

Agronomic evaluation of *Cannabis sativa* (L.) cultivars in northern Colombia

Evaluación agronómica de cultivares de *Cannabis sativa* (L.) en el norte de Colombia



HERMES ARAMÉNDIZ-TATIS^{1, 3}
CARLOS CARDONA-AYALA¹
MIGUEL ESPITIA-CAMACHO¹
ANDREA HERRERA-CONTRERAS²
ALISSON VILLALBA-SOTO²

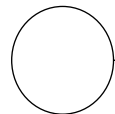
Cannabis plant.

Photo: A.C. Herrera-Contreras

ABSTRACT

Cannabis sativa (L.) is used to obtain fiber, seeds and phytocannabinoids for medicinal and recreational purposes. The commercial production of this species is limited by the lack of knowledge of the agronomic behavior and the content of phytocannabinoids, hence the need for evaluation of genetic diversity, for the selection of cultivars, in accordance with the legal provisions in force in Colombia. The objective of this work was to evaluate the agronomic characteristics and phytocannabinoid content of 10 cultivars, in Pueblo Bello-Cesar, northern Colombia. The study was conducted in 2022, under greenhouse conditions with polycarbonate cover and anti-aphid mesh. We evaluated 10 clones of territorial seed source, using cuttings of 13 cm in length, of female plants. The rooted cuttings were planted in 6 L bags, in a mesh house until harvest. The randomized complete block design was used, with 10 treatments and three repetitions. Each experimental unit consisted of 20 plants, with a density of 16 plants/m², both in the vegetative and reproductive phases, with distances between plants and rows of 14 cm. Genetic variability was estimated in both vegetative and reproductive characteristics and phytocannabinoid content. Three groups of genotypes were identified, according to the combinations of alleles coding for the phytocannabinoid content: high THC (tetrahydrocannabinol), similar THC-CBD ratio and high CBD (cannabidiol), which determines their potential use, mainly in medicine.

Additional keywords: genetic variability; seed source; medical cannabis; agronomic characteristics; biomass production; phytocannabinoids.



¹ Universidad de Córdoba, Montería (Colombia). ORCID Araméndiz-Tatis, H.: <https://orcid.org/0000-0002-2585-6273>; ORCID Cardona-Ayala, C.E.: <https://orcid.org/0000-0002-9607-3858>; ORCID Espitia-Camacho, M.: <https://orcid.org/0000-0001-7382-9643>

² Cultivate S.A.S. ZOMAC, Pueblo Bello (Colombia). Herrera-Contreras, A.C.: <https://orcid.org/0000-0003-0702-7065>; ORCID Villalba-Soto, A.L.: <https://orcid.org/0000-0003-0558-0801>

³ Corresponding author. haramendiz@correo.unicordoba.edu.co

RESUMEN

Cannabis sativa (L.) es utilizada para la obtención de fibra, semillas y fitocannabinoides con fines medicinales y recreativos. La producción comercial de esta especie está limitada por el desconocimiento del comportamiento agronómico y el contenido de fitocannabinoides, de allí que la necesidad de evaluación de la diversidad genética, para la selección de cultivares, de acuerdo con las disposiciones legales vigentes en Colombia. El objetivo de este trabajo fue evaluar las características agronómicas y el contenido de fitocannabinoides de 10 cultivares, en Pueblo Bello-Cesar, norte de Colombia. El estudio se realizó en 2022, bajo condiciones de invernadero con cubierta de policarbonato y malla antiáfidos. Se evaluaron 10 clones de fuente semillera territorial, utilizando esquejes de 13 cm de longitud, de plantas femeninas. Los esquejes enraizados se sembraron en bolsas de 6 L, en casa malla hasta su cosecha. Se utilizó el diseño de bloques completos aleatorizados, con 10 tratamientos y tres repeticiones. Cada unidad experimental estuvo conformada por 20 plantas, con una densidad de 16 plantas/m² tanto en la fase vegetativa como reproductiva, con distancias entre plantas e hileras de 14 cm. Se estimó variabilidad genética tanto en las características vegetativas y reproductivas como en el contenido de fitocannabinoides. Se identificaron tres grupos de genotipos, según las combinaciones de alelos codificantes del contenido de fitocannabinoides: alto THC (tetrahidrocannabinol), similar proporción THC-CBD y alto CBD (cannabidiol), lo que determina su uso potencial, principalmente en medicina.

Palabras clave adicionales: variabilidad genética; fuente semillera, cannabis medicinal, características agronómicas, producción de biomasa, fitocannabinoides.

Received: 01-03-2023 Accepted: 01-05-2023 Published: 09-05-2023

INTRODUCTION

Cannabis sativa (L.) is a species native to Eurasia where it was domesticated (Small, 2018); it is used to obtain fiber, seeds and phytocannabinoids for medicinal and recreational purposes (Busta *et al.*, 2022). In this sense, Ascrizzi *et al.* (2019), considered it with the greatest potential for use due to the cannabinoids present and also because of its low environmental impact, and its resilience to pests and diseases, making its organic production possible. This has allowed its distribution worldwide over the past two millennia, adapting to diverse environments and selecting diverse morphotypes in response to environmental conditions (Small, 2015; Naim-Feil *et al.*, 2021). After being stigmatized by the UN as dangerous in 1961 (Janatová *et al.*, 2018), this species has now been legalized in many countries due to legislative changes that have decriminalized its consumption and regulated the production of plant derivatives for therapeutic and, more restrictively, recreational purposes (Dufresnes *et al.*, 2017).

The Colombian state through different laws and decrees promulgated since 2016, have supported the policy of using cannabis as a plant species for medicinal purposes, under the fulfillment of parameters established in the said regulations and with this, has

ratified its willingness to place the medical cannabis sector in a priority and strategic way for the development of the country, within the framework of legality, entrepreneurship and equity. Since it is presumed that by 2024 the medical cannabis market will be worth 62.000 million dollars, of which Latin America will only take advantage of 9,1% (PINE, 2020); a lot of companies have become interested in venturing into this field and taking advantage of the market potential. However, the genetic improvement of this species was limited due to legal restrictions and today high-yield genotypes of phytocannabinoids and other secondary metabolites are required to enter the market.

The genus *Cannabis*, has a number of secondary metabolites, called cannabinoids, terpenoids, flavonoids, steroids, alkaloids, lignans, etc (Kojoma *et al.*, 2006), which are accumulated in the form of carboxylic acid in the glandular trichomes of female flowers (Potter, 2014) such as delta-9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), which by decarboxylation are transformed into tetrahydrocannabinol (THC) and cannabidiol (CBD) according to Welling *et al.* (2016). The most well-known and specific chemical group of secondary cannabis

metabolites includes cannabinoids, especially psychoactive Δ^9 -THC, for which Canada and the European Union countries have set a limit of 0.3 and 0.2%, respectively, and for their CBD modifier of 1.0% (Small, 2018). CBD is a non-psychoactive isomer with anti-inflammatory, anticonvulsant, analgesic, anticancer, and antioxidant properties (Hadener *et al.*, 2019).

Piluzza *et al.* (2013) evaluated the genetic diversity of domesticated and wild cultivars from different countries, through the molecular analysis of variance, reporting the existence of genetic divergences between the accessions in 74 and 26% within the accessions, and grouped the accessions into eight groups. Similarly, García-Tejero *et al.* (2020) evaluated five varieties under macro tunnel conditions, reporting significant differences in biomass production, cannabidiol (CBD) and cannabigerol (CBG) content.

Through decree 631 of 2018 (Minsalud, 2018), the Colombian government created the legal figure of 'seed source' and determined before December 31, 2018, the registration and legalization before the *Instituto Colombiano Agropecuario* (ICA), of the variety of seeds that already existed in the country, intended for the planting of cannabis. For this reason, it is essential to conduct agronomic evaluation tests of cultivars in the different subregions where it is intended for planting, to then register in the National Registry of Cultivars (*Registro Nacional de Cultivares*), and be able to produce and market.

The objective of this work was to carry out the agronomic and phytocannabinoid content evaluation of cultivars under the conditions of northern Colombia.

MATERIALS AND METHODS

Location

The study was conducted at the La Esperanza farm, located in the municipality of Pueblo Bello-Cesar, in the Caribbean natural region of Colombia, 10°41' N, 73°52' W and height of 1,044 m a.s.l. The evaluation of the genotypes was made under greenhouse conditions with a polycarbonate cover and anti-aphid mesh, average temperature of 22.8°C, minimum of 16.1°C and maximum of 33.6°C; average relative humidity of 72%, minimum of 53% and maximum of 84%.

Genotypes

We evaluated 10 clones (Tab. 1) obtained from pre-existing seed source in the Colombian territory. From the mother plants of each clone, the 13 cm cuttings were extracted, necessary for the establishment of a population of 60 for each clone.

Table 1. Origin and name of the genotypes used in the research.

Genotype	Origin	Name	Species
1	Magdalena	Mountain tradition	<i>C. sativa</i>
2	Magdalena	Old culture	<i>C. sativa</i>
3	Cundinamarca	Cundi Gold	<i>C. sativa</i>
4	Magdalena	Blondie Grl	<i>C. sativa</i>
5	Cauca	River cosmic1	<i>C. sativa</i>
6	Cundinamarca	Ice Nilo	<i>C. sativa</i>
7	Antioquia	High Paisa	<i>C. sativa</i>
8	Cauca	Timbiquí Skunk	<i>C. sativa</i>
9	Magdalena	Algarrobo CBD	<i>C. sativa</i>
10	Atlántico	No High	<i>C. sativa</i>

Experimental design

The randomized complete block design was used, with 10 treatments and three repetitions. Each experimental unit consisted of 20 plants per plot, with a density of 16 plants/m² in the vegetative and reproductive phases, with distances between plants and rows of 14 cm.

The vegetative response variables considered in the study corresponded to: Number of leaflets (NL), height of plant in female flowering (FFPH), length of internodes of the main stem (ILMS), length of the petiole (LP), length of the central leaflet (CLL), width of the central leaflet (WCL), number of stems per plant (NSPP); the reproductive variables correspond to: Days to female flowering (DFL); days to harvest (DH); harvested stem height (HHS); weight of 1,000 seeds (W100S) and Yield of dry flowers (DFY). For the determination of tetrahydrocannabinol (THC) and cannabidiol (CBD), representative samples of one gram of flower were taken from each experimental unit. The data for the analysis of variance and Tukey's mean test for the response variables were performed using the GLM procedure of SAS version 9.2 (SAS, 2008).

Management of cuttings

The cuttings were planted in rooting trays of 72 alveoli each, properly labeled and separated by clone, treated with root-stimulating hormones, nitrogen, phosphorus, potassium, and calcium, under permanent lighting of 24 h for a period of 15 d.

Once rooted, the cuttings were transferred to the home mesh and transplanted into 6 L geotextile bags, until harvest. A substrate composed of perlite, coconut fiber, peat, composite forest material, pumice, worm casting, bat guano, soybean meal, alfalfa meal, fishbone meal, seaweed meal, green sand and beneficial mycorrhizal fungi was used: *Funneliformis mosseae*, *Rhizophagus intraradices* and *Septoglomus deserticola*.

Vegetative phase: An integrated management of irrigation and fertilizers, and of pests and diseases was implemented, with a photoperiod of 18 light hours, of which 12 h were of natural light and six of 200 W reflectors. The radiation intensity control was performed using 80% silver polyshade. Irrigation was done with self-compensating drippers of 4 L h⁻¹, 1.5 L/plant, with contribution of nitrogen, phosphorus, potassium, sulfur, magnesium, boron, copper, manganese, zinc, and calcium.

Flowering phase: In this phase the photoperiod was 12:12 (light:dark), the control of radiation intensity, phytosanitary management, irrigation (2 L/plant) and fertilization were adjusted to the need of the plant. Harvesting was done by cutting one (1) inch from the neck of each plant; the total biomass of flowers was determined, and the relationships between variables were estimated. Subsequently, the proper drying and obtaining of the samples for cannabinoid analysis was carried out.

Obtaining phytocannabinoids

To obtain the THC and CBD contents, a sample of 10 plants for each experimental unit/clone were air-dried under dark conditions to a moisture content of 12%. Subsequently, they were ground to obtain a sample of 0.3 g for each experimental unit, and analyzed by gas chromatography according to the methodology of Poniatowska *et al.* (2022).

RESULTS AND DISCUSSION

The mean squares of the analysis of variance recorded in tables 2 and 3 highlight significant differences ($P < 0.01$) for all the characteristics evaluated, similar results were reported by Piluzza *et al.* (2013) and Baldini *et al.* (2020), in Italy. The statistical significance of the vegetative and reproductive characteristics, show the genetic variation of the cultivars under study, whose coefficients of variation ranged between 0.78% for W100S and 14.79% in DFY, being lower than those reported by Petit *et al.* (2020). This indicates that the dispersion of the data of each variable around the mean was reduced and reflects a good precision and experimental reliability in the estimation of the genetic differences between the cultivars.

The highest NL was observed in the Blondie Gr1 genotype, without significant statistical differences with respect to 'No High', 'Algarrobo CBD' and 'River cosmic1'. The CLL did not vary significantly in seven of the 10 genotypes. However, the Blondie Gr1 genotype presented longer CLL, without statistically differentiating from 'Timbiquí Skunk' and 'River cosmic 1'. This indicates that this characteristic does not vary much between the genotypes studied. On the other hand, WCL and LP, were highly variable in the 10 genotypes, with the cultivars Blondie Gr1, River cosmic1, Ice Nilo and Timbiquí Skunk having the highest WCL. Regarding LP, the highest were observed in 'Timbiquí Skunk', 'Blondie Gr1' and 'River cosmic1'. Both LP, CLL and WCL are allometric measures indicating that the leaf surface captures light for photosynthesis and therefore 'Timbiquí Skunk', 'Blondie Gr1' and 'River cosmic1' have larger leaves, while 'Algarrobo CBD', 'No High', 'High Paisa', 'Mountain tradition', 'Old culture' and 'Cundi Gold' present smaller leaves, with lower magnitudes of LP, CLL and WCL (Tab. 2).

The highest FFPH was observed in the Blondie Gr1, River cosmic1, No High and Algarrobo CBD genotypes, while those with the lowest height were 'Old culture', 'Mountain tradition' and 'Cundi Gold'; the highest NSPP was expressed in the High Paisa, No High, Mountain tradition, Algarrobo CBD, Cundi Gold and Old culture genotypes, while the lowest NSPP was expressed in the Blondie Gr1 cultivar (Tab. 2). The FFPH records in the present study were lower

Table 2. Mean squares (CM) of the analysis of variance and means of vegetative characteristics of 10 cultivars of *Cannabis sativa*.

FV	CM						
	NF	FFPH (cm)	ILMS (cm)	LP (cm)	CLL (cm)	WCL (cm)	NSPP
Blocks	0.15	1051.71	0.17	3.39	8.35	0.15	9.57
Genotypes	1.69 **	503.28**	1.60**	8.01**	14.45**	0.83**	17.03**
Error	0.097	53.78	0.064	0.55	1.44	0.049	1.75
CV (%)	7.85	5.26	5.6	11.67	9.03	7.45	9.74
R ²	0.89	0.87	0.92	0.88	0.84	0.89	0.84
Genotype	Mean						
Mountain tradition	3.26 d	127.7 dc	3.74 dc	5.13 de	11.48 b	2.39 c	15.43 ab
Old culture	3.51 cd	124.2 d	3.53 d	5.41 cde	10.95 b	2.50 c	14.10 abc
Cundi gold	3.93 bcd	127.7 dc	4.08 dc	6.12 cde	11.31 b	2.83 bc	14.11 abc
Blondie grl	5.22 a	160.0 a	5.50 a	8.41 ab	17.21 a	3.84 a	9.43 d
River cosmic1	4.99 a	153.6 ab	5.06 ab	7.38 abc	14.61 ab	3.42 ab	10.37 cd
Ice nilo	3.45 c	124.4 dc	5.24 a	7.07 bcd	12.65 b	3.44 ab	12.61 bcd
High paisa	3.04 d	138.8 bdc	3.89 dc	5.17 de	12.23 b	2.75 c	17.21 a
Timbiqui skunk	3.52 cd	142.3 abcd	5.40 a	9.42 a	16.94 a	3.46 ab	12.51 bcd
Algarrobo CBD	4.43 ab	147.5 abc	4.30 c	4.69 e	11.95 b	2.45 c	14.29 ab
No high	4.39 abc	148.2 abc	4.33 bc	4.88 e	12.55 b	2.56 c	15.54 ab
Media general	3.97	139.35	4.51	6.37	13.29	2.96	13.56

** : $P < 0.01$; * : $P < 0.05$; means with the same letters do not differ significantly, according to Tukey's 5% test; NL: number of leaflets; FFPH: female flowering plant height; ILMS: internode length of the main stem; LP, length of the petiole; CLL, central leaflet length; WCL: width of the central leaflet; NSPP: number of stems per plant.

Table 3. Mean squares of the analysis of variance and means of reproductive characteristics and phytocannabinoids of 10 cultivars from *Cannabis sativa*.

FV	CM						
	DFL (d)	DH (d)	HHS (cm)	W100S (g)	DFY (g)	CBD	THC
Blocks	8.15	3.43	1138.69	0.0008	1729.54	0.158	0.042
Genotype	15.54**	17.51**	571.73**	0.67**	4763.48*	103.570**	158.623**
Error	2.56	3.14	60.5	0.001	1896.92	0.154	0.925
CV (%)	3.17	2.21	5.88	0.78	14.79	7.91	10.6
R ²	0.77	0.74	0.86	0.97	0.58	0.88	0.98
Genotype	Mean						
Mountain tradition	49.27 ab	76.67 c	122.00 c	12.08 d	260.77 a	6.71 b	2.58 cd
Old culture	50.43 ab	77.00 bc	117.23 c	12.10 d	265.27 a	7.06 b	3.38 cd
Cundi gold	51.83 a	77.33 bc	119.37 c	13.00 b	251.83 a	0.04 c	13.59 b
Blondie grl	52.07 a	81.67 abc	155.78 a	13.50 a	319.15 a	0.04 c	16.10 ab
River cosmic1	51.83 a	80.00 abc	148.37 ab	13.08 b	308.39 a	0.04 c	15.88 b
Ice nilo	46.23 b	82.00 ab	119.07 c	13.02 b	341.10 a	0.05 c	18.82 a
High paisa	50.90 ab	79.00 abc	129.30 bc	12.50 c	251.08 a	6.95 b	4.69 c
Timbiqui skunk	46.93 b	80.33 abc	133.30 abc	13.00 b	263.16 a	0.04 c	14.42 b
Algarrobo CBD	53.00 a	86.67 a	139.03 abc	12.30 cd	336.67 a	14.79 a	0.65 d
No high	51.83 a	82.00 ab	139.70 abc	12.80 b	347.98 a	13.99 a	0.62 d
Media general	50.43	79.97	132.32	12.74	294.54	4.97	9.07

** : $P < 0.01$; * : $P < 0.05$; means with the same letters do not differ significantly, according to Tukey's 5% test; DFL: days to female flowering; DH: days to harvest; HHS: height of harvested stem; W100S: weight of a thousand seeds; DFY: dried flower yield; CBD: cannabidiol; THC: tetrahydrocannabinol.

than those of Schumann *et al.* (1999), who reported 173-254 cm. However, short plants have an agronomic advantage, since, having a shorter internode length, they allow a higher population density and with it a greater number of productive branches in a smaller area (Sarkar *et al.*, 2017; Small, 2018). On the other hand, the High Paisa and Mountain tradition genotypes not only presented higher NSPP, but also lower ILMS (Tab. 2), so the size of the mother plant and the architecture of the stem are determinants of the cloning rate by cuttings. In this regard, the effects of light on the size of the internodes and the number of meristems has been studied, with results showing that under far-red LED lighting the length of internodes increases, while metal halide light statistically reduces their length. However, the greater length of internodes achieved with far-red LED light was industrially insignificant (Campbell *et al.*, 2019).

For their part, Bevan *et al.* (2021), maintained that the yield of the inflorescence is related to the fresh weight of the aerial part, the growth rate of the plant and the dry weight of the root, so a taller plant would have higher NF, CLL and WCL and consequently higher inflorescence yield and cannabinoid production according to Bernstein *et al.* (2019); while Trancoso *et al.* (2022), expressed that there is not always a connection with a greater amount of phytocannabinoids.

The DFL ranged between 46.23 and 53.00 (Tab. 3), which indicates that the genotypes evaluated are much earlier than those recorded by Baldini *et al.* (2020), who in Italy reported between 97 and 107

d. The earliest genotypes in bloom are 'Ice Nilo' and 'Timbiquí Skunk', and the later are 'Algarrobo CBD', 'Blondie Gr1', 'River cosmic1', 'Cundi Gold' and 'No High' and this agrees with the positive and significant correlation between HHS with respect to DFL (Tab. 3), as previously reported by Faux *et al.* (2013), who also noted that this depends on the genetics of the cultivar, as well as variations in temperature and photoperiod.

The genotypes that needed more DH were 'Algarrobo CBD', 'No High' and 'Ice Nilo', while the earliest to harvest were 'Mountain tradition', 'Old culture' and 'Cundi Gold'. It was observed that the DFL do not correlate with the DH (Tab. 3), that is, the plants that flower first are not necessarily also harvested first, because although these two characteristics correlate in genotypes such as 'Algarrobo CBD', 'Mountain tradition' and 'No High'; in others like 'Ice Nilo' that flowered early, their harvest was later than expected. According to Faux *et al.* (2013) the duration from sowing to full flowering correlated better with stem and seed yield.

The highest HHS and W100S were observed in the Blondie Gr1 genotype (Tab. 3). Likewise, these variables presented positive and significant correlation with NF, FFPH, ILMS, CLL and DH (Tab. 4); in turn, DFY correlated positively with HHS, NL, FFPH and DH. This suggests that the genotypes that present female flowering and greater height at harvest are more yielding in flower biomass (Tab. 2 and 3), so these characteristics must be taken into consideration for

Table 4. Phenotypic correlations between the vegetative and reproductive characteristics of *Cannabis sativa* L.

Caract	NF	FFPH	ILMS	LP	CLL	WCL	NSPP	DFL	DH	HHS	W100S	DFY
NF	1	0.56**	0.46*	0.29 ^{ns}	0.47**	0.39*	-0.51**	0.47**	0.31 ^{ns}	0.59**	0.53**	0.39*
FFPH		1	0.49**	0.15 ^{ns}	0.38*	0.25 ^{ns}	-0.21 ^{ns}	0.49**	0.47**	0.98**	0.39*	0.38*
ILMS			1	0.71**	0.67**	0.77**	-0.62**	-0.19 ^{ns}	0.51**	0.49**	0.82**	0.33 ^{ns}
LP				1	0.88**	0.87**	-0.58**	-0.39*	0.13 ^{ns}	0.17 ^{ns}	0.67**	-0.04 ^{ns}
CLL					1	0.83**	-0.52**	-0.17 ^{ns}	0.22 ^{ns}	0.40*	0.60**	0.04 ^{ns}
WCL						1	-0.69**	-0.28 ^{ns}	0.27 ^{ns}	0.29 ^{ns}	0.81**	0.09 ^{ns}
NSPP							1	0.01 ^{ns}	-0.28 ^{ns}	-0.23 ^{ns}	-0.58**	-0.04 ^{ns}
DFL								1	0.10 ^{ns}	0.45*	-0.05 ^{ns}	-0.01 ^{ns}
DH									1	0.45*	0.34 ^{ns}	0.66**
HHS										1	0.41*	0.45*
W100S											1	0.25 ^{ns}
DFY												1

Caract: characteristic; NF: number of leaflets; FFPH: plant height in female flowering; ILMS: stem internode length major; LP: petiole length; CLL: length of the central leaflet; WCL: width of the central leaflet; NSPP: number of stems per plant; DFL: days to female flowering; DH: days to harvest; HHS: height of the harvested stem; W100S: thousand seed weight; DFY: dried flowers yield. **: $P < 0.01$; *: $P < 0.05$; ^{ns}: not significant.

the selection of cultivars with higher yields of dry flowers and heavier seeds.

On the other hand, the analysis of variance for DFY showed significant differences ($P=0.0465$) between cultivars; however, Tukey's post hoc test did not estimate such differences because it is a conservative test, with a greater requirement to establish a significant difference between two means, which occurs when the p-value is very close to the type I error ($\alpha=0.05$). However, the No High, Ice Nilo and Algarrobo CBD genotypes presented a difference of 103.7 g m^{-2} (35.2%) with respect to the rest. DFY varied between 251.8 g m^{-2} in High Paise and 347.9 g m^{-2} in No High (Tab. 3).

The differences in DFY performance are supported by Vanhove *et al.* (2011), on three factors: light intensity, population density and cultivar genetics. In this study, the short plants were not the most efficient in flower production, but rather the tallest, as they produced greater flower biomass, due to a better use of light (photosynthesis) related to allometric characteristics such as NL, CLL and WCL.

Phytocannabinoid content

The analysis of variance with the mean squares of the sources of variation of the model, corresponding to the cannabinoids CBD and THC, in their basic form, and their mean values, are recorded in table 3. It was observed that the genotypes evaluated showed highly significant differences between them ($P<0.01$). Differences between cultivars have also been reported in works carried out by Glivar *et al.* (2020) and Busta *et al.* (2022), emphasizing that this is due to genetic differences, as a result of the differential expression of the genes responsible for the biosynthesis of cannabinoids, with uniformity within each clone. However, there is less variation in the CBD determinations, given its lower coefficient of variation.

With regard to the accumulation of tetrahydrocannabinol (THC) and cannabidiol (CBD), the enzymes THCA synthetase (THCAS) and CBDA synthetase (CBDAS) are necessary for the production of the acid forms THCA and CBDA, which are synthesized from cannabigerolic acid (Yamamuro *et al.*, 2021), accumulate in the trichomes of the inflorescence and, when subjected to intense heat, undergo decarboxylation leading to the formation of CBD and THC (Welling *et al.*, 2016; Wróbel *et al.*, 2018; Cascini *et al.*, 2019).

According to the results, three groups are evident according to De Meijer *et al.* (2003) and Staginnus *et al.* (2014), the first, is made up of genotypes with the highest CBD content, which according to Cascini *et al.* (2019), correspond to the homozygous genetic combination $B_{(D)}/B_{(D)}$ represented by the cultivars Algarrobo CBD and No High, this result is consistent with that reported by Vanhove *et al.* (2011).

The second group is represented by the heterozygous genotype of the codominant alleles $B_{(T)}/B_{(D)}$, which includes the genotypes Old culture, High Paise and Mountain tradition, with an approximate ratio 1:1 in the accumulation of THC and CBD, which can be variable, and is related to the efficiency in the transformation of CBGA by THCA and CBDA synthetase (De Meijer *et al.*, 2003). The third group is represented by the homozygous $B_{(T)}/B_{(T)}$ genotype, which groups five genotypes with the highest THC and lowest CBD levels, similar results were reported by Mark *et al.* (2009); and correspond to 'Cundi Gold', 'Blondie Grl', 'River cosmic1', 'Timbiquí Skunk' and 'Ice Nilo', which explains the genetic differences between them and their potential use for medicinal purposes, since CBD is believed to have a therapeutic effect on addiction to cocaine, opioids and psychostimulants, as well as on Alzheimer's (Evren and Umut, 2019), while THC is mainly used for recreational purposes (Cascini *et al.*, 2019).

The precise characterization of the accumulation of CBD and THC in the cultivars is very important, since it allows discriminating the potential use to satisfy the needs of identifying parents in genetic improvement programs such as the pharmaceutical industry for the manufacture of cannabinoid-based products.

Considering the results obtained and given the importance of this species for both the primary and industrial sectors, this study allows for the selection of cultivars that respond to the agroecological conditions, needs, and demands of producers and the cannabis agroindustry in the country, which would promote the development and competitiveness of the cannabis agribusiness (Minsalud, 2018; PINE, 2020).

CONCLUSIONS

Genetic variability was identified both in the agronomic characteristics and in the cannabinoid content. Three groups of genotypes were identified based on

the combinations of alleles coding for phytocannabinoid content: high THC, similar THC-CBD ratio and high CBD, which determines their potential use, mainly in medicine.

BIBLIOGRAPHIC REFERENCES

- Ascrizzi, R., L. Ceccarini, S. Tavarini, G. Flamini, and L. Angelini. 2019. Valorisation of hemp inflorescence after seed harvest: Cultivation site and harvest time influence agronomic characteristics and essential oil yield and composition. *Ind. Crops Prod.* 139, 111541. Doi: <https://doi.org/10.1016/j.indcrop.2019.111541>
- Baldini, M., C. Ferfuaia, F. Zuliani, and F. Danuso. 2020. Suitability assessment of different hemp (*Cannabis sativa* L.) varieties to the cultivation environment. *Ind. Crops Prod.* 143, 111860. Doi: <https://doi.org/10.1016/j.indcrop.2019.111860>
- Bernstein, N., J. Garelick, and S. Koch. 2019. Interplay between chemistry and morphology in medical cannabis (*Cannabis sativa* L.). *Ind. Crops Prod.* 129, 185-194. Doi: <https://doi.org/10.1016/j.indcrop.2018.11.039>
- Bevan, L., M. Jones, and Y. Zheng. 2021. Optimisation of nitrogen, phosphorus, and potassium for soilless production of *Cannabis sativa* in the flowering stage using response surface analysis. *Front. Plant Sci.* 12, 2587. Doi: <https://doi.org/10.3389/fpls.2021.764103>
- Busta, L., I. Dweikat, S.J. Sato, H. Qu, Y. Xue, B. Zhou, L. Gan, B. Yu, T.E. Clemente, E.B. Cahoon, and C. Zhang. 2022. Chemical and genetic variation in feral *Cannabis sativa* populations across the Nebraska climate gradient. *Phytochemistry* 200, 113206. Doi: <https://doi.org/10.1016/j.phytochem.2022.113206>
- Campbell, L.G., S.G.U. Naraine, and J. Dufresne. 2019. Phenotypic plasticity influences the success of clonal propagation in industrial pharmaceutical *Cannabis sativa*. *PLoS ONE* 14(3), e0213434. Doi: <https://doi.org/10.1371/journal.pone.0213434>
- Cascini, F., A. Farcomeni, D. Migliorini, L. Baldassarri, I. Boschi, S. Martello, S. Amaducci, L. Lucini, and J. Bernardi. 2019. Highly predictive genetic markers distinguish drug-type from fiber-type *Cannabis sativa* L. *Plants* 8(11), 496. Doi: <https://doi.org/10.3390/plants8110496>
- De Meijer, E.P.M., M. Bagatta, A. Carboni, P. Crucitti, V.M.C. Moliterni, P. Ranalli, and G. Mandolino. 2003. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics* 163(1), 335-346. Doi: <https://doi.org/10.1093/genetics/163.1.335>
- Dufresnes, C., C. Jan, F. Bienert, J. Goudet, and L. Fumagalli. 2017. Broad-scale genetic diversity of *Cannabis* for forensic applications. *PLoS ONE* 12(1), e0170522. Doi: <https://doi.org/10.1371/journal.pone.0170522>
- Evren, C. and G. Umut. 2019. The good face of *Cannabis sativa*: Cannabidiol. *Düşünen Adam.* 32(4), 283-288. Doi: <https://doi.org/10.14744/DAJPN.2019.00041>
- Faux, A.-M., X. Draye, R. Lambert, R. d'Andrimod, P. Raulier, and P. Bertin. 2013. The relationship of stem and seed yields to flowering phenology and sex expression in monoecious hemp (*Cannabis sativa* L.). *Eur. J. Agron.* 47(1), 11-22. Doi: <https://doi.org/10.1016/j.eja.2013.01.006>
- García-Tejero, I.F., A. Hernández, C. Ferreiro-Vera, V.H. Duran, J. Hidalgo, C. Sánchez-Carnerero, and S. Casano. 2020. Yield of new hemp varieties for medical purposes under semi-arid Mediterranean environment conditions. *Comun. Sci.* 11, e3264. Doi: <https://doi.org/10.14295/cs.v11i0.3264>
- Glivar, T., J. Eržen, S. Kreft, M. Zagožen, A. Čerenak, B. Čeh, and E.T. Benkovič. 2020. Cannabinoid content in industrial hemp (*Cannabis sativa* L.) varieties grown in Slovenia. *Ind. Crops Prod.* 145, 112082. Doi: <https://doi.org/10.1016/j.indcrop.2019.112082>
- Hadener, M., S. Köning, and W. Weinmann. 2019. Quantitative determination of CBD and THC and their acid precursors in confiscated cannabis samples by HPLC-DAD. *Forensic Sci. Int.* 299, 142-150. Doi: <https://doi.org/10.1016/j.forsciint.2019.03.046>
- Janatová, A., A. Fraňková, P. Tlustoš, K. Hamouz, M. Božik, and P. Klouček. 2018. Yield and cannabinoid contents in different cannabis (*Cannabis sativa* L.) genotypes for medical use. *Ind. Crops Prod.* 112, 363-367. Doi: <https://doi.org/10.1016/j.indcrop.2017.12.006>
- Kojoma, M., H. Seki, S. Yoshida, and T. Murunaka. 2006. DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fiber - type" *Cannabis sativa* L. *Forensic Sci. Int.* 159(2-3), 132-140. Doi: <https://doi.org/10.1016/j.forsciint.2005.07.005>
- Marks, M.D., L. Tian, J.P. Wenger, S.N. Omburo, W. Soto-Fuentes, J. He, D.R. Gang, G.D. Weiblen, and R.A. Dixon. 2009. Identification of candidate genes affecting Δ^9 -tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *J. Exp. Bot.* 60(13), 3715-3726. Doi: <https://doi.org/10.1093/jxb/erp210>
- Minsalud, Colombia Ministerio de Salud y Protección Social. 2018. Decreto 631, Por el cual se modifica el artículo 2.8.11; 11.1 y se adiciona el numeral 15 al artículo 2.8.11.9.1. del Decreto 780 de 2016. In: https://www.minsalud.gov.co/Normatividad_Nuevo/Decreto%20631%20de%202018.pdf; consulted: January, 2023.
- Naim-Feil, E., L.W. Pembleton, L.E. Spooner, A.L. Malthouse, A. Miner, M. Quinn, R. Polotnianka, R.M. Baillie, G.C. Spangenberg, and N.O.I. Cogan. 2021. The characterization of key physiological traits of medicinal cannabis (*Cannabis sativa* L.) as a tool for precision breeding. *BMC Plant Biol.* 21, 294. Doi: <https://doi.org/10.1186/s12870-021-03079-2>

- Petit, J., E.M.J. Salentijn, M.-J. Paulo, C. Thouminot, B.J. van Dinter, G. Magagnini, H.-J. Gusovius, K. Tang, S. Amaducci, S. Wang, B. Uhrlaub, J. Müssing, and L.M. Trindade. 2020. Genetic variability of morphological, flowering, and biomass quality traits in hemp (*Cannabis sativa* L.). *Front. Plant Sci.* 11, 102. Doi: <https://doi.org/10.3389/fpls.2020.00102>
- Piluzza, G., G. Delogu, A. Cabras, S. Marceddu, and S. Bullitta. 2013. Differentiation between fiber and drug types of hemp (*Cannabis sativa* L.) from a collection of wild and domesticated accessions. *Genet. Resour. Crop Evol.* 60, 2331-2342. Doi: <https://doi.org/10.1007/s10722-013-0001-5>
- PINE, Colombia Proyecto de Interés Nacional Estratégico. 2020. Industria del cannabis medicinal. In: <https://www.portafolio.co/economia/gobierno-declara-proyecto-de-interes-nacional-a-la-industria-del-cannabis-544916>; consulted: January, 2023.
- Poniatowska, J., K. Panasiewicz, M. Szalata, L. Zarina, S. Zute, and K. Wielgus. 2022. Variability of cannabinoid yields of fibre hemp cultivars depending on the sowing density and nitrogen fertilization. *Plant Soil Environ.* 68(11), 525-532. Doi: <https://doi.org/10.17221/223/2022-PSE>
- Potter, D.J. 2014. A review of the cultivation and processing of cannabis (*Cannabis sativa* L.) for production of prescription medicines in the UK. *Drug Test. Anal.* 6(1-2), 31-38. Doi: <https://doi.org/10.1002/dta.1531>
- Sarkar, S., J. Banerjee, and S. Gantait. 2017. Sex-oriented research on dioecious crops of Indian subcontinent: An updated review. *3 Biotech* 7, 93. Doi: <https://doi.org/10.1007/s13205-017-0723-8>
- Schumann, E., A. Peil, and W.E. Weber. 1999. Preliminary results of a German field trial with different hemp (*Cannabis sativa* L.) accessions. *Genet. Resour. Crop Evol.* 46, 399-407. Doi: <https://doi.org/10.1023/A:1008696018533>
- Small, E. 2015. Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. *Bot. Rev.* 81, 189-294. Doi: <https://doi.org/10.1007/s12229-015-9157-3>
- Small, E. 2018. Dwarf germplasm: The key to giant *Cannabis* hempseed and cannabinoid crops. *Genet. Resour. Crop Evol.* 65, 1071-1107. Doi: <https://doi.org/10.1007/s10722-017-0597-y>
- Staginnus, C., S. Zörntlein, and E. de Meijer. 2014. A PCR marker linked to a THCA synthase polymorphism is a reliable tool to discriminate potentially THC-rich plants of *Cannabis sativa* L. *J. Forensic Sci.* 59(4), 919-926. Doi: <https://doi.org/10.1111/1556-4029.12448>
- Trancoso, I., G.A.R. Souza, P.R. Santos, K.D. Santos, R.M.S.N. Miranda, A.L.P.M. Silva, D.Z. Santos, I.F. García-Tejero, and E. Campostrini. 2022. *Cannabis sativa* L.: Crop management and abiotic factors that affect phytocannabinoid production. *Agronomy* 12(7), 1492. Doi: <https://doi.org/10.3390/agronomy12071492>
- Vanhove, W., P. van Damme, and N. Meert. 2011. Factors determining yield and quality of illicit indoor cannabis (*Cannabis* spp.) production. *Forensic Sci. Int.* 212 (1-3), 158-163. Doi: <https://doi.org/10.1016/j.forsciint.2011.06.006>
- Welling, M.T., L. Liu, T. Shapter, C.A. Raymond, and G.J. King. 2016. Characterisation of cannabinoid composition in a diverse *Cannabis sativa* L. germplasm collection. *Euphytica* 208 (3), 463-475. Doi: <https://doi.org/10.1007/s10681-015-1585-y>
- Wróbel, T., M. Dreger, K. Wielgus, and R. Słomski. 2018. The application of plant in vitro cultures in cannabinoid production. *Biotechnol. Lett.* 40(3), 445-454. Doi: <https://doi.org/10.1007/s10529-017-2492-1>
- Yamamuro, T., H. Segawa, K. Kuwayama, K. Tsujikawa, T. Kanamori, and Y.T. Iwata. 2021. Rapid identification of drug-type and fiber-type cannabis by allele specific duplex PCR. *Forensic Sci. Int.* 318, 110634. Doi: <https://doi.org/10.1016/j.forsciint.2020.110634>