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Report on natural occurrence and significance of syncephalium, secondary capitula development in *Calendula officinalis*

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Abstract
Calendula officinalis (pot marigold) typically produces a solitary capitulum, but a rare floral variation was observed where secondary capitula emerged within the primary capitulum, forming a syncephalium-like structure. Out of 50 plants grown at ICAR–Indian Agricultural Research Institute, Gauria Karma, Jharkhand, one exhibited this variation, with 14 smaller secondary capitula. These secondary heads had fewer disc florets and altered ray florets, leading to reduced seed yield. This phenomenon might be due to hormonal regulation, genetic factors, or evolutionary strategies in Asteraceae. Though reported in other Asteraceae species, this is the first documented case in *C. officinalis* under Eastern India's agro-climatic conditions. This report highlights a novel floral variation and its significance with potential implications for breeding, seed production, and ornamental traits.

Keywords: Syncephalium, *Calendula,* pot marigold, capitulum, ornamental

Introduction
Calendula officinalis, a member of the Asteraceae family, native to central Europe and the Mediterranean region, is often known as pot marigold or English marigold (Ashwlayan *et. al.* 2018) [1]. It is widely cultivated for its medicinal, ornamental, and cosmetic properties around the world. Plants are erect, bushy, grow up to 30–60 cm in height and produce bright orange or yellow flowers. Asteraceae, also known as Compositae or sunflower plant family, with 16 subfamilies and 50 tribes, with an estimation of 25000–35000 species account for 10% of all flowering plants (Fu *et al*., 2023) [9]. Being one of the largest plant families, the flowering diversity is huge, yet poorly understood. The inflorescence is a capitulum that emerges on an enlarged receptacle, which is a characteristic feature of the whole family. By layman, this composed structure is commonly considered as a single flower; this is scientifically a false flower or pseudanthium. Inflorescence composed of multiple florets consisting of ray florets at the outer margin or periphery and disc florets in the centre. Disc florets are bisexual, whereas ray florets are female or styliferous and sterile with zygomorphic corollas (Zhang, L., & Elomaa, P., 2016) [19, 8].

Asteraceae includes species that show a higher level of complexity in their inflorescences. As examples of simple capitula, calendula, sunflower and gerbera heads appear as solitary structures on top of a floral stem (Zhang, L., & Elomaa, P., 2018) [20, 8]. However, several capitula may also form synflorescences, being arranged in distinct branched systems, such as racemes (e.g., *Artemisia pycnocephala*), cymes (e.g., *Senecio vulgaris*), or corymbs (e.g., *Achillea millefolium*). Another hierarchical level of complexity is when flower heads are aggregated onto a single receptacle and form higher order structures, also known as capitulescence or syncephalium (e.g., Craspedia, Echinops, Lagascea) (Harris, E. M, 1995; Claβen-Bockhoff, 1996; Leins *et al*., 1979; Harris, 1994) [12, 13, 11]. Typically, *Calendula officinalis* flowers bear a single capitulum; however, in this report, secondary capitula emerge within the primary capitulum, leading to morphological variations. This report aims to analyse the structural aspects and significance of secondary capitula development in Calendula.

Materials and Methods

Seeds of *Calendula officinalis* were procured from the Division of Floriculture and Landscaping, ICAR- Indian Agricultural Research Institute, New Delhi and sown in the nursery bed on October 20, 2024. After the emergence of true leaves, seedlings were transplanted to the experimental farm of ICAR–Indian Agricultural Research Institute, Gauria Karma, Jharkhand, on 19 November 2024. The seedlings were established in demonstration plots at a spacing of 60 × 45 cm. Standard agronomic practices, including irrigation, fertilisation, and pest management, were followed to ensure optimal plant growth and development.

Results and Discussion

After the transplanting of around 50 plants of *C. officinalis* to the demonstration plots, the first flower bud appeared in the first week of January 2025. During the flowering phase, authors found an unusual floral variation in one plant where multiple capitula or heads (secondary capitula) emerged from a single capitulum (primary capitulum), forming a syncephalium-like structure. Unlike typical solitary capitula observed in Calendula remaining plants, this plant exhibited the fusion or clustering of capitula and is rarely reported in *Calendula* and suggesting an atypical flowering pattern within the Asteraceae family. The distribution of this flowering behaviour appeared random rather than genetically inherited, as it was not found in other plants within the population.

Morphological and Developmental Characteristics

The primary capitulum of *Calendula officinalis* exhibited normal development, with well-differentiated ray and disc florets arranged in a typical pattern. In contrast, the 14 secondary capitula that emerged from the primary capitulum were noticeably smaller and underdeveloped, often consisting of a reduced number of disc florets. The ray florets in these secondary heads were morphologically similar to those in the primary capitulum but appeared in fewer numbers. Additionally, some secondary capitula exhibited deformed or incomplete floral structures, suggesting possible disruptions in floral meristem development. These irregularities indicate an alteration in inflorescence differentiation, potentially influenced by genetic or environmental factors. The presence of secondary capitula directly influenced reproductive success in the affected plant. Some secondary capitula did not reach full maturity, resulting in non-viable or aborted floral structures. This incomplete development of secondary inflorescences may have further hindered seed production efficiency (Figure 1).

Possible reasons and significance

Hormonal influence

Hormonal regulation is a crucial factor that might have influenced this phenomenon. Earlier studies reported that exogenous treatment of gibberellic acid (GA_3) produced secondary inflorescences in *Calendula officinalis* (Bose, T. K. and Nitsch, J. P. 1970 and Ram, H. Y. M. and Mehta, U. 1978) [3, 16]. In contrast, in our report, there is no exogenous application of gibberellins. It might be due to variation in endogenous gibberellins, leading to the natural occurrence of secondary capitulum. An imbalance in key phytohormones, including auxins, gibberellins, and cytokinins, could have disrupted normal capitulum differentiation and led to the excessive formation of floral structures.

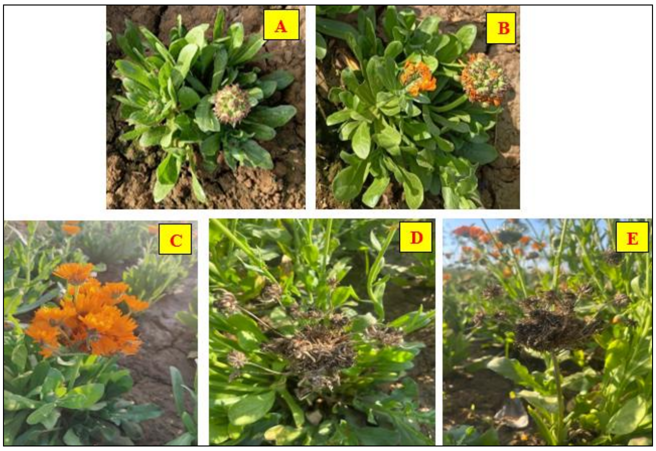
Genetic Factors

In all basic inflorescence types, flower meristem identity is controlled by homologs of at least three functionally conserved proteins – LEAFY (LFY), UNUSUAL FLORAL ORGANS (UFO) and SEPALLATA3 (SEP3) (Elomaa *et al*., 2018) [8]. Study of molecular-level understanding of capitulum development is hindered by the lack of mutant resources and efficient gene-editing techniques in relevant plant species (Zhang and Elomaa, 2024) [18]. One of the most studied species of capitulum development is *Gerbera hybrida*. Studies in *Gerbera hybrida* suggested that LFY function may play a role in regulating the development of syncephalium. (Zhao *et al.*, 2016) [22]. Additionally, environmental factors such as fluctuations in temperature, nutrient availability, and photoperiod may contribute to abnormal floral differentiation, further influencing capitulum development.

Evolutionary Factors

Fossil evidence has re-estimated the Asteraceae family origin to the late Cretaceous (~83 million years ago) in Southern South America, with subsequent dispersal to Asia and Africa likely via North America (Mandel *et al*., 2019) [15]. The variation in flower inflorescence, such as syncephalium, may further increase the size of the inflorescence for pollination (Harris, 1999) [10], while the development of complex inflorescence structures is not fully understood. It may represent an evolutionary strategy to attract a broader range of pollinators by mimicking larger floral displays. This variation in flower heads could contribute to the diversification and adaptive success of the Asteraceae family (Zhang and Broholm, 2024) [21]. Burtt (1961) [4] and Stebbins (1967) [17] proposed that a reduction in the number of florets per capitulum may enhance protection against insect predation. However, if the capitulum were reduced to a single floret, the nutritional advantage gained by minimising the supply route would be significantly diminished. Consequently, the development of a secondary head may serve as an adaptive mechanism that balances protection and nutrient distribution. In certain cases, the formation of a syncephalium may provide enhanced defence against herbivory compared to smaller capitula. This is achieved by shifting the protective role from the phyllaries to the secondary bracts and integrating ovules into the secondary receptacle, thereby resulting in the loss of the distinct identity of individual capitula.

Similar occurrences of syncephalium-like structures have been reported in other members of Asteraceae, *Dyssodia decipiens* (Classen-Bockhoff, 1992) [6], *Echinops bannaticus* (Bremer, 1994) [2], *Polyachyrus poeppigii*, *Moscharia solbrigii*, *Leucheria salina*, *Triptilion capillatum* (Katinas *et al*., 2008) [13]. However, such observations in *Calendula officinalis* are scarce, highlighting the need for further investigation.



(A) Bud stage showing early capitulum formation.
(B) Developing secondary capitulum with involucral bracts enclosing immature floret
(C) Fully opened secondary capitulum displaying ray and disc florets (Lateral view of the syncephalium).
(D-E) Drying stage showing seed formation and capitulum desiccation (Lateral and superior view of the syncephalium)

Fig 1: Developmental stages of secondary capitulum in *Calendula officinalis*

Conclusion

Most calendula research has been focused on the extraction and pharmaceutical properties of bioactive compounds from flowers. This study documents the first known report of syncephalium, secondary capitula development in *Calendula officinalis* under Eastern India's agro-climatic conditions. The occurrence of syncephalium, secondary capitula development in *Calendula officinalis,* represents a rare flower abnormality, a significant morphological adaptation within Asteraceae, driven by genetic regulation, evolutionary selection, and ecological advantages. Its occurrence enhances pollination, optimises reproductive success, and contributes to species diversification. This unusual floral structure may have implications for reproductive efficiency, seed production, and ornamental value. Further research on the genetic and physiological basis of this phenomenon could provide insights into floral development in *Asteraceae*.

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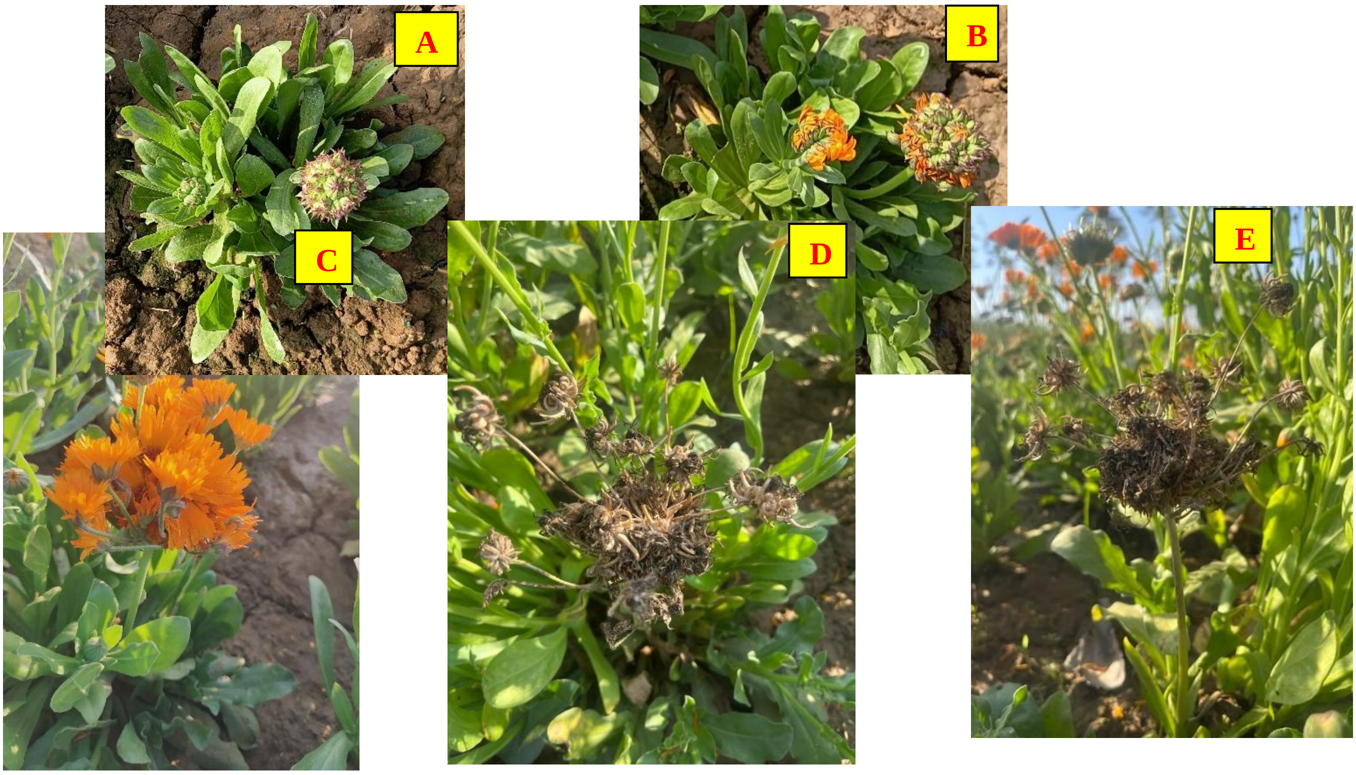

Figure 1. Developmental stages of secondary capitulum in *Calendula officinalis*-

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