

Original Research Paper

Resilience of French marigold (*Tagetes patula* L.) genotypes to cold stress: Morphological and biochemical insights

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ABSTRACT

French marigold cultivation in northern regions faces significant challenges from cold stress sensitivity, severely impacting plant growth and flowering. Understanding genotypic variations in cold tolerance is crucial for developing climate-resilient cultivars. This study investigated cold stress impact on four French marigold genotypes (Hisar Beauty, Hisar Jafri, Valencia Yellow, and Orange Winner) under open field and polyhouse conditions. Significant reductions in vegetative and floral traits were observed, with genotype-environment interactions affecting plant performance. The genotype Hisar Beauty exhibited highest cold resilience with moderate reductions in plant height (11.1%), plant spread (7.4%), and leaf area (8.2%), followed by Hisar Jafri, showing moderate plant spread decline (22.5%), minimum reduction in leaf area (5.6%), and longest flowering duration (51.20 days). Cold stress delayed bud initiation and reduced flower yield, while, Hisar Beauty maintained superior flowering with higher number of flowers (61.00), flower weight (3.89 g), and stable duration. The genotype Hisar Beauty retained highest chlorophyll content (11.17 mg/g FW) with only 15.9% reduction versus 32.5% and 35.9% decline in Valencia Yellow and Orange Winner, respectively. Total phenolics were recorded highest in Hisar Beauty (687.13 µg/g FW), exceeding Valencia Yellow (231.02 µg/g FW) and Orange Winner (226.10 µg/g FW). Principal component analysis (PCA) showed Hisar Beauty and Hisar Jafri clustering positively on PC1 with superior traits, distinguishing them from cold-susceptible genotypes. Both genotypes are promising for cold-prone regions.

Keywords: Cold stress, flowering traits, French marigold, phenolic compounds, principal component analysis

INTRODUCTION

Marigold (*Tagetes* spp.), is a popular annual ornamental crop, widely grown in India, as loose flower on 80.98 thousand hectares yielding about 941.46 MT (Anonymous, 2024). Of the 55 species in the genus *Tagetes*, French marigold (*Tagetes patula* L.) and African marigold (*Tagetes erecta* L.) are the most widely grown for different purposes including landscaping and as loose or potted flowers (Gregorio & Ham, 2007). It is native to the regions extending from the southwestern United States of America to South America, and has gained commercial importance due to attractive flowers and adaptability to diverse climatic conditions (Sumalatha et al., 2023). French marigold is well-regarded for its ornamental appeal, often grown in flower beds, pots, borders, window and porch boxes (Nair et al., 2023). Beyond its aesthetic value, the plant has significant applications in the pharmaceutical and poultry industries, as the essential

oils extracted possess antiparasitic, antispasmodic, antibiotic, antimicrobial, and antiseptic properties (Chowdhury et al., 2009; Sharma et al., 2024). French marigold is sensitive to abiotic stresses, particularly cold stress, which can substantially affect its growth, flowering and biochemical attributes. Cold stress is especially detrimental during the reproductive phase, leading to considerable reduction in flower yield and quality, particularly under the North Indian conditions during winter. This stress also impairs vegetative growth by disrupting cell division and nutrient uptake, resulting in stunted growth and reduced plant vigour. Additionally, low temperatures can induce chlorophyll degradation, thereby reducing the photosynthetic efficiency and overall plant health (Liu et al., 2018). Phenolic compounds, which are essential for stress tolerance, which also exhibit their altered bio-synthesis under cold conditions, potentially weakening the plant's antioxidant defences (Chaeikar et al., 2020).



Owing to increase in cultivation area of French marigold in regions susceptible to low temperatures, it is crucial to understand the impact of cold stress on vegetative growth, flowering and different biochemical attributes. This study aims to elucidate these effects and identify traits associated with cold tolerance, providing insights for formulating breeding strategies and also device management practices for French marigold plants under cold stress conditions.

MATERIALS AND METHODS

Healthy seeds of four French marigold genotypes, namely, Hisar Beauty, Hisar Jafri, Valencia Yellow and Orange Winner (Fig. 1) were sown in nursery beds in October 2021 and 2022. Irrigation and NPK fertilizer (2 g/L) were applied as per the recommended package of practices for raising healthy seedlings. After 30 days, well-rooted seedlings were transplanted into plastic pots containing a garden soil and farmyard manure mix (2:1) prepared a week before transplanting during November each year. This study aimed to assess the impact of low temperatures on flowering marigold plants during peak winter period (December and January). In open-field (cold stress) conditions, the maximum/minimum temperatures ranged from 28.0/0.8°C (2021-2022) and 26.4/1.2°C (2022-2023) with a 4–5°C diurnal variation compared to polyhouse conditions (33.7/3.4°C and 30.1/3.7°C, respectively). Growth, flowering, and yield parameters including plant height, spread, number of primary and secondary branches, leaf area, days to bud initiation and full bloom, flowering duration, flower diameter, number of flowers, single flower weight, flower yield per plant, and flower longevity were recorded following standard protocols (morphological data recorded at the time of full bloom stage) (Choudhary et al., 2014). Biochemical samples were collected from the leaves during the peak winter season (second week of January). Total chlorophyll content was assessed using the dimethyl sulfoxide extraction method (Hiscox &

Israelstam, 1979), while, total phenolics content was measured using the Folin-Ciocalteu method (Singleton & Rossi, 1965) using UV-Vis spectrophotometer (Lambda 365, PerkinElmer, Waltham, MA, USA).

Morpho-biochemical data obtained were analyzed using R software (version 4.3.2) in a factorial completely randomized block design with genotype and environment as factors. Each environment had three replications with twelve plants per replication. ANOVA was performed, and treatment means (mean of two consecutive years, 2021-2022 and 2022-2023) were compared using Duncan's multiple range test at the 5% significance level, using the Agricolae package (De Mendiburu, 2014). Line plots were conducted using ggplot2 and principal component analysis (PCA-Biplot) was performed with FactoMineR and Factoextra packages.

RESULTS AND DISCUSSION

Cold stress disrupts physiological, morphological, and biochemical processes in plants (Yadav, 2010). As evidenced in this study, the four French marigold genotypes raised under open field and polyhouse conditions, showed distinct response. Genotype-environment interaction ($G \times E$) was significantly influenced the plant performance. Plant height was more affected under open conditions, where Hisar Beauty showed moderate reduction (11.1%), indicating better resilience, while, Valencia Yellow and Orange Winner experienced substantial decline of 47.9 and 43.7%, respectively (Table 1 & 2). These findings are consistent with that of Babaei et al. (2021), who also observed reduction in plant height under drought stress. Plant spread also declined, while, genotype Hisar Beauty recorded the minimum decline (7.4%) compared to Hisar Jafri (22.5%). The number of primary branches was highest in Hisar Beauty (14.0), with only a 12.5% reduction, while Valencia Yellow

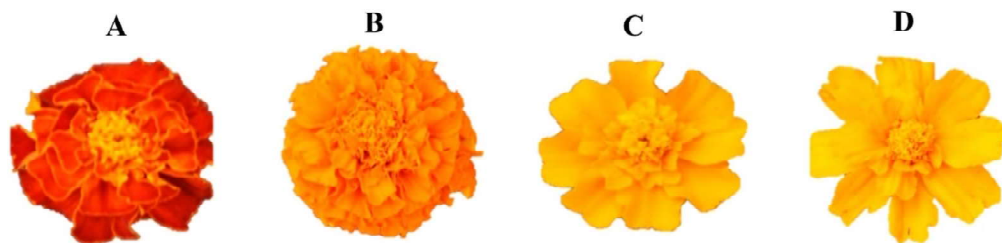


Fig. 1 : Comparative flower phenotypes of French marigold (*Tagetes patula* L.) genotypes
A. Hisar Beauty, B. Hisar Jafri, C. Valencia Yellow, D. Orange Winner

Table 1 : Impact of cold stress on vegetative growth traits in French marigold genotypes

Genotype	Plant height (cm)			Plant spread (cm)			No. of primary branches / plant			No. of secondary branches / plant			Leaf area (cm ²)		
	Open	Poly house	Mean	Open	Poly house	Mean	Open	Poly house	Mean	Open	Poly house	Mean	Open	Poly house	Mean
Hisar Beauty	26.78 ^b	30.12 ^a	28.45 ^a	27.12 ^b	29.30 ^c	26.93 ^b	14.00 ^b	16.00 ^a	15.00 ^a	22.23 ^b	24.34 ^a	23.29 ^a	23.12 ^d	25.19 ^c	24.16 ^b
Hisar Jafri	25.55 ^c	29.71 ^a	27.63 ^b	25.55 ^d	32.95 ^a	31.54 ^a	10.89 ^d	12.00 ^c	11.45 ^b	16.65 ^c	19.34 ^c	18.00 ^c	39.98 ^b	42.34 ^a	41.16 ^a
Valencia Yellow	6.67 ^g	12.80 ^c	9.74 ^d	10.05 ^g	14.30 ^f	12.18 ^c	7.12 ^g	9.80 ^c	8.46 ^d	18.43 ^d	21.76 ^b	20.10 ^b	2.40 ^f	5.51 ^e	3.96 ^c
Orange Winner	8.45 ^f	15.01 ^d	11.73 ^c	9.87 ^g	15.05 ^c	12.46 ^c	8.12 ^f	11.00 ^d	9.56 ^c	13.12 ^f	16.54 ^c	14.83 ^d	2.03 ^f	5.92 ^c	3.98 ^c
Mean [*]	16.86 ^B	21.91 ^A		18.90 ^B	22.65 ^A		10.03 ^B	12.20 ^A		17.61 ^B	20.50 ^A		16.88 ^B	19.74 ^A	
LSD	G						0.20			0.52			0.92		
(p d'' 0.05)	E						0.14			0.37			0.65		
	G × E						0.28			NS			NS		

Genotype (G), Environment (E), and their interaction (G × E).; NS: Non-significant differences.

Values (mean of two consecutive years, 2021-22 and 2022-23) followed by the same letter are not significantly different according to LSD ($p \leq 0.05$).

*Mean values in the same row followed by different uppercase letters are significantly different ($p \leq 0.05$) between environments (open vs. polyhouse)

Table 2 : Percentage reduction in morphological parameters under open field conditions compared to polyhouse conditions in French marigold genotypes

Genotype	Plant height (cm)	Plant spread (cm)	No. of primary branches/plant	No. of secondary branches/plant	Leaf area (cm ²)
Hisar Beauty	11.1	7.4	12.5	8.7	8.2
Hisar Jafri	14.0	22.5	9.3	13.9	5.6
Valencia Yellow	47.9	29.7	27.3	15.3	56.5
Orange Winner	43.7	34.4	26.2	20.7	65.7

Values represent percentage reduction calculated as: [(polyhouse value - open field value)/polyhouse value] × 100

and Orange Winner showed the significant decline in number of both primary and secondary branches. Leaf area, a critical indicator of photosynthetic capacity, was severely impacted in Valencia Yellow and Orange Winner, with reductions of 56.5 and 65.7%, respectively, while, Hisar Beauty maintained good vegetative growth (Table 1 & 2). These results are in line with the earlier studies of Mazhar et al. (2012) on chrysanthemum and El-Attar (2017) on antirrhinum.

Flowering traits were significantly influenced by the different environmental conditions (Fig. 2). Genotype Hisar Jafri took the longest time for bud initiation (47.45 days), while, Valencia Yellow and Orange Winner initiated buds earlier due to their genotypic characteristics (Fig. 2A). The genotype Hisar Beauty and Hisar Jafri, being late-flowering genotypes, had

the minimal variation in days to bloom across environments, indicating minimal sensitivity to cold stress (Fig. 2B). This finding aligns with the studies on salt-tolerant in *Arabidopsis* (Park et al., 2016) and *Schrenkiella parvula* (Oh et al., 2014), which had early flowering under stress compared to control conditions. Hisar Jafri and Orange Winner exhibited had longest flowering duration (51.20 days) and maximum flower diameter, respectively, while Hisar Beauty recorded the minimal environmental impact (Fig. 2C, D). Genotype Hisar Beauty also produced the highest number of flowers/plant (61.00) with minimal variation, highlighting its better cold tolerance for floral traits. In contrast, Valencia Yellow and Orange Winner showed 46 and 47% reduction in flower number under open growing conditions (Fig. 2E). Similar decline in floral traits under stress conditions was also reported by Garcia-Caparrós &

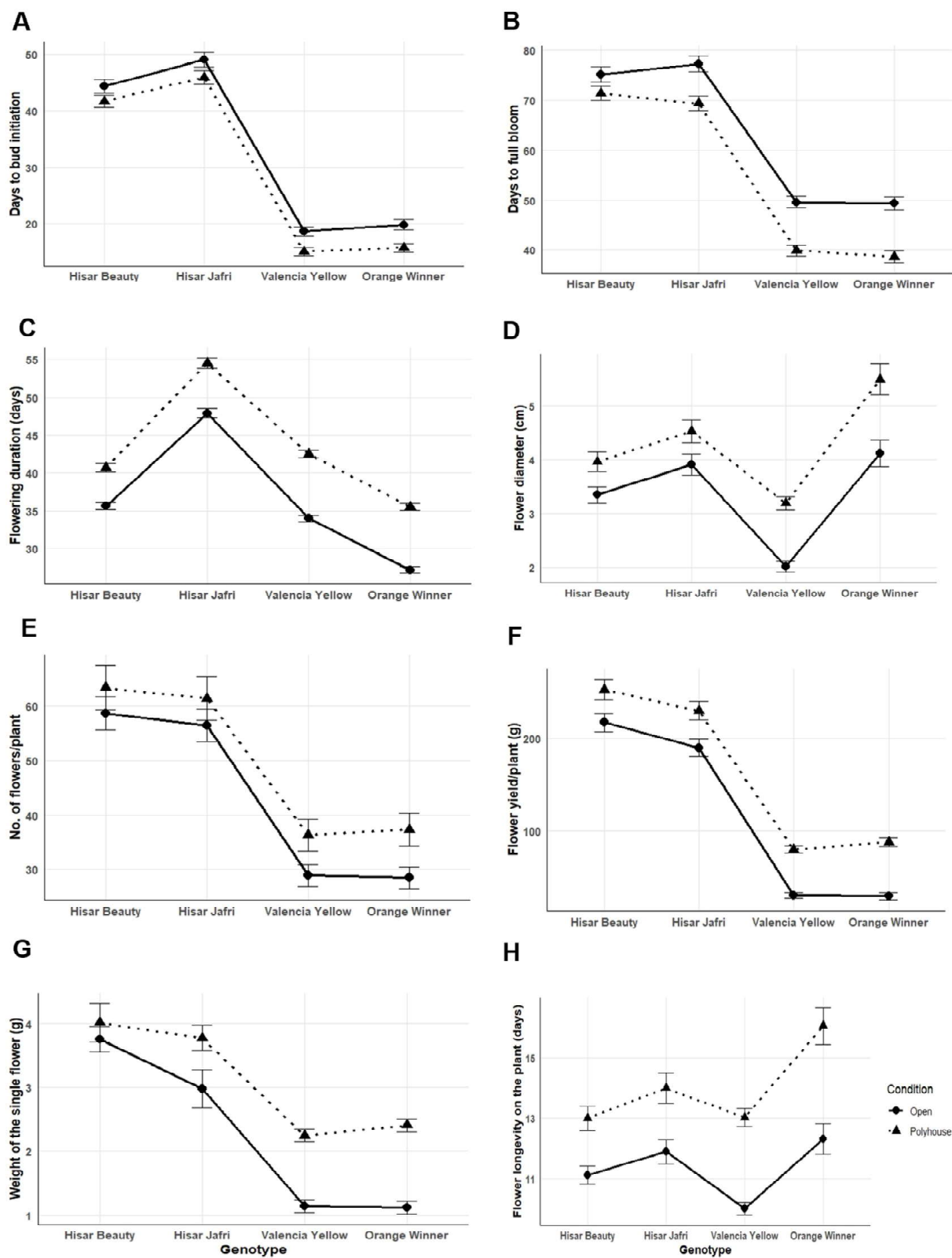


Fig. 2 : Effect of cold stress on floral traits of four French marigold genotypes

(A) Days to bud initiation, (B) Days to full bloom, (C) Flowering duration, (D) Flower diameter, (E) Number of flowers per plant, (F) Flower yield per plant, (G) Weight of single flower, and (H) Flower longevity on the plant under cold stress (solid line) and control conditions (dotted line). Error bars represent standard error of the mean

Lao (2018) in different ornamental plants. Hisar Beauty registered the highest single flower weight (3.89 g) and flower yield per plant (234.47 g) under open field conditions, outperforming genotypes Orange Winner by 45.4 and 87.2%, respectively. All the genotypes exhibited significant increase in flower yield under polyhouse conditions, with flower yield and single flower weight increasing by 38.9 and 27.3%, respectively. However, Valencia Yellow and Orange Winner recorded the substantial yield reductions of 61.5 and 65.7% under open conditions, indicating their stress susceptibility (Fig. 2F, G). These findings align with those of Mohamed et al. (2002), who reported significant yield reduction in wild marigold under drought stress. Cold stress also impacted the flower longevity, with about 23.7% increase under polyhouse conditions. Orange Winner had the maximum longevity (14.18 days), while Hisar Beauty showed stable performance, suggesting its resilience under adverse conditions (Fig. 2H). The genotypic variation in flower longevity reflects differential expression of senescence-associated genes and antioxidant defense mechanisms, with tolerant genotypes exhibiting delayed upregulation of catabolic processes. These results are consistent with those of Chrysargyris et al. (2018), who found that abiotic stress negatively impacted the flower longevity and weight. Overall, the flowering response patterns observed in this study provide valuable insights for developing climate-resilient marigold cultivars with enhanced reproductive stability under changing environmental conditions.

Cold stress negatively impacted the leaf chlorophyll content, a crucial determinant of photosynthetic

efficiency. Genotype Hisar Beauty maintained the highest leaf chlorophyll level under open conditions (11.17 mg/g FW) during winter, with only a 15.9% reduction compared to the polyhouse. In contrast, Valencia Yellow and Orange Winner experienced the highest decline, 32.5 and 35.9%, respectively (Fig. 3A). The degradation of chlorophyll pigments upon exposure to cold stress has been linked to the photo-oxidation leading to reduced photosynthetic capacity (Wu et al., 2019), thereby causing reduced vegetative growth and flower production. Amini et al. (2021) and Jan et al. (2018) observed decline in chlorophyll content in chickpea and calendula, respectively, due to cold stress, reinforcing the fact that cold-induced chlorosis amongst various plant species. The differential chlorophyll retention among genotypes reflects genotypic variation in chlorophyllase enzyme activity and antioxidant defense systems, wherein cold-tolerant genotypes like Hisar Beauty demonstrate superior chloroplast membrane stability and enhanced enzymatic protection against oxidative degradation. Carotenoids function as essential photoprotective compounds that quench excess photon energy through non-photochemical quenching mechanisms and scavenge singlet oxygen species, thereby preventing chloroplast photoinhibition and maintaining photosynthetic integrity under cold stress. Phenolic compounds play a key role in scavenging reactive oxygen species (ROS), and their levels varied under cold stress. The genotype Hisar Beauty recorded the highest total phenolics content (687.13 $\mu\text{g/g}$ FW) under open conditions, surpassing other genotypes. In contrast, Valencia Yellow and Orange Winner

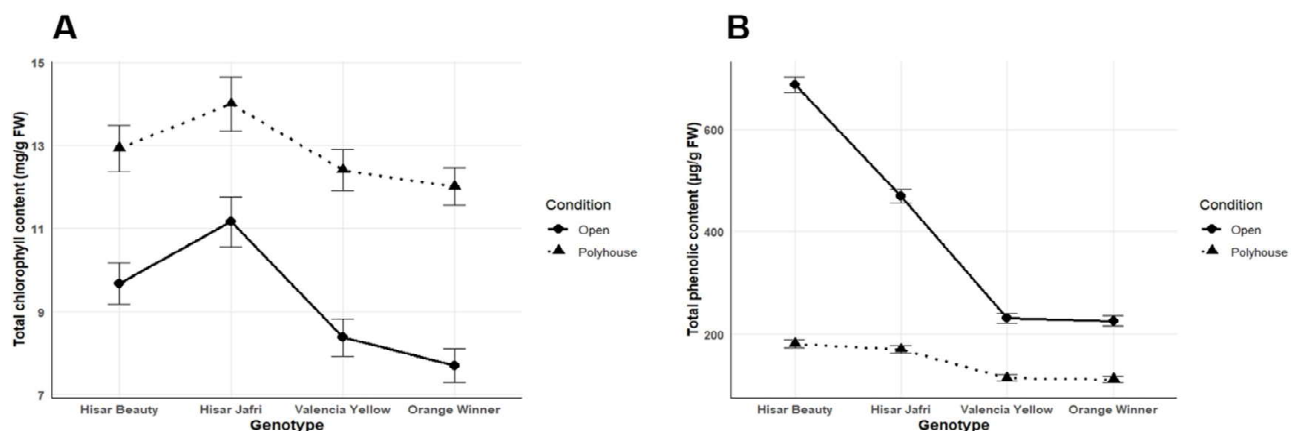


Fig. 3 : Effect of cold stress on biochemical traits in four French marigold genotypes

(A) Total chlorophyll content and (B) Total phenolic content on the plant under cold stress (solid line) and control conditions (dotted line). Error bars represent standard error of the mean

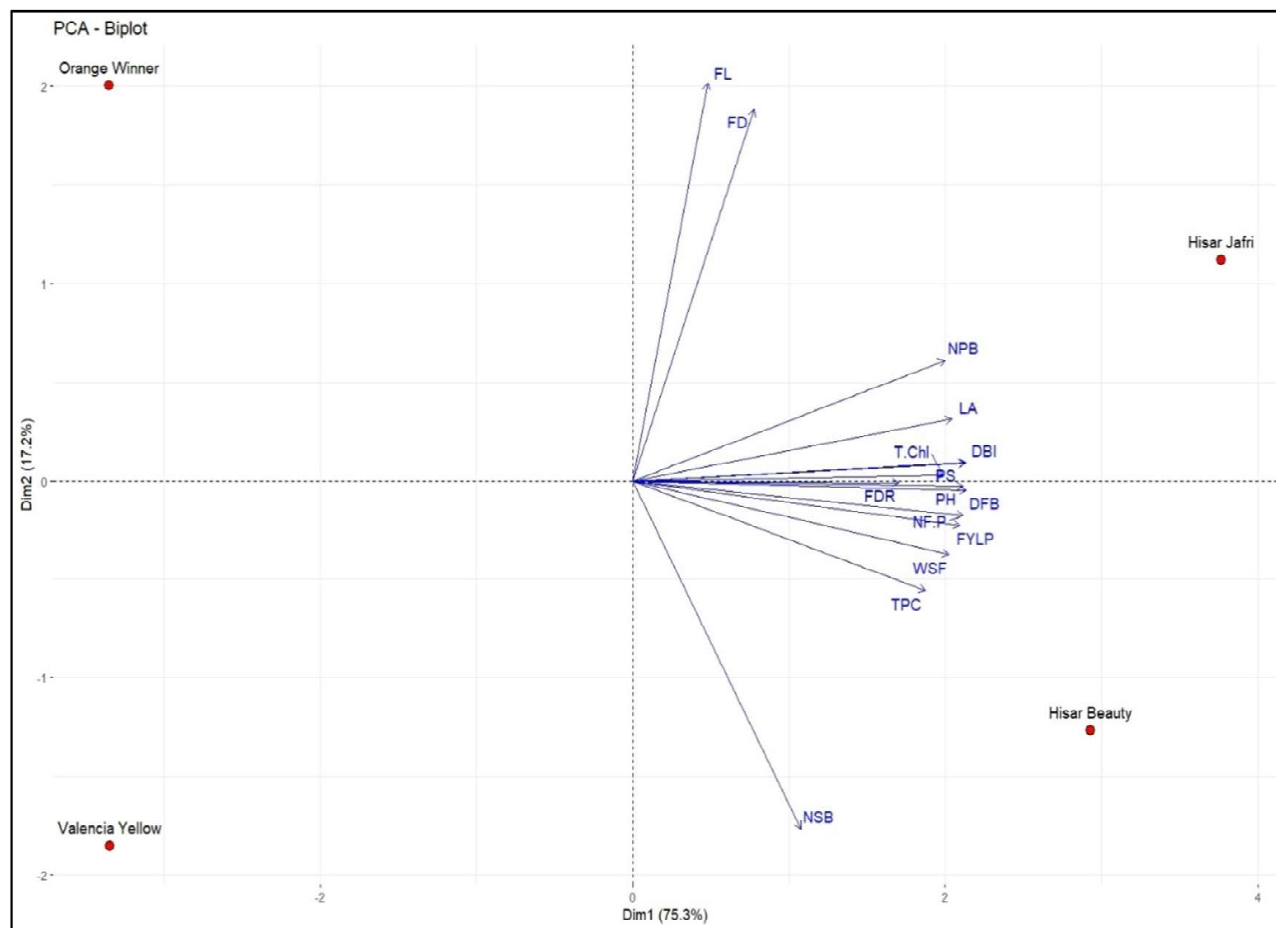


Fig. 4 : Principal component analysis (PCA-Biplot) of French marigold genotypes

Plant height, 'PH', Plant spread, 'PS', Number of primary branches, 'NPB', Number of secondary branches, 'NSB', Leaf area, 'LA', Days to bud initiation, 'DBI', Days to full bloom, 'DFB', Flowering duration, 'FDR', Flower diameter, 'FD', Number of flowers per plant, 'NF/P', Weight of single flower, 'WSF', Flower yield per plant, 'FYLP', Flower longevity, 'FL', Total chlorophyll, 'TChl' and Total phenolic content, 'TPC'

exhibited the lower phenolics levels (231.02 and 226.10 $\mu\text{g/g}$ FW, respectively) reflecting their cold susceptibility (Fig. 3B). Cold-tolerant genotypes like Hisar Beauty and Hisar Jafri accumulated higher phenolics, which may enhance their oxidative stress resilience. This finding is consistent with that of Babaei et al. (2021) and Selmar & Kleinwachter (2013), who found higher phenolic accumulation on exposure to stress in tolerant species. Conversely, the cold-sensitive genotypes might have suffered from degradation under stress, compromising their defense and thereby increasing the tissue necrosis.

The Principal component analysis revealed multidimensional genotype-trait relationships under cold stress, with PC1 and PC2 explaining 75.3% and 17.2% variance, respectively (92.5% total), indicating effective parameter selection for genotypic variation

capture. PC1's high Eigen value suggests cold tolerance is governed by co-varying trait complexes rather than individual parameters, with PC1 loaded by morphological (plant height, leaf area, branches) and yield components (flower number, weight), while PC2 represented physiological traits (chlorophyll content, flowering duration, longevity), indicating complementary cold adaptation mechanisms. Cold-tolerant genotypes (Hisar Beauty, Hisar Jafri) clustered positively on PC1 with superior morphological and yield traits, while susceptible genotypes (Valencia Yellow, Orange Winner) positioned negatively, demonstrating clear phenotypic divergence in stress responses (Fig. 4). This clustering pattern supports recent multivariate stress tolerance studies in wheat (Kumar et al., 2023) and grape rootstocks (Amulya et al., 2024) and confirming PCA's robustness in plant stress physiology research.

CONCLUSION

The study highlights the complex interaction between genotype and environment in marigold performance under cold stress. Genotype Hisar Beauty consistently outperformed others for traits such as plant height, branching, flowering, chlorophyll content, and total phenolics content; demonstrating its resilience. In contrast, Valencia Yellow and Orange Winner were identified having higher cold susceptibility, with significant decline in vegetative and floral traits. These findings underscore the importance of cold-tolerant genotypes like Hisar Beauty and Hisar Jafri having ability for sustained growth and productivity under cold stress, reinforcing the relationship between morphological and biochemical traits with stress tolerance.

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