The Pharma Innovation Journal 2022; 11(1): 518-522

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 518-522

www.thepharmajournal.com
Received: 19-11-2021
Accepted: 21-12-2021

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Influence of cold stress on morpho-physiological traits of African marigold (*Tagetes erecta*) genotypes during reproductive phase

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Abstract
The objective of this study was to see the effect of cold on the growth, flowering and physiological responses in different African marigolds to find out the cold tolerant genotypes so that they can be further used in crop improvement programme. During winter season, ten genotypes of African marigold were grown in pots in two different growing environments: open and polyhouse conditions, from November to February. The results revealed that marigold genotypes showed a severe drop in growth and yield parameters, decline in the relative water content (RWC) and photosynthetic rate due to cold stress conditions in open as compared to polyhouse conditions. The genotypes Af./W-4 was recorded with highest plant height, leaf area, maximum RWC, photosynthetic and transpiration rate and with less electrolyte leakage rate 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 assay of several morpho-physiological parameters.

Keywords: cold stress, African marigolds, open and polyhouse conditions, growth, flowering traits, ELR

Introduction
The Asteraceae family's marigold (*Tagetes* spp.) is a popular ornamental crop grown worldwide. It is a Mexican native that became naturalized in India about 350 years ago (Kumar *et al*, 2018) [9]. Marigold is a popular loose flower grown in India and ranked top most in loose flower area (56.04 m ha) and production (497.59 MT) (Indian Horticulture Data Base 2016-17). It is gaining popularity among Indian farmers due to its ease of cultivation, short duration, extensive adaptability, wide range of shape, size, and good flower maintaining quality. Marigold flowers are widely used as loose flowers for garlands, religious offerings, social functions, and other purposes such as pigment extraction (Gupta and Vasudeva, 2012) [5], as a feed additive for the poultry industry (Nuraini and Djulardi, 2017) [12]. The flowers are rich source of carotenoids (Panwar *et al*, 2014) [14] and serve as a potential leader in marigold exporter. Marigold flowers can be grown year-round under a variety of agro-climatic conditions. The key means of assuring quality marigold output has been temperature. Low temperature is a primary abiotic stress that affects plant growth and development, as well as limiting plant spread (John *et al*, 2016; Sanghera *et al*, 2011) [7, 17]. Plants that are sensitive to cold must be grown in expensive, heated greenhouses. However, most of the farmers cannot afford the high costs of greenhouses required for high-quality marigold cultivation while avoiding the negative effects of cold stress on flower yield. Farmers cultivate a wide variety of marigold varieties for different seasons, however during the winter, production of marigold is limited, especially in northern India, due to cold injury and farmers are left with no cold-tolerant varieties. It is vital to strengthen plants inherent stress tolerance in order to reduce heating expenses. Before that, it's critical to understand how certain stressors affect plant morphological and physiological changes as well as flower quality, so that breeding programs may be devised to select/ develop genotypes that are more tolerant to cold stress. Till now, no investigations on the morphological and physiological responses of African marigold genotypes to cold stress have been done thus, the objective of this study was to investigate the growth, flowering and physiological responses of well grown marigold genotypes under cold stress conditions.

Material and Methods

Morphological traits

The present work was carried at ICAR- Indian Agricultural Research Institute, Division of Floriculture and Landscape Architecture research farm, New Delhi-110012. Ten genotypes of African marigold (*Tagetes erecta* L.) suitable for the winter season were chosen for the present study which includes Pusa Bahar (PB), Pusa Narangi Gainda (PNG), Pusa Basanti Gainda (PBG), Af./W-1, Af./W-2, Af./W-4, Af./W-6, Af./W-7, Af./W-8 and Af./W-3-2. The genotypes were grown in pots during the winter season under two growing environments: open (O) and polyhouse (P) conditions. Seed sowing was done during mid-October and transplanted in mid-November 2020. Observations were recorded for traits like plant height (cm) (25, 50, 75 days after transplanting (DAT) and/or flowering stage), plant spread (cm), leaf area (m^2), number of days for flower bud initiation, flower bud showing color, full bloom, flower diameter (cm), flower yield/plant (g).

Physiological traits

Estimation of Relative Water Content (RWC)

RWC was estimated according to Barrs H D & Weatherley P E. (1962) [2] by using the formula

RWC (%) = [(Fresh weight–Dry weight)/ (Turgid Weight–Dry Weight)]x100.

Estimation of Electrolyte leakage rate (ELR)

Leakage of ions from the leaves was estimated according to Sairam *et al*. (1997) [16].

ELR (%) = [1-(C_1 / C_2)] ×100.

Photosynthetic and transpiration rate

The photosynthetic rate (μmolCO_2 m^2/sec) and transpiration rate (m. mol H_2O m^2/sec) was measured using an infrared gas analyser. The experiment was carried 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.

Results and Discussion

Vegetative traits influenced by cold stress Cold, as a major environmental factor, can affect plant vegetative and blooming processes. The results of this study revealed that the effect of cold stress on well-grown African marigold genotypes resulted in a considerable reduction in plant height under open compared to polyhouse conditions. At 25 DAT maximum and minimum plant heights were recorded in genotypes Af./W-4 (18.44 cm) and PBG (12.56 cm) respectively, whereas at 50 and 75 DAT, maximum and minimum plant heights were observed in genotypes Af./W-4 (21.67 cm), Af./W-6 (14.36 cm) and Af./W-4 (32.67 cm), Af./W-6 (21.48 cm) respectively (Table 1). Similar findings were reported in Zoysia grass, with decreased plant growth as shown by canopy height due to decreasing temperature by Li S *et al.* (2018) [11] and similarly, growth rate was lowered in cold treated plants compared to control in Bermuda grass by Fan J *et al.* (2014) [4]. The plant spread (cm) differed significantly between genotypes and between the two growing conditions. Under polyhouse conditions, the genotype Pusa Bahar followed by Af./W-4 showed considerably larger plant spread than open conditions, with 76 and 78% respectively (Table 1). Similar findings were noted by Lajayer H M *et al.* (2018) [10] under chilling and freezing sress, *Thymus kotschyanus* had a larger area coverage than *T. kotschyanus*. Maximum leaf area was reported in the genotype Af./W-4 (19.76 m2) whereas minimal leaf area was recorded in the genotype Af./W-8 (10.21 m^2) under cold stress conditions (Table 1). The results obtained are in agreement with those of Lajayer H M *et al.* (2018) [10] under chilling and freezing conditions in *Thymus* spp. In *Coffea arabica* L. it was reported that low temperature regimes caused a reduction in vegetative development by Barros *et al.* (1997) [1].

Flowering traits influenced by cold stress

One of the primary breeding objectives 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 genotype Af./W-1 (39.45) had the shortest flower bud initiation time while the genotype PBG (51.99) had the longest (Table 2). The genotypes PNG (62.99) and Af./W-4 (80.73) were recorded as the minimum and maximum number of days for flower bud showing color (Table 2). The time it took for different African marigold genotypes to fully bloom ranged from 73.30 (polyhouse) to 86 days (open) with minimum and maximum number of days for full bloom was recorded in the genotypes Af./W-8 (73) and Af./W-7 (85.50) respectively (Table 2). Among all the African marigold genotypes, Pusa Bahar (6.19 cm) followed by Af./W-4 (4.60 cm) had the largest flower diameter (Table 2). The mean flower yield/plant (g) for all African marigold genotypes ranged from 140.85 g/plant (under open conditions) to 205.68 g/plant (under polyhouse conditions). The genotypes Pusa Bahar (262.69 g) and Af./W-8 (94.48 g) had the highest and lowest flower yield/plant (g) correspondingly(Table 2). Similar effects have been documented, such as a decrease in green gram genotypes yield and productivity under cold stress conditions (Kabi M *et al.* 2017) [8].

Physiological traits influenced by cold stress

RWC and solute accumulation are good indicators of plant water stress and they are linked to different plant functions (Nayyar and Walia, 2003) [8]. Our findings revealed that exposure to low temperatures had an effect on water content, as evidenced by a decrease in RWC in the open compared to the polyhouse conditions. Among all the African marigold genotypes maximum and minimum RWC was found in the genotypes Af./W-4 (93.70%) and Af./W-8 (85.78%) respectively (Fig 1(a)). The results showed that the genotypes Af./W-8 (86.08%)and Af./W-4 (62.45%) had the highest the lowest ELR (Fig 1(b)). Our findings were also consistent with the literature since ELR was greatly elevated in Coffea plants under cold stress (Campos *et al.* 2003) [3]. Low temperatures had affected various components of the photosynthetic process. It reduced the activity of enzymes involved in the Calvin cycle and the formation of Reactive Oxygen Species in PS II and PS 1(Soitamo *et al.* (2008) [18]; Ruelland *et al.* (2009) [15]). In the current study, the genotype Af./W-8 (12.03 μ.molCO_2 m^2/sec) was observed with the reduced photosynthetic activity and the genotype Af./W-4 (22.41 μ.molCO_2 m^2/sec) shown higher photosynthetic activity under cold stress (Fig 1(c)). Similar trends of results were recorded in their investigation on the physiological performance of papaya genotypes under abiotic stress, Jeyakumar *et al.* (2005) [6] found that the leaf RWC had a substantial impact on

photosynthesis reducing the photosynthetic rate by more than 50% when the water content was less than 82%. The maximum and minimum transpiration rate was observed in the genotypes Af./W-4 (7.21 m.mol H_2O m^2/sec) and PNG (5.57 m.mol H_2O m^2/sec) respectively (Fig 1(d)).

Table 1: Effect of cold stress on vegetative traits of African marigold genotypes

	PH @ 25 DAT			PH@ 50 DAT			PH @ 75 DAT			Plant spread (cm)			Leaf area (m^2)		
Genotypes	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**
PB	11.24	18.74	14.99	15.69	24.48	20.09	24.78	32.00	28.39	30.67	40.05	35.36	9.17	21.45	15.31
PNG	14.47	17.44	15.95	17.92	21.66	19.79	23.47	30.00	26.74	20.95	21.35	21.15	12.27	17.27	14.77
PBG	11.75	14.06	12.91	12.56	18.87	15.72	24.06	29.00	26.53	23.40	34.29	28.85	13.08	21.38	17.23
Af./W-1	11.03	16.31	13.67	13.78	20.94	17.36	16.97	28.00	22.48	20.50	27.90	24.20	14.61	19.92	17.27
Af./W-2	14.00	16.19	15.09	17.94	22.83	20.38	21.78	29.50	25.64	20.85	21.60	21.23	11.63	18.27	14.95
Af./W-4	15.34	18.80	17.07	18.44	24.90	21.67	27.33	38.00	32.67	30.20	38.65	34.42	14.66	24.86	19.76
Af./W-6	10.78	13.12	11.95	13.07	15.65	14.36	18.96	24.00	21.48	22.80	30.50	26.65	14.37	17.98	16.17
Af./W-7	11.56	12.44	12.00	14.77	16.80	15.79	23.34	31.30	27.32	25.98	31.35	28.66	8.76	17.38	13.07
Af./W-8	15.31	18.40	16.86	16.40	22.13	19.26	20.43	29.00	24.72	17.56	23.20	20.38	7.48	12.93	10.21
Af./W-3-2	12.80	14.53	13.66	16.67	21.79	19.23	19.12	29.50	24.31	28.97	33.95	31.46	11.60	19.40	15.50
Mean	12.73	15.76	-	15.72	21.01	-	22.02	30.03	-	24.18	30.28	-	11.76	19.08	-
CD $_{(0.05)}$ Genotype (G)	0.49			0.56			0.80			0.25			0.29		
CD $_{(0.05)}$ Environment (E)	0.22			0.25			0.36			0.11			0.13		
CD $_{(0.05)}$ (G × E)	0.69			0.80			1.14			0.36			0.41		
SE ± m (G)	0.17			0.20			0.28			0.09			0.10		
SE ± m (E)	0.08			0.09			0.13			0.04			0.05		
SE ± m (G × E)	0.24			0.28			0.40			0.12			0.14		

Table 2: Effect of cold stress on flowering traits of African marigold genotypes

	Days taken for flower bud initiation			Days taken for flower bud showing color			Days taken for full bloom			Flower diameter (cm)			Flower yield/plant (g)		
Genotypes	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**	**O**	**P**	**Mean**
PB	48.56	43.89	46.22	77.50	70.00	73.75	88.00	78.00	83.00	5.48	6.89	6.19	213.78	311.60	262.69
PNG	44.67	42.78	43.72	65.98	60.00	62.99	85.00	69.00	77.00	4.88	6.21	5.54	176.9	235.59	206.25
PBG	56.44	47.54	51.99	72.45	68.33	70.39	88.00	75.00	81.50	3.87	5.79	4.83	179.87	265.20	222.54
Af./W-1	41.45	37.44	39.45	69.87	67.22	68.55	83.00	77.00	80.00	5.28	6.45	5.87	97.90	154.80	126.35
Af./W-2	41.89	39.67	40.78	67.54	62.44	64.99	84.00	68.00	76.00	5.38	5.75	5.57	102.43	176.89	139.66
Af./W-4	49.98	41.20	45.59	82.56	78.89	80.73	89.00	76.00	82.50	5.44	6.27	5.86	112.34	180.20	146.27
Af./W-6	43.33	40.22	41.78	74.67	65.11	69.89	87.00	80.00	83.50	4.39	6.61	5.50	167.84	226.05	196.95
Af./W-7	42.78	39.67	41.22	69.76	65.00	67.38	94.00	77.00	85.50	4.04	5.16	4.60	123.87	192.00	157.94
Af./W-8	44.00	40.89	42.45	68.89	63.33	66.11	82.00	64.00	73.00	4.96	5.66	5.31	76.75	112.20	94.48
Af./W-3-2	43.89	37.89	40.89	67.34	63.003	65.17	80.00	69.00	74.50	4.28	5.09	4.68	156.90	202.31	179.61
Mean	45.70	41.12	-	71.66	66.33	-	86.00	73.30	-	4.80	5.99	-	140.85	205.68	-
CD $_{(0.05)}$ Genotype (G)	1.03			2.13			2.23			0.16			6.41		
CD $_{(0.05)}$ Environment (E)	0.46			0.95			0.91			0.07			2.87		
CD $_{(0.05)}$ (G × E)	1.46			3.01			3.16			0.22			9.07		
SE ± m (G)	0.36			0.75			0.78			0.05			2.24		
SE ± m (E)	0.16			0.33			0.35			0.02			6.41		
SE ± m (G × E)	0.51			1.05			1.11			0.08			2.87		

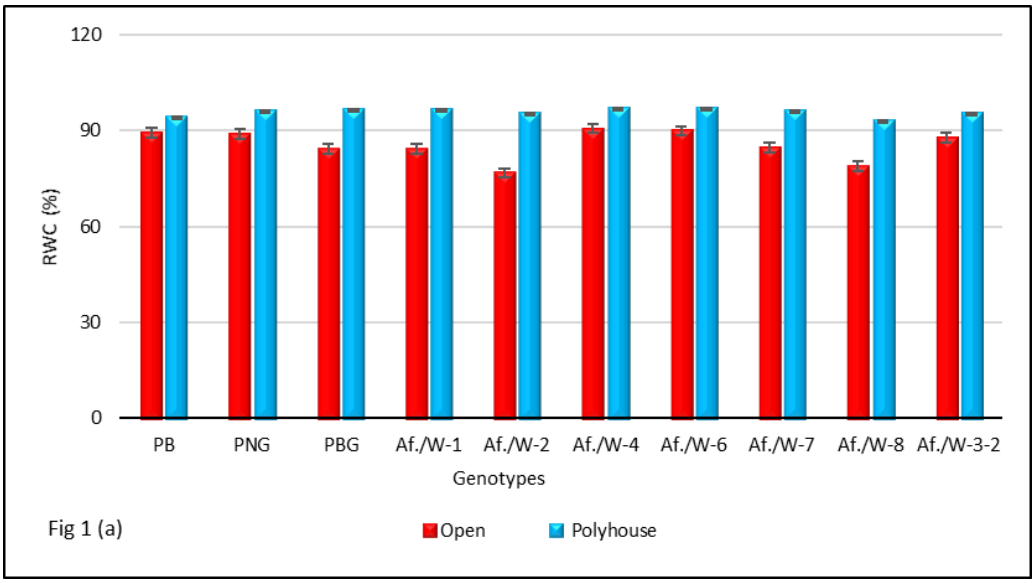

Fig 1 (a)

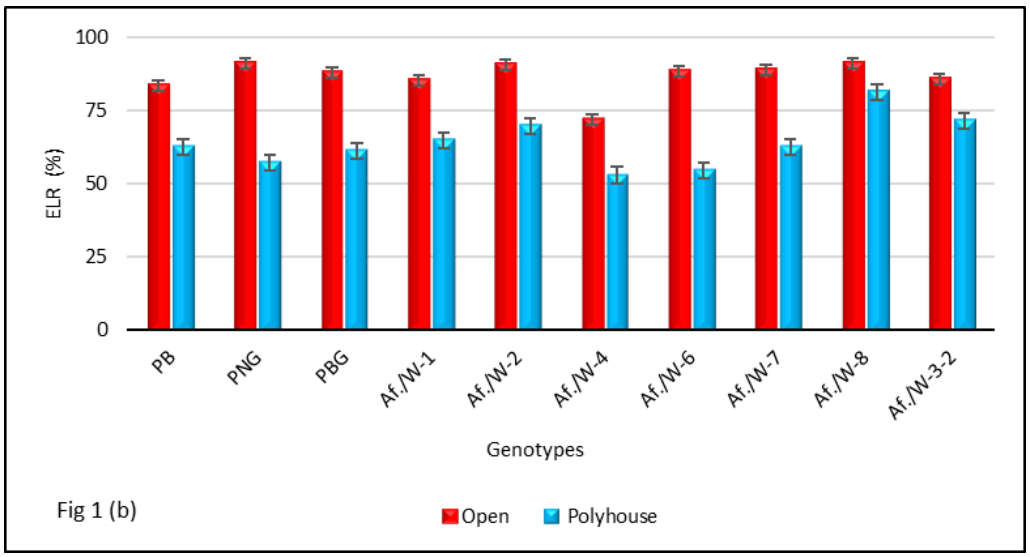

Fig 1 (b)

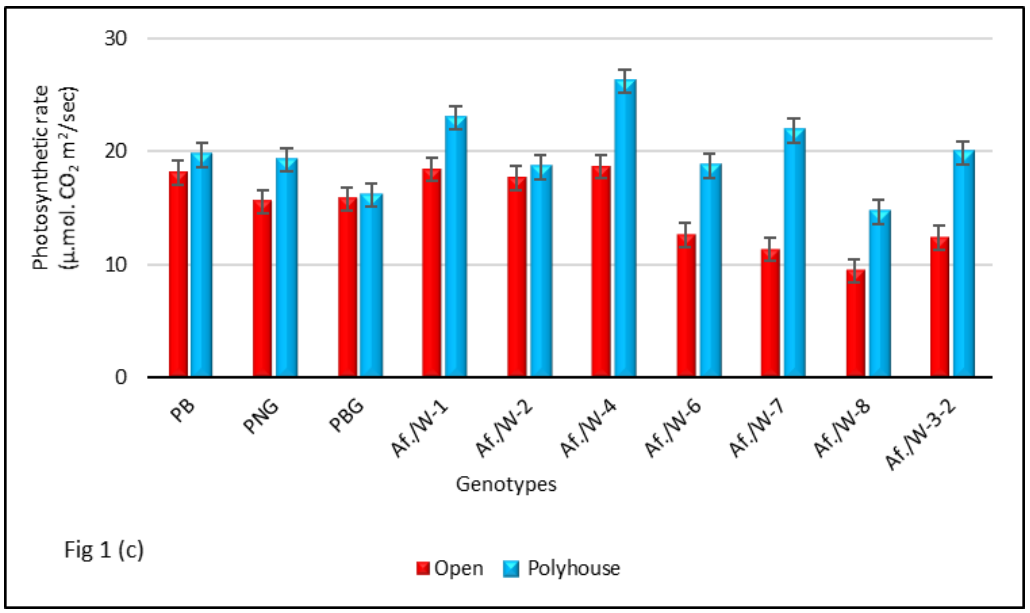

Fig 1 (c)

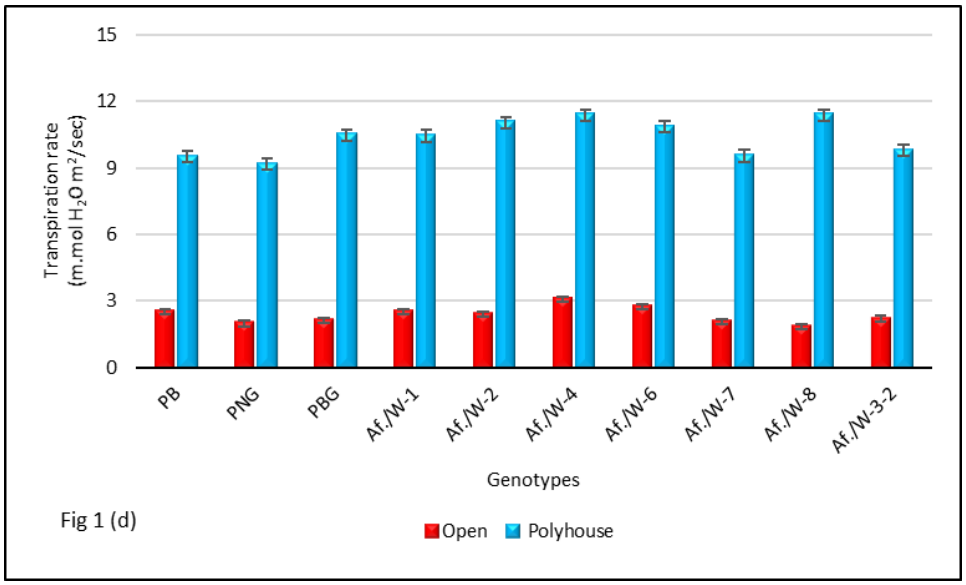

Fig 1: Effect of cold stress on physiological traits of African marigold genotypes

Conclusion

As a result, based on the morphological and physiological analysis, it may be concluded that the African marigold genotypes under polyhouse conditions outperformed the open condition genotypes, however the genotypes Af./W-4 followed by Pusa Bahar, were found to be the most promising cold tolerant genotypes.

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