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Multivariate analysis to identify drought responsive morpho-physiological traits in standard chrysanthemum genotypes

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ABSTRACT

Chrysanthemum is a major flower crop grown globally for its use as cut flowers, potted plants and landscape decoration. It is highly susceptible to drought; hence, it is necessary to identify the drought responsive key traits in chrysanthemum. In this study, 14 standard chrysanthemum genotypes were evaluated for drought tolerance under hydroponics and pot culture in three replications of a completely randomised design (CRD). The seedlings were exposed to osmotic stress with 10% PEG (Polyethylene Glycol). In pot culture, a drought stress of -60 kPa was applied for seven days during the vegetative growth and flower bud initiation stages. R statistical analysis was performed (PCA analysis, R2.15.1 by R Development Core Team) to evaluate several morphological and physiological traits. In hydroponics and pot culture drought conditions, RWC, MSI, and flower yield were highly correlated with chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, chlorophyll fluorescence, stem girth, flower yield, and biomass. As a result, these traits can be considered useful selection indicators for drought tolerance. According to PCA, the traits chlorophyll a, chlorophyll b, chlorophyll a/b, total chlorophyll, carotenoids, RWC, and chlorophyll fluorescence were the most crucial traits that involved in genotype variability under stress contributing significantly to PC1 in pots and hydroponics. In hydroponics and pot culture, the variability was categorized into three and five principal components, respectively. These results offered useful information for chrysanthemum breeders aiming to select and improve drought tolerance through targeted selection and marker-assisted breeding strategies.

Key words: Drought stress, multivariate analysis, morpho-physiological traits, total chlorophyll, PCA.

INTRODUCTION

Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.), a popular ornamental plant globally. These are known for their beautiful flowers with different colours including yellow, orange, orange-red, pink, red, etc. which are mainly contributed by carotenoids and anthocyanins (Ullas *et al*., 19; Ullas *et al*., 20). It is severely hampered by abiotic stresses, especially drought (Yang *et al*., 21). Plants experience intermittent water deficits, negatively impacting photosynthesis, water relations, nutrient uptake, and enzyme activities. This reduces growth, flowering, and ornamental quality (Muhammad *et al*.,15).Genotypic variation influences their potentials for water uptake, translocation, and retention, as well as metabolic activities in plants.(Joshi *et al*.,12)

Breeding for drought tolerance is challenging due to the polygenic nature of drought resistance and its interactions with various traits (Ahmed *et al*., 2). Understanding these interactions helps in selecting better genotypes with higher drought tolerance (Dahiya *et al*., 5). Quantitative and polygenic inheritance makes yield-based selection less effective, so understanding the quantitative link between yield-related traits is crucial for developing a successful breeding strategy, and correlations can measure the magnitude and direction of these associations (Greenacre *et al*., 8). Principal Component Analysis (PCA) is a statistical method used in plant breeding to identify determinant traits and their inter-relationships, and classify genotypes based on their performance under stress conditions (Jolliffe *et al*., 11; Macrum *et al*., 14).

The present study examines different standard chrysanthemum genotypes under drought stress and non-stress conditions in hydroponics and pot culture conditions using PCA. The findings aim to understand drought tolerance mechanisms and develop drought-tolerant cultivars through efficient selection strategies.

MATERIALS AND METHODS

The experimental research was conducted in greenhouse conditions at the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi during 2020-21 to 2022-23. This study used fourteen (14) standard chrysanthemum genotypes (Star White, Yellow Reflex, Discovery, Tata Century, Thai Chen Queen, Pusa Centenary, Pusa Arunodhaya, DFR-

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261, Pusa Kesri, DFR-75, Kajal, DFR Red, Star Yellow and Red Purple) obtained from the institute germplasm collection (Fig.1).

One-month-old rooted cuttings were transplanted into a hydroponic system with a Hoagland solution. After one week, the rooted cuttings were transferred to a medium containing Hoagland solution supplemented with 10% Poly Ethylene Glycol (PEG). The plants are tested for various drought-related indices on the seventh day. An experimental setup with a standard Hoagland solution was used as a control. On the seventh day of PEG treatment, leaf samples were collected to measure the physiological traits like relative water content (RWC), membrane stability index (MSI), carotenoids, and chlorophyll. At the same time points, canopy temperature depression (CTD) and chlorophyll fluorescence were also determined. All 14 genotypes were also grown in white coloured plastic pots. Seven days of moisture stress (-60 Kpa) were imposed in two cycles, one at the vegetative phase and the second during flower bud initiation.

Marcum *et al.* (14) approach was used to determine MSI. RWC calculation was performed using Barrs and Weatherley (4). Based on Arnon's formula given by Arnon (3) and Hiscox and Israelstam (10) carotenoids and chlorophyll contents were determined by dimethyl sulfoxide (DMSO) method. Using an infrared thermometer (Fluke 59 Mini), the canopy temperature was measured on the sixth day, and the mean temperature in the environment was subtracted from the canopy temperature to determine the canopy temperature depression (CTD). A FluorPen FP 100 fluorometer was used to measure chlorophyll fluorescence on the sixth day. Similarly, morphological traits such as biomass (g), plant height (cm), plant spread (cm), primary branches, secondary branches, stem girth (cm), flower weight (g), flower diameter (cm), number of flowers/plant and flower yield/plant (g) were measured at the time of flowering.

In this study, a completely randomised design (CRD) was used. Each genotype comprises three replications for the two treatments (standard Hoagland solution and the Hoagland solution with 10% PEG). The PCA of the traits and correlation analysis between physiological and morphological traits were carried out using the R statistical programme (PCA analysis, R2.15.1 by R Development Core Team).

RESULTS AND DISCUSSION

Both in hydroponics and pot culture experiments, the correlation among chlorophyll a/b, chlorophyll a, chlorophyll b, and total chlorophyll is similar under control (Fig. 2A and 2C) and drought (Fig. 2B and 2D) stress environments. Chlorophyll a strongly correlates with chlorophyll b and total chlorophyll, where chlorophyll a is the primary pigment and chlorophyll b is an energy-providing accessory pigment (Ghasemi *et al*., 7).Chlorophyll b is synthesized from chlorophyll a*via* the enzyme chlorophyllide an oxygenase (Dey *et al*., 6).So, the availability of chlorophyll a directly influences the production of chlorophyll b.Under drought stress condition, if a plant maintains or proportionally reduces chlorophyll a, chlorophyll b levels will likely follow the same trend leading to a positive correlation.

Under both hydroponics and pot stress conditions there is a considerable positive association between carotenoids and chlorophyll a, chlorophyll b, and total chlorophyll (Fig. 2B and 2D). Unlike the control condition, there is a substantial correlation between the carotenoid concentration and chlorophyll under water stress. This correlation between chlorophyll concentration and carotenoid content in the leaf shows that the photosynthetic activity level affects the carotenoid concentration. Lashbrooke *et al.* (13)

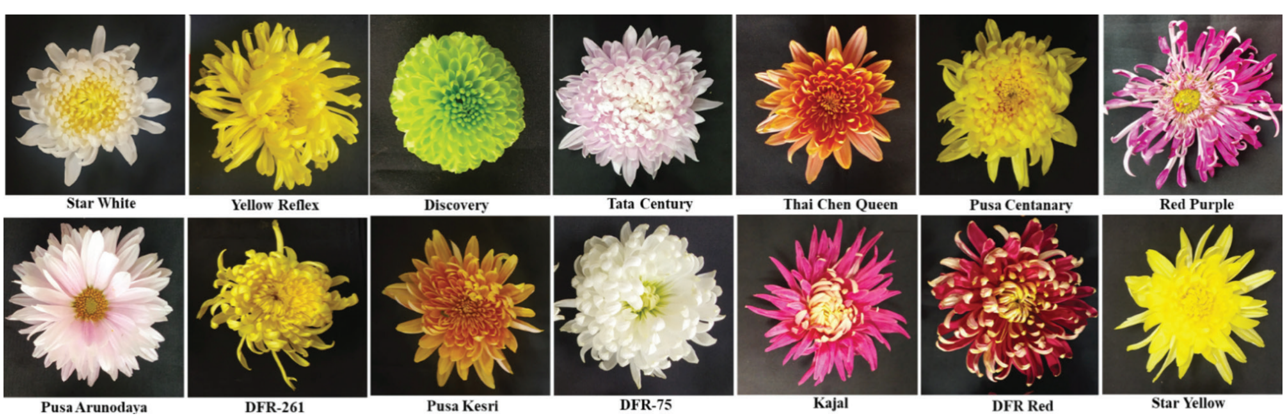

Fig. 1. Standard chrysanthemum genotypes used in the present study.

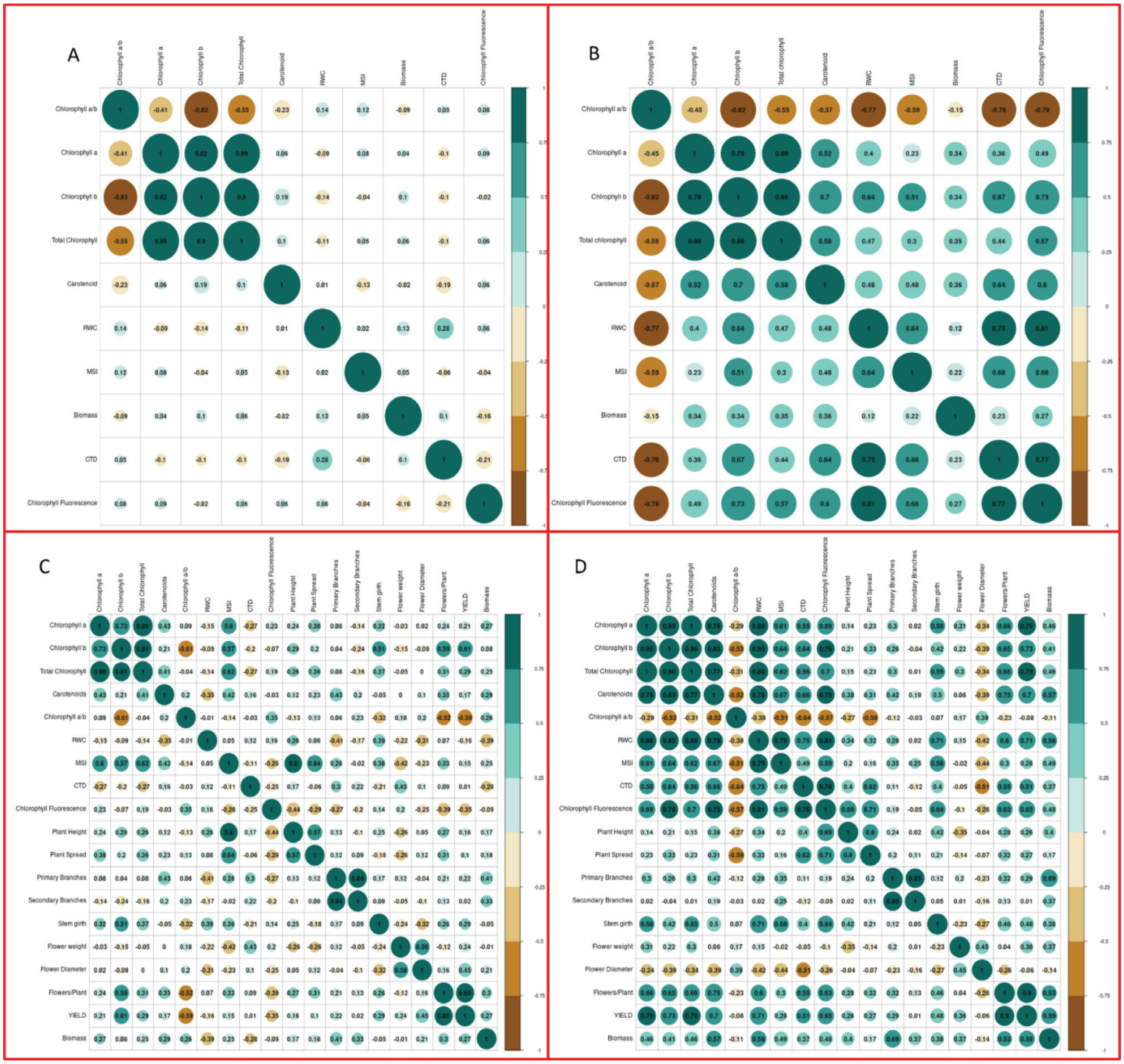

Fig. 2. Correlation matrix for non- drought stress (A)& droughtstress (B) under hydroponics and non-drought stress (C) & drought stress (D) under pot culture conditions for the morpho-physiological traits.

found a similar correlation in grapes. During drought, carotenoids act as antioxidants and help to protect chlorophyll from degradation leading to tightens their relationship. Plants that maintain more chlorophyll also need more carotenoids to guard it, hence, plants with higher chlorophyll may have higher carotenoids which is a strong, significant positive correlation. Under well-watered conditions, the lower levels of reactive oxygen species reduce the critical role of antioxidant carotenoids. Plants may produce carotenoids for light harvesting, but not necessarily in proportion to chlorophyll.

In our study, under both hydroponics and pot stress conditions, total chlorophyll showed an extremely favourable association with chlorophyll fluorescence (Fv/Fm), but not exhibited under well-watered conditions. These favourable associations reflect that chlorophyll fluorescence measurement can track changes in chlorophyll content and the degree of leaf senescence due to drought stress. Under both hydroponics and pot culture stress conditions, carotenoids have a strong positive correlation with canopy temperature depression (CTD) and chlorophyll fluorescence, although they are not significant under non-stress situations. Carotenoids scavenge ROS produced during photo-oxidative stress, protect plants from over-excitation in solid light, dissipate the excess of absorbed energy, and lessen the impact of severe temperatures (Strzałka *et al*., 18).

Relative water content (RWC) considerably positively correlates with CTD, Chlorophyll Fluorescence, and membrane stability index (MSI) under stress in both hydroponics and pot culture, whereas MSI significantly positively correlates with CTD and Chlorophyll Fluorescence. It was observed that the loss of chlorophyll happens simultaneously with mesophyll chloroplast injury, slowing the rate of photosynthetic activity. The membrane stability index (MSI), chlorophyll b, and chlorophyll fluorescence were found to be positively correlated with the relative water content. An influential favourable correlation was found between flower yield, number of flowers/plants, and total chlorophyll (Fig. 2C and 2D). Number of flowers/plants, flower yield, and biomass are significantly and highly associated with carotenoids.

Under drought stress in pot culture, RWC was highly positively correlated with chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, MSI, CTD, chlorophyll fluorescence, primary branches, stem girth, number of flowers per plant, flower yield, and biomass, but it was insignificant under control conditions. A change in the behaviour of varieties in various environments may be the cause of the differential behaviour of the indices under different conditions. CTD has a high positive correlation with plant spread, number of flowers per plant, and flower yield. Hence, a rise in canopy temperature causes a decrease in flower yield. Plant height, plant spread, stem girth, number of flowers per plant, and flower yield are highly positively correlated with chlorophyll fluorescence (Fv/Fm), indicating the effectiveness of PSII (Guo *et al*., 9).The positive association between flower diameter and fresh flower weight suggests that flower size directly influences flower weight. The similar observation was made in marigold by Poulose *et al.* (16).

Both stress and non-stress-induced screening and selection separately would not be the most efficient for increasing flower yield under drought stress. However, characteristics correlated with floweryield/plant, RWC and MSI are the best indices. These traits can be a helpful selection index for phenotyping drought tolerance in chrysanthemum. So, the traits associated with RWC, MSI, and flower yield under hydroponics and pot culture drought treatment are chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, RWC, MSI, chlorophyll fluorescence and stem girth, number of flowers per plant,flower yield and biomass. So, these traits are good selection indicators for drought tolerance. Additionally, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, RWC, MSI, and chlorophyll fluorescence behaved nearly identically in the seedling stage (hydroponics) and flowering stage (pot culture), making them highly reliable characteristics to screen chrysanthemum germplasm for drought tolerance in the seedling stage itself (Table 2).

Scree plots show the proportion of variation in each principal component, eigenvalues, and their relationship with percentage variation, illustrating the decreasing order of components or factors. (Fig. 3A, 3B, 3C and 3D).

Three principal components PC1, PC2, and PC3 together explained over 82% of variation under hydroponics stress conditions while PC1 and PC2 together contributed to 71.78% of the total variation. The traits chlorophyll a, carotenoids, chlorophyll b, chlorophyll fluorescence, chlorophyll a/b, RWC, and MSI, had high loading (>0.3) into PC1. In contrast, the traits CTD, chlorophyll a/b, chlorophyll b, MSI and chlorophyll fluorescence had high loading into PC2 irrespective of whether they were positive or negative. This means these traits strongly influence the respective PC. Similarly, four PCs were important for hydroponics non-stress conditions, accounting for 79.85% of the total variation, of which the first two PCs contributed 54.52%. Under non-stress conditions, variables chlorophyll b, total chlorophyll, chlorophyll a and chlorophyll a/b had high loadings into the PC1, while Biomass, CTD, MSI and RWC had a strong influence on PC2 (Table 1). It might be due to that chlorophyll plays a major role in the light reaction of plant photosynthetic process, which involves absorbing solar energy for the fixation of CO_2 (Ahmed *et al*., 1)

The percentage of variation explained by specific PCs (eigenvalue > 1) and its correlation with other traits under pot culture are shown in Table 2. Under pot stress conditions, five principal components accounted for nearly 87 % of the overall variation, with PC1 and PC2 having the largest cumulative influence on the total variation. RWC, chlorophyll fluorescence, chlorophyll b, total chlorophyll and carotenoids had high negative loading into PC1, while flower weight, chlorophyll a/b and plant spread strongly influenced PC2 under pot stress conditions. Similarly, seven PCs were found important for the non-stress conditions, accounting for 88.43% of the total variation, of which the first two PCs contributed 41.9 %. Under non-stress condition, the traits chlorophyll b, MSI, total chlorophyll, chlorophyll a and flowers/plant

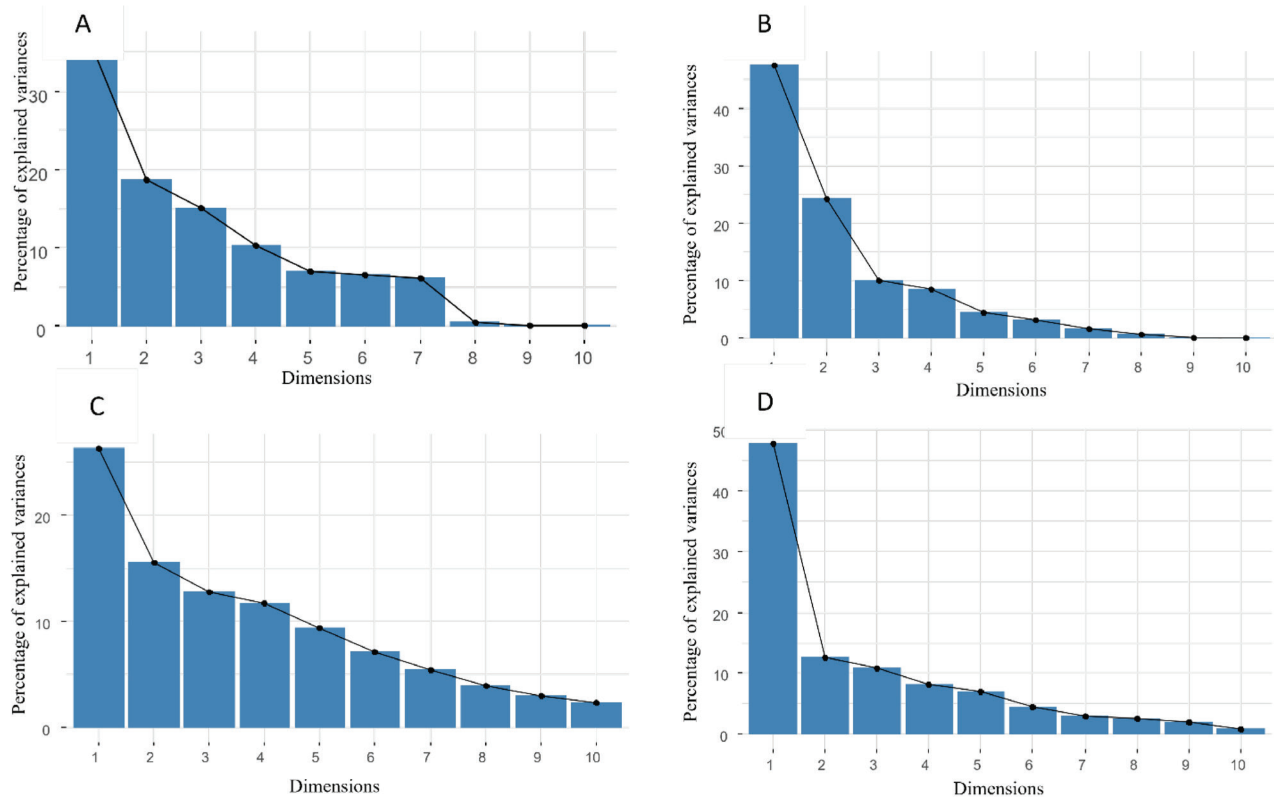

Fig. 3. Scree plot of PCA for non- drought stress (A) & drought stress (B) under hydroponics and non-drought stress (C) & drought stress (D) under pot culture conditions for the morpho-physiological traits.

Table 1: Rotated component matrix of 10 morpho-physiological traits of 14 chrysanthemum genotypes evaluated under drought stress and non-stress conditions in hydroponics.

Control					Stress			
	PC 1	PC 2	PC 3	PC 4		PC 1	PC 2	PC3
Eigenvalues	1.89	1.36	1.22	1.01	Eigenvalues	2.17	1.55	1.00
Percentage of variance	35.79	18.73	15.09	10.20	Percentage of variance	47.47	24.31	10.01
Cumulative % of the variance	35.79	54.52	69.61	79.85	Cumulative % of the variance	47.47	71.78	81.79
Traits		Latent vectors			Traits		Latent vectors	
Chlorophyll a (µg/g)	-0.43	-0.09	-0.33	0.03	Chlorophyll a/b	-0.32	0.43	0.02
Chlorophyll b (µg/g)	-0.51	-0.04	0.11	-0.03	Chlorophyll a	-0.43	0.16	-0.03
Total chlorophyll (µg/g)	-0.48	-0.08	-0.22	0.01	Chlorophyll b	-0.35	0.40	0.01
Carotenoids (µg/g)	-0.10	0.017	0.05	0.92	Total chlorophyll	-0.22	0.05	0.17
Chlorophyll a/b	0.37	-0.03	-0.44	0.16	Carotenoids	0.36	0.24	0.08
RWC (%)	0.26	-0.40	0.37	0.08	RWC	-0.32	-0.12	0.51
MSI	0.05	0.47	-0.33	-0.20	MSI	-0.31	-0.34	0.26
Biomass (g)	0.14	0.55	0.22	0.07	Biomass	-0.25	0.17	-0.69
CTD (˚C)	-0.08	0.48	0.01	0.22	CTD	-0.10	-0.52	-0.35
Chlorophyll fluorescence	0.23	-0.19	-0.56	0.13	Chlorophyll fluorescence	-0.34	-0.34	-0.12

Table 2: Rotated component matrix of 19 morpho-physiological traits of 14 chrysanthemum genotypes evaluated under drought stress and non-stress conditions in pot culture.

Stress						Control						
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 1	PC 2	PC 3	PC4	PC 5	PC6	PC7
Eigenvalues	3.01	1.54	1.43	1.24	1.15	2.23	1.71	1.55	1.49	1.33	1.16	1.01
Percentage of variance	47.8	12.63	10.84	8.21	6.99	26.34	15.56	12.80	11.75	9.38	7.18	5.43
Cumulative % of the variance	47.8	60.43	71.27	79.49	86.48	26.34	41.90	54.69	66.44	75.82	83.00	88.43
Traits			Latent vectors					Latent vectors				
Chlorophyll a (µg/g)	-0.29	-0.17	0.19	-0.11	-0.01	-0.29	-0.17	0.19	-0.11	-0.01	-0.29	-0.17
Chlorophyll b (µg/g)	-0.30	-0.05	0.18	-0.13	-0.16	-0.30	-0.05	0.18	-0.13	-0.16	-0.30	-0.05
Total chlorophyll (µg/g)	-0.30	-0.16	0.19	-0.11	-0.02	-0.29	-0.16	0.19	-0.11	-0.02	-0.29	-0.16
Carotenoids (µg/g)	-0.30	-0.02	-0.04	-0.06	-0.04	-0.30	-0.02	-0.04	-0.06	-0.04	-0.30	-0.02
Chlorophyll a/b	0.17	-0.34	0.08	0.15	0.52	0.17	-0.34	0.08	0.15	0.52	0.17	-0.34
RWC (%)	-0.31	-0.02	0.10	-0.09	0.09	-0.31	-0.02	0.10	-0.09	0.09	-0.31	-0.02
MSI	-0.24	0.02	-0.11	-0.34	0.06	-0.24	0.02	-0.11	-0.34	0.06	-0.24	0.02
CTD (˚C)	-0.26	0.25	0.07	0.00	-0.17	-0.26	0.25	0.07	0.00	-0.17	-0.26	0.25
Chlorophyll fluorescence	-0.30	0.21	0.03	0.20	0.02	-0.30	0.21	0.03	0.20	0.02	-0.30	0.21
Plant height (cm)	-0.15	0.29	-0.21	0.44	0.21	-0.15	0.29	-0.21	0.44	0.21	-0.15	0.29
Plant spread (cm)	-0.16	0.33	-0.14	0.37	-0.27	-0.16	0.33	-0.14	0.37	-0.27	-0.16	0.33
Primary branches	-0.14	-0.26	-0.54	0.00	-0.08	-0.14	-0.26	-0.54	0.00	-0.08	-0.14	-0.26
Secondary branches	-0.04	-0.21	-0.60	-0.10	-0.02	-0.04	-0.21	-0.60	-0.10	-0.02	-0.04	-0.21
Stem girth (cm)	-0.21	0.04	0.03	0.02	0.60	-0.21	0.04	0.03	0.02	0.60	-0.21	0.04
Flower weight (g)	-0.03	-0.49	0.16	0.16	-0.39	-0.03	-0.49	0.16	0.16	-0.39	-0.03	-0.49
Flower diameter (cm)	0.14	-0.20	0.13	0.54	-0.10	0.14	-0.20	0.13	0.54	-0.10	0.14	-0.20
Flowers/plant	-0.25	-0.10	0.04	0.15	0.08	-0.25	-0.10	0.04	0.15	0.08	-0.25	-0.10
Yield	-0.26	-0.23	0.16	0.25	0.06	-0.26	-0.23	0.16	0.25	0.06	-0.26	-0.23
Biomass (g)	-0.21	-0.25	-0.26	0.17	0.05	-0.21	-0.25	-0.26	0.17	0.05	-0.21	-0.25

contribute highly to PC1, and the traits, primary branches, secondary branches, RWC, and flower diameter contributes majorly to PC2. From PCA it is concluded that the traits chlorophyll a, chlorophyll b, chlorophyll a/b, total chlorophyll, carotenoids, RWC, and chlorophyll fluorescence are the most important traits which contributed to the variability in the genotypes under stress as it was the major contributor to PC1 under pot and hydroponics conditions. When stress is applied in the pot conditions (Fig. 4D), none of the characters positively contribute to PC1 and PC2.

Genotypes were subjected to biplot analysis followed byPCA to determine their correlation. PCA biplots can highlight the relationships among various variables, genotypes, and the PCs that correspond with them (Fig. 4A, 4B, 4C and 4D). Together with the observations, the plot also demonstrates the original variables as vectors. A vector's angle with a PC axis determines how much it contributes to that PC alone. The angle between the vectors reveals the correlation of the traits. Genotypes that excelled in a particular trait were frequently plotted near the vector line and further in that vector's direction on the edges of the convex hull. Chlorophyll a/b is the most critical feature under stress in hydroponics and positively impacts PC1 and PC2 when osmotic stress is applied (Fig. 3B). In alignment with our study several researchers have successfully employed PCA biplot analysis to evaluate drought-tolerant wheat varieties (Singh *et al.*, 17).

The study found that traits like RWC, MSI, and flower yield are linked to chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, chlorophyll fluorescence, stem girth, flower yield, and biomass, making them useful selection indicators for chrysanthemum breeding for drought stress tolerance.

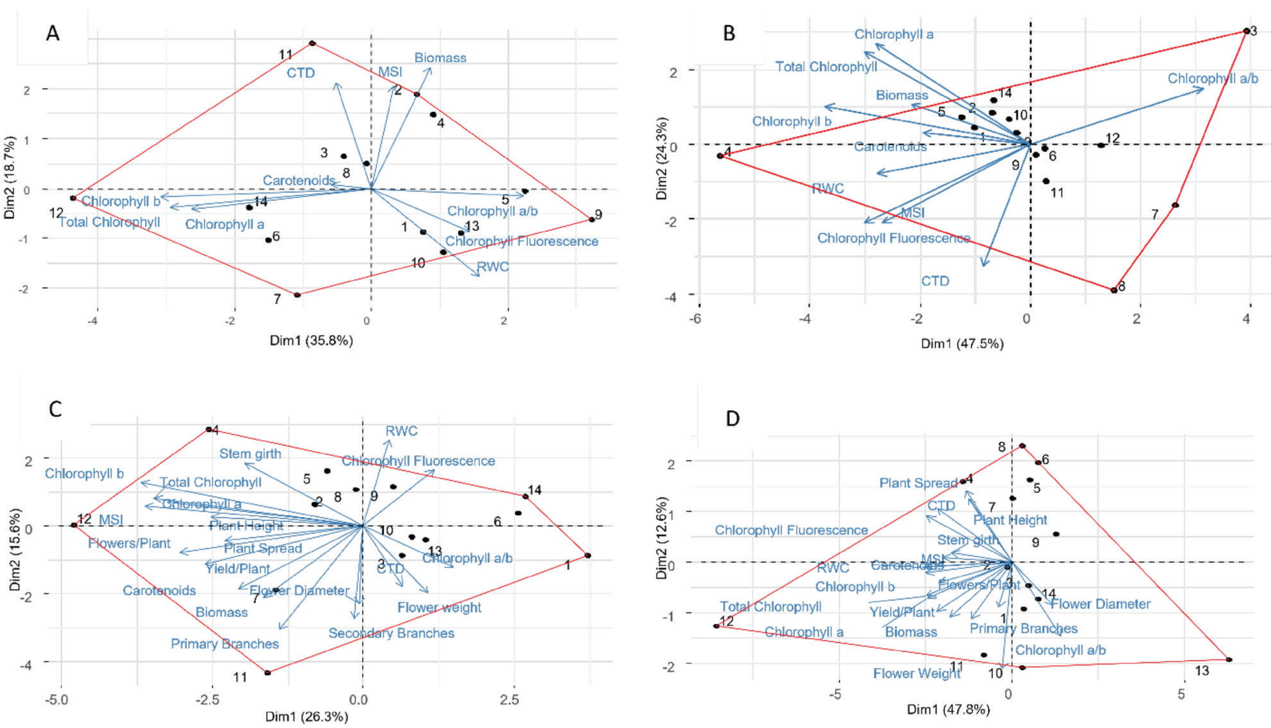

Fig. 4. Principal Component Analysis (PCA) biplot showing genotypic grouping on the basis of morpho-physiological traits under non- drought stress (A), drought stress (B) in hydroponics and non-drought stress (C), drought stress (D) in pot culture conditions.

AUTHORS' CONTRIBUTION

Conceptualization of research (N, BP, GK, AMS, GK), Designing of experiments (N, BP, SP), Contribution of experimental materials (N, GK, KPS), Execution of field/lab experiments and data collection (BP, N), Statistical analysis of data and interpretation (BP, N, SK), Preparation of manuscript (BP, N, GK)

DECLARATION

No conflicts of interest are disclosed by the authors.

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