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Integrating agro-morphological traits and chemical assays for genotype characterization in sesame (*Sesamum indicum* L.)

Narayana Reddy B[1*], Bharati D[2], Rupesh Kumar Reddy B[2], Mohan Naidu G[1]
[1] Sri Venkateswara Agricultural College, ANGRAU, Tirupati, Andhra Pradesh, India
[2] Regional Agricultural Research Station, ANGRAU, Tirupati, Andhra Pradesh, India
Corresponding Author: Narayana Reddy B

Abstract
Sesame (*Sesamum indicum* L.) is a nutritionally rich and economically important oilseed crop with considerable potential for genetic improvement. This review synthesizes current approaches for the characterization of sesame genotypes, integrating agro-morphological descriptors and chemical assays. Agro-morphological characterization remains a cornerstone for assessing genetic variability, encompassing key quantitative and qualitative traits such as plant height, branching pattern, days to flowering, capsule attributes, seed weight, and oil content. Substantial phenotypic variation observed within and among sesame populations highlights its utility in selection and breeding programs. However, reliance solely on morphological traits is constrained by environmental influences and the labor intensive nature of phenotypic evaluation.To enhance precision and efficiency in varietal identification, chemical assays including sodium hydroxide (NaOH), potassium hydroxide (KOH), gibberellic acid (GA_3) response, and 2,4-D soak tests—have been widely employed. These methods are rapid, reproducible, and effective for distinguishing genotypes, detecting admixtures, and classifying germplasm into distinct groups, thereby complementing morphological assessments. Furthermore, under the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001, genotype registration is governed by Distinctiveness, Uniformity, and Stability (DUS) criteria, underscoring the importance of robust characterization frameworks. Comprehensive characterization and conservation of sesame germplasm are therefore imperative for safeguarding genetic resources and enabling their strategic utilization. Such efforts provide a critical foundation for parental selection and the development of high-yielding, stable, and climate resilient sesame varieties.

Keywords: *Sesamum indicum*, agro-morphological characterization, chemical assays, genetic diversity, varietal identification, germplasm characterization, plant breeding

Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest domesticated oilseed crops, valued for its high nutritional quality and economic importance Its adaptability to marginal environments and resilience to abiotic stresses make it a crucial crop for smallholder farmers in developing countries (Dossa *et al.*, 2017) [9]. Africa is considered the primary center of origin of sesame, from where it spread to West Asia and subsequently to India, China, and Japan, which later became secondary centres of diversification (Weiss, 1983) [54]. The crop thrives in tropical and subtropical climates, requiring relatively low inputs and exhibiting tolerance to drought conditions.

Sesame seeds are rich in proteins, unsaturated fatty acids, vitamins (E and B), essential minerals such as calcium, iron, and magnesium, and bioactive antioxidants. The oil is highly stable and nutritionally valuable due to the presence of lignans such as sesamin, sesamol, and sesamolin, along with a favorable fatty acid profile (Suja *et al.*, 2004) [48]. Globally, sesame is cultivated across diverse agro-ecological regions, from semi-arid tropics to temperate zones. In India, it occupies about 10.39 lakh hectares with an annual production of 4.29 lakh tonnes and an average productivity of 413 kg ha^{-1} (India Stat, 2023[14]–2024).

Despite its importance, sesame productivity remains low and has shown a declining trend, mainly due to the lack of high-yielding and disease-resistant cultivars. Therefore, the development of climate-resilient and high-yielding varieties is imperative. The success of such breeding programmes largely depends on the identification and utilization of genetically diverse and superior parental lines. In this context, characterization plays a fundamental role in crop improvement.

Characterization refers to the systematic description of plant germplasm, encompassing the expression of highly heritable traits ranging from morphological and agronomic features to biochemical constituents and molecular markers (Nagabhushana *et al.*, 2025) [27]. It facilitates the identification of genotypes with desirable attributes such as high yield, disease resistance, drought tolerance, and improved nutritional quality. In addition, comprehensive characterization is essential for the protection of intellectual property rights associated with newly developed varieties and for assessing potential risks related to the introduction of new genotypes, including gene flow and environmental impacts. A thorough understanding of genetic variability and genotype performance enables the selection of suitable lines for specific agro-ecological conditions and management practices. Thus, well-characterized germplasm serves as a valuable reservoir of genetic diversity for the development of improved cultivars.

Genetic diversity in crop species can be assessed using agro-morphological, biochemical, and molecular markers (Reiter *et al.*, 1993; Liu, 1997) [23, 39]. Among these, agro-morphological characterization remains a primary and widely used approach; however, it is often labour-intensive,

time-consuming, and influenced by environmental conditions. To complement these limitations, several chemical assays—such as sodium hydroxide (NaOH), potassium hydroxide (KOH), gibberellic acid (GA_3) response, and 2,4-D soak tests—have been developed for rapid and reliable varietal identification (Savaliya *et al*., 2023) [42]. These assays are simple, reproducible, and effective in detecting varietal mixtures and grouping genotypes into distinct classes (Agrawal, 1987) [1], thereby providing supportive evidence to strengthen morphological characterization (Vanderburg and Vanzwol, 1991) [52].

Furthermore, establishing the genetic identity of genotypes is essential for their protection and registration under the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001 in India, which is based on Distinctiveness, Uniformity, and Stability (DUS) criteria. Thus, systematic characterization and conservation of sesame germplasm are crucial for safeguarding genetic resources and ensuring their effective utilization in breeding programmes.

In this context, the present review aims to critically synthesize existing knowledge on agro-morphological and chemical approaches for the characterization of sesame genotypes, highlighting their roles in assessing genetic diversity and ensuring varietal identification. Emphasis is placed on the complementarity of these methods in supporting DUS testing, germplasm management, and breeding strategies, while also identifying key challenges and future research directions for the development of high-yielding, stable, and climate-resilient sesame cultivars.

Agro Morhological Characterization

Agro-morphological characterization of *Sesamum* species has revealed substantial diversity and distinctive features across cultivated varieties and landraces. It involves the systematic evaluation of quantitative and qualitative traits associated with plant growth, development, and yield performance. In sesame (*Sesamum indicum* L.), important descriptors include plant height, number of branches, days to flowering, capsule length, number of seeds per capsule, seed weight, and oil content (Asma *et al*., 2024; Assefa *et al*., 2023) [2, 3]. These morpho-physiological traits provide valuable insights into phenotypic variability and facilitate the identification of superior genotypes for breeding programmes. Considerable variation observed within and among sesame populations highlights its rich genetic diversity, similar to patterns reported in other crops such as pearl millet (Pucher *et al*., 2015) [33]. Such variability forms the basis for effective germplasm utilization, parental selection, and the development of high-yielding and adaptable cultivars. Integration of agro-morphological traits with molecular markers such as RAPD and SSR further enhances the resolution of genetic diversity analysis and supports precision breeding.

1. Plant Height

Plant height is a key agronomic trait typically measured from the base or first leaf node to the uppermost capsule bearing viable seeds. Significant variation exists in global sesame germplasm, with more than 50% of uniculm varieties ranging between 120 and 150 cm. According to Langham (2019) [22], plant height may vary from 56 to 311 cm under normal growing conditions. The trait is strongly influenced by environmental factors such as moisture availability, temperature, soil fertility, light intensity, and plant density (Langham, 2007) [18], making it an important indicator of adaptability and yield potential.

2. Height to First Capsule

The height to the first capsule is measured from the ground or first leaf node to the lowest capsule on the main stem. Most sesame varieties exhibit a moderate height of 30–60 cm. This trait is particularly important for mechanical harvesting, as it determines the optimal cutter bar position. Extremely low capsule placement increases the risk of mechanical damage, whereas excessively high placement is often associated with taller plants and reduced yield. For efficient harvesting, the recommended height ranges from 15–20 cm for manual harvesting and 15–40 cm for mechanized systems, depending on field conditions (Langham and Wiemers, 2002) [17].

3. Branching Pattern

Branching pattern determines plant architecture and yield potential in sesame. Based on the extent of branching, genotypes are classified into categories ranging from uniculm (no branching) to highly branched types (Langham, 2019) [22]. Branching generally occurs below the capsule-bearing zone, and highly branched genotypes tend to produce more nodes and capsules. Branched varieties are advantageous for efficient space utilization, weed suppression, and increased capsule number (Beech, 2001) [4]. However, increased branching may reduce plant height and enhance susceptibility to lodging under strong winds. Therefore, branching is a critical trait for optimizing yield and mechanization suitability.

4. Leaf Characteristics

Leaves play a central role in photosynthesis and biomass accumulation, thereby influencing yield. Sesame exhibits extensive variation in leaf morphology, including shape, size, arrangement, and pubescence. Leaf shapes range from linear and lanceolate to ovate and cordate, with variations depending on genotype and position on the plant. Lower leaves may be entire, lobed, or deeply divided, while upper leaves are generally simpler in structure (Langham, 2018b) [21]. Leaf length varies from 4.4 to 42.5 cm, and petiole length from 0.2 to 17.0 cm. Leaf arrangement patterns include opposite, alternate, ternate, or mixed types. Such diversity contributes to efficient light interception and aids in designing ideotypes for improved productivity.

5. Floral Traits and Phenology

Sesame flowers are bisexual and borne in the leaf axils on short pedicels. The corolla is tubular and varies in color from white and pink to violet and purple, often exhibiting diverse pigmentation patterns due to anthocyanins and flavonols (Langham, 1947a) [16]. Floral diversity contributes to overall morphological variability and may influence pollination and reproductive success. The trait "days to 50% flowering," defined as the stage when half of the plants in a population bear at least one open flower, is a key phenological parameter used to assess variability and predict maturity duration (Manasa *et al.*, 2025).

6. Capsule Traits

Capsule characteristics are critical determinants of yield in sesame. Capsules are formed in the leaf axils, typically from

the 4th to 6th node pairs. Considerable variation exists in capsule size, shape, color, pubescence, and dehiscence. The number of capsules per leaf axil ranges from 1 to 7, with classifications based on single or multicapsular arrangements (Langham, 2017b [19]; Weiss, 2000). Higher capsule number per axil is generally associated with increased yield potential.

7. Number of Locules per Capsule

The number of carpels (locules) per capsule is another important yield-related trait. Sesame capsules are commonly classified as bicarpellate or tetracarpellate, although tricarpellate and other forms also occur (Langham, 2017a) [19]. While an increased number of locules theoretically enhances seed number, yield advantages may not always be realized due to limitations in assimilate partitioning (Yol and Uzun, 2012) [50].

8. Capsule Length

Capsule length is positively associated with seed number and yield. The global range varies from 1.3 to 7.0 cm, depending on genotype (Langham, 2017a) [19]. Measurements are typically taken from fully developed capsules in the middle portion of the plant to ensure consistency. This trait is frequently used as a selection criterion in breeding programmes.

9. Seed Coat Colour

Seed coat colour is one of the most polymorphic traits in sesame, ranging from white to black with numerous intermediate shades such as yellow, brown, grey, and red (Prasad and Gangopadhyay, 2011; Zhang *et al*., 2013) [32, 55]. It is an important agronomic and quality trait, often associated with nutritional composition, oil content, and disease resistance. Black seeds generally exhibit higher mineral and carbohydrate content but lower oil and protein levels compared to white seeds (Kanu, 2011) [15]. Additionally, seed colour has evolutionary and taxonomic significance and is widely used as a descriptor in germplasm classification (Shahidi *et al*., 2006; El-Bramawy *et al*., 2008) [10, 43].

Chemical Characterization Methods

Varietal identification based solely on morphological traits is often laborious, time-consuming, and influenced by environmental factors, limiting its reliability for precise genotype discrimination. To overcome these limitations, several rapid and reproducible chemical assays have been developed for varietal identification in crop species, including sesame. These methods—such as sodium hydroxide (NaOH) test, potassium hydroxide (KOH) test, gibberellic acid (GA_3) response test, and 2,4-dichlorophenoxyacetic acid (2,4-D) soak test—are simple, cost-effective, and provide consistent results (Agrawal, 1987) [1]. Importantly, they serve as complementary tools to morphological characterization, often providing confirmatory evidence for genotype differentiation (Vanderburg and Vanzwol, 1991) [52].

Plant growth regulators play a crucial role in these assays by influencing physiological responses at very low concentrations. These organic compounds regulate plant growth, development, and source–sink relationships, thereby affecting seedling vigor, biomass accumulation, and assimilate translocation. Variability in genotype-specific responses to such chemicals forms the basis for their application in varietal characterization.

1. Seedling Growth Response to Gibberellic Acid (GA_3)

Gibberellic acid (GA_3) is a key plant hormone involved in seed germination, cell elongation, and early seedling growth. The GA_3 response test evaluates differential growth responses among sesame genotypes, reflecting variation in hormonal sensitivity and physiological behavior.

In this assay, seeds are surface-sterilized and placed on blotter papers moistened with 25 ppm GA_3 solution, with distilled water used as control, following standard ISTA procedures (Anon., 1996). After incubation at 25 ± 1°C for seven days, coleoptile length is measured from randomly selected seedlings. The response is expressed as the percentage increase in coleoptile length over control:

$$\text{Percent increase over control} = \frac{\text{Coleoptile length in } GA_3 - \text{Coleoptile length in control}}{\text{Coleoptile length in control}} \times 100$$

Based on the response, genotypes are categorized as very low (<10%), low (10–30%), or moderate (>30%) responders (Nethra *et al*., 2007) [29]. Similar approaches have been successfully applied in sesame (Mesfin *et al*., 2013; Donga *et al*., 2018) [8, 25] and other crops such as groundnut, rice, wheat, and pigeon pea, demonstrating the robustness of this method for varietal discrimination.

2. Seedling Growth Response to 2,4-D

The 2,4-D assay utilizes 2,4-dichlorophenoxyacetic acid, a synthetic auxin, to assess genotype-specific sensitivity to growth inhibition. This test provides insights into hormonal regulation, growth suppression, and herbicide tolerance among genotypes.

Surface-sterilized seeds are incubated on blotter papers moistened with 2 ppm 2,4-D solution, with water-treated seeds as control (Anon., 1996). After seven days, coleoptile length is measured and the response is expressed as percentage reduction compared to control:

$$\text{Percent decrease over control} = \frac{\text{Coleoptile length in control} - \text{Coleoptile length in 2,4-D}}{\text{Coleoptile length in control}} \times 100$$

Genotypes are classified as susceptible (<85% reduction) or highly susceptible (>85% reduction) (Tiwari *et al*., 2013) [49]. This method has been widely validated across sesame and other crops, indicating its effectiveness in detecting physiological variability and supporting varietal classification.

3. Sodium Hydroxide (NaOH) Test

The NaOH test is a rapid biochemical assay based on the reaction between seed constituents and an alkaline solution. It primarily detects variations in phenolic compounds and related metabolites, resulting in distinct color changes that facilitate genotype differentiation.

In this method, seeds are soaked in 5% NaOH solution and incubated at room temperature for approximately five hours. The resulting colour change is visually assessed and genotypes are grouped into categories such as no change, pale yellow, or yellow (Vanangamudi *et al*., 1988) [51]. The simplicity and reproducibility of this test make it a widely used tool for varietal identification.

4. Potassium Hydroxide (KOH) Test

The KOH test operates on a similar principle to the NaOH assay, utilizing a strong alkaline solution to induce colour reactions in seed components. It provides complementary information for distinguishing genotypes based on biochemical composition.

Seeds are soaked in 5% KOH solution under controlled conditions, and the resulting color change is recorded. Based on the intensity and nature of the reaction, genotypes are classified into categories such as no change, pale yellow, yellow, or reddish-brown (Sripunitha and Sivasubramaniam, 2014) [45]. The KOH test has been extensively applied in sesame and other crops, demonstrating its reliability for varietal differentiation.

Overall, chemical characterization methods provide a rapid, cost-effective, and reproducible approach for distinguishing sesame genotypes. When used in conjunction with agro-morphological and molecular techniques, these assays enhance the accuracy of varietal identification, support DUS testing, and facilitate efficient germplasm management and crop improvement programmes

Integration of Morphological and Chemical Approaches

The characterization of crop germplasm has evolved from reliance on single-method approaches to integrated, multi-dimensional frameworks that combine morphological, chemical, and molecular tools. In sesame, such integration is essential for achieving precise, reliable, and comprehensive assessment of genetic diversity, varietal identity, and breeding potential. Each approach offers distinct advantages and limitations; however, their combined application provides a robust platform for germplasm evaluation and utilization.

Agro-morphological characterization forms the foundation of genotype assessment, enabling the evaluation of phenotypic traits directly associated with agronomic performance and yield. These descriptors are indispensable for field-level selection, DUS (Distinctiveness, Uniformity, and Stability) testing, and the identification of ideotypes suited to specific agro-ecological conditions. However, their expression is often influenced by environmental factors, leading to genotype × environment interactions that may reduce precision.

Chemical characterization methods complement morphological approaches by offering rapid, simple, and reproducible tools for varietal identification. Assays such as NaOH, KOH, GA_3 response, and 2,4-D sensitivity capture biochemical and physiological differences among genotypes, often with minimal environmental influence. These methods are particularly useful for detecting varietal mixtures, confirming genetic identity, and supporting seed quality assurance systems. However, their discriminatory power may be limited for closely related genotypes.

The integration of these approaches offers a comprehensive framework wherein morphological traits provide agronomic relevance, and chemical assays enable rapid validation, ensure precise genetic discrimination. Such multi-tiered characterization strengthens varietal identification, enhances germplasm conservation strategies, and supports efficient parental selection. Importantly, it aligns with DUS requirements under the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001, where robust and multi-evidence-based characterization is essential.

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