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Full Length Research Paper

Tolerance to freezing stress in *Cicer* accessions under controlled and field conditions

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Freezing tolerance was determined in 5 annual wild *Cicer* and 225 *Cicer arietinum* L. accessions, grown both in field and controlled conditions. In controlled conditions, the temperature was decreased 5°C daily to achieve -20°C. Field trial was conducted at Urmia, Iran. In general, 'kabuli' chickpeas were more susceptible to freezing stress than 'desi' chickpeas. Some 'kabuli' types such as FLIP 93-261C and x03TH21 which presented high freezing tolerance during early seedling stage, withstood -15.6°C without snow cover. Based on severity score data, the highest freezing tolerance sources were all accessions *of Cicer echinospermum* and *Cicer reticulatum* and 15 lines from *C. arietinum* germplasm. The results obtained in controlled conditions were approximately confirmed in the field conditions. The most resistant genotypes to freezing stress were wild accessions of ILWC 81, ILWC 106, ILWC 139, ILWC 181, ILWC 235, and cultivated lines, Sel 96 TH 11404, Sel 96 TH 11439, Sel 96 TH 11488, Sel 98 TH 11518, x03TH21 and FLIP 93-261C. Our results indicated the possibility of autumn sowing of chickpea in the high plateaus of Iran.

Key words: Autumn planting, chickpea, *Cicer* species, freezing tolerance.

INTRODUCTION

According to statistics database of Food and Agriculture Organization, chickpea (*Cicer arietinum* L.) is the third most important food legume grown in 11.6 million ha with 8.8 million ton productions in 2008. It is grown in over 45 countries in all continents of the world (FAOSTAT, 2009). It provides a high quality protein to the people in developing countries (Yadav et al., 2007). Although chickpea potential seed yield of about 5 t ha^{-1} has been reported, the low realized seed yield is a result of lack of widely adapted cultivars, susceptibility to several biotic and abiotic stresses and short period of growth at spring sowing. Its production increases near 100% at winter or autumn sowing. Freezing stress as a main factor has limited the time of cultivation (Toker et al., 2007a).

Field screening for stress response often involves growing germplasm lines in contrasting conditions and estimating a susceptibility index from the relative survival, proposed by Singh et al. (1998). A key limiting factor in screening for abiotic stress tolerance is environmental heterogeneity. Climatic stresses such as drought or frost may not occur in the year in which the trial is run or may be so severe that all accessions in the trial are killed. Soils vary in water retention capacity, compression, aeration and mineral content, in the space of a few centimeters. Thus, plants that appear to be tolerant or resistant to the stress have often escaped it instead (Stoddard et al., 2006).

During the germination phase, the chickpea seedlings are protected by the buffering role of soil. Indeed, even when air temperature (at 10 cm above soil surface) falls down to -12°C, it never became negative at 10 cm below the soil surface (Wery, 1990). After emergence, the increase in frost resistance could be explained by the hardening process: lowering of cells' freezing point by organic (mainly saccharose and glucose) and mineral compounds storage (Couvreur et al., 1979; Toker et al., 2007a).

While land for testing genetic materials is often cheap,

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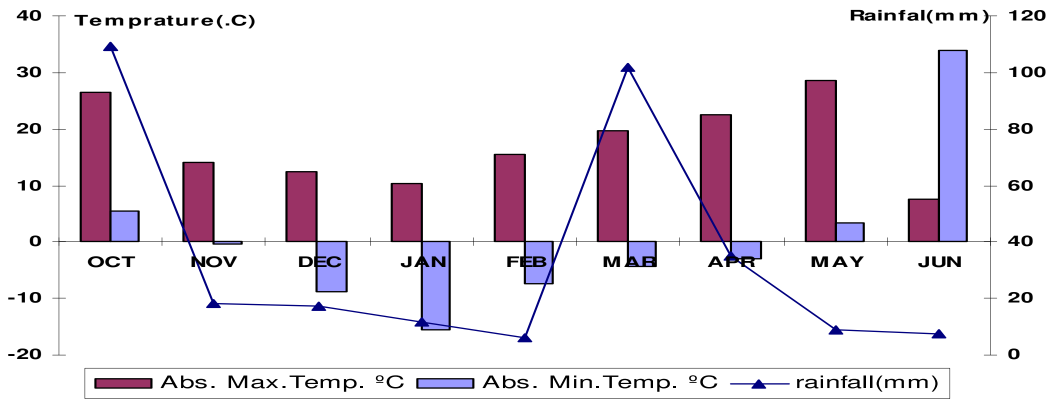

Figure 1. Weather conditions at Urmia rainfed farm in 2008 - 2009.

in-field modifications such as homogenized soil beds or rain-out shelters are limited in size and are expensive. Furthermore, field trials generally run the whole growing season. Therefore, it is often considered desirable to have a controlled-environment screening system, where the response may be evaluated uniformly and rapidly. A screening system may become generally acceptable when it is based on simple selection criteria, provides rapid and accurate screening of large numbers, is non-destructive, reproducible and relates to field performance (Saxena et al., 1994; Serraj et al., 2003).

Unlike the cultivated chickpea, wild *Cicer* species posses high level of resistance to biotic and abiotic stresses (Singh et al., 1998; Toker et al., 2007ab; Canci and Toker, 2009a). High levels of cold tolerance have been reported in local cultivars and other related species such as *Cicer bijugum*, *Cicer reticulatum*, *Cicer echinospermum*, *Cicer pinnatifidum* and *Cicer judaicum* (Singh et al., 1990; 1995; Malhotra and Saxena, 1993; Toker, 2005). Singh et al. (1984) conducted a trial to find sources of resistance to frost in the chickpea germplasm by screening 3158 genotypes in a high elevation plateau in Turkey, and reported several cold tolerance chickpeas. Singh et al. (1998) evaluated 228 accessions of eight annual wild *Cicer* species including cultigens for biotic and abiotic stresses. Some accessions of the annual wild *Cicer* species showed cold tolerance. Toker (2005) evaluated 43 accessions from eight annual wild *Cicer* species for cold tolerance at high elevations of the west Mediterranean region of Turkey. He found that the level of cold tolerance in some accessions was the same as reported by Singh et al. (1998).

The aim of this study was to evaluate a large number of genetically diverse chickpea genotypes for their reaction to freezing stress under field and controlled conditions.

MATERIALS AND METHODS

A total of 220 chickpea (*C. arietinum* L.) lines and 5 accessions of two annual wild *Cicer* species: three *C. echinospermum* P. H. Davis. and two *C. reticulatum* Ladiz. (Bge) M. Pop.; were used for freezing tolerance screenings. The first series of seeds was sown at pods in a completely randomized block design with four replications in greenhouse. After seedling and at 5 - 6 leaflet stage, they were moved to controlled condition (12/12) for (day/night). The temperature was decreased 5°C daily to achieve -20°C. This experiment was carried out at West Azerbaijan Agricultural and Natural Resources Research Center, Iran.

The second series of seeds were planted in a systematic design for two replications in October 2008 by using ILC533 as the check at every 5^{th} row at Urmia, Iran (45° 09′ E, 37° 12′ N and 1520 m from sea level). The accessions were grown in one row of 2 m length with inter- and intra-row spacing of 30 and 10 cm, respectively.

Plants were not irrigated because natural rainfall sufficed (110 mm). The experimental areas were fertilized at a rate of 20 kg N and 40 kg P_2O_5 per hectare. Weed control was done by hand three times at seedling stage and prior to flowering.

For the visual screening of freezing tolerance, we used a scale of 1 - 9, where 1 = free; no visible symptom of damage, 3 = tolerant; slight foliar damage (11 - 20% leaflets show withering) and up to 20% branches show withering and drying, no plant killing, 5 = intermediate; 41-60% leaflets and 21-40% branches show withering and drying, up to 5% plant killing, 7 = susceptible; 81 - 99% leaflets and 61 - 90% branches show withering and drying, 26 - 50% plant killing and 9 = highly susceptible; 100% plant killing (ICARDA, 2008).

In field experiment, we also recorded the number of plants germinated before onset of severe winter (PC1) and after the winter are over (PC2). Finally, for each genotype in replicate a frost resistance ratio (FRR) was calculated as follows (Wery 1990):

$$FRR = \frac{PLH}{PLE}$$

Where, PLH = Number of plant per line at harvest time; PLE = number of plants per line after emergence and before the first frost.

Evaluation for freezing tolerance was done after the death of the susceptible check. Seedlings were not covered by snow in 2008 - 2009. The number of days with freezing temperatures was 79. The lowest temperature was -15.6°C in January 2009 (Figure 1). Statistical analysis was completed using Microsoft Excel 2003.

RESULTS

Reaction of annual *Cicer* species and other lines to

Table 1. A total of 225 *Cicer* accessions evaluated for freezing tolerance under controlled conditions.

Genotype	Type	FTS*	Genotype	Type	FTS	Genotype	Type	FTS
ILWC 106	Desi	1	x03TH 29	Kabuli	5	X 94TH 151 K1	Kabuli	4
ILWC 139	Desi	1	x03TH 164	Kabuli	7	X 94TH 151 K5	Kabuli	5
Sel 95TH1 716	Desi	1	x03TH 165	Kabuli	5	X 94TH 174 K1	Kabuli	4
Sel 95TH1 745	Desi	2	x03TH 135	Kabuli	7	X 95TH 4 K1	Kabuli	7
Sel 96TH1 1403	Desi	2	ILC 3287	Kabuli	5	X 96TH 50 K3	Kabuli	3
Sel 96TH1 1404	Desi	1	Hashem	Kabuli	5	X 96TH 94 K1	Kabuli	5
Sel 96TH1 1406	Desi	1	X 96TH 3 K3	Kabuli	5	FLIP 92-113C	Kabuli	5
Sel 96TH1 1485	Desi	1	X 96TH 3 K4	Kabuli	4	FLIP 92-162C	Kabuli	5
Sel98TH1 1518	Desi	1	X 96TH 174K2	Kabuli	7	FLIP 92-164C	Kabuli	5
FLIP 93-261C	Kabuli	4	Sel 93TH2 4469	Desi	3	FLIP 92-169C	Kabuli	7
ILWC 81	Desi	1	FLIP 93-174C	Kabuli	5	FLIP 94-44C	Kabuli	5
Sel 95TH1 744	Desi	2	FLIP 95-46C	Kabuli	7	FLIP 95-55C	Kabuli	3
Sel 98TH1 744	Desi	1	FLIP 95-57C	Kabuli	5	FLIP 95-58C	Kabuli	5
ILWC 181	Desi	1	FLIP 95-61C	Kabuli	7	FLIP 95-60C	Kabuli	7
ILWC 235	Desi	2	FLIP 97-26C	Kabuli	4	FLIP 95-63C	Kabuli	7
Sel 93TH2 4477	Desi	4	FLIP 97-111C	Kabuli	5	FLIP 96-114C	Kabuli	5
Sel 96TH1 1439	Desi	1	FLIP 97-173C	Kabuli	7	FLIP 97-118C	Kabuli	5
Sel 96TH1 1484	Desi	2	FLIP 98-106C	Kabuli	5	FLIP 98-21C	Kabuli	5
Sel 96TH1 1488	Desi	1	FLIP 98-121C	Kabuli	5	FLIP 98-23C	Kabuli	7
x03TH 21	Kabuli	1	FLIP 98-206C	Kabuli	5	FLIP 98-131C	Kabuli	2
FLIP 98-258C	Kabuli	5	FLIP 98-134C	Kabuli	7	FLIP 99-25C	Kabuli	4
FLIP 03-112C	Kabuli	5	FLIP 99-01C	Kabuli	7	FLIP 99-46C	Kabuli	4
FLIP 93-255C	Kabuli	4	FLIP 99-34C	Kabuli	5	FLIP 99-48C	Kabuli	5
Sel 93TH2 4416	Desi	3	FLIP 99-45C	Kabuli	5	FLIP 00-40C	Kabuli	7
FLIP 96-116C	Kabuli	5	FLIP 00-6C	Kabuli	5	FLIP 02-47C	Kabuli	5
FLIP 98-91C	Kabuli	7	FLIP 00-17C	Kabuli	5	FLIP 02-89C	Kabuli	7
FLIP 98-178C	Kabuli	7	FLIP 00-25C	Kabuli	5	×2001TH 35	Kabuli	7
FLIP 99-58C	Kabuli	5	FLIP 01-16C	Kabuli	7	x03TH 2	Kabuli	5
FLIP 02-80C	Kabuli	7	FLIP 02-77C	Kabuli	5	x03TH 130	Kabuli	5
Sel 93TH2 4460	Desi	7	FLIP 03-107C	Kabuli	5	x03TH 134	Kabuli	7
FLIP 97-217C	Kabuli	5	FLIP 03-153C	Kabuli	5	x03TH 166	Kabuli	5
FLIP 00-44C	Kabuli	5	FLIP 03-143C	Kabuli	5	x03TH 173	Kabuli	5
x03TH 152	Kabuli	3	×2001TH 149	Kabuli	4	ILC 533	Kabuli	9
ILC 4134	Kabuli	5	×2001TH 152	Kabuli	5	ILC 3279	Kabuli	8
ILC 8262	Kabuli	3	x03TH 22	Kabuli	8	S 96078	Kabuli	8
FLIP 93-93C	Kabuli	4	x03TH 148	Kabuli	7	Gazvin	Kabuli	7
FLIP 99-47C	Kabuli	5	x03TH 1	Kabuli	5	Bivanij	Kabuli	5
FLIP 03-46C	Kabuli	5	ILC 6142	Kabuli	6	Djam	Kabuli	7
FLIP 03-142C	Kabuli	7	X 94TH 174 K2	Kabuli	3	X 96TH 44 K1	Kabuli	4
×200I TH 45	Kabuli	5	X 94TH 174 K6	Kabuli	6	FLIP 87-59C	Kabuli	7
x03TH 16	Kabuli	5	X 95TH 1 K1	Kabuli	4	FLIP 87-85C	Kabuli	5
x03TH 17	Kabuli	5	X 96TH 151K5	Kabuli	7	FLIP 93-48C	Kabuli	7
X 96TH 3 K5	Kabuli	7	FLIP 94-107C	Kabuli	9	FLIP 93-182C	Kabuli	7
X 95TH 5 K10	Kabuli	6	FLIP 95-59C	Kabuli	5	FLIP 94-25C	Kabuli	7
FLIP 82-150C	Kabuli	3	FLIP 95-64C	Kabuli	4	FLIP 94-108C	Kabuli	1
FLIP 93-254C	Kabuli	5	FLIP 95-67C	Kabuli	4	FLIP 95-62C	Kabuli	7
FLIP 94-123C	Kabuli	5	FLIP 97-21C	Kabuli	5	FLIP 97-102C	Kabuli	6
FLIP 99-61C	Kabuli	7	FLIP 97-85C	Kabuli	5	FLIP 98-16C	Kabuli	4
FLIP 01-6C	Kabuli	4	FLIP 97-131C	Kabuli	4	FLIP 98-22C	Kabuli	5
ILC 8617	Kabuli	1	FLIP 97-230C	Kabuli	1	FLIP 98-24C	Kabuli	7
S 96002	Kabuli	7	FLIP 97-258C	Kabuli	3	FLIP 98-107C	Kabuli	5

Table 1. Contd

S 96019	Kabuli	5	FLIP 97-211C	Kabuli	5	FLIP 98-108C	Kabuli	5
S 96027	Kabuli	7	FLIP 98-15C	Kabuli	6	FLIP 98-143C	Kabuli	5
FLIP 85-57C	Kabuli	4	FLIP 98-55C	Kabuli	5	FLIP 99-59C	Kabuli	1
FLIP 95-50C	Kabuli	3	FLIP 99-19C	Kabuli	5	FLIP 00-14C	Kabuli	5
FLIP 95-51C	Kabuli	5	FLIP 00-10C	Kabuli	4	FLIP 00-15C	Kabuli	5
FLIP 95-69C	Kabuli	5	FLIP 00-38C	Kabuli	5	FLIP 00-18C	Kabuli	1
FLIP 97-32C	Kabuli	7	FLIP 00-55C	Kabuli	7	FLIP 00-19C	Kabuli	5
FLIP 97-74C	Kabuli	4	FLIP 01-18C	Kabuli	5	FLIP 01-58C	Kabuli	7
FLIP 97-121C	Kabuli	5	FLIP 01-51C	Kabuli	7	FLIP 02-09C	Kabuli	5
FLIP 97-174C	Kabuli	7	FLIP 02-79C	Kabuli	4	FLIP 02-74C	Kabuli	5
FLIP 97-219C	Kabuli	5	FLIP 03-30C	Kabuli	5	FLIP 02-78C	Kabuli	5
FLIP 98-38C	Kabuli	5	FLIP 03-32C	Kabuli	7	FLIP 03-145C	Kabuli	6
FLIP 98-130C	Kabuli	2	FLIP 03-38C	Kabuli	4	×2001TH 38	Kabuli	5
FLIP 98-200C	Kabuli	6	×2001TH 76	Kabuli	5	×2001TH 39	Kabuli	9
FLIP 99-66C	Kabuli	5	×2001TH 103	Kabuli	3	×2001TH 40	Kabuli	7
FLIP 00-39C	Kabuli	5	×2001 TH 112	Kabuli	7	×2001TH 54	Kabuli	5
FLIP 00-69C	Kabuli	5	x03TH 19	Kabuli	5	x2001TH 148	Kabuli	7
FLIP 00-81C	Kabuli	5	x03TH 28	Kabuli	5	x2001TH 150	Kabuli	4
FLIP 01-4C	Kabuli	5	x03TH 150	Kabuli	5	x03TH 27	Kabuli	7
FLIP 01-9C	Kabuli	5	x03TH 153	Kabuli	5	x03TH 136	Kabuli	5
FLIP 01-54C	Kabuli	5	x03TH 177	Kabuli	7	x03TH 169	Kabuli	7
FLIP 02-02C	Kabuli	5	ILC 482	Kabuli	7	x03TH 180	Kabuli	5
FLIP 02-04C	Kabuli	5	ILC 3182	Kabuli	5	x03TH 3	Kabuli	5
×2001TH 34	Kabuli	5	Line 16	Kabuli	7	x03TH 24	Kabuli	5

* FTS: Freezing tolerance score; A score of 1 - 9 where: 1 = free, no visible symptom of damage; 3 = tolerant, slight foliar damage, no plant killing; 5 = intermediate, up to 5% plant killing; 7 = susceptible, 26 - 50% plant killing; 9 = highly susceptible, 100% plant killing.

freezing stress is given in Table 1, Figures 2 and 3. The level of resistance to freezing stress varied from highly resistant to partial resistant in the genotypes tested (Table 1). Almost 16% of the genotypes were highly resistant to freezing stress (with freezing tolerance score of 1 to 3) and a few genotypes (10%) were rated moderately or partially resistant (with freezing tolerance score of 4). The most highly resistant genotypes belonged to wild accessions (Table 1). However, some lines of *C. reticulatum* showed high freezing stress tolerance (Table 1).

Based on severity score data in filed trial, the best freezing tolerance sources were all accessions *of C. echinospermum* and *C. reticulatum* and 15 *C. arietinum* lines that showed high tolerant withstood - 15.6°C without snow cover (Figures 2 and 3). Most lines along with the sensitive check, ILC 533, were killed at all rows (Figures 2 and 3).

All the wild accessions and 29 *C. arietinum* lines had more than 50% PC2 whereas the plants of 50% *C. arietinum* lines were completely killed after frost. The results showed that the FRR for 28 *C. arietinum* lines and wild accessions except one accessions of *C. echinospermum* (ILWC 235) was 0.7 - 1.0 and for 27 lines was 0.5 - 0.7 (Figure 3). There is high accordance between results obtained by PC2 criteria and FRR (Figure 4).

The results obtained in controlled conditions were approximately confirmed in the field conditions (Table 1; Figures 2 and 3). However some lines such as ILC 6142, S 96078, x95TH 1 K1 and FLIP 94-108C having high freezing tolerance in controlled conditions showed intermediate or sensitive reactions at field conditions.

In general, our results showed that 'kabuli' chickpeas were more susceptible to freezing stress than 'desi' chickpeas (Table 1; Figures 2 and 3). In the seedling phase, 12 days after emergence, 'kabuli' lines showed greater vegetative growth than all other lines. Considering our two series of experiments conducted in filed and controlled conditions, the most resistant genotypes to freezing stress were wild accessions of ILWC 81, ILWC 106, ILWC 139, ILWC 181, ILWC 235, and cultivated lines, Sel 93 TH 24477, Sel 96 TH 11404, Sel 96 TH 11439, Sel 96 TH 11488, Sel 98 TH 11518, x03TH21 and FLIP 93-261C.

DISCUSSION

The comparison of freezing tolerance scores of *Cicer*

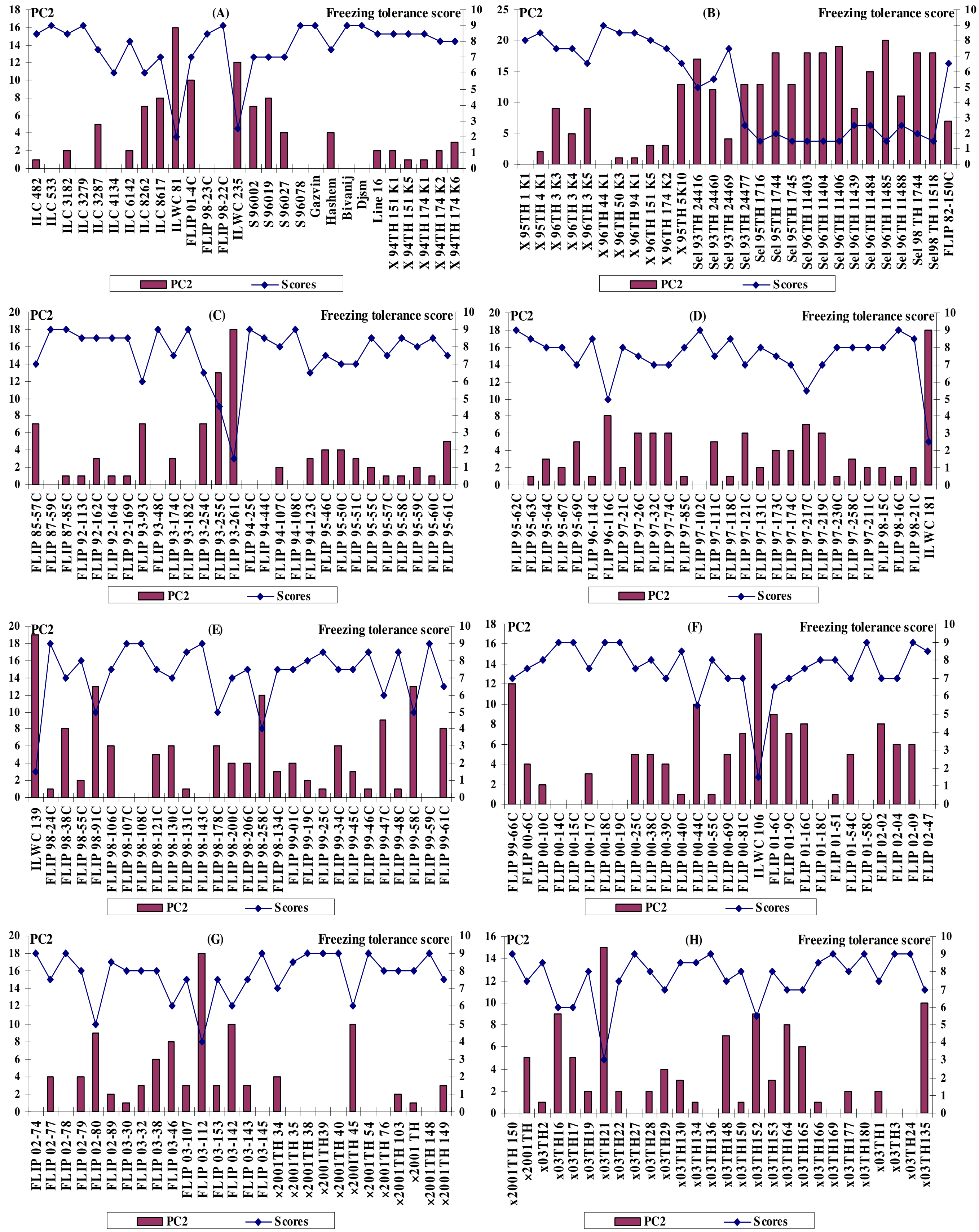

Figure 2. PC2 and freezing tolerance scores of 225 chickpea accessions in field conditions (A - H).

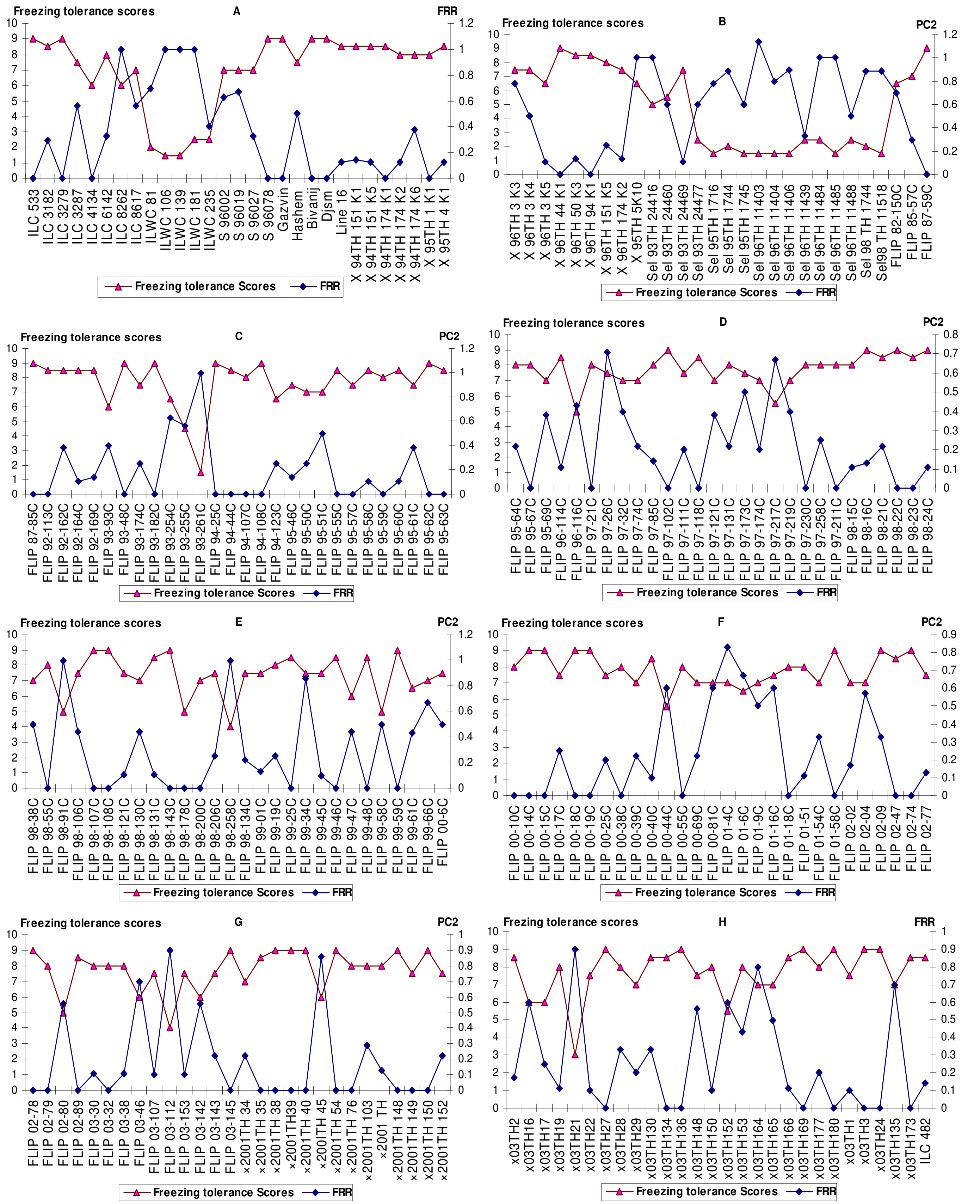

Figure 3. FRR and freezing tolerance scores of 225 chickpea accessions in field conditions (A - H).

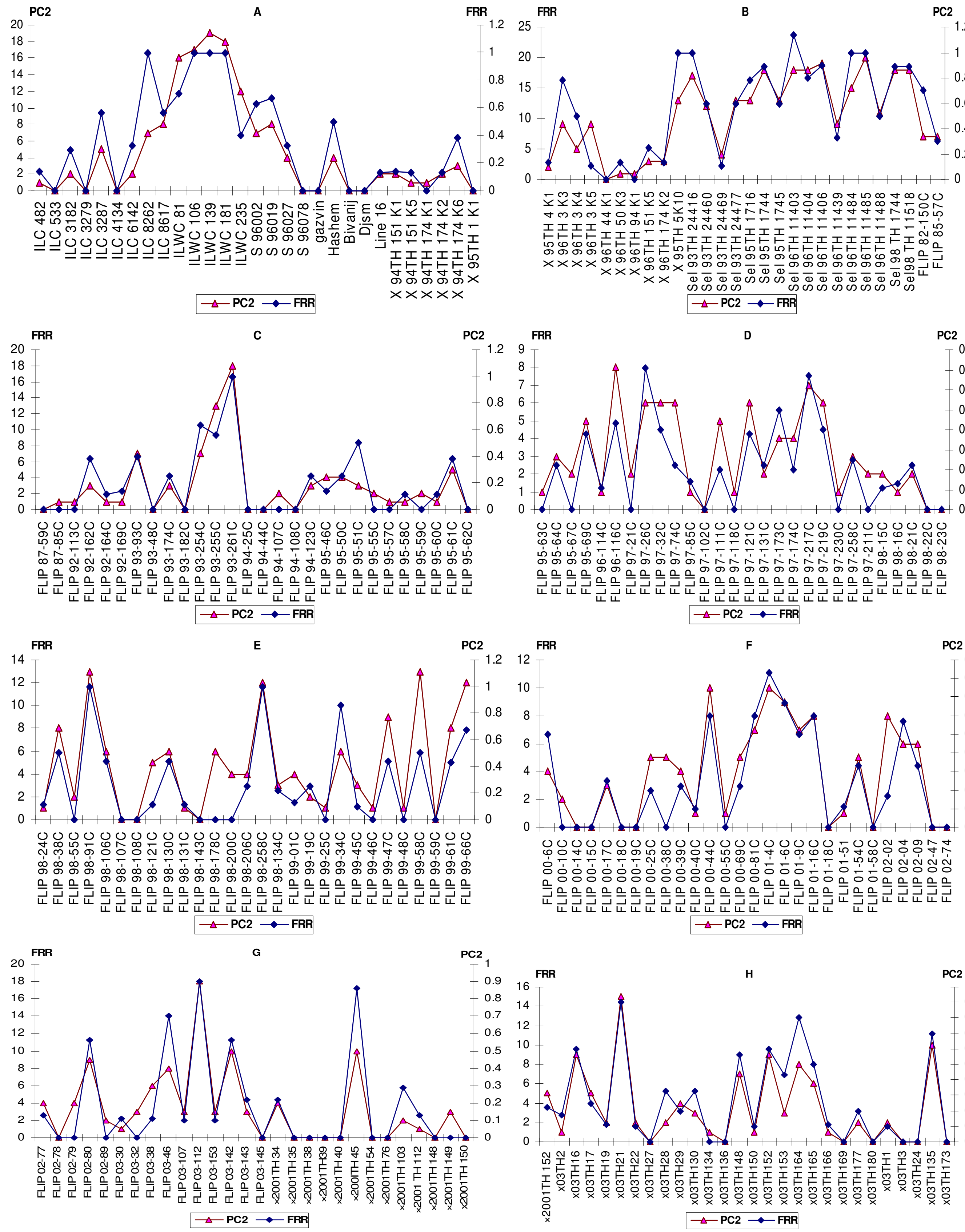

Figure 4. FRR and PC2 of 225 chickpea accessions in field conditions (A - H).

species indicated that the level of freezing tolerance in all accessions of *C. reticulatum* and *C. echinospermum* was superior to those well known cold tolerant varieties, ILC 8262 and ILC 8617 (Table 1; Figures 2 and 3). These results confirmed the findings of Singh et al. (1990, 1995), Toker (2001), Robertson et al. (1995) and Singh et al. (1998). They found that most of the accessions of *C. bijugum*, *C. echinospermum* and *C. reticulatum* were tolerant to cold and have significantly higher levels of cold tolerance than the cultivars (Singh et al., 1998; Toker, 2005). *C. reticulatum* Ladiz., which is an endemic species distributed in South-east Turkey and the wild progenitor of the cultivated chickpeas (Ladizinsky and Adler, 1976; Toker, 2009), possesses high level of cold tolerance since its seeds germinates in autumn and grows in winter after its pods are shattered and seeds separated (Toker, 2009). 'Desi' chickpeas were more tolerant to freezing stress than 'kabuli' chickpeas. Similar results were also outlined by Canci and Toker (2009b) for drought resistance.

The cross ability of *C. reticulatum* and *C. echinospermum* have already been reported (Ladizinsky and Adler, 1976; Pundir and van der Maesen 1983; Jaiswal et al., 1986; Singh and Ocampo, 1997; Gaur and Gour 2002; Toker, 2009). Recently, the cold tolerant genes of *C. reticulatum* and *C. echinospermum* have been transferred to cultivated chickpeas in the breeding programs of ICARDA (1999). For colder areas of highlands, cultivar suitable for winter or autumn sowing could be achieved by the use of genes for freezing tolerance from the wild *Cicer* species. However, Toker et al. (2009a) pointed out that cold tolerance in chickpea must be considered with resistance to ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. Considering our two series of experiments conducted in filed and controlled conditions, *C. arietinum* lines including Sel 93 TH 24477, Sel 96 TH 11404, Sel 96 TH 11439, Sel 96 TH 11488, Sel 98 TH 11518, x03TH21 and FLIP 93-261C along with the wild accessions, ILWC 81, ILWC 106, ILWC 139, ILWC 181, ILWC 235, have a great value for use in breeding programmes for freezing tolerance stress. According to our results, chickpeas having freezing resistance could be sown in autumn at the high plateaus of Iran and grown in winter in order to provide its yield advantages.

To further elucidate the genetic control of freezing tolerance in studied materials and usefulness of tolerant genotypes as breeding parents, a mating design programme is ongoing to estimate general and specific combining abilities as well as the mode of inheritance for this trait. Parental genotypes and their F1 hybrids will be evaluated in greenhouse as well as in field experiments in appropriate statistical design. These experiments encourage us to identify the most promising combination (F1) in order to produce a population for construction of the chickpea genetic map using molecular markers. Use of molecular markers technology will help to identify the quantitative trait locus (QTLs) controlling freezing tolerance in chickpea. The ultimate goal will be the use of molecular markers which are linked to freezing tolerance genes to improve breeding lines through marker-assisted selection.

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