

Original Research Article

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Identification of cold tolerant French marigold genotypes using morpho-biochemical parameters

Chandana Shivaswamy¹, Kanwar Pal Singh^{*1}, Sapna Panwar¹, Vishwanathan Chinnusamy², Kusuma M. V.¹, Gouthami Shivaswamy³, Rojina Wahengbam¹ and Shubham Jagga⁴

¹Division of Floriculture and Landscaping, ICAR-IARI, New Delhi (India)-110012

²Division of Plant Physiology, ICAR-IARI, New Delhi (India)-110012

³Department of Post-harvest technology, Amity Institute of Horticulture Studies and Research, Amity University, Noida, Uttar Pradesh (India)-201301

⁴Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi (India)-110012

ABSTRACT

French marigold (*Tagetes patula* L.) is a widely cultivated ornamental species, valued for its vibrant blooms and adaptability. However, low winter temperatures in northern India can adversely impact its vegetative growth and floral development, particularly during the early growth stages that coincide with peak winter. To assess cold stress tolerance, twenty French marigold genotypes were evaluated under natural winter conditions (November 2022–February 2023) in New Delhi. Various morphological traits (plant height, leaf length, and width), floral traits (days to visible flower bud colour, number of petals, petal dimensions, flower longevity), and biochemical parameters (chlorophyll a and b content) were studied. A significant genotypic variability was observed across all traits. Gulzafri Yellow (GY) and Gulzafri Orange (GO) recorded maximum plant heights (25.86 cm and 23.76 cm at 50 DAT, respectively), while 'IIHR-MO-4' showed the largest leaves (14.66 cm and 7.70 cm in length and width respectively). 'Fr./R-5' exhibited the highest petal count (180.58), chlorophyll a content (9.78 mg g⁻¹ FW), and longest flower longevity (17.81 days). Principal Component Analysis (PCA) revealed that leaf size, plant height, petal number, and chlorophyll a were key contributors to cold tolerance, explaining 74.18% of the total variation. Correlation analysis confirmed strong associations between vegetative vigour, chlorophyll retention, and floral traits. The study involved meticulous evaluation during the winter season to accurately record genotypic responses to cold stress under natural conditions, leading to the identification of promising cold-tolerant French marigold genotypes. It established a robust morpho-biochemical framework for genotype selection, highlighting that a combination of morphological, floral, and biochemical traits can serve as reliable indicators for screening cold-tolerant lines. Notably, 'Gulzafri Yellow' (GY), 'Gulzafri Orange' (GO), 'IIHR-MO-4', and 'Fr./R-5' emerged as potential candidates for winter breeding programs.

Keywords: French marigold, cold stress, morphological traits, flower longevity, biochemical traits, chlorophyll, cold-tolerant, principal component analysis, correlation analysis.

INTRODUCTION

French marigold (*Tagetes patula* L.), a member of the Asteraceae family is a widely cultivated ornamental plant valued for its vibrant flowers, short crop duration, and adaptability across diverse agro-climatic zones. Its bright blooms and compact growth habit make it a popular choice for landscaping, container gardening, borders, and the loose flower trade. Beyond its ornamental appeal, French marigold has significant commercial value due to its diverse applications in the pharmaceutical, food, and cosmetic industries. Various extracts from the plant are utilized for natural dyes, essential oils, and therapeutic compounds, contributing to its growing cultivation demand in many countries, including India [7],[3],[16],[12].

Despite its wide adaptability and economic importance, *T. patula* is vulnerable to environmental stresses, particularly abiotic factors such as cold temperatures, which restricts its performance during the winter season, especially in northern plains and elevated regions of India.

Cold stress, defined as exposure to temperatures below the optimal range required for normal growth and development, poses a major constraint to the cultivation of ornamental crops like French marigolds. This stress is particularly detrimental during the early vegetative and reproductive stages when plants are most susceptible. In regions where winter temperatures frequently fall below 10 °C, cold stress can severely impair germination, stunt vegetative growth, delay flowering, reduce flower quality, and in extreme cases, lead to plant mortality [4],[2]. Morphologically, cold stress disrupts critical growth processes such as cell division and elongation, resulting in reduced plant height, fewer branches, and smaller leaves, traits indicative of diminished vigour and plant health. Impairment in various reproductive traits was also seen due to cold stress including delayed flower bud coloration, decreased petal number, and shortened flower longevity reflecting a decline in

*Corresponding Author: **Kanwar Pal Singh**

DOI: <https://doi.org/10.21276/AATCCReview.2025.13.03.439>

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ornamental value. On a biochemical level, cold temperatures inhibit chlorophyll biosynthesis and destabilize chlorophyll *a* and *b* pigments, which are essential for photosynthesis. Reduced chlorophyll content is associated with lowered photosynthetic efficiency and physiological vitality under stress conditions. Therefore, a combined assessment of morphological traits, floral characteristics, and chlorophyll content offers a comprehensive approach to evaluating cold tolerance in French marigold genotypes.

In India, winter conditions in states such as New Delhi, Uttar Pradesh, Himachal Pradesh, and the north-eastern region significantly limit open-field marigold cultivation. Low temperatures during this season reduce flower yield and quality, causing economic losses to farmers and disrupting flower supply chains, especially during culturally important festivals and events. Currently, there is a lack of commercially available cold-tolerant marigold varieties, forcing growers to depend on protected cultivation methods that raise production costs and complexity.

The study was undertaken to evaluate twenty French marigold genotypes under natural cold stress conditions, focusing on early vegetative growth, floral traits, and chlorophyll content that collectively reflect cold tolerance. The objective was to identify resilient genotypes capable of maintaining superior performance despite exposure to low temperatures. Such genotypes can serve as valuable genetic resources for breeding programs aimed at developing cold-tolerant varieties. Ultimately, these efforts will support the expansion of French marigold cultivation into colder regions, stabilize flower production, improve farmer income, and ensure a consistent supply of flowers during the winter months, strengthening the floriculture industry and supporting rural livelihoods.

MATERIALS AND METHODS

Planting Material

Twenty genotypes of French marigold (*Tagetes patula* L.) i.e. 'Hisar Beauty' (HB), 'Hisar Jafri' (HJ), 'Gulzafri Orange' (GO), 'Gulzafri Yellow' (GY), 'Pusa Deep' (PD), 'Pusa Arpita' (PA), 'Pusa Parv' (PP), 'Valencia Yellow' (VY), 'Dainty Marietta' (DM), 'IIHR Fm-1', 'IIHR-MO-4', 'Fr./R-2', 'Fr./R-5', 'Fr./R-6', 'Fr./R-7', 'Fr./R-10', 'Fr./R-14-1', 'Fr./R-14-6', 'DH/Fr./R-1', and 'DH/Fr./R-2', were selected for the experiment.

Experimental Site

The experiment was conducted at the Research Farm of the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi (28°38'39" N, 77°9'25" E, 228 m amsl) from November 2022 to February 2023. Seeds were sown in open nursery beds in late October 2022 under average day/night temperatures of 30.6/16.2 °C and 39–96% relative humidity. After 30 days, healthy seedlings with 3–4 true leaves were transplanted into 10-inch pots containing garden soil and farmyard manure (1:2 v/v) in late November, when temperatures dropped to 26.5/10.5 °C and humidity ranged from 36–85%. The standard agronomic practices were followed, with no artificial cold protection to allow natural stress exposure. The study used a completely randomized design (CRD) with three replications; each genotype (treatment) was represented by three pots (Fig. 1). Morphological, floral, and biochemical (chlorophyll) traits were recorded at early vegetative and flowering stages to evaluate cold stress responses.

Meteorological Conditions

During the experimental period from November 2022 to February 2023, daytime temperatures ranged from 14.6 °C to 34 °C, while night-time temperatures dropped as low as 0 °C. The coldest months were December and January, which coincided with the peak flowering stage. Weather data were obtained from the Division of Agricultural Physics, ICAR-IARI, New Delhi (Fig. 2; Supplementary Table 1).

Estimation of early vegetative and floral Parameters

Plant height (cm): Plant height was recorded at 25 and 50 days after transplanting (DAT). It was measured from the base of the plant to the topmost growing point using a meter scale.

Leaf length and width (cm): Leaf length and width were measured during the vegetative phase using a standard meter scale. Fully expanded mature leaves were selected for measurement.

Days to visible flower bud colour (days): The number of days from transplanting to the appearance of flower bud colour was recorded as an index of floral initiation under cold stress.

Number of petals per flower (No.): Fully opened flowers were observed during the peak flowering phase, and the number of petals per flower was manually counted.

Petal length and width (cm): Petal length and width were measured using a digital vernier calliper from randomly selected fresh flowers at full bloom.

Flower longevity on the plant (days): Flower longevity was recorded as the number of days from flower opening to withering while still on the plant, indicating post-bloom sustainability under stress.

Estimation of Biochemical Parameters

Chlorophyll *a* and *b* content (mg g⁻¹ fresh weight):

Chlorophyll *a* and *b* contents were estimated during the flowering phase using the non-maceration method in DMSO (Fig. 3) as described by Hiscox and Israelstam (1979) [6]. Fresh leaf discs (100 mg) were incubated in 7 ml of DMSO at 65°C until complete chlorophyll extraction. Absorbance was measured at 645 nm and 663 nm using a UV-Vis spectrophotometer. Chlorophyll concentrations were calculated using the following formulae:

- Chlorophyll *a* (mg/g fwt) = $(12.7 \times A_{663}) - (2.69 \times A_{645})$
- Chlorophyll *b* (mg/g fwt) = $(22.9 \times A_{645}) - (4.68 \times A_{663})$

Statistical analysis

The results were expressed as the mean \pm standard error of the mean of 3 biological replicates. Pearson's correlation analysis and principal component analysis (PCA) were performed using Python software (version 3.13.2) with the scikit-learn 1.6.1 library. Variations among the treatments and their means were compared using the Tukey test at 5% probability ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of cold stress on early vegetative and floral parameters in French marigold genotypes

Plant height at 25 and 50DAT

The data presented in Table 1 indicates that the genotype 'GO' exhibited the maximum plant height (16.40 cm) at 25 days after

transplanting (DAT), which was at par with 'GY' (15.73 cm), while the minimum was recorded in 'Fr./R-14-1' (5.33 cm). The plant height among genotypes ranged from 5.33 to 16.40 cm at 25 DAT. Similarly, at 50 DAT, the genotype 'GY' recorded the maximum height (25.86 cm), followed by 'GO' (23.76 cm), whereas the lowest plant height was again noted in 'Fr./R-14-1' (7.40 cm). The plant height among genotypes ranged from 7.40 to 25.86 cm at 50 DAT. It was evident that the genotype 'Fr./R-14-1' exhibited a significant reduction in height under cold stress, indicating its susceptibility to low-temperature conditions. In contrast, the genotypes 'GY' and 'GO' showed robust vegetative growth even under sub-optimal temperatures, highlighting their potential suitability for cultivation in areas experiencing chilling weather. The observed variation in plant height among genotypes under cold stress conditions can be attributed to their differences in genetic makeup and physiological adaptability. Under cold stress, plants tend to divert energy towards survival mechanisms and maintaining basic physiological functions, rather than vegetative expansion, which results in shorter internodal lengths and reduced plant stature [4]. Similar reductions in vegetative parameters due to low temperatures were also reported by [1],[5],[14] in French marigolds.

Leaf length and width

The genotype 'IIHR-MO-4' exhibited the maximum leaf length (14.66 cm), whereas the minimum leaf length was observed in 'Fr./R-7' (1.93 cm) (Table 1). Similarly, the highest leaf width was also recorded in 'IIHR-MO-4' (7.70 cm), while the lowest was in 'Fr./R-7' (1.55 cm). These results clearly indicate that leaf growth in French marigold genotypes was markedly influenced under cold stress conditions. Genotypes like 'Fr./R-7' showed severely restricted leaf expansion, suggesting a high sensitivity to chilling temperatures, while 'IIHR-MO-4' maintained superior leaf dimensions, showcasing its tolerance to cold stress. Cold-induced reductions in leaf length and width can be attributed to diminished cell expansion and overall growth, as reported in mung bean [11]. Under low-temperature conditions, plant physiological processes such as photosynthesis and nutrient uptake are impaired, which in turn limits vegetative development. Despite these general trends, the extent of reduction varies across genotypes due to their inherent genetic traits and adaptability to stress environments. Similar reductions in leaf size under cold conditions have also been observed in *Thymus* spp. by [9] affirming the sensitivity of leaf growth to temperature fluctuations.

Days to visible flower bud colour (days)

Floral parameters play a critical role in assessing the performance of French marigold genotypes under cold stress conditions. In the present investigation, the earliest bud colour initiation was recorded (Table 1) in the genotype 'HJ' (77.71 days), whereas the latest was observed in 'Fr./R-6' (25.88 days), with the range across genotypes spanning from 25.88 to 77.71 days. Early colour expression in flower buds serves as a vital selection criterion for developing early-flowering cultivars suitable for North Indian agro-climatic zones. Advancing flowering prior to the peak of harsh winter months (December–January) is essential, as low temperatures during this period adversely affect flower quality and marketability [1]. Being a quantitative short-day plant, French marigold tends to initiate bud formation and flowering earlier under the influence of low temperatures and reduced photoperiods typically encountered during winter [13].

Number of petals per flower (no.), petal width and length

The data presented in Table 1 revealed that the maximum number of petals was recorded in the genotype Fr./R-5 (180.58), while the minimum was observed in DM (6). In terms of petal dimensions, the longest petals were recorded in IIHR-MO-4 (2.85 cm) and the shortest in Fr./R-14-1 (0.87 cm). The widest petals were also noted in IIHR-MO-4 (2.11 cm), whereas the narrowest were found in Fr./R-6 (0.89 cm). Although cooler temperatures generally reduce the overall vegetative growth of plants, they may simultaneously promote greater floral investment, including increased petal number, length, and width. This phenomenon can be attributed to a physiological shift wherein the plant allocates more assimilates towards reproductive structures under stress conditions, possibly as a survival mechanism to ensure reproductive success [15]. The improved petal development observed under cold stress may be due to genotype-specific responses aimed at maintaining flower traits even under low temperature conditions [5]. These findings are consistent with earlier reports by [1] in French marigold.

Flower longevity on the plant (days)

The data presented in Table 1 revealed that maximum flower longevity on the plant was recorded in genotype Fr./R-5 (17.81 days), while the minimum was observed in DM (11.18 days). The variation in flower longevity among genotypes could be attributed to differences in photosynthetic efficiency, which may have contributed to better assimilated production and prolonged floral retention [15]. Similar results were reported by [8] reported in chrysanthemum.

Effect of cold stress on biochemical parameters in French marigold genotypes

Chlorophyll *a* and *b* content (mg g⁻¹ fresh weight)

Data on chlorophyll *a* and *b* content of twenty genotypes, shown in Fig. 4, indicate that the highest chlorophyll *a* content was observed in genotype Fr./R-5 (9.78 mg g⁻¹ fresh weight), while the lowest was recorded in DM (4.10 mg g⁻¹ fresh weight). Regarding chlorophyll *b*, genotype HJ exhibited the maximum value (2.78 mg g⁻¹ fresh weight), whereas the minimum was found in Fr./R-7 (1.70 mg g⁻¹ fresh weight). An increase in chlorophyll *a* and *b* content was evident in the cold-tolerant genotypes Fr./R-5 and HJ, whereas a significant decline occurred in the susceptible genotypes DM and Fr./R-7. Cold temperatures adversely affect the photosynthetic system by disrupting thylakoid membrane integrity, which reduces chlorophyll biosynthesis and stability [10]. [17] reported significant genotypic differences in chlorophyll content of French marigolds during winter, attributing the variation to factors such as photosynthetic efficiency, cold tolerance, antioxidant potential, and metabolic adaptability. These findings align with reports by [11] in mungbean and [1] and [5] in French marigold.

Cold stress responses in French marigold genotypes using Principal Component Analysis and Pearson's correlation analysis

Pearson correlation analysis revealed important relationships among morphological and physiological traits in French marigold genotypes under cold stress (Fig. 5). Plant height at 25 and 50 days after transplanting showed a strong positive correlation ($r = 0.89$), indicating uniform growth. Leaf length was highly correlated with leaf width ($r = 0.96$), number of

petals ($r = 0.80$), and days to visible flower bud colour (BC; $r = 0.78$), suggesting that genotypes with larger leaves tend to have more floral structures but may take slightly longer to show flower colour under cold conditions. Flower length was positively correlated with number of petals ($r = 0.79$) and chlorophyll *a* ($r = 0.72$), showing a link between floral traits and photosynthetic capacity. Chlorophyll *a* also showed strong associations with leaf traits and chlorophyll *b* ($r = 0.88$), indicating pigment stability during stress. Petiole length and width were significantly correlated ($r = 0.75$), reflecting coordinated structural development. Overall, the results suggest that genotypes with better vegetative growth, higher chlorophyll content, and balanced flowering timelines are more likely to tolerate cold stress. These traits can be useful for identifying cold-tolerant genotypes in marigold breeding programs.

A principal component analysis (PCA) was performed to assess the variation among 20 French marigold genotypes based on morphological, physiological, and biochemical traits under low-temperature conditions (Table 2). The first two principal components (PC1 and PC2) together explained 74.18% of the total variation, with PC1 alone contributing 60.03% and PC2 contributing 14.15% (Table 3). PC1 was mainly associated with leaf length (LL), leaf width (LW), number of petals (NOP), plant height (PH 25DAT, PH 50DAT), days to visible flower bud colour (BC), and chlorophyll *a* (CHL A), indicating that these traits contributed significantly to cold tolerance. PC2 was influenced by petiole length (PL), petiole width (PW), and chlorophyll *b* (CHL B), reflecting plant structure and pigment composition. Based on the PCA biplot (Fig. 6), genotypes such as Gulzafri Orange, Hisar Beauty, and Pusa Deep were positively associated with PC1 traits, suggesting better cold tolerance due to vigorous growth and higher chlorophyll content. IIHR-MO-4 showed a high expression of petiole-related traits, indicating potential structural advantages. In contrast, genotypes such as Fr/R-6, Fr/R-7, Dainty Marietta, and Valencia Yellow were positioned on the negative side of PC1, reflecting poor performance in key traits and likely cold susceptibility. Genotypes near the origin, such as Fr/R-14-6, Fr/R-0, and Hisar Jafri, showed moderate trait expression, indicating an average cold response. This analysis effectively grouped the genotypes into tolerant, moderate, and susceptible categories, aiding in the selection of promising lines for cold-tolerant marigold breeding.

CONCLUSION

The present study revealed considerable genotypic variation in French marigolds (*Tagetes patula* L.) for cold stress tolerance under natural winter conditions. As the early growth stages of these genotypes coincided with peak winter temperatures, this period proved critical in determining their resilience and developmental success. Among the twenty genotypes evaluated, 'Gulzafri Yellow' and 'Gulzafri Orange' showed better early vegetative growth, while 'IIHR-MO-4' exhibited superior leaf development. Genotype 'Fr./R-5' performed best in reproductive traits, with higher petal number, chlorophyll *a* content, and flower longevity. Principal Component Analysis indicated that plant height, leaf traits, petal number, and chlorophyll *a* content contributed most to cold tolerance. Positive correlations were observed between vegetative traits, chlorophyll content, and floral performance further emphasized the role of early-stage performance in determining cold resilience. The study identifies promising genotypes such as GY, GO, IIHR-MO-4, and Fr./R-5, which can be used in breeding programs to develop cold-tolerant varieties.

Such genotypes will benefit farmers by enabling winter marigold cultivation in colder regions without protective structures, ensuring steady production and income during low-temperature periods.

Future scope

The identified cold-tolerant genotypes constitute a valuable genetic resource for the development of French marigold cultivars adapted to low-temperature environments. Building upon the established morpho-biochemical framework, future research may focus on multi-season evaluations and the integration of molecular tools to enhance selection precision. Such efforts are expected to facilitate the expansion of winter marigold cultivation, reduce dependence on protected cultivation systems, and contribute to the resilience of floriculture in temperate regions.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Acknowledgment

The author would like to thank Division of Floriculture and Landscaping, ICAR-IARI, New Delhi and Division of Plant Physiology, ICAR-IARI, New Delhi for providing materials and all the necessary resources required for conducting the research.

Author's contribution

Chandana Shivaswamy conducted the research experiment and analysed the data; Kanwar Pal Singh and Sapna Panwar conceptualized the research, guided throughout the experiment and main manuscript writing; Kusuma M. V. and Rojina Wahengbam helped in sample collection and biochemical analysis; Vishwanathan Chinnusamy helped in providing lab facility; Gouthami Shivaswamy and Shubham Jagga helped in software and data analysis.

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