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# Barbados gooseberry sprout production in a high-density plantation system

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## ABSTRACT

Barbados gooseberry (BGB) is a non-conventional vegetable highlighted for its nutritional value, particularly its protein content. However, there is a lack of scientific information for improving the plantation system of this plant. This research proposed to evaluate high-density systems to cultivate BGB plants for sprout production. The experiment was carried out in the Horticulture Sector of the Canguiri Experimental Station Farm from UFPR, located in the county of Pinhais, Paraná State, Brazil. BGB was planted in open field beds using distinctive plantation systems according to plant stands and pruning and thinning management. The experimental design was randomized blocks with three repetitions per treatment. Five high-density plantation systems (treatments) were tested: 10 (10×10): 10 × 10 cm; 2) (20×20): 20 × 20 cm; and 3) (30×30): 30 × 30 cm spacing with thinning to keep one sprout growing per primary branch per plant; 4) (30×30TS) was represented by 30 × 30 cm spacing with thinning to keep one sprout growing from each of two secondary branches grown from the primary branch to give two sprouts (TS) per plant; 5) (30×30FS) consisted of plants with 30 × 30 cm spacing with no thinning (FS = free growing sprouts). The first three treatments were thinned to one sprout per plant. Protein content of BGB sprouts was also determined to verify its real protein content. A high-density plantation system promoted BGB sprouts productivity with high protein content (28.8% from leaves of sprout). Treatment 5 produced the highest yield (21.7 t/ha per month).

**Keywords**: *Pereskia aculeata*, planting density, sprouting, productivity.

## RESUMO

**Produção de brotos de ora-pro-nóbis em sistema de cultivo superadensado**

O ora-pro-nóbis (OPN) é uma olerícola não convencional que se destaca pelo seu valor nutricional, especialmente, em relação a seu teor proteico. Porém, há escassez de informações técnico-científicas sobre sistemas de cultivo recomendados. Este trabalho teve por objetivo avaliar a produção de brotos de OPN em sistema superadensado. O experimento foi desenvolvido no Setor de Horticultura da Fazenda Experimental Canguiri da UFPR, em Pinhais-PR. Para isso, efetuou-se o plantio do OPN em canteiros a céu aberto em diferentes sistemas de cultivo que variaram conforme a densidade de plantas (espaçamento) e manejo de podas e desbrotas. O delineamento experimental foi em blocos ao acaso com três repetições por tratamento. Foram testados cinco sistemas de cultivo em alta densidade de plantio (tratamentos): 1) 10 cm × 10 cm, 2) 20 cm × 20 cm, 3) 30 cm × 30 cm, 4) 30 cm × 30 cm e 5) 30 cm × 30 cm de espaçamento de plantio; sendo os três primeiros com desbrotas para produção de um broto por planta por colheita, o quarto com desbrota para produção de dois brotos por planta por colheita e o último sem desbrotas para livre produção de brotos. Foi também avaliada a porcentagem de proteína nos brotos de OPN para verificar o seu real conteúdo proteico. Os resultados mostraram que sistema de cultivo superadensado do OPN promove alta produtividade ao mesmo tempo que oferece um produto de alto conteúdo proteico (28,8% em folhas de broto). O cultivo no espaçamento de 30 × 30 cm e sem desbrotas proporcionou a maior produção de brotos (27,1 t/ha por mês).

**Palavras-chave:** *Pereskia aculeata*, adensamento, brotação, produtividade.

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Unconventional food plants aroused the interest of consumers and growers worldwide, although their cultivation and cooking are still limited in certain regions, such as Brazil (Leal *et al*., 2018; Tuler *et al*., 2019; Terra & Ferreira, 2020). These plants are an excellent alternative source of important nutrients for the human diet and health, and their production increases horticulture business diversity (Barreira *et al*., 2015; Anacleto *et al*., 2018).

In Brazil exist uncountable native unconventional food plant species. However, the knowledge on the use of these plants is limited, especially in terms of cultivation and preparation. The lack of agronomic data contributes to the hesitation in using these plants as crops, which has precluded their presence from farms and markets, and consequently from consumer's tables (Tuler *et al*., 2019; Souza *et al*., 2020).

Barbados gooseberry (BGB) (*Pereskia aculeata*), a perennial tropical plant of indeterminate growth belonging to the Cactaceae family, is one of such plants known as "ora-pro-nóbis" in Brazil, also sometimes called "beef for poor", "green meat" or "vegetable meat," due to the high protein content of its leaves, the main marketable portion of the plant (Tofanelli & Resende, 2011; Mazon *et al*., 2020). In Brazil, BGB is found from northeastern (e.g. Bahia State) to the southern region (e.g. States of Minas Gerais, Paraná, Santa Catarina, and Rio Grande do Sul) mainly in the Brazilian ecosystems such as Caatinga, Savanna, and Atlantic Forest (Carvalho *et al*., 2019).

BGB leaves can be eaten as salad, in soups, omelets, and pies, as well as can be dried to produce flours usually added as ingredient to prepare bread, cake, and pasta or to manufacture compounded medicines (Duarte & Hayashi, 2005; Teixeira *et al*., 2021).

Higher nutrient levels of BGB are very well known, however it has other important properties such as biological activities, among them, anti-inflammatory action due to the presence of phytosterols (e.g. taraxerol, taraxasterol and sitosterol), antimicrobial and antioxidant activities due to presence of phenolic compounds, diuretic and hypotensive activities due to inhibition of arginine vasopressin release promoted by ingestion of aqueous extract of leaves (Colacite *et al*., 2022; Maciel *et al*., 2019; Santos *et al*., 2018). These biological activities have released BGB as an interesting vegetable raw material to be applied in pharmaceutical, nutraceutical and food industries. BGB also produces edible fruits, that are rich in carotenoids and vitamin C, which can be manufactured to produce juices, jams, liqueurs, and ice creams (Ribeiro *et al*., 2014).

Its tender, fleshy leaves are also rich in other nutrients, such as calcium, iron, lysine, and mucilage (Souza *et al*., 2020; Tofanelli & Mógor, 2021). BGB is also used as hedge plant, as well as ornamental plant due to its showy flower (Santos *et al*., 2012).

BGB is resistant, easy to grow, and requires only simple management for its cultivation. Despite these favorable features, BGB cultivation is hindered in part because of the presence of spines (young branch) and thorns (mature branch) which cause management to be slow and arduous, and present a risk of piercing the grower (Tofanelli & Mógor, 2021).

BGB sprouts are young and tender and have not yet developed spines. Thereby, to manage spineless portion of plant would decrease the risk of hurting the grower. In addition, BGB sprouts also have a distinctive flavor (Almeida & Corrêa, 2012; Souza *et al*., 2020) and possibly a higher protein content than the mature leaves that are generally used for cooking or feeding.

BGB crops are usually planted at 3.0 m × 3.0 m between plants in simple row planting system, as well as in double row planting system with 0.2 to 1.0 m between plants on row × 0.6 to 1.0 m between simple rows within double rows and 2.0 m to 2.5 m between double rows (Madeira *et al*., 2022; Souza *et al*., 2018; Tofanelli & Resende, 2011). All these planting systems have as purpose the production of mature leaves to be harvested from plants grown practically without pruning for canopy management, where their yields range from 0.32 to 1.28 t/ha per harvest and up to eight harvests per year (Madeira *et al*., 2016; Tofanelli & Resende, 2011). However, to produce sprouts from a spineless canopy we have suggested a planting system that can raise its crop productivity. Thereby, we essayed here a high-density planting system managed by successive pruning. Crop managements to cultivate BGB we have never seen before.

To our knowledge, there is no literature on the development or investigation of a BGB plantation system for sprout production, or an efficient method for obtaining high quality and quantity of BGB sprouts. Thus, this research aimed to evaluate production of sprouts from BGB plants cultivated in high-density stands as a novel plantation system, as well as to determine the protein content of the BGB sprout.

## MATERIAL AND METHODS

The experiment was conducted in the Horticulture Sector of Canguiri Experimental Station Farm of Federal University of Paraná (UFPR), Pinhais, Paraná State, Brazil (25°26'S, 49°16'W, 947 m altitude), where the climate is Cfb according to the Köppen's scale, average maximum and minimum temperatures 24 and 11°C, and 1500 mm the annual average rainfall.

### Plant material

The BGB plants were grown in the vegetable garden from January 2020 to May 2021. Plantation was established using seedlings propagated by semihardwood stem cuttings collected in November 2019 from tree BGB mother plants, six years old, cultivated in the Canguiri Experimental Station Farm. Cuttings were sized to 1.0 cm in diameter and 15 cm in length and planted in 128 $cm^3$ capacity tubes, filled with a soil base substrate (soil + manure + sand at 4:1:1 v/v). After planting, the cuttings were placed in a greenhouse covered with 150 micra thick plastic film where they were grown for 90 days, irrigated by a microsprinkler system, after which they were planted in the vegetable garden.

The BGB seedlings were planted in raised beds previously plown with a hoe. Each bed was 0.9 m wide and 8.0 m long with 0.4 m wide furrows (paths) between the beds. Prior to plowing, ovine manure fertilizer was applied at 20 $L/m^2$ on beds and incorporated into the soil, according to fertilization recommended by Madeira *et al*. (2016), adapted to the planting systems essayed here. Once the beds were prepared, the seedlings were planted according to each plant spacing (treatment). Irrigation on the beds was provided three times per week applying a water layer of 5 mm (5 $L/m^2$) every irrigation when rain was absent for one month, at which time seedling survival observation was completed. The primary branch of BGB plants was pruned at 10 cm height, 60 days after planting, to stimulate budding and later, thinned according to each treatment.

Management was performed only to form the base canopy from April to July 2020. Thus, hand thinning was performed bi-weekly using prune shears to produce only one sprout per plant to be harvested when it reached

20 cm in length. After harvest, budding was stimulated and plants were thinned to again leave one sprout per plant. This routine was repeated after every harvest. In the lots where BGB plants were cultivated to produce two sprouts per plant, two distal sprouts remained per plant after the first pruning to later form two secondary branches growing diagonally from the primary branch. When the secondary branches reached 30 cm in length, they were pruned at 20 cm to stimulate budding for subsequent thinning to one sprout per secondary branch according of the same procedure described earlier. There was no thinning on the plants cultivated to produce several sprouts per plant. No data were collected in this phase.

On August 2020, after intense cold weather that damaged the plants, plants were pruned in all experimental lots according to the procedure previously described, followed by thinning and harvesting. This initiated the first data collection season on September 13, 2020, which continued for 90 days. The second season was from March to May 2021, when this initiated the second data collection season on August 3, 2021, which continued for 90 days. In this period, BGB plants were managed according to the same procedure described for the first season, except for beds containing plants cultivated to produce sprouts freely, where pruning was performed using a brush cutter at 20 cm height. This was necessary because these plants formed a tall and wide canopy with thicker branches where dangerous thorns could grow that are difficult to prune using shears. There was no plant management between the data collection seasons (January to February, 2021) in order to allow plants to grow freely and prepare bed canopies for late pruning just to simulate an eventual season production scheduling, as well as to know how to proceed pruning after fallow. Therefore, we had also two collected data seasons in order to obtain temporal replicate.

In the second data collection season, BGB cultivated to produce one or two sprouts per plant were pruned using prune shears, while those cultivated to grow freely were thinned with grass shears due to the density of the canopy grown in those lots where there was no longer an architectured base. Subsequently, thinning by hand or with prune shears was performed weekly to leave one or two sprouts per plant depending on the treatment. There was no thinning on the plants that freely produced sprouts.

BGB cultivation was based on an organic system of fertilization only with ovine manure topdressing, using 20 L/m$^2$ (Madeira *et al.*, 2016) in August 2020 and March 2021 after pruning to begin each data collection season, and weed control by mechanical weeding followed by the application of mulched straw at 2 cm height. Mechanical weeding was bimonthly, when weeds were observed growing into assay blocks, and straw mulch was applied twice in each assessment season, once after manure topdressing fertilization and again after 45 days, except for lots with freely sprouting plants because the dense canopy did not allow light to penetrate, which inhibited weed growth, thereby dismissing both weed control and the second mulch application.

## Experiment description

The experimental design consisted of random blocks with three repetitions per treatment and 1 m$^2$ bed per plot, with the 0.25 cm$^2$ central sections chosen for analysis. Five treatments were tested using different stands (spacing) and different canopy bases as follows: 1) (10×10): 10 × 10 cm; 2) (20×20): 20 × 20 cm; and 3) (30×30): 30 × 30 cm spacing with thinning to keep one sprout growing per primary branch per plant; 4) (30×30TS) was represented by 30 × 30 cm spacing with thinning to keep one sprout growing from each of two secondary branches grown from the primary branch to give two sprouts (TS) per plant; 5) (30×30FS) consisted of plants with 30 × 30 cm spacing with no thinning (FS, free growing sprouts).

## Data collection

After ten months from transplanting the seedlings to field conditions (beds), we initiated the data collection. In both seasons, data were collected every 6 days from all sprouts that reached 20 cm in height on the evaluated area on the beds (0.25 m$^2$). We measured number of sprouts, length, and width of basal, median, and apical leaves, weighed total fresh matter of sprouts, total fresh matter of leaves, and fresh matter from a sprout, and finally concentration of protein. Some data were used to calculate the mean fresh matter of sprouts, leaf mean fresh matter per sprout, leaf fresh matter percentage per sprout, sprout yield, leaf yield, leaf fresh matter index, and leaf size index.

Three leaves (basal, median, and apical) were extracted from a randomly selected sprout in each plot every collection day for respective weighing and measuring, to obtain the length and width of the central portion. The 15$^{th}$ leaf before the last developed leaf was used as the apical sample.

Leaf matter index (*LMI*) and leaf size index (*LSI*) were calculated according to following equations (Sampaio, 1998; Paiva & Oliveira, 2006):

$$LMI = \frac{BLI + MLI + ALI}{3}$$

$$BLI = \frac{BLFM}{BLL\ x\ BLW}\ x\ 1000$$

$$MLI = \frac{MLFM}{MLL\ x\ MLW}\ x\ 1000$$

$$ALI = \frac{ALFM}{ALL\ x\ ALW}\ x\ 1000$$

$$LSI = (BLL\ x\ BLW) + (MLL\ x\ MLW) + (ALL\ x\ ALW)$$

Where *BLI* = basal leaf index; *MLI* = median leaf index; *ALI* = apical leaf index; *BLFM* = mean of basal leaf fresh matter; *BLL* = mean of basal leaf length; *BLW* = mean of basal leaf width; *MLFM* = mean of median leaf fresh matter; *MLL* = mean of median leaf length; *MLW* = mean of median leaf width; *ALFM* = mean of apical leaf fresh matter; *ALL* = mean of apical leaf length; *ALW* = mean of apical leaf width.

Sprout and leaf yields were calculated for each season by converting the 0.25 m$^2$ plot evaluated to tons per hectare (t/ha), and considering a 20% loss of area for the furrows/paths between beds.

## Protein determination

The samples were dried in an oven with forced air ventilation at 65°C for ≥3 days until constant weight and BGB protein content was determined according to the Kjeldahl method. Protein concentration was calculated using 6.25 as conversion factor (Association of Official Analytical Chemists, 2012).

**Statistical analysis**

Analysis of variance (Anova) was used to determine effect of factors and comparison of means was performed using Tukey's test at 5% probability. Polynomial regression was applied to verify monthly harvest trends across data collection season. Analyses were performed using the Sisvar® statistical program (Ferreira, 2011).

## RESULTS AND DISCUSSION

The high-density plantation system influenced all parameters analyzed, including when in combination with growing season, except for the leaf percentage per sprout and leaf size index. The greatest number of sprouts (158.2) was obtained by high-density cultivation where thinning was not practiced (30×30FS) in the 2020 season (Table 1). This result was expected, because there was no thinning on this planting system, consequently, sprouting was higher. However, the quantity produced and the biometric quality of sprouts in this treatment, such as leaf size and sprout weight, were undetermined, as well as its performance in comparison with the other assays.

The greater number of sprouts obtained in the 2020 season (Table 1) may be related to the climate during this period, particularly the higher temperatures, longer days, and increased overall light (data not shown). Season 2020 occurred from late winter to late spring and season 2021 from late summer to late autumn. During the first period, temperature and light generally rise, which may increase plant growth, while during the second period, mainly milder temperatures and lower light conditions prevail in the region, which corresponds to decreased growth (Anselmini *et al*., 2006; Andreacci *et al*., 2017).

The greater fresh matter of sprouts (1,618.0 g/0.25 $m^2$) was obtained in 2020 with plants at 30 × 30 cm spacing without thinning management (Table 1). The second highest result (1,014.0 g/0.25 $m^2$) for the same parameter was higher in season 2020 than 2021 (832.5 g/0.25 $m^2$), probably due to the greater matter accumulation in this period. Fresh matter of leaves showed similar

**Table 1.** Agronomic parameters evaluated from Barbados gooseberry (BGB) plants cultivated on high-density systems for sprout production in two growing seasons. Curitiba, UFPR, 2022.

| Parameters | CV (%) | Spacing (cm) | Season 2020 | Season 2021 |
|---|---|---|---|---|
| Number of sprouts | 16.9 | 10×10 | 84.0 bA | 79.5 bA |
| | | 20×20 | 36.0 cA | 41.5 cA |
| | | 30×30 | 7.2 dA | 10.5 dA |
| | | 30×30TS | 12.5 dA | 16.0 dA |
| | | 30×30FS | 158.2 aA | 111.7 aB |
| Fresh matter of sprouts (g/0.25 $m^2$) | 15.0 | 10×10 | 1,014.0 bA | 832.5 aB |
| | | 20×20 | 603.2 cA | 581.4 bA |
| | | 30×30 | 152.7 dA | 162.1 cA |
| | | 30×30TS | 226.6 dA | 227.3 cA |
| | | 30×30FS | 1,618.0 aA | 1,019.5 aB |
| Fresh matter of leaves (g/0.25 $m^2$) | 13.3 | 10×10 | 813.4 bA | 646.9 aB |
| | | 20×20 | 493.7 cA | 450.1 bA |
| | | 30×30 | 127.9 dA | 121.9 cA |
| | | 30×30TS | 186.6 dA | 174.4 cA |
| | | 30×30FS | 1,132.8 aA | 669.9 aB |
| Sprout fresh matter (g) | 9.5 | 10×10 | 12.1 cA | 10.5 bA |
| | | 20×20 | 16.9 bA | 14.0 aB |
| | | 30×30 | 21.1 aA | 15.4 aB |
| | | 30×30 TS | 18.2 bA | 14.2 aB |
| | | 30×30 FS | 10.2 cA | 9.2 bA |
| Leaf fresh matter per sprout (g) | 11.4 | 10×10 | 9.7 cA | 8.1 bA |
| | | 20×20 | 13.8 bA | 10.8 aB |
| | | 30×30 | 17.7 aA | 11.6 aB |
| | | 30×30TS | 15.0 bA | 11.0 aB |
| | | 30×30FS | 7.1 cA | 6.0 bA |
| Sprout yield (t/ha) | 15.0 | 10×10 | 32.4 bA | 26.6 aB |
| | | 20×20 | 19.3 cA | 18.6 bA |
| | | 30×30 | 4.9 dA | 5.2 cA |
| | | 30×30TS | 7.2 dA | 7.3 cA |
| | | 30×30FS | 51.8 aA | 32.7 aB |
| Leaf yield (t/ha) | 13.2 | 10×10 | 26.0 bA | 20.7 aB |
| | | 20×20 | 15.8 cA | 14.4 bA |
| | | 30×30 | 4.1 dA | 3.9 cA |
| | | 30×30TS | 6.0 dA | 5.6 cA |
| | | 30×30FS | 36.2 aA | 21.4 aB |

Means followed by different lowercase letters in the column and uppercase letters in the row, differ from each other by Tukey's test at 5% probability; CV (%) = coefficient of variance.

results to those described previously, with the 30×30FS treatment promoting greater leaf matter (1,132.8 g/0.25 $m^2$) and better results in 2020 than 2021 (813.4 g/0.25 $m^2$).

In a way, these results so far were expected. However, the question still remains about the quality of the BGB sprouts collected from these plantation systems. This will be discussed later.

Although the 10×10 treatment had the highest number of plants per unit area (100 plants/$m^2$), it showed lower number of sprouts and fresh matter, as well as less fresh matter of leaves per area in comparison to the 30×30FS system (Table 1) because it had multiple base stems that produced many sprouts. Souza *et al.* (2020) estimated that there would be 1,182.5 g/0.25 $m^2$ (as less as value converted from original units of t/ha/yr) of fresh matter from all developed leaves harvested from the entire top of the plants for 90 days on a BGB plantation stand of 41.4 plants/$m^2$, which was 26.9% lower than the 30×30FS (11.1 plants/$m^2$) and 14.2% higher than the 10×10 (100 plants/$m^2$) treatment presented here (Table 1). We emphasize that only sprouts were harvested in the present work and that the 10×10 system endured frequent thinning to leave one sprout per plant, which may account for the difference in our results from those of Souza *et al*. (2020).

The higher fresh matter of sprouts (21.1 g) was obtained by BGB cultivated with 30 × 30 cm spacing with one sprout left per plant (Table 1), showing that this growing system promoted better quality sprouts because it was larger/heavier than sprouts collected from the other tested assays. Vegetable size (length, width, weight) is consistently relevant for consumers (Amorim *et al*., 2017; Jardina *et al*., 2017; Yokoro & Pereira, 2020). Thus, if a specific market required larger or heavier sprouts, this cultivation system would be recommended.

BGB planted with 30 × 30 cm spacing to produce one sprout per plant per harvest promoted the greatest amount of leaf matter per sprout (17.7 g) (Table 1). Plants spaced further apart in the plantation receive more light and nutrients, which promotes maximum growth, and consequently, higher fresh weight per plant (Hasan *et al*., 2017).

The 30×30TS and 10×10 plantations produced the least fresh matter of sprout (12.1 g and 10.2 g in season 1, 10.5 g and 9.2 g in season 2, respectively) and leaf matter per sprout (9.7 g and 7.1 g in season 1, 8.1 g and 6.0 g in season 2, respectively) (Table 1); probably the denser canopy and higher stand of these respective cultivation systems induced etiolation on sprouts that were elongated with thinner stems and smaller leaves as result of higher competition for light (Frölech *et al*., 2020). We also have to consider here source-sink relationship, seeing that stronger sink from planting systems was expected, which had higher density of plants or no thinning, because in these cases sprouting of them was higher; consequently, they produced higher number of sprouts, which means higher strong sink and lower strong source in comparison with the other planting systems. It is widely known that assimilated compounds produced by mature leaves during the photosynthesis process are crucial to supply carbon and energy demanded for the formation of


**Figure 1.** Barbados gooseberry (BGB) cultivated with 30×30 cm spacing without thinning management for free sprouting. (A) = Density canopy formed on the bed. (B) = High sprouting from the dense canopy showing spineless sprouts. Curitiba, UFPR, 2022.

**Table 2.** Agronomic parameters evaluated from Barbados gooseberry (BGB) plants cultivated on different high-density systems for sprout production. Curitiba, UFPR, 2022.

| Treatment | Leaf per sprout (%) | Leaf size index |
|---|---|---|
| 10×10 | 79.0 a | 93.4 a |
| 20×20 | 79.5 a | 102.7 a |
| 30×30 | 79.5 a | 105.0 a |
| 30×30TS | 79.7 a | 105.8 a |
| 30×30FS | 68.1 b | 59.9 b |
| Season | | |
| 2020 | 79.7 a | 103.4 a |
| 2021 | 74.6 b | 83.3 b |
| CV (%) | 3.2 | 12.0 |

Means followed by different lowercase letters in the column differ from each other by Tukey's test at 5% probability; CV (%) = coefficient of variance.

**Table 3.** Protein content of portions of Barbados gooseberry (BGB) sprouts in comparison with mature leaves. Curitiba, UFPR, 2022.

| BGB Product | Protein (%) |
|---|---|
| Leaves from sprout | 28.8 a |
| Stems from sprout | 20.3 b |
| Mature leaves(1) | 16.2 c |
| CV (%) | 3.5 |

(1)Conventionally marketed. Means followed by different lowercase letters in the column differ from each other by Tukey's test at 5% probability; CV (%) = coefficient of variance.

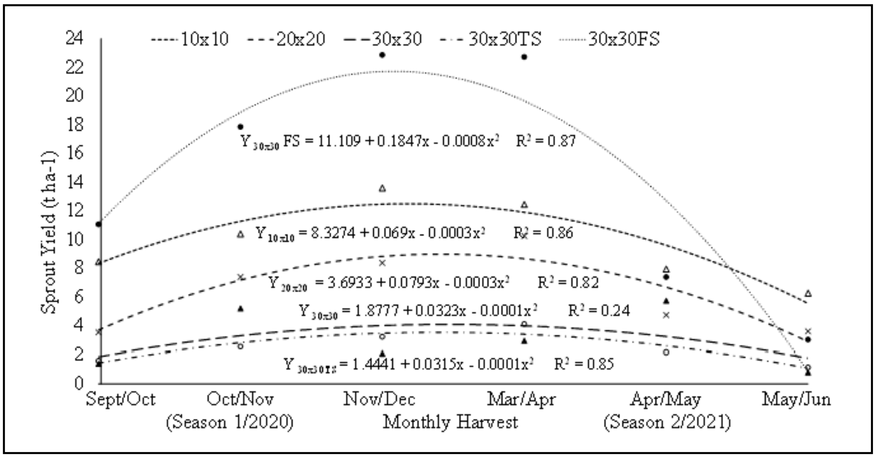


**Figure 2.** Sprout yield of Barbados gooseberry (BGB) cultivated on high-density stand systems during two seasons. Values are related to total harvest for every 5 data collection dates (monthly). Curitiba, UFPR, 2022.

new tissues, as well as for the growth of vegetable organs (Taiz & Zeiger, 2017).

Considering only yield as a result, the superior plantation system would be based on the no thinning practice on BGB plants spaced at 30 × 30 cm because of the high sprout yield (58.1 t/ha). In growing season 2021, this plantation system showed a greater yield (32.7 t/ha), although the 10×10 treatment had a statistically equal result (26.6 t/ha; Table 1).

The free growing system for BGB planted with 30 × 30 cm spacing also showed a superior result for leaf yield collected from sprouts in the season 2020 (36.2 t/ha). In season 2021, this assay was also greater (21.4 t/ha), as was the 10×10 cultivation system (20.7 t/ha) (Table 1).

Overall, the plantation based on no thinning management of BGB planted with 30 × 30 cm spacing (Figure 1) appears to be the most productive system for BGB cultivated in a high-density stand.

Along with the higher sprout and leaf yields, and a greater number of sprouts, the 30×30 plantation system without thinning also promoted horticultural advantages in vegetable management during the assay, and we highlighted three of these.

First, weed control in the free budding and sprouting BGB planted with the 30 × 30 cm spacing was simplified compared to other assays (data not shown), because a dense canopy was formed on the beds a few months after pruning, which did not allow sunlight to penetrate, thereby inhibiting weed growth (Figure 1A).

Second, harvesting was easier than other plantation systems tested (data not shown) because the canopy had a 30 cm height overall and sprouts grew outside the canopy (Figure 1B) allowing for straightforward harvesting by hand by breaking off the stems. Harvesting in this plantation system was safer because the grower did not touch spines or thorns that were located inside the canopy.

Finally, production costs would be lower due to less labor required because of better weed control and elimination of thinning.

The lowest percentage of leaves per sprout (68.1%) and the lowest leaf size index (59.9) were obtained when BGB was planted with 30 × 30 cm without thinning (Table 2), which is probably related to the high competition for light, nutrients, and water among leaves due to excessive sprouts growing in this plantation system that may inhibit leaf growth.

Leaf matter index was only affected by the growing season, with 2020 showing better results (30.9) than 2021 (29.4), which relates to the larger length and width of leaves in 2021.

Sprout yields were estimated for all high-density plantation systems and, as expected, the higher yields tended to concentrate in the middle of summer (Figure 2). BGB production increases in the spring, peaks in the summer, and decreases from autumn to winter. BGB stop growth and production at low temperatures, and frost or snow in south region of Brazil can extremely damage the plants, causing leaf burn (Silva *et al*., 2018). However, affected plants can be late-pruned to start the next growing season. In the present study, climate injury occurred in the middle winter, before and after experimental period, mainly in 2021 when the low temperatures minimally damaged plants (data not shown). The two high-density plantation systems that obtained higher sprout yields showed estimated maximum values of 21.7 t/ha for 30×30FS and 11.2 t/ha for 10×10 (Figure 2), both harvested from December 22, 2020 to January 01, 2021, which coincides with summer.

Protein is one of the most important substance present in BGB leaves; however, the protein content exclusively in sprouts has not been evaluated. The analyses revealed that leaves from sprout had greater protein contents (28.8%) and that the stems had higher levels of protein (20.3%) than mature leaves (16.2%) collected from plants cultivated in the conventional plantation (Table 3). According to Malta *et al*. (2002), young leaves tend to show high protein content due to their accelerated metabolic activity; therefore, the tissues of the tender portions likely have higher protein content than those of the mature portions of plants. Protein content of mature leaves has been described as variable, with ranges from 17.4 to 28.4% (Almeida Filho & Cambraia, 1974; Almeida & Corrêa, 2012).

We proposed a new cultivation system for the production of BGB sprouts as a novel marketable product, and the protein value revealed here is conducive for marketing, along with the favorable distinctive flavor these sprouts present.

In conclusion, a plantation with 30 × 30 cm spacing without thinning or 10 × 10 cm with thinning to produce one sprout per plant per harvest promoted higher yields. We suggest 30 × 30 cm spacing to produce one or two sprouts per plant per harvest or 20 × 20 cm to produce one sprout per plant per harvest to increase quality. In addition, high-density planting of BGB produced sprouts with high protein contents.

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