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Evaluation of Ethiopian chickpea (*Cicer arietinum* L.) genotypes for frost tolerance

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Abstract: Frost stress is one of the most significant abiotic factors affecting chickpea (*Cicer arietinum* L.) production in the Ethiopian highlands. To investigate the frost tolerance of chickpea, 673 genotypes were characterized using an augmented design at Bakelo, Debre Berhan, Ethiopia for two years. A significant ($p < 0.01$) variability amongst genotypes was recorded for all agronomic traits considered. A considerable number of accessions better performing over the frost susceptible genotypes were identified for agronomic traits. Stem/leaf pigmented genotypes showed a better reaction for frost stress than non-pigmented genotypes. The majority of black seeded chickpea adapted well under frost stress when compared to with brown and white seeded genotypes. According to the freezing tolerance rate (FTR) and plant survival rate (SR), 83 (12.3 %) and 85 (12.6 %) genotypes were identified as frost tolerant. There was a strong correlation ($p < 0.01$) in grain yield with FTR, SR, seed shriveling score, stem/leaf pigmentation and seed color. Based on our findings, Ethiopian chickpea landraces has a good genetic potential for frost resistance traits for use in breeding programs.

Key words: chickpea; Ethiopian landraces; frost survival rate; frost tolerance; germplasm characterization

Ovrednotenje etiopskih genotipov čičerke (*Cicer arietinum* L.) za toleranco na mraz

Izvleček: Mrazni stres je eden izmed najznačilnejših abiotskih dejavnikov, ki vpliva na pridelavo čičerke (*Cicer arietinum* L.) v etiopskem višavju. Za preučevanje tolerance na mraz je bilo v izboljšanem poskusu analiziranih 673 genotipov čičerke v Debre Birhan, Etiopija, v obdobju dveh let. Med genotipi je bila ugotovljena značilna variabilnost ($p < 0{,}01$) za vse preučevane agronomske lastnosti. Prepoznano je bilo znatno število akcesij, ki so se izkazale boljše v preučevanih agronomskih lastnostih kot tiste občutljive na mraz. Genotipi z obarvanimi stebli ali listi so se boljše odzvali na mrazni stres kot neobarvani. Večina genotipov čičerke s črnimi semeni je bila bolje prilagojena na mrazni stres v primerjavi s tistimi z rjavimi ali belimi semeni. Glede na toleranco na mraz (FTR) in preživetje rastlin (SR), je bilo 83 (12,3 %) in 85 (12,6 %) genotipov na mraz toleratnih. Ugotovljena je bila močna povezava ($p < 0{,}01$) med pridelkom semena in FTR, SR, nagubanostjo semena, obarvanostjo stebla in listov ter barvo semena. Na osnovi teh ugotovitev imajo etiopske tradicionalne sorte čičerke dober genetski potencial za odpornost na mraz in so lahko uporabne v žlahtniteljskih programih.

Ključne besede: čičerka; tradicionalne etiopske sorte; lastnosti tolerance na mraz; ovrednotenje genotipov

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) cultivation and utilization are profoundly notable within Ethiopian culture and produced by smallholder farmers under rainfed condition (Ferede et al., 2018). The cultivation is so profound that chickpea production in Ethiopia is one of the most widespread legume in terms of both area and volume. Across Ethiopia chickpea cultivation occupies ~1,620,497.30 hectares of land annually with an estimated production of 30,113,480570 kg (CSA, 2019). Both the land dedicated to chickpea production and the volume of production itself has been increasing over the last decade in Ethiopia (Fikre & Bekele, 2020; Fikre et al., 2018). Ethiopia is thus the largest producer, consumer, and exporter of chickpea in Africa, and is among the top ten most vital chickpea producers in the world (FAOSTAT, 2020). Chickpea production is suited to areas having vertisol-dominated soil with an altitudinal range of 1400 to 2300 meters above see level (Bejiga et al., 1996). Nevertheless, it is cultivated across a wide selection of zone (Fikre et al., 2018). Moreover, Ethiopia is considered to be the second greatest diversity hotspot of chickpea amongst major chickpea growing countries (Van der Maesen, 1987). Taking into consideration both immense variability among the chickpea germplasm and many agroecological zones as well as the increased demand for animal feed and processed foods (Fikre et al., 2020; Muoni et al., 2019; Shiferaw &Hailemariam, 2007), Ethiopia features great potential to expand chickpea production within the highland areas if the chickpea varieties are resistance to frost stress.

Chickpea is important for Ethiopian highland cultivation and is preferably sown in early- to mid-September. Previously, mid-August was considered the appropriate sowing date, but due to the "*belg*" rainy season, chickpea cultivation was heavily impacted by root rot. Root rot issues can be avoided by planting in mid-September, leading to higher yields. However, the later sowing date presents a new issue, due to the elevation of highlands, which is frost stress. The frost stress takes place late in the podding and flowering stages. Frost stress during these stages causes issues such as flower abortion, poor pod set, and impaired pod filling, leading to a drastic reduction in yield and quality (Croser et al., 2003). These stressors can be classified as chilling (0 °C to 12 °C) or freezing/frost (< 0 °C) temperatures (Gogoi et al., 2018; Toker et al., 2007). Moreover, temperatures lower than 10 °C at flowering can reduce grain yield by 15–20 % (Chaturvedi et al., 2009). Therefore, the need for improving frost-tolerance in chickpea has become evident which requires characterization of chickpea germplasm for frost tolerance.

Determining the nature of genetic diversity and variability existing among chickpea genotypes for frost resistance is mandatory to identify promising genotypes that are productive in Ethiopian highlands with late sowing dates. However, few studies have been conducted so far in this regard. Hence, research is needed to further understand the optimal utilization of landraces as sources of novel traits for frost resistant chickpea variety development. Therefore, the aim of this research is to identify chickpea genotypes that are both highly productive and frost resistant through use of field screening of genotypes for frost-tolerance. The long-term goal is to establish highly productive and frost tolerant chickpea varieties supporting Ethiopian highland farmers by enhancing food security and improving rural livelihoods.

2 MATERIAL AND METHODS

2.1 EXPERIMENT SITE

The experiment was conducted at Bakelo, Debre Berhan Agricultural Research Center experimental site (Debre Berhan, Ethiopia) for two consecutive growing seasons (2018/19 and 2019/20). The experimental site is located 147 km away from Addis Ababa at a N 09°41'42" latitude and E 39°37'20" longitude. Its altitude is 2,837 meter above sea level and receives an annual mean precipitation of 965.25 mm. The temperature ranges from 6.5 °C to 20.1 °C with mean annual temperature of 13.3 °C. The dominant soil type of Bakelo is black vertisol. The daily minimum and maximum temperature values are indicated in Fig 1.

2.2 PLANT MATERIALS

A total of 673 genotypes (559 Ethiopian genotypes from the Ethiopian Biodiversity Institute (EBI), 83 elite frost resistant genotypes from the International Center for Agricultural Research in the Dry Areas (ICARDA), three susceptible local checks and 28 improved chickpea varieties released from Ethiopian Agricultural Research Centers were screened for their tolerance against frost stress under field condition at Bakelo, Debre Brehan, Ethiopia, which is a frost prone area (see Supplementary Table S1 for further details) using freezing tolerance rate, plant survival rate and other frost resistant-related agronomic traits. The geographical origin of the Ethiopian chickpea germplasm used in the study is indicated in Fig. 2.

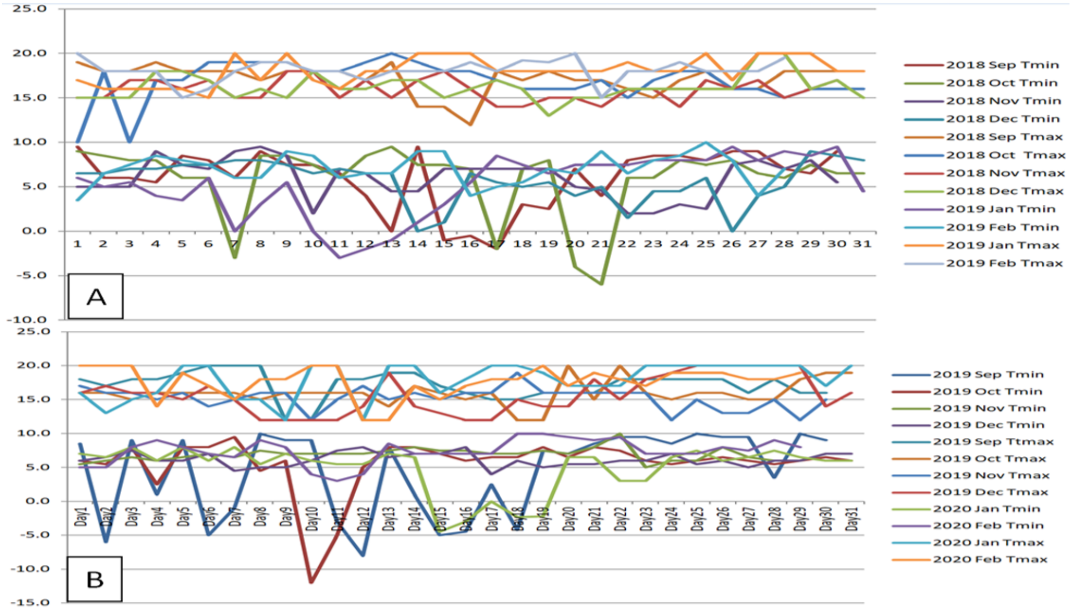

Figure 1: Daily maximum and minimum temperature of Bakelo, Debre Berhan during 2018/2019 (A) to 2019/2020 (B) growing seasons (Source: Debre Berhan Agricultural Research Center)

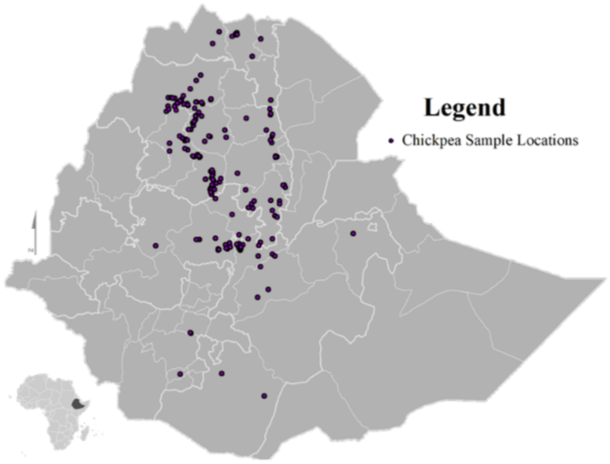

Figure 2: Map showing the geographical distribution of Ethiopian chickpea germplasm

2.3 EXPERIMENTAL DESIGN

Augmented design without replication was used. Each genotype was sown in two rows with 3 m row length and 0.2 m spacing between rows and 0.1 m between plants. Diammonium phosphate fertilizer (100 kg ha^{-1}) and other appropriate management practices were applied. Five individual plants were tagged randomly from each genotype per plot and they were used for morphological data collection. Recording agronomic characteristics were conducted following the procedure described by chickpea descriptor (IBPGR, ICRISAT and ICARDA 1993).

2.4 DATA COLLECTED

Qualitative and quantitative morphological traits were recorded as per described in Table 1.

Table 1: List of qualitative and quantitative characters recorded, their codes and descriptions

Characters	Description
Qualitative traits	
Stem/Foliage Pigmentation (SLM)	0 = No Anthocyanin, 1 = Low Anthocyanin 2 = Medium Anthocyanin, 3 = High Anthocyanin
Seed Color (SC)	1 = Black, 2 = Brown, 3 = White
Flower Color (FC)	0 = White, 1 = Pink
Quantitative traits	
Plant Height (cm) (PLH)	Average canopy height of five representative plants taken at maturity stage
Days to 50 % Flowering (DTF)	Number of days from sowing until 50 % of the plants have started to flower
Days to 50 % Podding (DTP)	Number of days from sowing until 50 % of the plants have started to podding
Days to 90 % Maturity (DTM)	Number of days from sowing until 90 % of the pods have matured and turned yellow
Number of Primary Branches (NPB)	Average number of basal primary branches per plant taken from five representative plants
Number Secondary Branches (NSB)	Average number of secondary branches per plant taken from five representative plants
Number of Fertile Pods per Plant (NIPPP)	Average number of fertile pods taken from five representative plants taken at maturity stage
Number of Infertile Pods per Plant (NIPPP)	Average number of infertile pods taken from five representative plants taken at maturity stage
Thousand Seed Mass (TSM)	Thousand seeds were counted and weighted at 12 % moisture content on a 0.1 g sensitive balance in milligram
Grain Yield (GY in kg ha^{-1})	Dried mass (kg) of seed per plot at 12 % moisture content
*Freezing tolerance rate (FTR)	Scored on 1-9 scale bases (Singh et al., 1989): where, 1 = No visible symptoms of damage; 2 = Highly tolerant, up to 10 % leaflets show damage; 3 = Tolerant, 11-20 % leaflets show damage; 4 = Moderately tolerant, 21-30 % leaflets and up to 20 % branches show withering and drying, but no killing; 5 = Intermediate, 41-60 % of leaflets and 21-40 % branches show withering and drying, up to 5 % plant killing; 6 = Moderately susceptible, 61-80 % leaflets and from 41-60 % branches show withering and drying, 6-25 % plant killing; 7 = Susceptible, 81-99 % leaflets and 41-80 % branches show withering and drying, 26-50 % plant killing; 8 = Highly susceptible, 100 % leaflets and 81-99 % branches show withering and drying, 51-99 % plant killing; and 9 = 100 % plant killing
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)
Seed shriveling score (SSS)	Visual measurement and estimating the kernel's condition (1 = plump, 3 = intermediate and 5 = shriveled

*= Frost score was recorded when susceptible checks showed sign for frost damages or completely died.

2.5 DATA ANALYSIS

The collected data for each trait were subjected to statistical analysis of variance using augmentedRCBD R Packages version 0.1.3 (Aravind et al., 2020). The analysis helps us to partition the variance into different sources (phenotypic, genotypic and environmental variance) and genetic parametrs to see if the difference among genotypes is statistically significant or not for each trait considered (Singh & Chaudhary, 1977). Pearson correlation coefficients between variable was estimated and tested for significance using MINTAB 10 statistical package (Wild, 2005)

3 RESULTS AND DISCUSSION

The performances of the chickpea genotypes in response to frost stress were assessed in natural condition and the results obtained are discussed.

3.1 THE EFFECT OF FROST ON AGRONOMIC TRAITS

ANOVA was performed for the two seasons separately because the intensity of frost stress was different for both years. There was a significant difference ($p < 0.01$) among genotypes for plant canopy height, number of primary branches, number of secondary branches, fertile pods per plant, infertile pods per plant, days to 50 % flowering, days to 50 % podding, days to 90 % maturity, thousand seed mass, and grain yield (Table 2) in 2018/2019 and 2019/2020 growing seasons. These differences in performance indicate the existences of variability among genotypes for frost tolerance. Similar finding was reported by Mir et al. (2019).

Based on Fisher's least significant difference (LSD) result indicated that there was a significant difference ($p < 0.05$) among genotypes for the mean value of agronomic traits examined in this study. A wide range value of the means was recorded for the traits recorded. The LSD means and range of values of the traits for chickpea genotypes examined is presented in Supplementary Tables S2 and S3 for further details. The LSD means value differences and the mean rage value of the traits further confirms the existence of variable responses to frost stress among genotypes. The responses of genotypes to the effect of frost stresses at each crop stage are discussed below because the genotypes responses to the frost damage were variable at each stage.

3.1.1 Seedling and vegetative stage

The frost stress occurred in both seasons and genotypes had shown uniform germination and seedling establishment (Fig 3A). The lowest temperature recorded during this stage was -2.0 °C in Sept 2018 and -8.0 in Sept 2019 growing seasons. All genotypes did not show any symptoms or damage in response to frost stress, which means that these genotypes had shown good tolerance to frost stress at seed germination and seedling development stages. However, most authors agreed that germination percentage and seedling development are sensitive to frost stress which results in poor crop stand establishment, and reduced seedling vigor with stunted seedlings (Croser et al., 2003; Maphosa et al., 2020; Srinivasan et al., 1998). During the vegetative stage, 43 (6.4 %) genotypes (One improved variety, one EBI genotypes and 54 ICARDA genotypes) were killed by frost (Fig 3B) in both growing seasons. The list of genotypes killed by frost is indicated in the Supplementary Table S4. Theses genotypes were identified as a highly susceptible to frost stress because they could not resist the frost stress when the minimum temperature of -6.0 °C and -12 °C were recorded in Oct 2018 and Oct 2019, respectively. These genotypes showed poor growth development, wilting, chlorosis, necrosis and finally death of the whole plant, which was the manifestation of frost injury. Similar observations were reported by Croser et al. (2003) and Mahajan & Tuteja (2005). The remaining genotypes had shown medium to good reactions to frost stress at vegetative stage because the impact of frost stress at this stage was minimal in both growing seasons.

3.1.2 Number of branches and plant height

The number of primary and secondary branches has been significantly affected by frost in both seasons where a wide range was recorded. The range of number of primary branches was 0 to 16.1 in 2018/2019 and 0 to 27.2 in 2019/2020 growing season and for number of secondary branches it was 0 to 25.6 in 2018 and 0 to 46.5 in 2019. The majority of the accession scored below five for primary and secondary branch in both growing seasons. However, 69 (10.3 %) and 71 (10.6 %) genotypes produced better number of primary branch (> 7) in 2018/2019 and 2019/2020 growing seasons, respectively. The response of genotypes to the effect of frost stress for plant height development was variable. A wide range of plant height was observed in both cropping seasons (20.3 to 58 cm in 2018/2019 and 17.2 to 57 cm in 2019/2020). One hundred two (15.2 %) and 89 (13.2 %) genotypes had a record of less than 35 cm plant height in 2018/2019 and 2019/2020 cropping season, respectively. Genotypes 132663 (58 cm) and 140294 (57.04 cm) had shown better plant height. In this experiment, most genotypes gave good positive reaction for plant height to the frost effect though frost significantly reduced plant height. This is probability because of the duration of time that frost occurred is not sufficient to have a negative impact to the plant development.

3.1.3 Reproductive stages

Seventeen genotypes (2.5 %) (Seven EBI genotypes and 10 from ICARDA) were killed by frost stress during reproductive stages (Fig 3C and 3D). Moreover, the effect of frost was clearly examined in the delay of number of days to flower, pod and mature in the remaining genotypes with different degree. The range of 47.7 to 87.54, 54.2 to 89.6 and 118.7 to 160 days was recorded for days to flower, days to pod and mature for 2018/2019 growing season, respectively, while 48 to 77.7, 55 to 99.6 and 99.9

to 171.2 days for 2019/2020 cropping season, respectively. The range of fertile pods per plant was 0 to 237.5 and 0 to 162.7 for 2018/12019 and 2019/2020 cropping seasons, respectively. The range of infertile pods per plant was 0 to 77.3 and 0 to 116 for 2018/2019 and 2019/2020 cropping seasons, respectively. The genotypes 227152-A (237.5) and 41301-B (162.7) produced the highest number of fertile pods in 2018/2019 and 2019/2020 cropping seasons, respectively (Fig 3F). The minimum temperature recordered during reparative stage especially at flowering and podding stages was below 5 °C in both seasons (Fig 1) which caused flower abortion and pod dropping for genotypes having poor response to frost stress. These frost symptoms were observed in most frost susceptible genotypes and they produced either empty pods or pods containing small shriveled seeds. Similar observation was reported by Gogoi et al. (2018) stating that temperature falls below 15 °C causes flower and pod abortions. Various authors agreed that the reproductive stage is more susceptible to frost stress than seedling stages because frost stress negatively affects pollen fertility, pod set, number of aborted flowers, total number of pods per plant, seed number, size and shape, rate and duration of seed filling which consequently reduced biomass and grain yield (Berger et al., 2012; Croser et al., 2003; Gogoi et al., 2018; Kumar et al., 2010; Nayyar et al., 2007; Srinivasan et al., 1999). Low temperature stress during reproductive development is responsible for the induction of flower abscission, pollen sterility, pollen tube distortion, ovule abortion and reduced fruit set leading to reduction in seed yield (Sharma & Nayyar, 2014).

3.1.4 Thousand seed mass and grain yield

Seed development of all genotypes was severely affected by frost because the minimum temperature of -3.0 °C and -4.5 °C were recorded during seed dvelopment stage in Jan 2019 and Jan 2020, respectively (Fig 1). The majority of the genotypes produced shrived seed (Fig 4). Most genotypes that performed well till seed development became affected at seed development stage. The range of 0 g to 300 g and 0 kg ha^{-1} to 2531 kg ha^{-1}were recorded for thousand seed mass and grain yield for 2018/2019 cropping season respectively, while for 2019/2020 cropping season the range was, 0 to 297 g and 0 kg ha^{-1} to 2604 kg ha^{-1}, respectively. Wu et al. (2014) indicated that the prolonged period of chilling range temperatures (0 °C to 12 °C) at any phenological stage of development in chickpea has detrimental effects on final seed yield. Low temperature has negative impact on yield and 15-20 % yield loss was estimated and temperature below 15 % during flowering leads to flower and pod abortion leading to poor yield (Croser et al., 2003). Frost stress affects the source-sink balance by markedly decreasing the source of assimilates for grain filling which, in turn, reduces potential yield (Maphosa et al., 2020). Chaturvedi et al. (2009) estimated a yield loss of 15-20 % has been associated with low temperature. Low temperature during vegetative stage leads to decreased vegetative growth, biomass production and yield in north India (Mir et al., 2019; Singh et al., 1993).

3.1.5 Seed color

The majority of the frost susceptible genotypes showed a seed color fade up. Some of the genotypes had shown plumped seed with fade up seed color. This indicates that frost causes seed size and seed discoloration in chickpea. Similar observation was made for faba bean (Sallam et al., 2015). This happened because frost affects the mobilization of plant resources in to seed setting (Croser et al., 2003).

3.2 PLANT SURVIVAL RATE (SR)

Frost tolerance was assessed using plant survival rate (SR) for 673 diverse chickpea germplasms for two growing seasons under field condition (Table 3). It was observed that the SR values ranged from 0.0 (60 genotypes) to 0.86 (genotypes 16341-A, 24159-C and 30290-A) and 0.0 (60 genotypes) to 0.87 (genotype 41167-C) for 2018/2019 and 2019/2020 growing seasons, respectively. The value of SR score for the two growing seasons showed variation because of the different duration and intensity of frost stress occurred in the different seasons. The frost intensity and length of occurrence were more sever in 2019/2020 growing season than in 2018/2019. So, high value of SR was recorded in 2018/2019 than in 2019/2020 growing season (Fig 1). One hundred fifty seven and 87 chickpea genotypes had shown above 0.8 SR score, while the remaining 516 and 586 genotypes were below 0.8 SR score for 2018/2019 and 2019/2020 growing seasons, respectively. Eighty five genotypes were consistently given SR score value above 0.8 in both growing seasons. In the experimental site, frost occurred consistently throughout the life cycle of the crop's development, and hence, the frost survival score were taken at the end of each crop stages. The fluctuation of minimum temperature of two different growing seasons exhibited a similar pattern of SR value change for all genotypes. Minimum temperature of 2019/2020 growing seasons was lower than that of 2018/2019 growing season. It is clear that the SR of chickpea is closely associated to the temperature changes. Similar patterns were observed also in field pea (Liu et al., 2017). This approach has been employed to screen frost tolerance in rapeseed/canola (Fiebelkorn & Rahman, 2016) and field pea (Liu et al., 2017).

Table 2: Mean square and mean for the tested traits of 673 (562 EBI genotypes, 83 exotic and 28 improved chickpea) genotypes grown at Bakelo, Debre Berhan, Ethiopia from 2018-2020 growing seasons (I for 2018/2019 and II for 2019/2020)

Sources of Variation	Degree of freedom	Type III Mean Squares									
		PLH	NPB	NSB	FPPP	IPPP	DTF	DTP	DTM	TSM	GY
(I)											
Block	9	0.6^{ns}	0.3^{ns}	1.6^{ns}	168.3^{ns}	35.9^{ns}	4.55^{ns}	14.9^{ns}	3.51^{ns}	5.0^{ns}	7224^{ns}
Treatment	612	23.5^{***}	2.2^{***}	4.9^{***}	429.4^{***}	47.6^{*}	8.15^{*}	17.4^{*}	28.57^{*}	814.8^{***}	154550^{***}
Treatment: check	2	0.99^{ns}	0.3^{ns}	0.6^{ns}	35.3^{ns}	277^{***}	41.6^{**}	61.9^{**}	21.43^{ns}	13.1^{ns}	174411^{***}
Treatment: test and test vs. Check	610	24***	2.2^{***}	4.9^{*}	430.7^{***}	46.8^{*}	4.04^{*}	17.2^{*}	28.59^{*}	817.4^{***}	154485^{***}
Residuals	18	0.78	0.43	0.53	84.8	21.6	4.82	7.8	11.59	6.8	8976
CV		2.32	13.32	20.06	22.88	41.34	4.16	4.57	2.56	3.07	8.72
Mean		38.35	4.89	3.65	40.31	10.9	52.72	61.22	133.0	87.11	1118.9
(II)											
Block	9	8.74	2.29^{ns}	0.9^{ns}	295^{ns}	59.6^{ns}	2.1^{ns}	34.4^{ns}	50^{ns}	10^{ns}	7435^{ns}
Treatment	612	38.4^{***}	2.2^{*}	3.6^{*}	513^{**}	180^{***}	22.5^{***}	29.45^{*}	49.13^{*}	1177^{***}	253659^{***}
Treatment: check	2	8.59^{ns}	0.8^{ns}	1.8^{ns}	211^{ns}	27^{ns}	7.03^{ns}	$103.^{**}$	65.1^{ns}	75.8^{*}	65909^{**}
Treatment: test and test vs. Check	610	38.5^{***}	2.2^{*}	3.6^{*}	514^{**}	180^{***}	22.6^{***}	29.21^{*}	49.08^{*}	1180^{***}	254275^{***}
Residuals	18	2.57	1.38	2.56	184	26.3	5	14.49	49.36	16.7	9573
CV (%)		3.89	21.47	41.33	25.47	16.0	4.09	5.29	5.36	6.78	14.0
Mean		41.39	5.49	3.87	53.76	31.75	54.72	72.19	130.66	61.39	720.0

Symbols for level of significance:'***' 0.001 '**' 0.01 '*' 0.05, ns is none significant, PLH = Plant Canopy Height (cm), NPB = Number of primary branches, NSB = Number secondary branches, FPPP = Fertile pods per plant, IPPP = Infertile pods per plant, DTF = Days to 50 % flowering, DTP = Days to 50 % podding, DTM = Days to 90 % maturity, TSM = Thousand seed mass, and GY = Grain yield in kg ha^{-1}

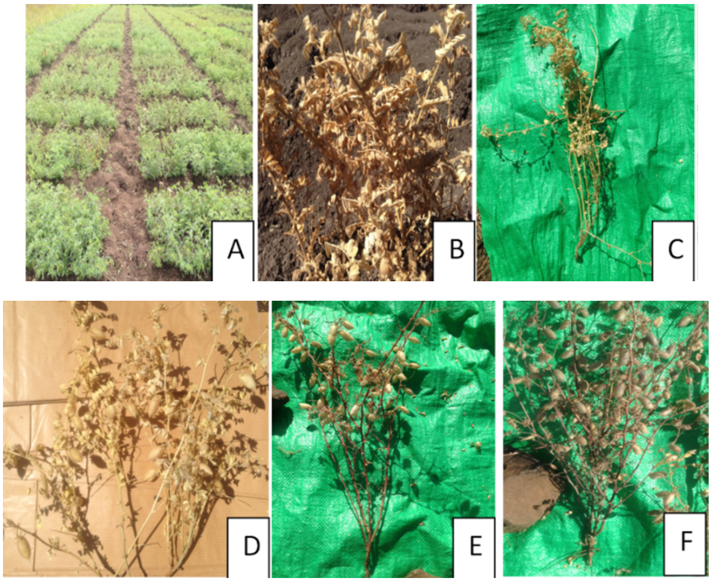

Figure 3: Frost response in chickpea at different growing stages: chickpea genotypes seedling coverage (A), plant death during pre-flowering stage (B), reduced pod setting (C and D) and better pod setting (E and F)

Table 3: Frost survival rate (SR) of 562 Ethiopian chickpea, 83 exotic and 28 improved chickpea genotypes tested at Bakelo, Debre Berhan, Ethiopia, 2018 to 2020 growing seasons

No	SR Rating	2017/2018		2019/2020		Common genotypes for both years	
		No of genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	≥ 0.8	157	23.3	87	12.9	85	19.6
2	≥ 0.6 to < 0.8	273	40.6	199	29.6	155	35.8
3	≥ 0.4- < 0.6	136	20.2	213	31.7	96	22.2
4	≥ 0.2- < 0.4	33	4.9	60	8.9	23	5.3
5	< 0.2	74	11.0	114	16.9	74	17.1
Total		673		673		433	

Table 4: Freezing tolerance rate (FTR) of 673 (562 Ethiopian chickpea, 83 exotic and 28 improved) chickpea genotypes tested at Bakelo, Debre Berhan, Ethiopia from 2018 to 2020 growing seasons

No	FTR Rating	2017/2018		2019/2020		Common genotypes for both years	
		No of genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	1	27	4.0	0	0		
2	2	32	4.8	27	4.0		
3	3	110	16.3	57	8.5		
Sub Total		169	25.1	84	12.5	83	15.5
4	4	261	38.8	154	22.9		
5	5	82	12.2	131	19.5		
6	6	50	7.4	118	17.5		
Sub Total		393	58.4	403	59.9	341	63.9
7	7	29	4.3	57	8.5		
8	8	20	3.0	25	3.7		
9	9	62	9.2	104	15.5		
Sub Total		111	16.5	186	27.6		
Grand Total		673		673		424	

3.3 FREEZING TOLERANCE RATE (FTR)

Freezing tolerance rate with a rating scale of 1-9 has been used for measuring cold stress injury during early vegetative stage or seedling stage in earlier studies (Singh et al. 1989). Based on FTR, 169 (1-3 score) and 84 (2-3 score) genotypes were described as tolerant to highly tolerant, while 504 (4-9) and 590 (4-9) were described as moderately tolerant to highly susceptible genotypes during 2018/2019 and 2019/2020 growing seasons, respectively (Table 4). Eighty three genotypes were rated within the score of 1-3 consistently in both growing season. In this experiment, it is observed that the majority of the genotypes that were resistant at seedling stages failed to resist frost that occured late at reproductive stage. From this result we can conclude that FTR score must be taken throughout the crop stages. Generally, single FTR score may work for areas where frost occurs once in the life cycle of the crop stages, however, in areas where, frost occurs consistently throughout the life cycle of the crop, FTR should be score frequently. In addition, genotypes that showed better FTR value gave either shriveled seeds or empty pods. So, FTR is not able to evaluate the capacity of frost resistance at reproductive stages and the suscep-

Table 5: Seed shriveling score (1-5) of 673 (562 Ethiopian chickpea, 83 exotic and 28 improved chickpea) genotypes tested at Bakelo, Debre Brehan, Ethiopia from 2018 to 2020 growing seasons

No	SSR Rate	2017/2018		2019/2020		Common genotypes for both years	
		No of genotypes	Percentage	No of genotypes	Percentage	No of genotypes	Percentage
1	1	145	21.6	47	7.0	33	12.6
2	2	128	19.0	83	12.3	33	12.6
3	3	126	18.7	154	22.9	42	16.0
4	4	177	26.3	194	28.8	78	29.8
5	5	97	14.4	195	29.0	76	29.0
Total		673		673		262	

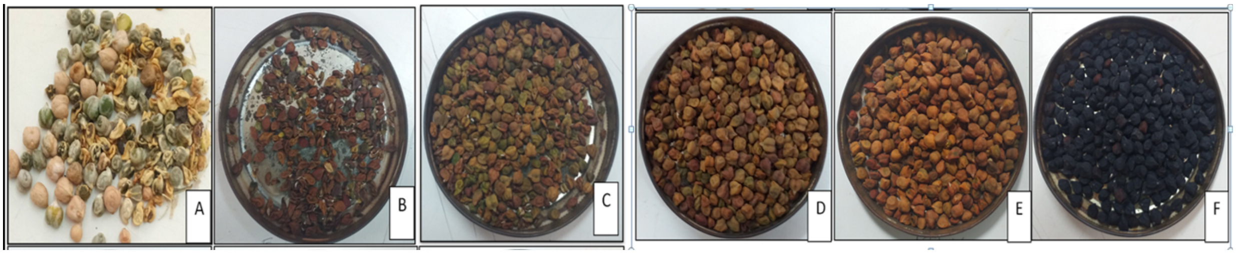

Figure 4: Seeds of chickpea genotypes showing different reaction to frost stress (A and B are very shriveled (Score of 5), C is Shriveled (Score of 4), D is intermediate (Score of 3), E is medium plumped (Score of 2) and F is plumped (Score of 1)

tible genotypes will be overlooked by this approach. FTR is the most important indices used for freezing screening for different crops tested at seedling stage (Badeck et al., 2015; Nezami et al., 2012; Srinivasan et al., 1998; Toker, 2005).

3.4 SEED SHRIVELING SCORE (SSS)

Visual assessment of seed damage by frost stress was done for all the genotypes for both seasons (Table 5). One hundred forty five and 47 genotypes produced plumped seeds (Score of 1: Fig 4E and 4F) in 2018/2019 and 2019/2020 cropping seasons, respectively, while the remaining genotypes gave medium to shriveled seeds (Fig 4A to 4D). Genotypes that were rated as frost resistant based on SR and FTR indices failed to produce plumped seeds, which means that all genotypes that had a better SR and FTR score did not produce plumped seed. However, all genotypes that produced plumped seed had a better SR and FTR value. From this result, it is possible to conclude that SR and FTR indices can indicate frost resistances at seedling or vegetative stages alone. Therefore, SR and FTR indices will not be applicable to screen genotypes for frost resistance at reproductive stage. Visual assessment of frost damaged seed has been applicable also to screen faba bean genotypes for frost resistance variability (Henriquez et al., 2018).

In general to select the frost tolerant promising genotypes, it is advisable to consider frost tolerance related traits and agronomic traits together. Genotypes that are consistently selected by all the parameters are considered as a promising frost tolerant genotype which can be directly taken by farmers or serve as a breeding material for further breeding activities. The selected frost tolerant genotypes will help to stabilize yield and expand the chickpea production areas into Ethiopian highland where chickpea production is not a common practice because of frost damage. In this study, 94 (51 black, 29 brown and 14 white) genotypes were selected as frost tolerant, the remaining genotypes as intermediate to susceptible. The promising frost resistant genotypes were selected with the following criteria i.e. Frost survival rate (>0.75), seed shriveling score (1-2), and freezing tolerance rate (1-4). The selected genotypes are listed in table 6.

Table 6: List of eighty two frost resistant chickpea genotypes selected based on SR (> 0.75), FTR (score of 1,2,3) and seed score (1 and 2)

No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source	No	Genotype	Seed Color	Source
1	16341-A	Black	EBI	33	208994-A	Brown	EBI	65	30293-A	Brown	EBI
2	207674	Black	EBI	34	235036-A	Brown	EBI	66	207739-B	Brown	EBI
3	30336-A	Black	EBI	35	209016-B	Black	EBI	67	71875	Brown	ICARDA
4	30336-B	Black	EBI	36	209022-A	Black	EBI	68	75095	Brown	ICARDA
5	41004-C	Black	EBI	37	209026-A	Black	EBI	69	140941	Brown	ICARDA
6	41036-B	Black	EBI	38	212589-B	Black	EBI	70	116451	Brown	ICARDA
7	41051-A	Black	EBI	39	212914-B	Black	EBI	71	126302	Brown	ICARDA
8	41081-A	Black	EBI	40	214731-B	Black	EBI	72	9427	Red	ICARDA
9	41107-B	Black	EBI	41	214734-A	Black	EBI	73	128699	White	ICARDA
10	41133-A	Black	EBI	42	215067-A	Black	EBI	74	9632	White	ICARDA
11	41167-C	Black	EBI	43	215190-A	Black	EBI	75	10163	White	ICARDA
12	41206-B	Black	EBI	44	215289-B	Black	EBI	76	140394	White	ICARDA
13	207608	Black	EBI	45	236196-B	Black	EBI	77	7339	White	ICARDA
14	207622	Black	EBI	46	236459-B	Black	EBI	78	70753	White	ICARDA
15	207638	Black	EBI	47	236479-C	Black	EBI	79	73395	White	ICARDA
16	207640	Black	EBI	48	237054-B	Black	EBI	80	69420	White	ICARDA
17	207648	Black	EBI	49	207686	Black	EBI	81	132663	White	ICARDA
18	207652	Black	EBI	50	207664-A	Black	EBI	82	9415	White	ICARDA
19	207668	Black	EBI	51	30349-B	Black	EBI	83	Yelebe	White	EARCs
20	207670	Black	EBI	52	30348-B	Black	EBI	84	Akaki	Red	EARCs
21	207684	Black	EBI	53	41127-B	Black	EBI	85	mariye	Red	EARCs
22	207688-A	Black	EBI	54	207746	Black	EBI	86	Natoli	Red	EARCs
23	207692	Black	EBI	55	207173-C	Black	EBI	87	Teketay	Red	EARCs
24	207712	Black	EBI	56	41075-C	Brown	EBI	88	kutaye	Brown	EARCs
25	207714	Black	EBI	57	41093-B	Brown	EBI	89	Teji	White	EARCs
26	207728-A	Black	EBI	58	41255-B	Brown	EBI	90	Shola	White	EARCs
27	207730	Black	EBI	59	207175-A	Brown	EBI	91	Worku	Red	EARCs
28	207748	Black	EBI	60	207635-C	Brown	EBI	92	Harbu	White	EARCs
29	208988-A	Red	EBI	61	30350-B	Red	EBI	93	Dalota	Brown	EARCs
30	209026-B	Red	EBI	62	41301-B	Red	EBI	94	Mastewal	Brown	EARCs
31	227152-B	Red	EBI	63	207766	Black	EBI				
32	30334-C	Red	EBI	64	207770	Black	EBI				

EBI = Ethiopian Biodiversity Institute, ICARDA is International International Center for Agricultural Research in the Dry Areas, EARCs = Ethiopian Agricultural Research Centers

Table 7: Phenotypic Pearson's correlation matrix for 9 traits in chickpea 673 (562 Ethiopian chickpea, 83 exotic and 28 improved chickpea) genotypes tested at Bakelo, Debre Berhan, Ethiopia from 2018/2019 (above diagonal) to 2019/2020 (below diagonal) growing seasons

	PLH	NPB	NSB	FPPP	IPPP	DTF	DTM	TSM	GY	SR	FTR	SSS	FC	SLP	SC
PLH	0	0.65**	0.43**	0.51**	-0.3**	0.13**	0.13**	0.64**	0.59**	0.68**	-0.66**	-0.48**	-0.52**	0.36**	-044**
NPB	0.64**	0	0.71**	0.68**	0.01**	0.12**	0.12**	0.35**	0.39**	0.43**	-0.40**	-0.31**	0.41**	0.28**	-0.33**
NSB	0.47**	0.68**	0	0.69**	0.04^{ns}	0.13**	0.19**	0.18**	0.23**	0.25**	-0.25**	-0.18**	0.20**	0.16**	-0.17**
FPPP	0.52**	0.69**	0.69**	0	-0.0^{ns}	-0.1^{ns}	0.12**	0.28**	0.33**	0.37**	-0.36**	-0.3**	0.28^{ns}	0.22**	-0.26**
IPPP	-0.2**	0.17**	0.19**	0.22**	0	0.08*	0.06^{ns}	-0.4**	-0.7**	-0.6**	0.56**	0.70**	0.1^{ns}	-0.3**	0.11**
DTF	0.01^{ns}	0.06^{ns}	0.15**	-0.2**	-0.1^{ns}		0.28**	-0.0^{ns}	-0.2**	-0.2**	0.16**	0.15**	-0.4**	-0.2**	0.30**
DTM	0.02^{ns}	0.00^{ns}	0.06^{ns}	-0.2**	-0.1^{ns}	0.30**	0	-0.0^{ns}	-0.08*	-0.08*	0.03^{ns}	0.01^{ns}	-0.2**	-0.1^{ns}	0.11**
TSM	0.47**	0.24**	0.22**	0.20**	-0.5**	0.14**	0.13**	0	0.69**	0.77**	-0.76**	-0.60**	-0.27**	0.32**	-0.27**
GY	0.42**	0.20**	0.18**	0.21**	-0.6**	0.0^{ns}	0.00^{ns}	0.72**	0	0.86**	-0.84**	-0.8**	0.43**	0.59**	-0.52**
SR	0.65**	0.4**	0.3**	0.38**	-0.4**	0.0^{ns}	-0.1**	0.66**	0.73*	0	-0.90**	-0.79**	0.41**	0.47**	-0.47**
FTR	-0.6**	-0.36**	-0.3**	-0.3**	0.44**	-0.1^{ns}	0.09*	-0.6**	-0.7**	-0.9**	0	0.77**	-0.37**	-0.6**	0.53**
SSS	-0.5**	-0.18**	-0.2**	-0.2**	0.54**	-0.1**	-0.1^{ns}	-0.8**	-0.8**	-0.8**	0.75**	0	-0.32^{ns}	-0.5**	0.44**
FC	0.57**	0.46^{ns}	0.25**	0.41**	0.22**	-0.4**	-0.2**	0.11*	-0.21*	-0.3**	-0.20**	-0.11**	0	0.57**	-0.79**
SLP	0.43**	0.30**	0.18**	0.31**	-0.4**	-0.2**	-0.1^{ns}	0.37**	0.48**	0.47**	-0.49**	-0.51**	0.60**	0	-0.80**
SC	-0.51*	-0.4**	-0.2**	-0.3**	0.11**	0.30**	0.18**	-0.2^{ns}	-0.3**	-0.5**	0.40**	0.32**	-0.79**	-0.8**	0

^{ns} = non significant; **=Correlation is significant at the 0.01 level (2-tailed); *=Correlation is significant at the 0.05 level (2-tailed), PLH = Plant Canopy Height (cm), NPB = Number of primary branches, NSB = Number secondary branches, FPPP = Fertile pods per plant, IPPP = Infertile pods per plant, DTF = Days to 50 % flowering, DTP = Days to 50 % podding, DTM = Days to 90 % maturity, TSM = Thousand Seed Mass, GY = Grain yield in kg ha^{-1}, SR = Frost survival rate, FTR = Frost tolerance rate, SSS = Seed shriveling score, FC = Flower Color, SLP = Stem/leaf pigmentation, and SC = Seed color

3.5 PHENOTYPIC CORRELATION COEFFICIENT

The phenotypic association of agronomic and frost tolerance related traits were analyzed for each genotype and the following result were obtained (Table 7). Most of the frost tolerance related traits have shown a strong significant relationship with agronomic traits. Grain yield was positively and significantly correlated ($p < 0.01$) with fertile pod per plant (0.33 and 0.21), thousand seed mass (0.69 and 0.72), SR (0.86 and 0.73), and stem/leaf pigmentation (0.59 and 0.48), while a strong negative correlation was seen for infertile pod per plant (-0.7 and -0.6), FTR (-0.70 and -0.6), SSS (-0.8 and -0.8), seed color (-0.52 and -0.30), and flower color (-0.43 and -0.21) for 2018/2019 and 2019/2020 growing seasons, respectively. It was observed that genotypes having strong stem/leaf pigmentation had shown a good performance in all agronomic traits and had also a better SR and FTR score. Similarly, flower and seed color had shown also a strong correlation with agronomic performances. Genotypes having pink flower and black seed color had better performances than the ones with white flower and white seed colored ones. From this result, the selection of genotypes having strong stem/leaf pigmentations and genotypes with black seeded chickpea types and pink flower would greatly assist plant breeders to develop frost resistant variety to reduce the risk of frost damages. The majority of black seeded chickpea performed well in agronomic traits and SR and FTR score was higher than brown and white seeded chickpea types. The majority of white seeded chickpea types were highly susceptible to frost stress. Brown seeded chickpea with strong stem/leaf pigmentation exhibited better reaction to frost stress than the one with brown seeded with week stem/leaf pigmentation. The result agree with previous findings in faba bean where genotypes with white flower being susceptible to frost stress, while tannin-containing genotypes and wild relatives are more tolerant (Henriquez et al., 2018; Inci & Toker, 2011). Frost stress causes accumulation of anthocyanins in the basal part of the stem, branches and leaves (Croser et al., 2003). Bhasker et al. (2018) indicated that the accumulation of anthocyanin due to high temperature has a positive relation with high grain yield because of the induction of antioxidant defense system. Frost damage has strong correlation with lower yield (Henriquez et al., 2018; Kanouni et al., 2009).

From this result it is possible to conclude that the presence of pigmentation induced by frost stress can be a good indicator for frost tolerance mechanism.

4 CONCLUSIONS

This experiment has shown that the degree of frost damage varied at different crop stages. The effect of frost was not seen on seed germination and seedling establishment. However, considerable frost damage was observed at vegetative and reproductive stages for most genotypes. The capacities of genotypes for frost tolerance were estimated using freezing tolerance rate (FTR) and frost survival rate (SR) and their agronomic performances. Eighty three and 85 genotypes were selected based on FTR and SR respectively. However, both indices are not able to evaluate frost resistance of the genotypes at reproductive stage, if the frost occurs consistently throughout the crop stages. Genotypes having good SR and FTR value produced shriveled seed and empty pods due to frost stress that occurred later at flowering and seed development stages. Therefore, to select the frost tolerant potential genotypes, it is advisable to consider SR and FTR values, pod setting, seed shriveling score, and grain yield together. Genotypes that are consistently selected by all these parameters are considered as promising frost tolerant genotypes. In addition, in areas where frost occurs consistently during the seedling and vegetative stages of the crop only, the selection of frost resistance at these stages by considering less FTR and high SR values are enough to select the frost resistant promising genotypes. The effect of frost stress to chickpea genotypes are variable depending on seed color type, presence and absence of stem/leaf pigmentation and different level of stem/leaf pigmentation. Chickpea genotypes with black seeded and/or having strong stem/leaf pigmentation performed well for frost stress reaction. From these observations, it is possible to conclude that stem/leaf pigmentation and black seeded color might be linked to a gene that confers frost resistance in chickpea. From this experiment, 94 genotypes were identified to be frost tolerant genotypes which can be taken by plant breeder for frost tolerant chickpea variety development program attesting that Ethiopian checkpea genotypes have a potential source for frost tolerance trait. Identification of the mechanism of stem/leaf pigmentation and black seed color for frost resistance is required. Also, identification of quantitative trait loci (QTLs) associated with gene controlling frost tolerances in chickpea is equally important.

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