

Genetics parameters and path analysis in *Cannabis sativa* L.

Parámetros genéticos y análisis de sendero en *Cannabis sativa* L.



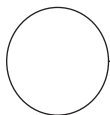
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Inflorescence of *C. sativa*.

Photo: I. Pastrana-Vargas

ABSTRACT

The ostracism to which the species was subjected in the last century generated a weak use of genetic variability in the genetic improvement of characteristics of interest. This study aimed to estimate genetic parameters, correlation, and path analysis for 13 agronomic traits, cannabidiol (CBD) and tetrahydrocannabinol (THC) content in 10 cannabis genotypes from different departments of Colombia. The study was conducted under greenhouse conditions with a polycarbonate cover and anti-aphid mesh at the La Esperanza farm in Pueblo Bello, Cesar (North Colombia). A randomized complete block design with 10 treatments (genotypes) and three replicates was used. Each experimental unit consisted of 20 plants obtained from mother plants and transplanted at 14 cm between rows and between plants. Significant differences ($P \leq 0.01$) were detected between genotypes for all traits and THC and CBD content. Heritability in a broad sense showed values higher than 82% for all the traits studied. Genetic variability between genotypes was detected for number of leaflets, internode length of main stem, length of petiole, central leaflet-length, width of central leaflet, number of stems per plant, CBD, and THC, which allowed obtaining genetic gains higher than 30%. There was a high, inverse, and significant phenotypic and genotypic correlation between the percentage of CBD and THC ($r = -0.93^{**}$). Overall, width of central leaflet direct and indirect effects explains the association level between CBD and THC with the correlated traits. It is possible to increase CBD and THC by selecting genotypes with higher width of central leaflet.



Additional keywords: cannabinoids; correlations; heritability; genetic gain; genetic variability.

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RESUMEN

El ostracismo a que fue sometida en el siglo pasado la especie, generó un débil aprovechamiento de la variabilidad genética en el mejoramiento genético de características de interés. El objetivo del estudio fue estimar los parámetros genéticos, correlación y análisis de sendero para 13 caracteres agronómicos, el contenido de cannabidiol (CBD) y tetrahidrocannabinol (THC) en 10 genotipos de cannabis procedentes de diferentes departamentos de Colombia. El estudio se realizó en invernadero con cubierta de policarbonato y malla antiáfido, en Pueblo Bello, Cesar (Colombia). Se utilizó un diseño de bloques completos al azar con 10 tratamientos (genotipos) y tres repeticiones. Cada unidad experimental estuvo conformada por 20 plantas obtenidas de plantas madre, y trasplantadas a 14 cm entre surcos y entre plantas. Se detectaron diferencias significativas ($P \leq 0,01$) entre genotipos para todas las características investigadas. La heredabilidad en sentido amplio acusó valores, superiores al 82% para todas las características. Se detectó variabilidad genética entre los genotipos para el número de folíolos, longitud de entrenudos del tallo principal, longitud del peciolo, longitud del foliolo central, anchura del foliolo central, número de tallos por planta, THC y CBD, lo cual permitió obtener ganancias genéticas superior al 30%. Existió correlación fenotípica y genotípica alta, inversa y significativa entre el porcentaje de CBD y THC ($r = -0,93^{**}$). En general, el efecto directo e indirecto a través de la anchura del foliolo central explican el nivel de asociación entre CBD y THC con los caracteres correlacionados. Es posible aumentar el CBD y THC, a través de la selección de genotipos con mayor ancho del foliolo central.

Palabras clave adicionales: cannabinoides; correlaciones; heredabilidad; ganancia genética; variabilidad genética.

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INTRODUCTION

Cannabis sativa L. is an annual species native to Asia, of great importance in the production of fiber, nutrition and health due to the presence of phytocannabinoids, the most relevant being cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA), which accumulate in the female inflorescences (Richins *et al.*, 2018), which when subjected to decarboxylation are transformed into CBD (cannabidiol) and THC (tetrahydrocannabinol) (Burgel *et al.*, 2020; Martínez *et al.*, 2020).

The changes in recent years in the global policy of the United Nations (UN), regarding the elimination of *C. sativa* from the list of highly dangerous species with low application in medicine (CND, 2020) and recognizing its medicinal properties. It allowed changes in legislation in many countries, generating expectations of cannabis cultivation and the creation of companies, due to its decriminalization of its consumption and regulation of the production of derivatives for therapeutic purposes (Dufresnes *et al.*, 2017), especially in medicine, given that THC acts as a psychoactive agent and has anti-inflammatory, appetite stimulant, anthelmintic and analgesic properties; while CBD regulates the euphoric effects of THC, is antipsychotic, anticancer and antidiabetic (Burgel

et al., 2020); these two secondary metabolites are of the greatest interest for agroindustrial exploitation in Colombia (Minsalud, 2018).

The estimation of genetic parameters is of great interest in plant breeding (Weldemichael *et al.*, 2017; Pessoa *et al.*, 2023) as it allows us to know genetic variability, enables the increase of quantitative characteristics through direct or indirect selection (Hallauer *et al.*, 2010). Therefore, knowledge of the CBD, THC content and the most important agronomic characteristics in cannabis require the highest priority (García-Tejero *et al.*, 2020) given the few studies reported in bibliographic databases about this species. Therefore, the objective of this research was to estimate the genetic parameters in agronomic traits and CBD and THC, to improve the selection criteria in the genetic improvement of the species.

MATERIALS AND METHODS

This study was conducted at the La Esperanza farm in the municipality of Pueblo Bello, Cesar, in the Caribbean natural region of Colombia, 10°41' N – 73°52' W and elevation of 1,044 m a.s.l. 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%.

A total of 10 genotypes from different departments of Colombia were evaluated: Magdalena (Mountain tradition, Old culture, Blondie Grl, Algarrobo CBD); Cundinamarca (Cundi Gold, Ice Nilo); Cauca (River cosmic1, Timbiquí Skunk); Antioquia (High Paisa) and Atlántico (No High).

The experimental design applied was Randomized Complete Blocks with 10 treatments and three replications. Each experimental unit consisted of 20 plants obtained from mother plants and transplanted at 14 cm between rows and between plants (Araméndiz-Tatis *et al.*, 2023).

The vegetative response variables considered in this study correspond to number of leaflets (NF), height of plant in female flowering (FFPH), length of internodes of the main stem (ILMS), length of the petiole (LP), central leaflet-length (CLL), width of central leaflet (WCL), number of stems per plant (NSPP). The reproductive traits correspond to days to female flowering (DFL), days to harvest (DH), harvested stem height (HHS) and dried flower yield (DYF).

For the determinations of tetrahydrocannabinol (THC) and cannabidiol (CBD), representative samples of 1 g of flower were taken from each experimental unit. Subsequently, a sample of 0.3 g for each experimental unit, and analyzed by gas chromatography according to the methodology of Poniatowska *et al.* (2022).

One-way analysis of variance and Tukey's mean comparison tests at 5% statistical probability, were conducted to estimate variation between cultivars. The following genetic parameters were estimated: phenotypic coefficient of variation (CV_p), genotypic coefficient of variation (CV_g), the variability index $CV_g/CV_e = (b)$, mean phenotypic variance between genotypes (σ_p^2), mean environmental variance between genotypes (σ_e^2), mean genetic variance between genotypes (σ_g^2), broad sense heritability (h^2_A), expected genetic gain (AG) and AG expressed as a percentage of the mean (AG%), for each of the response variables considered.

The statistical analyzes and genetic parameters will be carried out using the free access computer program GENES, Windows version (1990.2020.15), developed by Cruz (2020).

The broad sense heritability (h^2_A) for each variable was estimated in the classical way as described in the following formula: $h^2_A = (\sigma_g^2/\sigma_p^2) \times 100$, where: σ_g^2 represents genetic variance and σ_p^2 is the phenotypic variance. Genetic advance (GA) was estimated for each variable, according to: $AG = k\sigma_p h^2_A$ (Johnson *et al.*, 1955), where k = selection differential, it is a constant for a given selection intensity (2.06 at 5%), σ_p = phenotypic standard deviation, and h^2_A = heritability in the broad sense.

Genetic advancement was expressed as a percentage of the mean according to Robinson *et al.* (1949), classified as: low (<10%), moderate (10-20%) and high (>20%).

Estimates of the coefficients of phenotypic and genotypic correlations were made using the following equations (1 and 2):

$$rF_{(xy)} = \frac{COV_{(xy)}}{[\sigma^2_{(x)} \cdot \sigma^2_{(y)}]^{1/2}} \quad (1)$$

$$rF_{(xy)} = \frac{COV_{(xy)}}{[\sigma^2_{(x)} \cdot \sigma^2_{(y)}]^{1/2}} \quad (2)$$

where: $r_{(xy)}$ and $COV_{(xy)}$ are the phenotypic and genetic correlations and covariances between traits X and Y, respectively; $\sigma^2_{(x)}$ and $\sigma^2_{(y)}$ are the phenotypic and genetic variances of X and Y, respectively.

Path analyses were performed, with phenotypic correlations and genotypic correlations obtained for CBD and THC for their medicinal importance. Each of them served as an effect variable (Y) depending on the causal variables: ILMS (X1), LP (X2), WCL(X3), NSPP (X4), THC (X5), with the use of phenotypic and genotypic correlation matrices between these variables. In the path analysis, the direct effects (path coefficients P_i) were estimated from the phenotypic and genotypic correlation matrix, which decomposes and organizes it into the following matrix system:

$P = A^{-1}R$, where: A^{-1} is the inverse of the correlation matrix (between each of the cause variables), R is the

vector of correlation coefficients between the cause variables with the effect variable, and P is the path coefficients vector.

The path coefficient due to residual effects or other variables not considered in the study (h) is estimated by the equation (3):

$$h = [1 - (P_{1.r_{1y}}) - (P_{2.r_{2y}}) - (P_{3.r_{3y}}) - (P_{4.r_{4y}}) - (P_{5.r_{5y}})]^{1/2} \quad (3)$$

RESULTS AND DISCUSSION

The evaluated genotypes showed significant differences ($P \leq 0.01$) for vegetative and reproductive characteristics, THC and CBD content, except for days to harvest (DH). The genetic differences suggest the possibility of selecting at least one cultivar with better agronomic characteristics (Tab. 1). The presence of genetic variability is highly desired in genetic improvement programs since it allows significant genetic advances to be achieved in agronomic characteristics such as those associated with cannabinoid

content, results that are consistent with those reported by Richins *et al.* (2018) and one of the reasons is geographic origin given that climate and genetics influence its phenotypic response (Babaei and Ajdani, 2020; Tsaliki *et al.*, 2021).

The variance components (Tab. 2) highlight that the phenotypic variance (σ_p^2) was greater in magnitude than the genetic variance (σ_g^2) in all the characteristics evaluated. Similarly, the genetic variance was higher than the environmental variance (σ_e^2), results are consistent with those reported by Manggoel *et al.* (2012) and Weldemichael *et al.* (2017). Consequently, the little environmental influence due to their control allowed the detection of genetic differences between the genotypes and the clonal selection of cultivars with a higher percentage of CBD and/or THC.

According to Weldemichael *et al.* (2017), the phenotypic variation coefficients (CV_p) and genotypic variation coefficients (CV_g) are considered high if they are greater than 20%, intermediate 10-20%, and low if they are less than 10%. In this study LP, CBD

Table 1. Mean squares of the analysis of variance of vegetative, reproductive and cannabinoid characteristics of 19 *Cannabis sativa* cultivars.

SV	Vegetative						
	NF	FFPH (cm)	ILMS (cm)	LP (cm)	CLL (cm)	WCL (cm)	NSPP
Blocks	0.15	1051.7	0.17	3.39	8.35	0.15	9.57
Genotype	1.69**	503.2**	1.60**	8.01**	14.45**	0.83**	17.03**
Error	0.09	53.78	0.06	0.55	1.44	0.04	1.75
Mean	3.97	139.3	4.51	6.37	13.29	2.96	13.56
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
SV	Reproductive and cannabinoids						
	DFL (d)	DH (d)	HHS (cm)	DFY (g)	CBD (%)	THC (%)	
Blocks	8.15	3.43	1138.6	1279.5	0.158	0.042	
Genotype	15.54**	17.51**	571.7**	4763.4**	103.57**	158.62**	
Error	2.56	3.14	60.5	1896.9	0.15	0.92	
Mean	50.43	79.97	132.3	294.5	4.97	9.07	
CV (%)	3.17	2.21	5.8	14.7	7.91	10.6	
R^2	0.77	0.74	0.97	0.58	0.88	0.98	

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, DFY: dried flowers yield, CBD: cannabidiol, THC: tetrahydrocannabinol **: $P < 0.01$.

and THC presented CV_p greater than 24%; while NF, ILMS, CLL, WCL and NSSPP, recorded intermediate values (Tab. 2), corroborating the existence of genetic variability, which can be taken advantage of through clonal selection accompanied by a nitrogen fertilization program, as indicated by Poniatowska *et al.* (2022), since this allows greater photosynthetic efficiency and therefore greater accumulation of the phytocannabinoids CBD and THC, in female inflorescences.

The estimated heritability in the broad sense (Tab. 2) had values higher than 82.1%, for all variables, highlighting those of CBD and THC with values between 99.9 and 99.4%, respectively, so they are considered high (Weldemichael *et al.*, 2017), because the environmental effect was small in the expression of phenotypic values. Heritability estimates in the narrow sense are more relevant than heritability in the broad sense, for genetic progress in the genetic improvement of populations since the former allows the predominance of additive gene action in the characters and be efficient with individual selection (Manggoel *et al.*, 2012).

The most important expected genetic advance AG (Tab. 2) was achieved for the characteristics NF, ILMS, LP, CLL, WCL, NSPP, CBD and THC, with values greater than 30% and considered high according to Johnson *et al.* (1955).

The most important expected genetic advance AG (Tab. 2) was achieved for the characteristics NF, ILMS, LP, CLL, WCL, NSPP, CBD and THC, with values greater than 30% and considered high according to Johnson *et al.* (1955). The advances achieved for phytocannabinoids indicate that Algarrobo CBD and No high genopytes for their high CBD content and Cundi gold, Blondie grl, River cosmic, Ice nilo and Timbiki skunk genopytes for greater THC accumulation, can be used as female parents to improve the percentage of phytocannabinoids, which it depends on the biotic and abiotic effects of pollination and its content can be reduced by 75 and 60%, respectively, due to seed formation and the genetic composition of the pollinator chemotype, given that co-dominant alleles control the synthesis of THCA and CBDA, in this way the BD allele codes for CBDA synthetase and the BT allele for THCA synthase (Small, 2018); so recurrent selection would be a good strategy according to Feder *et al.* (2021) to improve populations by taking advantage of additive genetic effects (Campell *et al.*, 2020) or *in vitro* micropropagation as an alternative to overcome these limitations and manage to maintain the genetic identity and the desired phytochemical profile of the selected plant (Atehortua, 2018).

Genotypic correlations (r_G) were of greater magnitude than phenotypic correlations (r_P), which is consistent with the preponderance of genetic variation (Tab. 3) results are consistent with Hemavathy *et al.*

Table 2. Estimate of genetic parameters for 13 characteristics from 10 cultivars of *Cannabis sativa* L.

Parameters	NF	FFP (cm)	ILMS (cm)	DFL (d)	LP (cm)	CLL (cm)	WCL (cm)	NSPP	DH (d)	HHS (cm)	DFY (g)	CBD (%)	THC (%)
Mean	3.97	139.30	4.51	50.43	6.37	13.29	2.96	13.56	79.97	132.30	294.54	4.97	9.08
σ_p^2	0.56	167.70	0.53	5.17	2.66	4.81	0.27	5.67	5.83	177.24	14.79	34.52	52.87
σ_e^2	0.03	17.90	0.02	0.85	0.18	0.48	0.01	0.58	1.04	20.16	10.49	0.05	0.30
σ_g^2	0.53	149.80	0.51	4.32	2.48	4.33	0.26	5.09	4.79	157.07	0.71	34.47	52.56
CV_p	7.85	5.26	5.60	3.17	11.67	9.03	7.45	9.74	2.21	5.88	1587.83	7.91	10.60
CV_g	18.32	8.78	15.87	4.12	24.75	15.67	17.19	16.64	2.74	9.47	632.31	118.06	79.88
b	2.34	1.67	2.84	1.30	2.12	1.74	2.31	1.71	1.24	1.61	955.52	14.93	7.54
h_A^2	94.2	89.30	96.00	83.50	93.10	90.00	94.10	89.8	82.10	88.60	60.20	99.90	99.40
AG	1.5	23.80	1.40	3.90	3.10	4.10	1.00	4.4	4.10	24.30	49.40	12.10	14.90
AG (%)	36.7	17.10	32.00	7.80	49.20	30.60	34.40	32.5	5.10	18.40	16.80	243.20	164.00

NF: number of leaflets, FFP: 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, DFY: dried flowers yield, CBD: cannabidiol, THC: tetrahydrocannabinol, σ_p^2 , σ_e^2 , σ_g^2 : phenotypic, environmental and genetic variance, CV_p and CV_g : phenotypic and genetic variation coefficient, b: (CV_g/CV_p), h_A^2 : heritability in a broad sense, AG: genetic advance, AG (%): genetic advance in the percentage of the mean.

(2015) and may be due to the pleiotropic action of a gene or due to gene linkage.

The NF presented positive and significant phenotypic and genotypic correlations ($P < 0.01$) with FFPH, HHS and DFY, and significant positive genetic correlations ($P < 0.05$) with DFL, which indicates that a greater NF gives the plant a greater capacity for photosynthesis (Saloner and Bernstein, 2020) to benefit vegetative growth and the reproductive capacity of the plant to produce a greater number of female flowers. However, NF is reduced when the plant develops several stems, which is evident in the magnitude of the negative correlations with NSPP: -0.71^* to -0.81^{**} (Tab. 3).

On the other hand, FFPH showed positive and significant genotypic correlations ($P < 0.05$) with CLL and DH and positive and significant phenotypic correlations ($P < 0.01$) with CLL and HHS, which indicates that taller plants have greater CLL and later (DH). In this sense, it would be much more beneficial to select smaller and earlier flowering plants, in such a way that the increase in population density would compensate for the yield of female flowers according to Babaei and Ajdarian (2020).

Positive and significant phenotypic and genotypic correlations ($P < 0.01$) were found between ILMS with LP, CLL, WCL and THC and positive and significant genetic correlations ($P < 0.05$) were found

Table 3. Phenotypic and genetic correlations and level of significance between 13 variables in the evaluation of 10 genotypes of *Cannabis sativa*.

VAR	R	FFPH	ILMS	DFL	LP	CLL	WCL	DH	HHS	NSPP	DFY	CBD	THC
NF	rP	0.82**	0.49	0.61	0.26	0.47	0.43	0.50	0.85**	-0.71*	0.58	-0.05	0.24
	rG	0.87**	0.50	0.67*	0.26	0.48	0.45	0.62	0.91**	-0.80**	0.78**	-0.05	0.26
FFPH	rP		0.55	0.51	0.32	0.66*	0.44	0.60	0.99**	-0.49	0.46	0.07	0.10
	rG		0.56	0.57	0.38	0.75*	0.52	0.72*	1.00**	-0.59	0.57	0.08	0.11
ILMS	rP			-0.31	0.85**	0.83**	0.92**	0.60	0.58	-0.79**	0.46	-0.56	0.78**
	rG			-0.35	0.89**	0.88**	0.98**	0.68*	0.61	-0.89**	0.59	-0.58	0.80**
DFL	rP				-0.44	-0.17	-0.29	0.10	0.49	0.03	0.14	0.44	-0.40
	rG				-0.50	-0.20	-0.32	0.14	0.55	0.03	0.33	0.48	-0.44
LP	rP					0.89**	0.91**	0.16	0.36	-0.77**	-0.03	-0.80**	0.83**
	rG					0.90**	0.93**	0.15	0.43	-0.82**	0.01	-0.83**	0.86**
CLL	rP						0.84**	0.35	0.68*	-0.75*	0.13	-0.51	0.57
	rG						0.85**	0.41	0.79**	-0.81**	0.26	-0.54	0.60
WCL	rP							0.33	0.49	-0.84**	0.21	-0.78**	0.90**
	rG							0.36	0.59	-0.87**	0.35	-0.81**	0.93**
DH	rP								0.59	-0.30	0.86**	0.26	0.10
	rG								0.70*	-0.28	1.00**	0.30	0.11
HHS	rP									-0.57	0.48	0.01	0.16
	rG									-0.68*	0.55	0.01	0.17
NSPP	rP										-0.33	0.63*	-0.75*
	rG										-0.57	0.66*	-0.79**
DFY	rP											0.30	0.05
	rG											0.39	0.07
CBD	rP												-0.93**
	rG												-0.93**

VAR: variables; NF: number of leaflets; FFPH: plant height in female flowering; ILMS: stem internode length major; DFL: days to female flowering; LP: petiole length; CLL: length of the central leaflet; WCL: width of the central leaflet; DH: days to harvest; HHS: height of the harvested stem; NSPP: number of stems per plant; DFY: dried flowers yield; CBD: cannabidiol; THC: tetrahydrocannabinol **: $P < 0.01$; *: $P < 0.05$; rP and rG: phenotypic and genotypic correlations.

with DH, while negative and significant phenotypic and genotypic correlations ($P < 0.01$) were recorded with NSPP, this indicates that plants with longer internodes have greater advantages in attributes related to leaves and THC accumulation and a lower number of stems per plant, making it an important criterion in the genetic improvement of this species, when an increase of this phytocannabinoid is desired.

Positive and significant phenotypic and genotypic correlations ($P < 0.01$) were detected between LP with CLL, WCL and THC; while negative and significant phenotypic and genotypic correlations ($P < 0.01$) were recorded with NSPP and CBD, which is important to take into consideration when selecting plants with larger leaf area, because they favor the accumulation of THC and reduce the accumulation of CBD due to being associated with homozygous $B(\tau)/B(\tau)$ genotypes, which groups cultivars with higher levels of THC and lower levels of CBD, similar results were reported by Marks *et al.* (2009).

The phenotypic and genotypic correlations between CLL with respect to WCL and HHS were positive and significant ($P < 0.01$), indicating that taller plants at the time of harvest have longer and wider leaflets, which favors greater leaf area and capacity for photosynthesis. Meanwhile, the WCL showed negative and significant phenotypic and genotypic correlations ($P < 0.01$) with NSPP and CBD but positive correlations with THC, which corroborates genetic control, as stated by Marks *et al.* (2009).

The DH recorded significant positive phenotypic and genotypic correlations ($P < 0.01$) with DFY and positive genetic correlations having significance ($P < 0.05$) with HHS, so the height of the plant at the time of harvest is important, and it is possible to obtain more female flowers through the selection of short plants with branches, which favors the production of floral biomass.

The NSPP showed positive and significant phenotypic and genotypic correlations ($P < 0.05$) with respect to the percentage of CBD and the opposite with respect to the percentage of THC, these results are consistent with Bevan *et al.* (2021), therefore, a good indicator is the selection of plants with more branches, which favors the action of a higher concentration of CBDA synthetase (CBDAS), necessary for the production of the acid form CBDA, which is synthesized from cannabigerolic acid (Yamamuro *et al.*, 2021), accumulates in the trichomes of the inflorescences and, by decarboxylation, forms CBD (Cascini *et al.*, 2019).

The inflorescences have the highest concentration of cannabinoids, however, the higher yield of flowers is not always related to higher yields of secondary metabolite (Tab. 3), this situation demands great caution, given the important influence of the environment. There appears to be a poor relationship between inflorescence and phytocannabinoid yield, and these concentrations decrease as the yield of the plant's inflorescence increases, apparently due to a distribution effect in other parts of the plant as argued by Bevan *et al.* (2021); Naim-Feil *et al.* (2022) and Trancoso *et al.* (2022).

The phenotypic and genotypic correlation between the percentage of CBD and THC was high, inverse, and significant ($P < 0.01$), results agree with Vergara *et al.* (2021), who also maintain that the enzyme THCA synthetase (THCAS) is much better in its action on the precursor cannabigerolico acid than CBDA synthetase.

Path analysis (Tab. 4) showed the direct (diagonal in bold) and indirect (horizontal) effects of the phenotypic and genotypic correlations of CBD and THC, in relation to ILMS, LP, WCL, NSPP and THC, highlighting that these values were greater when considering CBD in relation to THC.

When considering the direct effects of the phenotypic and genotypic correlations of CBD (Tab. 4A and B) with the variables of interest, it can be seen that ILMS, LP and THC registered higher values of the direct genotypic effects with respect to the phenotypic ones, highlighting WCL, whose direct and indirect effects explain the level of association of the variables with CBD because they present the highest magnitudes, this suggests that pathway analysis with the use of genetic correlations is simpler and more reliable for CBD selection via WCL (Tab. 4B). Therefore, when the purpose is to improve the CBD content, the WCL characteristic is decisive in increasing the CBD content and equally, the selection of plants with less ILMS, LP and THC.

The direct effects on the phenotypic and genotypic correlations of THC (Tab 4C and D) with the variables under study highlight that at the phenotypic level the ILMS variable has a positive direct effect while for LP and CBD, its direct effect is negative. At the genotypic level, both LP and CBD exert a negative effect and, WCL, a high and positive direct effect. It can be detected again that WCL presents the most important direct and indirect effects that explain the

level of association of the variables with THC because it presents the highest values, this allows us to infer that path analysis with the use of genetic correlations, offers reliability for the selection of TCH through WCL. Based on the above, it can be deduced that the selection of plants and/or genotypes with higher WCL leads to the increase of TCH in a more agile and economical way due to the ease in measuring WCL compared to the other variables.

CONCLUSION

There is genetic variability between the genotypes studied in the agronomic characteristics number of leaflets, length of internodes of the main stem, length of the petiole, central leaflet length, width of central leaflet, number of stems per plant and in the phyto-cannabinoids CBD and THC, which allowed to obtain high genetic gains.

Table 4. Path analysis for CBD and THC with phenotypic and genetic correlations of *Cannabis sativa* L.

A) CBD with phenotypic correlations						
VAR	ILMS	LP	WCL	NSPP	THC	rP (CBD)
ILMS	0.77	-0.43	-0.20	0.02	-0.72	-0.56
LP	0.66	-0.51	-0.19	0.02	-0.77	-0.80
WCL	0.71	-0.46	-0.21	0.02	-0.83	-0.78
NSPP	-0.61	0.39	0.18	-0.02	0.70	0.63
THC	0.60	-0.42	-0.19	0.02	-0.93	-0.93
$R^2 = 0.98$ - Residual effect = 0.02						
B) CBD with genotypic correlations						rG (CBD)
ILMS	-1.16	-0.71	2.89	0.19	-1.79	-0.58
LP	-1.04	-0.79	2.75	0.18	-1.92	-0.83
WCL	-1.13	-0.74	2.96	0.19	-2.08	-0.81
NSPP	1.03	0.65	-2.56	-0.22	1.76	0.66
THC	-0.93	-0.68	2.75	0.17	-2.24	-0.93
$R^2 = 0.87$ - Residual effect = 0.13						
C) THC with phenotypic correlations						
VAR	ILMS	LP	WCL	NSPP	CBD	rP (THC)
ILMS	0.70	-0.40	-0.08	0.02	0.54	0.78
LP	0.60	-0.47	-0.08	0.02	0.77	0.83
WCL	0.65	-0.43	-0.09	0.02	0.75	0.90
NSPP	-0.56	0.37	0.07	-0.02	-0.61	-0.75
CBD	-0.39	0.38	0.07	-0.01	-0.96	-0.93
$R^2 = 0.98$ - Residual effect = 0.02						
D) THC with genotypic correlations						rG (THC)
ILMS	-0.12	-0.31	0.89	0.04	0.30	0.80
LP	-0.11	-0.35	0.85	0.04	0.43	0.86
WCL	-0.12	-0.33	0.91	0.04	0.42	0.93
NSPP	0.11	0.29	-0.79	-0.04	-0.35	-0.79
CBD	0.07	0.29	-0.74	-0.03	-0.52	-0.93
$R^2 = 0.97$ - Residual effect = 0.03						

VAR: variables; ILMS: stem internode length major; LP: petiole length; WCL: width of the central leaflet; NSPP: number of stems per plant; CBD: cannabidiol; THC: tetrahydrocannabinol; R^2 : determination coefficient.

There is a high, inverse, and significant phenotypic and genotypic correlation between the percentage of CBD and THC.

The width of central leaflet showed the most important direct and indirect genetic effects on the accumulation of CBD and THC, so it was suggested that the selection process be considered to increase cannabinoids.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

- Araméndiz-Tatis, H., C. Cardona-Ayala, M. Espitia-Camacho, A. Herrera-Contreras, and A. Villalba-Soto. 2023. Agronomic evaluation of *Cannabis sativa* (L.) cultivars in northern Colombia. *Rev. Colomb. Cienc. Hortic.* 17(1), e15695. Doi: <https://doi.org/10.17584/rcch.2023v17i1.15695>
- Atehortua, L. 2018. Nuevas fronteras científicas y tecnológicas para el mejoramiento y la producción de cannabis. *Canna World Cong.* 1(2), 41-50.
- Babaei, M. and L. Ajdanian. 2020. Screening of different Iranian ecotypes of cannabis under water deficit stress. *Sci. Hortic.* 260, 108904. Doi: <https://doi.org/10.1016/j.scienta.2019.108904>.
- 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, 764103. Doi: <https://doi.org/10.3389/fpls.2021.764103>
- Burgel, L., J. Hartung, A. Pflugfelder, and S. Graeff-Höninger. 2020. Impact of growth stage and biomass fractions on cannabinoid content and yield of different hemp (*Cannabis sativa* L.) genotypes. *Agronomy* 10(3), 372. Doi: <https://doi.org/10.3390/agronomy10030372>
- Campell, L.G., J. Dufresne, and S.A. Sabatino. 2020. Cannabinoid inheritance relies on complex genetic architecture. *Cannabis Cannabinoid Res.* 5(1), 105-106. Doi: <https://doi.org/10.1089/can.2018.0015>
- Cascini, F., A. Farcomeni, D. Migliorini, L. Lucini, L. Baldassarri, I. Boschi, S. Martello, S. Amaducci, 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>
- CND, Commission on Narcotic Drugs. 2020. Statements following the voting on the WHO scheduling recommendations on cannabis and cannabis-related substances. E/CN.7/2020/CRP.24. In: https://www.unodc.org/documents/commissions/CND/CND_Sessions/CND_63Reconvened/ECN72020_CRP24_V2007524.pdf; consulted: February, 2024.
- Cruz, C. 2020. Programa GENES V.1990.2020-15 - Aplicativo computacional em genética e estatística. In: Universidade Federal de Viçosa, <https://www.ufv.br/dbg/genes/genes.htm>; consulted: July, 2020.
- 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>
- Feder, C.L., O. Cohen, A. Shapira, I. Katzir, R. Peer, O. Guberman, S. Procaccia, P. Berman, M. Flaishman, and D. Meiri. 2021. Fertilization following pollination predominantly decreases phytocannabinoids accumulation and alters the accumulation of terpenoids in cannabis inflorescences. *Front. Plant Sci.* 12, 753847. Doi: <https://doi.org/10.3389/fpls.2021.753847>
- García-Tejero, I., A. Hernández, C. Ferreira-Vera, V.H. Duran Zuazo, 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>
- Hallauer, A.R., J.B. Miranda Filho, and M.B. Carena. 2010. Quantitative genetics in maize breeding. pp. 577-653. In: *Quantitative genetics in maize breeding. Handbook of plant breeding.* Vol. 6. Springer, New York, NY. Doi: https://doi.org/10.1007/978-1-4419-0766-0_12
- Hemavathy, A.T., N. Shunmugavalli, and G. Anand. 2015. Genetic variability, correlation and path co-efficient studies on yield and its components in mungbean [*Vigna radiata* (L.) Wilczek]. *Legume Res.* 38(4), 442-446. Doi: <https://doi.org/10.5958/0976-0571.2015.00050.8>
- Johnson, H.W., H.F. Robinson, and R.E. Comstock. 1955. Estimates of genetic and environmental variability in soybeans. *Agron J.* 47(7), 314-318. Doi: <https://doi.org/10.2134/agronj1955.00021962004700070009x>
- Manggoel, W.N., M.I. Uguru, O.N. Ndam, and M.A. Dasbak. 2012. Genetic variability, correlation, and path coefficient analysis of some yield components of ten cowpea [*Vigna unguiculata* (L.) Walp] accessions. *J. Plant Breed. Crop Sci.* 4(5), 80-86.
- 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>
- Martínez, V., A.I. De-Hond, A. Borelli, R. Capasso, M.D del Castillo, and R. Abalo. 2020. Cannabidiol and other non-psychoactive cannabinoids for prevention and treatment of gastrointestinal disorders: useful nutraceuticals? *Int. J. Mol. Sci.* 21(9), 3067. Doi: <https://doi.org/10.3390/ijms21093067>

- 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. Bogota.
- Naim-Feil, E., E.J. Breen, L.W. Pembleton, L.E. Spooner, G.C. Spangenberg, and N.O.I. Cogan. 2022. Empirical evaluation of inflorescences' morphological attributes for yield optimization of medicinal *Cannabis* cultivars. *Front. Plant Sci.* 13, 858519. Doi: <https://doi.org/10.3389/fpls.2022.858519>
- Pessoa, A.M.S., C.H.C. Bertini, E.B.L. Castro, L.M. Freitas, and L.B.R. Araújo. 2023. Genetic parameters and selection indices of cowpea genotypes for green grain production. *Rev. Caatinga* 36(2), 310-319. Doi: <http://doi.org/10.1590/1983-21252023v36n208rc>
- 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 fertilisation. *Plant Soil Environ.* 68(11), 525-532. Doi: <https://doi.org/10.17221/223/2022-PSE>
- Richins, R.D., L. Rodriguez-Uribe, K. Lowe, R. Ferral, and M.A. O'Connell. 2018 Accumulation of bioactive metabolites in cultivated medical *Cannabis*. *PLoS ONE* 13(7), e0201119. Doi: <https://doi.org/10.1371/journal.pone.0201119>
- Robinson, H., R.E. Comstock, and P.H. Harvey. 1949. Estimates of heritability and degree of dominance in corn. *Agron. J.* 41(8), 353-359. Doi: <https://doi.org/10.2134/agronj1949.00021962004100080005x>
- Saloner, A. and N. Bernstein. 2020. Response of medical cannabis (*Cannabis sativa* L.) to nitrogen supply under long photoperiod. *Front. Plant Sci.* 11, 572293. Doi: <https://doi.org/10.3389/fpls.2020.572293>
- 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>
- 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, 1492. Doi: <https://doi.org/10.3390/agronomy12071492>
- Tsaliki, E., A. Kalivas, Z. Jankauskiene, M. Irakli, C. Cook, I. Grigoriadis, I. Panoras, I. Vasilakoglou, and K. Dhima. 2021. Fibre and seed productivity of industrial hemp (*Cannabis sativa* L.) varieties under Mediterranean conditions. *Agronomy* 11(1), 171. Doi: <https://doi.org/10.3390/agronomy11010171>
- Vergara, D., C. Feathers, E.L. Huscher, B. Holmes, J.A. Haas, and N.C. Kane. 2021. Widely assumed phenotypic associations in *Cannabis sativa* lack a shared genetic basis. *PeerJ* 20(9), e10672. Doi: <https://doi.org/10.7717/peerj.10672>
- Weldemichael, G., S. Alamerew, and T. Kufa. 2017. Genetic variability, heritability, and genetic advance for quantitative traits in coffee (*Coffea arabica* L.) accessions in Ethiopia. *Afr. J. Agric. Res.* 12(21), 1824-1831. Doi: <https://doi.org/10.5897/AJAR2016.12059>
- 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>