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Identification of Climate-Resilient Desi Chickpea Genotypes

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Abstract

The autumn planting of chickpea via climate-resilient genotypes can be considered a management strategy for addressing the constraints imposed by climate change. In this regard, an integrated method including two years of field trials (conditions in which plants faced subzero temperatures), membrane stability and freezing temperature (-10°C) screening was used to evaluate the cold/freezing tolerance of 38 desi chickpea cultivars in the primary vegetative growth phase. The cold-tolerant cultivar Katran and the international sensitive line ILC533 were also included in the experiments as controls. Different indices, including the average relative yield and frost resistance, mean ranks and sum rank indices computed and the total rank assigned to the genotypes, were estimated. Principal component analysis (PCA) and genotype × yield trait (GYT) graphical display were used to determine high-yield genotypes with greater relative frost resistance. A total of 16 genotypes (217240, 37707, 218992, 218170, 217450, 217379, 218029, 217251, 217242, 218923, 218194, 217327, 218195, 218767, 218142, 217471) were identified as cold/freezing tolerant with high yield performance in field trials. The field trial results were approximately confirmed by the results of the freezing tolerance evaluation under controlled conditions. On the other hand, the assessment of membrane stability was not fully consistent with the field trial results, as only four of the genotypes identified as tolerant in the field trials (37707, 217471, 218149 and 218195) presented high membrane stability. The PCA results revealed that more than 85% of the observed variation among the genotypes was attributed to the first (66.8%) and second (18.4%) components. The mean relative performance, mean ranks and rank sum index had the highest coefficient factors in component 1. The cold/freezing-tolerant high-yielding genotypes 37707, 217450, 218170, 218923, 218029, 218195, 218992, and 217379, as well as the cold-tolerant check cultivar, presented the greatest amounts of both components. The results revealed the usefulness of the GYT biplot for distinguishing cold/freezing-tolerant genotypes. On the basis of these results, the harvest index, plant canopy height and first pod height traits could be attributed to desi chickpea germplasms with proper productivity and cold/freezing tolerance.

Keywords: Autumn planting, Chilling tolerance, Desi cultivar, Frost resistance, Genetic diversity, Native germplasm.

Introduction

Climate change and its negative effects on crop production are among the challenges facing humanity over the next 50 years. Chickpea is a valuable crop in the changing climatic scenario, where the crop yield increases with increasing temperature and CO₂ levels [1]. Most importantly, unpredictable weather conditions resulting from climate change, such as erratic rainfall and the frequency of drought and extreme temperatures, i.e., high (> 30°C) and low (< 15°C) temperatures, constitute major constraints on chickpea production [2].

Climate change adaptation and the management of its impacts are essential strategies for sustainable agricultural production [3]. Shifting the planting date from spring to autumn is considered a promising approach for mitigating the effects of climate change on chickpea production. The seed yield performance of chickpea is 57.1% greater in the winter sowing season than in the spring sowing season [4].

However, better establishment, proper use of precipitation and avoidance of terminal drought and heat stresses resulted in greater production and yield stability in autumn [5]. However, winter kill can limit the productivity of fall-sown chickpea. Suboptimal temperatures (temperature <15°C) in both the freezing and chilling ranges can be detrimental to chickpea production because they hamper chickpea growth and vigor at all phenological stages [6].

The limited genetic diversity available to breeders requires extensive screening of crop germplasms, including various gene pools, to identify cold-tolerant genotypes [7, 8]. Therefore, one of the main goals of rainfed legume research in Iran is to achieve cold-tolerant chickpea genotypes to develop autumn planting.

The goals of cold tolerance breeding vary among regions; i.e., genotypes should be selected for freezing tolerance (below 0°C) during early growth and chilling tolerance (up to 0°C) during reproductive growth [9].

Two main types, kabuli and desi, have been defined for cultivated chickpea. Desi chickpea accounts for more than eighty percent of the world production [10] and is cultivated mainly in India, Pakistan, Bangladesh, Mexico, Ethiopia and Iran. Desi-type chickpea generally has a bushy growth habit, varying degrees of anthocyanin pigments, pink and purple flowers, angular seeds with thick testae and diverse color spectra from cream, yellow, green, brown to black.

In Iran, desi chickpea is often used for splitting and dahl production. To date, cold-tolerant cultivars suitable for autumn planting have not been introduced. Therefore, desi chickpea has been cultivated in spring from traditional landraces or old cultivars. Hence, terminal drought and heat stress severely affect productivity.

The screening of local landraces and wild relatives of chickpea to identify chilling-tolerant accessions is a prerequisite [7]. An old study on screening for cold tolerance using 2408 accessions originating from 31 different countries revealed that 68% of the cold-tolerant genotypes belonged to four countries: Chile, India, Iran and Morocco. Therefore, to identify sources of resistance, further searches should be conducted in accessions originating from these countries [11].

The chickpea collection of the National Plant Gene Bank of Iran (NPGBI), which is one of the largest chickpea collections in the world [12], includes over 3,000 accessions of desi-type

chickpea [13]. The results of different studies clarify the existence of suitable genetic diversity in this collection to achieve breeding goals, some of which are mentioned further. The screening of 812 entries of this collection, which originated from high and cold regions of the country under natural cold conditions prevailing at the Maragheh Rainfed Agricultural Research Station during the 2018–2019 cropping season (with an average minimum temperature of -14.5°C and an average of 25 frost days in the winter months) and in conditions where the cold-sensitive international line, ILC533, was completely dead, led to the selection of cold-tolerant plants [13]. Additionally, the evaluation of 335 accessions of this collection under autumn planting conditions at the Sararoud station in Kermanshah via multiple trait genotype–ideotype indices (MGIDIs) led to the identification of 59 superior accessions in terms of agronomic traits [14].

Visual screening under field conditions and measurement of cell membrane stability under controlled conditions were used to screen 366 accessions of chickpea to identify the most promising cold-tolerant genotypes in the western Himalayas of Jammu and Kashmir [7].

The identification of superior genotypes is a key part of plant breeding. However, a genotype does not necessarily have all the desirable traits together. Therefore, identifying unknown and unfavorable relationships between key traits is challenging. Selection on the basis of multiple traits has been proposed to address this challenge. Genotype $\times$ yield $\times$ trait (GYT) biplot analyses are useful graphical tools that provide an efficient overview of the relationships between yield, different traits and genotypes. Any traits, including agronomic traits, grain quality, nutritional quality or disease resistance, could be used in combination with yield to identify superior genotypes. The GYT biplot ranks genotypes on the basis of their ability to combine yield with other target traits and simultaneously shows their trait profiles, i.e., their strengths and weaknesses [15]. This method has been used to evaluate genotypes in various crops, including sunflower [16], wheat [17, 18], sugar beet [19], rapeseed [20], and barley [21].

Many chickpea genetic resources have been collected and conserved in gene banks. The comparison of genotypic responses to cold/freezing stress could be useful for identifying superior genotypes with desirable agronomic traits that are suitable for autumn planting. The utilization of these resources is possible by selecting a unique germplasm and ultimately using chickpea breeding programs for cold tolerance.

To that end, three approaches were used in the present study to compare the genotypic differences of 38 desi chickpea genotypes purified from local landraces. 1) A two-year trial was conducted in the field under natural conditions (at subzero temperatures) to evaluate the winter survival rate and agronomical traits. Genotypic variation was compared on the basis of survival rate and performance; diverse indices were computed and used to group the genotypes through multivariate analysis.

2) Genotype $\times$ yield $\times$ trait (GYT) biplot analyses were used to identify the relationships between evaluated traits as well as to introduce superior genotypes. 3) A complementary experiment was conducted under controlled conditions to compare injury symptoms after freezing stress and the MSIs of the understudied genotypes to identify superior genotypes.

Materials and methods

Cold/Freezing Tolerance Evaluation in Field Tests

Plant Materials, Experimental Design and Sowing

The study was conducted as two-year trials at the National Plant Gene Bank of Iran (NPGBI), Seed and Plant Improvement Institute, at 35 48°N latitude and 51 10°E longitude in Karaj (at an altitude of 1321 m above sea level). The study employed a complete block design with three replications. Thirty-eight purified genotypes from NPGBI-sourced desi chickpea accessions selected on the basis of our previous project [13] were included in the study. The codes and collection sites of the genotypes are detailed in Supplementary Table S1. To compare the cold tolerances of the genotypes, a cold-tolerant cultivar, Katran, and a cold-susceptible international line, ILC533, were also included in the study as check genotypes.

Each experimental plot consisted of four rows 4 m in length, with inter- and intrarow spacings of 50 and 10 cm, respectively. Planting was performed during the first week of November during the two cropping seasons 2022–23 and 2023–24. The sowing date and simultaneous irrigation after planting for rapid emergence [11] allow plants to reach early vegetative growth before the winter setting; the growth stage, which is susceptible to freezing injury and is associated with stages in which plants usually experience subzero temperatures in the target environment of the country. Standard agronomic practices were followed to raise a good crop. The weed control was generated by hand three times during the growth period.

Table 1. The Number and the code of under studied chickpea genotypes

Entry	Code *	Entry	Code	Entry	Code	Entry	Code
G1	ILC533	G11	217331	G21	218028	G31	218335-1
G2	37707	G12	217340	G22	218029	G32	218394
G3	217223	G13	217379	G23	218087	G33	218554
G4	217240	G14	217389	G24	218142	G34	218767-1
G5	217242	G15	217410	G25	218149	G35	218768
G6	217251	G16	217442	G26	218151	G36	218803
G7	217274	G17	217450	G27	218166	G37	218923
G8	217276	G18	217471	G28	218170	G38	218991
G9	217309	G19	217489	G29	218194-2	G39	218992
G10	217327	G20	217499	G30	218195	G40	Katran

* Indicates the under studied genotypes were selected and purified from which desi chickpea accessions of the National Plant Gene Bank of Iran.

Weather Conditions at the Experimental Site

The weather conditions of the experimental site during the two cropping seasons are shown in Figure 1. The weather conditions from December to February were found to be ideal for screening the cold/freezing stress tolerance of chickpea. During the years 2022--2023 (December–February), the average minimum temperatures ranged from -1 to -4°C. While six snow days were recorded, the plants experienced 70 frost days, and the minimum temperature was as low as -9°C in February. The average maximum recorded temperature ranged from 4 to 7°C. During the fall to winter (December–March) of the year 2023--2024, the average minimum temperatures ranged from 1 to 5°C. A total of 10 snow days were recorded, while a cumulative total of 30 frost days and a minimum temperature of -8°C were recorded in February. The average maximum recorded temperature ranged from 6 to 12°C. The amount of

snowfall during February was recorded as 29 cm. In total, on the basis of the statistics, the weather conditions in the first year trial were harsher than those in the second year.

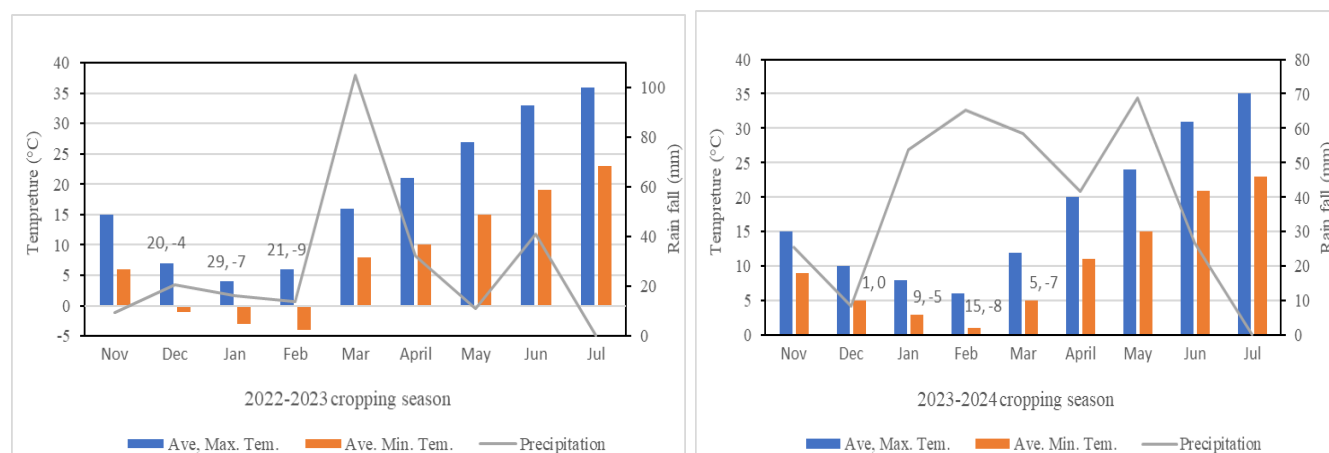


Figure 1. Weather condition statistics of the experimental site, including the average maximum and minimum temperatures and the rainfall in different months of the two cropping seasons, were obtained. The labels show the number of frost days as well as the absolute minimum temperature during the winter months.

Data Collection, GYT Table and Estimation of Indices

Seedling establishment was recorded in the experimental plots before the onset of winter frost (S1). After exposure to cold/freezing temperatures in the fall or winter, the number of remaining plants in each experimental plot (S2) was recorded again in the month of March. The survival ratio (SR) was estimated on the basis of the ratio of the number of remaining plants after the onset of frost to the initial number [22] and was used to calculate the relative frost resistance of the genotypes.

During the growing season, various phenological and agronomic traits, such as days to maturity (DM), primary (PBN) and secondary branch number (SBN), first pod height (FPH), plant canopy height (PH), and pod number per plant (PNP), were recorded. For quantitative traits, data were gathered from five randomly selected plants in each plot. To determine the grain yield (Y) as well as the harvest index (HI), the plants in the two middle rows of each experimental plot were harvested after full maturity and weighed. The grains were threshed and separated from the straw and stubble, and the grain yield was estimated in tons per hectare. The harvest index was calculated by dividing the seed weight by the biomass weight, and the weight of one hundred grains was estimated by weighing three replicates of 250 seeds from each plot.

The relative frost resistance (RFR) and relative performance (RP) of each genotype were separately computed for each year of the trial. The mean relative performance (MRP) [23] and the mean relative frost resistance (MRFR) of the two years were also computed and used to rank the genotypes. In addition, on the basis of the average rank assigned to each genotype in terms of these indices and the standard deviation of the assigned ranks, the rank sum index (RS) was estimated [24] and used to determine the genotypes with greater performance and frost resistance (Table 2).

To form the genotype × yield × trait (GYT) table, all the traits were multiplied by the grain yield for each genotype (e.g., Y*HI). For days to maturity, for which a larger value means less desirable, the GYT was obtained by dividing the yield value by days to maturity for each genotype (e.g., Y/DM). This ensures that in the GYT table, a larger value is always more desirable. As desi chickpea with intermediate seed weight is preferred for dahl production in the country [13], the 100-grain weight was not included in the GYT table. Given that the studied traits had different units and scales, data standardization was performed via equation (1) to eliminate units [15].

$$P_{ij} = \frac{T_{ij} - \bar{T}_j}{S_j} \quad (1)$$

where P_{ij} is the standardized yield–trait j combination for genotype i , T_{ij} is the data of genotype i for yield–trait j combination, $\bar{T}_j$ is the mean of all genotypes for yield–trait j combination, and S_j is the standard deviation of yield–trait j combination across all genotypes. The superiority index (SI) was calculated by averaging all the standardized values of the trait–yield combination.

Table 2. Indices for frost resistance and performance and their estimation method

Indices	Method of estimation
Relative frost resistance (RFR)	Survival rate of a given genotype divided into mean of survival rate of all
Relative performance (RP)	Yield performance of a given genotype divided into mean yield performance of all
Mean relative performance (MRP)	Relative performance of a genotype averaged across two years
Mean relative frost resistance	Relative frost resistance of a genotype averaged across two years
Mean Rank	The average of RFR, RP, MRFR and MRP Ranks of a given genotype
Rank Sum (RS)	Mean Rank + standard deviation of the ranks

Cold/Freezing Tolerance Evaluation under Controlled Conditions

To evaluate the freezing tolerance of genotypes under controlled conditions, an experiment was implemented in a factorial arrangement on the basis of a completely randomized design with three replications as follows:

Seed Planting

Seeds were surface sterilized with 1% sodium hypochlorite (NaClO), rinsed with distilled water and placed on moist filter paper for germination. After three days, uniformly germinated seeds were transferred into pots containing a mixture of field soil, perlite or peat moss at a 1:1:1 volume ratio. Each genotype was sown in three sets; each set consisted of three biological replicates with three healthy seeds per replicate. Pots were maintained in a greenhouse under natural light at $20 \pm 2^\circ\text{C}$ with a 16/8 h light/dark photoperiod and 65% relative humidity until 5 weeks were reached.

Freezing tolerance evaluation

Freezing tolerance was evaluated at -10°C using a thermogradient freezer (the temperature was selected on the basis of the results of preexperiments at -10 , -14 and -18°C , which revealed that none of the understudied genotypes could stand at -14 or -18°C).

Initially, one set of 5-week-old plants (with three replicates of each genotype) was transferred from the greenhouse to a thermogradient freezer and exposed to 4°C for 18 hours in the dark to induce cold acclimation. Holding plants in the dark restricts the loss of water through transpiration and results in a more favorable plant water balance [6]. After that, the temperature was decreased at a rate of 2°C per hour until it reached –10°C, after which it was held for 2 hours at this temperature. The temperature was subsequently increased to 4°C, and the pots were maintained for 12 hours. After that, the plants were transferred to the original greenhouse conditions to recover. Freezing stress injury was visually ranked after two weeks on a rating scale of 1–9 (Table 3), as described by Fiebelkorn [25].

Table 3. Freezing tolerance ratings developed by Fiebelkorn [25]

Score	Visual identification	Freezing tolerance description
1	Plant completely green	-
2	Plant with minimal freezing damage	Highly tolerant
3	Plant at least 75% green	tolerant
4	Plant between 50 and 75% green tissue	Moderately tolerant
5	Plant 50% green	Intermediate
6	Plant between 25 and 50% green tissue	Moderately susceptible
7	Plant 25% green	susceptible
8	Plant almost dead but has minimal green	Highly susceptible
9	Plant completely dead	-

Membrane Stability Assessment

The membrane stability of the genotypes was evaluated under cold (4°C) and control conditions (20 ± 3°C) via the electrolyte leakage method [26, 27]. To that end, one set of 5-week-old plants (with three replicates of each genotype) was transferred to a growth chamber at 4°C. Another set of plants was kept in a greenhouse under control conditions (20 ± 3°C). After 12 hours, the plants from both treatments were sampled, washed with deionized water and incubated in 10 ml of deionized water at 25°C for 24 hours with constant shaking on a rotary shaker at 100 revolutions per minute (rpm). The initial electrical conductivity (L1) of the solution was recorded. The samples were then autoclaved for 15 minutes at 120°C and cooled to room temperature, after which the final electrical conductivity (L2) was recorded. Electrolyte leakage (EL %) in both treatment groups was calculated via the formula $(L1/L2) \times 100$. This represents the leakage of electrolytes from damaged plant tissues in both treatments as a percentage of the leakage from tissues was completely destroyed after autoclaving.

The membrane injury index was estimated as the relative increase in the EL % of each genotype via equation (2). where ELs is the EL % under cold treatment and ELc is the EL % under control conditions.

$$\text{Membrane injury index} = \frac{\text{ELs} - \text{ELc}}{\text{ELc}} * 100 \quad (2)$$

Statistical analysis

A combined analysis of variance (ANOVA) for data recorded during two-year trials was conducted to assess variations among treatments. The mean comparison of traits was performed via the least significant difference (LSD) method in comparison with the tolerant cultivar Katran. All the statistical analyses were performed via SPSS ver. 24 and SAS ver. 9.2 software.

Principal component analysis (PCA) was performed to identify indices that have greater contributions to variations and could distinguish frost-tolerant and high-yielding genotypes, employing the Kaiser criterion to retain components with an eigenvalue of ≥ 1 [28]. A scatter plot diagram was constructed on the basis of the coefficients of the two major components. PCA and scatter plot drawing were performed via Stratigraphic Ver. 19. In addition, using the standardized data, a GYT biplot was drawn via GGE biplot software ver.6.3 on the basis of the first and second principal components. The column chart was generated via Excel and used for comparison of the means $\pm$ SEs of the traits evaluated under controlled conditions.

Results

Field trial evaluation of cold/freezing tolerance and selection of superior genotypes

Analysis of Variance and Descriptive Statistics

The combined analysis of variance of the data obtained from the two cropping seasons revealed that the effect of genotype was not significant for day to maturity, yield, branch number or pod number per plant. The effect of year was significant for all the studied traits except for plant canopy height and first pod height. The interaction effect of year and genotype was significant for all the studied traits except the number of pods per plant (Table 4).

Table 4. The combined analysis of variance for evaluated traits in chickpea genotypes at two cropping seasons

SOV	df	Mean Square									
		Survival Ratio	Grain Yield (t/h)	Plant canopy Height (cm)	Frist Pod Height (cm)	Days to Maturity	100 Grains Weight (g)	Harvest Index	Primary Branch Number	Secondary Branch Number	Pod Number per plant
Year (Y)	1	28231.7**	227957.6**	959.6 ^{ns}	17.1 ^{ns}	297.0**	42.0*	11263.8**	0.1 ^{ns}	248.8*	130913.6*
Block	2	685.9	1077.8	105.0	100.6	22.4	3.5	145.9	1.2	5.7	1992.5
Genotype (G)	39	647.5**	1797.8 ^{ns}	98.7*	30.3*	45.5 ^{ns}	103.2**	201.2**	0.9 ^{ns}	74.4 ^{ns}	563.9 ^{ns}
G* Y	39	272.8**	1805.3**	48.7**	48.7**	28.9**	5.1**	101.3*	0.6 ^{ns}	50.6**	425.8 ^{ns}
Error	156	67.5	494.7	13.6	13.6	4.7	1.7	68.9	0.4	11.9	376.8

^{ns} and ** ,respectively shows not significant and significant at the 0.01% probability level.

The descriptive statistics for the evaluated traits in both cropping seasons are presented in Table 5. Traits such as pod number per plant, yield performance, branch number, and 100-grain weight presented greater variability in both cropping seasons. Conversely, day to maturity displayed lower variability, with CVs of 1.3 and 1.8 during the two cropping seasons, respectively.

The coefficients of variation were 13 and 15 for plant canopy height and 15 and 17 for first pod height during the two cropping seasons, respectively. The survival ratio ranged from 16.3 to 82.5%, with a mean of 61.9% and a coefficient of variation of 25 from the 2022--2023 cropping

seasons. This trait ranged from 54.2 to 96.3%, with a mean of 83.5% and a coefficient of variation of 9 in the 2023--2024 cropping season (Table 5).

Table 5 - Statistical parameter of evaluated traits in chickpea genotypes at two cropping seasons

Trait (unit)	2022-2023				2023-2024			
	Min.	Max.	Mean	C.V*(%)	Min.	Max.	Mean	C.V(%)
Survival Ratio (%)	16.3	82.5	61.86	24.95	54.17	96.25	83.55	9.89
Grain Yield (t/h)	0.03	0.23	0.14	31.52	0.02	1.61	0.76	46.07
Plant Canopy Height (cm)	22.0	42.7	31.52	15.86	25.53	46.53	35.52	13.85
Frist Pod Height (cm)	11.4	23.2	16.87	17.28	11.77	23.87	17.40	15.72
Days to Maturity	219	234	226.63	1.79	217	230	224.46	1.26
100 Grains Weight (g)	10.9	30.6	20.28	23.60	13.3	27.5	19.4	18.4
Harvest Index	26.5	47.4	40.45	11.22	16.53	41.4	25.9	25.65
Primary Branch Number	2.3	4.6	3.28	14.34	2.67	4.67	3.32	15.59
Secondary Branch Number	7.8	28.3	16.47	29.75	7	23.67	14.44	29.07
Pod Number per Plant	14.3	53.5	29.84	30.75	50	108.33	76.56	20.48

*, C.V. stand for Coefficient of Variation

Genotypic differences in agronomic traits as well as cold/frost tolerance

Supplementary Table S2 presents the means of the traits for each genotype across two years of field trials, providing a comparative overview of genotypic differences in the evaluated traits. The significant differences between the studied genotypes suggest that suitable genetic materials could be used to advance breeding goals in desi-type chickpea as follows:

Survival ratio at different cold/freezing temperatures:

On the basis of the results of the 2022--2023 cropping season, under conditions where the SR of cold tolerance of the Katran variety was 56%, the SR of the sensitive line ILC533 was 16%, and line 218195, with an SR of 82.5%, presented the highest cold/frost resistance. Genotypes 218194, 218142, 218923, 217240, 217251, 218028, 217389, 217274, 217242, 218029, 217327 and 37707, with SRs between 70 and 80%, presented the highest cold/frost resistance. Compared with genotype 218195, which had the highest cold/frost resistance, the SR was significantly lower in the cultivar Katran and the lines ILC533 and 217489, 218166, 218767-1 and 218768.

In the 2023--2024 cropping season, the SRs were 86% and 54% for the Katran cultivar and the sensitive line ILC533, respectively. Genotypes 218087, 218195 and 218149 presented the highest cold resistance, with SRs greater than 95%. Genotypes 218028, 217274, 218394, 217327 and 218554, with SRs of over 90%, were in the next ranks.

Yield Performance

On the basis of the results, in terms of grain yield, genotypes 217471, 217240, 217499, 218394, 217410, 21751, 217223, 218194 and Katran presented the highest performance during the 2022--2023 cropping season. In terms of genotype numbers 218992, 37707, and 218991 as well as the cold-tolerant variety, Katran had the highest performance during the 2023--2024 cropping season. Genotype numbers 218170, 217450, 218923 and 217240 were in the next ranks. The lowest grain yield in both years of the trial was observed for the sensitive line ILC533.

Plant Canopy and First Pod Height

Genotype numbers 218195, 217389 and 217240 had the highest plant canopy heights among the studied genotypes, with a canopy height of 42 cm. Genotype numbers 218087 and 218149 were in the next ranks, with canopy heights of 38 and 37 cm, respectively. Genotype numbers 217331, 217389, 217410, 217450, 218029, 218087, 218149, 218170, 218194, 218195 and 218923, whose first pod heights were above 19 cm, were significantly different from those of the other genotypes in terms of this trait during the 2022--2023 cropping season.

In the 2023--2024 cropping season, genotypes 217450, 217331, and 37707 presented the highest plant canopy heights, with canopy heights of 46 and 43 cm, respectively. The first pod heights of these genotypes were 15, 21 and 23 cm, respectively. Genotype numbers 217442, 218335-1 and 218923 were next in rank, with canopy heights above 40 cm. The first pod height of these genotypes was approximately 19 cm. The first pod heights of genotypes 217327, 217389, 218166 and 218803 were greater than 20 cm. The canopy heights of these genotypes were 38, 39, 37 and 35 cm, respectively.

Harvest Index

Genotype numbers 218991, 218767-1, 218394, 218142, 217274 and Katran presented the highest harvest indices from the 2022--2023 cropping season. Genotype numbers 218029, 218767-1, 218991, 218992 and Katran presented the highest harvest indices during the 2023--2024 cropping season.

Day to maturity

In the 2022--2023 cropping season, the early mature genotype was 218992. In the 2023--2024 cropping seasons, 218767--1 and 218768, with a maturity of 218 days, were the earliest, and 217309, with a maturity of 231 days, was the latest mature genotype among the studied genotypes.

Branch number

Although there were no statistically significant differences in terms of primary and secondary branch numbers between genotypes, the number of secondary branches was affected by cropping season or its interaction with genotype. The highest number of secondary branches was observed at 218087, 218195, 218335--1 and 218554 in the 2022--2023 cropping season. In the 2023--2024 cropping season, the greatest number of secondary branches was observed for genotypes 217309, 217223, 217450, and 217410.

Pod number per plant

Genotypes 218187, 218151, 37707, 218803, 218767-1, 218335-1 and 218394-1 produced the highest number of pods per plant during the 2022--2023 cropping season. In the 2023--2024 cropping season, genotype 217309, with 108 pods per plant, presented the greatest number of this trait. Genotype numbers 218992, 218151, 218029, 217450 and 217223, with approximately one hundred pods per plant, were in the next ranks.

100-grain weights

Genotype numbers 217331 and 217442, with 100-grain weights of 27.5 and 26 grams, respectively, presented the highest 100-grain weights among the genotypes. In total, 16 genotypes had suitable grain weights (between 17 and 21 grams). Grain size is an important characteristic in determining the market price and economic importance of chickpea.

Selection of superior genotypes on the basis of indices

A variety of indices were computed in this study, including relative frost resistance (RFR), relative performance (RP), mean relative performance (MRP), mean relative frost resistance (MRFR), mean ranks, rank sum (RS) and the superiority index. The diversity of these indices and the ranking of the genotypes based on them in two years of field trials are shown in Supplementary Table S3. Genotypic differences on the basis of these indices are as follows:

Relative frost resistance (RFR)

Genotypes 218142, 218195 and 218194 presented the highest relative frost resistance index values from the 2022--2023 cropping season. In the 2023--2024 cropping season, genotypes 218087, 218149 and 218195 presented the highest relative frost resistance indices.

Relative performance (RP)

Genotype numbers 217240, 217471 and 217499 presented the greatest relative performance during the 2022--2023 cropping season. In the 2023--2024 cropping season, genotypes 218992, 30707 and 218991 presented the highest relative performance.

Mean RP and RFR

On the basis of the mean relative frost resistance index in the two years, genotype numbers 218195, 217274 and 218028 ranked the best. On the basis of the mean relative performance index at two years, genotypes 217240, 30707 and Katran ranked the best (Table S3).

The graphical display of the genotypes on the basis of the mean relative performance and mean relative frost resistance during the two-year trial (Figure 2) demonstrated that the susceptible line (ILC533) was located at the lower corner of the fourth quarter (IV) of the plot. The 12 genotypes that were placed on the second quarter (II) of the scatter plot and brushed in green presented indices greater than the mean. The Katran cultivar (cold tolerance check), 217471, 218767-1, 218142 and 218195 genotypes were placed near the mean line of both indices. Among these genotypes, genotypes 37707 and 217240 presented greater relative performance, and genotype 218195 presented the greatest relative frost resistance (Figure 2).

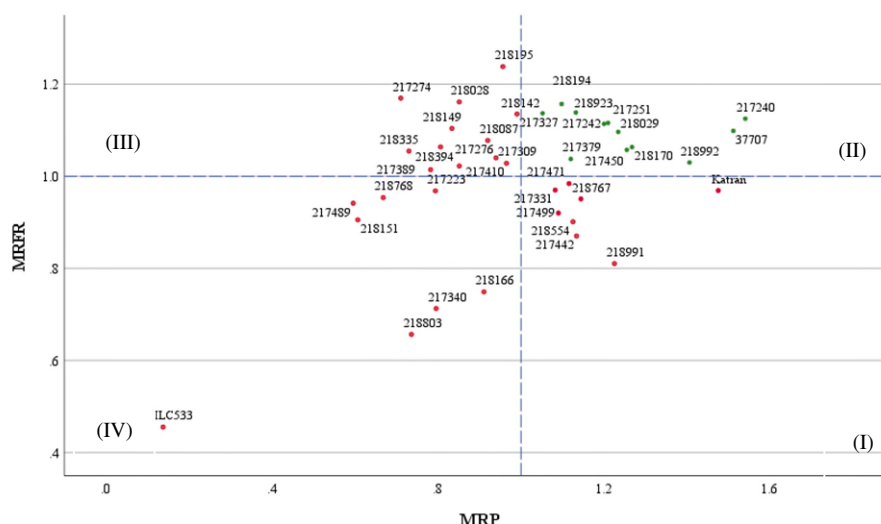


Figure 2. Biplot graphical display of the genotypes based on the MRFR (mean relative frost resistance) and MRP (mean relative performance) during the two cropping seasons. The blue lines represent the average values of both indices among all the understudied genotypes.

Superiority Index

Genotype numbers 217240, 217471 and 218394 presented the highest superiority indices from the 2022--2023 cropping season. In the 2023--2024 cropping season, genotypes 30707, 218992 and Katran presented the highest superiority indices (Table S3).

Mean Ranks

Genotype numbers 217240, 37707, 218029, 217242, 217251, 218170, 218923, 217450, and Katran (cold tolerance check cultivar) had the lowest mean ranks of indices. These findings indicate that these genotypes were superior on the basis of all the calculated indices (Table S3).

Rank Sum Index

A comparison of the standard deviations of the ranks of the genotypes based on different indices revealed that the 217242 and 218029 genotypes, with standard deviations of 1.48 and 1.83, respectively, had the lowest standard deviations of the ranks; thus, they had the lowest rank in terms of the rank sum index (Table S3). Genotype numbers 217240, 218029, 217242, 37707, 218170, 217251, 217450, 218142, 217379, 218194, 218923 and Katran (cold tolerance check cultivar) had the lowest rank sum indices. The sensitive check line, ILC533, had the lowest number of all the estimated indices and thus had the highest rank as well as the highest rank sum index among all the understudied genotypes.

Principal component analysis

The results of principal component analysis using the correlation coefficient matrix of the evaluated indices and considering the eigenvalues greater than 1 revealed that two components explained 85.23% of the variation between genotypes (Table 6). The mean relative performance with a positive sign and mean ranks of all estimated indices, as well as the rank sum index with a negative sign, had the highest coefficient factor in Component 1, with 66.84% of the variation. Component 2 accounted for 18.4% of the variation in the data and had the

highest relationship with the superiority index in both years of the trial. The Kaiser–Meyer–Olkin measure of the sampling adequacy amount (KMO = 0.593), which is nearly 0.6, indicates that principal component analysis provides interesting information about the underlying component (Table 6).

Table 6. Eigen value, percentage of explained variance, component loading in chickpea genotypes

	Component 1	Component 2
Eigen value	4.01	1.1
%Proportional Variance	66.84	18.39
% Cumulative Variance	66.84	85.23
Sum Rank	-0.45	-0.01
Superiority index2	0.35	0.67
Mean Relative Frost Resistance (MRFR)	0.36	-0.36
Mean relative performance (MRP)	0.45	0.26
Mean Rank	-0.49	0.07
Superiority index1	0.33	-0.59

KMO =0.593; KMO coefficient shows Kaiser–Meyer–Olkin measure of sampling adequacy.)

Mean Rank: the average ranks of a given genotype based on all calculated indices. Rank Sum: The Mean Rank of a given genotype + the standard deviation from the average, 1 and 2 shows superiority index calculated from result of the first (2022-2023) and the second (2023-2024) cropping seasons, respectively.

A biplot was created via the first two principal components to compare different genotypes and investigate the relationships between the studied cold tolerance indices (Figure 3). Indices with acute angles between their vectors showed strong positive correlations. Positive and strong correlations were observed between the mean relative frost resistance (MRFR) and the superiority index in the first year of the trial. On the other hand, the superiority index in the second-year trial was strongly correlated with the mean relative performance (MRP).

On the basis of Figure 2, the genotypes are placed on the right side of the biplot, with more component 1, which is blue in color, resulting in greater relative performance and frost resistance as well as a lower mean and sum of the ranks. The genotypes placed at the top of the right part of the biplot also had a greater superiority index. As shown in Figure 3, genotypes 37707 and 217240 have the maximum amounts of MRP and MRFR, respectively.

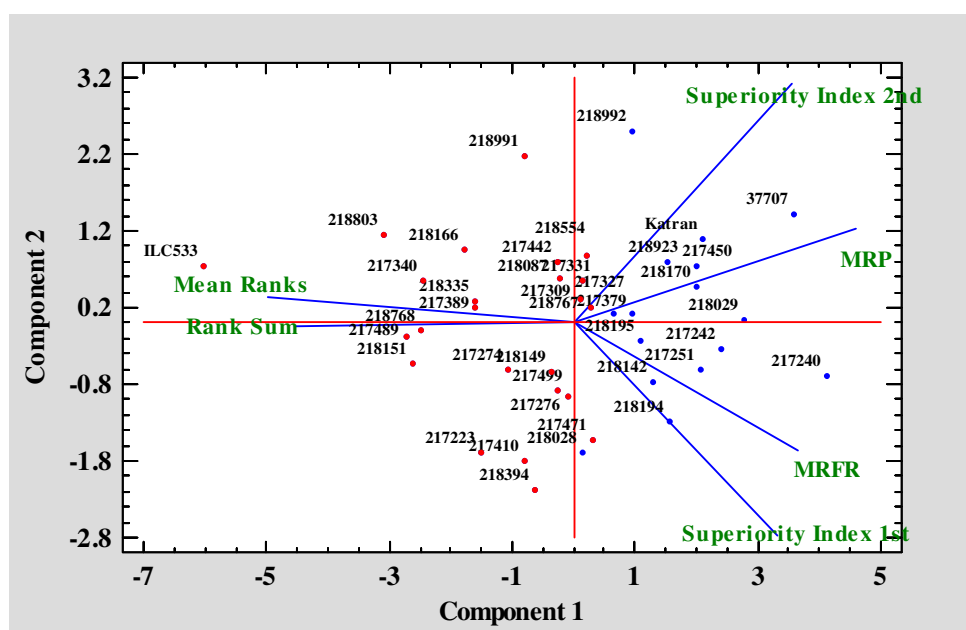


Figure 3. Biplot graphical display of the genotypes based on the two principal components extracted from the principal component analysis. MRFR: mean relative frost resistance, MRP: mean relative performance, mean ranks: the average ranks of a given genotype on the basis of all calculated indices. Sum Rank: The mean ranks of a given genotype + the standard deviation from the average; 1 and 2 show the superiority indices calculated from the results of the first (2022--2023) and second (2023--2024) cropping seasons, respectively. The red lines represent the means for both principal components.

GYT and selection of superior genotypes on the basis of the GGE biplot

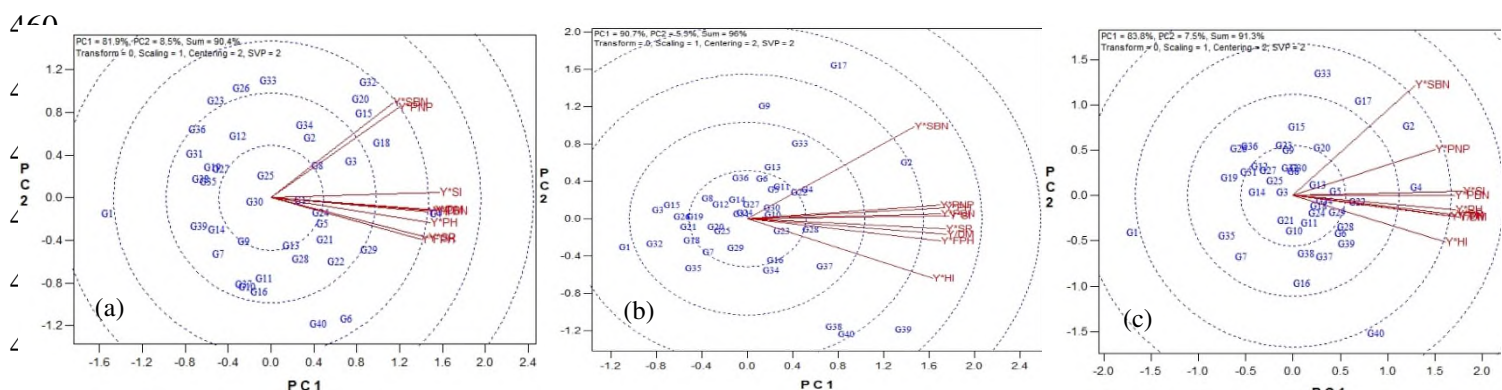
The GYT values for the understudied genotypes in two-year field trials are shown in Supplemental Table S4. The GGE biplot analysis of the standardized GYT revealed that the first two principal components explained 90.4% of the total variation, with PC1 accounting for 81.9% and PC2 accounting for 8.5% of the variation. This high proportion of explained variance indicates that the biplot adequately represents the genotype $\times$ yield $\times$ trait relationships and provides a reliable basis for interpretation.

Associations among various yield–trait combinations

Since all yield–trait combinations have yield as a component, they tend to be positively correlated with each other, as indicated by the acute angles in the biplot (Figure 4). Traits with acute angles between their vectors showed strong positive correlations. On the basis of the GGE plot, in the first year of the trial, positive and strong correlations were observed between Y*PNP and Y*SBN. Moreover, Y*SR and Y* FPH also have strong correlations. In addition, Y*PBN, Y*DM and Y*HI are strongly correlated, as their vectors are completely tangent to each other. Moreover, Y*SI has a strong correlation with Y*PBN, Y*DM, Y*HI, Y*PH, Y*FPH, and Y*SR (Figure 4a). In the second year, all yield combination traits were strongly correlated with each other. The only exception was the correlation of Y*SBN with Y*HI, as indicated by the wide angle between their vectors (Figure 4b).

On the basis of the mean yield–trait combination of both years, strong correlations were observed between Y*PBN and Y*SI. Moreover, Y*SR, Y*PH and Y* FPH also have strong correlations. In addition, Y*SR, Y*PBN, Y*PH, Y*FPH and Y*SI are strongly correlated.

457 Moreover, Y*SI and Y*SR are strongly correlated with Y*HI (Figure 4c). Among the studied
 458 yield–trait combinations, only the correlation of Y*HI with Y*SBN was weaker than that of
 459 the other traits (Figure 4).



466 Figure 4. Tester vector view of the genotype × yield × trait (GYT) biplot. (a), (b) and (C) show the results
 467 of associations among the yield–trait combinations in the first (2022--2023) and second (2023--2024)
 468 cropping seasons and the means of both years' trials. The trait abbreviations are as follows: PBN: primary
 469 branch number; SBN: secondary branch number; DM: days to maturity; HI: harvest index; PNP: pod
 470 number per plant; PH: plant canopy height; FPH: first pod height from ground; Y: yield; SR: survival
 471 ratio; SI: superiority index.

472

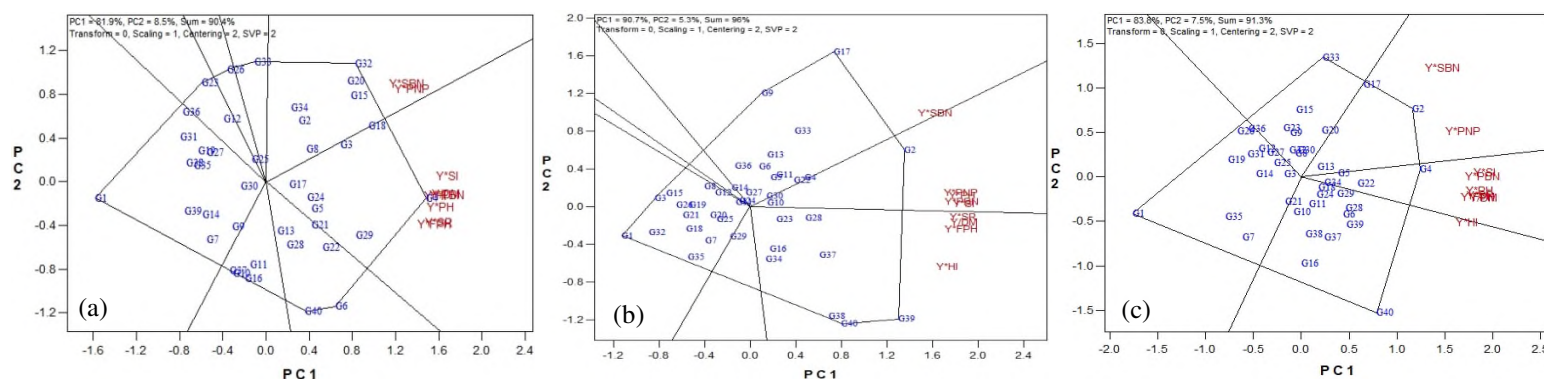
473 Trait profiles of the genotypes

474 The polygon view of the GGE biplot was used to identify the which-won-where pattern and to
 475 determine superior genotypes for different traits or groups of traits. The biplot was divided into
 476 several sectors, each representing a distinct pattern of genotype superiority. The clear
 477 separation of genotypes in the which-won-where view confirms the existence of differential
 478 genotype responses, emphasizing the importance of understudied genotypes for trait-oriented
 479 selection.

480 On the basis of the GGE plots depicted in Figure 5, in the first year of the trial, genotypes
 481 217240 (G4), 217251 (G6), the Katran cultivar (G40), and 218394 (G32) and the genotypes
 482 near it, including 217410 (G15) and 217499 (G20) as well as ILC533 (G1), which are located
 483 at the vertices of the polygon, were identified as the most responsive genotypes. 218394 (G32),
 484 217410 (G15) and 217499 (G20) were superior in yield when they were combined with SBN
 485 and PNP. Genotypes 217240 (G4), 217251 (G6), and the Katran cultivar (G40) were won on
 486 the basis of yield combinations with other measured traits (Figure 5a). In the second year of
 487 the trial, genotypes 37707 (G2), 217450 (G17) and 217309 (G9) were superior in yield when
 488 combined with SBN, PNP, Ph, and PBN traits and the SI. The yields of 218991 (G38), 218992
 489 (G39) and the Katran cultivar G40 were determined on the basis of the combination of yield
 490 with the SR, DM, FPH and HI (Figure 5b).

491 On the basis of the combination of yield and trait data from both years, 37707 (G2), 217450
 492 (G17) and 218554 (G33) were superior in yield in combination with the SBN and PNP traits.
 493 The yields of the 217240 (G4) and Katran (G40) cultivars were determined on the basis of
 494 combinations of the SR, DM, FPH, PH, PBN, and HI traits as well as the Y*SI (Figure 5c).

495 Genotype G1 (ILC533, cold susceptible line) is located on the left side of the GGE plots,
 496 indicating that it has no desirable yield trait combination (Figure 5).



503 Figure 5. The which-won-where view of the genotype $\times$ yield $\times$ trait (GYT) biplot. (a), (b) and (C)
 504 Plants with outstanding profiles in the first (2022--2023) and second (2023--2024) cropping seasons
 505 and the means of both years' trials. The trait abbreviations are as follows: PBN: primary branch number;
 506 SBN: secondary branch number; DM: days to maturity; HI: harvest index; PNP: pod number per plant;
 507 SR: survival ratio; PH: plant canopy height; FPH: first pod height from ground; Y: yield; SR: survival
 508 ratio; SI: superiority index.

509 Superiority rank of the genotypes on the basis of their yield–trait combinations

510 The average tester coordination view of the genotype by yield-trait (GYT) biplot was used to
 511 rank the genotypes on the basis of their overall superiority and their strengths and weaknesses.
 512 The small circle in the biplot represents the placement of the “average yield-trait combination”.
 513 The double-row line separates genotypes on the basis of the yield–trait combination average.
 514 The genotypes placed on the right side of the line have more yield–trait combinations than
 515 average does, and those placed on the left side of the line have yield–trait combinations that
 516 are poorer than average [15].

517 On the basis of the GGE plots depicted in Figure 5, in the first year of the trial, genotypes
 518 217240 (G4), 217471 (G18), 218394 (G32), 217410 (G15), 217499 (G20) and 217223 (G3)
 519 and 218194 (G29) were superior in yield combination with traits and had higher ranks (Figure
 520 6a). In the second year of the trial, 37707 (G2), 218992 (G39), the Katran cultivar (G40),
 521 218991 (G38), 217450 (G17), 218170 (G28) and 218923 (G37) were superior in terms of yield
 522 in combination with traits (Figure 6b). On the basis of the mean yield-trait combination of both
 523 years of trials, 37707 (G2), 217240 (G4), 217450 (G17), and 2188029 (G22) and the Katran
 524 cultivar (G40) were superior (Figure 6c). Genotype G1 (ILC533, cold susceptible line), placed
 525 on the far-left side of the biplot, was ranked as the weakest genotype.

526 Genotypes 37707 (G2) and 217240 (G4) are placed close to the average tester axis (the red
 527 line) with short projections to the double-angled line, which means that these genotypes have
 528 balanced trait profiles. In addition, regardless of their overall superiority, all the genotypes
 529 placed below the average tester axis tended to have relatively good levels of yield in
 530 combination with the PBN, PH, FPH, DM, HI and SR traits. The opposite is true for genotypes
 531 placed above the average tester axis, which are good for yield combination with SBN as well
 532 as PNP.

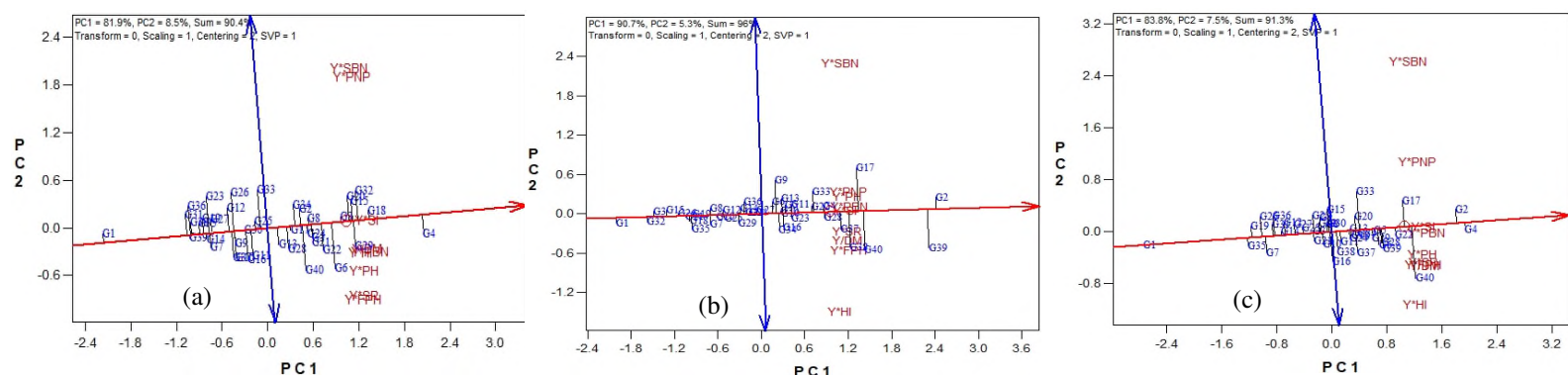


Figure 6. The average tester coordinate view of the genotype $\times$ yield $\times$ trait (GYT) biplot. (a), (b) and (C) Ranks of the genotypes on the basis of their overall superiority and strengths and weaknesses in the first (2022–2023) and second (2023–2024) cropping seasons and the means of both years’ trials. The small circle in the biplot represents the placement of the “average yield-trait combination”. The red line passes through the biplot origin, and the average yield–trait combination is called the average tester axis (ATA). Genotypes placed close to the ATA line with short projections to the double-arrow line tend to have balanced trait profiles, whereas those placed away from the line in either direction tend to have obvious strengths and/or weaknesses in yield trait combinations.

Controlled ConditionsCold/Freezing Tolerance Evaluation under

Freezing tolerance evaluation

The evaluation of plant leaf tissue damage percentage revealed that only genotypes 217389 (G14), 218142 (G24) and 218923 (G37) received a code of three (3), meaning that 75% of the leaf tissue was green; therefore, they were classified as tolerant genotypes. Genotype numbers 218149 (G25), 217274 (G7), 218194 (G29), 217327 (G10), 218335 (G31), 218170 (G28), 218028 (G21), 217240 (G4) and 218195 (G30) received a code of five (5), meaning that 50% of the plant leaf tissue was green; therefore, they were classified as intermediate genotypes. In total, 22 genotypes received a code of 6–8, meaning that they were classified as moderately to highly susceptible genotypes. The Katran cultivar has a code of 6, meaning that it was classified as moderately susceptible in our study under freezing temperatures (-10°C). Genotype numbers 218803 (G36), 218166 (G27), 217340 (G12), 218992 (G39) and ILC533 (G1; susceptible check line) received a code of nine (9), meaning that 100% of the plant leaf tissue was damaged or completely dead (Figure 7).

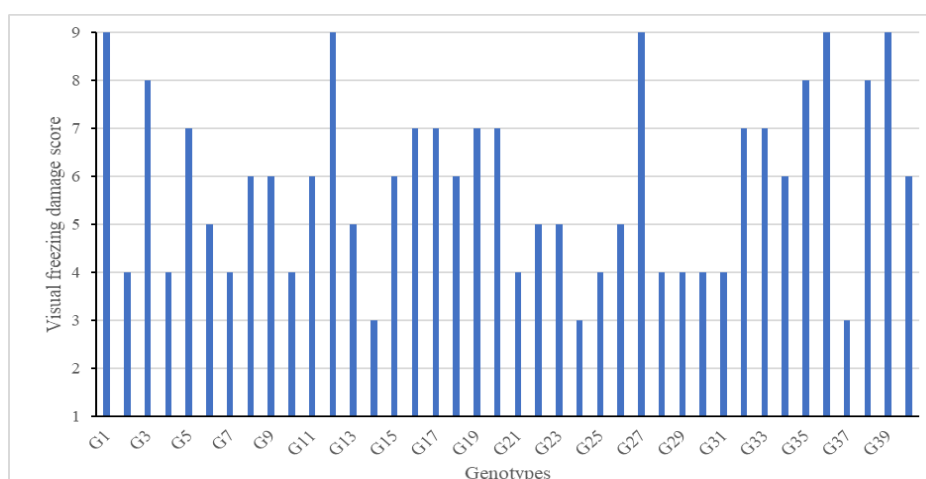


Figure 7. Visual stress injury of desi chickpea genotypes after two weeks of recovery following freezing at -10°C in comparison with cold-tolerant (G40; Katran) and cold-sensitive (G1; ILC533) checks, based on a rating scale of 1–9 (where 1 = no visible damage, 2 = highly tolerant, 3 = tolerant, 4 = moderately tolerant, 5 = intermediate, 6 = moderately susceptible, 7 = susceptible, 8 = highly susceptible, and 9 = 100% damage or dead).

Membrane Stability Assessment

The results of the evaluation of the effect of cold treatment on the percentage of electrolyte leakage (EL%) revealed that the range of EL% increased under cold stress. EL% varied between 7.5 (G9) and 24.8 (G39) among the genotypes under the control conditions. The range varied from 10.3 (G16) to 30.23 (G37) under cold stress. The results revealed diverse responses to cold stress among the genotypes. The minimum EL% under both treatments was recorded for genotype 217442 (G16), as the EL% varied from 9.86 to 10.3 in this genotype in the control and cold stress treatments, respectively. The maximum EL% (30.23%) was found in G37 under cold stress treatment (Figure 8a). The EL% varied from 9.3 to 11.5 in Katran, whereas it varied from 14.5 to 20.5 in ILC533 under the control and cold stress treatments.

The EL% increased in all of the genotypes due to cold stress; the percentage of this increase varied from 0.34% (G15) to 150% (G22 and G23). However, the increase in EL was 42% and 23% in ILC533 and Katran, respectively (Figure b). The percentage of EL increase or membrane injury index was less than 6% and statistically nonsignificant for genotypes 37707 (G2), 217223 (G3), 217331 (G11), 217389 (G14), 217410 (G15), 217442 (G16), 217471 (G18), 217489 (G19), 218149 (G25) and 218195 (G30), indicating greater membrane stability of these genotypes under cold stress (Figure 8b).

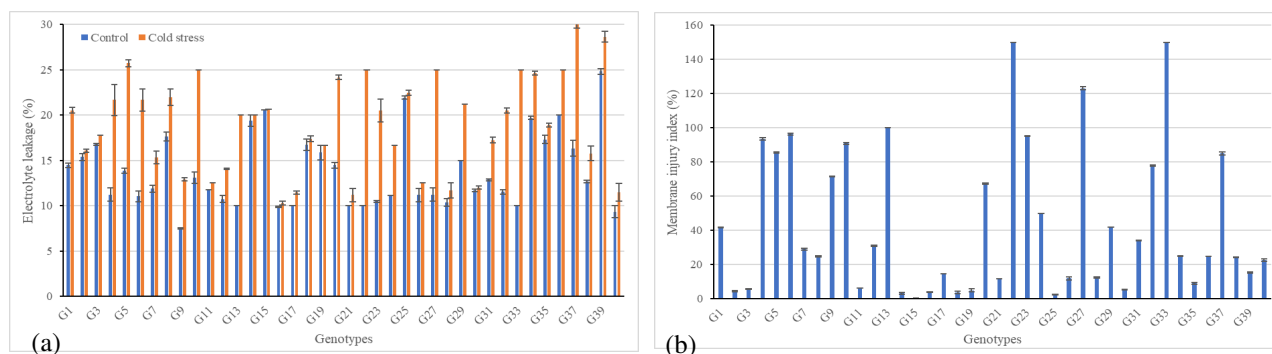


Figure 8. Electrolyte leakage percentage of desi chickpea genotypes under control and cold treatments (a) and the membrane injury index (b) in comparison with those of the cold-tolerant (G40; Katran) and cold-sensitive (G1; ILC533) controls.

Discussion

Field trial evaluation of cold/freezing tolerance and selection of superior genotypes

Genotypic differences in agronomic traits and cold/freezing tolerance

The combined analysis of variance revealed that the cropping season significantly influenced all the studied traits except plant canopy height and first pod height. The interaction effect of cropping season and genotype was also significant for all the studied traits except for the number of pods per plant (Table 3). Consistent with the results of this study, Güngör [4] reported that chickpea plant height did not significantly differ between years.

A comparison of the results of the two years clearly revealed harsh conditions during the 1st cropping season, which caused genotypic differences to be more obvious. The range of yield performance was lower, whereas the range of the survival ratio was greater in the first-year trial (Table 5). It is also clear from weather conditions (Figure 1) that there was a difference in frost duration of 40 days as well as the absolute minimum temperature that the plants experienced between the two-year trials. This clearly highlights the need for multiyear evaluation of genotypes to exploit the interaction between genotypes and environments to develop cold-tolerant desi chickpea genotypes. This issue has been mentioned in several studies, including [29] and [7].

The descriptive statistics for the evaluated traits in both cropping seasons (Table 5) revealed that traits such as the number of pods per plant, yield performance, branch number, and 100-grain weight presented greater variability in both cropping seasons. Conversely, day to maturity displayed lower variability. High genetic diversity for grain yield, 100-grain weight and canopy height in desi chickpea collection originating from Iran has also been reported in other studies [14, 30].

Genotypes 218195 and 217389 presented relatively high plant canopy heights and first pod heights during both cropping seasons. These results revealed the potential of these genotypes for mechanical harvesting. Significant differences ($P < 0.05$) in plant height were reported between ICRISAT desi chickpea genotypes [31]. High variation in grain weight was observed in the present study. This result is consistent with the results of Dwivedi *et al.* [32], who reported large variations in grain size and weight in chickpea germplasm collections. On the

basis of the results of the present study, 16 genotypes had suitable grain weights (between 17 and 21 grams). In desi-type varieties marketed in Iran, small and medium grains, with hundred grains weighing 16–20 g and yellow to light brown seed coat color, are preferred for dahlia production [13].

The results of the trait mean comparison revealed that there was a significant difference in terms of all evaluated traits as well as the survival ratio between genotypes. Consistent with these results, the evaluation of chickpea accessions under natural field conditions for cold/frost stress revealed highly significant differences between genotypes [7, 33].

Genotype numbers 218195, 218028, 217327 and 217274 presented high survival ratios in both years. These genotypes were therefore declared the most promising/candidate genotypes for desi chickpea cold/frost tolerance breeding. This finding highlights the potential of the desi chickpea collection of NPGBI for accessing cold-tolerant varieties suitable for autumn planting. The results of this study are inconsistent with the findings of Singh *et al.* [34], who suggested that the absence of freezing tolerance in desi-type chickpea could be because they have traditionally been grown and selected in relatively warm climates, including the Indian subcontinent and Ethiopia.

In accordance with these results, the results of studies conducted by Zaferanieh *et al.* [35] and Pouresmael *et al.* [13] confirmed the existence of high genetic diversity in terms of cold/frost resistance in desi chickpea genotypes. Saeed *et al.* [33] reported that desi chickpea species are more tolerant to freezing stress than kabuli chickpea species. On the basis of the results of these studies, approximately 39% of 36 genotypes that were identified as highly tolerant to freezing stress (tolerance score of 1--3) were desi type.

The results of an early assessment of the survival ratio of 3158 kabuli lines in a field trial at Ankara (altitude of 1055 m), where the air temperature reached -26.8°C and there was 47 days of snow cover, revealed that while 90% of the accessions did not survive, three of the landrace lines from India (ILC 2636, 2479, and 2491) and one from Iran (ILC 410) 100% survived [36]. Moreover, another study on a chickpea germplasm collection revealed that 15 tolerant to semitolerant accessions were selected from 872 Iranian accessions included in the study [11]. This result highlights the potential of chickpea germplasm originating from Iran for accessing cold/frost-resistant genotypes. The results of the current study have also been validated.

Selection of superior genotypes on the basis of indices and the GYT GGE biplot

On the basis of the results of the evaluation of various indices, each index calculated in each trial year directs us toward specific genotypes, which are not necessarily the same across different indices or different years. Therefore, multivariate analyses, including principal component analysis, have been suggested to integrate these indices and ultimately select the desired genotypes by considering all indices simultaneously [37, 38].

The results of principal component analysis using the correlation coefficient matrix of the evaluated indices and considering the eigenvalues greater than 1 showed that two components explained 85.23% of the variation between genotypes. A biplot was created via the first two principal components to compare different genotypes, and the relationships between the

studied cold tolerance indices revealed that genotypes 218992, 37707, 218923, 217450, 218170, 218029, 218195, 217379, and 217242. In addition, 217251, 218142, 217240, 218194 and the Katran cultivar (cold tolerance check), which have relatively high amounts of component 1, have relatively high relative performance and frost resistance as well as relatively low mean and sum values of the rank indices (Figure 2).

The graphical analysis of genotype $\times$ yield $\times$ trait (GYT) makes trait-oriented selection of desi chickpea genotypes suitable for autumn planting possible. The yield, traits and survival ratio under autumn planting were combined simultaneously. In accordance with these results, the GYT biplot proved to be an effective tool for visualizing complex genotype–trait interactions and identifying both broadly superior and trait-specific genotypes [15, 18, 38]. The GYT biplot approach is based on the paradigm that genotypes should be ranked by their performance in combination with other traits rather than individually [39].

On the basis of the results of associations among various yield–trait combinations, Y^*SR has a strong correlation with Y^*PBN , Y^*HI , Y^*PH , Y^*FPH , and Y^*SI . This result implies that improvement in one trait is likely to result in simultaneous improvement in the others. Such relationships are highly desirable in breeding programs, as they enable indirect selection for multiple traits through a single selection criterion.

Therefore, to identify germplasms suitable for autumn planting instead of measuring the survival ratio (SR), which requires two measurements of the established plants (before and after winter onset) and is time consuming, it is possible to replace each primary branch number (PBN), harvest index (HI), plant canopy height (PH) or first pod height (FPH) measurement. This is very important for screening high numbers of germplasm conserved in gene banks. The benefits of better water availability and lower temperatures during autumn planting resulted in greater height as well as more flowers and pods [40]. The height of chickpea plants is considered an effective indicator of vegetative growth. Genotypes with greater heights have a greater capacity for developing photosynthetic organs. This leads to increased production of dry material and, in the reproductive stage, the creation of a source for the development of pods and seeds, resulting in increased yield [41, 42]. It has been reported that any improvement in primary branches, biomass or the harvest index has contributed to a substantial increase in grain yield in recently released desi-type chickpea cultivars in Ethiopia [43]. On the other hand, varieties with more secondary branches have a high number of pods and seeds per plant, small seed sizes and low yields [43]. Sharma and Saini [44] reported that primary branches were the most important characteristic that was highly correlated with grain yield. In contrast, no significant differences in PBN were detected among the genotypes in the current study (Table 4). Thus, the ability of the $Y \times PBN$ interaction to discriminate among genotypes appears to result solely from differences in the yield component rather than from genotypic variation in PBN.

According to the polygon view of the GGE biplot drawn on the basis of the mean yield–trait combination of both years' trials, genotypes 37707 (G2), 217450 (G17) and 218554 (G33) were superior in yield in combination with secondary branch number (SBN) and pod number per plant (PNP) traits. The yields of the 217240 (G4) and Katran (G40) cultivars were determined on the basis of combinations of the SR, DM, FPH, PH, PBN, and HI traits as well

as the Y*SI (Figure 4c). On the basis of the mean yield-trait combination of both years of trials, the genotypes 37707 (G2), 217240 (G4), 217450 (G17), 2188029 (G22) and the Katran cultivar (G40) were superior. These findings indicate that these strains have high genetic potential for yield improvement and can be considered promising candidates for use in breeding programs. These results highlight the usefulness of the GGE biplot approach for evaluating desi chickpea genotypes while considering their trait profiles to identify superior genotypes.

Assessment of cold/freezing tolerance under controlled conditions

Freezing tolerance

The evaluation of plant leaf tissue damage percentage after freezing treatment (exposure to -10°C) revealed that only three genotypes, 217389 (G14), 218142 (G24) and 218923 (G37), were classified as tolerant. Nine genotypes were identified as intermediate susceptible, 22 genotypes were classified as moderately to highly susceptible, and five genotypes completely died at freezing temperatures. The identification of freezing-tolerant genotypes in the cultivated gene pool is considered very important for chickpea breeding since these genotypes can be easily crossed with released cultivars to produce fertile hybrids/segregating populations without linkage drag.

In accordance with this result, the results of screening 9095 accessions, landraces and breeding lines of chickpea revealed that no genotypes with a freezing tolerance rating of less than three were identified, and 86% of the genotypes were rated as moderately to completely susceptible [11]. The results of a cold tolerance analysis of cultivated germplasm on a rating scale of 1–9 revealed that 11 chickpea accessions presented very good cold tolerance and received a cold tolerance score of three [7]. Early studies have shown that some chickpea accessions can survive at temperatures as low as 8°C and 12°C during early growth stages [22, 6], suggesting the potential of the existing chickpea germplasm to select for cold-tolerant cultivars during seedling growth.

Although some studies reported lines that withstand temperatures of -12°C in the vegetative stage, the minimum temperature at which chickpea generally seems to survive is -8°C [22]. Freezing-sensitive plants are damaged or killed by temperatures below -1.5°C. Damage from freezing commonly occurs because ice forms within intercellular spaces [6]. In a comparison of the responses of 11 wild (*Cicer reticulatum*) and two cultivated chickpea species to freezing temperatures, [45] reported that chickpea cultivars did not survive freezing temperatures (-6°C) and died after 24 hours, with an average rating of 9.

On the basis of the results of the present study, Katran, a cold tolerance check cultivar, classified as moderately susceptible under freezing temperatures (-10°C), was used. Similar results were also reported by Mir *et al.* [7], who reported that two cold-tolerant cultivars (ICC 96029 and ICC 96030) did not perform well under field conditions.

Damage from freezing commonly occurs because ice forms within intercellular spaces. Increased membrane fluidity, osmotic adjustment and supercooling without ice nucleation have been reported as mechanisms associated with freezing tolerance at the cellular level. Antifreezing proteins and ice nucleators control the initial formation of ice [6].

All three mentioned freezing-tolerant genotypes, 217389, 218142 and 218923, as well as genotype numbers 217274, 218194, 217327, 218028, 217240 and 218195, which received a code of 5 for leaf damage and were classified as genotypes with high survival rates (SRs $\geq$ 70%) in the first-year field trial. Additionally, genotypes 218028, 218195, 217274 and 217327 were classified as genotypes with high survival rates (SR $\geq$ 90%) in the second-year field trial. This result further supports the findings of the field trials, particularly under the environmental conditions prevailing during the first year of the experiment, when more severe temperature stress was observed, with a cumulative 70 frost days and an absolute minimum temperature of -9°C .

Membrane stability as an indicator of cold/freezing tolerance

The results of cold stress evaluation on the basis of EL% and membrane injury estimation revealed that the mean value of EL% under controlled conditions was less than that under cold stress conditions. Similarly, Heidarvand *et al.* [46] and Mir *et al.* [7] reported less EL% in chickpea accessions under control conditions than under cold stress conditions.

The results revealed that genotypes 217379 (100%), 218166 (123%), 218554 (150%) and 218029 (150%) presented the greatest degree of membrane injury (Figure 8b), indicating their greater sensitivity to cold stress. In contrast, membrane injury was less than 6% and statistically nonsignificant in genotypes 37707, 217223, 217331, 217389, 217410, 217442, 217471, 217489, 218149 and 218195, indicating greater membrane stability of these genotypes under cold stress (Figure 8b). In accordance with these results, genotypes (IC 116783, ICC 15200 and AGBL-G-170004) with mean cold tolerance rates of three and estimated mean ELs of 21.26%, 21.58% and 21.94%, respectively, were identified as cold-tolerant genotypes [7].

Cold stress results in damage to membrane integrity, leading to solute leakage; hence, EL is widely used as a test for stress-induced injury to plant tissues. Wery *et al.* [47] considered membrane stability, an important mechanism of cold tolerance used as a physiological index to identify the magnitude of injury between different genotypes after plants are exposed to cold stress conditions in the field. A leakage score of more than 50% is considered lethal to plants. The greater the leakage of electrolytes is, the lower the tolerance of plants to cold stress [7].

In accordance with the results of the present study, an increase in EL at 4°C in chickpea genotypes was reported by other researchers [27, 48]. Less electrolyte leakage in chickpea accessions that are able to flower and set pods when subjected to cold stress conditions has also been reported [49]. Similarly, EL% was significantly greater in the leaves of chilling-sensitive chickpea genotypes than in those of tolerant chickpea genotypes [50].

The increase in EL% in the leaf tissues of cold-stressed plants was associated with increased oxidative stress [27, 51]. Chilling is reported to promote the formation of lipid peroxides in membranes, leading to their disorganization and hence leakage [52, 53].

On the basis of the results of the present study, the results of the membrane stability assessment confirmed the results of the field trials. A genotype with relatively high membrane stability was placed near or on top of the average line of the MRFR, as shown in Figure 2. However,

several exceptions have been reported; for example, genotypes 217379 and 218029, which are highly prone to membrane injury but have high cold/freezing tolerance under field conditions, were identified. Genotype 217389 was also classified as an intermediate genotype in terms of freezing tolerance under controlled conditions. Conversely, ILC533, a sensitive check line that has the highest cold/freezing sensitivity under field conditions, presented lower membrane injury than many other genotypes, which presented better results in field trials (Figures 2 and 3). This clearly demonstrates the diversity in genotypic responses in terms of the type, duration and intensity of stress.

Upon exposure to cold stress, a reduction in plasma membrane fluidity leads to increased membrane rigidity [54] and disruption of membrane structure and function. These changes in membrane properties can act as early signals that trigger the expression of cold-responsive genes and activate downstream physiological and molecular responses to cold stress. This highlights the importance of exploiting genetic diversity among genotypes to identify genes and favorable alleles involved in different components of cold tolerance and to pyramid these alleles in breeding programs to develop genotypes with enhanced and potentially more stable cold tolerance.

Conclusions

Many chickpea genetic resources have been collected and conserved worldwide with the aim of preserving the available diversity for posterity. The utilization of these resources is possible by selecting unique germplasms. The methods used to assess cold and freezing tolerance have several limitations and drawbacks. On the one hand, laboratory freezing methods may not reflect those experienced by plants in the field as other stresses imposed by overwintering, such as snow cover or ice heaving, have been omitted in these methods. On the other hand, field methods may fail to ensure the target range of low temperatures because of unpredictable severity as well as the inability to separate the effects of cold/freezing stress from those of other methods.

The findings of this study demonstrate that integrating field evaluation with controlled-condition assessment can effectively discriminate desi chickpea genotypes with different levels of cold/freezing tolerance.

Overall, the results revealed that significant variation in cold/freezing tolerance as well as agronomical traits exists among desi chickpea germplasms. The evaluation of cold/freezing tolerance in the field in two studies (2022--2023, in which the absolute minimum temperature reached -9°C with a cumulative 70 frost days), 2023--2024, in which the absolute minimum temperature reached -8°C with a cumulative 30 frost days), and cropping seasons, as well as the evaluation of freezing tolerance (-10°C) and cell membrane stability via EL% under controlled conditions, led to the identification of promising cold/freezing-tolerant desi chickpea genotypes.

On the basis of the estimation of different indices and multivariate analysis, out of 38 understudied genotypes, 16 (217240, 37707, 218992, 218170, 217450, 217379, 218029, 217251, 217242, 218923, 218194, 217327, 218195, 218767, 218142, 217471) contribute to cold/freezing tolerance as well as high yield performance in field trials. This highlights the

successful use of strict approaches during primary evaluations for the initial selection of genotypes.

Genotypes 217389, 218142 and 218923, with a freezing tolerance score of 3, identified as freezing tolerant under control conditions, were also classified as genotypes with high survival rates in both years of field trials. This result provides further confirmation of the field assessments. Although some freezing-tolerant genotypes, such as 218095 and 217389, presented relatively high membrane stability, this association was not observed in many of the other genotypes. Therefore, the membrane injury index may not be a reliable indicator for identifying cold/freezing-tolerant desi chickpea genotypes. This inconsistency may be attributed to variation in the underlying mechanisms employed by different genotypes to cope with cold and freezing stress. This issue requires further investigation at the cellular and molecular levels.

The results of the present study revealed the usefulness of GYT for distinguishing cold/freezing-tolerant desi chickpea genotypes and its optimal combination with yield performance as well as other agronomical traits, such as days to maturity, primary and secondary branch numbers, first pod height, plant canopy height, pod number per plant and the harvest index. On the basis of the results of the harvest index, plant canopy height or first pod height may serve as an index for identifying cold/freezing-tolerant desi chickpea genotypes with relatively high grain yield and proper for autumn planting.

Given the moderate to very high broad-sense heritability observed for cold tolerance, the cold- and freezing-tolerant genotypes identified in the present study could be valuable genetic resources for future chickpea breeding programs. These genotypes could also be used as parental materials for genetic mapping of cold tolerance and for transcriptomic analyses to identify DEGs involved in the response to cold and freezing stress.

The identified genotypes have been submitted for further evaluation in multilocation trials under different agroclimatic conditions in cold regions of Iran. These trials aimed to assess the stability of their performance for agricultural production under autumn planting conditions, as well as their resistance to *Ascochyta* blight.

Although several studies have suggested that the wild relatives *C. echinospermum* or *C. reticulatum*, the species of the primary gene pool that are crossable with the cultivar, are good sources of cold/freezing tolerance. However, the results of the current study revealed that chickpea cultivars, especially desi-type ones originating from Iran, are valuable resources for exploiting and determining cold/freezing stress tolerance. These results validate those of early studies on this issue by Sing *et al.* (11), who reported that Iranian germplasms are suitable resources for exploiting and identifying cold/freezing-tolerant germplasms.

On the basis of our information, the current research is limited in that it was performed on this subject via genetic resources conserved in the chickpea collection of the National Plant Gene Bank of Iran (NPGBI). On the basis of the results of the present study, desi chickpea cultivars originating from Iran are valuable resources for exploiting and identifying appropriate resilience genotypes on the basis of breeders' desire. Therefore, more benefits could arise from the increased exploitation of this collection and its use in the breeding and introgression of

cold-tolerant chickpea cultivars. The incorporation of cold tolerance in autumn-sown chickpea will lead to early flowering and maturity, a strategy that would allow the avoidance of terminal drought and high temperatures due to global warming and increase the reproductive period, leading to increased productivity.

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Author contributions

F.R. and M.P.; Supervision

M. P. and F. R.; Conceptualization and Methodology

M.P.; Selection of plant material

M. P. and Z. G.; Planting, data collection, and data curation.

M. P. and Z. G.; Data analysis

M. P. and Z. G.; Writing—original draft preparation

M. P. and F.R.; Writing—review and editing

Data availability

All data generated or analysed during current study are available from the corresponding author on reasonable request.

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