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# Perspective Advancements in Plant Science through Segregating Sorghum Gametophyte Developmental Stages

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## Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is an important C4 crop that holds significant promise for sustainable agriculture and bioenergy production. Recent breeding and biotechnology advances on this multipurpose crop were aimed at enhancing the yield, quality, and resilience. Microspores, which can be described as precursors of pollen grains have a pivotal role in plant reproduction for angiosperms. The molecular mechanisms underlying progression of these male gametophytes, from their initiation within the anthers to the formation of mature pollen grains, have functional significance in plant reproduction and evolution. Regulatory pathways in microspore development play an important role for evolutionary adaptation of this potential biofuel crop. This manuscript provides a comprehensive overview on the use of *S. bicolor* microspores while delving into the developmental pathways influencing their fate per genetic factors.

**Keywords:** Sweet sorghum; sorghum bicolor; sustainable agriculture; crop breeding; pollen development; bioenergy; reproductive biology; plant breeding; genetic regulation; evolutionary adaptation; microspores; pollen development

## Introduction

As a versatile C4 crop, sweet sorghum (*Sorghum bicolor* L. Moench) has a wide range of applications, ranging from food and feed to bioenergy and industrial uses [1,2]. Its sugar-water is processed into syrup, molasses, and other food products while the plant is excellent feedstock for animal fodder and agro-industrial value chains. For the reproductive cycle its microsporocytes, the precursor cells located within the sporogenous tissue, undergo meiosis to generate haploid microspores [3,4]. Microspores or the male gametophytes in flowering plants, undergo crucial intermediates from their initiation within the anthers to their maturation into functional pollen grains [5]. The successful transfer of male gametes towards fertilization and genetic diversity depends on the process of pollen development which encompasses their ontoge-

ny, differentiation, and functional significance in plant fertility and evolution [3,4]. For exploring the genetic modification approaches, microspores are ideal plant materials since have under developed cell wall and can allow simple transformation protocols such as PEG and electroporation to be used, which have been established for animal cells [6]. In this review the intermediate sorghum microspore developmental stages are highlighted, when they essentially free float as natural protoplasts, towards breeding and genetic manipulation approaches.

## Genetic Characteristics, Adaptation and Uses

Sweet sorghum exhibits well-suitability for cultivation in diverse agroecological regions and under varying climatic conditions to yield high sugar content in the stalks, suitable for bioethanol pro-



duction [7-9]. Traits such as drought tolerance, heat tolerance, pest resistance, and adaptability in varying climatic conditions are influenced by genetic factors and environmental conditions [10]. Targeted trait selection such as those for high sugar content, biomass yield, and stress tolerance can lead to the development of improved cultivars tailored to specific agroecological niches and production systems. Furthermore, the incorporation of genetic resources from wild relatives offers opportunities for introgression of novel traits for further enhancing the adaptability and sustainability of sweet sorghum [11]. Traditional breeding approaches, complemented by modern biotechnological tools such as molecular markers and genomic selection via microspore culture [12-14], offer promising avenues for accelerating genetic gain in sweet sorghum. Mass isolated sweet sorghum microspores can be targeted in their natural protoplast state towards genetic analysis and modification as well as tissue culture.

### Microsporogenesis and Molecular Mechanisms

Microspores originate from the microsporocytes or pollen mother cells within the anthers of sorghum plants through the series of intricate cellular events tightly regulated by a network of genetic and hormonal cues, orchestrating the spatial and temporal expression of key developmental genes in a process called microsporogenesis [15,16]. Transcriptional profiling studies have identified several transcription factors, including MYB, bHLH, and MADS-box proteins, as well as epigenetic modifications, such as DNA methylation and histone acetylation being key regulators of the fine-tuning of gene expression during microsporogenesis [17,18]. Moreover, small RNA-mediated gene regulation, plays a pivotal role in controlling the expression of target genes involved in microspore development and differentiation or maturation process which culminates with the deposition of sporopollenin, a complex polymer essential for pollen wall formation and pollen viability [19]. Sweet sorghum microspores of early uninucleate to mid binucleate stages have underdeveloped cell wall and as natural protoplasts can allow the use of genetic transformation protocols that have been established for animal cells [6].

### Applications in Plant Breeding and Biotechnology

The microspore differentiation processes per the regulation of gene expression involve the reorganization of cellular structures and till pollen wall formation through biopolymer synthesized by the tapetum cells [15], the sorghum gametophytes remain as natural protoplast. The microspores can be induced in vitro to develop into viable, mature pollen capable to fertilize the female gametes, with potentials to accelerate the breeding process, enhance genetic diversity, and overcome cross-incompatibility limitations associated with traditional breeding methods [20]. After genetic manipulation/transformation of the microspores as natural protoplasts, the subsequent mature pollen obtained through in vitro maturation on modified MS media [21] can substantially accelerate plant breeding programs. Another way of realizing genetic manipulation potentials for microspores per their development stages, can be through the production of haploid plants, for enhancing crop yield, stress tolerance, and reproductive traits [1,14]. Environmental cues

and hormonal signals modulate the timing and progression of microsporogenesis, and engineered microspores at different stages can either be induced to mature in vitro or produce haploid plant towards genetic enhancements.

### Future Perspectives and Conclusion

Pollen grains serve as vehicles for the transfer of male gametes, facilitating fertilization and seed formation while the immature microspores as natural protoplast have profound implications for plant fertility, evolution, and agricultural productivity. After meiosis, uninucleate microspores are released and continue developmental through mitosis I (PMI) and mitosis II towards binucleate and trinucleate stages, respectively. For elucidating the molecular mechanisms underlying microspore development especially the pollen-enriched and pollen-specific genes, the development stages after release till bicellular gametophytes would be most accessible per being in natural protoplast state. For unraveling the molecular mechanisms and regulatory networks governing microspore development, over 1000 gene expressions have been detected, which can allow deeper insights into plant reproductive biology, evolution, and adaptation. Sweet sorghum single panicle holds all microspore developmental windows from basal to proximal end offering a great promise of contributing significantly to the improvement of multipurpose crops through gene editing, in vitro maturation and androgenesis of gametophytes [22].

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