



Physiochemical and morphological responses of marigold (*Tagetes erecta*) genotypes under cold stress

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The marigold (*Tagetes* spp.) of the Asteraceae family is a popular ornamental crop grown all over the world. It's a Mexican native and got naturalized 350 years ago (Kumar *et al.* 2018). Marigold is a common loose flower in India where it ranks first in terms of loose flower area (56.04 m ha) and production (497.59 MT) (Indian Horticulture Data Base 2016–17). Marigold flowers are widely used as loose flowers and other purposes such as carotenoid pigments (Akshaya *et al.* 2017). Farmers grow a range of marigold varieties for different seasons however, cold injury limits marigold production throughout the winter especially in northern India and farmers are left with no cold-tolerant cultivars. There have been no studies on the physiological, biochemical and morphological responses of marigold genotypes to cold stress hence the objective of this study was to look into the responses of marigold genotypes under cold stress in order to identify the cold tolerant genotype.

Pusa Bahar (PB), Pusa Narangi Gaiinda (PNG), Pusa Basanti Gaiinda (PBG), Af./W-1, Af./W-2, Af./W-4, Af./W-6, Af./W-7, Af./W-8, and Af./W-3-2 were 10 genotypes of African marigold (*Tagetes erecta* L.) grown in pots in two different growing environments: open (O) and polyhouse (P) during winter (*rabi*) season (2020) at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi. Seeds were sown in mid-November and transplanted in the last week of December 2020. During the peak winter, leaf samples were obtained 20 days after transplanting (DAT) and all the assays were performed in the PG-II lab at ICAR-Division of Floriculture and Landscaping, IARI, New Delhi.

Relative Water Content (RWC) was estimated according to Barrs H D and Weatherley P E (1962) as:

$$\text{RWC (\%)} = \frac{[(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100}{1}$$

Electrolyte leakage rate (ELR) was estimated according to Sairam *et al.* (1997) as:

$$\text{ELR (\%)} = [1 - (C_1 / C_2)] \times 100.$$

The photosynthetic rate ($\mu\text{mol CO}_2\text{m}^2/\text{sec}$) and transpiration rate ($\text{m.mol H}_2\text{O m}^2/\text{sec}$) was measured using an infrared gas analyzer.

Chlorophyll a, b were determined using the protocol described by Hiscox and Israelstam (1979):

$$\text{Chl-a} = 12.7 A_{663} - 2.69 A_{645}; \text{Chl-b} = 22.9 A_{645} - 4.68 A_{663} \text{ (Arnon (1949))}$$

where A is the absorbance of light at wavelengths of 663, 645 nm.

The end product of lipid peroxidation, malondialdehyde (MDA) was quantified using the method established by Heath and Packer (1968).

Observations were recorded for traits like plant height (PH) (cm) (75 Days After Transplanting (DAT), plant spread (cm), days for full bloom (DFB), flower diameter (FD) (cm), flower yield (FY)/plant. The experiment was laid out in Completely Randomized Design (CRD) with two treatments and 3 replications and the data were analyzed by using the software Windostat ver.9.3.

Physiological responses under cold stress: Our findings revealed that exposure to low temperatures had an effect on water status, as evidenced by a decrease in RWC in the open compared to the polyhouse conditions. Among all the African marigold genotypes maximum RWC was found in the genotype Af./W-4 (92.14%) (Table 1). The results showed that the genotypes Af./W-8 (92.03%) and Af./W-4 (73.42%) had the highest and lowest ELR respectively (Table 1). Our findings were also consistent with the literature since ELR was greatly elevated in Coffea plants under cold stress (Campos *et al.* 2003). The genotypes Af./W-8 (4.90 $\mu\text{mol CO}_2\text{m}^2/\text{sec}$) and Af./W-4 (9.99 $\mu\text{mol CO}_2\text{m}^2/\text{sec}$) showing the reduced and higher photosynthetic activity respectively under cold stress (Table 1). The maximum and minimum transpiration rate was observed in the genotypes Af./W-4

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Table 1 Influence of cold stress on physiological and biochemical parameters of African marigold genotypes

Genotype	RWC (%)			ELR (%)			Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$)			Transpiration rate ($\mu\text{mole H}_2\text{O m}^{-2} \text{ sec}^{-1}$)			Chl 'a' (mg/g fwt)			Chl 'b' (mg/g fwt)		
	O	P	Mean	O	P	Mean	O	P	Mean	O	P	Mean	O	P	Mean	O	P	Mean
PB	75.34	93.68	84.51	89.91	71.19	80.55	6.19	10.20	8.20	0.17	0.88	0.53	6.19	7.13	6.66	1.27	1.57	1.42
PNG	82.26	91.59	86.93	91.94	62.13	77.04	5.26	8.86	7.06	0.14	0.77	0.46	6.29	6.81	6.55	1.21	1.53	1.37
PBG	74.29	89.30	81.79	92.65	69.67	81.16	7.36	8.49	7.93	0.21	1.27	0.74	5.65	9.54	7.60	0.93	1.99	1.46
Af./W-1	82.80	94.02	88.41	91.87	72.11	81.99	8.34	9.10	8.72	0.37	0.85	0.61	4.57	8.75	6.66	1.10	1.52	1.31
Af./W-2	71.86	91.47	81.66	92.56	76.77	84.66	7.55	8.60	8.07	0.35	1.19	0.77	7.67	7.98	7.83	1.18	2.03	1.60
Af./W-4	86.76	97.52	92.14	87.26	59.57	73.42	9.50	10.47	9.99	0.59	1.31	0.95	7.90	10.57	9.23	1.35	2.33	1.84
Af./W-6	86.43	95.75	91.09	91.96	64.64	78.30	5.55	7.81	6.68	0.27	1.20	0.74	5.47	8.10	6.79	1.27	1.75	1.51
Af./W-7	77.85	93.00	85.43	90.61	67.56	79.09	6.55	7.26	6.90	0.58	0.71	0.65	4.89	9.15	7.02	1.08	2.08	1.58
Af./W-8	70.04	90.20	80.12	94.67	89.39	92.03	4.79	5.01	4.90	0.44	0.53	0.49	4.42	6.03	5.22	0.14	1.75	0.95
Af./W-3-2	75.33	94.53	84.93	90.07	74.42	82.25	8.05	10.42	9.24	0.43	0.84	0.64	4.83	7.93	6.38	1.20	1.79	1.49
Mean	78.30	93.10		91.35	70.75		6.92	8.62		0.96	0.36		5.79	8.20		1.07	1.83	
CD (0.05) Genotype (G)		2.70			2.33			0.24			0.02			0.26			0.05	
CD (0.05) Environment (E)		1.21			1.04			0.11			0.01			0.12			0.02	
CD (0.05) (G×E)		3.83			3.29			0.35			0.03			0.37			0.07	
SE $\pm$ m (G)		0.95			0.81			0.09			0.01			0.09			0.02	
SE $\pm$ m (E)		0.42			0.36			0.04			0.00			0.04			0.01	
SE $\pm$ m (G×E)		1.34			1.15			0.12			0.01			0.13			0.03	

Table 2 Influence of cold stress on vegetative and flowering parameters of African marigold genotypes

Genotype	PH @75 DAT			Plant spread (cm)			DFB			FD (cm)			FY/plant (g)		
	O	P	Mean	O	P	Mean	O	P	Mean	O	P	Mean	O	P	Mean
PB	42.00	57.90	49.95	19.80	25.80	22.80	94.00	87.00	90.50	3.99	4.78	4.39	200.80	280.98	240.89
PNG	49.00	60.80	54.90	24.35	38.90	31.62	97.00	93.00	95.00	4.29	4.55	4.42	102.34	190.87	146.61
PBG	51.83	62.00	56.92	17.00	32.60	24.80	95.00	90.00	92.50	3.57	4.28	3.93	186.87	224.76	205.82
Af./W-1	34.92	47.69	41.30	14.90	20.05	17.47	95.00	87.00	91.00	4.17	4.86	4.51	98.90	120.09	109.50
Af./W-2	43.50	55.86	49.68	15.90	20.50	18.20	94.00	89.00	91.50	4.05	4.09	4.07	93.34	121.90	107.62
Af./W-4	56.23	75.48	65.85	19.20	29.90	24.55	98.50	92.00	95.25	4.52	5.11	4.81	100.12	132.87	116.50
Af./W-6	33.24	40.72	36.98	20.30	37.90	29.10	97.00	87.000	92.00	4.28	4.84	4.56	175.67	212.76	194.22
Af./W-7	34.67	41.13	37.90	21.90	30.06	25.98	97.00	83.00	90.00	3.90	4.05	3.98	103.45	156.89	130.17
Af./W-8	31.50	36.26	33.88	11.95	22.20	17.08	92.90	80.00	86.45	3.63	4.22	3.93	67.78	90.12	78.95
Af./W-3-2	21.50	35.25	28.38	17.50	31.80	24.65	97.00	86.00	91.50	3.84	4.44	4.14	112.87	187.56	150.22
Mean	39.84	51.31		18.28	28.97		95.74	87.40		4.03	4.52		124.21	171.88	
CD (0.05) Genotype (G)		1.51			0.60			3.19			0.11			4.59	
CD (0.05) Environment (E)		0.68			0.27			1.42			0.05			2.05	
CD (0.05) (G×E)		2.14			0.85			4.51			0.15			6.49	
SE _{±m} (G)		0.53			0.21			1.11			0.03			1.61	
SE _{±m} (E)		0.24			0.09			0.49			0.01			0.72	
SE _{±m} (G×E)		0.75			0.30			1.57			0.05			2.27	

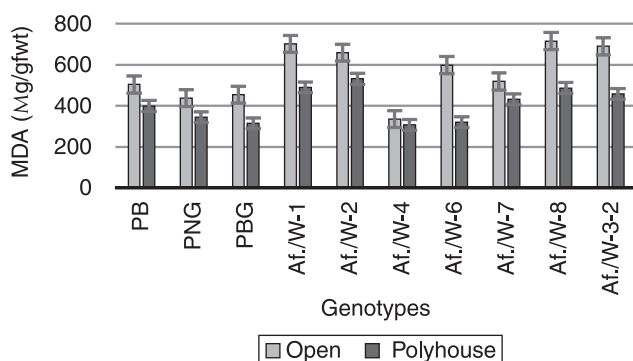


Fig 1 Effect of cold stress on MDA content of African marigold genotypes.

(0.95 $\mu\text{mol H}_2\text{O m}^2/\text{sec}$) and PNG (0.46 $\mu\text{mol H}_2\text{O m}^2/\text{sec}$) respectively (Table 1).

The chlorophyll pigments in the leaves reduced significantly after exposure to low temperature in all the African marigold genotypes. Highest chlorophyll 'a', 'b' was observed in the genotype Af./W-4 with 9.23, 1.84 mg/g fwt respectively (Table 1). Lipid peroxidation increased after exposure to low temperature in the open compared to the polyhouse conditions, indicating membrane damage. The highest lipid peroxidation was observed in the genotype Af./W-8 (601.61 $\mu\text{g/g fwt}$) and the genotype Af./W-4 (321.45 $\mu\text{g/g fwt}$) with the lowest one (Fig 1). MDA levels were greater in wheat seedlings exposed to 4°C (Hou *et al.* 2010) and these findings support our results.

Morphological parameters: Cold, as a major environmental factor, can alter plant vegetative and blooming processes. The results of this study revealed that the effect of cold stress on African marigold genotypes resulted in a considerable reduction in PH under open compared to polyhouse conditions. At 75 DAT, maximum and minimum PH was observed in genotypes Af./W-4 (65.85 cm) and Af./W-6 (28.38 cm) respectively (Table 2). Similar findings were obtained in Zoysia grass, with decreased plant growth as shown by canopy height due to decreasing temperature (Li S *et al.* 2018). Under polyhouse conditions, the genotype PB followed by Af./W-4 showed significantly larger plant spread than open conditions (62.59% and 53.56% respectively) (Table 2).

Flowering characteristics: One of the primary breeding aims of marigold in North Indian conditions is to breed for early flowering in order to avoid the harsh winter temperatures that prevail in December and January. The time it took for different African marigold genotypes to fully bloom ranged from 86.45 days (Af./W-8) to 95.25 days (Af./W-4) (Table 2). Among all the African marigold genotypes, PB (4.81 cm) had the largest FD. The mean FY/plant (g) for all African marigold genotypes ranged from 124.21 g/plant (open) to 171.88 g/plant (polyhouse). The genotypes PB (240.89 g) and Af./W-8 (78.95 g) had the highest and lowest FY/plant (g) correspondingly (Table 2). Similar results have been documented, such as a decrease in green gram genotypes yield and productivity under cold

stress conditions (Kabi M *et al.* 2017).

As a result, it may be concluded that the African marigold genotypes under polyhouse conditions outperformed than the genotypes grown in open conditions. However, the genotypes Af./W-4 followed by PB were found to be the most promising cold tolerant genotypes based on physiological, biochemical and morphological assay.

SUMMARY

The objective of this study was to look into the physiochemical and morphological responses of marigold genotypes under cold stress, in order to identify the cold tolerant genotype. During the winter (*rabi*) season, 10 genotypes of African marigold were grown in pots in open and polyhouse growing environments from December 2020 to April 2021 at the research farm of ICAR-IARI, New Delhi. The results revealed that marigold genotypes showed a severe drop in RWC, photosynthesis, transpiration, chlorophyll pigments and increased ELR and MDA content as the cold sensitive genotypes and vice versa was found in the Af./W-4 genotype. There was a significant decrease in the growth and yield parameters due to cold stress conditions in open as compared to polyhouse conditions. The genotypes Af./W-4 and Pusa Bahar recorded highest plant height and maximum plant spread, flower diameter, higher yield/plant respectively among all other African marigold genotypes. Therefore, genotypes Af/W-4 followed by Pusa Bahar are deemed the most tolerant under cold stress conditions, based on the basis of physiochemical and morphological responses.

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