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Morphological study of safflower pollen for crop improvement

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Abstract

Safflower (*Carthamus tinctorius* L.) oilseed crop known for its abundance of (PUFA) polyunsaturated fatty acid content, making it beneficial for heart health. Cultivated primarily for edible oil, dyes, and bird feed. Analyzing the floral biology and pollen morphology of safflower can offer valuable understanding into its reproduce mechanisms, important for breeding novel varieties. Field observations conducted at Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, revealed variations in anthesis timing influenced by environmental factors like dew accumulation and foggy weather. Such insights underscore the significance of considering environmental conditions in breeding programs to develop resilient safflower varieties. Microscopic examination of safflower pollen unveiled yellow, egg-shaped grains with varying sizes from 33-56 μm . Detailed analysis revealed viable pollen with characteristic morphological features such as a thin intine, thick exine with tri-laminate tectum, and germinating pollen tubes. Study offer valuable information on pollen viability and fertility, essential for optimizing pollination techniques and selecting parental lines to improve reproductive success and yield.

Keywords: Aceto-carmine, breeding program, environmental factors, pollen viability, reproductive behavior

Introduction

Pollen grains serve as the male sex cells in flowering plants and are crucial for the fertilization process. These microscopic bodies vary in shape and size across different plant species. Typically, pollen grains are composed of two layers: the outer exine and the inner intine. The intine is a thin and uniform layer primarily consisting of pectolase, which can be broken down by acetolization. Conversely, the exine layer is highly resistant to physical and biological degradation. This unique property allows pollen grains to be preserved for extended periods. The morphological characteristics of pollen grains play a crucial part in comprehending their functional aspects, particularly in pollination biology. The morphological characteristics of pollen grains are significant for understanding their functional roles, particularly in pollination biology. Pollen grains' structural features, such as their apertures, serve as crucial points for interactions with the environment. These apertures act as entry and exit points for pollen exchange, facilitating pollination and fertilization processes in plants (Mallick, 2019) ^[14]. The main aims of the current studies are to determine pollen size, shape, intine and exine sculpture of safflower pollen, and also to focus on safflower's pollen morphology, which serves as an essential basis for comprehending its reproductive mechanisms, which is essential for breeders aiming to create novel varieties with enhanced characteristics such as yield, disease resistance, and adaptation to diverse environmental conditions.

Safflower (*Carthamus tinctorius* L.), an oilseed crop commonly known as "Kusum" with its Sanskrit origin as "Kusumbh" is grown mainly for edible oil, dyes and for bird feed in the arid regions (Dajue *et al.*, 1996) Safflower is basically Asteraceae family. There are twenty-five species in this genus, out of which only *Carthamus tinctorius* L. (2n = 24) is cultivated (Pavithra *et al.*, 2016) ^[18]. Safflower contain 78 percent of polyunsaturated fatty acids (Linoleic Acid) which is mostly useful for heart patients as it reduces blood cholesterol levels (Singh, 2008) ^[22].

Additionally, it has 8% saturated fatty acid, 16-20% mono saturated fatty acid (oleic acid), 2-3% of stearic acid, and 6-8% of palmitic acid (Hamdan *et al.*, 2011) ^[7]. Safflower is a drought tolerant crop that grows best on heavy soils with limited moisture content (Pushvali and Kumar 2017) ^[19]. In 2021-22 Total safflower production of India is 0.437 lack tonnes, leading safflower producing state is Maharashtra, which contribute 0.149 lack tonnes of the total production. (Directorate of oilseed development ministry of Agriculture and Farmers Welfare, Government of India 2022). Safflower oil is valued for its excellent cooking properties, typically comprising 75% linoleic acid along with tocopherols, recognized for their antioxidant properties and abundant vitamin E content. (Ergonul and Ozbek 2020) ^[6]. Due to this reason, safflower oil is incorporated into the diets of individuals with cardiovascular conditions as it promotes circulation. (Jhajharia *et al.*, 2013) ^[10]. The safflower enhancement breeding initiative in India commenced in 1931, and the first variety, N-630, was made available in 1942. The ICAR-Indian Institute of Oilseeds Research recently developed DSH-185, the first public sector cytoplasmic-genetic male sterility (CGMS)-based safflower hybrid. It yields on average, 21 q/ha under irrigated condition (ICAER-IIOR Hyderabad). The orange petals of this plant are a key component in the production of carthamin dye, a natural coloring ingredient that is additionally employed to make "saffo-tea." It can also be made into silage or hay (Rathod *et al.*, 2021) ^[20].

In terms of flower biology, (Leininger and Urie, 1964) ^[11] found that, in their varieties and growth environment, the optimal conditions for peak dry matter accumulation, oil content, germination, and the hull percentage reaches its lowest point approximately 28 days following fertilization of a floret, with seed moisture levels ranging from 22% to 25%. Variability in leaf size is notable across safflower varieties, typically measuring between 2.5 and 5 cm in width and 10 to 15 cm in length. Leaves situated on the lower stem display pronounced serrations, gradually transitioning to shorter and stiffer, ovate to obovate shapes around the inflorescence, where they form the involucre bracts. While lower leaves typically lack spines, spines begin to develop higher up the stem during the bud stage, becoming robust and hard by full flowering. Certain varieties, especially in regions like China and unconventional regions of India, have been selectively bred to be almost entirely free of spines, facilitating manual harvesting of floral components and seeds.

Emasculation of flowers occurs during the late bud stage by extracting the anther tubes, which are the upper section of the corolla tubes, and the petal lobes in order to guarantee planned crossing (Knowles 1980) ^[8]. The emasculated florets are pollinated with pollen from a chosen flower or head on the subsequent day, after the styles have elongated. This breeding method is relatively time-intensive and is utilized in numerous breeding programs.

Pollens of safflower are yellowish in color with average diameter of 53-56 µm. According to (Li and Mundel 1996) ^[2, 12], wind does not greatly transport it. In 1950, Claassen studied the outcrossing rates of safflower plants cultivated with and without cages to exclude insects.

The anther wall in safflower is made up of one epidermis (outer layer), endothecium (outer layer), middle layer and tapetum at maturity. Tapetum is mainly made up of one layer of cells.

Pollination and cross-pollination are dependent on the dispersal of pollen and the duration of viability of the pollen grain. In general, viability of pollen depends on several factors such as temperature and humidity. Safflower is cultivated in dry areas where the pollen is anticipated to desiccate rapidly (USDAAPHIS, 2006) ^[23]. There is limited information on the viability of safflower pollen. However, a study showed that safflower pollen has a very short life span. The viability of safflower pollen decreased as low as 73.6% as soon as 24 hours after anthesis (Pandey and Kumari, 2008) ^[17].

Pollen conservation is an important component for the maintenance of plant genetic resources and it can promote improved efficiency in breeding programs and germplasm conservation and exchange (Dinato *et al.*, 2020) ^[15].

Material and Methods

The investigation was carried out at Central research farm of Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture Science and Technology (SHUATS), Naini, Prayagraj, UP, during Post-Rainy 2022-23. In conducting the research on SFF-708 genotype of safflower, pollen viability an assortment of materials was employed. Safflower flowers served as the source for pollen extraction, ensuring the collection of authentic and representative samples. The essential tools included a standard slide for mounting the pollen, forceps for delicate handling, a brush for the gentle collection of pollen grains, a needle for precise manipulation, and cover slips for sealing the prepared slides. Additionally, the staining process utilized 1% acetocarmine (1 to 2 drops) and glycerin (one drop), providing the necessary contrast for viability assessment. The research also involved the use of a spirit lamp for controlled heat and a light microscope for detailed observation, enabling a comprehensive analysis of safflower pollen viability.

The methodology adopted for the research integrated a staining method, building upon the technique originally described by (Zirkle, 1937) ^[26]. The safflower pollen grains were gathered in the morning at 8 AM using a brush and placed onto a petri dish., and then mounted on slides. The staining process involved the careful application of 1 percent of Acetocarmine, the pollen sample initiating the staining procedure. Subsequently the prepared slide is subjected to gentle heating on a spirit lamp, which improve overall staining efficiency. Following the application of heat, A single drop of glycerin is meticulously applied to the slide, followed by covering it with a cover slip in the subsequent step, providing protection against contaminants, and preventing the pollen. Finally, the fully prepared slide is placed on the microscope stage for observation. Viability determination followed the criteria established by Zirkle, 1937 ^[26] where in well stained and pollen grains were identified as viable, while unstained and shriveled ones were categorized as non-viable. The effectiveness of this approach and its adaptability across various plant species have been showcased through its successful implementation in numerous studies, including those on sorghum by (Ingle *et al.*, 2023) ^[9], guava, pomegranates, and mango by (Balasubramanyam, 1959) ^[1], pomegranates by (Nirmalandunath and Randhawa, 1959) ^[16] and mango by (Singh 1961) ^[21]. His comprehensive approach ensured a robust methodology for evaluating safflower pollen viability in this study.

Results & Discussion

Safflower is usually self-pollinating oilseed crop as its disc florets are hermaphrodite and the stigma is encased within five anthers, united and releasing pollen onto their own receptive stigma. which further elongates with the exposure of sunlight in the morning (Panday and Kumari, 2008) ^[17]. The floral biology of Safflower is such that during anthesis, the receptive stigma emerges from the corolla tube, which allows out-crossing to some extent through the insect pollinators like bees etc. Normally, the anthesis in Safflower begins just after Sunrise i.e., between 6:30-8:00 AM but the anthesis of Safflower in Prayagraj, Uttar-Pradesh was observed between 8:00 to 9:00 AM during normal days and between 10:30 to 11:30 AM during foggy days in the month of March, 2023. The variation in the Anthesis period was observed because of the accumulation of heavy dewdrops on the disc florets of Safflower and low intensity of sunlight in morning accompanied by foggy weather. The observed variation in anthesis timing due to factors like dew accumulation and foggy weather underscores the importance of environmental factors in reproductive processes. Understanding how environmental factors impact anthesis can assist breeders in creating safflower cultivars that can withstand changes in climate and weather patterns. This involves examining the timing of anthesis and morphology of safflower pollen, Gaining insights into its reproductive behavior under various environmental conditions. This knowledge allows breeders to optimize pollination techniques, timing of hybridizations, and selection of parental lines to maximize outcrossing and genetic diversity. The Pollen morphology of Safflower studied under light microscope revealed that it has yellow-colored pollens which are egg shaped and have size ranging between 33-56 μm . Observations revealed that the mature viable pollen of safflower had a thin intine and thick exine with tri-laminate tectum, columellae, together with a endexine similar to that observed by (Yeung *et al.*, 2011) ^[25]. This analysis also

found a germinating pollen tube that resembled the one (Lin., 1967) ^[13] had seen in his study. detailed examination of safflower pollen morphology provides breeders with valuable information about pollen viability and fertility. Viable pollen, with intact cellular structures and metabolic activity, is crucial for successful pollination and seed set. In contrast, dead pollen, arising from different stressors such as extreme temperatures or pollutants, cannot participate in fertilization processes. The presence of germinating pollen tubes indicates the potential for successful fertilization and seed development. This information can aid breeders in choosing traits linked to superior pollen quality, such as increased pollen germination rates and pollen tube growth, leading to improved reproductive success and yield.



Fig A: Male Part of Flower

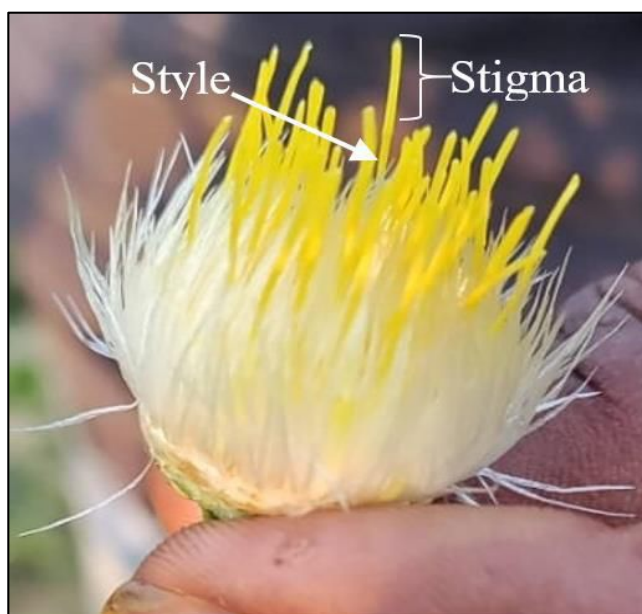


Fig B: Female Part of Flower

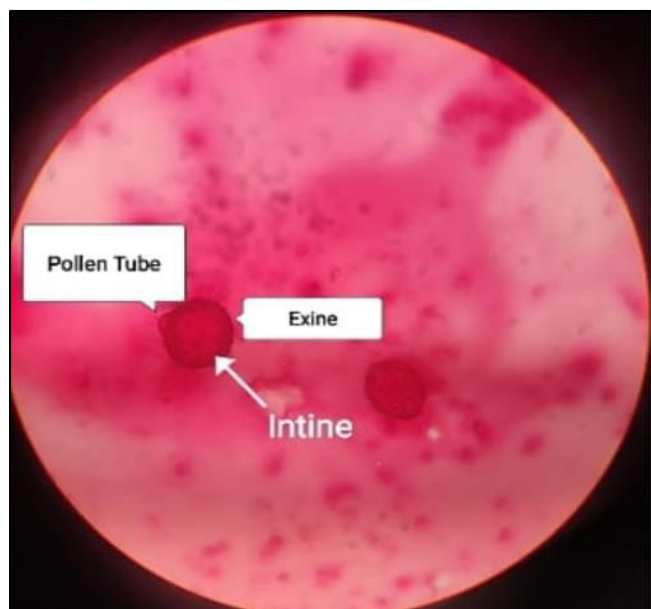


Fig C: Microscopic View of Viable pollen

Conclusion

It can be concluded that the Safflower's pollens are easy to collect and their general morphology can be studied through staining method by using acetocarmine under light microscope. The study provides illumination on safflower's floral biology and pollen morphology, granting valuable understanding of its reproductive processes. The observed variation in anthesis timing due to environmental conditions, emphasizes the significance of considering environmental factors in safflower breeding programs. By understanding the factors influencing pollen viability and fertility, breeders can optimize pollination techniques and select parental lines to enhance outcrossing and genetic diversity. Moreover, the identification of germinating pollen tubes highlights the potential for successful fertilization and seed development. The study lays a sturdy groundwork for future investigations and aids in the advancement of safflower cultivars with enhanced characteristics and resilience to environmental challenges.

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