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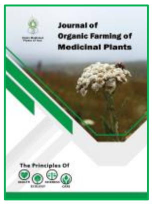


Original Article

# Planting density on growth, yield and quality of bushy growth habit hull-less seed pumpkin (*Cucurbita pepo* var. Styriaca)

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ABSTRACT

The seeds and oil obtained from Styrian pumpkin contain valuable compounds that can be used in the treatment of various diseases. An experiment was conducted using a split design in the form of a randomized block with three replications to determine the optimal planting distance for achieving maximum seed and oil yield, as well as efficient resource utilization. The study involved four Styrian pumpkin hybrids (23×8, 2×23, 23×11, 7×14) and two planting densities: 20,000 and 10,000 plants·ha$^{-1}$. The research took place during the spring and summer of 2023 at the Faculty of Agricultural Sciences, University of Guilan. Several plant characteristics were evaluated, including initial and final plant length, number of leaves, number of side branches, leaf area, 1000 seed weight, vegetative biomass, fruit yield, seed yield, oil yield, and the amount of some fatty acids, total sterol, and beta sterol content. The results indicated significant differences between genotypes and planting densities. The highest yield per plant observed at the 10,000 plants·ha$^{-1}$. However, the yield per hectare was greater at the 20,000 plants·ha$^{-1}$. In high-density planting, plants exhibited more vegetative growth, fewer leaves, and fewer side branches, but the overall yield increased due to the greater number of plants compensating for the reduction in individual plant yield. High planting densities effectively utilized environmental resources, as dense vegetation cover maximized leaf area index and photosynthetically active radiation absorption. Consequently, the rate of dry matter accumulation and plant growth increased. Based on the results, a planting density of 20,000 plants·ha$^{-1}$ is recommended for the all of investigated hybrids. Future research should explore the feasibility of further increasing this density.



## 1. Introduction

Medicinal pumpkin (*Cucurbita pepo* var. Styriaca) is an important and valuable medicinal plant from the Cucurbitaceae family and originated from Austria and Slovenia (Tanska et al., 2020). Hull-less seeds in this type of pumpkin were created as a result of random and natural mutation, leading to morphological changes and the formation of seeds with a thin shell (Habibi et al., 2011). Each fruit of this pumpkin contains 400 to 500 seeds, equivalent to 150 to 200 grams of seeds. The seeds are dark green or olive green in color (Nomikos et al., 2009).

Research conducted in different locations shows the high oil content in its seeds. One investigation revealed a high percentage of two important unsaturated fatty acids, namely oleic and linoleic acid, along with other compounds such as alpha-linoleic acid, phytosterols, omega-3 fatty acids, vitamin E, and tocopherols in the seeds. This plant has positive clinical effects on the prostate and also has bladder-strengthening effects (Heim et al., 2018). Tocopherol isomers in the seed oil of this plant have antioxidant capacity (Broznić et al., 2016). Research on mice confirmed the antiparasitic, antioxidant, and anticoagulant properties of pumpkin seed oil (Beshay et al., 2019). Many pharmacological studies confirm the effects of medicinal pumpkin on liver protection, antioxidant activity, anti-cancer, antimicrobial, anti-inflammatory, analgesic properties, and inhibiting the activities of benign-malignant hyperplasia of the prostate (Perez Gutierrez, 2016).

Regarding the effect of plant density on the performance of agricultural and medicinal plants, several experiments have been conducted. All the results indicate that the response of different plants to plant density varies according to the type of plant species, variety, and climatic and soil conditions (Kulture et al., 2001). Closer plant spacing decreases soil exposure, thereby reducing evaporation rates. Reducing row spacing and increasing plant density are effective strategies for enhancing water and energy efficiency in crop production, ultimately leading to higher yields. Research indicates that these practices minimize evaporation and optimize resource utilization, which is crucial in arid regions (Deressegn, 2017). Plants at higher densities produced maximum fruit yield compare to wider spacing. This was possibly due to increase in plant number per unit area, which might contribute to the production of more yield per unit area and leading to higher yield (Kavut et al., 2014).

In recent decades, the cultivation of hybrid cultivars has become increasingly prominent in cultivated species, especially *C. pepo* species. Hybrid cultivars should be as efficient as standard open-pollinated cultivars and also possess other important characteristics such as high quality, disease resistance, and suitable adaptation for industrial processing. The advantages of pumpkin hybrids include a semi-limited growth habit, wider adaptation, resistance to biological and environmental stresses, increased edible quality, and enhanced nutritional efficiency, such as high carotenoid content and higher yield (López, 2004; Elias et al., 2020). Therefore, having a hull-less seed pumpkin with limited plant growth obtained through inter- or intra-species crossing can lead to the production of varieties with much higher production capacity and provide the opportunity to export this valuable product. Usually, the progeny resulting from the crossing of two dissimilar parents exhibit more heterosis, known as hybrid vigor (Ranjbar, 2012). A group of researchers concluded that a population of 5000 plants per hectare will have the highest yield in *Cucurbita pepo* var. Styriaca (Cushman et al., 2002). In previous research the reduction planting density leads to an increase in fruit size. The highest amount of fruit production was 10746 per hectare. while the size of fruits decreased (Heagy et al., 2023). The purpose of this experiment was to achieve the best planting density in *Cucurbita pepo* var. Styriaca cultivation and its effect on quality attributes.

## 2. Materials and Methods

This research was carried out on the research farm of the Faculty of Agricultural Sciences at the University of Guilan in the spring and summer of 2023. The study was conducted using a split-plot design in a randomized complete block with 3 replications. Two planting densities, 10,000 and 20,000 plants·ha$^{-1}$, were considered as the main factor, with hybrids at four levels as the secondary factor. Hybrids were obtained from the crossing between pure lines that breeded in University of Guilan after crossing of zucchini and *Cucurbita pepo* var. styriaca (Davoodi et al., 2021). Genotype descriptions and characteristics are listed in table 1.

**Table 1**. Hybrids descriptions and characteristics

| Hybrid | Growth habit | Plant picture | Fruit picture |
|---|---|---|---|
| 7×14 | Semi-determinate |  |  |
| 23×11 | Determinate |  |  |
| 2×23 | Semi-determinate |  |  |
| 23×8 | Indeterminate |  |  |

To conduct the experiment, seeds of the hybrids were transferred to the field after germination in petri-dish. Before planting, the field was appropriately plowed and disked. Plants cultivated in blocks with a distance of 1 meter on the row and 1 meter between row at a planting density of 10,000 plant·ha$^{-1}$ and for planting density of 20,000 plant·ha$^{-1}$, a distance of 0.5 meter on the row and 1 meter between row was considered. Fertilization was done using fully decomposed animal manure (60 ton·ha$^{-1}$). To homogenize fertilization on both planting densities 1200 and 600 gram of fully decomposed animal manure was mixed with the soil at each planting site in 10,000 and 20000 plant·ha$^{-1}$ respectively before planting the seeds. A drip irrigation system was used to water the plants evenly once a week. All necessary crop care, including weed control, was performed from planting to harvest.

After the fruits fully ripened, they were harvested from each plot (8 m$^2$) to determine yield and yield components. Measurements included plant height one month after planting and before harvest (in centimeter), number of leaves, number of branches, leaf area (in square millimeter per plant), percentage of leaf disease using the G.C.L. BUBBLE ETCH TANKS model (in square millimeter), thousand seed weight (in gram), seed yield per plant (in gram), yield per unit area (in g·m$^{-2}$), yield per hectare (in ton·ha$^{-1}$), oil yield (in ton·ha$^{-1}$), and biomass of different plant parts (in gram per plant and ton·ha$^{-1}$).

Fifty grams of hull-less pumpkin seeds were used for oil extraction with n-hexane solvent was performed using a Soxhlet device, model SOXTEC SYSTEM HT 1046. To analyze the oil, the oil was obtained with 20 milligrams of potassium hydroxide, 1600 microliters of methanol, and 400 microliters of a borane trifluoride derivative compound. This mixture was poured into a vial and placed in a Bain-Marie machine at 60 degrees Celsius for one hour. The vial was shaken for five seconds every ten minutes. Next, the sample was brought to room temperature, and one milliliter of deionized water and

250 microliters of hexane solvent were added. The mixture was then centrifuged at 4400 rpm at room temperature (Milinsk et al., 2008).

In the conducted experiment, the derived samples of each hybrid were injected into the device without considering the densities, and the linoleic, oleic, and palmitic fatty acids in the hybrids were compared. To identify and quantify fatty acids, the prepared sample, was analyzed using a GC FID device, model TRACE GC from ThermoQuest-Finnigan, with N2 carrier gas at a flow rate of 1.1 ml/min. The detector was an FID type, operating at 280 degrees Celsius, with detector gas flow rates of Air: 350 ml/min, H2: 35 ml/min, and Make-up: 30 ml/min. The sample was injected with a special syringe, and its peaks were analyzed with x-caliber software and the amount of total sterol and beta-sitosterol (mg/ml) was detected (Rabrenovic et al., 2014; Kostadinović Veličkovska et al., 2015).

Data analysis was performed using SAS software version 9.1, and mean data comparisons were made using Tukey’s test at the probability level corresponding to ANOVA table.

## 3. Results and Discussion

The results of the analysis of variance (Table 2) indicated that the studied genotypes exhibited significant differences in response to different crop densities for all investigated traits, except for vegetative biomass. For vegetative biomass, the simple effects of genotype and planting density were significant.

The mean comparison results for the initial plant length trait, measured 30 days after planting, showed that all hybrids at a planting density of 20,000 plant·ha$^{-1}$ exhibited higher vegetative growth than at a planting density of 10,000 plants·ha$^{-1}$ (Table 3). At the higher planting density, the hybrid 23×8, with an average of 33.83 cm, and the hybrid 7×14, with an average of 34.13 cm, had the highest vegetative growth. In contrast, the hybrid 2×23, with an average of 31.83 cm, and the hybrid 23×11, with an average of 30.04 cm, had the lowest vegetative growth.

At the lower planting density, the hybrid 7×14, with an average of 31.87 cm, and the hybrid 23×8, with an average of 29.43 cm, showed the highest growth. The hybrids 2×23 and 23×11, with averages of 26.13 cm and 24.06 cm, respectively, had the lowest plant length. Overall, at the lower planting density, the hybrid 23×11 had the lowest growth, while the hybrid 7×14 had the highest vegetative growth. At the higher planting density, the hybrids 23×11 and 23×8 had the lowest and highest averages, respectively (Table 3). A study showed that shorter planting distances limit shoot growth space, resulting in longer plant lengths (Abdel-Rahman et al., 2012).

However, the results of the comparison of the means for the final plant length trait, measured before harvesting the fruits, showed that at high planting density, hybrids 23×8 and 2×23 had the highest averages of 289.33 cm and 268.65 cm, respectively, while hybrids 7×14 and 23×11 had the lowest plant lengths. At a planting density of 10,000 plant·ha$^{-1}$, hybrids 23×8 and 2×23 had the highest plant lengths with averages of 256.87 and 226.33 cm, respectively, while hybrids 7×14 and 11×23 had the lowest plant lengths with averages of 217.97 and 195.88 cm (Table 3). At high planting density, due to competition with other plants for nutrients, light, and water, the plants focused more on vegetative growth and the production of vegetative organs (Bahlgerdi et al., 2014). This suggests the presence of genes responsible for delayed growth development in plants, which is the second gene controlling the growth pattern in pumpkins (Hassan et al., 2016).

Based on the mean comparison results, all hybrids at a planting density of 10,000 plant·ha-$^{1}$ had a greater number of leaves than at a planting density of 20,000 plant·ha$^{-1}$ (Table 3). At the 10,000 plant·ha$^{-1}$density, hybrids 23×11 and 7×14 had the highest number of leaves, with averages of 144.66 and 131.81, respectively. In contrast, hybrids 2×23 and 23×8 had the fewest leaves, with averages of 125.16 and 111, respectively, at the end of the season (Table 3). At the 20,000 plant·ha$^{-1}$ density, hybrids 23×11 and 7×14 again had the highest number of leaves, with averages of 125.50 and 124.50, respectively. Hybrids 2×23 and 23×8 had the fewest leaves, with averages of 109.16 and 93.33, respectively. The results indicated that increasing planting density decreases the number of leaves. At both densities, the hybrid 23×11 had the most leaves, while the hybrid 23×8 had the fewest (Table 3). The number of nodes and leaves is directly related to plant length, and increasing density causes a decrease in the number of leaves per plant (Bahlgerdi et al., 2014).

All the hybrids had more lateral branches at the planting density of 10,000 plant·ha$^{-1}$ than at the planting density of 20,000 plant·ha$^{-1}$. At the planting density of 10,000 plant·ha$^{-1}$, hybrids 23×11 and 7×14 had the most lateral branches, with averages of 41.65 and 32.44, respectively. In contrast, hybrids 2×23 and 23×8 had the fewest lateral branches, with averages of 28.10 and 25.92, respectively, at the end of the growth period (Table 3). At the planting density of 20,000 plant·ha$^{-1}$, hybrids 23×11 and 7×14 again had the most lateral branches, with averages of 29.81 and 25.94, respectively. Hybrids 2×23 and 23×8 had the fewest lateral branches, with averages of 21.24 and 16.58, respectively. Overall, at both planting densities, the hybrid 23×11 had the most lateral branches,

**Table 2.** Effect of plant density on *Cucurbita pepo* measured characteristics

| Source of variation | df | Mean of square | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant length after one month | Plant length before harvest | Number of lateral branches | Leaf area | leaf area with disease symptoms | Number of leaves |
| Replication (R) | 2 | 6.69* | 69.17* | 9.83* | 59079ns | 4.83ns | 79.54nS |
| Density (D) | 1 | 286.50* | 22460.59* | 900.20* | 612305** | 603.004** | 2715.02** |
| R×D | 2 | 1.96ns | 205.86* | 7.39* | 56936ns | 0.87ns | 42.38ns |
| Genotype (G) | 3 | 89.90** | 28089.93** | 469.39** | 84585** | 320.98** | 2466.98** |
| G×D | 3 | 6.99* | 904.93** | 20.28* | 72830** | 1.69* | 71.57* |
| Error | 12 | 1.46 | 45.76 | 4.05 | 1.22 | 0.419 | 20.38 |
| C.V% | - | 4.30 | 5.47 | 7.86 | 5.60 | 5.03 | 4.15 |

ns, ** and *, non-significant, significant at P≤0.01 and P≤0.05, respectively

**Table 2-** Continue

| Source of variation | df | Mean of square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Seed biomass per plant | Seed biomass per surface ($m^2$) | Seed biomass per ha (ton) | Weight of thousand seeds | Oil yield per ha (ton) | Vegetative biomass per plant | Vegetative biomass per ha (ton) |
| Replication (R) | 2 | 6.24ns | 17.19*ns | 0.01ns | 62.43ns | 0.0039ns | 100.97ns | 0.034ns |
| Density (D) | 1 | 1493** | 1132.96* | 0.113* | 6745.02* | 1.006** | 1007.21* | 9.038** |
| R×D | 2 | 2.43ns | 9.58ns | 0.0009ns | 82.99ns | 0.0026ns | 49.712ns | 0.024ns |
| Genotype (G) | 3 | 631.8** | 1290.38** | 0.129* | 3097.677** | 0.056** | 1200** | 11909** |
| G×D | 3 | 43.51** | 113.72** | 0.011** | 123.4190** | 0.001* | 4.4869ns | 1100ns |
| Error | 12 | 4.86 | 18.65 | 0.001 | 19.66 | 0.001 | 44.08 | 0.014 |
| C.V% | - | 9.57 | 13.11 | 13.11 | 4.73 | 8.45 | 9.439 | 12.77 |

**Table 2-** Continue

| Source of variation | Degree of freedom | Mean of square | | | |
|---|---|---|---|---|---|
| | | Fruit biomass per plant | Fruit biomass per ha (ton) | Total biomass per plant | Total biomass per ha (ton) |
| Replication (R) | 2 | 76.427ns | 0.072ns | 297.68ns | 0.0516ns |
| Density (D) | 1 | 36146** | 17.609** | 38822** | 59.071** |
| R×D | 2 | 185.49ns | 0.075ns | 52.55nS | 0.0026ns |
| Genotype (G) | 3 | 16452** | 3.117** | 11909** | 2.3359** |
| G×D | 3 | 960.48* | 0.190* | 1100* | 0.1741** |
| Error | 12 | 182.10 | 0.047 | 198.48 | 0.058 |
| C.V% | - | 4.69 | 5.88 | 3.20 | 3.80 |

ns, ** and *, non-significant, significant at P≤0.01 and P≤0.05, respectively

**Table 3.** Interaction effects of genotypes and plant densities on measured characteristics of hull-less pumpkin

| Genotype | Density | Plant length after one month (cm) | Plant length before harvest (cm) | Number of lateral branches | Number of leaves | leaf area with disease symptoms (%) | leaf area ($mm^2$) |
|---|---|---|---|---|---|---|---|
| 7×14 | 10000 | 31.87c | 217.97c | 32.44c | 131.81c | 7.806c | 1118c |
| 7×14 | 20000 | 34.13* | 247.52** | 25.94** | 124.50* | 14.002** | 9592** |
| 23×11 | 10000 | 24.06c | 195.88c | 41.65c | 144.66c | 3.733c | 1379c |
| 23×11 | 20000 | 30.04** | 229.22** | 29.81** | 125.50** | 10.97c | 1094** |
| 2×23 | 10000 | 26.51c | 226.33c | 28.10c | 125.16c | 9.69c | 916c |
| 2×23 | 20000 | 31.83** | 268.65** | 21.24** | 109.16** | 17.95** | 698** |
| 23×8 | 10000 | 29.43c | 256.87c | 25.92c | 111c | 16.71c | 749c |
| 23×8 | 20000 | 34.83** | 289.83** | 16.58** | 93.33** | 23.06** | 505** |

C= control, ns, ** and *, non-significant, significant at P≤0.01 and P≤0.05, respectively

**Table 3.** Continue

| Genotype | Density | Seed biomass per plant (g) | Seed biomass per surface (g) | Seed biomass per ha (ton) | weight of one thousand seeds (g) | Oil yield (ton $ha^{-1}$) | Fruit biomass per plant (g) | Fruit biomass per ha (ton) | Total biomass per plant (g) | Total biomass per ha (ton) |
|---|---|---|---|---|---|---|---|---|---|---|
| 7×14 | 10000 | 35.88$^{c}$ | 35.88$^{c}$ | 0.358$^{c}$ | 12.94$^{c}$ | 0.44$^{c}$ | 234.35$^{c}$ | 2.34$^{c}$ | 282.33$^{c}$ | 3.37$^{c}$ |
| 7×14 | 20000 | 19.42$^{**}$ | 38.85$^{**}$ | 0.388$^{**}$ | 10.38$^{**}$ | 0.75$^{**}$ | 186.19$^{**}$ | 3.72$^{**}$ | 233.01$^{**}$ | 5.64$^{**}$ |
| 23×11 | 10000 | 40.83$^{c}$ | 40.83$^{c}$ | 0.408$^{c}$ | 146$^{c}$ | 0.48$^{c}$ | 268.86$^{c}$ | 2.68$^{c}$ | 365.29$^{c}$ | 3.56$^{c}$ |
| 23×11 | 20000 | 29.52$^{**}$ | 59.04$^{**}$ | 0.590$^{**}$ | 12.83$^{**}$ | 0.79$^{**}$ | 189.19$^{**}$ | 3.78$^{**}$ | 282.37$^{**}$ | 5.64$^{**}$ |
| 2×23 | 10000 | 30.035$^{c}$ | 30.31$^{c}$ | 0.300$^{c}$ | 106$^{c}$ | 0.40$^{c}$ | 189.16$^{c}$ | 1.73$^{c}$ | 280.56$^{c}$ | 2.80$^{c}$ |
| 2×23 | 20000 | 19.65$^{**}$ | 39.31$^{**}$ | 0.393$^{**}$ | 89.50$^{**}$ | 0.67$^{**}$ | 135.98$^{**}$ | 2.71$^{**}$ | 243.68$^{**}$ | 4.87$^{**}$ |
| 23×8 | 10000 | 21.12$^{c}$ | 21.12$^{c}$ | 0.211$^{c}$ | 11.33$^{c}$ | 0.55$^{c}$ | 264.33$^{c}$ | 2.64$^{c}$ | 359.33$^{c}$ | 3.59$^{c}$ |
| 23×8 | 20000 | 14.22$^{**}$ | 28.44$^{*}$ | 0.284$^{**}$ | 87.50$^{**}$ | 0.84$^{**}$ | 208.80$^{**}$ | 4.17$^{**}$ | 306.06$^{**}$ | 6.12$^{**}$ |

The means followed by same letters are not significantly different at p<0.05.

**Table 4.** Effects of genotypes on vegetative biomass

| Genotype | Vegetative biomass per plant(g) | Vegetative biomass per ha(ton) |
|---|---|---|
| 7×14 | 71.43$^{b}$ | 1.093$^{b}$ |
| 23×11 | 59.676$^{c}$ | 0.915$^{c}$ |
| 2×23 | 82.608$^{a}$ | 1.266$^{a}$ |

C= control, ns, ** and *, non-significant, significant at P≤0.01 and P≤0.05, respectively

**Table 5.** Percentage of some fatty acids of hybrid

| hybrid | Linoleic acid | Oleic acid | Palmitic acid |
|---|---|---|---|
| 7×14 | 42/95 | 8/51 | 33/2 |
| 23×11 | 37/83 | 10/2 | 7/03 |
| 2×23 | 45/59 | 5/89 | 0/9 |
| 23×8 | 45/4 | 7/23 | 0/51 |

Table 6. Effect of genotypes and plant density on sterols compounds of hull-less seed pumpkin

| Hybrid | Density | ß-sitosterol (mg/ml) | total sterols (mg/ml) |
|---|---|---|---|
| 7×14 | 10000 | 0.03 | 0.81 |
| 7×14 | 20000 | 0.06 | 0.91 |
| 23×11 | 10000 | 0.05 | 0.97 |
| 23×11 | 20000 | 0.05 | 0.78 |

while the hybrid 23×8 had the fewest at the end of the growth period (Table 3).

In a similar study, the maximum number of lateral branches was obtained at a density of 2 plants·$m^{-2}$, which is consistent with our results (Bahlgerdi et al., 2014). However, the increase in density caused a decrease in the leaf area of all hybrids. At a planting density of 10,000 plant·$ha^{-1}$, hybrids 23×11 and 7×14 had the largest leaf areas, with averages of 1379 and 1118 square millimeter, respectively. In contrast, hybrids 2×23 and 238 had the smallest leaf areas, with averages of 916 and 749 square millimeter, respectively (Table 3). At a planting density 20,000 plant·$ha^{-1}$, hybrids 23×11 and 7×14 again had the largest leaf areas, with averages of 1094 and 9592 square millimeter per plant, respectively. Hybrids 2×23 and 23×8 had the smallest leaf areas, with averages of 698 and 505 square millimeter per plant, respectively. Overall, at both planting densities, the hybrid 23×11 had the largest leaf area, while the hybrid 23×8 had the smallest (Table 3).

The leaf area in all hybrids decreased at a planting density of 20,000 plant·$ha^{-1}$, and some hybrids reacted more and some less to the planting density, but on a large scale, the increase in the number of cultivated plants compensated for this decrease in leaf area at a high planting density. Increasing planting density can increase the competition for light and lead to a decrease in the rate of photosynthesis (Kong et al., 2023).

The percentage of the surface infected with disease at a planting density of 20,000 plant·$ha^{-1}$ was higher in all genotypes compared to a planting density of 10,000 plant·$ha^{-1}$. At the planting density of 10,000 plant·$ha^{-1}$, hybrids 23×8 and 2×23 had the highest disease percentages, with averages of 16.716% and 9.926%, respectively, while hybrids 7×14 and 23×11 had the lowest disease percentages, with averages of 7.806% and 3.837%, respectively (Table 3). At planting density of 20,000 plant·ha$^{-1}$, hybrids 23×8 and 2×23 had the highest disease percentages, with averages of 23.066% and 17.958%, respectively. In contrast, hybrids 7×14 and 23×11 had the lowest disease percentages, with averages of 7.806% and 3.738%, respectively. Overall, at both planting densities, the hybrid 23×8 had the highest disease percentage, while the 23×11 hybrid had the lowest (Table 3).

The primary goal of hybridization between Cucurbita species is to combine growth habits with resistance to diseases and insects. An increase in plant density may prevent the spread of insects or reduce the percentage of plants damaged by insects, but it may also accelerate the spread of pathogens (Speight, 1983). In fact, with increased planting density, competition for light, nutrients, and water will intensify. Additionally, reduced planting intervals can increase leaf humidity, promoting the spread of diseases, which are significant factors in reducing crop yield (Riggs & Reiners, 1999).

The average comparison results showed that the seed yield per plant was higher at a planting density of 10,000 plant·ha$^{-1}$ in all genotypes compared to a planting density of 20,000 plant·ha$^{-1}$. Hybrids 23×11 and 7×14 had the highest seed yields per plant, with averages of 40.83 and 35.88, respectively, while hybrids 2×23 and 23×8 had the lowest seed yields per plant, with averages of 30.035 and 21.12, respectively. At the planting density 20,000 plant·ha$^{-1}$, hybrids 23×11 and 7×14 again had the highest seed yields, while hybrids 2×23 and 23×8 had the lowest (Table 3).

The results also showed that, despite the increase in plant length at a density of 20,000 plant·ha$^{-1}$, the number of lateral branches and leaf surface area, which are directly related to yield, decreased due to competition among the plants. However, the increased number of cultivated plants not only compensated for this decrease in yield but also maximized the production and use of land and available resources especially in organic cultivation systems. According to the results, hybrid 23×11 had the highest yield per unit area and ha, with averages of 59.04 g·m$^{-2}$ and 0.59 ton·ha$^{-1}$, respectively. In contrast, hybrid 23×8 had the lowest yield, with averages of 28.44 g·m$^{-2}$ and 0.284 ton·ha$^{-1}$ (Table 3). A previous report also indicated the highest yield at a planting density of 3 plants per square meter (Bahlgerdi et al., 2014).

In plants such as pumpkins, where the reproductive part is harvested for economic purposes, an excessive increase in density causes photosynthetic materials to be used for vegetative growth or respiration instead of seed growth (Ball et al., 2000). In pumpkins and melons, it has been proven that increasing plant density significantly increases fruit yield, primarily due to the number of fruits per unit area (Nerson, 2005).

The weight of 1000 seeds were higher at a planting density of 10,000 plant·ha$^{-1}$ in all genotypes compared to a density of 20,000 plant·ha$^{-1}$. At the planting density of 10,000 plant·ha$^{-1}$, hybrids 23×11 and 7×14 had the highest thousand-seed weights, with averages of 146 and 126.94 g, respectively. In contrast, hybrids 2×23 and 23×8 had the lowest thousand-seed weights, with averages of 106 and 119.33 g, respectively (Table 3). At the planting density of 20,000 plant·ha$^{-1}$, hybrids 23×11 and 7×14 had the highest thousand-seed weights, with averages of 121.83 and 106.38 g, respectively. Hybrids 2×23 and 23×8 had the lowest thousand-seed weights, with averages of 89.50 and 87.50 g, respectively. Overall, at both planting densities, hybrids 23×11 and 7×14 had the highest thousand-seed weights, while hybrids 2×23 and 23×8 had the lowest (Table 3).

The increased competition for the absorption of photosynthetic substances between the fruits and the formed seeds causes the seeds to not fill well, resulting in a lower weight per thousand seeds (Kermani et al., 2014).

The mean comparison results showed that the yield of oil per hectare at the planting density of 20,000 plant·ha$^{-1}$ in all hybrids was higher than the planting density of 10,000 plant·ha$^{-1}$. At planting density of 20,000 plant·ha$^{-1}$, hybrids 23×8 and 23×11 had the highest oil yield with an average of 0.84 and 0.79 ton, respectively, while hybrids 7×14 and 2×32 had the lowest oil yield with an average. 0.75 and 0.67 ton, respectively (Table 3).

At planting density of 10,000 plant·ha$^{-1}$, hybrids 23×11 and 23×8 had the highest oil yield with an average of 0.55 and 0.48 ton, respectively, while hybrids 7×14 and 2×23 had the lowest oil yield. In general, in both densities, hybrids 23×11 and 23×8 had the highest and lowest oil yields, respectively (Table 3).

Hull-less seed pumpkin is an indeterminate plant (Omidbeygi, 1995), adjusting the distance between rows to control vegetative growth and the number of reproductive reservoirs (fruits) is crucial (Rylski, 1974; Robinson, 1993). Reducing the distance between rows limits the plants’ access to resources, especially space and water, which prevents excessive vegetative growth. This establishes a proper balance between vegetative and reproductive growth, leading to more fruit production and ultimately increasing seed and oil yields in hull-less seed pumpkins (Rylsky, 1974; Robinson, 1993).

A planting density of 20,000 plant·ha$^{-1}$ resulted in higher total biomass per ha compared to planting density of 10,000 plant·ha$^{-1}$. Hybrid 7×14 in planting density 10,000 plant·ha$^{-1}$, produce 35.88, 234.35, and 282.33 g, seed, fruit, and total biomass respectively, and in planting density 20,000 plant·ha$^{-1}$, produce 19.42, 186.19, and 233.01 g, seed, fruit, and total biomass respectively (Table 3). In general, the experiment results indicate that increasing plant density decreases the yield of seeds and fruits per plant, while increasing the yield of fruits and seeds per ha (Kermani et al., 2014).

Increasing plant density decreases the yield of individual plants but increases the yield per unit area (Ball et al., 2000). As plant density rises, the seed and fruit yield per plant declines because the canopy closes, leading to increased competition for nutrients and light, which are essential for photosynthesis (Kultur et al., 2001). Doubling the density increases yield per area but reduces the average size of individual plants (Table 4). This reduction in biomass is mainly due to shading, which lowers the photosynthetic rate. At high densities, shading has a greater impact on plants than nutrient depletion (Postma et al., 2020). In crops like pumpkins, where the reproductive part is harvested for economic purposes, excessive density causes photosynthetic materials to be used for vegetative growth or respiration instead of seed growth (Ball et al., 2000). In pumpkins and melons, increasing plant density significantly boosts fruit yield, primarily due to the higher number of fruits per unit area (Nerson, 2005). In other hands plant densities effect on the quantities of phytosterols in vegetable oils that have been used for the classification and assessment of their quality (Rabrenovic et al., 2014; Kostadinovic et al., 2015). Cultivation and environmental conditions influence the concentration of phytosterols (Rabrenovic et al., 2014).

The results showed that the content of fatty acids in each hybrid is different. The hybrid 2×23 had 45.59%, 5.89%, and 0.9% of linoleic, oleic, and palmitic acids, respectively. The hybrid 23×11 had 37.83%, 10.2%, and 7.03%, and the hybrid 7×14 had 42.95%, 8.51%, and 33.2%. The hybrid 8×23 had 45.4%, 7.23%, and 0.51% of linoleic, oleic, and palmitic acids, respectively. The results showed that the hybrid 2×23 had the highest linoleic acid content, while the hybrid 23×11 had the highest oleic and palmitic acid contents (Table 5).

Based on the seed and oil yield results, hybrids 2×23 and 23×8 had low yields, so we did not proceed to the qualitative phase for these two hybrids. For hybrids 7×14 and 23×11, after oil extraction and derivatization, sample was injected into the GC device. The results showed that hybrid 7×14 had 0.03 and 0.06 mg/ml ß-sitosterol in low and high plant densities, respectively. It also had 0.81 and 0.91 mg/ml total sterols, respectively. Hybrid 23×11 had 0.05 mg/ml ß-sitosterol in both low and high plant densities (Table 6).

## 5. Conclusion

It seems that at higher densities, due to the reduction in the number of branches and consequently the number of flowers in the vine, and on the other hand, due to the faster closing of the canopy and the lack of proper penetration of light into the canopy, pollination of flowers is not done well and the finally number of fruits decreased. High plant density cultivation is possible by using bushy growth habit that led to high resource use efficiency that is very important in organic cultivation systems.

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