



Characterization of cannabis varieties and the intrinsic and extrinsic factors affecting cannabis germination and seedling establishment: A descriptive review

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ABSTRACT

Cannabis sativa L. is the utmost consumed, grown, and produced illicit drug worldwide. The psychotropic activity of the component (-)-*trans*- Δ^9 -tetrahydrocannabinol (Δ^9 -THC), however, led to the banning of cannabis worldwide for many centuries. Besides being the most popular crop for recreational purposes, cannabis is grown globally as a multifunctional agricultural crop for its oily seeds and long, durable fibers. These properties have led to cannabis application in various industries, including agriculture, textile, bio-composite, papermaking, automotive, construction, and biofuel. Furthermore, medicinal cannabis constitutes of high concentration of cannabinoids, which have been discovered relevant in the pharmaceutical industry. As a result, various countries worldwide have relaxed regulations on cannabis. However, the cannabis products' final use depends on the cultivar. Yet, the crop has hundreds of cultivars owing to constant breeding and human selection, which has altered the original plant with the spread of the crop worldwide. Generally, these cultivars possess chemical composition that varies genetically, and with the growing environmental conditions. Therefore, the current descriptive review of literature highlights the techniques available for classifying cannabis varieties, given the ongoing debate over classifying cultivars into distinct taxonomic groups. The effect of intrinsic and extrinsic factors on cannabis germination and seedling establishment are also highlighted.

1. Introduction

Cannabis sativa L., commonly known as marijuana, dagga, or hemp, is an annual dioecious and dicotyledonous plant belonging to the *Cannabaceae* family (Chaohua et al., 2016; Lata et al., 2016; Flores-Sanchez et al., 2009). Cannabis which originated from Central Asia, is one of the earliest plants domesticated in eastern Asia since 3 500 BC (Decorte et al., 2011). Furthermore, it is one of the most consumed, grown, and produced illicit drug worldwide, with about 151 countries cultivating cannabis plants from 2010 to 2018 (Shao et al., 2023; Duvall, 2019; Citti et al., 2018). South Africa being among the world's largest producers of *Cannabis*, with its cultivation occurring mainly in remote rural areas of the Eastern Cape and KwaZulu-Natal provinces (Peltzer and Ramlagan, 2007; Gastrow, 2003).

Cannabis classification as an illicit drug owes to the intoxicating effects of the psychotropic activity of the component (-)-*trans*- Δ^9 -

tetrahydrocannabinol (Δ^9 -THC) (Citti et al., 2018). This compound then led to the ban of cannabis worldwide for centuries (Citti et al., 2018). Until recently when many countries across the world considered uplifting the regulations controlling the cultivation of cannabis, particularly for hemp and drug type for medicinal purposes (Allaf et al., 2023; Citti et al., 2018; Gouws, 2017). Since regulatory changes have been made in various countries worldwide, the global cannabis market has seen significant growth, making it a lucrative industry (Qobo and Sihlobo, 2019). In 2023, the legal cannabis market is estimated to be worth \$57.18 billion, with a projected value of \$147 billion by the end of 2027 (Stefkov et al., 2022).

The growing interest, particularly in medicinal cannabis, follows the recent discovery of the relevance of cannabinoids in the pharmaceutical industry (Citti et al., 2018). It is suggested that cannabinoids usually interact with cannabinoid (CB) receptors (CB₁ and CB₂) and other receptor systems to show their pharmacological effects in humans

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(Lewis-Bakker et al., 2019). As a result, various medicines known to activate cannabinoid CB₁ and CB₂ receptors have been manufactured from cannabis derivatives (Pertwee, 2009). Amongst these medicinal products include Cesamet® (nabilone), Marinol® (dronabinol; Δ^9 -tetrahydrocannabinol), and Sativex® (Δ^9 -tetrahydrocannabinol with cannabidiol) (Harrison and Simpson, 2021; Gaisey and Narouze, 2021; Turri et al., 2018; Pertwee, 2009).

On the other hand, hemp has been grown globally as a multifunctional agricultural crop for its oily seeds and long, durable fibers (Smith et al., 2019; Salentijn et al., 2015). It has been used in many industries, including agriculture, papermaking, textile, automotive, construction, bio-composite, and biofuel (Schlutenhofer and Yuan, 2017; Chaohua et al., 2016; Salentijn et al., 2015). Hemp seed oil has desirable quantities of omega-6 and omega-3 lipids for human and animal diets, cooking, or processing cosmetics and fuels (Schlutenhofer and Yuan, 2017; Chaohua et al., 2016; Salentijn et al., 2015). The rediscovery of the multifunctional properties of cannabis has, thus, sparked scientific interest in this plant (Citti et al., 2018; EMCDDA, 2018).

Scientific research studies in this crop are critical even for developing medicinal products in many countries, including South Africa. Particularly, considering that variation in chemical composition amongst cannabis cultivars or species is generally prevalent (Procaccia et al., 2022; Jin et al., 2017; Farag and Kayser, 2015). This is largely owed to the existence of hundreds of different cannabis cultivars as a result of the long history of hybridization by humankind (Birenboim et al., 2022; Procaccia et al., 2022; Jin et al., 2017). These cultivars are known to have varying chemical composition resulting not only from genetic factors, environmental conditions, morpho-spatial position in the plant and crop management practices (Song et al., 2023; De Prato et al., 2022; Danziger and Bernstein, 2021a; Toth et al., 2021; Piluzza et al., 2013). Therefore, the growing cannabis industry and the accessibility of cannabis products for medical and other industrial purposes demand improved product analysis, which is essential for improving product standardization and quality assurance (Monti et al., 2022; Jin et al., 2017).

Furthermore, despite the increased interest in hemp and medicinal cannabis, the physiology of cannabis germination and seedling establishment is not very well understood. Seed germination and seedling establishment are crucial phases for crop production and plant density and are also the most sensitive stages of plant growth (Lamichhane et al., 2018; Jorenush and Rajabi, 2015). Generally, the germination of seeds can occur in the field if certain conditions triggering the germination process exist, including sufficient temperature, moisture, and light (Çanak et al., 2020). Hence, seed germination is considered a quick and preliminary screening as germination patterns provide insight into plant establishment and favourable conditions (Hu et al., 2018). Therefore, this descriptive review seeks to provide insights into the chemical composition of cannabis to determine optimal analytical procedures for the quantitative determination of compounds in samples. This is so that the discovered compounds provide useful information for differentiating cannabis varieties (i.e., medicinal and drug-type varieties) for choosing planting material based on the desired end-use product. It also seeks to give an overview of the physiology and biochemistry of cannabis seed germination and plant development and the influence of internal and external factors in these growth processes.

2. Classification of cannabis varieties

Plant physiological features determine crop yield and quality; hence, they are critical in breeding and crop management (Sarsenbaev et al., 2013). Those of cannabis are even more important as it is known for its significant degree of heterogeneity, which is believed to be partly caused by its sexual dimorphism (Sarsenbaev et al., 2013). Sexual dimorphism of cannabis is witnessed through the rate of growth and development as they differ significantly between female and male plants (Sarsenbaev et al., 2013). Male plants are known for flowering and senescing earlier

than female plants (Sarsenbaev et al., 2013). Besides, cannabis has spread globally, and its dispersion from its natural habitat has altered the original plant through constant human selection (Thomas, 2012).

Human selection has resulted in cannabis having enormous phenotypic diversity observed in various physiological features, including leaf shape, seed size, fiber quality, branching, height, phytochemistry, phenology, and ecology (Vergara et al., 2016). Consequently, it has been difficult to classify cultivars taxonomically, and it is still unclear if the existing cultivars belong to two or more species (Fischedick et al., 2010). Even though taxonomic classification is still under evaluation, scientists have identified three subspecies under the species *Cannabis sativa*, namely *C. sativa* subs *sativa* and *C. sativa* subs *indica*, and *C. subs ruderalis* (Procaccia et al., 2022; Simiyu et al., 2022; Thomas, 2012). Otherwise, the cultivars classified thus far were categorized through cannabinoid composition (chemical composition) and non-chemical characteristics (de Meijer, 1994), as briefly described in subsections 2.1 and 2.2.

2.1. Morphological and physiological (non-chemical) classification

Non-chemical attributes generally involve classifying cannabis varieties through morphological and physiological features (Salentijn et al., 2015; de Meijer, 1994). These characteristics are mostly resultants of domestication and are based on the climatic adaptation of the plant (de Meijer, 1994). Under this classification, plants are distinguished based on: (a) population types, which are wild and naturalized plants, landraces, and cultivars; (b) end-use of the plant, such as fiber cultivars, seed cultivars, drug strains, and ornamentals; (c) time of flowering, namely, short- and long-day flowering, intermediate flowering varieties (d) gender of the plant which are monoecious and dioecious cultivars; and (e) geographic origin.

De Meijer (1994) discovered three demarcated groups using the morphological and physiological traits, namely, drug hemp, oilseed hemp, and fibre hemp. The drug hemp varieties were categorized as short, highly branched plants, and their leaves' distinct features were small in size and dark green colour (de Meijer, 1994). The oilseed hemp varieties were also categorized as short but were early maturing plants and rich in seed production (de Meijer, 1994). Whereas fibre hemp varieties were categorized as tall unbranched plants with poor seed production (de Meijer, 1994). On the other hand, tall plants with slender morphology and short life cycles were categorized into male plants (Lubell and Brand, 2018; Small et al., 1975).

However, this review identified that categorizing *C. sativa* plants using sex morphology is flawed. This is considering that sex morphology can be modified or reversed by exogenously applying plant growth regulators (PGRs) and chemicals, such as ethylene and cytokinin, gibberellins, silver nitrate, and silver thiosulfate (STS) (Lubell and Brand, 2018). For instance, female flower formation can be promoted in male plants by applying exogenous ethylene and cytokinin (Lubell and Brand, 2018). Whereas hormones and chemicals such as gibberellins, silver nitrate, and STS promote male flowers to be formed on female plants (Lubell and Brand, 2018).

However, reversing the sex morphology from male to female cannabis plants is prevalent, following the great importance of female cannabis plants in various industrial applications. This is associated with the fact that unfertilized female plants achieve pure chemical compounds and produce high metabolite content compared to male, as well as fertilized female and monoecious plants (Lata et al., 2016). As a result, there has been an increasing interest in feminized seeds and vegetative female clones (Kurtz et al., 2020). However, feminized seeds and vegetative production are mostly not easily accessible to growers and are costly compared to normal seeds (DiMatteo et al., 2020). Therefore, the subsections 2.1.1 and 2.1.2 discusses the options available for classification of varieties as a seed level, which should provide useful information that will minimize the costs of cannabis production.

2.1.1. Seed morphology for variety classification

The macro- and microstructure of seeds are very significant for classifying Angiosperm taxa, particularly (Rashid et al., 2018; Kaya et al., 2011). Among species, diversity exists in seed shape, size, texture, hilum size, and colour, which can help study systemic relationships in a large variety of plant families and, subsequently, distinguish taxa (Rashid et al., 2021; Rashid et al., 2018). However, the morphology of the seed coat, in particular, is categorized as the most important part of the seed for studies related to taxonomy and evolution as it generally has stable parameters and is unlikely affected by external environmental factors during seed development and ripening within the fruit (Waheed et al., 2021; Karaismailoglu, 2019; Rashid et al., 2018; Kaya et al., 2011). The seed coat patterns provide information that solves classification problems, establishes evolutionary relationships, elucidates the seed coat's adaptive significance, and serve as genetic markers for discovering genotypes in segregating hybrid progenies (Kaya et al., 2011).

Usually, stereomicroscopes are used to examine plant species to identify features that characterize them into taxa (Candan et al., 2016). In cannabis, such studies are very limited, with only Naraine et al. (2020), who studied the morphological divergence of seeds among ruderal, fibre, oilseed, dioecious/monoecious, and marijuana variants using a stereoscope. Naraine et al. (2020) found that the seeds of hemp cultivars are larger and lighter in colour compared to marijuana seeds because they have been subjected to more selection for these features. Fibre cultivars have larger seeds compared to oilseed cultivars, associated with the former having poor seed production, and thus, more energy is channelled into the seeds (Naraine et al., 2020; de Meijer, 1994). The seeds of monoecious oilseed cultivars were characterized as slimmer with a more pronounced elongated base compared to those of dioecious cultivars. As mentioned previously, oilseed varieties are rich in seed production, and thus less energy is channelled into them; hence, they are slimmer (Naraine et al., 2020; de Meijer, 1994).

However, optical microscopy often fails to reveal qualitative differences in the research objects, of which scanning electron microscopy (SEM) has been useful in identifying these differences (Majeed et al., 2023; Candan et al., 2016). According to Candan et al. (2016), scanning electron microscopy (SEM) has been useful in identifying differences in micromorphological characteristics of leaves, pollens, fruit, and seeds when there are disagreements on the identity of taxa. This is because SEM detects ultrastructural characteristics by combining extreme magnification to reveal qualitative differences that optical microscopy cannot detect (Majeed et al., 2023). These ultrastructural characteristics include exo- and endomorphic, macro- and micromorphological, seed coat morphology, and microsculpturing features (Kaya et al., 2011). As a result, SEM has been widely adopted for reviewing phenetic linkages and species classification (Rashid et al., 2018; Kaya et al., 2011), but no such information is available on cannabis.

2.1.2. Near-infrared and Raman spectroscopy for classification of varieties

Differentiating the sex of the cannabis seed before sowing is a key factor in minimizing the yield losses, required labour, and high costs (DiMatteo et al., 2020; Kurtz et al., 2020). This is associated with the fact that the sex of the cannabis plant is often indistinguishable until the onset of flowering (DiMatteo et al., 2020; Toth et al., 2020). Cannabis cultivation may always be associated with yield losses, following that male and monoecious plants are removed at a later stage of development so that unfertilized female plants can be produced (DiMatteo et al., 2020; Toth et al., 2020; Lata et al., 2016). This ensures the production of an end product with a highly consistent chemical profile from unfertilized female plants (Toth et al., 2020; Lata et al., 2016).

Generally, traditional methods such as manual inspection, protein electrophoresis, DNA molecular marker technique are used for discrimination of different seed varieties (Liu et al., 2022; Bai et al., 2020). In cannabis, Cascini et al. (2019) revealed that genetic markers can differentiate between cannabis drug-type and fiber-type varieties,

both in seeds and during the early stages of plant development, well before cannabinoids accumulate. In their study they conducted sequencing of tetrahydrocannabinolic acid synthase (THCAS) and cannabidiolic acid synthase (CBDAS) genes from 21 *C. sativa* L. varieties (Cascini et al., 2019). From this study, Cascini et al. (2019) were able to successfully distinguish between drug-type and fiber-type cannabis samples, which was achieved with a perfect discrimination level of AUC 100%. This distinction was subsequently validated through chemical analyses to confirm the actual cannabinoid concentrations (Cascini et al., 2019). However, studies similar to the one conducted by Cascini et al. (2019), requires that a standard DNA fingerprint of seeds must be constructed first and the establishing a complete DNA molecular marker identification system requires professional technicians and is expensive.

As a result, technology devices such as near-infrared hyperspectral imaging (NIR-HSI) have been identified as a prospective approach for variety characterization at the seed level as it provides detailed spatial information and spectral information inside the research object (Jin et al., 2022; Cui et al., 2020; He et al., 2019; Zhang et al., 2018). The spatial and spectral information is then used for physicochemical and external morphological characterization of the sample (Jin et al., 2022; Cui et al., 2020; He et al., 2019; Zhang et al., 2018). NIR-HIS analysis allows to obtain results within a short period without any variations and destructing the sample, unlike protein electrophoresis and DNA molecular marker technologies where variations are prevalent (Bai et al., 2020; He et al., 2019; Zhang et al., 2019). Additionally, protein electrophoresis and DNA molecular marker approach are found only suitable for application in detection on a small scale as they involve reagent-dependent treatment processes and are destructive and time-consuming (Wu et al., 2019). However, these technologies must be coupled with machine learning methods for deep mining the information in hyperspectral images (Jin et al., 2022). Deep learning has been the most preferred method because of its self-learning feature (Jin et al., 2022).

2.2. Chemical classification of cannabis

It is well known that *Cannabis sativa* comprises of about 441 non-cannabinoid compounds and 125 phytocannabinoids (Kopustinskiene et al., 2022; Radwan et al., 2021; Thomas and ElSohly, 2016). However, the current literature review focuses on cannabinoids because they have been and potentially are still to be used to identify different cannabis varieties, particularly Δ^9 -THC and cannabidiol (CBD). CBD and Δ^9 -THC remain the most prevalent cannabinoids found in cannabis plants (Burnier et al., 2019; Citti et al., 2018). Δ^9 -THC is known to possess about 90% of the psychoactive effects of cannabis and was isolated and characterized by Raphael Mechoulam in 1964 (Burnier et al., 2019; Citti et al., 2018). Whereas CBD, an isomer of THC, is a non-psychoactive compound (Lata et al., 2016). Cannabinoids are the most active secondary metabolism compounds uniquely found in cannabis (Agarwal et al., 2018; Bonini et al., 2018; Kill et al., 2016; Radwan et al., 2008). The isolation and characterization of these compounds date back to the 1940s, and over about 125 cannabinoids have been discovered since that year (Kopustinskiene et al., 2022; Radwan et al., 2021; Citti et al., 2018).

However, generally, cannabis chemical composition tends to vary with varieties; hence, chemotaxonomic classification involves distinguishing different cannabis genotypes through cannabinoids and their concentration into different chemotypes (Piluzza et al., 2013; Broseus et al., 2010; de Meijer, 1994). Apart genotypes, the production and accumulation of cannabinoids are influenced by environmental conditions, morpho-spatial position in the plant and crop management practices, such as light, fertilizer regimes, water availability, temperature, plant architecture, plant density and biotic stresses (Song et al., 2023; De Prato et al., 2022; Danziger and Bernstein, 2021a; Toth et al., 2021). For instance, Wei et al. (2021) found that a higher proportion of blue light promoted the synthesis and accumulation of THC and CBD

cannabinoids in the 'Xinma' hemp variety. UV-B irradiation within a wavelength range of 280–320 nm has also been shown to increase THC content in certain cannabis varieties (Wei et al., 2021; Potter and Duncombe, 2012; Lydon et al., 1987).

The application of nitrogen (N), potassium (K) and phosphorus (P) fertilizers have also been found to alter the accumulation of secondary metabolites (Song et al., 2023; Saloner and Bernstein, 2022; Saloner and Bernstein, 2021; Shiponi and Bernstein, 2021). Generally, higher N and K availability tends to decrease the concentration of cannabinoids and terpenoids (Song et al., 2023; Saloner and Bernstein, 2022). To maintain a high secondary metabolite profile in medical cannabis cultivars, it is recommended to use an N level of 160 mg/L, a K level of 60 mg/L, and a P level ranging between 5 and 15 mg/L (Song et al., 2023; Saloner and Bernstein, 2022; Saloner and Bernstein, 2021; Saloner and Bernstein, 2020).

Pruning techniques have also been shown to impact the chemical composition of medical cannabis plants, particularly in 'Topaz' cultivar, as noted by Danziger and Bernstein (2021b). However, the increased floral yield biomass appears to be the primary factor in cannabinoid production per plant, not the specific chemical changes induced by the pruning methods (Danziger and Bernstein, 2021b). In addition, salinity has also shown impact on the accumulation of secondary metabolites in cannabis as Yep et al. (2020) reported that the yield and potency of cannabis cultivar 'Nordle' decreased as cannabis roots were exposed to increasing NaCl concentrations.

Amongst the cannabinoids, Δ^9 -THC and CBD are the most important chemotaxonomic markers. This is because the chemotaxonomic markers involve categorizing varieties using the relative proportions of Δ^9 -THC and CBD (Piluzza et al., 2013; Broseus et al., 2010). The use of the relative proportions of Δ^9 -THC and CBD is associated with the fact that these compounds are hardly affected by external factors, including environmental conditions. Therefore, it is often relatively constant throughout the plant's life, which makes it advantageous distinguishing cannabis genotypes using chemotaxonomic markers (Staginnus et al., 2014). Over and above, the THC: CBD ratio of the cannabis raw material is critical for industrial applications and thus assists in selecting genotypes suitable for developing plant material for industrial applications or medicinal purposes (Fischedick et al., 2010).

Chemotaxonomy was used to identify five distinct chemotypes that currently exist in cannabis, which are chemotypes I, II, III, IV and V (Cerrato et al., 2021). Chemotype I represents high THC varieties, possessing CBD of less than 0.5% and Δ^9 -THC content ranging between 1 and 20% or more (Cerrato et al., 2021; Soorni et al., 2017; Khan et al., 2014; Piluzza et al., 2013; Broseus et al., 2010). Chemotype III represents high CBD varieties, possessing Δ^9 -THC content below 0.3% (Schlottenhofer and Yuan, 2017; Khan et al., 2014; Broseus et al., 2010). Chemotype II represents the intermediate type, which possesses an equal amount of Δ^9 -THC and CBD (Khan et al., 2014; Piluzza et al., 2013). Chemotype IV represents cannabigerol (CBG) predominant varieties, and chemotype V represents varieties with no detectable amount of phytocannabinoids (Cerrato et al., 2021).

Therefore, concerning chemotaxonomy, determining the chemical composition of cannabis plays a crucial part in selecting genotypes suitable for developing plant material for industrial applications or medicinal purposes. Various effective techniques have been used to analyze cannabis plant metabolites, including cannabinoids and terpenes (Koo et al., 2023; Birenboim et al., 2022). Nonetheless, these techniques come with certain challenges during sample analyses. Therefore, subsection 2.2.1 briefly discusses the existing techniques and highlight the challenges that need to be overcome to ensure that chemical classification yields the desired results.

2.2.1. Techniques for analyzing cannabis composition for the classification of varieties

Direct analyses of the cannabis plant metabolites have been critical for chemotaxonomy, and this has been achieved through the application

of various analytical techniques (Koo et al., 2023; Birenboim et al., 2022; Cicaloni et al., 2022; Ramos-Guerrero et al., 2022; Gaafar et al., 2018; Leghissa et al., 2018). These techniques include gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS or MS/MS), GC coupled with flame ionization detection (FID), high-performance liquid chromatography (HPLC), HPLC coupled with diode-array detector (DAD), Thin-layer chromatography (TLC), Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry and Ultra-Performance Liquid Chromatography-Mass Spectrometry (SPME-GC-MS and UPLC-MS). (Koo et al., 2023; Birenboim et al., 2022; Cicaloni et al., 2022; Ramos-Guerrero et al., 2022; Stefkov et al., 2022; Gaafar et al., 2018). Among these techniques, vibrational spectroscopy methods, including nuclear magnetic resonance (NMR), infrared (IR), near-infrared (NIR), Fourier transform infra-red (FTIR), Raman, and Fourier transform near infra-red (FT-NIR), are also used for analysis of cannabis secondary metabolites (Birenboim et al., 2022; Ramos-Guerrero et al., 2022; Stefkov et al., 2022).

Nonetheless, GC-FID and HPLC with UV detection remain the most commonly used methods for analysing the phytocannabinoids in cannabis extracts (Koo et al., 2023; Procaccia et al., 2022; Berman et al., 2018; Happyana and Kayser, 2016). The GC analytical method, together with principal component analysis (PCA), has been successfully used to categorize the chemotaxonomic of the cannabis varieties (Happyana and Kayser, 2016). Moreover, the GC analytical method has been used to control the quality of plant materials (Happyana and Kayser, 2016). Cannabis terpenoids, on the other hand, are volatile in nature, and GC coupled with either FID or MS is suggested as the most suitable technique for analysing such compounds (Shapira et al., 2019). Terpene volatility also requires a careful selection of extraction methods, and liquid extraction is commonly used in cannabis (Shapira et al., 2019). The liquid extraction is usually conducted using a variety of solvents, such as ethanol, *n*-pentane, petroleum ether, and chloroform (Birenboim et al., 2022; Shapira et al., 2019).

However, the analytical methods mentioned above pose some challenges during sample analyses. One of the challenges associated with GC coupled with FID and HPLC coupled with UV detection is the required reference compounds to prepare a calibration curve, which is generally unavailable for many cannabinoids (Hazeekamp et al., 2004). Both analytical methods, GC with FID and HPLC with UV detection, can also be affected by chlorophyll or lipids impurities in the sample (Hazeekamp et al., 2004). As a result, these methods require a sample clean-up step before analysis (Hazeekamp et al., 2004). Moreover, GC with FID requires prior derivatization to distinguish between cannabinoids and their carboxylic counterparts (Procaccia et al., 2022; Hazeekamp et al., 2004). Using the GC analysis method, measuring both acid and neutral cannabinoids is challenging because decarboxylation may occur in the injection ports (Berman et al., 2018). As a result, only decarboxylated phytocannabinoids are measured (Berman et al., 2018). It has also been reported that cannabinoids may degrade (oxidize, isomerize) in the injector port and column (Berman et al., 2018).

Berman et al. (2018) suggest that metabolite extracts can be derived through silylation for measuring both acid and neutral phytocannabinoids; however, obtaining a complete derivatization yield is still challenging. Hence, LC is mostly preferred over GC as it allows for determining the cannabinoid composition of both acid and neutral derivatives without a derivatization step (Citti et al., 2018). HPLC has been identified as the only most suitable technique for the simultaneous analysis of acid and neutral compounds; however, it must still be coupled with UV detection to obtain the desired results (Hazeekamp et al., 2004). However, it is also flawed as it is usually challenging to separate the entire components and thus requires multiple chromatographic runs (Hazeekamp et al., 2004). It also becomes difficult to detect contaminations with UV detection (Hazeekamp et al., 2004). Proton nuclear magnetic resonance (^1H NMR) has been identified as the solution to this challenge as it allows analysing cannabinoids without conducting any chromatographic purification, chromatographic separation

or use of certified reference standards (Stefkov et al., 2022; Hazekamp et al., 2004).

A proton nuclear magnetic resonance (^1H NMR) analytical method has been identified as a novel approach for characterizing cannabis cultivars and is suitable for metabolic profiling as it can describe the observable chemical profile (Lesma et al., 2014; Happyana and Kayser, 2006). Over and above, ^1H NMR is suggested to have high accuracy, highly reproducible within a short analysis time, and effective in analyzing large samples and requires simple steps for sample preparation (Stefkov et al., 2022; Gaafar et al., 2018; Lesma et al., 2014; Hazekamp et al., 2004).

Recently, there has been a growing importance in characterizing the chemical profile of cannabis inflorescence throughout its development, before harvesting, as it plays a crucial role in determining the time of chemical maturation of the plants (Harpaz et al., 2022). This is associated with the fact that the cannabinoid concentration tends to increase as the inflorescence and overall plant development progress (Harpaz et al., 2022). Consequently, this information is critical for determining the optimal stage with the aim of achieving the highest possible cannabinoid levels (Harpaz et al., 2022). However, conventional and commonly used techniques such as GC-FID and HPLC with UV detection use lab-based non-portable equipment and require sample preparation and a complex, time-consuming approach that demands specialized expertise and thus, do not allow its use for on-site analysis (Monari et al., 2023; Harpaz et al., 2022). This has resulted to numerous methods, such as electrochemical detection, being proposed as novel approach over conventional techniques for rapid screening of cannabinoids in cannabis plants (Monari et al., 2023; Pholsiri et al., 2022).

Electrochemical detection, which includes portable biosensor essays, provide on-site analysis capabilities with high sensitivity, rapid and low-cost analysis, and has proven to yield the desired results (Harpaz et al., 2022; Pholsiri et al., 2022). The study by Monari et al. (2023) compared two electrochemical sensors, namely screen-printed electrodes (SPEs) modified with carbon black (CB) (SPEs-CB) and SPE modified with poly-(3,4-ethylenedioxythiophene) (PEDOT) (SPE-PEDOT) electrodes, with the HPLC for detection and quantification of cannabinoids in different types of cannabis inflorescences. Their study revealed eminent correlation between the results obtained by the electrochemical method and those obtained by HPLC (Monari et al., 2023). Furthermore, electrochemical detection allows monitoring the THC levels to ensure that the plant material consist of the permitted levels (Pholsiri et al., 2022).

However, the electrochemical detection has also faced some challenges, primarily stemming from the closed oxidation potential of THC and CBD due to their similar chemical structures, which resulted to overlapping peaks (Pholsiri et al., 2022). Additionally, other cannabinoids such as cannabigerol (CBG) and cannabinol (CBN) were interfering with the quantification of THC and CBD (Pholsiri et al., 2022). However, these issues have been effectively resolved through the combination of large-pore silica gel coated on paper chromatography, which enables the high-efficiency separation of THC and CBD while minimizing interference from other cannabinoids (Pholsiri et al., 2022). As a result, the paper chromatography procedure has been integrated with electrochemical detection for detection and quantification of THC and CBD in cannabis oil (Pholsiri et al., 2022). Despite these challenges, the advantages of electrochemical detection have established it as a highly suitable on-site tool for detection and quantification of cannabinoids (Harpaz et al., 2022).

3. Factors critical for cannabis seed germination and seedling development

3.1. Internal factors

3.1.1. Seed quality

Seed quality generally comprises viability, seed lot purity, health, mechanical damage, physiology, germinability, and physical

characteristics (Finch-Savage and Bassel, 2016; Rahman and Cho, 2016). In the field production, a high-quality seed has strong vigour and steady germination and establishes quickly in the field (Kandpal et al., 2016). Furthermore, a high-quality seed is more tolerant of biotic and abiotic stresses and more efficiently utilizes available radiation and nutrients (Bianchini et al., 2021). As a result, these seeds produce seedlings that provide rapid soil coverage, which is advantageous for minimizing the potential negative effects of weed-crop competition (Bianchini et al., 2021).

However, seeds get exposed to various threats during production, including freezing and heat damage, mechanical damage, fungal infection, mildew, and insect damage (Zhang et al., 2020). These factors normally result in a decline in seed vitality; consequently, their germination and emergence get affected, directly impacting yield (Medeiros et al., 2020; Zhang et al., 2020). Seed viability is also influenced by various features, including plant species, seed development and maturation environmental conditions, the physiological status of the matured seed, and techniques for seed storage (discussed in depth in 3.2.1 of subsection 3.2) (Parihar et al., 2014).

Furthermore, seeds are prone to damage during drying, processing, and storage after harvest for later sale, which may decrease seed viability and vigour (Feng et al., 2018). For instance, it is reported that quality indicators of cannabis seeds are influenced by various drying factors, such as layer thickness, air flow rate, flow temperature above 60 °C, and layer mixing (Oseyko et al., 2021). Developing hybrids from different seed varieties also leads to severe seed purity degradation, consequently decreasing crop yield (Zhang et al., 2020; Huang et al., 2016). The development of hybrids is a serious issue in cannabis, as there are currently hundreds of cultivars due to a long history of hybridization (Jin et al., 2017).

Hence, successful plant breeding and production requires high-quality seeds (Feng et al., 2019; Goodarziian Ghahfarokhi et al., 2014). Using high-quality seeds is advantageous in plant breeding as it minimizes the costs of conducting field trials and increases the likelihood of discovering a superior crop variety (Medeiros et al., 2020). High-quality seed is a good start for plant growth in plant production as it can increase yield significantly, indicating an abundant harvest (Feng et al., 2019; Rahman and Cho, 2016).

Certification of seed quality attributes is usually accomplished by measuring seed germinability or physicochemical attributes through widely used methods, germination and vigour tests (Medeiros et al., 2020; Feng et al., 2018). However, it is difficult to screen and categorize viable and non-viable seeds in large quantities using these conventional methods or naked eye as they are time-consuming, subjective, and destructive (Medeiros et al., 2020; Ambrose et al., 2016). Over and above, they are inadequate in detecting any changes in the chemical composition and internal anatomical features of seeds, which are characteristics that relate to seed viability and vigour (Medeiros et al., 2020). Following the shortcomings linked to conventional methods, there has been a desire for a method that can provide detection of seed quality in a quick, reliable, non-destructive, and objective manner (Medeiros et al., 2020; Feng et al., 2019; Feng et al., 2018).

Rahman and Cho (2016) suggest machine vision, spectroscopy, hyperspectral imaging, and X-ray imaging for seed quality testing. These techniques are highly recommended for collecting information on complex traits related to seed quality (Medeiros et al., 2020). Fourier Transform Near-Infrared (FT-NIR) spectroscopy has been previously used to determine the viability of seeds, and it detects seed compounds by acquiring a large number of spectral data details (Medeiros et al., 2020). Near-infrared (NIR) spectroscopy is a rapid technology previously used to examine the chemical properties of complex food matrices and intact seeds or grains (Caporaso et al., 2018).

On the other hand, the hyperspectral imaging technique has recently been recognized as a great analytical tool for assessing seed quality and safety in a non-destructive manner (Mukasa et al., 2020; Feng et al., 2019). It has been widely used to assess seed viability, vigour, defect,

disease, and cleanness and determine seed composition (Feng et al., 2019). Moreover, NIR-HSI is being explored for its benefits in the seed industry to evaluate seed viability against traditional methods (He et al., 2019). NIR-HSI technology is also perceived to have high detection speed and is inexpensive for seed viability detection (Cui et al., 2020).

However, such studies on cannabis seed quality are still limited, and this has been associated with its ban for many centuries. Chumchu and Patil (2023) recently used computer vision and machine learning to classify cannabis seeds into 17 categories according to their external features, such as colour, shape, and size.

3.1.2. Seed dormancy

Seed dormancy and germination are interrelated physiological traits significantly impacting seed plant adaptation and survival (Yan and Chen, 2020). When orthodox seeds are produced, they develop desiccation tolerance and remain dormant until the ambient environment favours germination (Yan and Chen, 2020). However, a dormant seed may not germinate even under favourable conditions (Longo et al., 2021; Yan and Chen, 2020; Chahtane et al., 2017). Seed dormancy is categorized based on the embryo's developmental state during seed dispersal, seed physical features, and/or physiological responses to environmental factors (McGinty et al., 2021).

According to Longo et al. (2020), dormancy may start during seed maturation and then be finely regulated by many transcription factors interacting in a complex molecular network that regulates hormonal levels and signaling. Plant hormones known as abscisic acid (ABA) and gibberellic acid (GA) are critical for inducing, maintaining, and releasing seed dormancy (Longo et al., 2021). These hormones act antagonistically; while ABA promotes dormancy during seed maturation and inhibits seed germination, GA promotes seed germination (Longo et al., 2021; Yan and Chen, 2020). Thus, when the level of ABA is reduced, and GA is increased, the seed dormancy gets released, and subsequently, germination commences (Yang et al., 2020). Increased GA levels after imbibition are required for the rupture of the testa and endosperm, as well as the emergence of the radicle (Yang et al., 2020). Thus, dormancy breaking encompasses a net shift to increased GA biosynthesis and ABA degradation, resulting in a low ABA/GA ratio (Xu et al., 2020).

Independent of how seeds are released from dormancy, there have been various studies on the release of seed dormancy using different methods, and some assessing and quantifying the dormancy level of seed lots (Pausas and Lamont, 2022; Buijs et al., 2018). Among these methods are cold stratification, in which seeds imbibe cold for a period of time, and dry after-ripening (AR), in which the seeds are stored in a dry environment (Chen et al., 2020; Elias et al., 2020; Buijs et al., 2018). However, environmental factors such as temperature and soil moisture are critical in dormancy release (Pausas and Lamont, 2022). Previously, it has been demonstrated that cold stratification releasing seed dormancy varies with soil moisture content and temperature and the duration of the cold stratification exposure (Chen et al., 2020). It is reported that a temperature of about 0–10 °C of cold stratification is effective for seed dormancy release (Elias et al., 2020). However, it is highly dependent on the species as there are also differences between species and varieties in relation to the degree of dormancy (Elias et al., 2020).

Studies on cannabis seed dormancy and dormancy release, though very limited owing to illegality issues in the past centuries, have revealed that cannabis also experiences a certain level of dormancy (Jovićić et al., 2019). Wild cannabis, also known as feral or ditch weed, was reported to stay dormant in soils for up to 10 years and still be viable (Jovićić et al., 2019). The study by Elias et al. (2020) showed dormancy was at its peak in newly harvested seeds of 'Merlot' and 'Berry Blossom' hemp varieties, which they could break with wet prechilling treatment at 10 °C within 5 days. Prechilling is considered hydropriming because it activates germination enzymes to initiate the breakdown and movement of stored seed reserves into embryonic axes during the treatment (Elias et al., 2020).

In another study, Islam et al. (2021a) found that wet prechilling at 4 °C for 5 days decreased the germination of 'Ferimon', 'Han NW', and 'Morpeth' hemp varieties compared to control but enhanced shoot and root growth. Thus, even though the study by Elias et al. (2020) recommended prechilling seeds at 10 °C for breaking dormancy and attaining maximum germination, assessing its effect on early seedling growth parameters, such as shoot and root growth, is still critical. Besides prechilling, Islam et al. (2021a) found that other pre-treatment methods, such as GA₃ at 500 and 1000 mg·L⁻¹ and chlorine dioxide (ClO₂) at 500 and 1000 mg·L⁻¹ also decreased the germination of 'Ferimon', 'Han NW' and 'Morpeth' hemp varieties. Similar to prechilling at 4 °C, GA₃ and (ClO₂) enhanced the shoot and root growth of these varieties compared to the control. Over and above, it was revealed that research studies assessing the germination performance of different cannabis varieties under various pre-treatments, including phytohormones and scarification are still essential (Önol and Yildirim, 2021; Utami et al., 2021).

3.2. External factors

3.2.1. Seed storage conditions

Seeds are not always intended for immediate use after collection and are often stored for varying amounts of time before being used in propagation programs (De Vitis et al., 2020). During prolonged storage, the seed quality, viability, and vigour decrease gradually with a change in lipid peroxidation, which is known as seed deterioration (Feng et al., 2018; Kandpal et al., 2016; Zheng and Ma, 2014). Like any other seed, the compounds, including lipids and proteins in cannabis seed, as well as other quality characteristics deteriorate during storage, entering an aging process after natural maturity and consequently affecting their further processing (Oseyko et al., 2021; Feng et al., 2018; Kandpal et al., 2016). Seed degradation is largely caused by free radicals-mediated lipid peroxidation, enzyme inactivation or protein degradation, cellular membrane disruption, and damage to genetic (nucleic acid) integrity (Zheng and Ma, 2014). Seed deterioration is mainly influenced by genetics and environmental interactions during seed maturation and harvests, in which temperature, seed moisture, and relative humidity are the leading influential environmental factors (Feng et al., 2018; Suriyong et al., 2015; Zheng and Ma, 2014; Mohammadi et al., 2011).

In addition, crops with high oil content have been found prone to seed deterioration due to the lower chemical stability of lipids in relation to starch (Prudente and Paiva, 2018; Suriyong et al., 2015). The oil content of the oil crops, such as soybean, peanut, and sunflower, is a major contributor to the reduction in seed storage potential (Suriyong et al., 2015). The high protein content also contributes to the reduction in seed storage potential due to the high affinity of this substance to water (Prudente and Paiva, 2018). Thus, cannabis seed is no exception as it contains an oil content of 25 – 35%; in comparison, sunflower possesses an oil content of 35–42%, peanut has 45 – 56%, and soybean has 18 – 22% (Suriyong et al., 2015). Cannabis also contains a protein content of 20 – 25%, whereas sunflower has a protein content of 20–40%, peanut has 22 – 33%, and soybean has 38– 42% (Suriyong et al., 2015).

Cannabis seed is orthodox; thus, it can be stored in seed banks for an extended time, and its viability may not be impacted (Suriyong et al., 2015; Parihar et al., 2014). However, seeds require storage with lower temperature and humidity control as it often delays physiological and physiochemical changes and, ultimately, seed aging, thereby leading to an extended viability period (Feng et al., 2018; Kandpal et al., 2016; Mohammadi et al., 2011). Previous studies suggest that 5 °C and 5–7% relative humidity are the ideal conditions for the preservation of cannabis seed viability under long-term storage of 5 years and 15 °C for a storage period of up to 12 months; however, seeds must have a moisture content of 6% (Oseyko et al., 2021; Oseyko et al., 2020). Seed moisture content reportedly plays a significant role in cannabis seed storage as an 11% moisture content at 20 °C significantly reduced the germination capacity within 18 months compared to when seeds had a

moisture content of 6% (Oseyko et al., 2021).

Some farmers may afford to maintain such favourable conditions, with the right seed moisture content, humidity, and temperature level for storing seeds for further processing. However, such conditions are not always available for small resource-poor farmers in developing countries; hence, issues of seed deterioration have become a serious problem (Mohammadi et al., 2011). Resource-poor farmers from developing countries often save seeds from harvest under normal conditions for use in the following planting season (Hlatshwayo et al., 2021). This form of seed saving is disadvantageous as it is reported that normal storage conditions resulted in a sharp decline in seed germination energy and capacity of Ukrainian cannabis varieties, which was completely lost after four years of storage (Oseyko et al., 2021). Small and Brookes (2012) also reported that the longevity of cannabis seeds decreases rapidly to about 70–80% after 2 years under uncontrolled storage conditions.

3.2.2. Water/soil moisture

Water availability is a paramount limiting factor in crop development as seed germination begins with water uptake (López et al., 2021; Wolny et al., 2018). Therefore, an optimum moisture level is essential for imbibition, enzyme activation, embryo stimulation, reserve mobilization, and plumule emergence and elongation (Wijewardana et al., 2018). Reduced soil moisture leads to a reduced percentage and rate of germination and, subsequently, low seed germination (Rahmah et al., 2020; Wijewardana et al., 2018). Reduced water availability also results in high mortality rates and low crop performance during the initial stages of crop development (Tounekti et al., 2020). Cannabis is sensitive to soil moisture conditions and requires high moisture during its growing season, especially throughout the first six weeks of plant establishment (Adesina et al., 2020; Williams and Mundell, 2018; Cole and Zurbo, 2008). Williams and Mundell (2018) suggested that a lack of soil moisture at planting hinders germination and affects the overall cannabis yield. Therefore, adequate moisture must be ensured at the germination stage to achieve maximum seed germination and early canopy closure, which is beneficial for the plant as weeds can be suppressed effectively (Cole and Zurbo, 2008).

Reduced soil moisture or water deficit, also known as drought, is largely determined by various factors, including rainfall patterns, the moisture-holding capacity of the soil, and water losses through evapotranspiration (Fahad et al., 2017). It threatens the success of plant establishment in areas with low rainfall, xeric soil, and high temperatures during warm seasons such as spring and summer (López et al., 2021). Poor germination and impaired seedling establishment are the initial manifestations of drought (Fahad et al., 2017). Thereafter, the extreme water deficit disrupts growth, nutrient and water relations, photosynthesis, and assimilate partitioning in plants (Babaei and Ajdarian, 2020). It eventually leads to the death of the plant and, ultimately, a significant reduction in crop yields through the negative impacts of water stress on plant growth, physiology, and reproduction (Babaei and Ajdarian, 2020; Fahad et al., 2017).

In cannabis, Du et al. (2022) reported that water stress induced through 20% PEG reduced the germination rate and germination potential and delayed radicle and hypocotyl growth in hemp seedlings of 'Yunma 1' YM and 'Bamahuoma' BM. In 'Black Label', an industrial hemp cultivar, water deficit reduced biomass, seed yield, and water potential (Gill et al., 2022). Notably, 'Black Label' did not increase the root production relative to shoot under water stress like other plants (Gill et al., 2022). Thus, hemp may have yet undiscovered alternative mechanisms for optimizing water use within the plant without allocating a higher proportion of biomass to the roots (Gill et al., 2022). Water stress, together with continuous lighting, has been found beneficial for seed ripening in cannabis (Schilling et al., 2023).

3.2.3. Salinity

Salinity is a serious soil degradation arising from natural causes and

human-mediated activity, including irrigation in arid and semi-arid regions (Mohammad et al., 2014). Salinity severely affects plant growth and productivity through water deficit and ionic toxicity (da Silva et al., 2019; Al-Musa et al., 2012; Voigt et al., 2009). The first effect of high salinity is a decrease in soil water potential and thus restricting water uptake by the seeds, which delays seed imbibition and radicle protrusion (da Silva et al., 2019; Voigt et al., 2009). As seeds germinate, they absorb saline ions, which trigger toxic effects on several biochemical and physiological processes, delaying the emergence of seedlings and increasing mortality (Voigt et al., 2009). Salinity stress also leads to overproduction of reactive oxygen species (ROS) and eventually to toxicity which is linked to their reactions with numerous cell components (Sharma et al., 2013; Wang et al., 2012). This toxicity then causes a cascade of oxidative reactions and the consequent inactivation of enzymes, lipid peroxidation, protein degradation, and DNA damage (Wang et al., 2012). Together, these effects restrict growth and development and thus reduce crop yields (Sarabi et al., 2017; Krasensky and Jonak, 2012).

Hemp is known for its high tolerance to saline environment stress due to its wide distribution, deep root system, fast growth, high biomass, strong resistance to stress, and ease of cultivation (Cheng et al., 2016). However, salt stress tolerance in various species, including hemp, varies with the salt type and salt concentration and among varieties and with plant growth and development stages, affecting alterations in plant morphology, anatomy, and physiology (Di Mola et al., 2021; Hu et al., 2018; Cheng et al., 2016). It also varies with plant growth and development stages, affecting alterations in plant morphology, anatomy, and physiology (Di Mola et al., 2021; Cheng et al., 2016). Furthermore, salt tolerance is associated with seed reserve mobilization, and the tolerant seeds reportedly tend to accumulate more reserves (Hu et al., 2018). For instance, salt stress negatively affected seed germination and seedling development of the 'Yunma 5' and 'Bamahuoma' hemp varieties through inhibition of germination rate and the length of the radical and hypocotyl in the study conducted by Hu et al. (2018). However, 'Yunma 5' was found to be more salt tolerant as the germination rate of this variety was not significantly reduced by the salinity levels up to 150 mM NaCl and Na₂SO₄, whereas that of 'Bamahuoma' was significantly reduced at salinity level as low as 100 mM NaCl and 50 mM Na₂SO₄ (Hu et al., 2018).

Furthermore, radicles and hypocotyls at each salt treatment of Na₂CO₃, NaHCO₃, Na₂SO₄, and NaCl were longer in 'Yunma 5' compared to in 'Bamahuoma'. However, the radicle and hypocotyl growth were more salt-sensitive compared to the germination rate as the salt concentration increased to 300 mM (Hu et al., 2018). The salt stress tolerance also varied with salt type among the varieties, such as saline and alkaline; however, both varieties exhibited alkali sensitivity (Hu et al., 2018). This is because sodium ions in salt stress cause ion toxicity and reduce the osmotic potential, leading to osmotic stress and, eventually, plant metabolic disorders (Fang et al., 2021). On the other hand, alkali stress induced by NaHCO₃ and Na₂CO₃, the most commonly used compounds, results in similar effects as saline stress. However, it further increases the pH, which typically disturbs the cell pH stability, destroys cell membrane integrity, and reduces root vitality and photosynthetic function (Fang et al., 2021). Moreover, Yep et al. (2020) found that the yield and potency of the drug-type cultivar 'Nordle' decreased with continuous root exposure to increasing NaCl concentrations.

3.2.4. Temperature

The environmental conditions to which the plants are exposed often influence the ecophysiological responses, and among the key factors for seed germination is the temperature (Zucareli et al., 2015). Temperature influences seed germination rate and maximum seed germination in three ways: seed aging, dormancy loss, and germination (Gajanayake et al., 2011). Temperature is also a modifying factor as it influences the germination percentage and rate by affecting the imbibition rate and mobilization of storage reserves (Reis et al., 2020; Zucareli et al., 2015;

Sikder et al., 2009). Furthermore, it influences the supply of substrates necessary for seed reserve mobilization, including water, and consequently affects plant growth and development (Sikder et al., 2009). The mobilization of seed reserves and seed germination also vary in different temperature regimes; hence, the temperature is a modifying factor in germination (Sikder et al., 2009).

Cannabis is an annual summer crop; thus, it grows well in warm temperatures averaging between 15 and 27 °C (Humphries and Florentine, 2019). The study by Kumar et al. (2020) reports that the optimum germination temperature for the *Cannabis sativa* of 'Kausani' accession was 25 °C. On the other hand, a study by Byrd (2019) found that the tested hemp cultivars showed a difference in temperature and origin as germination percentage decline at temperatures above 25 °C was observed for Canadians and at about 30 °C for Northern European cultivars. Byrd (2019) also found that the germination percentage declined rapidly for all cultivars at temperatures near 40 °C and displayed limited germination capacity at temperatures as high as 45 °C. Generally, extreme and fluctuating temperatures are critical in the sense that they restrict plants' distribution, adaptability, and yield potential (Gajanayake et al., 2011). High temperature can reduce the germination potential of the seeds as a result of damage to the proteins, interrupted protein synthesis, inactivation of major enzymes, and damage in membranes, resulting in poor germination and stand establishment (Fahad et al., 2017). High temperature can also majorly affect the process of cell division and favour oxidative damage (Fahad et al., 2017). Furthermore, it is reported that high temperatures may accelerate maturity by inducing flowering, resulting in stunted cannabis growth (Humphries and Florentine, 2019). Low soil temperatures at sowing also tend to influence plant populations and may result in poor standing, and notably, cannabis is intolerant to frost (Humphries and Florentine, 2019; Gajanayake et al., 2011; Cole and Zurbo, 2008).

Over and above, temperature is also a limiting factor in cannabis growth as it affects net photosynthesis (P_N) (Jin et al., 2019). Low temperatures slow P_N , and the P_N stops when there is excessive heat, resulting in plants using up energy in cooling under high temperatures by acquiring water and transpiring it through the stomata (Jin et al., 2019).

3.2.5. Lighting

Light influences germination and other aspects of plant development and physiology and the content of its pigments and other metabolites (Jin et al., 2019; Namdar et al., 2019; Chandra et al., 2015). Light features, such as light quantity (intensity), light quality (spectrum), and photoperiod (the duration of exposure to light), affect the functions of the plant through the regulation of photosynthesis, photoperiodism, and photomorphogenesis (Cheng et al., 2023; Wei et al., 2021; Magagnini et al., 2018). Cannabis is a photophilic plant, and thus, its growth and development are directly influenced by light intensity and quality variations (Li et al., 2023; Cheng et al., 2022). For instance, the study conducted by Rodriguez-Morrison et al. (2021) found that the yield in relation to harvest index and the size and density of the apical inflorescence in cannabis increased with the increasing light intensity. Light intensity also greatly influences the synthesis of plant metabolites. Notably, Rodriguez-Morrison et al. (2021) also found that light intensity had no effects on the potency of cannabinoids and had minor effects on the potency of terpenes (Rodriguez-Morrison et al., 2021).

The cannabis plant is sensitive to photoperiod and is a short-day plant, which strongly influences the productivity of different crop varieties (Ahrens et al., 2023; Adesina et al., 2020). Cannabis requires 2 days of darkness for radicle protrusion, whereas seedlings require continuous light for 2 weeks to promote maximum biomass accumulation (Pierroz, 2023; Schilling et al., 2023; Bilodeau et al., 2019). The cannabis plant also measures the length of daylight falling onto its leaves to regulate its development and flower production (Thomas, 2012). It is reported that cannabis requires 12 h of uninterrupted darkness to transition from the vegetative to the flowering stage (Schilling et al.,

2023; Bilodeau et al., 2019; Chandra et al., 2017; Thomas, 2012). The light must then be reduced when the seedlings have fully grown into a flowering stage. After that, continuous lighting is required for seed ripening (Schilling et al., 2023).

However, the effect of light generally varies with several factors, including the photoperiod sensitivity of a genotype. Schilling et al. (2023) found that photoperiod-insensitive cannabis cultivars of 'Finola' flowered under different light regimes of ultra-short days (8:16), short days (12:12), long days (16:8), and continuous light (24:0). While on the other hand, the photoperiod-sensitive cannabis plants of 'Fedora 17' and 'Felina 32' cultivars flowered shortly after exposure to 12 h of uninterrupted darkness, with reduced or no flowering under continuous light and long days. Furthermore, light impacted the physiology of the photoperiod-sensitive cannabis plants, as plants produced under ultra-short days were shorter and feeble than those grown under short days, long days, and continuous light (Schilling et al., 2023). Moreover, 'Fedora 17' reportedly yielded the highest number of seeds under short-day conditions with no seed setting under continuous light. On the contrary, 'Finola' yielded the highest number of seeds under continuous light compared to the other light regimes.

Notably, specific light wavelengths enable plants to adapt and modify their biological cycle, and plants use different types of photoreceptors, including phytochromes, to perceive changes in light quality (Demotes-Mainard et al., 2016). Blue light has been recognized as the best quality light for hemp as it has been found to promote growth parameters, such as fresh and dry biomass of the shoot, number of leaves per plant, stem diameter, root length, and chlorophyll content (Cheng et al., 2023; Cheng et al., 2022). It also promotes net photosynthesis, stomatal conductance, and transpiration (Cheng et al., 2023; Cheng et al., 2022). Supplemental blue light has also been found to increase cannabinoid concentration (% w/w), and green light leads to the accumulation of both cannabinoids and terpenes (Morello et al., 2022). Wei et al. (2021) also found that the higher proportion of blue light promoted the synthesis and accumulation of cannabinoids in hemp.

As a result, most commercial production of cannabis occurs under greenhouses with supplemental electric lighting or growth chambers with sole source electric lighting to ensure sufficient light is provided for the plants (Trancoso et al., 2022; Hawley et al., 2018). Light wavelengths/spectrum is one of the important factors in controlled cultivation as it regulates growth, development, and the accumulation of metabolites (Sng et al., 2021). In the cultivation of cannabis under a controlled environment, various lamps, such as high-pressure sodium (HPS) lamps and light-emitting diodes (LEDs), have been used to provide the required light spectrum (Trancoso et al., 2022). However, LEDs have been widely used for their versatile mobility, narrow spectrum range, energy efficiency, and longevity compared to HPS lamps (Morello et al., 2022; Wei et al., 2021).

Artificial lighting and light spectrum influence plant development and the profile of terpenes and cannabinoids (Morello et al., 2022; Danziger and Bernstein, 2021a; Potter and Duncombe, 2012). Terpenes represent the largest group of cannabis phytochemicals found abundantly on the surface of the female inflorescence (Calvi et al., 2018). They are responsible for the unique aromatic properties and influence the flavour quality of cannabis inflorescence and its therapeutic effect (Calvi et al., 2018). Lighting has been recognized for producing cloned female cannabis yielding a drug product of consistently high potency (Morello et al., 2022; Potter and Duncombe, 2012). Higher cannabinoid concentration has been found under LED light compared to HPS treatment (Magagnini et al., 2018). As previously mentioned in subsection 2.2, an irradiation with UV-B at a wavelength of 280–320 nm has also been found to increase the THC content of drug-type cannabis varieties (Potter and Duncombe, 2012; Lydon et al., 1987). This is associated with the fact that plants use the wavelength of blue light and UV to synthesize carotenoids and anthocyanins (Islam et al., 2021b). Generally, UV-B radiation penetrates plant tissues, inducing DNA damage, photo-inhibition, and lipid peroxidation, and resulting in growth, physiology,

and productivity retardation (Islam et al., 2021b; Chen et al., 2018; Lydon et al., 1987). The plants then produce secondary metabolites in higher concentrations, in this case, cannabinoids, which absorb and prevent actinic UV-B radiation (Takshak and Agrawal, 2019; Chen et al., 2018; Ning et al., 2012; Lydon et al., 1987). However, the response of cannabis to spectral changes varies with cultivars (Li et al., 2023; Danziger and Bernstein, 2021a).

Various studies have investigated the influence of light intensity, spectrum, and photoperiodism on cannabis growth. This includes the study conducted by Livadariu et al. (2019), where they reported that LED treatments were most effective in promoting fresh weight and sprouting in hemp compared with the control (sunlight). However, such information is lacking, or little is known with respect to the effect of light on cannabis germination parameters. Yet, plants generally respond to light variations for the completion of their life cycle, including seed germination, while different colors of light influence differently on plant growth stages (Cheng et al., 2022). Furthermore, the light requirement may act as a mechanism that indicates conditions suitable for seed germination (Motsa et al., 2015). This is why some seeds germinate better under only light or darkness, and some seeds can germinate either under light or darkness (Flores et al., 2016; Motsa et al., 2015). Meeting the light requirement is also important as it prevents the depletion of stored seed reserves and, thus, aids the plant species to survive (Motsa et al., 2015).

4. Conclusion and future prospects

The physiology of cannabis germination and seedling establishment is not very well known due to limited research studies on the crop resulting from being banned for centuries owing to its psychoactive compound THC. The review provides useful information for choosing varieties for application in different industries to meet the growing demand of the pharmaceutical industry and assist growers in meeting the purity standards of the target market. It also highlights the effect of intrinsic and extrinsic factors on seed germination and seedling development and their impact on the final product in terms of yield and chemical composition. Furthermore, it highlights the progressive initiatives in detection and quantification of cannabis chemical profile from inflorescence and plant development to harvested material. Through this overview, it is clear that research on the classification of different varieties is still much needed, not only to determine the end-use product but to determine the response of varieties to intrinsic and extrinsic factors during germination and seedling development on the morphological and biochemical level.

Notably, few studies have quantified the chemical composition of cannabis using highly sought-after advanced technologies such as ¹H NMR. Thus, this overview suggests that these technologies should also be used for classifying cannabis varieties, as it is known that chemical profiling has been useful in classifying cannabis varieties. The use of technology devices such as NIR-HSI for cannabis classification is also recommended, as they have the potential to differentiate varieties and determine sex at a seed level effectively. This is critical in cannabis to minimize the yield losses, labour required, and high costs of cannabis cultivation. Furthermore, this overview suggests that SEM is incorporated into the investigations on cannabis classification. This follows that SEM reportedly has a high potential for reviewing phenetic linkages and classification of various crop species. This is because cannabinoid composition and non-chemical characteristics have been previously used to categorize cannabis species, and there is still ongoing evaluation. Therefore, seed morphology seems prospective to solve taxonomy-related issues, hence the suggestion of SEM research.

Determining seed quality before planting is also an important factor, just as variety classification, for determining seed vitality, germination, and emergence and achieving high yields. Thus, the use of advanced non-destructive techniques is recommended to collect information on complex traits related to seed quality and to reduce the turnaround time.

Therefore, this overview recommends investigating the efficiency and effectiveness of machine learning, spectrometry, X-ray imaging, FT-NIR, NIR spectroscopy, and hyperspectral imaging in detecting cannabis seed quality. The investigations can further incorporate different storage conditions to study their influence on seed quality, germination, and plant development. However, these investigations should be mindful of inequalities among cannabis growers in relation to access to controlled storage conditions and the rising climate change, and therefore, must consider a wide spectrum of storage environmental variations.

Besides seed quality, cannabis germination is influenced by the embryo's developmental state, nutrients stored in the endosperm or embryo, and environmental factors. However, there are very limited studies on these factors in cannabis. Therefore, this overview recommends investigations on cannabis dormancy and different techniques for releasing seed dormancy and their interaction with environmental conditions. It also recommends studies on hydrolysis and mobilization of seed reserves during germination and how these processes are influenced by intrinsic factors as well as extrinsic factors, especially considering the ever-increasing climate change.

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Declaration of Competing Interest

None.

Data Availability

No data was used for the research described in the article.

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