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Evaluation of chickpea (*Cicer arietinum* L.) genotypes for tolerance to Frost in controlled environment

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ABSTRACT: The study aimed to evaluate the frost tolerance variability of Ethiopian chickpea (*Cicer arietinum* L.) germplasm under controlled environment using growth chamber. A total of 72 genotypes were screened for frost tolerance using complete randomized design with two replications. The analysis of variance result indicated that there was a significant (P<0.01) difference amongst genotypes for plant height, number of foliage, number of primary branch, growth rate, and fresh biomass weight. Based on plant survival rate (SR), 31 (43.1%) genotypes scored above 0.8 values. Based on Freezing tolerance rate (FTR), 37(51.4%) and 31(43.1%) genotypes were rated at a score of 1 to 3 in freezing test 1 (T1) and freezing test 2 (T2), respectively. There was a strong negative correlation between fresh biomass yields with SR (-0.75** for T1 and -0.71** for T2 at p<0.01), while a strong positive correlation with FTR value (0.74** at p<0.01). Based on the combined result of FTR and SR scores, 26 genotypes were found to be frost-tolerant genotypes at a temperature level as low as -5^{o}C at seedling stage. Based on our findings, Ethiopian chickpea germplasm has a genetic potential for frost-tolerance traits for use in breeding programs.

Key words/phrases: Chickpea, Freezing test, Frost screening, Frost tolerant

INTRODUCTION

Plant genetic resources play a significant role in the variety development program by serving as a reservoir for enormous genes that confer tolerance to abiotic and biotic stresses and potential sources of gene for most important agronomic traits (Rao, 2004). The maintenance of a wide array of genetic pool for different crops is the main target for gene bank manager. Recognizant of this, the Ethiopian Biodiversity Institute (EBI) has been collecting and maintaining plant genetic resources in its genebank. And, extensive genetic characterization and evaluation of the germplasm for agronomic and quality traits are required to make it more useful to breeders and farmers (Castañeda-Álvarez *et al*., 2016).

Chickpea (*Cicer arietinum* L.) is currently the third largest food legume crop in Ethiopia in terms of area covered and production volume next to faba bean and haricot bean, occupying roughly 239,786.13 ha of land annually and producing 459,173,187 Kg with an average productivity of 2025 kg/ha for desi and 1682 kg/ha for kabuli type chickpea (CSA, 2019). Currently, chickpea is introduced to lowland areas using irrigation and also to select areas of the Southern Nation and Nationality People Region (Nigusie Girma *et al*., 2017) contributing to the steady increase of chickpea production. Furthermore, chickpea production could be expanded into highland locations (>2500masl) where frost is a typical occurrence. The highland constitutes 2/3rd of the total cultivated land in Ethiopia (Mulugeta Assefa *et al*., 2014) and chickpea production can be extended to these areas if frost-tolerant chickpea varieties are available to the farmers.

Chickpea is a cold sensitive legume crops and cold stress is the second most important limiting factor in its production (Sassenrath *et*

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al., 1990). Cold stress is classified as Chilling injury (0 °C to 15 °C) and freezing/frost injury (below -1.5 °C) (Croser *et al*., 2003; Jha *et al*., 2014), both of which have and overlapping effects on chickpea growth and production (Croser *et al*., 2003; Jha *et al*., 2014). Low field temperatures causes poor seed germination, poor crop stand establishment, chlorosis, wilting, necrosis of leaf tips, reduced plant height and branches, full leaf curling, and plant death (Croser *et al*., 2003; Kumar *et al*., 2010). Moreover, frost stress lowers leaf water status and chloroplast membrane stability, resulting in the loss of respiration and photosynthesis (Croser *et al*., 2003; Yadav, 2010).

Frost-tolerance is one of the most important pre-requisites to grow cool season legumes in frost prone areas. The degree of frost damages varies among genotypes due to the differences in frost-tolerance capacity of the genotypes. Evaluation of plant germplasm for frost-tolerance variability is very crucial to identify resistant genotypes. For example genetic variation of frost-tolerance has been reported for field pea seedlings (Bourion *et al*., 2003), chickpea (Kanouni *et al*., 2009; Nezami *et al*., 2012; Mir *et al*., 2019). However, there was no documented information regarding the potential of Ethiopian chickpea genotypes for frost-tolerance variability.

Two types of chickpea frost screening protocols; field screening under natural condition and under controlled environment using growth chamber have been used by various breeders. The natural field screening method is expensive and time consuming, there is unpredictable frost severity and irregular low temperature frequency (Maqbool *et al*., 2010), the lowest temperature is not controlled and there are large temporal and spatial variations in the field (Nezami *et al*., 2012). However, a controlled environment (using freezing chamber) screening method offers much more precise control of the timing and intensity of frost treatment (Wu *et al*., 2014). It is also inexpensive, quick and highly reproducible (Nezami *et al*., 2012). Therefore, the objective of the study was to screen Ethiopian chickpea genotypes for frost-tolerance variability in control environment using growth chamber and identify frost-tolerant genotypes to be used in chickpea breeding program.

MATERIAL AND METHODS

Plant materials

The study was conducted using 72 genotypes constituting 51 Ethiopian chickpea genotypes, 13 genotypes from the International Center for Agricultural Research in the Dry areas (ICARDA), and 13 improved chickpea varieties form Deber Zeit Agricultural Research Centers. The genotypes were selected based on the field performances of genotypes showing poor to better reaction to frost stresses at vegetative and grain filling period (Sintayehu Admas *et al*., 2021).

Experimental design

Ten seeds of each genotype were planted in pots with 20 cm internal diameter and 20 cm depth. The pots were filled with homogeneous soil mixture which was prepared by mixing the sub-surface (0-30cm) soil thoroughly collected from Debre Zeit Research Center chickpea farm. The seedlings were thinned to five plants per pot at four leaf stage. A complete randomized design with two replications was used. DAP (100 kg/ha) and other management practices were applied. Each morphological and physiological data were collected from all the five individual plants.

A modified frost screening protocol using freezing chamber designed by Nezami *et al*. (2012); Zhu *et al*. (2014), and Mugabe *et al*. (2019) were used. The plants were grown in green house for two weeks at Ethiopian Biodiversity Institute and moved to the controlled environment chamber (Snijders labs climate chambers) for five weeks. Seedlings were subjected to a gradual low temperature acclimation protocol for four weeks. Acclimation started at seven days with 7°C days/5°C nights, 11-h a photoperiod (PP) followed by 5°C days/2°C nights, 10-h PP for 7 days and then at 2°C days/0°C nights, 9-h PP for 14 days with 250 mmol m^2s^1 levels of irradiance. Subsequently, the frost treatment test took place for seven days under 5°C days/-2°C night, 10-h PP for 3 days, 5°C days/-3°C nights, 10-h PP for 2 days and finally 5°C days/-5°C nights, 10-h PP for 2 days. Finally, the pots were allowed to thaw overnight at 4°C and the plants were moved back to the green house for one week for scoring to conduct freezing test 1: to evaluate the re-growth potential of both the foliage and auxiliary buds of the genotypes and freezing test 2: to test the viability of the foliage using 1-9 scale as indicated below.

Data Collected

Plant Height (cm): Average canopy height of five representative plants taken before and after frost treatment.

Number of foliage leaf per plant: Average number of foliage leaf per plant taken from five representative plants before and after frost treatment.

Number of primary branches per plant: Average number of basal primary branches per plant taken from five representative plants before and after frost treatment.

Plant height growth rate: The ratio of the difference between plant height before and after frost stress to plant height after frost stress.

Number of foliage leaf growth rate: The ratio of the difference between number foliage leaf per plant before and after frost stress to number foliage leaf per plant.

Number of primary branches plant growth rate: The ratio of the difference between number of primary branches per plant before and after frost stress to number of primary branches per plant.

Fresh biomass yield (g/plant): Average fresh weight of five plants.

Freezing tolerance rate (FTR): Visual identification of viability of the foliage and foliage and auxiliary buds re-growth scored on 1-9 scale bases (Fiebelkorn, 2013 cited by Mugabe *et al*., 2019), where, 1=Plant completely green, 2= Plant with minimal freezing damage, 3= Plant at least 75% green, 4= Plant between 50 and 75% green tissue, 5= Plant 50% green, 6= Plant between 25 and 50% green tissue, 7= Plant 25% green, 8= Plant almost dead but has minimal green, and 9= Plant completely dead.

Plant survival rate (SR): Calculated by dividing the number of surviving plants after the frost period by the number of emerged plants after sowing was calculated (Heidarvand *et al*., 2011).

Statistical Analysis

The performances of genotypes were tested for significance by performing an analysis of variance (ANOVA) in a complete randomized design using R-4.1.0 statistical software (Thomas *et al*., 2013). Treatment mean comparison was performed for significance, using a Fisher's least significant difference (LSD) test at 5% probability using R software. Pearson correlation coefficients between variable was estimated and tested for significance using MINITAB release 14 statistical software (MINITAB, 1998).

RESULTS AND DISCUSSIONS

In Ethiopia, chickpea is commonly grown in areas having vertisols soil type with an altitude range of 1400 to 2300 meters above sea level (Geletu Bejiga *et al*. 1996). There is still an immense potential to introduce chickpea as a new crops in highland area (> 2300 masl). In highland area, however, the existence of frost stress limits crop production. So to bring chickpea as an alternative crop in this area; it requires the improvement of chickpea towards the capacity of chickpea for frost stress tolerance. This requires an extensive germplasm screening to identify frost tolerant genotypes. Studying frost tolerance and breeding for frost-tolerant chickpea varieties play a fundamental role in increasing chickpea production in frost prone areas. In the present study, chickpea genotypes were evaluated under controlled environment using growth chamber. The experiment has shown the response of 72 chickpea genotypes with respect to frost stress tolerance, which occurred during the two weeks old chickpea seedlings under controlled conditions.

The analysis of variance raveled significant differences at $P \leq 0.01$ for frost-tolerance traits variability amongst genotypes for plant height, number of foliage, number of primary branch, growth rate, and fresh biomass weight (Table 1). This indicated that the differences in the genotypes to the reaction of frost damage were variable, which is an indicator of the existence of variability amongst genotypes for frost tolerances.

Table 1. Analysis of variance and mean squares for tested traits grown under controlled environment at Ethiopian Biodiversity Institute, 2021.

Sources of Variation	Degree of freedom	Mean Squares								
		PLH BF	PLH AF	GR PLH	NF BF	NF AF	GR NF	NPB AF	GR NPB	FW
Genotypes	71	8.0**	55.78**	0.04**	2.22**	45.43**	0.06**	31.55**	0.27**	26.27**
Error	72	2.12	7.48	0.01	1.45	3.95	0.01	2.58	0.02	2.94

PLHBF=Plant height before frost treatment in cm, PLHAF= Plant height after frost treatment in cm, GRPLH= Growth rate of plant height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight in gm

Fisher's least significant difference (LSD) result indicated that the differences among the means of the genotypes for a given trait were significant ($P<0.05$). Wide mean ranges were observed for all the collected traits in the genotypes (Table 2). The LSD value and range values confirmed the presence of a variable response among genotypes for frost stress. Similarly Mir *et al.* (2018) reported presence of variability in chickpea germplasm for frost stress.

Table 2. Mean value of quantitative traits of 72 chickpea genotypes grown under controlled environment at Ethiopian Biodiversity Institute, 2021.

No	Acc	Types	Source	PLH BF	PLH AF	GR PLH	NF BF	NF AF	GR NF	NPB AF	GRN PB	FW	SR	FT1	FT2
1	207674	Desi	EBI	11.3	22.6	0.50	5.9	18.7	0.7	12.2	1.0	14.7	0.9	2	3
2	30350-B	Desi	EBI	10.6	21.1	0.49	5.4	14.8	0.6	7.4	1.0	10.5	1.0	2	3
3	41133-A	Desi	EBI	9.6	21.1	0.55	5.3	18.5	0.7	4.7	1.0	10.6	0.8	2	3
4	207173-B	Desi	EBI	13.1	25.3	0.48	5.4	17.5	0.7	10.4	1.0	13.6	1.0	1	1
5	207175-A	Desi	EBI	11.2	20.5	0.46	7.3	19.0	0.6	7.4	1.0	7.5	0.8	1	2
6	207766	Desi	EBI	11.9	22.5	0.47	6.6	20.2	0.7	14.7	1.0	12.0	0.8	1	2
7	209026-B	Desi	EBI	8	17.2	0.53	5.8	11.1	0.5	3.0	1.0	13.2	1.0	1	1
8	227152-B	Desi	EBI	10.3	19.3	0.47	5.2	16.7	0.7	7.3	1.0	10.9	0.8	1	2
9	41301-B	Desi	EBI	8.1	16.4	0.50	5.2	13.7	0.6	6.8	1.0	11.1	1.0	1	2
10	207746	Desi	EBI	7.5	14.5	0.48	5.5	8.0	0.3	1.5	1.0	10.7	1.0	2	3
11	Teketay	Desi	DZARC	8.3	16.1	0.48	6.2	17.5	0.7	11.2	1.0	11.1	1.0	1	1
12	Natoli	Desi	DZARC	5.0	9.7	0.48	5	8.6	0.4	0.8	0.5	10.2	1.0	2	3
13	Akaki	Desi	DZARC	9	19.9	0.55	5.7	13.5	0.6	4	1.0	11.6	1.0	1	1
14	9427	Kabuli	ICARDA	9.1	16.6	0.46	6	12.3	0.5	5.8	1.0	9.2	1.0	1	1
15	69420	Kabuli	ICARDA	7.1	17.2	0.59	5.5	9.9	0.5	0	0.0	11.9	1.0	1	1
16	Worku	Desi	DZARC	8.6	20.2	0.58	5.1	10.7	0.5	2.8	1.0	8.8	0.9	3	3
17	Mariye	Desi	DZARC	6.2	12.2	0.50	6.0	8.05	0.3	0	0.0	9.0	1.0	1	1
18	DBB	Desi	EBI	8.8	17.9	0.51	6.2	13.0	0.5	4.3	1.0	10.0	0.8	2	3
19	ENR	Desi	EBI	11.2	21.4	0.48	6.2	19.1	0.7	8.3	1.0	9.1	1.0	2	2
20	TEGR	Desi	EBI	9.9	20.8	0.52	6.2	18.1	0.7	10.0	1.0	9.1	1.0	2	3
21	30334-C	Desi	EBI	12.8	22.5	0.44	7.7	18.9	0.6	10.9	1.0	13.6	0.5	1	2
22	207648	Desi	EBI	12.2	19.1	0.36	6.3	19.1	0.7	16.0	1.0	13	1.0	1	1
23	207728-A	Desi	EBI	10.6	18.0	0.41	5.6	14.0	0.6	5.2	1.0	12.1	1.0	2	2
24	208988-A	Desi	EBI	12.1	19.9	0.39	6.4	15.5	0.8	12.4	1.0	14.6	1.0	1	1
25	Kutaye	Desi	DZARC	8.3	15	0.45	5.4	14.5	0.6	8.9	1.0	9.3	1.0	1	1
26	Yelbie	Kabuli	DZARC	8.4	13.8	0.39	5.9	8.9	0.3	5.6	1.0	9.8	1.0	2	3
27	Teji	Kabuli	DZARC	5.7	12.8	0.56	4.2	8.9	0.5	0.5	0.5	10.8	0.7	1	2
28	Mastewal	Desi	DZAR	8.9	18.3	0.52	6.4	12.5	0.5	3.5	1.0	12.8	0.7	1	2

			C												
29	Shahso	Kabuli	DZARC	8.1	19.2	0.58	5.5	15.0	0.7	6.9	1.0	9.4	0.6	1	1
30	30300-A	Desi	EBI	12.3	24.2	0.49	6.5	13.5	0.5	7.3	1.0	13.5	0.9	4	5
31	30309-A	Desi	EBI	8.1	17.8	0.26	6.1	13.8	0.6	7.0	1.0	5.5	0.6	6	6
32	41075-C	Desi	EBI	11.9	20.1	0.41	6.1	12.0	0.5	11.4	1.0	9.4	1.0	4	4
33	41078-B	Desi	EBI	10.3	20.6	0.50	5.6	16.5	0.7	8.8	1.0	9.1	0.6	3	5
34	41094-C	Desi	EBI	7.3	14.0	0.47	5.1	15..	0.7	6.8	1.0	7.9	0.8	4	5
35	41153-A	Desi	EBI	11.2	22.4	0.49	5.1	16.6	0.7	5.9	1.0	12.0	0.7	3	4
36	41282-B	Desi	EBI	11.4	20.1	0.44	6.0	16.9	0.6	8.2	1.0	13.8	0.7	3	3
37	41323-A	Desi	EBI	10.0	19.9	0.50	5.5	17.0	0.7	9.1	1.0	11.2	0.7	3	5
38	207167-A	Desi	EBI	11.8	20.9	0.43	6.9	18.9	0.6	6.5	1.0	10.3	0.8	4	4
39	207640	Desi	EBI	10.4	14.2	0.27	5.1	12.7	0.6	3.8	1.0	10.3	0.7	5	6
40	207652	Desi	EBI	9.5	17.4	0.46	4.5	16.0	0.7	6.7	1.0	9.2	0.9	4	5
41	207670	Desi	EBI	11.4	21.4	0.42	8.0	18.9	0.6	13.5	1.0	12.1	0.7	5	6
42	209026-A	Desi	EBI	10.1	19	0.47	5.9	16.3	0.6	6.8	1.0	9.1	0.8	4	5
43	212477-A	Desi	EBI	11.0	17.7	0.38	5.8	12.4	0.5	3.9	1.0	12.5	0.6	1	2
44	241800-A	Desi	EBI	9.9	18.9	0.48	5.6	15.8	0.7	5.5	1.0	9.8	0.9	3	5
45	30339-A	Desi	EBI	10.1	19.3	0.48	6.3	13.1	0.5	5.8	1.0	10.1	1.0	4	5
46	Minjar	Desi	DZARC	8.8	17.5	0.50	4.3	12.4	0.7	4.3	1.0	11.2	0.6	3	4
47	140941	Kabuli	ICARDA	10.3	16.6	0.38	6.2	14.2	0.6	9.3	1.0	11.7	0.7	4	4
48	141693	Kabuli	ICARDA	8.6	18.7	0.54	6.5	8.2	0.2	0	0.0	1.5	0.5	8	8
49	125187	Kabuli	ICARDA	8.3	14.4	0.71	5.1	10.4	0.5	2.9	1.0	3.7	0.5	8	8
50	Dubie	Desi	DZARC	8.1	15.7	0.48	3.9	17.3	0.9	4.2	1.0	10.8	0.6	6	6
51	140294	Kabuli	ICARDA	9.1	16.9	0.47	4.5	7.1	0.4	0	0.0	8.2	0.6	3	5
52	132663	Kabuli	ICARDA	6.6	14.1	0.53	5.3	11.0	0.5	3.6	1.0	5.8	0.8	4	4
53	Kasech	Kabuli	DZARC	10.5	0	0	2.2	0	0	0	0.0	0	0.0	9	9
54	16341-A	Desi	EBI	12.3	24.1	0.49	5.9	16.8	0.7	10.3	1.0	13.2	0.7	5	6
55	41081-A	Desi	EBI	9.2	16.9	0.46	5.1	11.7	0.6	6.1	1.0	5.2	0.5	7	7
56	207608	Desi	EBI	8.3	17.4	0.52	3.9	10.1	0.6	0	0.0	5.1	0.4	8	9
57	207638	Desi	EBI	9.3	19.8	0.53	4.4	14.4	0.8	6.8	1.0	10.4	0.4	7	7
58	207649-A	Desi	EBI	6.8	15	0.56	5.0	7.7	0.3	2.1	1.0	8.0	0.5	6	7
59	207668	Desi	EBI	9.7	21.8	0.56	6.1	13.5	0.6	3.7	1.0	10.1	0.7	5	5
60	209008-A	Desi	EBI	9.2	18	0.49	5.8	16.6	0.7	9.4	1.0	11.4	0.6	5	5
61	209016-B	Desi	EBI	11.3	20.7	0.46	6.0	15.6	0.6	5.9	1.0	9.5	0.6	5	7
62	212688-C	Desi	EBI	10	23.2	0.57	5.4	14.4	0.6	4.7	1.0	10.4	0.7	5	6
63	215190-A	Desi	EBI	9.2	19.1	0.53	5.1	12.0	0.6	5.8	1.0	12	0.6	6	7
64	237054-B	Desi	EBI	6.4	16.9	0.62	4.9	11.5	0.6	2.8	1.0	6.9	0.4	7	7
65	Dimtu	Desi	DZARC	6.5	16.6	0.61	5.1	11.5	0.6	0.7	0.5	9.6	0.5	6	6
66	141720	Kabuli	ICARDA	11.5	21.4	0.46	6.2	19.0	0.7	9.1	1.0	3.2	0.3	8	8
67	75095	Kabuli	ICARDA	5.6	13.4	0.58	4.3	6.8	0.4	0.0	0.0	6.2	0.0	9	9
68	209026-A	Desi	EBI	10	18.9	0.47	5.1	13.0	0.6	4.0	1.0	4.8	0.4	7	8
69	10163	Kabuli	ICARDA	3.7	10.4	0.65	3.0	5.6	0.5	0.0	0.0	1.5	0.4	8	8
70	8191	Kabuli	ICARDA	8.2	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	9	9
71	139930	Kabuli	ICARDA	6.1	0.0	0.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0	9	9
72	73221	Kabuli	ICARDA	8.2	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	9	9
	Mean			9.3	17.3	0.5	5.4	13.1	0.5	5.6	0.8	9.2	-	-	-
	±SE			1.5	2.8	0.1	1.2	2.0	0.1	1.6	0.2	1.9	-	-	-
	LSD (5%)			2.9	5.5	0.2	2.4	3.9	0.2	3.0	0.3	3.8	-	-	-
	Range			3.7-13.1	0-25.3	0-0.71	1.7-8	0-20.2	0-0.8	0-16.	0-1	0-14.7	0-1.0	1-9	1-9

PLHBF=Plant height before frost treatment, PLHAF= Plant height after frost treatment, GRPLH= Growth rate of plant height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost *treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight, SR=Survival rate, FT1 =Freezing test 1, and FT2=Freezing test 2. SE=pooled standard deviation, LSD=Least square difference, EBI=Ethiopian Biodiversity Institute, DZAR=Deber Zeit Agricultural Research Centers, ICARDA=International Center for Agricultural Research in the Dry areas*

The reaction of genotypes to frost as indicated by plant survival rate (SR) is given in Table 3. Thirty one genotypes (43.1%) rated more than 0.8 score, while the remaining genotypes rated less than 0.8 score (56.9%). Five genotypes scored 0 which means that they were killed by frost stress since it had poor reaction to frost stress. SR and FTR results showed that differences in the levels of frost tolerance between different genotypes were highly variable. Based on FTR and SR scores most genotypes were found to exhibit moderate frost tolerance at a temperature of -5°C. A plant survival score was used as an index to describe genotypes tolerance to low temperature (Heidarvand *et al.*, 2011). The susceptible four genotypes were killed at -2°C, the remaining genotypes had shown variable number of plant deaths which indicates the different capacity of genotypes for frost reactions.

Table 3. Plant survival rate (SR) of 72 chickpea genotypes grown under controlled environment at Ethiopian Biodiversity Institute, 2021.

No	SR Rating	No of genotypes
1	Δ0.8	31 (43.1 %)
2	Δ0.6 to <0.8	22 (30.6 %)
3	Δ0.4 to <0.6	9 (12.5 %)
4	Δ0.2 to <0.4	5 (6.9 %)
5	<0.2	5 (6.9 %)
Total		72

The result of foliage and auxiliary buds re-growth (Freezing test 1), and foliage viability (Freezing test 2) is indicated in Table 4. The majority of the genotypes showed recovery from frost damage. Four genotypes did not recover because they were killed by frost. The remaining genotypes recovered with low to high rate (Fig. 1). The records of leaf damage of the frost-susceptible genotypes showed severely damaged genotypes and all plant leaves died, while in resistant genotypes the leaf damage were nil to medium level (Fig. 1). Although the foliage of these genotypes had injured foliage following a frost, re-growth occurred from auxiliary buds at the stem (Fig. 2). The scores were done visually using freezing tolerance rate (FTR). The FTR scores were taken at one week after the end of the frost treatments. Thirty seven (51.4%) and 31 (43.1%) genotypes showed no or little leaf damage due to frost injury for freezing test 1 and freezing test 2 respectively. The remaining genotypes, 35 (48.6%) and 41 (56.9%) genotypes scored from 4 to 9 at freezing test 1 and freezing test 2, respectively. Theses genotypes were moderately frost-tolerant to highly frost-susceptible genotypes.

Table 4. Freezing tolerance rate (FTR) of 72 chickpea genotypes grown under controlled environment at Ethiopian Biodiversity Institute, 2021.

No	FTR Rating	No. of genotypes (Freezing Test 1)	No. of genotypes (Freezing Test 2)
1	1	19 (26.4%)	11 (15.3%)
2	2	10 (13.9%)	10 (13.9%)
3	3	8 (11.1%)	10 (13.9%)
Subtotal		**37 (51.4%)**	**31 (43.1%)**
4	4	9 (12.5%)	6 (8.3%)
5	5	7 (9.7%)	11 (15.3%)
6	6	5 (6.9%)	7 (9.7%)
7	7	4 (5.6%)	6 (8.3%)
8	8	5 (6.9%)	5 (6.9%)
9	9	5 (6.9%)	6 (8.3%)
Subtotal		**35 (48.6%)**	**41 (56.9%)**
Total		**72**	**72**

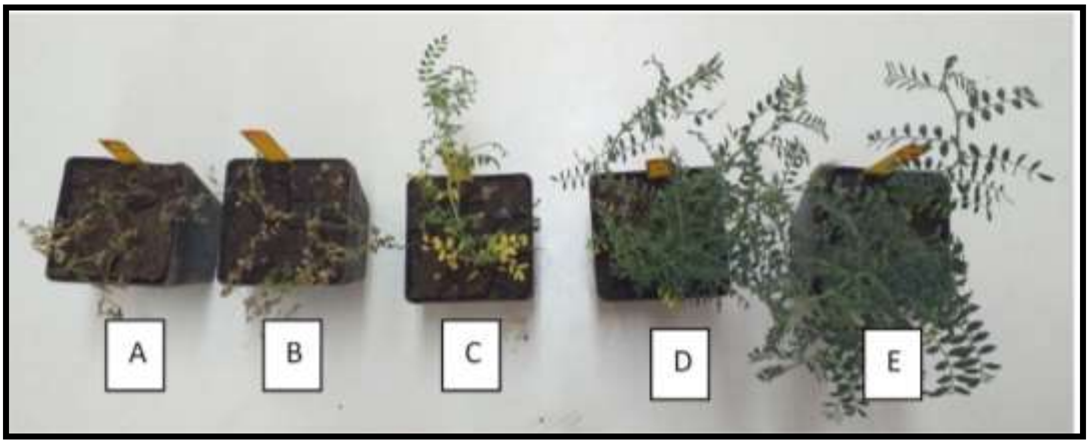

Figure 1. The reactions of genotypes to frost injury [A (139930) and B (73221) highly frost-susceptible genotypes, C (30339-A) moderately frost-tolerant genotypes, D (209026-A) and E (Teketay) frost-tolerant genotypes].

Figure 2. The variable potential of genotypes to the re-growth of the foliage and auxiliary buds one week after the end frost treatments [A (973221), B (141693), and C (207649-A) genotypes did not show foliage and auxiliary buds re-growth, D (Minjar) genotypes had shown foliage re-growth, E (Akaki) and F (41282-B) genotypes had shown foliage and auxiliary buds re-growth. The arrow indicates the growing of primary branches.

A rating scale of 1-9 has been used for measuring frost stress injury during early vegetative stage or seedling stage in earlier studies (Singh *et al*., 1989). The score was done by visual observation of the viability of the foliage, and buds re-growth of foliage and auxiliary. The susceptible genotypes were not showed foliage and auxiliary bud re-growth at all, in addition the percent of damage on foliage and auxiliary buds were severe. However, the frost-tolerant genotypes were gave better reaction to frost stress; moderate to high foliage and auxiliary bud re-growth rate were observed. Freezing and/or chilling range temperatures cause poor establishment, reduced vigor resulting in stunted seedlings and retarding plant growth and, in extreme cases, may lead to plant death (Croser *et al* 2003; Maphosa *et al*., 2020). However, an expected result was observed for 19 genotypes (Table 2, genotype listed from no 54 to 72) in which they showed better growth rate of plant height, foliage leaf and primary branch, while their reaction to frost stress were poor with FTR score of 5 and above. This happened because these genotypes had performed well during a frost treatment of -2°C and -3°C, however when frost treatment temperature continued to drop at a level to -5°C, then, these genotypes could not withstand the frost stress and whole plant death started which were manifested a week after the end of frost treatment.

The phenotypic association of agronomic and frost tolerance related traits were analyzed based on the mean values of the recorded traits of all genotypes and the result is given in Table 5. SR and FTR scores were strongly correlated with plant height, number of foliage, number of primary branch, growth rate, and fresh biomass weight at p<0.01. FTR score showed a negative strong correlation between the traits considered and SR score, while SR score showed a positive strong correlation between the recorded traits and FTR value. A strong positive correlation between two traits means that increasing one trait would be accompanied by an increasing in the other trait also. But, if the correlation is negative, increasing one trait would result in the reduction of the other. Such types of traits are governed by a pleiotropic effect of genes or linkage of genes controlling the inheritance of two or more characters (Dabholkor, 1992). Similar findings were reported by Mugabe *et al.* (2019).

Table 5. Phenotypic Pearson's correlation matrix for 11 traits of 72 chickpea grown under controlled environment at Ethiopian Biodiversity Institute, 2021.

Traits	PLH AF	GR PLH	NF BF	NF AF	GR NF	NPB AF	GR NPB	FW	SR	Test 1	Test 2
PLHBF	0.59**	-0.12*	0.51**	0.62**	0.41**	0.71**	0.50**	0.44**	0.29**	-0.26**	-0.22**
PLHAF		0.65**	0.68**	0.84**	0.79**	0.60**	0.66**	0.69**	0.56**	-0.45**	-0.42**
GRPLH			0.31**	0.46**	0.59**	0.08ns	0.34**	0.41**	0.41**	-0.31**	-0.29**
NFBF				0.68**	0.42**	0.61**	0.55**	0.56**	0.61**	-0.52**	-0.51**
NFAF					0.89**	0.80**	0.75**	0.66**	0.57**	-0.51**	-0.48**
GRNF						0.65**	0.70**	0.62**	0.47**	-0.4**	-0.40**
NPBAF							0.67**	0.55**	0.47**	-0.41**	-0.39**
GRNPB								0.60**	0.54**	-0.45**	-0.43**
FW									0.74**	-0.75**	-0.71**
SR										-0.87**	-0.85**
Test 1											0.98**

PLHBF=Plant height before frost treatment, PLHAF= Plant height after frost treatment, GRPLH= Growth rate of plant height during frost treatment, NFBF=Number of foliage before frost treatment, NFAF=Number of foliage after frost treatment, GRNF=Growth of number of foliage during frost treatment, NPBAF=Number of primary branch after frost treatment, GRNPB=Growth rate of number of primary branch during frost treatment, FW=Fresh weight, SR=Survival rate, Test 2=Freezing test 2, and Test 1=Freezing test 1

As a conclusion, frost tolerant screening of chickpea seedlings in controlled environment using growth chamber has enabled the identification of frost tolerant genotypes at an early growth stage. The frost-tolerant genotypes were selected based on SR and FTR values (Freezing test 1 and freezing test 2). Genotypes that were consistently rated as frost-tolerant genotypes in both indices (SR value of ≥ 0.8 and FTR score of 1 to 3) were selected. Twenty six chickpea genotypes (Table 2, genotypes listed from no. 1 to 26) were identified as frost-tolerant genotypes tested at seedling stage which can withstand a temperature as low as -5°C. Based on these findings, Ethiopian chickpea landraces have genetic potential for frost resistance traits. It is recommended that these genotypes can be used for future frost-tolerant cultivar development program through implementing multi-locations and multi-year field trails to test the frost tolerance adaptation to a more wide range of chickpea growing environments.

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