

## RESEARCH ARTICLE

# Planting density: Key factor for crop management of *Cassia angustifolia* Vahl

Emilia Constantinescu<sup>1</sup>, Sorina Nitu-Nastase<sup>2, 3\*</sup>, Dorina Bonea<sup>1\*</sup>, Liviu Aurel Olaru<sup>1</sup>, Vasile Adrian Blaj<sup>3</sup>, Ion Saracin<sup>1</sup>, and Virgil Nitu<sup>2</sup>

<sup>1</sup>University of Craiova, Faculty of Agronomy, 200421 Craiova, Dolj County, Romania.

<sup>2</sup>National Institute of Research and Development for Potato and Sugar Beet Braşov, 500470 Braşov County, Romania.

<sup>3</sup>Institute of Research and Development for Grassland Braşov, 500128 Braşov County, Romania.

\*Corresponding authors (nastasesorina03@gmail.com; dorina.bonea@edu.ucv.ro).

Received: 24 July 2024; Accepted: 21 October 2024, doi:10.4067/S0718-58392025000200245

## ABSTRACT

Medicinal plants (MP) are used in various forms (teas, tinctures, creams, food supplements, etc.), with a single purpose, that of improving the quality of human life from birth to senescence. The choice of the species *Cassia angustifolia* Vahl, to be introduced into culture and for the development of mechanized cultivation technology is argued by the high demand for ecological raw material, correlated with climate changes. Establishing the optimal planting distance is very important in crop management to improve plant growth and yield. In this study, the effects of planting density on the growth and yield of *C. angustifolia* plants were evaluated in the edaphoclimatic conditions of Brasov, Romania. The experiment included three factors: Factor A – experimental year: 5 April 2016 (A1), 11 April 2017 (A2), 4 April 2018 (A3). Factor B – Distance row by row (RR): 25 (B1), 50 (B2), and 70 cm (B3). Factor C – Distance plant by plant (PP): Continuous row (C1), 15 cm (C2), and 25 cm (C3). Three years' data indicated that plant density of 25 (RR) × continuous row (PP) resulted in obtaining the highest yield of fresh (48.33 t ha<sup>-1</sup>) and dry (7.93 t ha<sup>-1</sup>) biomass. Increasing planting density increased individual plant development (height, number of branches and weight), but reduced yield of biomass.

**Key words:** *Cassia angustifolia*, dry biomass, height, mechanized technology, planting distance.

## INTRODUCTION

Nature provides medicinal plants (MP), an untapped and boundless treasure trove of active chemical constituents with significant therapeutic potential, these plants thus become a beneficial source in the development of phytomedicines.

Other considerations also arise, such as distrust of allopathic medicine, the ineffectiveness of allopathic therapy, or the belief that herbal therapies do not produce side effects (Brousse and Gallé, 2017; Niţu et al., 2022).

According to numerous scientific reports, the use of medicinal plants in the form of crude extracts or chemical components is largely associated with a reduced risk of degenerative diseases, which are associated with oxidative stress, because they contain antioxidants such as: Phenol, carotenoids, vitamins and flavonoids (Hafez et al., 2019, Maury et al., 2020). Medicinal plants have been widely used as therapeutic medicine to treat health disorders since ancient times. Natural plant extracts are commonly used in the pharmaceutical, cosmetic and food industries (Al-Ghamdi et al., 2020).

In Romania, the concern for curing diseases with the help of plants has an old tradition. This is due to the fact that Romania has one of the most varied flora on the European continent, being the meeting point of the Central European, Mediterranean and Euro-Asian flora. This explains the fact that in Romania there are about 4000 species of plants, of which more than 20% are medicinal plants. About 25% of these medicinal plants are already used to obtain botanical products on a large scale (Segneanu et al., 2019).

*Cassia angustifolia* Vahl is a traditional medicinal plant that belongs to the *Cassia* genus, subtribe Cassiinae, tribe Cassieae, subfamily Caesalpinioideae, family Fabaceae, order Fabales, subclass Rosidae, class Magnoliopsida, division Magnoliophyta, commonly classified as *Cassia senna* (Lal et al., 2023). No species of *Senna* is native to Europe, although many have long been used in European medical tradition and it was introduced into European medicine by the Arabs in the 9th or 10th century (Satti, 2020).

This plant is accepted in the pharmacopoeias, both in the UK and in USA, and the leaves and pods of *C. angustifolia* are used for anthelmintic treatment (powder), decoction for intestinal worms, as a laxative, antipyretic in typhoid fever, splenomegaly, cholera, anemia, toxicity and genotoxicity induced by *Escherichia coli* (Ahmed et al., 2016; Albrahim et al., 2021).

*Cassia angustifolia*, commonly known as "senna", is used in different traditional systems of medicine against several diseases. Senna is a powerful medicine against anemia, cancer, typhoid fever, cholera, biliousness, jaundice, rheumatism, tumors, bad breath, bronchitis, leprosy, etc. (Singh et al., 2018; Boonhok et al., 2021; Natarajan et al., 2022). It is one of the most widely used herbal laxatives (Rama-Reddy et al., 2015; Ramchander et al., 2017).

The plant contains anthraquinone compounds, especially senidols and senoids, as well as flavonoids and mucilages. The leaves or pods contain glycosides, sennosides A, sennosides B, sennosides C and sennosides D (Jnanesha et al., 2018; Nilofer et al., 2018; Kumar et al., 2022; Lal et al., 2023; Thaker et al., 2023). The flavonol-yellow coloring matter kaempferol and its glucosides kaempferin and isorhamnetin have also been reported in *C. angustifolia* leaves (Zibae et al., 2023).

In Romania, the culture of medicinal and aromatic plants (MAP) has been revived in the last decade, obtaining high and quality productions presupposing technical knowledge, interest, passion and a lot of work.

The choice of the species *C. angustifolia* to be introduced into culture and for the development of cultivation technology is argued by the high demand for ecological raw material, correlated with the trends of climate change. The technologies applied in the organic farming system use inputs without hormones, genetically modified organisms, pesticides, antibiotics and other synthetic substances and all of these allow both the preservation of the bioavailability of active compounds during processing and the elimination of toxicological risks in final food products (Savescu and Popescu, 2022).

General knowledge about the cultivation of medicinal plants is not enough, because they present a very large variety of species, have a different lifespan (annual, biennial or perennial), the method of propagation is different from one species to another (seeds, cuttings, splitting the bush, stolons), the size of the plant differs greatly, and harvesting, drying and conditioning are done according to the part of the plant used (flowers, leaves, roots, stolons, buds, etc.) Improving crop cultivation technology by establishing the optimal planting distance in local climatic and edaphic factors is very important for successful production (Nițu et al., 2022; Betancur et al., 2024). Thus, in this paper we aimed to investigate the effects of different planting density on the growth and yield of *C. angustifolia* in the edaphoclimatic conditions of Brașov, Romania, the main objective being to establish the optimal planting density. The knowledge obtained in the present study can serve as a reference for the strategic establishment of new *C. angustifolia* crops under the conditions of sustainable agriculture.

## MATERIALS AND METHODS

### Edaphoclimatic characteristics

The experiment was carried out at the National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB), Brașov, Romania, within the Laboratory of Technology and Good Agricultural Practices, Department of Medicinal and Aromatic Plants. The experimental area is geographically positioned in Brașov County (45°42' N, 25°45' E; 520 m a.s.l.)

The requirements for climate and soil, fertilization, the place in the rotation, are very important aspects that must be known when aiming to establish a medicinal plant culture.

The climate belongs to the temperate one, and the region is located at the transition between the West-European climate, oceanic in nature, and the excessively continental one. The precipitation regime is low (500-600 mm), the average number of rainy days per year being 142.

During the winter period, in 2015-2016, 149.2 mm rainfalls were recorded, a level 27.8 mm lower than the multi-year average (MYA) (177.0 mm). At the beginning of the vegetation period, April was warmer than normal, the monthly average air temperature exceeding the MYA by 2.8 °C. The amount of rainfalls that fell in April (98.4 mm) was almost double that of MYA. In May, the air temperature was lower by 1.2 °C compared to the MYA, and the amount of precipitation exceeded the MYA value by 22.4%. The climatic conditions in June favored a good growth of plants and the development of a foliage rich in medicinal plant experiences, thanks to temperatures higher on average by 2.5 °C and precipitations that totaled 121.2 mm (148.2% compared to MYA). August came close to the characteristic value of the area, with an average air temperature higher by 0.9 °C and with the amount of rainfalls reduced by 14.5 mm, and September was slightly warmer (+1.4 °C) and with less rainfalls (-14.5 mm).

In the agricultural year 2016-2017 the amount of rainfalls (688.2 mm) in Brasov exceeded the MYA value by 53.8 mm (8.5%). The level of monthly rainfalls was close to the MYA, more significant deviations were recorded only in October 2016. The winter was characterized by significant rainfalls, reaching 237 mm, exceeding the MYA value by 60 mm. The precipitation ensured a good supply of water to the soil in the spring. During the growing season, the rainfalls totaled 451.2 mm, a value very close to the MYA. The distribution was uniform in the vegetation period of 2017. This year rain fell in every decade of the period, unlike previous years with frequent decades without rain. Between April and September, the decadal amounts of rainfalls varied between 8.4 mm (April, decade I) and 48 mm (July, decade I). The level and distribution of rainfalls in the spring of 2017 favored a good grip of seedlings and plant development.

The agricultural year 2017-2018 was warmer and richer in precipitation. In the autumn-winter period, the average air temperature was higher by 1.5 °C compared to MYA (0.7 °C). Average monthly temperatures were higher, compared to the MYA values, throughout the October-March interval, with deviations between 0.5 and 3.3 °C. The highest thermal deviation was registered in January +3.3 °C. During the vegetation period (April-August), the air temperature was higher on average by 2.7 °C, compared to MYA. Average monthly temperature deviations were between 0.5 and 5.5 °C, the maximum deviation being recorded in April. The sum of rainfalls exceeded the MYA value of 177.0 mm by 141.3 mm. It is worth noting that in all months of plant growth (October-March) the amount of precipitation was higher compared to MYA. Between April and September, the rainfalls fell was generally below the MYA values, except for June and July, when they exceeded the MYA values, especially in June, by more than 100 mm.

The soil texture in the upper horizons is sandy and loamy-sandy, and at depth it is sandy-loamy, with a pH of 6.7. The soil of the experimental site contains 27% clay, 4.68% humus, total N 3.15%, P<sub>2</sub>O<sub>5</sub> 7.36 mg 100 g<sup>-1</sup> soil and K<sub>2</sub>O 12.67 mg 100 g<sup>-1</sup> soil. Soil samples were analyzed using spectrophotometry and atomic absorption. The predominant soil is Chernozem, with a high humus content in the first horizon, a very high N supply in the first centimeters and a moderate acid reaction of the soil solution. Nitrification conditions are unbalanced due to the acid reaction in the soil (Moldovan et al., 2022).

### **Description of the biological material**

The biological material used in the experiment was obtained from Agricultural Research and Development Station (ARDS) Secuieni (Neamț county, Romania) and belongs to the local population "De Secuieni" having a germination of 85%, a physical purity of 92% and 2.4 g weight of 1000 grains (WTG).

*Cassia angustifolia*, commonly known as "senna", has flowers of type 5, with free elements, slightly zygomorphic, pedicels 3-4 cm long, grouped in terminal or axillary racemes, up to 15 cm long. The sepals are slightly uneven, yellow-green in color, 10-13 mm long and 6-9 mm wide. The petals are yellow and slightly uneven, 14-17 mm long and 7-10 mm wide. The 10 stamens are free. The ovary is hairy and stipitate. The plant blooms in April-June. The fruit is a dehiscent, slightly hairy pod, 5-6 cm long and 1.7-2.3 cm wide, slightly curved, with approx. 10 seeds. The fruits turn black when ripe. The anatomy of the stem and the leaf are similar to those of species from the Fabaceae family, being reconfirmed in specialized works (Santhan, 2014; Savulescu et al., 2018; Nițu et al., 2022).

## Experimental design

The experience was threefold. Factor A – experimental year: 5 April 2016 (A1), 11 April 2017 (A2), 4 April 2018 (A3). Factor B – Row by row distance (RR): 25 (B1), 50 (B2), and 70 cm (B3). Factor C – Plant by plant distance (PP): Continuous row (C1), 15 (C2) and 25 cm (C3).

Nine variants resulted from the combination of factors B × C, each of them presenting a distinct number of plants per m<sup>2</sup>: B1 × C1 = 60; B1 × C2 = 26.6; B1 × C3 = 16; B2 × C1 = 30; B2 × C2 = 13.33; B2 × C3 = 8; B3 × C1 = 21.5; B3 × C2 = 9.5; B3 × C3 = 5.7. The study of the interaction between the factors involved the analysis of 27 variants.

The experiments were established according to the method of randomized blocks, with three replicates, each replicate having a length of 2 m, with an isolation distance of 1 m between replicates, on an area of 70 m<sup>2</sup> cultivated in an ecological system.

*Cassia angustifolia*, behaving like an annual species, is recommended to be introduced in rotation. In the first crop year (2016), the preceding plant was durum wheat *Triticum turgidum* L. in the second year (2017) the preceding plant was *Malva silvestris*, and in the third year (2018) the preceding plant was *Dracocephalum moldavica* L.

After freeing the land from plant residues, 30 t ha<sup>-1</sup> well-fermented manure (from bovines) were administered, in order to quickly mineralize and avoid weeding, then plowing was carried out at 28 cm, on which occasion both the organic fertilizer and plant resources were incorporated. A work was carried out with a disk harrow, in order to keep the soil clean of weeds and to avoid compaction, and in the spring, the work of preparing the germinal bed was carried out with the combiner, work carried out 2 d before sowing, achieving a germinal bed well shredded, but placed at the depth of embedding the seeds.

The need for nutrients came from the decomposition of manure, through the mineralization of vegetable remains (contribution of the preceding plant) and from the natural fertility of the soil, which is from the Chernozem category with a high percentage of humus.

The experience was located in an ecological system, so the application of other types of fertilizers was avoided for the basic fertilization, as well as foliar fertilizers, taking into account that, from this species, the aerial part (leaves, flowers, seeds) is used. Sowing was carried out differently during the 3 yrs of experimentation, dynamically (4, 5, 11 April), taking into account the influence of temperature and humidity on the seed germination process, by reducing the interval from sowing to emergence. The seeds, being small (WTG of 2.4 g) were sown at a depth of 2 cm, when the soil temperature exceeded 10-12 °C, thus ensuring a uniform and rapid emergence.

Throughout the vegetation period, the crop was kept clean of weeds by row weeding, four manual harrows and two mechanical harrows, with the motor cultivator, for the variants sown at 50 cm, respectively 70 cm RR. Due to its ecological nature, the experience did not allow the administration of pesticides (herbicides, fungicides, insecticides, growth stimulants, stabilizers, desiccants).

During the three experimental years, no pathogenic agents were reported, which would cause significant economic damage, because this species is cultivated only experimentally in Romania.

## Statistical analysis

Statistical analysis of experimental data was performed in Excel (Microsoft) and SPSS program version 17 (SPSS, Chicago, Illinois, USA), means were processed by ANOVA test of significant differences ( $p \leq 0.05$ ) for fully randomized multifactorial experiments, theoretical t values and DL limit differences for 5%, 1% and 0.1%. Principal component analysis and cluster analysis were performed using SPSS.

# RESULTS AND DISCUSSIONS

## Influence of distance row by row and experimental year on *C. angustifolia* growth and development parameters

The plant height varied between 79.11 and 112.22 cm, maximum height been recorded under the conditions of 50 cm row by row (RR) distance in the third experimental year (A3B2). The lowest value for height were recorded in the second experimental year at the distance 25 cm RR (A2B1). The assessment of this parameter shows the unsuitability of RR distances of 25 cm, low values been registered in the first 2 yr.

The branching potential presented significant differences, the highest values being in the first year for all variants (from 25.67 to 25 cm RR to 28.44 to 70 cm RR) and the lowest in the second experimental year (from 17.89 cm to 25 cm RR to 19.22 to 70 cm RR), unfavorable climatic conditions having an overall influence on the development of plants.

Also, the weight of the plants varied a lot, from 128.11 g in the first year for the variant A1B1 to 244 g for the variant A2B3 in the second year. In general, in all 3 yrs of experimentation, variant B3 (70 cm RR) presented the highest values, a greater distance positively influencing the weight of the plants.

The analysis of fresh biomass variations shows a contrasting image compared to the weight analysis. The distance 25 cm RR presented the highest biomass yield, with value between 35.06 t ha<sup>-1</sup> in the first year (A1B1) and a maximum 37.97 t ha<sup>-1</sup> in the third year (A3B1). The dry biomass yield follows the same trend as the fresh one, the highest yield (6.84 t ha<sup>-1</sup>) being to distance 25 cm RR (A3B1) (Table 1).

**Table 1.** Influence of experimental year × distance row by row (RR) factors on *Cassia angustifolia* parameters. Factor A—experimental year: 2016 (A1), 2017 (A2) and 2018 (A3). Factor B—distance RR: 25 (B1), 50 (B2) and 70 cm (B3). Means ± se followed by different letters were considered significant at  $p < 0.05$  according to LSD test.

Factor combination	Height cm	Branch nr	Weight g	Fresh biomass t ha <sup>-1</sup>	Dry biomass t ha <sup>-1</sup>
A1B1	93.67 ± 1.58 <sup>c</sup>	25.67 ± 0.58 <sup>bc</sup>	128.11 ± 20.07 <sup>a</sup>	35.06 ± 1.65 <sup>d</sup>	6.75 ± 0.38 <sup>d</sup>
A1B2	101.89 ± 3.24 <sup>d</sup>	27.56 ± 1.00 <sup>cd</sup>	170.56 ± 12.67 <sup>bc</sup>	26.60 ± 3.18 <sup>bc</sup>	5.10 ± 0.62 <sup>c</sup>
A1B3	101.33 ± 2.04 <sup>e</sup>	28.44 ± 1.17 <sup>d</sup>	221.00 ± 14.22 <sup>d</sup>	25.02 ± 3.31 <sup>ab</sup>	4.79 ± 0.60 <sup>ab</sup>
A2B1	79.11 ± 3.15 <sup>a</sup>	17.89 ± 0.98 <sup>a</sup>	132.22 ± 25.63 <sup>a</sup>	34.85 ± 2.08 <sup>d</sup>	6.49 ± 0.39 <sup>d</sup>
A2B2	86.89 ± 2.20 <sup>b</sup>	18.89 ± 0.77 <sup>a</sup>	160.33 ± 18.92 <sup>b</sup>	23.07 ± 1.65 <sup>a</sup>	4.43 ± 0.20 <sup>a</sup>
A2B3	88.67 ± 1.98 <sup>bc</sup>	19.22 ± 0.49 <sup>a</sup>	244.00 ± 20.58 <sup>e</sup>	27.03 ± 3.17 <sup>bc</sup>	5.01 ± 0.49 <sup>bc</sup>
A3B1	110.56 ± 4.82 <sup>e</sup>	23.22 ± 1.30 <sup>b</sup>	178.33 ± 18.87 <sup>bc</sup>	37.97 ± 2.68 <sup>e</sup>	6.84 ± 0.39 <sup>d</sup>
A3B2	112.22 ± 4.34 <sup>e</sup>	25.22 ± 1.30 <sup>bc</sup>	181.78 ± 13.47 <sup>c</sup>	28.13 ± 3.20 <sup>c</sup>	5.30 ± 0.52 <sup>c</sup>
A3B3	110.33 ± 2.39 <sup>e</sup>	25.22 ± 0.88 <sup>bc</sup>	212.67 ± 8.92 <sup>d</sup>	24.86 ± 3.72 <sup>ab</sup>	4.68 ± 0.60 <sup>ab</sup>

#### Influence of distance plant by plant and experimental year on *C. angustifolia* growth and development parameters

The plants height, like in the case of the distance RR, varied with the plant by plant (PP) distance. The lowest value was in the second experimental year at the variant A2C1 (77.78 cm) and the highest in the third year at the variant A3C2 (113.11 cm). It results that the height of the plants is not a stable character, it can be significantly influenced by the distance PP.

The number of branches is a more stable character not being significantly influenced by the distance RR or between the distance PP, but by the climatic conditions.

In all years, the weight of the plant in the C3 variants (25 cm PP) was higher, unlike the C1 (continuous row), which presented the lowest values.

Both types of biomasses show differences induced by the combination of PP distance and experimental year. The highest values recorded in all the 3 yrs were for C1 (continuous row), with dry biomass yield between 34.97 (A2C1) and 41.96 t ha<sup>-1</sup> (A3C1) (Table 2).

#### Influence of planting density on *C. angustifolia* growth and development parameters

The maximum weight recorded was 254.89 g under the variant B3C2 (70 cm RR × 15 cm PP). Compared to this value, significantly lower value was recorded for the variant B1C1 (25 cm RR × continuous row). It is important to mention that the individual weight of plants is not relevant in the case of biomass yield.

The maximum value recorded for fresh biomass was 43.33 t ha<sup>-1</sup> for the variant B1C1 (25 cm RR × continuous row). The dry biomass yield showed a similar trend, the plants in continuous rows performed better than the variants within-row distances (Table 3).

**Table 2.** Influence of experimental year × distance plant by plant (PP) factors on *Cassia angustifolia* parameters. Factor A—experimental year: 2016 (A1), 2017 (A2) and 2018 (A3). Factor C—distance PP: continuous rows (C1), 15 (C2) and 25 cm (C3). Means ± se followed by different letters were considered significant at  $p < 0.05$  according to LSD test.

Factor combination	Height cm	Branch nr	Weight g	Fresh biomass t ha <sup>-1</sup>	Dry biomass t ha <sup>-1</sup>
A1C1	94.11 ± 2.47 <sup>bc</sup>	25.78 ± 0.64 <sup>def</sup>	120.89 ± 15.03 <sup>ab</sup>	38.27 ± 0.98 <sup>e</sup>	7.32 ± 0.29 <sup>e</sup>
A1C2	101.22 ± 2.45 <sup>cd</sup>	27.56 ± 1.07 <sup>ef</sup>	182.44 ± 19.23 <sup>c</sup>	27.64 ± 1.31 <sup>c</sup>	5.50 ± 0.29 <sup>c</sup>
A1C3	101.56 ± 2.44 <sup>cd</sup>	28.33 ± 1.11 <sup>f</sup>	216.33 ± 11.19 <sup>d</sup>	20.77 ± 2.99 <sup>a</sup>	3.83 ± 0.48 <sup>a</sup>
A2C1	77.78 ± 2.25 <sup>a</sup>	16.78 ± 0.95 <sup>a</sup>	110.11 ± 15.15 <sup>a</sup>	34.97 ± 1.81 <sup>d</sup>	6.21 ± 0.34 <sup>d</sup>
A2C2	86.33 ± 2.58 <sup>ab</sup>	18.89 ± 0.72 <sup>ab</sup>	185.78 ± 30.34 <sup>c</sup>	26.55 ± 1.29 <sup>c</sup>	5.06 ± 0.21 <sup>bc</sup>
A2C3	90.56 ± 1.68 <sup>b</sup>	20.33 ± 0.58 <sup>bc</sup>	240.67 ± 7.70 <sup>e</sup>	23.43 ± 3.50 <sup>b</sup>	4.66 ± 0.64 <sup>b</sup>
A3C1	111.44 ± 4.79 <sup>e</sup>	23.00 ± 0.99 <sup>cd</sup>	129.78 ± 14.27 <sup>b</sup>	41.96 ± 1.74 <sup>f</sup>	7.38 ± 0.26 <sup>e</sup>
A3C2	113.11 ± 3.52 <sup>e</sup>	25.78 ± 0.98 <sup>ef</sup>	183.67 ± 17.67 <sup>c</sup>	28.00 ± 1.36 <sup>c</sup>	5.36 ± 0.24 <sup>c</sup>
A3C3	108.56 ± 3.39 <sup>de</sup>	24.89 ± 1.51 <sup>de</sup>	215.33 ± 4.76 <sup>d</sup>	21.00 ± 3.10 <sup>b</sup>	4.08 ± 0.53 <sup>ab</sup>

**Table 3.** Influence of distance row by row (RR) × plant by plant (PP) factors on *Cassia angustifolia* parameters. Factor B— distance RR: 25 (B1), 50 (B2) and 70 cm (B3). Factor C—distance PP: continuous row (C1), 15 (C2) and 25 cm (C3). Means ± se followed by different letters were considered significant at  $p < 0.05$  according to LSD test.

Factor combination	Height cm	Branch nr	Weight g	Fresh biomass t ha <sup>-1</sup>	Dry biomass t ha <sup>-1</sup>
B1C1	89.00 ± 6.78 <sup>a</sup>	21.00 ± 1.84 <sup>a</sup>	72.22 ± 2.40 <sup>a</sup>	43.33 ± 1.44 <sup>e</sup>	7.93 ± 0.23 <sup>f</sup>
B1C2	97.89 ± 6.20 <sup>ab</sup>	23.00 ± 1.37 <sup>abc</sup>	108.00 ± 3.26 <sup>b</sup>	30.24 ± 0.91 <sup>d</sup>	5.80 ± 0.21 <sup>d</sup>
B1C3	96.44 ± 2.75 <sup>ab</sup>	22.78 ± 1.27 <sup>abc</sup>	214.44 ± 5.80 <sup>e</sup>	34.31 ± 0.93 <sup>e</sup>	6.35 ± 0.24 <sup>e</sup>
B2C1	97.67 ± 6.07 <sup>ab</sup>	21.44 ± 1.43 <sup>ab</sup>	115.67 ± 5.69 <sup>b</sup>	34.70 ± 1.71 <sup>e</sup>	6.30 ± 0.35 <sup>e</sup>
B2C2	101.11 ± 4.49 <sup>b</sup>	24.78 ± 1.71 <sup>bc</sup>	189.00 ± 7.64 <sup>d</sup>	26.46 ± 1.07 <sup>c</sup>	5.21 ± 0.22 <sup>c</sup>
B2C3	102.22 ± 4.04 <sup>b</sup>	25.44 ± 1.49 <sup>c</sup>	208.00 ± 6.61 <sup>e</sup>	16.64 ± 0.53 <sup>b</sup>	3.32 ± 0.14 <sup>b</sup>
B3C1	96.67 ± 4.06 <sup>ab</sup>	23.11 ± 1.37 <sup>abc</sup>	172.89 ± 2.84 <sup>c</sup>	37.17 ± 0.61 <sup>f</sup>	6.67 ± 0.15 <sup>e</sup>
B3C2	101.67 ± 3.23 <sup>b</sup>	24.44 ± 1.68 <sup>bc</sup>	254.89 ± 13.44 <sup>f</sup>	25.49 ± 1.34 <sup>c</sup>	4.91 ± 0.23 <sup>c</sup>
B3C3	102.00 ± 3.78 <sup>b</sup>	25.33 ± 1.86 <sup>c</sup>	249.89 ± 7.35 <sup>f</sup>	14.24 ± 0.42 <sup>a</sup>	2.90 ± 0.08 <sup>a</sup>

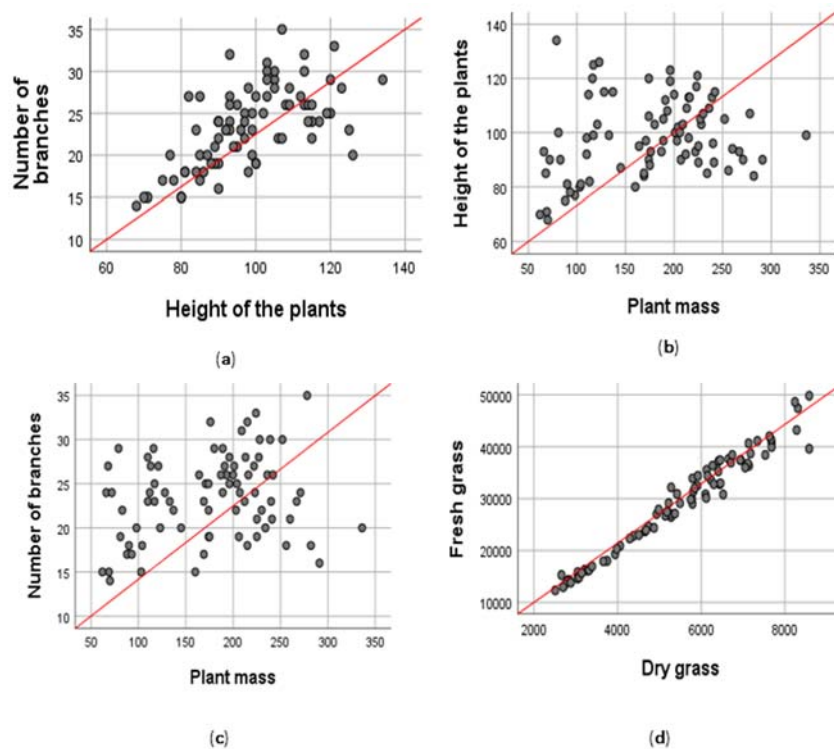
### Interrelations between *C. angustifolia* growth and development parameters

A positive linear relationship does appear between the plant height and the number of branches. As the height of plants increase, the number of branches increases as well (Figure 1a).

The relation between the weight and height of plants shows a positive correlation, with data in the area of 100-110 cm in height. Values between 160 and 220 g for plant weight can be considered the closest to the general linear model (Figure 1b).

A similar correlation between the number of branches and the weight of the plant shows that most plants were in the area of 180-240 g plant<sup>-1</sup> which developed especially on those with 22-28 branches (Figure 1c).

A strong positive correlation between fresh and dry biomass is observed in most variants, regardless of the distance between rows and between plants per row. A large yield of fresh biomass entails a, large yield of dry biomass. In different climatic years, the yields are different, the climatic conditions influencing the yield as a whole (Figure 1d).



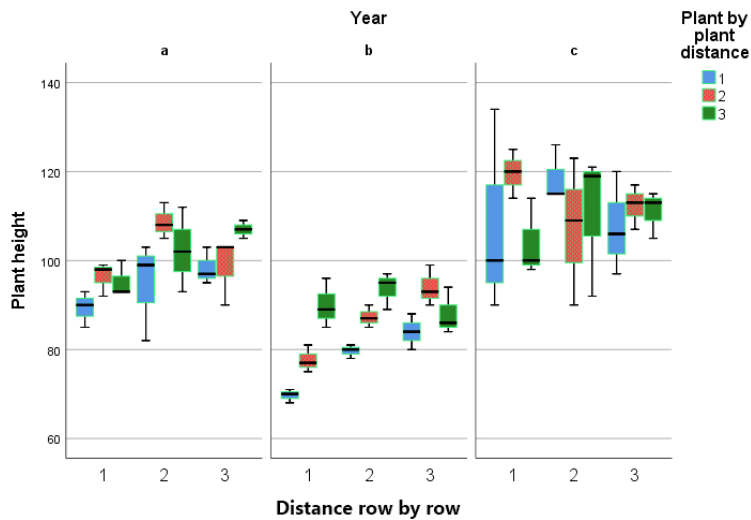
**Figure 1.** Interdependence between *Cassia angustifolia* growth and development parameters: Number of branches × height of the plants (a), height of the plant × weight (plant mass) (b), number of branches × weight (plant mass) (c), fresh × dry biomass (grass) (d).

#### Detection of factor importance on *C. angustifolia* growth and development

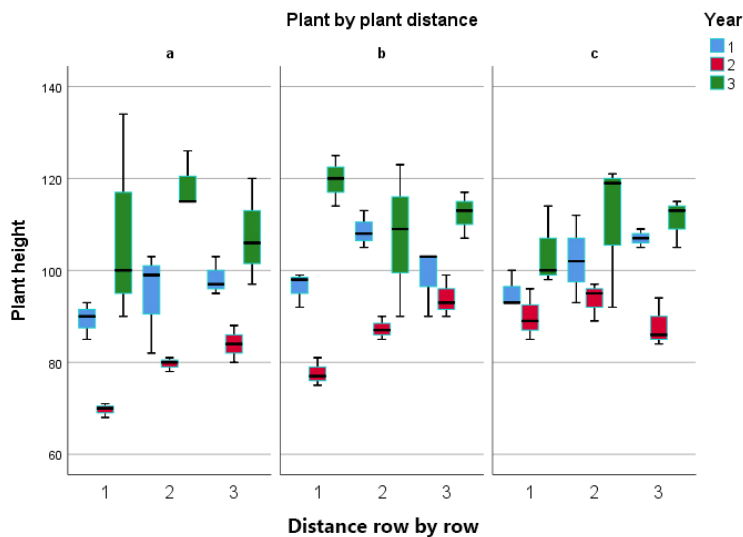
The distance of plants B1C1 in the first year (median was 90 units) showed less dispersed data than in second year when was a large variability (with the median in the third quartile, 98 units) and in the third year the median was in the lower quartile (96 units). First year to the distance C2B2 suggest that the plants height have a high agreement with the both planting distance (RR and PP). To the distance plant by plant C2 variant the plants height varied significantly, the distribution of the data was not symmetric. Also, at the distance B1C3 the data distribution is positively skewed, the median was very low, but in the second year the median was in the middle and the value symmetric. The same situation was in the third year, with the data closer and the median higher (112 units) (Figure 2).

The graph in the second year reveals that data distribution is higher than in the other 2 yrs. This is supported by the numerical measures. The median varied from 72 units to B1C1 till 96 units to B3C3. The variability is very tight within plots but significantly higher in the distance between rows. In the third year all the medians were above the values of 100 units. The plants height at the distance B1C1 show high variability, the median being between 100 and 116 units. Also, at the distance B3C3 the variability was high, from 100 to 119 units. It should be noted that as the planting distance increased, so did the height of the plants. We therefore conclude that in general, the plants height is influenced by the climatic conditions.

All of the variants from each RR distance provide similar results independently of the experimental year. The highest plant height was recorded in the first year at 70 cm RR. The 50 cm RR distance shows its intermediary character, with maximum of 110 units. The 25 cm RR presents the lowest value, but all the data hover around the center values. In the second year the values reported to PP distance showed an increase of plant height in all the variants, at 70 cm being registered the highest value (125 units). In the third year at 70 cm RR the median was in the upper quartile for 15 and 25 cm PP and in the lower quartile for the continuous row. Large amplitude presents the plants height at 25 cm RR (85-104 units) while at 50 cm RR the score is tighter (98 units maximum value) for all the variants (Figure 3).



**Figure 2.** Analysis of plant height and experimental year interaction. Plants height analysis to Experimental year  $\times$  Distance plant by plant (PP) interaction. Factor A—experimental year: 2016 (a), 2017 (b) and 2018 (c). Factor B—distance RR: 25 (1), 50 (2) and 70 cm (3). Factor C—distance PP: continuous row (1), 15 (2) and 25 cm (3).

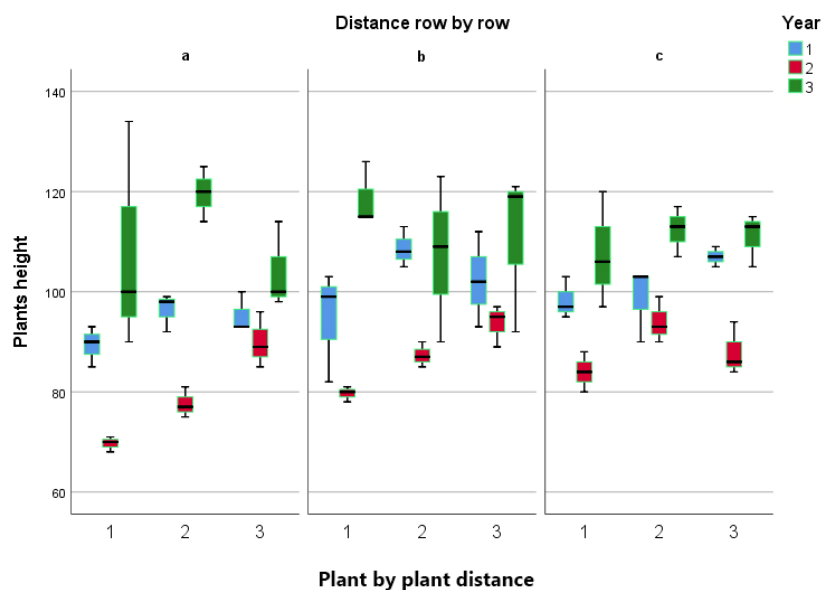


**Figure 3.** Analysis of plant height and plant by plant (PP) distance interaction grouped by experimental year. Plants height analysis to Distance row by row (RR)  $\times$  Experimental year interaction. Factor B—distance RR: 25 (a), 50 (b) and 70 cm (c). Factor A—experimental year: 2016 (1), 2017 (2) and 2018 (3). Factor C—distance PP: continuous row (1), 15 (2) and 25 cm (3).

The height of the plants in the first year was at a maximum level at B2C2 (116 units), the greater amplitude being recorded at the distances B2C1 and B2C3. B1C1 presented the lowest amplitude, with median to extreme values (96 units). In the second year, the plant height values were the lowest, their amplitude was also low, the highest values being recorded for the variants with a PP distance of 25 cm and a RR distance of 70 cm. In the third year, the maximum value was observed for the B1C1 variant (136 units), with a very large amplitude between variants and generally a median towards the extremes.



In summary, two foremost responses were observed: High variation of interquartile (median to extremes) and the length of the whiskers (Figure 4).



**Figure 4.** Analysis of plant height and row by row (RR) distance interaction grouped by experimental year. Plants height analysis to Distance row by row (RR) × Experimental year interaction. Factor C—distance plant by plant (PP): continuous row (a), 15 (b) and 25 cm (c). Factor A—experimental year: 2016 (1), 2017 (2) and 2018 (3). Factor B—distance RR: 25 (1), 50 (2) and 70 cm.

The performance of any crop is dependent on several environmental factors like temperature, humidity, rainfall, sunshine, and topographic condition of the cultivation area. Sowing time imposes a great role to obtain maximum yield. Therefore, it is important to find out the suitable date at which the sowing of senna seed is best for better yield, as well as the quality of this high-value medicinal crop (Nițu et al., 2017; Nilofer et al., 2018).

The technological requirements of *C. angustifolia* in temperate climate conditions have not yet been fully evaluated, the information present in the international literature being scarce. Due to the current climate changes, more information is needed to develop a sustainable technology for this plant.

In this study, in the first experimental year the temperature between April-August was 1.4 °C higher than the multiannual average, and additional rainfall of 29.7 mm. The second year had the approximately the same thermal value (16.3 °C), but the amount of rainfall was 122.1 mm lower than the average. The third experimental year had a temperature 2.7 °C higher than average and a supplementary amount of rainfall of 25.7 mm. These conditions explain the differences recorded between the experimental variants during the three years of experimentation. The small amount of rainfall in the second year influenced negatively the plants growth even if the temperatures were similar to those of the previous year. These results are contrary to the findings by Jnanesha et al. (2018) who reported that senna is drought tolerant crop thrive well in less water and require less management practices as compared to other crops in India.

The widest distance of 25 cm (C3) gave the highest plants and height number of branches compared to the other distance values of continuous row (C1) and 15 cm (C2), indicating that wider spacing could provide more favorable environment for branching due to improvement interception of sunlight by plants.

The biomass yield, both fresh and dry, is sensitive to PP distance. Continuous row at C1 (for PP) distance leads to potential fresh biomass of over 43.33 t ha<sup>-1</sup> and a dry biomass of 7.93 t ha<sup>-1</sup>. An increase in the RR distance and PP distance results in a decrease in both fresh and dry biomass yield of 37.17 t ha<sup>-1</sup>, respectively 6.67 t ha<sup>-1</sup> and significant biomass differences being observable.

## CONCLUSIONS

The studies and research carried out between 2016 and 2018 in Braşov, Romania, regarding the aspects of biology and technology for the introduction of the *Cassia angustifolia* into culture, showed that this species finds good climate and soil conditions for growth and development. Planting distances, row by row (RR) and plant by plant (PP), are very important factors for the growth and development of *C. angustifolia*, also the climatic conditions are very important for the development of the plants, obtaining larger plants in warmer years and with a greater volume of precipitation.

The maximum height being recorded at distance of 50 cm RR in the third experimental year. Plant height is not a stable character, it can be significantly influenced by the PP distance, showing differences induced by the combination of PP distance and experimental year. The branching potential showed significant differences, the highest values being in the first year for all variants. The number of branches is a more stable character, not being significantly influenced by the distance RR or PP, but by climatic conditions.

For biomass yield, the results showed that planting at distance of 25 cm (RR) × continuous row (PP) was most effective in improving fresh and dry biomass yield. Increasing the space between plants in a row leads to increased individual plant development, but reduces biomass yield.

### Author contribution

Conceptualization: E.C., S.N-N., D.B. Methodology: S.N-N., V.N., L.A.O. Software: S.N-N., V.A.B., V.N. Validation: E.C., S.N-N., D.B., I.S. Formal analysis: S.N-N., L.A.O., I.S. Investigation: S.N-N., V.N. Data curation: S.N-N., V.N. Writing-original draft preparation: E.C., S.N-N., D.B. Writing-review & editing: S.N-N., D.B., L.A.O., I.S. Visualization: V.A.B., V.S. Supervision: E.C., S.N-N., I.S. All co-authors reviewed the final version and approved the manuscript before submission.

### Acknowledgements

This research was funded by funds from the “Maintaining biodiversity in medicinal and aromatic plants by conserving and enriching the collection of genetic resources and producing seeds from higher biological categories for species representative of the hilly and mountainous areas”. Grant-ADER 2.4.1 granted by the Romanian Ministry of Agriculture and Rural Development.

### References

- Ahmed, S.I., Hayat, M.Q., Tahir, M., Mansoor, Q., Ismail, M., Keck, K., et al. 2016. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. BMC Complementary and Alternative Medicine 16(1):460. doi:10.1186/s12906-016-1443-z.
- Albrahim, J.S., Alosaimi, J.S., Altaher, A.M., Almulyfi, R.N., Alharbi, N.F. 2021. Employment of *Cassia angustifolia* leaf extract for zinc nanoparticles fabrication and their antibacterial and cytotoxicity. Saudi Journal of Biological Sciences 28(6):3303-3308.
- Al-Ghamdi, A.D., Zaheer, Z., Aazam, E.S. 2020. Sennoside A drug capped biogenic fabrication of silver nanoparticles and their antibacterial and antifungal activities. Saudi Pharmaceutical Journal 28(8):1035-1048.
- Betancur, M., Retamal-Salgado, J., López, M.D., Vergara-Retamales, R., Schoebitz, M. 2024. Planting density: Key strategy for optimizing soil health and fruit antioxidant activity in a calafate orchard. Chilean Journal of Agricultural Research 84:439-453.
- Boonhok, R., Sangkanu, S., Norouzi, R., Siyadatpanah, A., Mirzaei, F., Mitsuwani, W., et al. 2021. Amoebicidal activity of *Cassia angustifolia* extract and its effect on *Acanthamoeba triangularis* autophagy-related gene expression at the transcriptional level. Parasitology 148(9):1074-1082.
- Brousse, C., Gallé, J.B. 2017. Analysis of the results of the consumer survey put online by the Fédération des Paysans Herbalists. Final report. France AgriMer, Paris, France. <https://paysans-herboristes.org/wp-content/uploads/2019/10/Rapport-complet-de-lanalyse-de-lenquete-consommateurs-de-la-FPH.pdf>
- Hafez, S.A., Osman, S.M., Ibrahim, H.A., Seada, A.A., Ayoub, N.A. 2019. Chemical constituents and biological activities of *Cassia* genus: Review. Archives Pharmaceutical Sciences Ain Shams University 3(2):195-227.
- Jnanesha, A.C., Kumar, A., Vanitha, T.K., Verma, D.K. 2018. Opportunities and challenges in the cultivation of senna (*Cassia angustifolia* (Vahl.)). International Journal of Herbal Medicine 6(4):41-43.
- Kumar, A., Gupta, A.K., Siddiqui, S., Siddiqui, M.H., Jnanesha, A.C., Lal, R.K. 2022. An assessment, prospects, and obstacles of industrially important medicinal crop Indian Senna (*Cassia angustifolia* Vahl.): A review. Industrial Crops and Products 187(9):115472. doi:10.1016/j.indcrop.2022.115472.
- Lal, R.K., Chanotiya, C.S., Kumar, A. 2023. The prospects and potential of the horticultural and pharmacological medicinal herb senna (*Cassia angustifolia* Vahl.): A review. Technology in Horticulture 3:20. doi:10.48130/TH-2023-0020.

- Maury, G.L., Rodríguez, D.M., Hendrix, S., Arranz, J.C.E., Boix, Y.F., Pacheco, A.O., et al. 2020. Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants* 9(11):1048. doi:10.3390/antiox9111048.
- Moldovan, C., Nițu, S., Hermezi, M., Vidican, R., Sandor, M., Gadea, S., et al. 2022. Growth characteristics of *Dracocephalum moldavica* L. in relation to density for sustainable cropping technology development. *Agriculture* 12(6):789. doi:10.3390/agriculture12060789.
- Natarajan, S., Balachandar, D., Senthil, N., Paranidharan, V. 2022. Interaction of water activity and temperature on growth, gene expression, and aflatoxin B1 production in *Aspergillus flavus* on Indian senna (*Cassia angustifolia* Vahl.) *International Journal of Food Microbiology* 361:109457. doi:10.1016/j.ijfoodmicro.
- Nilofer, Singh, A.K., Singh, A., Singh, S. 2018. Impact of sowing and harvest times and irrigation regimes on the sennoside content of *Cassia angustifolia* Vahl. *Industrial Crops and Products* 125:482-490.
- Nițu, S., Hermezi, M., Chelmea, C. 2022. Study of some growth and reproductive elements in the *Cassia angustifolia* species. *Scientific Papers. Series A. Agronomy* 65(1):461-465.
- Nițu, S., Stefan, F.M., Chelmea, C.M., Hermezi, M. 2017. Preliminary aspects concerning the introduction into crop of the *Cassia angustifolia* Vahl. (Senna) species. *Journal of Horticulture, Forestry and Biotechnology* 21(1):98-104.
- Rama-Reddy, N.R., Mehta, R.H., Soni, P.H., Makasana, J., Gajbhiye, N.A., Ponnuchamy, M., et al. 2015. Next generation sequencing and transcriptome analysis predicts biosynthetic pathway of sennosides from Senna (*Cassia angustifolia* Vahl.), a non-model plant with potent laxative properties. *PLOS ONE* 10(6):e0129422. doi:10.1371/journal.pone.0129422.
- Ramchander, J.P., Jalwal, P., Middha, A. 2017. Recent advances on senna as a laxative: A comprehensive review. *Journal of Pharmacognosy and Phytochemistry* 6(2):349-353.
- Santhan, P. 2014. Leaf structural characteristics of important medicinal plants. *International Journal of Research in Ayurveda and Pharmacy* 5(6):673-679.
- Satti, N.M.E. 2020. Morphological, and some physiochemical properties of subtropical senna plant (*Cassia angustifolia* & *Cassia obtusifolia*) from Sudan. *International Journal of Botany Studies* 5(3):90-96.
- Savescu, P., Popescu, G. 2022. The ecological agricultural products-sources of raw materials for quality food products. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series* 52(1):334-339.
- Savulescu E., Georgescu, M., Popa, V., Luchian, V., 2018. Morphological and anatomical properties of the Senna Alexandrina Mill. (*Cassia angustifolia* Vahl.) *Agriculture for Life Life for Agriculture. Conference Proceedings* 1(1):305-310. doi:10.2478/alife2018-0045.
- Segneanu, A.E., Cegan, C., Grozescu, I., Cziple, F., Olariu, S., Ratiu, S., et al. 2019. Therapeutic use of some Romanian medicinal plants. In Perveen, S., Al-Taweel, A. (eds.) *Pharmacognosy - Medicinal plants*. IntechOpen. doi:10.5772/intechopen.82477.
- Singh, A.K., Singh, A., Singh, S. 2018. Impact of sowing and harvest times and irrigation regimes on the sennoside content of *Cassia angustifolia* Vahl. *Industrial Crops and Products* 125:482-490.
- Thaker, K., Patoliyaa, J., Rabadiyab, K., Rama-Reddy., N.R., Joshia, R. 2023. Senna (*Cassia angustifolia* Vahl.): A comprehensive review of ethnopharmacology and phytochemistry. *Pharmacological Research – Natural Products* 1:100003. doi:10.1016/j.prenap.2023.100003.
- Zibae, E., Javadi, B., Sobhani, Z., Akaberi, M., Farhadi, F., Amiri, M.S., et al. 2023. Cassia species: A review of traditional uses, phytochemistry and pharmacology. *Pharmacological Research - Modern Chinese Medicine* 9:100325. doi:10.1016/j.prmcm.2023.100325.