Indian Journal of Agricultural Sciences **95** (9): 1120–1126, September 2025/Article
https://doi.org/10.56093/ijas.v95i9.156579

Application of non-destructive imaging techniques to evaluate cold tolerance in French marigold (*Tagetes patula*) genotypes

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Received: 14 September 2024; Accepted: 29 July 2025

ABSTRACT

Climate change-induced erratic weather patterns necessitate the development of cold-tolerant marigold cultivars for sustainable floricultural production. The present study was carried out during winter (*rabi*) season 2021–22 and 2022–23 at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the efficacy of high-throughput, non-destructive image-based phenotyping techniques, including Red-Green-Blue (RGB), Near-Infrared (NIR), and Infrared (IR) imaging, for quantitative assessment of essential plant traits such as plant area, greenness, water content, and temperature. Ten French marigold (*Tagetes patula* L.) genotypes (Pusa Deep, Pusa Arpita, Dainty Marietta, Valencia Yellow, Orange Winner, Hisar Beauty, Hisar Jafri, Gulzafri Orange, Fr./W-20, Fr./W-21) were evaluated. The experiment was laid out in a complete randomized design (CRD) with two factors (genotype and environment) and three replications, with 18 plants/environment and 6 plants/replication. Technologies were applied to assess cold tolerance during the early reproductive phase of French marigold genotypes, grown under contrasting environments: Controlled conditions (polyhouse, 30.1°-33.7°C/3.4°-3.7°C) and cold stress (open field, 26.4°-28°C/0.8°-1.2°C) during winter season. Comparative analysis revealed that cold stress significantly impacted morpho-physiological parameters: Plant area decreased by 1.38-fold, caliper length by 1.07-fold, and compactness by 2.10-fold compared to the polyhouse environment. Convex hull area and circumference were reduced by 1.22-fold and 1.05-fold, respectively. Additionally, greenness and plant temperature decreased by approximately 1.03-fold, roundness by 2.07-fold, and plant water content by 1.44-fold. Statistical analysis revealed that open field conditions significantly decreased all measured morpho-physiological parameters, with plant compactness showing the greatest reduction compared to controlled conditions. Notably, genotypes including 'Hisar Beauty' and 'Hisar Jafri ' exhibited superior cold tolerance, demonstrating the least reductions in measured parameters under cold stress, while maintaining higher water content (NIR reflectance, 140.98%) and lower plant surface temperatures (19.06°C) compared to other genotypes. These findings underscore the potential of non-destructive image-based phenotyping as an efficient tool in screening for cold tolerance in marigold breeding programmes, offering a viable and precise alternative to traditional screening methods for accelerated cultivar development.

Keywords: Cold tolerance, French marigold, High throughput phenotyping, Infrared imaging, Near-infrared, Red-Green-Blue spectra

Marigold (*Tagetes* spp.), native to Mexico, is a widely cultivated ornamental plant, particularly in India (Kumar *et al.* 2024), where it leads in loose flower production with an area of 80.98 thousand hectares and a yield of 941.46 MT (Anonymous 2024). Among the 55 species within the genus, French marigold (*Tagetes patula* L.) and African marigold (*T. erecta* L.) are the most commonly cultivated, both in landscape design and for loose or potted flower production (Goody-Hernandez and Miranda-Ham 2007). French marigold, known for their vibrant colours and compact growth habit, are highly valued for use in landscaping and religious ceremonies (Choudhary *et al.* 2014). Cold stress severely affects marigold growth and productivity, particularly during the early reproductive phase when low temperature sensitivity peaks. Marigold exhibits cardinal temperatures of 10–12°C (minimum/base), 18–25°C (optimum), and 35–38°C (maximum), which define its thermal growth boundaries and physiological framework (Choudhary *et al.* 2014). Study temperatures were selected below optimal thresholds to induce sub-optimal stress conditions, enabling quantification of cold-induced physiological perturbations during this critical

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developmental stage. In the face of climate change induced erratic weather patterns, there is an urgent need for breeding cold-tolerant genotypes that can maintain flower yield and quality under sub-optimal temperatures. However, traditional cold tolerance screening methods are destructive and inefficient for large-scale breeding (Golzarian *et al.* 2011). Advanced high-throughput phenomics, using technologies like 3D imaging, sensors, NIR, fluorescence imaging, and LemnaTec, enables rapid, non-destructive assessment of traits linked to cold stress, such as leaf colour and chlorophyll content. Despite its potential, the application of image-based phenotyping in French marigold genotypes, particularly for cold tolerance screening, remains under explored.

It was hypothesized that image-based phenotyping can reliably differentiate between cold-tolerant and susceptible marigold genotypes, providing a valuable screening tool for breeding programmes. The study builds on recent research, which has demonstrated the utility of image-based methods in identifying stress-resilient traits in various crops like wheat, rice, maize and *Arabidopsis* (Granier *et al.* 2006, Golzarian *et al.* 2011). The objective of this study was to utilize non-destructive, image-based phenotyping to screen and identify French marigold genotypes that exhibit cold tolerance, thereby providing a rapid and reliable method for improving cold stress resilience in marigold breeding programmes.

MATERIALS AND METHODS

Plant material and experimental conditions: The present study was carried out during winter (*rabi*) 2021–22 and 2022–23 at ICAR-Indian Agricultural Research Institute, New Delhi. Ten French marigold genotypes (Pusa Deep, Pusa Arpita, Dainty Marietta, Valencia Yellow, Orange Winner, Hisar Beauty, Hisar Jafri, Gulzafri Orange, Fr./W-20, Fr./W-21) were evaluated for the low temperature effects on the early reproductive phase, i.e. during December and January. Plants were grown from seeds sown in October and transplanted into 14-inch white PVC pots containing a standardized potting mixture (soil: farmyard manure: sand in 2:1:1 ratio) with regular fertilization following recommended practices. Plants were grown under two conditions: A polyhouse (controlled) and an open field (cold stress) during November. In the first week of January, coinciding with the peak winter season, approximately three-month-old marigold plants at the early reproductive stage, grown under both polyhouse (controlled) and open field (cold stress) conditions, were transferred to the Nanaji Deshmukh Plant Phenomics Centre, ICAR-Indian Agricultural Research Institute, New Delhi, for high-throughput phenotyping (HTP) to assess the effects of cold stress. Maximum and minimum temperatures were recorded in the open field were 28°/0.8°C (2021–22) and 26.4°/1.2°C (2022–23), with a 3–4°C difference in diurnal temperatures compared to polyhouse conditions, where temperatures reached 33.7°/3.4°C and 30.1°/3.7°C during the respective years.

RGB imaging and extraction of image-based parameters: In this study, RGB imaging (Prosilica GT6600, Allied Vision Technologies GmbH, Germany; spectral band: 400–700 nm, sensor: ON Semi KAI-29,050) was employed to capture phenome data, including plant area, calliper length, greenness, compactness, convex hull area, convex hull circumference, and roundness (Arvidsson *et al.* 2011). An automated imaging unit captured three side-view images of plants at angles of 0°, 120°, and 240° to improve coverage and data accuracy, as side views provided more comprehensive information compared to top views. A uniform background was used to enhance the separation of plant regions from the background.

Application of NIR imaging for measuring plant water content: The hydration status of the plants was assessed using a NIR camera (Gold Eye P-032 SWIR Cool camera, Allied Vision Technologies GmbH, Germany; spectral band: 900–1700 nm, sensor: InGaAs). The NIR images were processed using a grayscale NIR image (12-bit) following a developed procedure (Acosta-Gamboa *et al.* 2016). Due to the challenge of delineating the region of interest using NIR grayscale images under cold stress, the responses were acquired by aligning NIR and RGB images. Two NIR images were captured per pot: One from a top-view angle and another from a side-view angle. The images were first aligned and then cropped to the size of the NIR images. Only the plant areas were used to calculate the average plant water content (%) from the RGB images.

Application of thermal imaging for measuring plant temperature: The IR camera (Pearl Eye P-030 LWIR camera, Allied Vision Technologies GmbH, Germany; spectral band: 8000–14,000 nm, sensor: uncooled microbolometer) was positioned 1.5 m away from the plants, capturing raw greyscale images with a 14-bit depth and a matrix array size of 480 (x) × 640 (y) pixels (Singh *et al.* 2023). The IR images were analysed utilizing a thermal image processing pipeline constructed with Lemna Grid software (Mazis *et al.* 2020). The IR images captured were demosaiced and transformed into binary images applying a universal conversion factor of $A/255 \times 16384 \times 0.0075 - 30$, as outlined in the operating manual (Allied Vision Technology, Stadtroda, Germany) to derive plant temperature (°C) values, where 'A' represented the grey value acquired from the image processing.

A complete randomized design (CRD) with two factors (genotype and environment) and three replications was used, with 18 plants/environment and 6 plants per replication. Data from the 2021–2022 and 2022–2023 seasons were analyzed via two-way ANOVA in R (Version 4.3.2), with means separated by the LSD test ($p \leq 0.05$). Regression analyses were conducted using ggplot2, and principal component analysis (PCA-Biplot) was performed with FactoMineR and Factoextra packages.

RESULTS AND DISCUSSION

Quantitative analysis of cold-stressed phenotypes using RGB image-based traits: Plant area represents the total pixel count of the segmented plant canopy extracted from RGB images through automated image segmentation algorithms.

This metric was computed using RGB imaging (Prosilica GT6600, Allied Vision Technologies GmbH, Germany; spectral band: 400–700 nm, sensor: ON Semi KAI-29,050) by applying colour-based thresholding to distinguish plant pixels from background, followed by morphological operations to refine segmentation boundaries. The biological relevance of plant area lies in its direct correlation with photosynthetic surface area and overall plant vigour, making it a reliable proxy for growth performance under stress conditions. Data revealed significant variation in RGB traits among genotypes, environments, and their interactions. 'Hisar Beauty' showed the largest mean plant area (116.76 Kilo Pixels), a 371.9% increase over 'Valencia Yellow' (24.78 Kilo Pixels) (Table 1, 2). The polyhouse environment had a 37.6% higher mean plant area than the open environment, with 'Hisar Beauty' showing the smallest reduction (29.3%). This substantial variation in plant area under cold stress conditions reflects the differential adaptive mechanisms employed by various genotypes, where tolerant cultivars maintain cellular integrity and continue metabolic processes despite low-temperature exposure (Abro *et al.* 2025). Caliper length, representing the maximum distance across the plant canopy, serves as an indicator of overall plant architecture and growth habit under stress. 'Hisar Beauty' and 'Pusa Arpita' had the highest calliper length (0.87 Kilo Pixels), while 'Valencia Yellow' had the lowest (0.61 Kilo Pixels), reflecting a 42.53% decrease. 'Hisar Beauty' and 'Hisar Jafri' exhibited the highest compactness (0.61 pixels), 27% higher than Fr./W-21, with compactness increasing by 109% in the polyhouse compared to the open environment. 'Hisar Beauty' also had the highest convex hull area, 34.55% greater than 'Hisar Jafri'. The polyhouse environment showed a 22.48% increase in convex hull area over the open environment. These differences highlighted genetic variation in cold stress responses, with 'Hisar Beauty' and 'Hisar Jafri' showing greater resilience.

'Hisar Beauty' and 'Hisar Jafri' had the highest mean convex hull circumference (2.50 and 2.49 Kilo Pixels, respectively), with the polyhouse environment showing a higher value (2.19 Kilo Pixels) than the open environment (2.08 Kilo Pixels) (Table 1). 'Hisar Beauty' also had the highest mean roundness (294.95 Kilo Pixels), significantly surpassing 'Valencia Yellow' (24.22 Kilo Pixels). These findings align with studies on rice (Yang *et al.* 2015), Arabidopsis (Awlia *et al.* 2016), *Quercus* spp. (Mazis *et al.* 2020), and chickpea (Pappula-Reddy *et al.* 2024), highlighting that stress conditions affect image-based morphological traits. Greenness decreased with increasing cold stress, with 'Hisar Beauty' and 'Hisar Jafri' exhibiting the highest greenness (55.93 and 55.30 Pixels, respectively) compared to 'Orange Winner' (44.98 Pixels). Greenness was higher in the polyhouse (51.22 Pixels) than in the open environment (49.67 Pixels)

Table 1 Mean comparison of morphological traits in French marigold genotypes under open and polyhouse (cold stress) conditions

Genotype	Plant area (Kilo Pixels)			Calliper length (Kilo Pixels)			Compactness (Pixels)			Convex hull area (Kilo Pixels)			Convex hull circumference (Kilo Pixels)		
	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
Pusa Deep	58.50^{f}	54.11^{g}	56.30^{e}	0.89^{ab}	0.78^{d}	0.84^{c}	0.65^{c}	0.32^{h}	0.48^{d}	357.00^{d}	294.35^{f}	325.67^{e}	2.13^{e}	2.08^{e}	2.11^{c}
Pusa Arpita	120.00^{b}	90.41^{d}	105.21^{b}	0.90^{ab}	0.87^{b}	0.89^{ab}	0.76^{b}	0.41^{fg}	0.58^{b}	392.51^{c}	326.42^{e}	359.47^{c}	2.51^{abc}	2.30^{d}	2.41^{b}
Dainty Marietta	48.07^{h}	32.10^{j}	40.08^{f}	0.77^{d}	0.64^{fg}	0.70^{e}	0.64^{c}	0.30^{h}	0.47^{d}	304.11^{f}	261.81^{g}	282.96^{f}	2.13^{e}	2.07^{e}	2.10^{c}
Valencia Yellow	26.15^{kl}	23.42^{l}	24.78^{i}	0.64^{fg}	0.61^{h}	0.63^{g}	0.58^{de}	0.22^{j}	0.40^{f}	231.42^{h}	178.72^{i}	205.07^{h}	1.89^{f}	1.83^{f}	1.86^{e}
Orange Winner	30.18^{j}	25.76^{kl}	27.97^{h}	0.66^{fg}	0.63^{fgh}	0.64^{fg}	0.60^{d}	0.27^{i}	0.43^{e}	225.78^{h}	168.40^{i}	197.09^{h}	1.83^{f}	1.79^{fg}	1.81^{e}
Hisar Beauty	131.67	101.84^{c}	116.76^{a}	0.91^{a}	0.87^{b}	0.89^{a}	0.80^{a}	0.42^{f}	0.61^{ab}	460.72^{a}	415.70^{b}	438.21^{a}	2.59^{a}	2.45^{cd}	2.50^{a}
Hisar Jafri	104.37^{c}	65.91^{e}	85.14^{d}	0.80^{cd}	0.77^{d}	0.79^{d}	0.79^{a}	0.42^{f}	0.61^{a}	422.28^{b}	332.69^{e}	377.49^{b}	2.56^{ab}	2.39^{bc}	2.49^{a}
Gulzafri Orange	118.83^{b}	67.18^{e}	93.01^{c}	0.90^{ab}	0.82^{c}	0.86^{bc}	0.76^{b}	0.33^{h}	0.54^{c}	388.80^{c}	305.87^{f}	347.33^{d}	2.42^{c}	2.30^{d}	2.36^{b}
Fr./W-20	39.54^{i}	29.01^{jk}	34.28^{g}	0.70^{e}	0.64^{fgh}	0.67^{f}	0.57^{e}	0.21^{jk}	0.39^{f}	270.35^{g}	223.76^{h}	247.06^{g}	2.02^{e}	1.87^{f}	1.95^{d}
Fr./W-21	30.67^{j}	25.99^{kl}	28.33^{h}	0.66^{f}	0.62^{gh}	0.64^{g}	0.38^{g}	0.19^{k}	0.29^{g}	291.12^{f}	223.58^{h}	257.35^{g}	1.87^{f}	1.70^{g}	1.78^{e}
Mean*	70.90^{A}	51.57^{B}		0.78^{A}	0.73^{B}		0.65	0.31		334.41^{A}	273.13^{B}		2.19^{A}	2.08^{B}	
LSD G		2.69			0.03			0.02			10.51			0.08	
($p \leq 0.05$) E		1.20			0.01			0.01			4.70			0.04	
G × E		3.80			0.36			0.03			14.87			0.11	

Values are presented as the mean of two consecutive years (2021–2022 and 2022–2023). Different letters within each column indicate significant differences at $p \leq 0.05$. G, Genotype; E, Environment; G × E, Genotype × Environment interaction; NS, Non-significant. *, Indicates significant differences between group means under two growing conditions (polyhouse and open)

(Table 2). These differences suggest varied stress responses, with ‘Hisar Beauty’ and ‘Hisar Jafri’ showing better greenness and higher chlorophyll content, indicating greater stress tolerance. Genotypes with higher greenness also demonstrated improved photosynthetic rates under stress (Panda *et al.* 2023). The retention of chlorophyll content under cold stress is a critical adaptive trait that enables continued photosynthetic activity despite adverse conditions. Recent studies on common bean have indicated that cold-tolerant genotypes typically exhibit a more robust antioxidant defence system, which plays a crucial role in safeguarding chlorophyll molecules against oxidative damage under low-temperature stress (Ali *et al.* 2025). The strong correlation observed between greenness and actual chlorophyll content measured via spectrophotometric analysis confirms that RGB-based greenness is a reliable, non-destructive proxy for photosynthetic capacity (Pappula-Reddy *et al.* 2024).

Changes in the water content determined by NIR imaging: Genotypes with higher plant water content exhibited lower NIR intensity, which increased under cold stress. NIR reflectance (%) represents the percentage of near-infrared light reflected by leaf tissues relative to a reference standard, serving as an indirect measure of plant water status where higher reflectance indicates lower water content. NIR reflectance ranged from ~96.05–154.13% in polyhouse conditions to ~140.98–155.76% under cold stress among French marigold genotypes. Cold stress induced substantial increases in NIR reflectance changes across all genotypes, with percentage changes ranging from 46.9% (Hisar Beauty) to 57.0% (Fr./W-21), demonstrating variable water loss responses. The overall mean NIR reflectance changed by 52.2% from polyhouse (97.95%) to open field conditions (149.12%), indicating significant water content reduction under cold stress. The mechanism underlying cold-induced water loss involves disruption of membrane integrity and reduced aquaporin activity, leading to impaired water uptake and increased cellular water efflux. ‘Hisar Beauty’ and ‘Hisar Jafri’ maintained higher water content, suggesting better cold stress tolerance (Table 2). These genotypes also showed higher membrane stability index (MSI), further indicating improved stress resilience. Notably, these tolerant genotypes exhibited the smallest relative changes in NIR reflectance (46.9% and 48.3%, respectively), confirming their superior water retention capacity under cold stress. Higher water content helps maintain cell turgor and metabolic functions, reducing stress effects. This finding aligns with Chen *et al.* (2014) and Kim *et al.* (2020), who reported reduced water content in drought-stressed rice genotypes.

Plant temperature changes determined using IR thermal imaging: Infrared imaging showed increased

Table 2 Mean comparison of morphological and physiological traits in French marigold genotypes under open and polyhouse (cold stress) conditions

Genotype		Greenness (Pixels)			Roundness (Kilo Pixels)			NIR reflectance (%)			Plant temperature (°C)		
		Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean	Polyhouse	Open	Mean
Pusa Deep		51.15^{c}	50.36^{cd}	50.75^{c}	159.82^{f}	51.87^{k}	105.84^{e}	97.22^{h}	150.22^{bcd}	123.72^{cd}	18.96^{def}	19.41^{bcd}	19.19^{bc}
Pusa Arpita		56.06^{ab}	53.70^{b}	54.88^{a}	190.80^{d}	77.14^{i}	133.97^{c}	96.78^{h}	145.45^{def}	121.12^{def}	18.49^{efg}	19.14^{cde}	18.82^{cd}
Dainty Marietta		48.53^{de}	47.86^{e}	48.19^{d}	103.85^{h}	37.69^{l}	70.77^{f}	96.92^{h}	149.65^{bcd}	123.28^{cde}	18.86^{cd}	19.25^{def}	19.05^{bcd}
Valencia Yellow		47.98^{e}	45.27^{fg}	46.63^{de}	25.56^{mn}	22.88^{n}	24.22^{j}	102.62^{g}	153.95^{ab}	128.29^{b}	19.26^{cd}	19.78^{abc}	19.52^{ab}
Orange Winner		45.39^{fg}	44.57^{g}	44.98^{e}	59.20^{j}	29.39^{m}	44.29^{h}	97.59^{gh}	151.60^{abc}	124.59^{bcd}	19.29^{cd}	19.79^{abc}	19.54^{ab}
Hisar Beauty		56.32^{a}	55.55^{ab}	55.93^{a}	338.20^{a}	251.69^{c}	294.95^{a}	96.05^{h}	140.98^{f}	118.51^{f}	18.15^{g}	19.06^{def}	18.60^{d}
Hisar Jafri		56.07^{a}	54.53^{ab}	55.30^{a}	314.59^{b}	116.29^{g}	215.44^{b}	96.48^{h}	143.26^{ef}	119.87^{ef}	18.36^{fg}	19.07^{def}	18.71^{cd}
Gulzafri Orange		54.34^{ab}	51.26^{c}	52.80^{b}	173.14^{e}	73.94^{i}	123.54^{d}	96.79^{h}	146.80^{cde}	121.80^{def}	18.53^{efg}	19.18^{cde}	18.85^{cd}
Fr./W-20		48.05^{de}	47.38^{ef}	47.72^{d}	71.93^{i}	30.31^{m}	51.12^{g}	99.96^{gh}	153.57^{ab}	126.76^{bc}	19.33^{cd}	20.08^{ab}	19.70^{a}
Fr./W-21		48.26^{de}	46.20^{efg}	47.23^{d}	43.50^{l}	25.41^{mn}	34.46^{i}	99.13^{ab}	155.76^{a}	127.44^{a}	19.45^{bcd}	20.18^{a}	19.82^{a}
Mean*		51.22^{A}	49.67^{B}		148.06^{A}	71.66^{B}		97.95^{B}	149.12^{A}		18.87^{B}	19.49^{A}	
LSD ($p \leq 0.05$)	G		1.68			4.12			3.81			0.50	
	E		0.75			1.84			1.70			0.22	
	G × E		2.37			5.83			5.39			0.71	

Values are presented as the mean of two consecutive years (2021–2022 and 2022–2023). Different letters within each column indicate significant differences at $p \leq 0.05$. G, Genotype; E, Environment; G × E, Genotype × Environment interaction; NS, Non-significant. *, Indicates significant differences between group means under two growing conditions (polyhouse and open)

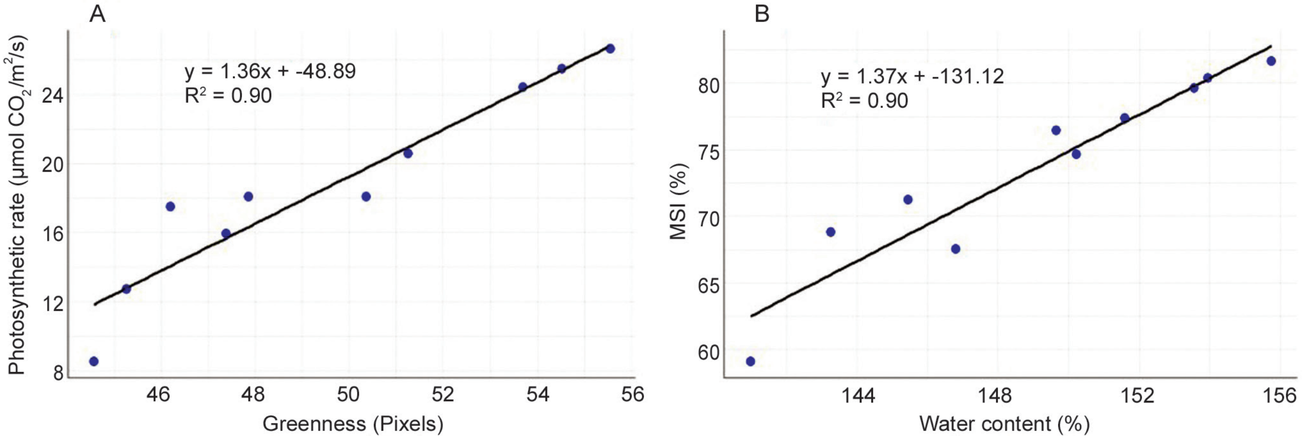

Fig. 1 Linear regression relationships between physiological traits under cold stress. (A), Relationship between photosynthetic rate and greenness; (B) Relationship between Membrane stability index (MSI) and water content.

plant temperature under cold stress. Thermal imaging has been used to assess crop responses to abiotic and biotic stresses (James and Sirault 2012, Ballester *et al.* 2013). In this study, cold-tolerant genotypes exhibited lower temperatures than susceptible ones, likely due to higher water content. Plant surface temperatures ranged from 18.15–19.4°C in polyhouse conditions to 19.06–20.18°C under cold stress. Cold stress induced an absolute temperature increase of 0.62°C (3.3% relative increase) across genotypes, with individual genotype responses ranging from 0.61°C (Hisar Beauty) to 1.03°C (Fr./W-21). The lower surface temperatures in cold-tolerant genotypes are attributed to enhanced cellular water retention and maintained transpiration rates under stress, which facilitate evapotranspiration cooling. Conversely, susceptible genotypes exhibit reduced transpiration due to stomatal closure and cellular dehydration, resulting in elevated surface temperatures and impaired thermoregulation. 'Hisar Beauty' had the lowest temperature (19.06°C) at the early reproductive stage, while 'Fr./W-21' had the highest (20.18°C). Similar findings were reported by Wang *et al.* (2013) in rice.

Regression and Principal Component Analysis (PCA) under cold stress conditions: The linear regression analysis (Fig. 1) revealed significant relationships between physiological traits under cold stress. Photosynthetic rate

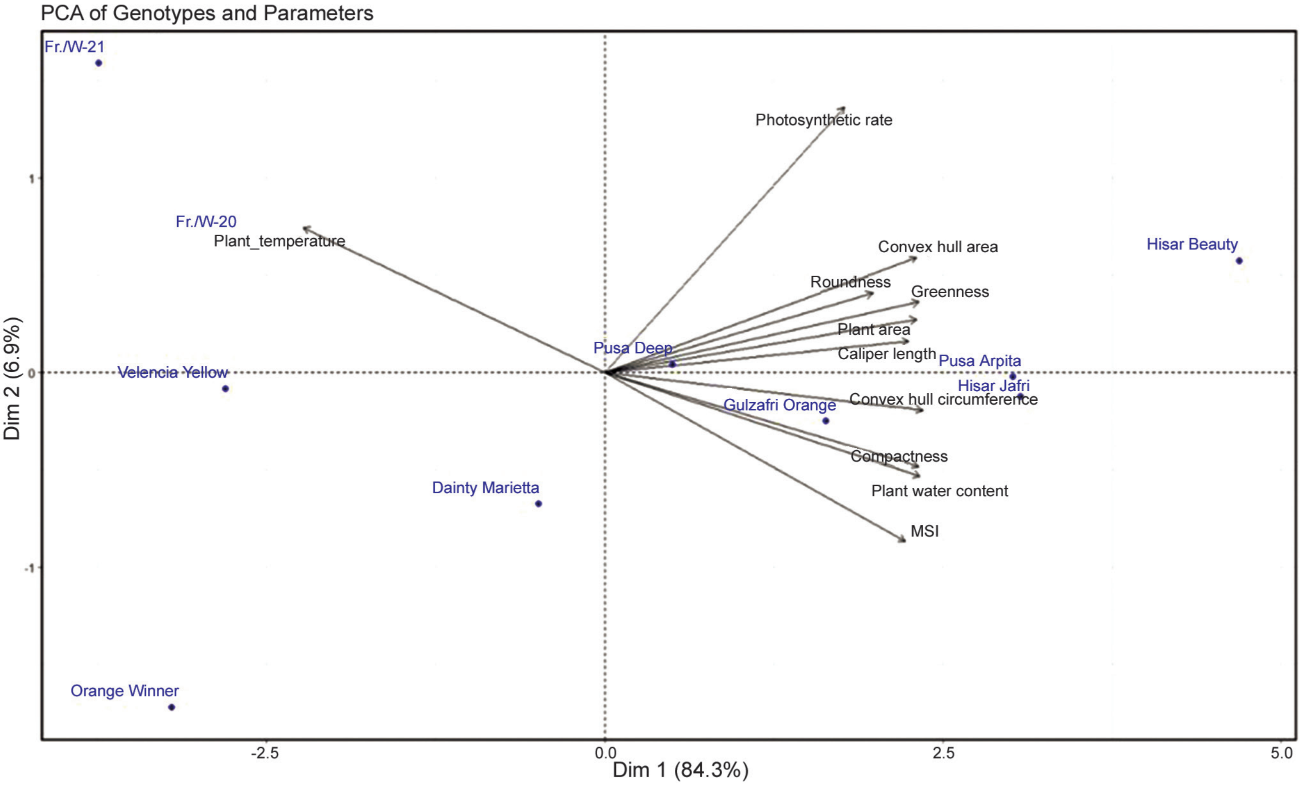

Fig. 2 Principal Component Analysis (PCA-Biplot) of genotypes and morphological and physiological traits under cold stress conditions.

increased with leaf greenness (Fig. 1A), while MSI was positively correlated with water content (Fig. 1B). These findings align with studies on rice (Hairmansis *et al.* 2014), and barley (Chen *et al.* 2018), showing similar correlations under stress. PCA analysis of French marigold genotypes under cold stress explained 84.3 and 6.9% of the variance for Dim 1 and Dim 2, respectively. Dim 1 was positively correlated with traits such as photosynthetic rate, greenness, plant area, and MSI, while Dim 2 had negative coefficients for plant temperature. The high variance explanation by the first principal component indicates strong co-variation among stress-related traits, suggesting that cold tolerance in marigold is determined by a coordinated set of physiological and morphological responses rather than individual traits (Zheng *et al.* 2024). Cold-tolerant genotypes clustered on the right side of the PCA plot, while cold-susceptible ones appeared on the left (Fig. 2). Similar genotype screenings using PCA have been applied to assess drought tolerance in rice (Das e*t al.* 2018), and cold tolerance in pistachio rootstocks (Goharrizi *et al.* 2021).

Significant reductions in key traits under cold stress demonstrated the adverse effects of low temperatures on plant performance. Genotypes like 'Hisar Beauty' and 'Hisar Jafri' showed enhanced cold tolerance, maintaining higher water content, lower temperatures, and better greenness. These findings validate the use of high-throughput imaging methods for rapid, accurate screening of cold tolerance, facilitating the development of resilient marigold varieties in response to climate change.

ACKNOWLEDGEMENT

The senior author acknowledges Indian Council of Agricultural Research, New Delhi for grant of Senior Research Fellowship.

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