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Review

An overview on the optimal methods of cultivating chickpea (*Cicer arietinum* L.) at negative temperatures

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ABSTRACT:

The advantages of winter planting of chickpea cultivation (*Cicer arietinum* L.) has led the selectivity of cold tolerant lines to be very important. Considering the large number of chickpea germplasm stored in seed banks, the appropriate diversity is available for selection of tolerant varieties. However, achieving this goal needs to design an appropriate screening program. In this regard, the different studies have already conducted to investigate the possibility of selectivity of cold tolerant lines in the field, greenhouse and *in vitro* by using various selective criteria such as viability, assessing the damage, electrolyte leakage, TTC test and biochemical markers. Evaluating the results of these studies show that each of these methods has its advantages and disadvantages and accordingly it seems that in selectivity program in the early stages of selection that the number of samples is high, the techniques based on *in vitro* planting and TTC selection criteria are more appropriate for selectivity. In the later stages that the number of samples decreases due to the selection, the methods such as selection in the controlled conditions and selection in the field by using the selection criteria such as viability can be used, respectively.

Keywords:
Selectivity, Cold tolerance, chickpea.

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INTRODUCTION

Among the cereals, chickpea (*Cicer arietinum* L.) is of great importance in terms of the area under cultivation and production (Katz, 1952). Chickpeas as an excellent source of protein play an important role in human food diet, especially in the food diet of low income classes of society (Saxena and Singh, 1987). In the country, this crop is often cultivated in the late winter or early spring as dry farming, so the plant face an increase in temperature and drought stress during its vegetative period and especially during the reproductive period that in most cases this leads to decrease the yield (Nezami, 2002). In such circumstances, it seems that the change of its planting date from the spring planting to winter planting is an appropriate strategy to overcome this problem. However, the change of planting date from the spring to autumn requires to achieve the freezing and cold tolerant lines. Given the presence of more than 20 thousand different chickpea genotypes at International Center for Agricultural Research in the Dry Areas (ICARDA) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) research centers (Jana and Singh 1993), the need to design a selective program with proper efficiency for the selectivity for freezing and cold tolerance is felt. In this regard, the studies have done on the selectivity of tolerant varieties in greenhouse and field conditions *in vivo* and *in vitro* condition with the different selection criteria that then the capabilities, advantages and disadvantages of each one were evaluated.

Selection *in vivo*

Selection in the field conditions

The research to study the possibility of winter planting of chickpea in the Mediterranean areas and also the selection of tolerant variety began by (Singh, 1990) for the first time in 1977-1978 on the Kabuli type of chickpea. The results of this study showed that if the disease of blight is controlled, a significant increase in the yield as a result of winter planting will be obtained (Toker and Çanci, 2003). Between 1981 and 1988, 4535 chickpea germplasms were studied and 15 tolerant lines were selected (Singh, 1990). Despite the success in planting the winter chickpea in low to medium Mediterranean areas, the autumn-winter planting of chickpea in the medium to high areas has not been very successful (Toker and Çanci, 2003). In this regard, to evaluate the possibility of winter planting of chickpeas in Khuzestan a study was conducted to determine the appropriate planting date. The results of this research showed that for doing this type of cultivation in the studied area the varieties were needed that tolerated -15°C (Eberhart and Russell, 1966). Then, to evaluate the cold tolerance, 530 varieties of chickpea samples in the seed bank of Ahvaz University were evaluated during two agricultural years and 30 samples were selected as tolerant genotypes (Nezami *et al.*, 2012). Although the field studies are useful to select the tolerant genotypes, the effect of unwanted factors and also the need for high cost and time decrease its ability to select the cold tolerant lines in large scale.

Accordingly, the selection for cold tolerance in field conditions is very difficult, especially when the selection between many samples is considered (Singh, 1990), considering that in fact, the researcher does not have the ability to control the environmental variables such as temperature. A severe drop in temperature beyond the expectation or lack of appropriate temperature below zero for the selection of varieties during one agricultural year may completely destroy the results of an experimental period (Nezami, 2002). The crop damage due to other parameters such as high precipitation that can cause lodging, hailing, and occurrence of diseases such as blight and weed damage are other factors that can make the selection for tolerant varieties at farm level difficult (Eberhart and Russell, 1966; Singh, 1990). In addition, in such studies due to lack of uniformity of conditions during two experiments in two different locations or in two or more consecutive years, very different results can be obtained. For example, genotype ILC481

has been introduced sensitive to cold (Mafakheri *et al.*, 2010) in one study and cold tolerance in another study (Singh, 1990). However, despite these problems, this method can be used as the final stage in the regional experimental stage to introduce the cold tolerant varieties in a selective program.

The selection under controlled conditions in a greenhouse

Problems and limitations of selection under field conditions led the studies in controlled conditions to be considered. According to Nezami *et al.* (2012), the effectiveness of the method for selection of tolerant varieties from sensitive varieties in the chickpea was studied. Their results showed that the results of field experiments were consistent with the results of experiments under controlled conditions (Nezami, 2002). This method also increases the accuracy of selection in addition to decreasing the time needed for experiment compared to the field conditions. The advantages of using this method of selection include the simultaneous effect of morphological characteristics affecting the cold tolerance and the amount of tolerance of cells in the selection of tolerant varieties. However, the use of this method on a large scale is problematic because of the need to extensive greenhouse and cold storage facilities. Thus, the use of faster and less costly methods are felt to achieve this goal.

Selection *in vitro*

The results of various studies showed that the selection *in vitro* condition can be used to select the living and non-living stress tolerant plants (Scheuerell and Mahaffee, 2002). The use of this method leads to save the time and cost, in addition to eliminating the environment variables affecting the selection. Accordingly, in order to investigate the possibility of using this method for selecting the cold tolerant lines in chickpea, since 2012 the studies began in the University of Agriculture, in Islamic Azad University during which the selectivity of tolerant lines was studied during three stages of selection at the levels of micro-cutting of branch (Nayyar *et al.*, 2005), callus (Tomes, 1985; Kerem *et al.*, 2005) and embryogenic cell suspension culture (Tomes, 1985) in chickpea. In addition, in these studies the effectiveness of different methods of measuring the cold tolerance such as measuring the survival rate (Kerem *et al.*, 2005). Damage index (Nayyar *et al.*, 2005; Kerem *et al.,* 2005), Triphenyl Tetrazolium Chloride test (TTC test) (Tomes, 1985) and electrolyte leakage (Kerem *et al.*, 2005) and the molecular markers including the proline content and soluble protein (Kerem *et al.*, 2005) were evaluated. The results of these studies showed that despite the efficiency of using this method in the selection of tolerant lines, the cultivation conditions should be chosen carefully to achieve good results (Tomes, 1985). The results showed that some components in the growth medium such as sugar (sucrose) and growth regulators (Nayyar *et al.*, 2005) and glutamic acid (Tomes, 1985) are effective factor in increasing or decreasing cold tolerance in the sample studied. For example, the genotype 505 MCC that is sensitive to cold had more freezing tolerance in the medium containing glutamic acid (Table 1). On the other hand, the somaclonal phenomenon, especially the changes in ploidy levels in callus culture and embryogenic suspension culture, can be effective in the selection. Although the studies conducted in this regard on the varieties evaluated suggested little effect of these changes on the selection (Tomes, 1985). However, considering the whole authority of researcher to change the composition of medium and also the simple control of environmental conditions and freezing treatment, as well as the appropriate decrease in the experimental size compared to field and greenhouse conditions, it will be a good method for selecting the cold tolerant lines in chickpea, If its stages are closely designed.

Cold tolerance measurement criteria

Selection of appropriate 'selection criteria' to measure the level of cold tolerance plays an important role in the efficiency of the methods used in the selectivi-

Table 1. LT_{50} values in different genotypes and treatments studied by using different methods in chickpea

Experimental sample	Time	LT_{50} (°C)			
	Acclimation	M99	M505	M252	283
	day	CC	CC	CC	MCC
Embryogenic cell suspension	12	-12.4	-12.6	-10.5	-11.4
Callus (Wu *et al.*, 2004)	12	-13.2	-12.9	-12.4	-11.4
Callus (Nokhodchi *et al*., 2003)	24	-	-10.4	-12.8	-13.2
Micro-sample of branch *in vitro* condition (Wu *et al*., 2004)	20	-	-11.6	-13.8	-
Whole plant in the pot (Nezami, 2002)	24	-	-9	-10	-

* Media containing glutamic acid

ty of cold tolerant varieties. So far, in the studies of selectivity criteria for selectivity of cold tolerant varieties in chick pea the different selection criteria were evaluated that the efficiency of some of these methods had been determined. In this regard, the following selection criteria may be mentioned:

Survival rate

Determining the survival rate is the most common and most accurate method to measure the level of cold tolerance *in vitro* and *in vivo*. In this method, the survival rate of samples will be examined after cold treatment and the results are presented as LT_{50} (the temperature at which 50% of samples maintain their survival). The studies show the efficacy of this method in the selectivity studies for cold tolerant varieties in chickpea (Nayyar *et al.*, 2005; Nezami, 2002; Kerem *et al.*, 2005). Although this method has good accuracy compared to other methods, it requires more time and cost than other methods.

Damage assessment

Assessment of damage to the plant or tissue studied is one of the most common methods to determine the level of cold tolerance that is applicable *in vitro* and *in vivo* (Nayyar *et al.*, 2005; Singh, 1990). In this method, the assessment of damage is done by observation and grading. Despite the efficiency in selectivity of tolerant lines in one experiment, the method had no enough stability due to individual error of results among the different experiments.

Triphenyl Tetrazolium Chloride test (TTC test)

This test is based on enzymatic activity and a way to quantify the dead cells in the plant tissue exposed to stress (Pelah *et al.*, 2003). This method is an efficient method to determine LT_{50} and also a simple and least expensive and is conducted in a short time. In addition, there is also the possibility of automation. Despite the advantages, the dependence of this method on dehydrogenase activity can make errors in determining the survival rate in different treatments of freezing temperatures (Tomes, 1985).

Electrolyte Leakage (EL)

This method is based on measuring the amount of exiting electrolytes from damaged cells into the deionized water. The studies conducted to use this method for selecting the freezing tolerant varieties in chickpea showed that this method does not have the efficiency required for the selectivity of tolerant varieties due to the lack of generality of this characteristic for all cold sensitive varieties (Kerem *et al.*, 2005). In addition, it needs more samples than TTC test.

Biochemical markers

In the studies on selectivity in cold tolerance in chickpea, the proline content and soluble protein were evaluated. The studies have shown that in the studied varieties the proline content has a direct relationship with freezing tolerance while such relationship is not true regarding the soluble protein (Kerem *et al.*, 2005). However, due to the cumulative role of each of these factors in cold tolerance, the selectivity by reference to

the results of a test is not enough and some biochemical markers affecting the cold tolerance should be evaluated on the varieties studied simultaneously and the selection will be done based on the outcome of these studies.

CONCLUSION

Based on the results of different studies, it seems that each of these methods can be used in a part of selection program for the selectivity of cold tolerant varieties in chickpeas. In this regard, the selection in the cultivation condition *in vitro* and with the selection criteria for TTC test can be used in the early stages of selection for selecting tolerant lines among a large amount of varieties due to being fast and low cost. In the later stages of experiment, by decreasing the sample size, the possibility of selection in controlled conditions and in greenhouse and by measuring the survival rate and determining the amount of damage is provided to more accurately study the cold tolerance in the varieties and also study the morphological characteristics affecting it. Finally, the field experiments and evaluating the yield of best genotypes can be introduced for the winter planting of chickpea.

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