commonly sold in the form of bunches. These cultivars stood out in the fall for the number of leaves per plant, except for LG Maia, demonstrating the stability of these cultivars in the growing season (Table 1).

Ágatha, Astro and Giovana cultivars presented higher plant height values in the summer and autumn (Table 1). However, it is noteworthy that two plants can be used per hole, with similar results for plant height, thus increasing bunch yield, as found by Reghin et al. (2005), who tested the cultivar “Cultivada”.

There were no statistical differences in yield (Y) between cultivars in summer and fall crops, as well as shoot fresh weight (SFW) (Table 1). Plants with a larger number of leaves are expected to have higher yield per plant and area, but this was not found in this work. This may be explained by the plant architecture of each cultivar, which was different from one another. However, the average value is within commercial standards, when the results reported above are compared with those of the organic system and even with the conventional system, as found by Oliveira et al. (2010), who studied the

| Cultivar          | NL (Summer) | PH (cm) | SFW (g.plant⁻¹) | Y (kg.m⁻²) | SDW (g.plant⁻¹) |
|-------------------|-------------|---------|-----------------|------------|-----------------|
| Ágatha            | 8.4 a       | 9.1 a   | 30.3 a          | 30.4 a     | 42.9 a          |
| Apreciatta Folha Larga | 6.9 b     | 7.5 c   | 27.5 b          | 27.7 c     | 36.6 a          |
| Astro             | 8.2 a       | 9.0 a   | 29.4 a          | 31.3 a     | 37.2 a          |
| Cultivada         | 6.8 b       | 8.3 b   | 25.7 c          | 27.4 c     | 33.1 a          |
| Folha Larga       | 8.1 a       | 9.5 a   | 27.4 b          | 29.3 b     | 36.2 a          |
| Giovana           | 8.3 a       | 9.2 a   | 30.1 a          | 31.1 a     | 44.0 a          |
| LG Maia           | 7.9 a       | 8.3 b   | 28.2 b          | 27.2 c     | 39.0 a          |
| Rococó            | 7.3 b       | 8.0 b   | 26.0 c          | 26.6 c     | 35.1 a          |
| CV (%)            | 8.44        | 6.51    | 5.03            | 5.16       | 20.51           |
| F                 | 7.79        | 12.30   | 12.15           | 12.63      | 1.84            |

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability of error.
behavior of arugula with organic and conventional fertilization.

Another important factor, in addition to production, is quality, because consumers appreciate organic products, not only because they do not use pesticides in their cultivation, but also because they believe in the higher nutritional value of these products (Srinijaig and Thapa, 2018), which indicates the importance of the search for cultivars with higher nutrient and vitamin content. Arugula, for example, which was evaluated in the present work, is a vegetable rich in vitamin C and iron (Pignone, 1997) and the cultivars Folha Larga and Rococó presented the highest vitamin C contents, both in the summer and autumn (Table 2). It is noteworthy, however, that some environmental factors may affect the concentration of this vitamin in the leaves: when light, temperature and water availability, for example, are inadequate, they may lead to increased concentration of vitamin C (Antoniou et al., 2009). Hall et al. (2014) and Nurzyńska-Wierdak (2015) found average values between 64 to 115 mg.100 g-1. These values are higher than those found in this study, but different methodologies were used to obtain the concentration of this vitamin in arugula leaves, which may bring divergent results; therefore, the methods have to be standardized.

Iron content only differed statistically among cultivars in the fall, and the mean values were numerically lower than those found in the summer assay (Table 2), possibly due to the lower temperature, which induces a reduction in their absorption, as suggested by Bozokalfa et al. (2009). The authors stated that this trait may also be related to the genotype and the availability of this element in the soil. Iron availability in the soil was similar for all cultivars, but the response of each cultivar to the lowest temperature may have varied.

Nutritional properties are important for breeding programs, as diet plays a key role in promoting and maintaining health. Consumption of vegetables such as arugula, which is a source of vitamins and minerals, is a significant way of compensating for nutritional deficiency (Carvalho et al., 2006) and biofortification of vegetables is aimed at improving nutritional properties (Chomchan et al., 2017).

As for the reproductive phase, LG Maia and Astro flourished earlier than the other cultivars, with 50% of the plants flowered at 55 DAS (Figure 1). Apreciatta Folha Larga was the last cultivar to complete flowering, with 24 days longer than the earliest cultivars. The cultivars Rococó and Cultivada did not bud (did not produce seeds), possibly because of specific requirements, as some cultivars require flowering induction by low temperatures followed by long days and high temperatures (Mohamedien, 1994; Morales and Janick, 2002). Thus, the cultivars Rococó and Cultivada may require lower temperatures as well as different photoperiods under the local conditions of the experiment for induction, of flowering.

For silique length (SL), one thousand seed weight (TSW) and number of seeds per silique (NSeed.silique\(^{-1}\)), Apreciatta Folha Larga and Folha Larga presented the lowest values (Table 3). Silique length is directly related to number of seeds per silique, because the larger the silique, the greater the potential number of seeds. These factors can also be determined by cultivar, since number of seeds per silique, according to Fazili et al. (2010), is genetically determined, with little influence on crop treatments, indicating the importance of choice of cultivar for better seed yield. There was no statistical difference in the parameters number of siliquae per plant and number of branches per plant.

For seed yield, LG Maia produced 3.6 times more seeds than the cultivar Folha Larga. Cultivars Astro, Giovana and

| Cultivar   | Vitamin C (mg ascorbic acid.100 g\(^{-1}\) sample) Summer | Fall | Ion content (mg. Kg\(^{-1}\)) Summer | Fall |
|------------|--------------------------------------------------------|------|-----------------------------------|------|
| Ágatha     | 11.06 b                                                | 9.4 b| 510.5 a                           | 113.7 b |
| Apreciatta | 13.19 b                                                | 12.3 a| 760.0 a                           | 222.0 a |
| Folha Larga| 15.87 b                                                | 13.9 a| 653.0 a                           | 143.7 b |
| Astro      | 13.22 b                                                | 12 a | 706.0 a                           | 112 b |
| Cultivada  | 19.05 a                                                | 13.8 a| 874.5 a                           | 124.2 b |
| Folha Larga| 18.67 a                                                | 8.2 b | 408.7 a                           | 292.2 a |
| Giovana    | 15.14 b                                                | 7.2 b | 554.2 a                           | 204.5 a |
| LG Maia    | 23.27 a                                                | 14 a | 578.7 a                           | 258.0 a |
| CV (%)     | 26.46                                                  | 15   | 48.93                             | 44.17 |
| F          | 3.42                                                   | 10.06| 0.92                              | 2.98   |

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability of error.

Figure 1. Flowering time of arugula cultivars and seed harvest in days after sowing.
LG Maia had best performance, with similar results to those found by Lazzeri et al. (2004) with 2.0 t.ha⁻¹ (autumn) and 1.55 t.ha⁻¹ (summer) and Esiyok et al. (2013) with 1.3 t.ha⁻¹, both in the conventional system, which demonstrates the production potential in the organic system.

The difference in water content (Table 4) of seeds from different cultivars did not exceed the two percentage points, as advisable. This information is important for seed testing, as uniform water content is required for physiological potential assessments to achieve consistent results (Marcos-Filho, 1999).

The environment where the seeds were formed has great influence on germination, vigor and health. In this work, management and place of crop production were the same; however, there was a difference between cultivars: Ágatha and Giovana had the lowest results for germination and first germination count, indicating the influence of genotype on physiological potential, as reported by Marcos-Filho (2015).

The health test, performed immediately after harvest (Table 5), detected the incidence of fungi *Alternaria* sp, *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp. and *Sclerotinia* sp. LG Maia and Folha Larga presented a higher incidence of fungi, although not expressive, and this result did not interfere with germination, but it may reduce storage vigor or affect seedling development, since fungi *Alternaria* sp., *Cladosporium* sp. and *Sclerotinia* sp. are transmitted to seedlings (Vieira et al., 2015).

In this research, production occurred in a protected environment, a condition that provides lower humidity than

### Table 3. Number of branches per plant (NB plant⁻¹), silique length (SL), total number of siliquae per plant (NSil plant⁻¹), one thousand seed weight (TSW), number of seeds per silique (NSeed.silique⁻¹) and seed yield (Y) of arugula cultivars conducted in an organic system.

| Cultivar       | NB (g.plant⁻¹) | PH (cm) | NSil.plant⁻¹ | TSW (g) | NSeed.silique⁻¹ | Y (ha⁻¹)* |
|----------------|----------------|---------|--------------|---------|-----------------|-----------|
| Ágatha         | 7.58 a         | 2.11 a  | 277.70 a     | 1.67 a  | 24.75 a         | 0.74 b    |
| Apreciatta Folha Larga | 6.90 a         | 1.48 b  | 354.02 a     | 1.34 b  | 17.54 b         | 0.61 b    |
| Astro          | 7.28 a         | 1.96 a  | 403.96 a     | 1.65 a  | 24.74 a         | 1.21 a    |
| Folha Larga    | 7.12 a         | 1.4 b   | 344.59 a     | 1.23 b  | 17.68 b         | 0.46 c    |
| Giovana        | 6.61 a         | 2.07 a  | 355.80 a     | 1.80 a  | 23.76 a         | 1.11 a    |
| LG Maia        | 6.56 a         | 2.16 a  | 466.21 a     | 1.64 a  | 23.84 a         | 1.69 a    |
| CV (%)         | 9.7            | 4.64    | 24.01        | 18.06   | 15.67           | 8.21      |
| F              | 0.6            | 28.91   | 1.04         | 4.79    | 8.04            | 7.89      |

The averages followed by the same letter do not differ statistically from each other. The Scott-Knott Test was applied. *Data transformed into √x.

### Table 4. Water content, germination test (G) and first germination count (1st GC) of seeds of different freshly harvested arugula cultivars.

| Cultivar       | Water content (%) | G (%)* | 1st GC (%)* |
|----------------|-------------------|--------|-------------|
| Ágatha         | 7.23              | 91 c   | 89 c        |
| Apreciatta Folha Larga | 7.13            | 100 a  | 100 a       |
| Astro          | 7.17              | 98 a   | 96 a        |
| Folha Larga    | 6.70              | 99 a   | 98 a        |
| Giovana        | 6.81              | 95 b   | 94 b        |
| LG Maia        | 6.51              | 98 a   | 97 a        |
| CV (%)         | -                 | 1.2    | 2.4         |
| F              | -                 | 8.07   | 9.68        |

*Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability of error. *Data transformed into √x.

### Table 5. Incidence of fungi (%) *Alternaria* sp (Al), *Aspergillus* sp (As), *Cladosporium* sp (Cl), *Penicillium* sp (Pe) and *Sclerotinia* sp (Sc) in seeds of different cultivars of freshly harvested arugula.

| Cultivar       | Al  | As  | Cl  | Pe  | Sc  |
|----------------|-----|-----|-----|-----|-----|
| Ágatha         | 0.5 | 1.0 | 4.0 | 1.5 | 0.0 |
| Apreciatta Folha Larga | 0.0 | 0.0 | 2.5 | 0.5 | 0.0 |
| Astro          | 1.0 | 1.5 | 1.0 | 1.0 | 0.0 |
| Folha Larga    | 6.5 | 2.0 | 6.5 | 1.0 | 0.0 |
| Giovana        | 2.0 | 0.0 | 1.0 | 0.0 | 0.0 |
| LG Maia        | 9.0 | 0.0 | 9.0 | 4.5 | 1.0 |
| CV (%)         | 93.56 | 146.72 | 83.75 | 138.15 | 482.51 |
| F              | 6.23 | 2.35 | 3.67 | 2.43 | 0.94 |

The means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability level.
in the open field, and, consequently, it may have contributed to the low incidence of fungi. But as seeds can act as a vehicle for fungal dissemination in disease-free areas, their control and the search for alternative treatments are important, since the legislation that regulates the production of organic seeds (Brasil, 2011), does not allow the use of chemicals for phytosanitary control for the purpose of seed treatment.

During storage, there was a reduction in seed germination and vigor, in general. However, Apreciatta Folha Larga, Astro and LG Maia presented higher values for germination, first germination count, germination speed index (GSI) and emergence in comparison to the other cultivars, and they did not differ from one another (Table 6). Folha Larga showed reduced germination and vigor in comparison to other cultivars during storage, possibly because of the increase in associated fungi, especially *Penicillium* sp., and genetic factors, as suggested by Suma et al. (2013). Moreover, the seeds were stored in paper bags, which allows the exchange of gases and water with the environment; therefore, it may have contributed to the reduction of germination and increase of fungi.

Regarding the incidence of fungi after storage, only *Penicillium* sp. and *A. alternata* were found to show differences between cultivars (Table 7). The seeds of the cultivar Astro presented lower incidence of *Penicillium* sp. and its germination, first germination count, GSI and emergence values were higher. LG Maia increased the percentage of fungi after storage; however, its initial germination contributed to maintaining vigor.

In the production of brassica seeds, it is recommended that in flowering and seed maturation, the environment is dry and free from precipitation, in order to obtain high yields of seeds with high vigor (Contreras et al., 2014). When the location cannot be chosen, a protected environment is an alternative. The results of this research showed that arugula seeds can be produced in the organic system, but water content before storage needs to be adjusted and airtight packaging needs to be used, allowing producers and companies to produce arugula seeds of acceptable quality in alternative production systems in protected environments, as long as farmers use the appropriate cultivar for each region.

**Conclusions**

The cultivars Giovana, Ágatha and Astro have good productive performance in the organic system.

Organic arugula seeds can be produced with high values for yield, germination, vigor and health in a protected environment. The cultivars Astro and LG Maia stand out in the production of seeds with high germination, vigor and health in the organic system.

| Cultivar | G (%) | 1st GC (%) | ESI | E (%) |
|----------|-------|------------|-----|-------|
| Ágatha   | 87 b  | 87 b       | 11.9 b | 78.5 b |
| Apreciatta Folha Larga | 94 a | 93 a | 12.9 a | 91.5 a |
| Astro    | 93 a  | 92 a       | 13.9 a | 95.5 a |
| Folha Larga | 81 b | 80 b | 10.8 b | 76.0 b |
| Giovana  | 85 b  | 84 b       | 12.0 b | 86.0 a |
| LG Maia  | 94 a  | 94 a       | 12.7 a | 92.5 a |
| CV (%)   | 3.1   | 5.7        | 8.1  | 7.6   |
| F        | 3.99  | 4.94       | 4.61 | 5.84  |

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability of error.

| Cultivar | Fungal incidence (%) |
|----------|----------------------|
|          | *Penicillium* sp.    | *Alternaria alternata* |
| Ágatha   | 12.5 a              | 0.5 a               |
| Apreciatta Folha Larga | 33.5 b | 1.0 a |
| Astro    | 23.5 a              | 5.5 b               |
| Folha Larga | 59.0 c | 0.0 a |
| Giovana  | 35.5 b              | 1.0 a               |
| Maia     | 30.0 b              | 2.0 a               |
| CV (%)   | 37.40               | 103.92              |
| F        | 6.56                | 5.28                |

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability of error.

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