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Identifying dryland-resilient chickpea genotypes for autumn sowing, with a focus on multi-trait stability parameters and biochemical enzyme activity

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Abstract

Background Chickpea is a key pulse crop grown in the spring in dryland regions. The cold resistance potential of chickpeas allows for the development of genotypes with varying sowing dates to take advantage of autumn and winter rainfall, particularly in dryland regions. In this study, we assessed grain yield, plant height, 100-seed weight, days to maturity, and days to flowering of 17 chickpea genotypes in five autumn-sown dryland regions from 2019 to 2021. Additionally, the response of selected chickpea genotypes to cold stress was examined at temperatures of -4 °C, 4 °C, and 22 °C by analyzing biochemical enzymes.

Results Mixed linear model of ANOVA revealed a significant genotype × environment interaction for all traits measured, indicating varying reactions of genotypes across test environments. This study reported low estimates of broad-sense heritability for days to flowering (0.34), days to maturity (0.13), and grain yield (0.08). Plant height and seed weight exhibited the highest heritability, with genotypic selection accuracies of 0.73 and 0.92, respectively. Moreover, partial least square regression highlighted the impactful role of rainfall during all months except of October, November, and February on grain yield and its interaction with environments in autumn-planted chickpeas. Among the genotypes studied, G9, G10, and G17 emerged as superior based on stability parameters and grain yield. In particular, genotype G9 stood out as a promising genotype for dryland regions, considering both MTSI and genotype by yield*trait approaches. The cold assay indicated that -4 °C is crucial for distinguishing between susceptible and resistant genotypes. The results showed the important role of the enzymes CAT and GPX in contributing to the cold tolerance of genotype G9 in autumn-sown chickpeas.

Conclusions Significant G×E for agro-morphological traits of chickpea shows prerequisite for multi-trial analysis. Chickpea's direct root system cause that monthly rainfall during plant establishment has no critical role in its yield interaction with dryland environment. Considering the importance of agro-morphological traits and their direct and

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indirect effects on grain yield, the utilization of multiple-trait stability approaches is proposed. Evaluation of chickpea germplasm reaction against cold stress is necessary for autumn-sowing. Finally, autumn sowing of genotype FLIP 10–128 C in dryland conditions can lead to significant crop performance.

Keywords Chickpea, Cold stress, Biochemical response, WAASB stability parameter, MTSI index, Genotype by yield*trait

Introduction

The chickpea is considered the second most important pulse crop among legumes because it possesses a high seed protein content and, therefore, is a predominant nutrient resource for human being and animals [1]. From an agronomic point of view, chickpea with a key role in nitrogen fixation and enrichment of soil can be applied in the rotation schedule of strategic crops such as wheat, especially in rain-fed conditions [2]. As proven, the major challenge of the present century is increasing plant yield, especially when they are facing environmental stresses. The important role of chickpea in providing plant-based proteins [3], as well as the distribution of arid and semi-arid regions worldwide [4], led to several studies about the reaction of chickpea germplasm in water-deficient conditions [5–8]. Likewise, chickpea genotypes have varied responses to climate change, so it is necessary to introduce adaptable genotypes. In Iran, the majority of chickpea production occurs through spring sowing in dryland conditions. This can result in drought stress at the end stage of plant development, particularly seed filling, which adversely impacts chickpea yield. In addition to water scarcity, the distribution of rainfall in several dryland regions may be uneven, limiting crop development [9]. In such circumstances, the autumn-sowing of chickpea (November to December) in dryland conditions has been proposed as a means of extending the growth period of chickpea and, consequently, its yield. Autumn-sowing of chickpea needs to check the cold resistance response of chickpea genotypes. Regarding the cold resistance potential of chickpea even to $-8\text{ }^{\circ}\text{C}$ [10], it is possible to develop and introduce genotypes with a broad range of sowing dates to benefit from autumn and winter rainfall, especially in the drylands of Iran.

Chickpea yield has polygenic and quantitative characteristics that are affected by environmental factors, and so, inspection of genotype \times environment interaction (GEI) in chickpea breeding programs to achieve genotypes with general and specific compatibility and also stable genotypes across multi-years and multi-locations is vital. In this way, several researchers have used different statistical procedures to study GEI and identify stable genotypes of chickpea [11, 12]. As a multivariate method, the AMMI (Additive main effect and multiplicative interaction) method, which is described by Gauch [13] has been frequently implemented for yield stability analysis of several crops, particularly chickpea [14]. Apart

from the AMMI method, researchers have also applied the best linear unbiased prediction (BLUP) method to investigate the adaptability and stability of target genotypes in multi-trial settings. While AMMI and BLUP aim to extract the genotype \times environment interaction from random error, their unique characteristics allow to differentiate these methods from each other. As proven by Gauch, 2013 [15], AMMI analysis primarily captured the Genotype \times Environment (G \times E) interaction pattern within the first interaction principal component axis (IPCA), whereas the subsequent IPCAs accounted predominantly for random error. Conversely, the BLUP method rates the genetic value of the genotypes that were studied by figuring out the average yield of genotypes in mixed models very accurately [16]. Olivoto et al. [17] presented a new method named WAASB (weighted average absolute scores of BLUPs), which leverages both AMMI and BLUP methods. In summary, an LMM implements singular value decomposition on a BLUP matrix to analyze the genotype-environment interaction. Afterward, the biplot of WAASB \times trait mean (Y), which is defined as WAASBY, could be used to jointly interpret stability and trait productivity.

On the other hand, selection based on only one attribute, such as yield, is not considered the most appropriate strategy because yield could be influenced by other traits. Therefore, plant breeders are going to incorporate several traits into an identified stable genotype. In this way, it is challenging to gather multiple traits into one genotype, especially when interesting traits have negative correlations with each other. For solving this challenge, two approaches, including genotype by yield*trait [18] and multiple traits stability index (MTSI) [19] were proposed. In genotype by yield*trait analysis, a genotype is deemed ideal if it exhibits optimal levels of each target trait, effectively balancing any negative associations among traits and consistently performing well across various environments. The MTSI is calculated based on the distance from the ideal genotype estimated through factor analysis [19]. This index provides the possibility of selecting stable genotypes with a positive selection differential for traits that are intended to increase and a negative selection differential for traits that are intended to decrease. There are some reports that utilized genotype by yield*trait to identify superior and stable genotypes in oat [18], wheat [20], maize [21], and sorghum [22]. The literature review also showed several studies using the

Table 1 List of chickpea genotypes used in the multi-environment experiments

Number	Code	Origin	Number	Code	Origin
G1	FLIP10-374 C	ICARDA	G10	FLIP 10-169 C	ICARDA
G2	FLIP11-19 C	ICARDA	G11	FLIP 10-171 C	ICARDA
G3	FLIP11-85 C	ICARDA	G12	FLIP 10-202 C	ICARDA
G4	FLIP11-86 C	ICARDA	G13	FLIP 10-294 C	ICARDA
G5	FLIP11-127 C	ICARDA	G14	OKER2011-29	IRAN
G6	FLIP11-136 C	ICARDA	G15	IDKMAR-2011-17	IRAN
G7	FLIP 10-67 C	ICARDA	G16	Ana	IRAN
G8	FLIP 10-114 C	ICARDA	G17	Nosrat	IRAN
G9	FLIP 10-128 C				

MTSI index in field crops, including maize [23, 24], lentil [25], sugar beet [26]. To our knowledge, no studies have employed the aforementioned multi-trait simultaneous approaches in the chickpea germplasm.

The present study evaluated the agro-morphological characteristics of 17 chickpea genotypes in five dryland regions of Iran over two consecutive years. The objective was to: (i) identify the monthly rainfall that affects chickpea performance in autumn-sowing in dryland

conditions. (ii) examine the efficiency of genotype by yield*trait and MTSI indices as simultaneous multi-trait stability approaches in identifying high-productive, stable chickpea genotypes that are well adapted to the test environments on autumn-sowing. (iii) test the efficacy of selected genotypes in response to cold stress using biochemical enzyme measurement.

Materials and methods

Multi-environment trials and agro-morphological trait measurement

A suit of 15 chickpea inbred lines kindly provided by ICARDA (International Center for Agricultural Research in the Dry Areas) accompanied by two check genotypes (Ana and Nosrat as cold resistant genotypes in dryland regions) (Table 1) were used in this study.

The field study was conducted at five locations (Fig. 1), including Maragheh, Zanjan, Hamadan, Kurdistan, and Urmia, over two consecutive years (2019–2020, 2020–2021). These locations, situated in five geographically diverse provinces of Iran, were categorized as cold, dryland regions. The climatic conditions of the

**Fig. 1** Geographical coordinates of inspected regions. In each province, test location was highlighted

five experimental locations was presented in Table S1. In each environment (year \times location), the experimental design utilized a randomized complete block design (RCBD) with four replications. Each plot comprised four rows, each 4 m long, with 25 cm of spacing between rows. The seeds were sown in December and the seeding rate was consistently maintained at 40 brush per m² across all environments. Field practices, including weed control, were executed manually during crop growth and development. Various traits, such as days to flowering (DF), days to maturity (DM), and plant height (PH) were recorded. After harvesting, traits such as the weight of 100 seeds (SW) and grain yield (GY) were measured.

The field data were assessed for normality using the Anderson-Darling test and checked for outliers. Levene's test was then applied to verify the homogeneity of variance, confirming the uniformity of individual error mean squares. To evaluate genotype stability across environments, a linear mixed model was employed [27]. The significance of each effect on the studied traits was determined using the likelihood ratio test (LRT) with a two-tailed chi-square test (one degree of freedom). Initially, for each environment, traits were modeled with a linear mixed-effects model, treating environment and environment-by-genotype interaction as random effects and genotype as a fixed effect [17]. This was done using the "gamem_met" function from the metan, an R-package [27], adhering to the standard linear mixed model [16]. The model can be expressed as:

$$y = Xb + Zu + \epsilon$$

where yy is a vector of response variables, bb is a vector of fixed effects, uu is a vector of random effects, XX is a design matrix of 0s and 1s relating yy to bb , ZZ is a design matrix of 0s and 1s relating yy to uu , and $\epsilon\epsilon$ is a vector of random errors.

Following the analysis of variance, genotype and genotype-by-environment interaction (GEI) are assumed to be random effects [17] to predict genetic parameters using the argument "genpar" in the function gamem_met. Subsequently, stability analysis was conducted by calculating the Weighted Average of Absolute Scores Parameter (WAASB) using the function "waasb" in the metan package. In this process, WAASB was estimated based on a singular value decomposition of the G \times E interaction effects from the matrix of the Best Linear Unbiased Predictions (BLUPs) as follows:

$$WAASB_i = \frac{\sum_{k=1}^p |IPCA_{ik} \times EP_k|}{\sum_{k=1}^p EP_k}$$

Where $WAASB_i$ is the weighted average of absolute scores of the i th genotype or environment, $IPCA_{ik}$ is

the absolute score of the i th genotype or environment in the k th IPC, and EP_k is the magnitude of the variance explained by the k th IPC.

As shown, $WAASBY_i$ is the superiority index with different weights between yield and stability for the g th genotype, Θ_Y and Θ_S are the weights for yield and stability, respectively; rG_g and rW_g are the rescaled values of the g th genotype for yield and WAASB, respectively.

$$WAASBY_i = \frac{(rG_g \times \theta_Y) + (rW_g \times \theta_S)}{\theta_Y + \theta_S}$$

In this study, MTSI was utilized to assess both the average performance and the concurrent stability of various traits having significant G \times E interaction comprising DM, PH, DF, SW, and GY, considering that higher values for studied traits except DM and DF are suitable. In this regard, the vector of trait importance as c (l, h, l, h, h, h) was defined and incorporated into the WAASB analysis before the MTSI approach [27]. Next, the MTSI analysis was conducted using the MTSI function within the "metan" package, implemented as outlined below:

$$MTSI_i = \left[\sum_{j=1}^f (\gamma_{ij} - \gamma_j)^2 \right]^{0.5}$$

Where $MTSI_i$ is the multi-trait stability index of the genotype i , γ_{ij} is the score of the genotype i in the factor j , and γ_j is the score of the ideal genotype in the factor j . Scores were computed through factor analysis for both genotypes and traits.

Rainfall, used as a covariate for explaining GEI, was incorporated through partial least squares (PLS) regression analysis in GEA-R software [28]. Hence, monthly rainfall data from October to May served as the environmental covariable. The PLS model model comprises an independent matrix X (rainfall data), a dependent matrix Y (yield), and the latent variables t represented as follows:

$$x = t_1p'_1 + t_2p'_2 + \dots + E = TP' + E$$

$$y = t_1q'_1 + t_2q'_2 + \dots + E = TQ' + E$$

where matrix T contains X -scores, the matrix P contains the X -loadings, matrix Q contains the Y -loadings, and F and E represent the residual matrices. Ultimately, the results of the PLS analysis were visualized in the form of a biplot.

To analyze genotype by yield*trait combinations, mean data for each trait of each chickpea genotype over two years were computed. Grain yield was adjusted by multiplying or dividing it by the respective trait value based on breeding objectives [18]. For PH and SW, yield trait

combinations were derived by multiplying the grain yield by the corresponding trait value, while for traits like DH and DM (where lower values are preferable), the yield trait combinations were calculated by dividing the grain yield by these traits. Values were standardized using the equation proposed by Yan and Fregeau-Reid [18]:

$$P_{ij} = (T_{ij} - T_j) / S_j$$

P_{ij} is equal with the standardized value of i^{th} individual for the character or character* yield mixture j in the standardized table, T_{ij} is the raw value of individual i for character or character* yield mixture j in the genotype by yield*character tables, T_j is the mean across individuals for character or character* yield mixture j , and S_j is the standard deviation for character or character* yield mixture j . Standardized data were handled to calculate the superiority index (SI) and mean SI values, recognizing superior individuals based on several characters. GYT-biplot analysis was performed using GGE-biplot software v.8.2 to deliver a graphic sight of the results.

Biochemical response of stable genotype to cold stress during autumn-sowing

The genotype FLIP 10–128 C (G9), identified as a superior and stable genotype among those studied, was subjected to a cold stress experiment beside cold tolerant (Ana) and sensitive (ILC533) genotypes to assess its capacity to thrive under such conditions. Therefore, a factorial experiment was performed using a completely randomized design with three replications in a growth chamber. The growth chamber conditions were set to 16 h of light and 8 h of darkness, a light intensity of 500 $\mu\text{mol}/\text{m}^2/\text{s}$, a relative humidity of 75%, and a temperature of 22 °C. Firstly, the seeds of the genotypes were disinfected with Vitex (sodium hypochlorite) for ten minutes. Following this, they were washed with distilled water and placed on filter paper in a petri dish with the necessary humidity. Once germination had occurred, the seedlings were transferred to pots. The indirect transfer of the plants to the soil was necessary for two reasons: firstly,

to ensure uniform growth, and secondly, to guarantee the precise implementation of the treatments into samples. In order to investigate the biochemical responses of plants to cold stress, a temperature gradient program was employed. This involved reducing the temperature of the growth chamber to 4 °C on the 21st day of the seedlings. Leaf sampling was then conducted, after which the temperature of the growth chamber was brought to -4 °C within 48 h. This process was repeated. It should be noted that a portion of the seedlings was maintained as a control within the growth chamber at a temperature of 22 °C. All leaf specimens sampled were handled in accordance with the methodology described in detail by Demirel et al. (2020) to measure biochemical constituents, including catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2).

After collecting antioxidant enzymes data, the existence of outlier data was checked and then two factor analysis of variance was done using “aov” command in “Agricolae” package. The Duncan multiple range test was implemented for mean comparison via “duncan.test” command at 5% probability level. All analysis was handled by R software. Preparation of graphs was undertaken using excel tool of Microsoft office 10.0.

Results

MLM analysis of variance and PLS regression

It is inferred from the mixed linear analysis of variance (Table 2) that the environment effect is highly significant for all of the studied traits, and so there is a difference among the tested environments. According to the P-value related to genotype \times environment interaction (GEI) (Table 2), its effect was also significant for all of the measured traits. In this study, the phenotypic variance varied between 27,033 (GY) and 4.02 (DF). The coefficient of variation of GEI (Table 2) as an indicator of trait reaction in response to the environment was positive for recorded traits as well. Regarding calculated statistics through REML (Table 2), low estimates of broad-sense heritability were observed for DF, DM and GY. It was also

Table 2 Mixed linear model analysis of variance and genetic parameters for agro-morphological traits of 17 chickpea genotypes across 10 environments

Trait	P-value			σ_p^2	GEI _{r2}	h _{2mg}	Accuracy of selection
	Env	Rep (Env)	Gen \times Env				
DF	2.54E-35	5.63E-27	1.45E-20	4.02	0.34	0.34	0.58
DM	3.47E-34	9.07E-14	1.25E-06	6.25	0.18	0.13	0.36
PH	3.18E-18	1.41E-14	2.75E-14	11.60	0.27	0.54	0.73
SW	3.36E-13	3.08E-03	8.54E-18	11.20	0.26	0.84	0.92
GY	4.66E-15	1.60E-24	1.33E-02	27033.00	0.09	0.08	0.29

P-values correspondence with sources of variations depicting significant differences when its value drops under threshold of 0.01 and 0.05. Here σ_p^2 as phenotypic variance, GEI_{r2} as the coefficient of variation for GEI effects, h_{2mg} as heritability on the entry-mean basis and accuracy of selection were calculated. DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: 100-Seed weight, GY: Grain yield

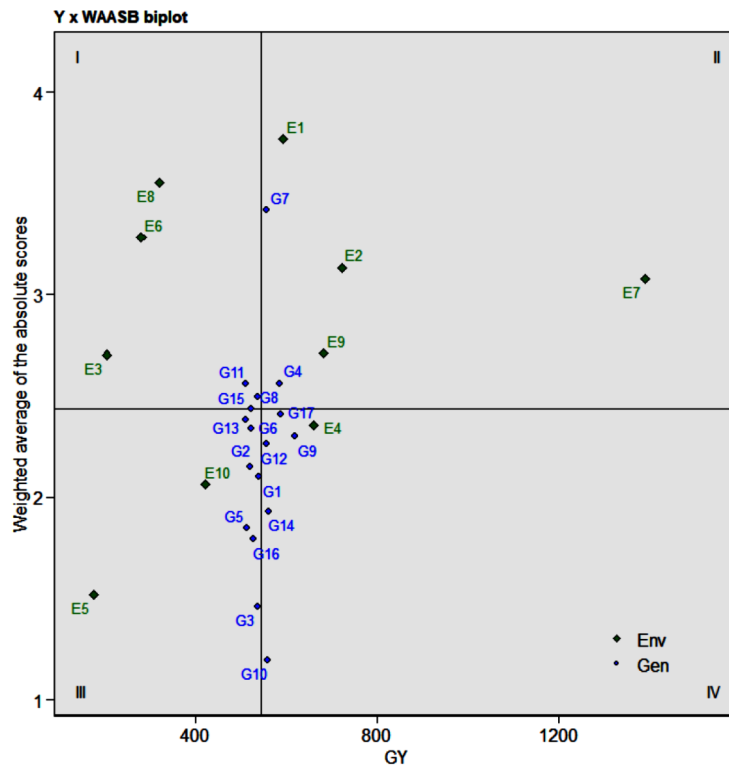


Fig. 3 The plot of response variable (GY) × WAASB across 10 environments Genotype ranking according to the different weighting of stability and seed yield

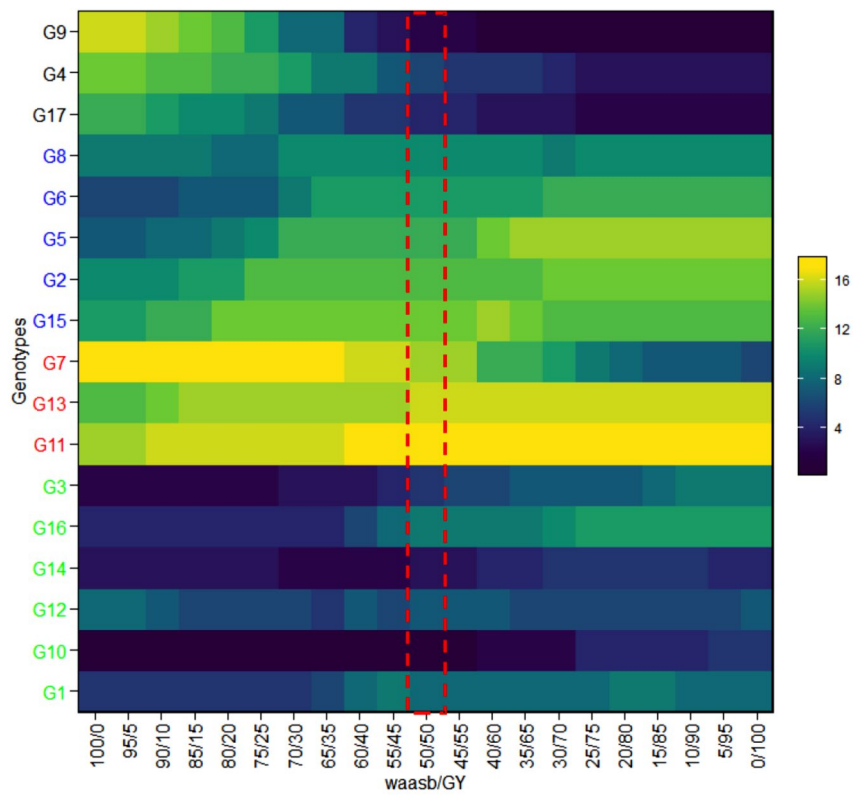


Fig. 4 Heatmap showing the rank of 17 studied chickpea genotypes based on different weights for GY versus WAASB stability index

in the ranking of genotypes considering the weight of GY and the stability index (WAASB) was recorded (Fig. 4). In the first column on the left side (Fig. 4), the ranking of genotypes based solely on the WAASB index (0/100) indicated that $G14 < G3 < G10$ were the most yield-stable genotypes. In the last column on the right side, the rankings of genotypes were based solely on GY (100/0), and revealed $G9 > G17 > G4$ as the most superior genotypes with stable yield. The red rectangle (Fig. 4) is ranking the genotypes based on their equal weight for stability and the responsible variable (GY). Hence, $G10 > G9 > G17$ were identified as the best genotypes when GY and stability parameters had equal weights (50/50).

Multi-trait simultaneous stability approaches

Although GY is an economic part of the chickpea, it could be influenced by several agro-morphological traits. So, regardless of yield and its stability, it is important to enhance plant superiority based on other characteristics. In the present work, with a sense of increasing GY, PH, and 100-SW and also decreasing DM, and DF, MTSI analysis was done (Table 3). In this process, factor analysis after scaling the trait using BLUP for genotype mean performance resulted in two factors with eigenvalues greater than 1 that explained 60% of total variation (Table 3). This finding suggests that the two factors were successful in capturing a substantial amount of variability in the traits. Moreover, the communality values for the variables ranged from 0.43 for the DF trait to 0.74 for the PH trait, with a mean of 0.60. These values suggest that a significant portion of the variability of each variable was explained by these factors. Considering the loading coefficients in correspondence to each trait in each factor (Table 3), the studied agro-morphological attributes of the chickpea panel could be classified. Hence, in FA1 and FA2, traits DM and GY had positive loadings, while traits PH, DF, and 100-SW possessed negative loadings. Results showed that genotype's rank is varied regarding MTSI value (Fig. 5) and genotype with the highest MTSI value is positioned at the center, while the genotype with the lowest MTSI value is situated at the outermost circle. It is concluded from MTSI analysis (Fig. 5) that G8 attained the top rank, succeeded by G9 and G14, establishing them as the most desirable and stable genotypes. Notably, the average values of all traits, except

for DM, demonstrated an increase in the selected genotypes, aligning with the intended objectives. Overall, the selected genotypes led to a favorable selection differential (SD) for DM and GY, fulfilling their intended purposes (Table 3).

In the following, as a novel approach, the GYT analysis was applied to combine (multiply or divide) all the agromorphological traits of chickpea genotypes with grain yield depending upon the breeding objectives. In this process, firstly the genotype by trait (GT) table (Table 4, left hand) through raw data from 10 environments was calculated. Afterward, GYT table (Table 4, right hand) was constructed, in which each column contained yield*trait combinations. In this study, the combinations yield*DM (days to maturity) and yield*DF (days to flowering) had the division operator ("/"), as opposed to the multiplication operator ("*") in other trait combinations, to manifest that more days to maturity is less desirable in dryland conditions. The "/" operator indicates that the values of the trait were reversed before being multiplied by the yield values. Consequently, in the GYT table (Table 4, right hand) a larger value is always more desirable. Results showed that the first three ranks of chickpea genotypes based on GY/DF and GY/DM are as follows $G9 > G4 = G17$ (Table 4). The order of first three ranks of studied genotypes regarding GY*PH was $G9 > G10 > G17$ (Table 4). As a result, the order of $G17 > G4 > G9$ was detected for studied genotypes based on GY*100-SW (Table 4). By using such yield*trait combinations it is possible to know the strengths and weaknesses of the selected genotypes. So, after standardization of yield*trait combinations (Table 5), the polygon view of GYT biplot (Fig. 6) and superiority indices (Table 5) were computed. The polygon view of GYT biplot represents the trait profile of 17 chickpea genotypes (Fig. 6). As illustrated in Fig. 6, the GYT biplot explained 94.7% of the total variation by graphically describing the first two principal components (PC1=86.4% and PC2=8.8%). The GYT biplot (Fig. 6) showed a strong correlation between all yield*trait combinations. The biplot illustrated seven radiation lines perpendicular to the sides of the polygon, dividing it into seven sectors. Among these sectors, only one contained the yield*trait combinations. This specific sector encompassed all yield*trait combinations and featured three chickpea genotypes (G7, G9, and G17), with

Table 3 Selection differential for the mean of the traits

Trait	Factor	Xo	Xs	SD	Communality	sense
DF	FA1	190	190	-0.213	0.43	decrease
DM	FA1	226	226	0.174	0.54	decrease
SW	FA1	32.9	32.5	-0.431	0.67	increase
PH	FA2	28.9	27.8	-1.14	0.74	decrease
GY	FA2	544	571	27	0.61	increase

DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: 100-Seed weight, GY: Grain yield

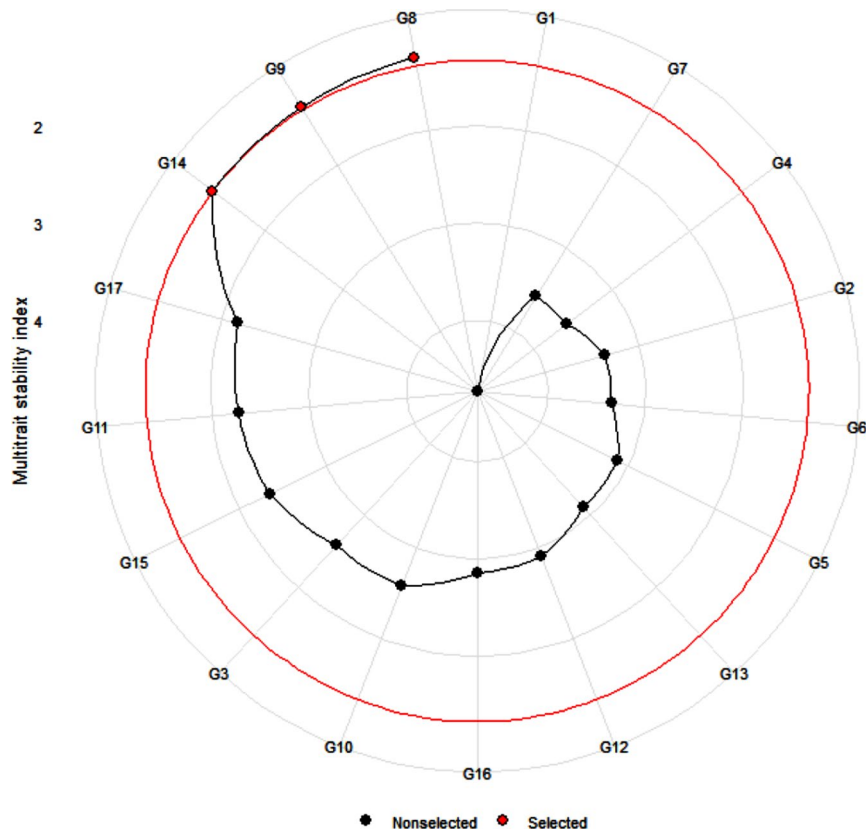


Fig. 5 Genotypes ranking based on the multi-trait stability index. The genotypes determined by the red dots were selected based on their MTSI values at 20% selection intensity

Table 4 Genotype by trait data for 17 chickpea genotypes for five traits (left hand). Each value is the mean across 10 trials for all traits. Right hand is genotype by yield*trait (GYT) data for 17 chickpea genotypes

Gen.	DF	DM	PH	SW	GY	GY/DF	GY/DM	GY*PH	GY*SW
G1	189.33	225.30	27.87	31.92	537.91	2.84	2.39	14989.48	17172.57
G2	189.78	225.05	29.64	30.53	519.58	2.74	2.31	15397.75	15863.45
G3	190.09	225.98	28.24	34.33	535.59	2.82	2.37	15122.30	18386.86
G4	190.30	226.00	27.64	34.20	584.58	3.07	2.59	16157.68	19993.84
G5	190.55	225.36	29.57	29.85	511.38	2.68	2.27	15118.80	15262.86
G6	190.36	226.35	28.80	33.18	522.98	2.75	2.31	15060.46	17354.97
G7	190.69	227.00	28.66	33.02	556.24	2.92	2.45	15941.34	18367.31
G8	189.80	225.96	27.24	33.37	535.47	2.82	2.37	14583.61	17869.86
G9	190.63	226.13	28.43	32.22	617.08	3.24	2.73	17540.56	19884.48
G10	190.76	226.55	31.40	34.05	557.02	2.92	2.46	17489.00	18968.89
G11	190.09	225.85	29.47	32.10	508.93	2.68	2.25	14999.29	16338.73
G12	191.35	226.65	28.94	35.60	555.26	2.90	2.45	16070.23	19765.55
G13	189.60	226.05	29.04	32.61	510.85	2.69	2.26	14835.08	16658.97
G14	189.66	226.75	27.72	31.89	560.26	2.95	2.47	15530.46	17866.14
G15	189.69	226.03	30.27	31.04	522.15	2.75	2.31	15806.73	16205.44
G16	190.53	226.46	29.45	35.67	526.23	2.76	2.32	15494.75	18772.20
G17	190.91	226.33	29.46	34.14	585.78	3.07	2.59	17257.05	19999.90

DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: 100-Seed weight, GY: Grain yield

G9 emerging as the superior genotype. It implied that G9 is the best genotype for PH, DF, DM, and 100-SW in connection with grain yield (Fig. 6). In GYT analysis, superiority indices (Table 5), which were calculated by

integrating all the standardized yield-trait combination values, ranked genotypes by the mean of all traits, where high values of SI indicate the best genotypes. Considering superiority indices relevant to the studied chickpea

Table 5 Standardized genotype by yield*trait (GYT) data and superiority index for the genotypes

GEN	GY/DF	GY/DM	GY*PH	GY*SW	Mean (Superiority index)
G1	-0.12	-0.17	-0.80	-0.49	-0.40
G2	-0.76	-0.78	-0.36	-1.35	-0.81
G3	-0.27	-0.31	-0.66	0.30	-0.23
G4	1.32	1.36	0.46	1.35	1.13
G5	-1.10	-1.08	-0.66	-1.74	-1.15
G6	-0.70	-0.77	-0.72	-0.37	-0.64
G7	0.36	0.31	0.23	0.29	0.30
G8	-0.24	-0.31	-1.24	-0.04	-0.46
G9	2.36	2.45	1.96	1.28	2.01
G10	0.37	0.37	1.91	0.68	0.83
G11	-1.14	-1.20	-0.79	-1.04	-1.04
G12	0.26	0.31	0.37	1.20	0.54
G13	-1.04	-1.15	-0.97	-0.83	-1.00
G14	0.59	0.47	-0.22	-0.04	0.20
G15	-0.67	-0.77	0.08	-1.13	-0.62
G16	-0.61	-0.66	-0.25	0.55	-0.24
G17	1.30	1.37	1.65	1.36	1.42

DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: 100-Seed weight, GY: Grain yield

genotypes (Table 5), genotypes G4, G9, and G17 had a positive SI higher than 1, while genotypes G7, G10, G12, and G14 had a SI below 1. In cases of G7 and G14, the

SI value was near zero, indicating these genotypes had intermediate values.

Capability of stable chickpea genotype at autumn-sowing
ANOVA results showed that genotype and cold stress had a significant effect on all biochemical enzymes that were measured. These enzymes were CAT, SOD, MDA, H₂O₂, and GPX. Hence, mean comparison (Fig. 7) by the method of Duncan was applied to clarify in detail the difference between treatments. As illustrated in Fig. 7, regardless of the of the cold stress level, MDA and H₂O₂ as indicators of plant stress had low values in both the resistant chickpea genotype (Ana) and the newly selected genotype (FLIP 10–128 C) compared with the susceptible check (ILC533). In this study, the activity of the enzymes CAT, SOD, and GPX varied across chickpea genotypes and cold temperatures as well (Fig. 7). In general, the cold-susceptible genotype (ILC533) depicted low values of CAT, SOD, and GPX enzymes, while FLIP 10–128 C and Ana (resistant genotype) had the same enzymatic reaction in response to cold stress (Fig. 7). These discrepancies were remarkable, especially at hard cold stress (-4 °C) condition that of FLIP 10–128 C had also higher values of GPX, and CAT than Ana (resistant check). Results pertaining to CAT, SOD, and GPX enzymes (Fig. 7) showed studied chickpea genotypes could be screened considering both -4 °C and 22 °C treatment.

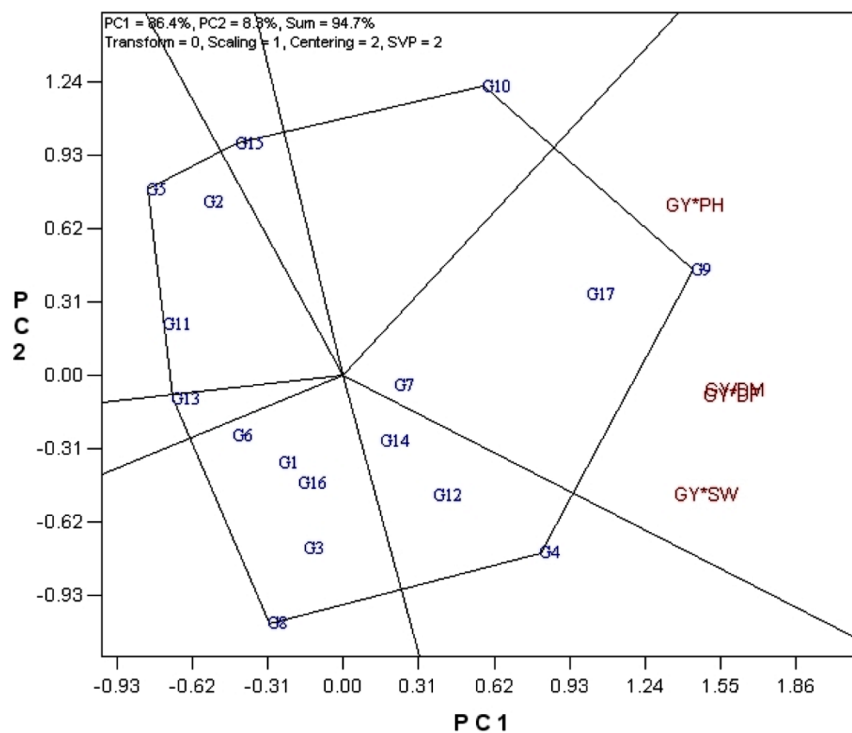


Fig. 6 The “which-won-where” perspective of the genotype by yield*trait (GYT) biplot was utilized to emphasize genotypes with exceptional profiles. This biplot was constructed using singular value decomposition of the standardized GYT table (with scaling = 1 and centering = 2). The trait codes correspond to: GY for grain yield, DM for days to maturity, PH for plant height, and SW for seed weight

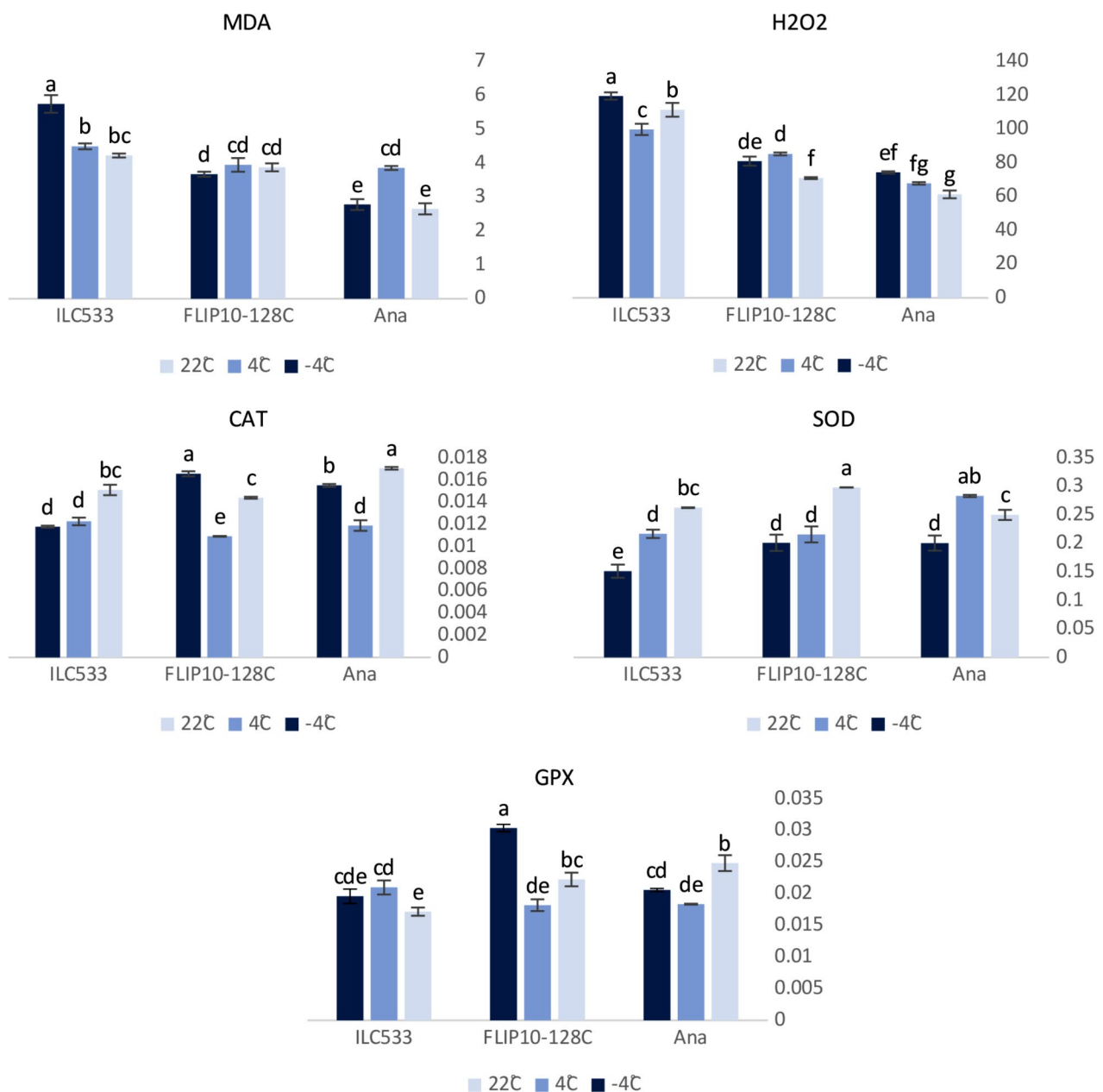


Fig. 7 Means comparison for biochemical enzymes activity in chickpea genotypes including Ana (tolerant check), ILC533 (susceptible check), and FLIP 10–128 C (superior stable genotype) at varied temperatures of cold stress. CAT: catalase, GPX: glutathione peroxidase, SOD: superoxide dismutase, MDA: malondialdehyde, and H₂O₂: hydrogen peroxide. Different letters above bars show a significant difference based on DNMRT mean comparison test

Discussion

Chickpea is one of the well-known crops that is grown in dryland regions and, depending on the region, is traditionally planted in the spring. Nowadays, with the expression of cold-resistant chickpea genotypes [29], it is possible to do early planting (November to December) in rain-fed regions. As an advantage of early planting, it increases plant yield following an increasing plant growth period. In this study, a sample of 17 cold-resistant

chickpea genotypes was evaluated via early planting at five locations as representative of cold dryland points during two consecutive years. The analysis of variance showed a significant difference among the studied chickpea genotypes regarding their response to environmental change. Similarly, Pouresmael et al. (2018) [30] and Karimizadeh et al. (2023) [12] also reported significant genotype × environment interaction for yield and the majority of agro-morphological traits of chickpea. Literature review [31] have shown that rainfall from other

climate factors could incredibly influence crop performance in rain-fed conditions and interfere in the interaction of a given genotype with the environment. So, in this study, monthly rainfall from crop season until harvesting time across 10 environments was considered a covariate and applied by partial least squares regression. The present study showed that rainfall in autumn, especially November and December, as early planting times accompanied by one month before harvesting chickpea could affect its grain yield in cold rain-fed regions.

Similar to our output, Karimizadeh et al. (2023) [12] revealed that rainfall in autumn and spring is involved in the genotype-environment interaction of chickpea. Also, Momenpour et al. (2023) [32] using statistical-geographical modeling for estimation of the yield of rainfed chickpeas in its major cultivation areas in Iran, manifested that province precipitation indices are the most effective indices of yield variability during the flowering stage of chickpea.

Although the identification of the effective climate factor is vital in rainfed conditions, it is considerable that rainfed circumstances are varied over years and locations, so meticulous identification of well-adapted and stable genotypes is significant in such a state. Accordingly, WAASB values as a robust stability parameter based on a linear mixed-effect model were calculated for each chickpea genotypes and the $GY \times WAASB$ biplot depicted genotypes G9, G12, G14, and G17 as promising genotypes for early planting in rain-fed regions. Regarding the WAASB/GY heatmap as a distinguishing feature of WAASB analysis, the aforementioned genotypes will be screened again depending on chickpea breeder aims. For instance, assuming equal weight (50/50) for grain yield and stability parameters of WAASB, genotype G10 is the winner, while by increasing the weight of grain yield opposite to WAASB (for example, 60/40), genotype G9 will be the winner. According to the literature review [12], the majority of studies about yield stability analysis of chickpea have utilized methods of GGE biplot [33], non-parametric [34] and AMMI [35]. Meanwhile, it is possible to take advantage of WAASB as a new method derived from AMMI and BLUP methods for identifying superior chickpea genotypes for rain-fed conditions.

Grain yield of chickpea also influenced by agro-morphological traits that are correlated with each other [36]. Hence, to achieve improved yield and stability in future crop varieties, other agro-morphological traits of interest could be incorporated into the background of chickpea [37]. Regarding the interaction of traits or genotypes with the environment and the negative association among the interested traits [20], simultaneous evaluation of multi-traits across environments is unavoidable. Although the selection of superior genotypes in context with multiple traits was done in some crops such as lentil [25], maize

[21], sugar beet [26], sweet potato [38], and sesame [39], it is not addressed in chickpea. As a goal of chickpea farmers and breeders, it will be profitable for chickpea plants to get more precipitation and also finish their growth period before later warm conditions in rain-fed regions. So, in this study, after measurement of agro-morphological traits of chickpea, decreasing of traits including days to flowering (DF) and days to maturity (DM) and increasing of traits including grain yield (GY), plant height (PH), and 100-seed weight (SW) were aimed at. Our findings showed that all goals had been reached by simultaneous multi-trait stability analysis using the MTSI index, and selection differential values calculated for each trait verified this finding. As inferred by MTSI values, genotypes $G8 > G9 > G14$ could be considered multi-trait stable superior genotypes for early planting in rain-fed regions. But, focusing on the WAASB/GY heatmap showed that, with equal weight for WAASB and grain yield, genotype G8 is not suitable for early planting in rain-fed regions. Hence, it is included that MTSI have to be implemented accompanied with WAASB/GY to get concise results.

Recently, Yan and Fregeau-Reid (2018) [18] and Yan (2024) [40] have extended a novel method named genotype by yield*trait (GYT), which also considers all the traits simultaneously with yield to identify superior genotypes. Even the generated GYT biplots could clarify the genotypes' strengths and weaknesses. In the present study, a standardized genotype by yield*trait table (with decreasing objectives for traits DF and DM) was constructed and utilized through a polygon biplot. As a finding, the polygon view of the GYT biplot showed that when chickpea traits were combined with grain yield, their correlation pattern became significantly positive, depicting that each trait gains its worth only when combined with yield [18, 20]. In the present work, similar to the MTSI index, the genotype G9 was detected as a multi-trait superior chickpea genotype for early planting in a rain-fed region. This finding is remarkable and proved by the results of Yan (2024) [40], who found that the $G+GE$ biplot of the GYT index graphically displays the mean and stability of the genotypes, especially when the same breeding objectives are assumed in the GYT analysis. The unique features of the GYT approach are the determination of the weaknesses and strengths of interested genotypes. In this regard, the superiority index [18] for each genotype across studied traits could be considered. Here, superiority indices showed that G9 had no weakness, while genotypes such as G8 and G14, which were selected by means of MTSI, had some weakness.

To sum up, genotype such as G9 (FLIP 10–128 C) has remarkable yield potential as well as agro-morphological performance in autumn-sowing regarding yield stability and multi-trait indices. Accordingly, it seems to have a

tolerant reaction against cold stress during the autumn and winter seasons. So, a complementary test of this genotype (FLIP 10–128 C) accompanied by tolerant (Ana) and susceptible (ILC533) chickpea genotypes was done under -4 °C, 4 °C, and 22 °C in controlled conditions. This temperature gradient has also been applied previously by Lotfi et al. (2023) for screening chickpea germplasm against cold stress. The different reactions of the studied genotypes (FLIP 10–128 C, ILC533, and Ana) to the inspected temperature gradient depicted the effectiveness of the selected temperature in chickpea germplasm screening. Herein, the cold tolerant or susceptible reaction of the aforementioned genotypes was measured via MDA (as a product of the peroxidation of unsaturated fatty acids by reactive oxygen species) and H_2O_2 as a type of ROS under stress [41]. In this way, low values of MDA and H_2O_2 , which were detected for FLIP 10–128 C resembled a tolerant check (Ana) and verified the cold resistance reaction of the superior stable genotype of FLIP 10–128 C. According to Mir et al. (2021) [42], such a cold-tolerant reaction leads to the suitable establishment of the plant and, finally, a high yield. In addition, resistance to different stresses depends on the antioxidant ability of the plant [10, 43] and a change in the antioxidant levels can prevent the damage caused by the stress. In this regard, high levels of SOD, GPX, and CAT as antioxidant enzymes were seen for tolerant genotypes (FLIP 10–128 C and Ana) at 4 °C and 22 °C. It is concluded that the chickpea genotypes even susceptible control (ILC533) doesn't show any response at 4 °C. The enzymes CAT and GPX, which were detected in maximum values for genotype FLIP 10–128 C at -4 °C, are more likely responsible for the cold tolerance of this genotype considering the average temperature of minus zero during December, January, February, and March in the studied environments.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-024-05463-0>.

Supplementary Material 1

Acknowledgements

The authors gratefully thanks from Dryland Agricultural Research Institute of Iran for supplying field trials facilities.

Author contributions

H.H.M.: Software and Formal analysis, Data curation, Writing-Original draft preparation. H.H.K.: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. H.K.: Data curation, Investigation, Methodology. S.S.S.: Data curation, Investigation, Methodology. B.M.A.: Data curation, Investigation, Methodology.

Funding

This work was supported by Dryland Agricultural Research Institute (DARI) of Iran (Project number: 0-15-15-017-990817).

Data availability

Data supporting the findings of this study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 19 June 2024 / Accepted: 29 July 2024

Published online: 06 August 2024

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