

Morphological analysis of glandular trichomes in six *Cannabis sativa* L. cultivars collected from the Rif Mountains (Northern Morocco)

Ismail El Bakali^{1*}, Ilham Rahmouni², Abderrahman Moukhles³, Aboubakr Boutahar¹, Samir El Bakali^{4,5}, Mhammad Houssni¹, Soufian Chakkour⁶, Mohamed Kadiri¹, Abderrahmane Merzouki¹

¹Laboratory of Applied Botany, Department of Biology, Faculty of Sciences of Tetuan, Abdelmalek Essaâdi University, Mhannech II. 93002, Tetuan, Morocco

²National Institute of Agronomy Research (INRA), Rabat Regional Research Center, Plant Breeding Research Unit, Morocco

³Laboratory of Applied Organic Chemistry, Department of Chemistry, Faculty of Sciences of Tetuan, Abdelmalek Essaâdi University, M'hanech II. 93002, Tetuan, Morocco

⁴Marine Ecotoxicology Team, Department of chemistry, Faculty of Sciences of Tetuan, Abdelmalek Essaâdi University, Mhannech II. 93002, Tetuan, Morocco

⁵Laboratory of Biology, Ecology, and Health, Faculty of Sciences of Tetuan, Abdelmalek Essaadi University, Mhannech II. 93002, Tetuan, Morocco

⁶Laboratory of Botany, Biotechnology, and Plant Protection, Department of Biology, Faculty of Science, Ibn Tofail University, Kenitra, Morocco

*Corresponding author: elbakali.ismail-etu@uae.ac.ma

Submitted:
01/03/2025

Revised:
14/06/2025

Accepted:
11/07/2025

Abstract: *Cannabis sativa* L. is a multipurpose crop with growing interest due to its medicinal properties and industrial applications. Trichomes specialized epidermal structures categorized as glandular or non-glandular play a crucial role in the biosynthesis of pharmacologically active compounds such as cannabinoids, terpenes, and flavonoids. Despite the global attention to *C. sativa*, Moroccan cultivars remain underexplored. This study investigates trichome morphology and density in six cultivars grown under controlled greenhouse conditions: 'Beldiya', 'Mexicana', 'Khardala', 'Avocat', 'Critical Plus', and 'Industriel'. Bract samples were collected at maturity and analyzed using light microscopy. Thirty images per cultivar were examined to characterize trichome types and quantify their density. The results revealed significant inter-cultivar variation. 'Mexicana' exhibited the highest density of non-glandular trichomes (17%), while 'Industriel' showed the lowest (12%). Conversely, 'Khardala' and 'Industriel' recorded the highest proportions of glandular trichomes (88%). Principal component analysis effectively distinguished among cultivars based on trichome traits. Overall, both glandular and non-glandular trichomes were present across all cultivars, with 'Beldiya' and 'Industriel' showing lower trichome performance in terms of type and density. These findings highlight the morphological diversity among Moroccan cannabis cultivars and underscore the potential for further biochemical and agronomic characterization. 'Khardala' and 'Mexicana' show promising trichome traits and are recommended for further valorization, breeding, and phytochemical studies.

Keywords: Hemp cultivars; Glandular trichomes; Non-glandular trichome; Density; Cultivars discrimination.

Abbreviations: Hamp; Trichomes; Moroccan cultivars; Glandular and Non-glandular trichomes; Medicinal cannabis.

Introduction

Cannabis sativa L. (*C. sativa*) is one of the oldest domesticated plants in human history. It has probably been used for at least 10,000 years (Schultes et al., 1974; Merlin, 2003). Hemp is widely considered to be indigenous to Central Asia, where it is confined to an area stretching from Turkestan in the west, Pakistan in the east, China in the north and the Himalayas in the south (Wills, 1998). This plant was cultivated and selected for multiple uses. The fibre from its stem is used in the production of rope and clothing. The oil from its achene is used in food, as a source of energy and to make medicines. However, the use of the resin extracted from its glands as a psychoactive substance is by far the most sought-after activity (Schultes et al., 1974). Interest in growing cannabis for medical and recreational purposes is increasing worldwide. Numerous biological and pharmacological effects are exhibited by the compounds found in *C. sativa* L. The plant produces essential oils and resins from inflorescence and leaves, nutrient-rich oil and proteins from seeds, and the industrially important high-quality fibre from the stem (Carus and Sarmiento, 2016; Baldini et al., 2018; El Bakali et al., 2022). *C. sativa* essential oil is very rich in terpenes. Terpenes are vital components for applications using plant products from a variety of plant species (Polito and Lange, 2023). *Cannabis* cultivars have different compositions of essential oils and terpenes, and this oil is widely known for its antibacterial properties, which can speed up the healing process of wounds (Booth et al., 2017; El Ghacham et al., 2023). Small (1979) reported that hemp can be

used in three forms: Its fibre is used to make clothing, paper and rope; Cannabinoids are used in the manufacture of medicines for certain illnesses (e.g. depression); using the oil from its achene for nutrition.

The biosynthesis of these key compounds is closely linked to specialized structures called trichomes. These microscopic outgrowths, primarily located on floral and foliar surfaces, are the main sites of resin production in *C. sativa* (Andre et al., 2016). Historically, the resin secreted by glandular trichomes was manually harvested to produce traditional preparations such as hashish (El Bakali et al., 2024a). Today, trichomes are central to both scientific research and industrial applications due to their phytochemical richness and their ecological role in plant defense against environmental stressors (Wagner, 1991; Clarke, 1998; Livingston, 2020). Most of the aerial parts of *C. sativa* are covered with different types of glandular and non-glandular trichomes (Dayanandan and Kaufman, 1976). For the non-glandular, cystolithic non-glandular trichomes are restricted to the upper side of vegetative fan leaves, and pointed non-glandular biomineralized trichomes of various types are located at different positions on each organ (Bar and Shtein, 2019). The non-glandular trichomes, such as cystolithic hairs, have been extensively and meticulously studied by light microscopy (Asahina et al., 1967; Shimomura et al., 1967; Nakamura, 1969; Nordal, 1970). These studies were further developed using scanning electron microscopy (SEM) to establish positive microscopic identification of marijuana (Bradford and Devaney, 1970; Hunter, 1971). Indeed, the use of scanning electron microscopy later enabled Hammond and Mahlberg (1973) to describe three types of glandular hair, which appear in particular on the bracts of female plants.

Trichomes of *C. sativa* have been studied for over a century, with early work by Briosi and Tognini (1894) providing some of the first detailed descriptions. While most early studies focused on mature trichomes, Mohan Ram and Nath (1964) explored their development. Research in the 1960s and 1970s, using light and scanning electron microscopy, primarily aimed to support the forensic identification of illicit cannabis products (e.g., Shimomura et al., 1967; Nakamura, 1969; Hammond and Mahlberg, 1973). In this context, we carried out an anatomical study of the bracts from six *C. sativa* cultivars grown in central-northern Morocco. This work is original in its focus on local Moroccan cultivars. Trichome type and density are directly related to resin production and can serve as distinguishing traits among cultivars, aiding in the selection of cultivars for quality or yield. The objectives of this study were to (i) describe and quantify the trichome types and morphology in each cultivar and (ii) assess their distribution to classify cultivars based on trichome characteristics.

Results

This investigation into the bract trichomes of cannabis revealed variability in morphology, density, and trichome type composition, which are characteristics specific to each cultivar. To compare the different *C. sativa* cultivars, we chose hair morphology as the discriminating characteristic. The trichome typology in the bracts of the cultivars 'Beldiya', 'Mexicana', 'Khardala', 'Avocat', 'Critical Plus' and 'Industriel' was discussed.

In all the cultivars studied, we identified four types of trichomes in the bracts analysed: non-glandular trichomes (NGT) were represented solely by the conical trichome type. Glandular trichomes were categorized into three types of hairs: glandular bulbous trichomes (GBT), glandular capitate-stalked trichome (GCStT) and glandular capitate sessile trichome (GCSeT) (Tanney et al., 2021) (Figure 1).

Comparison by type of trichome

Non-glandular trichomes (NGT)

The percentage of non-glandular trichomes was almost similar across all cultivars (Table 1). However, the cultivar 'Mexicana' showed a slightly higher proportion (17%), followed by the cultivar 'Beldiya' (14%), then 'Avocat' (13%) and 'Critical Plus' (13%). The lowest levels were observed in the cultivars 'Khardala' and 'Industriel', both at 12%.

Glandular capitate-stalked trichome (GCStT)

The cultivars 'Beldiya', 'Avocat' and 'Industriel' stand out from the others cultivars due to their high levels of glandular capitate-stalked trichomes (32%, 32%, 34% respectively). The other cultivars 'Mexicana', 'Khardala' and 'Critical Plus' have lower average rates of 25%, 24% and 28% respectively (Table 1).

Glandular bulbous trichomes (GBT)

This type of trichome shows approximately the same rate in all cultivars (Table 1). However, the proportion of this type of glandular bulbous trichome on 'Khardala' and 'Critical Plus' is slightly higher at 23%, followed by 'Industriel' and 'Avocat' at 21% and 20% respectively. In contrast, glandular bulbous trichomes are the least represented in the cultivars 'Beldiya' and 'Mexicana', both at 18%.

Glandular capitate sessile trichome (GCSeT)

The cultivars 'Mexicana' and 'Khardala' were distinguished from the other cultivars by their high levels of glandular capitate sessile trichome (40% and 41% respectively). On the other hand, the cultivars 'Beldiya', 'Critical Plus' and 'Avocat' exhibited very similar rates, 36%, 36% and 35% respectively. In 'Industriel', this type of trichome is the least represented at 33% (Table 1).

Comparison by trichome density

The four trichome types were identified in each cultivar, but with varying densities. The highest average density of GCSeT was found in the cultivars 'Critical Plus', 'Mexicana', 'Avocat' and 'Khardala', with rates of 451.40 T/mm², 421.33 T/mm², 417.64 T/mm² and 409.46 T/mm² respectively. However, the cultivars 'Beldiya' and 'Industriel' exhibited lower average densities of 258.55 T/mm² and 130.07 T/mm², respectively (Table 2).

The cultivars 'Avocat' and 'Critical Plus' showed a maximum average density of glandular capitate-stalked trichome (GCStT) at 374.10 T/mm² and 349.57 T/mm² respectively. Moreover, moderate densities were recorded in 'Mexicana' (269.89 T/mm²), 'Khardala' (242.98 T/mm²), and 'Beldiya' (229.79 T/mm²), whereas 'Industriel' exhibited the lowest density at 130.59 T/mm². For GBTs, the greatest densities were found in 'Critical Plus' (278.86 T/mm²), followed by 'Avocat' (237.18 T/mm²) and 'Khardala' (230.32 T/mm²). 'Mexicana' and 'Beldiya' showed lower densities, at 193.65 T/mm² and 123.73 T/mm², correspondingly. The cultivar 'Industriel' recorded the lowest density, with only 83.37 T/mm². The maximum average density of the non-glandular trichomes (NGT) type is found in the cultivars 'Mexicana', 'Critical Plus' and 'Avocat' with rates of 183.89 T/mm², 156.71 T/mm² and 147.21 T/mm² individually. However, the cultivars

'Khardala' and 'Beldiya' show an average density of 117.93 T/mm² and 98.93 T/mm² respectively. A minimum average density of 45.64 T/mm² characterises 'Industriel' (Table 2; Figure 2).

Table 1. Percentage of glandular and non-glandular trichomes in cultivars 'Beldiya'; 'Mexicana'; 'Khardala'; 'Avocat'; 'Critical Plus'; 'Industriel'.

Percentage	NGT (%)	GCStT (%)	GBT (%)	GCSeT (%)
'Beldiya'	14.00	32.00	18.00	36.00
'Mexicana'	17.00	25.00	18.00	40.00
'Khardala'	12.00	24.00	23.00	41.00
'Avocat'	13.00	32.00	20.00	35.00
'Critical Plus'	13.00	28.00	23.00	36.00
'Industriel'	12.00	34.00	21.00	33.00

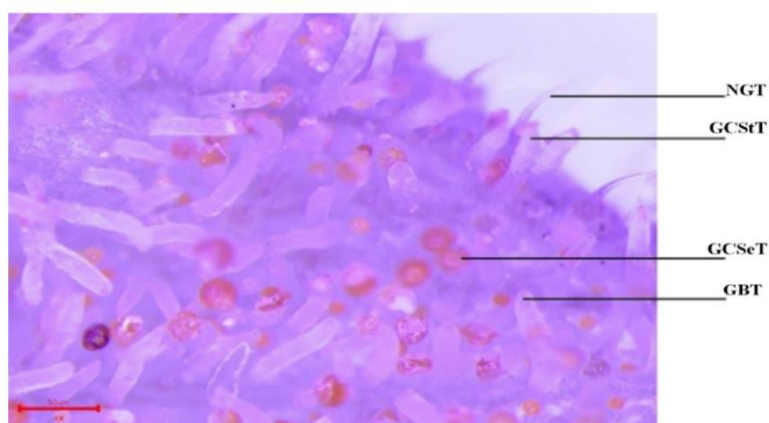


Fig. 1. Trichome types observed on *Cannabis sativa* bracts under light microscopy: non-glandular trichomes (NGT), glandular bulbous trichomes (GBT), glandular capitate-stalked trichomes (GCStT), and glandular capitate sessile trichomes (GCSeT).

Table 2. Mean values of trichome types across six *Cannabis sativa* cultivars grown in northern Morocco. Within each column, means sharing the same letter are not significantly different at $P < 0.05$. NGT: non-glandular trichomes, GBT: glandular bulbous trichomes GCStT: glandular capitate-stalked trichome and GCSeT: glandular capitate sessile trichome.

Cultivars	NGT	GCStT	GBT	GCSeT
'Avocat'	147.22 b	374.10 a	237.18 b	417.64 b
'Beldiya'	98.94 d	229.79 d	123.73 d	258.55 c
'Critical Plus'	156.71 b	349.57 b	278.86 a	451.40 a
'Industriel'	45.64 e	130.59 e	83.37 e	130.07 d
'Khardala'	117.93 c	242.98 d	230.32 b	409.46 b
'Mexicana'	183.87 a	269.89 c	193.65 c	421.33 b

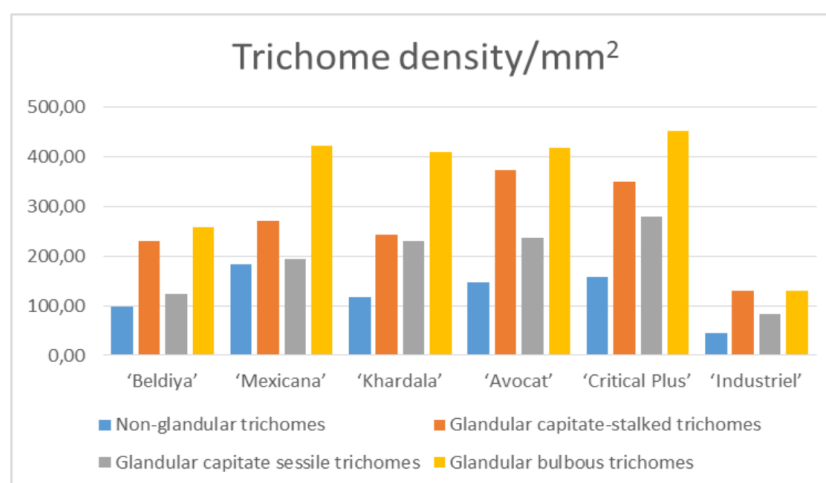


Fig. 2. Mean density of four trichome types across the six *Cannabis sativa* cultivars studied.

Table 3. Correlation coefficients between the parameters studied, NGT: non-glandular trichomes GBT: glandular bulbous trichomes GCStT: glandular capitate-stalked trichome and GCSeT: glandular capitate sessile trichome, in the six *Cannabis* cultivars studied.

	NGT	GCStT	GBT	GCSeT
NGT	–	0,64*	0,54	0,71**
GBT		–	0,67*	0,64*
GCStT			–	0,69**
GCSeT				–

* Significant at 0.05 probability level; ** Significant at 0.01 probability level; *** Significant at 0.001 probability level.

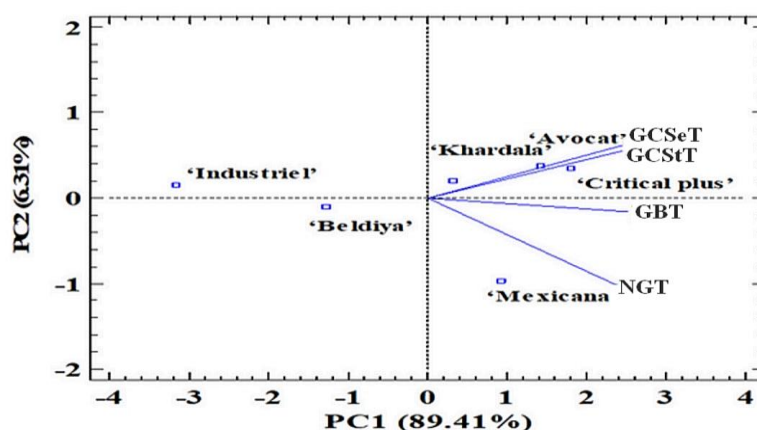


Fig. 3. Principal component analysis (PCA) of the cultivar-trichome matrix. Blue segments indicate eigenvalues representing the most significant parameter variations. NGT: non-glandular trichomes, GBT: glandular bulbous trichomes GCStT: glandular capitate-stalked trichome and GCSeT: glandular capitate sessile trichome.

Mean comparison among cultivars

Table 2 presents the ANOVA results for trichome variability across six *C. sativa* cultivars, revealing significant differences ($p < 0.05$). Pairwise comparisons showed that most cultivars differed significantly in non-glandular trichome (NGT) density, except 'Avocat' and 'Critical Plus'. NGT density ranged from 183.87 T/mm² in 'Mexicana' to 45.64 T/mm² in 'Industriel'.

Overall, glandular trichome densities were higher than non-glandular ones. No significant differences were observed in GCStT between 'Beldiya' and 'Khardala'. 'Critical Plus' showed the highest densities of GCSeT (451.40 T/mm²) and GBT (278.86 T/mm²), while 'Avocat' had the highest GCStT density (374.10 T/mm²). In contrast, 'Industriel' consistently recorded the lowest values across all trichome types. Overall, 'Critical Plus' had approximately twice the total trichome density of 'Beldiya', and 'Mexicana' showed the highest NGT density among all cultivars.

Correlations among parameters

Table 3 summarizes the correlation matrix among the trichome parameters analyzed. Significant positive correlations were observed between most variables. Notably, GBT showed positive associations with all other trichome types, except for non-glandular trichomes (NGT), where the correlation was not significant ($p < 0.05$).

For NGT, all remaining correlations were positive and significant. The strongest associations ($p < 0.01$) were found between GCSeT and NGT ($r = 0.706^{**}$), and between GCSeT and GCStT ($r = 0.694^{**}$), indicating a strong tendency for these traits to vary together.

Principal component analysis (PCA)

PCA was conducted using four dependent variables (NGT, GBT, GCStT, and GCSeT) to assess the potential for discriminating among the six studied *C. sativa* cultivars. The first two principal components explained approximately 96% of the total variance. PC1 accounted for 89% of the variation and showed strong positive loadings for all four variables, indicating their overall contribution to this axis. PC2 explained 6% of the variance, characterized by positive loadings for GCSeT and GCStT, and negative loadings for GBT and NGT.

The Figure 3 revealed clear separation patterns. 'Beldiya' and 'Industriel' were positioned on the negative side of PC1, reflecting low values for all trichome parameters. In contrast, 'Khardala', 'Avocat', and 'Critical Plus' were clustered on the positive side of PC1, indicating higher trichome densities. 'Mexicana' appeared near the center of the plot, mainly associated with low NGT values but moderate levels of other trichome types.

Discussion

The identification of plant species often relies on morphological traits, with anatomical and microscopic features gaining importance, especially in species with high variability. Among these, trichome analysis is a key tool for understanding plant adaptation and classification.

In *Cannabis sativa*, two main characteristics of floral bract trichomes are typically evaluated: trichome density and the proportion of different trichome types. According to Small and Naraine (2016), higher THC content is generally associated with denser hair coverage and a greater abundance of large-headed glandular trichomes (up to 119 μ m).

In our study, inter-cultivar variability was evident. 'Khardala' showed a high proportion of glandular trichomes, while 'Mexicana' was dominated by non-glandular types, confirming that trichome traits are strongly cultivar-dependent (Sangwan et al., 2001). Glandular trichome density, especially of stalked types, is associated with higher resin production (Fairbairn, 1972; Sirikantaramas et al., 2005).

Trichome-based hairiness proved to be a useful discriminating trait for cultivar identification and classification. For instance, 'Critical Plus' displayed nearly double the trichome density of 'Beldiya', while 'Mexicana' had the highest proportion of NGT, and 'Avocat' showed the highest proportion of GCStT.

Similarly, Punja et al. (2023) found significant differences in capitate trichome number and stalk length between two cannabis genotypes, with "Space Queen" exhibiting greater values than "Moby Dick" during flower development. These differences highlight the influence of genotype and plant age on trichome type, density, and maturation.

Moreover, Livingston et al. (2020) observed that sessile trichomes on vegetative leaves consistently contain eight secretory cells, while capitate-stalked trichomes on mature flowers have 12 to 16 cells patterns consistent across fiber and drug-type cultivars. This insight allows for more accurate estimation of glandular trichome density and may help predict trichome abundance in mature floral tissues (Tanney et al., 2021).

Among glandular types, GCSeT reached the highest average density in 'Critical Plus' (451.40 T/mm²). GCStT were most abundant in 'Avocat' and 'Critical Plus', but showed significantly lower density in 'Industriel' (130.59 T/mm²).

GBT density varies among cultivars, being lowest in 'Industriel' (83.37 T/mm²) and higher in 'Critical Plus', 'Avocat', and 'Khardala'. NGT density also shows variation, with 'Industriel' having the lowest value (45.64 T/mm²) and much higher densities in 'Mexicana', 'Critical Plus', and 'Avocat'. This variation in trichome type and density influences metabolite accumulation, as glandular trichomes differ in fluorescence, secretory cell number, and terpene profiles (Livingston et al., 2020). Notably, increased THC levels during flowering correlate with higher cannabinoid synthase in glandular trichome exudates from mid to late flowering (Aizpurua-Olaizola et al., 2016; Rodziewicz et al., 2019).

Correlation analysis showed that GBT were positively and significantly correlated with all other variables except NGT. The strongest positive correlations were found between GCSeT and NGT ($r = 0.706^{**}$), and between GCSeT and GCStT ($r = 0.694^{**}$), suggesting coordinated development possibly due to genetic linkage or pleiotropy as noted by Iezzoni and Pritts (1991). The significant correlations between traits allow for indirect prediction, enabling more rapid and cost-effective measurement of one trait based on another (Fairbairn, 1972; Hammond and Mahlberg, 1973; Dayanandan and Kaufman, 1976; Lanyon et al., 1981; Kim and Mahlberg, 2003; Livingston et al., 2020).

In this study, notable variation was observed among cultivars, with 'Critical Plus' exhibiting the highest densities of GCSeT (451.40 T/mm²) and GCStT (278.86 T/mm²), while 'Avocat' showed the highest GBT density (374.10 T/mm²). In contrast, 'Industriel' consistently showed the lowest trichome densities—GCStT (130.59 T/mm²), GCSeT (130.07 T/mm²), GBT (83.37 T/mm²), and NGT (45.64 T/mm²)—suggesting it likely produces the lowest phytocannabinoid content. This aligns with findings by Ghosh et al. (2023), who reported that higher densities of capitate sessile and stalked glands in female flowers correlate with greater phytocannabinoid synthesis. The cultivar 'Mexicana' had the highest density of non-glandular trichomes (183.89 T/mm²).

PCA based on four trichome traits explained 96% of variability among six hemp cultivars, effectively distinguishing groups and aiding cultivar cataloging. 'Beldiya' and 'Industriel' showed low trichome densities, while 'Khardala', 'Avocat', and 'Critical Plus' had high GBT and GCStT densities. 'Mexicana' was notable for its high NGT density. These findings align with previous studies confirming PCA's utility in differentiating hemp cultivars (El Bakali et al., 2022, 2024a,b; Ghosh et al., 2023).

Overall, trichome density and type effectively revealed morphological differences among cultivars grown in greenhouse conditions. 'Critical Plus' stands out as the most prolific cultivar, largely due to its high GCStT density, which correlates with commercial resin production.

The significance of the results, their limitations, and the directions for further study

Our findings demonstrated a number of detrimental effects of modernization, such as the increased fragility of morphological variability brought on by the new hybrids that are cultivated and the lack of use of Scanning Electron Microscopy (SEM) equipment to photograph the bract trichomes at the level of the studied cultivars. New hybrids are still being grown and used, but farmers are no longer as inclined to plant the highly prized local cultivar 'Beldiya', which is of lower quality. Given that this genetic resource is well suited to the local environment, it is crucial to support farmers in preserving the local variation. Our results offer important insights into the sustainable usage of all cultivars under agricultural conditions as well as the farmed variety. The aforementioned results hold significant value as they provide a broad comprehension of the existing circumstances, hence aiding in the development of changeable morphological traits. Additionally, conducting the same study in the future would provide insights into the future of biomass variability in hemp (*C. sativa*) cultivars grown in Morocco, including local cultivar, disappeared. The National Agency for the Regulation of Activities Related to Cannabis should support the maintenance of traditional cultivar by cultivation farmers. In addition to addressing the effects of the morphological traits of the plants, this strategy guarantees that cannabis is used therapeutically and permits industrial, medicinal, and cosmetic use. It also fosters employment opportunities for the younger generation and supports the long-term viability of this traditional agricultural practice. This method offers a more cohesive and locally based approach by giving local knowledge and practices precedence over ideas brought in from outside the community.

The used technique, based on morphology and distribution of trichomes on the collected bracts, provides a wealth of information and a high level of precision, allowing us to deepen our understanding of the overall status of each cultivar in morphological characteristics of plants. The adopted methodology has yielded significant results, revealing a remarkable diversity in all cultivar.

C. sativa L. is used by farmers as a basis for characterising parameters and documenting part of the varietal diversity. However, relying solely on use for medicinal purposes does not provide full utilisation of some practices or enable us to identify local cultivar. For a comprehensive interpretation of how farmers perceive, think about and manage new hybrids, it was necessary to understand the various genotypic and environmental factors that directly or indirectly influence their choices. The clear difference that was sometimes observed between cultivated cultivars was unclear and they were often similar to each other.

Prospecting is often most successful when each cultivar of cannabis is well investigated. One picture of the various trichome kinds on the plants should not be relied upon. Considering the purpose and extent of the investigation, further images of the plant bracts must be added. The presentation of trichome on plants is of great value because it revitalises the economic cycle, confirms identifications, stimulates discussions, and sometimes opens new possibilities for exploration and research on important uses of cannabis for therapeutic, industrial, medicinal and cosmetic purposes.

Material and methods

Plant material and growing conditions

Six commercial cultivars were studied: the local Moroccan cultivar 'Beldiya', native to the Rif Mountains; four high-THC, high-yielding cultivars 'Avocat', 'Critical Plus', 'Khardala', and 'Mexicana'; and 'Industriel', a Spanish cultivar primarily grown for fiber production. All cultivars were identified by their local names and sourced from farmers in Bab Taza, Chefchaouen province, northwestern Morocco (El Bakali et al., 2025).

Seeds of each cultivar were sown in black plastic pots (25 cm diameter, 30 cm height), with one seed per pot and a total of 15 pots prepared by cultivar. Each pot was filled with 10 liters of commercial peat substrate. In April 2018, pots were placed in a greenhouse at the Faculty of Sciences of Tétouan (35°33'38"N, 5°21'46"W, altitude 10 m). Throughout the growth period, natural photoperiod and relative humidity (60–80%) were maintained, with day/night temperatures ranging from 20–45°C and 10–30°C, respectively. Seedlings were irrigated every other day with three two-minute sessions.

Preparation of samples and trichome density measurements

The six *C. sativa* L. cultivars under study were germinated and grown under glass. After maturation (approximately 16 weeks), representative samples of each cultivar were placed in 10% formulated water for preservation and to maintain the tissue and morphology during observation. After careful sorting, a total of 180 samples were selected for analysis. Specifically, 30 bracts from each cultivar were selected for observation of the trichomes under a light microscope (x4). Fresh bracts were harvested from flowering plants, and the samples were photographed using a camera running Toup view 3.7 software. The morphology and distribution of trichomes on the adaxial surface of the collected bracts were evaluated. Trichomes were visually categorized based on their structural characteristics. Trichome density (number of hair types / surface area in ($\mu\text{m}^2 \times 10^6$) existing in each mm^2 of the bract was estimated by counting all trichome types in 30 separate photographs. An average value was calculated for each cultivar. Images were captured using ToupView 3.7, which offers full camera control and advanced image processing features. This software enabled clear visualization of trichome types, allowing accurate identification, detailed morphological description, and quantification of trichome density for each *C. sativa* cultivar.

Statistical analysis

All the determinations and measurements were carried out with 30 replicates for bracts. The mean was calculated to express values. Tukey's test was performed after a general linear procedure to evaluate quantitative differences. Generalized linear models were used to perform statistical analyses of the data (GLM). The 5% probability level was used to determine the significance of differences in mean values. The correlation between both types of trichomes was also evaluated (Pearson coefficient).

To investigate whether *Cannabis* cultivars could be distinguished from each other, the mean values of the variables were subjected to principal component analysis (PCA). The STATGRAPHICS version XVIII software program (Statpoint Technologies, Inc., Virginia, USA) was used to perform these statistical analyses.

Conclusion

Trichome morphology is a valuable trait for distinguishing *Cannabis sativa* cultivars, particularly given the species' high morphological variability resulting from domestication, hybridization, and selective breeding. Our findings demonstrate significant differences in trichome type and density among the six cultivars studied, confirming their usefulness as discriminative markers.

Analysis of our results shows that the levels of each type of trichome vary from one cultivar to another. The following breakdown shows the importance of each type of trichome in the different cultivars in descending order.

Non-glandular trichomes (NGT): 'Mexicana' > 'Critical Plus' > 'Avocat' > 'Khardala' > 'Beldiya' > 'Industriel'

Glandular capitate-stalked trichomes (GCStT): 'Avocat' > 'Critical Plus' > 'Mexicana' > 'Khardala' > 'Beldiya' > 'Industriel'

Glandular capitate sessile trichomes (GCSeT): 'Critical Plus' > 'Mexicana' > 'Avocat' > 'Khardala' > 'Beldiya' > 'Industriel'

Glandular bulbous trichomes (GBT): 'Critical Plus' > 'Avocat' > 'Khardala' > 'Mexicana' > 'Beldiya' > 'Industriel'

These results confirm that trichome traits are useful in distinguishing cultivars and can support breeding programs targeting specific morphological or phytochemical profiles.

Ethical statement

Research did not include any human subjects and animal experiments

* Credit authorship contribution statement

I. El Bakali: Field work, most of the laboratory work, analysis and interpretation of data and manuscript writing of this research. **A. Moukhles, S. El Bakali, I. Rahmouni:** Drafted the manuscript and critically revising it for important intellectual. **A. Boutahar, S. Chakkour, M. Houssni:** Field work, formatting, editing according journal guidelines. **M. Kadiri:** Analysis and interpretation of data. **A. Merzouki:** Read and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

References

- Andre CM, Hausman JF, Guerriero G (2016). *Cannabis sativa*: the plant of the thousand and one molecules. *Front Plant Sci*, 7: 19.
- Aizpurua-Olaizola O, Soydaner U, Öztürk E, Schibano D, Simsir Y, Navarro P, Etxebarria N, Usobiaga A (2016) Evolution of the cannabinoid and terpene content during the growth of *Cannabis sativa* plants from different chemotypes. *J Nat Prod*. 79(2) : 324-331.
- Asahina H, Ono M, Takahashi K, Ono Y (1967) Identification of *Cannabis* resin. *Bulletin of the National Institute of Hygienic Sciences Tokyo*. 85: 123-125.
- Baldini M, Ferfua C, Piani B, Sepulcri A, Dorigo G, Zuliani F, Danuso F, Cattivello C (2018) The Performance and Potentiality of Monoecious Hemp (*Cannabis sativa* L.) Cultivars as a Multipurpose Crop. *Agronomy*. 8(9): 162.
- Bar M, Shtein I (2019) Plant trichomes and the biomechanics of defense in various systems, with Solanaceae as a model. *Botany*. 97(12): 651-660.
- Booth JK, Page JE, Bohlmann J (2017) Terpene synthases from *Cannabis sativa*. *PLoS One*. 12:e073911. [https://doi: 10.1371/journal.pone.0173911](https://doi.org/10.1371/journal.pone.0173911).
- Bradford LW, Devaney J (1970) Scanning electron microscopy applications in criministics. *J Forensic Sci*. 15(1):110-119.
- Briosi G, Tognini F (1894) Intorno alla anatomia della canapa (*Cannabis sativa* L.). *Atti dell'Istituto Botanico dell'Università di Pavia*. 3:91-209.
- Carus M, Sarmento L (2016) The European Hemp Industry: Cultivation, processing and applications for fibres, shivs, seeds and flowers. *European Industrial Hemp Association*, 5: 1-9.
- Clarke, R. C. (1998). *Hashish!* (p. 387). Los Angeles: Red Eye Press.
- Dayanandan P, Kaufman PB (1976) Trichomes of *cannabis sativa* L. (*Cannabaceae*). *Am J Bot*. 63(5): 578-591.
- El Bakali I, Chakkour S, Boutahar A, El Bakali S, Kadiri M, Merzouki A (2025) Effect of temperature on seed germination of five hemp (*Cannabis sativa* L.) cultivars from Rif Mountains (northern Morocco). *POJ*. 17(01),1-9. [https://doi: 10.21475/POJ.17.01.25.p06](https://doi.org/10.21475/POJ.17.01.25.p06).
- El Bakali I, Hassoun M, Boutahar, A, El Bakali S, Sakar EH, Kadiri M, Merzouki A (2024a) A comparative evaluation of biomass and resin by-products attributes of six hemp (*Cannabis sativa* L.) cultivars grown in Rif Mountains (northern Morocco). *Vegetos*. 1-13. <https://doi.org/10.1007/s42535-024-01077-x>.
- El Bakali I, Chakkour S, El Bakali S, Boutahar A, Kadiri M, Merzouki A (2024b) Quantitative eco-anatomical analysis reveals distinctive stem traits of six *Cannabis sativa* cultivars in Rif Mountains (Northern Morocco). *Aust J Crop Sci*.18(09): 539-546. <https://doi.org/10.21475/ajcs.24.18.09.p65>.
- El Bakali I, Sakar EH, Boutahar A, Kadiri M, Merzouki A (2022) A comparative phytochemical profiling of essential oils isolated from three hemp (*Cannabis sativa* L.) cultivars grown in central-northern Morocco. *Biocatal Agric Biotechnol*, 42: 102327. <https://doi.org/10.1016/j.bcab.2022.102327>.
- El Ghacham S, El Bakali I, Zarouki MA, Aoulad El Hadj Ali Y, Ismaili R, El Ayadi A, Souhail B, Tamegart L, Azzouz A (2023) Wound healing efficacy of *Cannabis sativa* L. essential oil in a mouse incisional wound model: A possible link with stress and anxiety. *S Afr J Bot*. 163: 488-496. <https://doi.org/10.1016/j.sajb.2023.11.005>.
- Fairbairn JW (1972) The trichomes and glands of *Cannabis sativa* L. *Bulletin on Narcotics*, 24, 29-33.
- Ghosh D, Chaudhary N, Shanker K, Kumar B, Kumar N (2023) Monoecious *Cannabis sativa* L. discloses the organ-specific variation in glandular trichomes, cannabinoids content and antioxidant potential. *J Appl Res Med Aromat Plants*. 35: 100476.
- Hammond CT, Mahlberg PG (1973) Morphology of glandular hairs of *Cannabis sativa* from scanning electron microscopy. *Amer J Bot*. 60(6): 524-528.
- Hunter G (1971) *Metalogic: An introduction to the metatheory of standard first order logic*. Univ of California Press. Macmillan International Higher Education.
- Iezzoni AF, Pritts MP (1991) Applications of principal components analysis to horticultural research. *HortScience*. 26:334-338. <https://journals.ashs.org/hortsci/downloadpdf/journals/hortsci/26/4/article-p334.xml>.
- Kim ES, Mahlberg PG (2003) Secretory vesicle formation in the secretory cavity of glandular trichomes of *Cannabis sativa* L. (*Cannabaceae*). *Mol Cell*. 15(3): 387-395.
- Lanyon VS, Turner JC, Mahlberg PG (1981) Quantitative analysis of cannabinoids in the secretory product from capitate-stalked glands of *Cannabis sativa* L. (*Cannabaceae*). *Bot gaz*. 142(3): 316-319.
- Livingston SJ, Quilichini TD, Booth JK, Wong DC, Rensing KH, Laflamme-Yonkman J, Castellarin SD, Bohlmann J, Jonathan EP, Samuels AL (2020) *Cannabis* glandular trichomes alter morphology and metabolite content during flower maturation. *Plant J*. 101(1): 37-56. [https://doi: 10.1111/tip.14516](https://doi.org/10.1111/tip.14516).
- Merlin MD (2003) Archaeological evidence for the tradition of psychoactive plant use in the old world. *Econ Bot*. 57(3): 295-323. [https://doi.org/10.1663/0013-0001\(2003\)057\[0295:AEFTTO\]2.0.CO;2](https://doi.org/10.1663/0013-0001(2003)057[0295:AEFTTO]2.0.CO;2).
- Mohan Ram HY, Nath R (1964) The morphology and embryology of *Cannabis sativa* Linn. *Phytomorphology*, 14: 414-429.
- Nakamura GR (1969) Forensic aspects of cystolith hairs of *Cannabis* and other plants. *J Assoc Off Anal Chem*. 52(1): 5-16.
- Nordal A (1970) Microscopic detection of *Cannabis* in the pure state and in semi-combusted residues, p. 61-68. In C.R.B. Joyce and S.H. Curry [ed.], *the botany and chemistry of Cannabis*. Churchill, London.
- Polito JT, Lange BL (2023) Standard operating procedures for the comprehensive and reliable analysis of cannabis terpenes. In Jez J (Ed.): *Methods in Enzymology*. Academic press, 680: 381-419.
- Punja ZK, Sutton DB, Kim T (2023) Glandular trichome development, morphology, and maturation are influenced by plant age and genotype in high THC-containing *cannabis* (*Cannabis sativa* L.) inflorescences. *J Cannabis Res*. 5(1): 12.
- Rodziewicz P, Lorocho S, Marczak Ł, Sickmann A, Kayser O (2019) Cannabinoid synthases and osmoprotective metabolites accumulate in the exudates of *Cannabis sativa* L. glandular trichomes. *Plant Sci J*. 284: 108-116.
- Sangwan N.S., Farooqi A.H.A., Shabih F., Sangwan R.S. 2001. Regulation of essential oil production in plants. *Plant Growth Regul*. 34: 3-21. [https://doi:10.1023/A:1013386921596](https://doi.org/10.1023/A:1013386921596).
- Schultes RE, Klein WM, Plowman T, Lockwood TE (1974) *Cannabis*: an Example of taxonomic neglect. *Botanical Museum Leaflets*. Harvard University. 23(9): 337-367.
- Shimomura H, Shigehiro M, Kuriyama E, Fujita M (1967) Studies on *Cannabis*. I. Microscopical characters of their internal morphology and spodogram (Japanese). *Y akugakuZasshi: J Pharm Soc Jpn*. 87(11): 1334-1341.
- Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y (2005) Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol*.46(9): 1578-1582. [https://doi: 10.1093/pcp/pci166](https://doi.org/10.1093/pcp/pci166).

- Small E, Naraine SGU (2016) Size matters: evolution of large drug-secreting resin glands in elite pharmaceutical strains of *Cannabis sativa* (marijuana). *Genet Resour Crop Evol.* 63: 349-359.
- Small EP (1979) The species problem in *cannabis*: Science and semantics. Vol1. Science. Corpus eds. Toronto Canada.
- Tanney CA, Backer R, Geitmann A, Smith DL (2021) *Cannabis* glandular trichomes: A cellular metabolite factory. *Front Plant Sci.* 1923.
- Wills S (1998) *Cannabis* use and abuse by man: an historical perspective. Harwood Academic, Amsterdam.
- Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. *Plant Physiology.* 96(3): 675–679.
<https://doi.org/10.1104/pp.96.3.675>