# MUTATION STUDIES IN CHICKPEA (*CICER ARIETINUM* L.). 2. SCREENING FOR STRESS TOLERANCE

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Studies were undertaken to induce genetic variability in Kabuli chickpea genotypes viz. ILC's 482, 3279 and 6104 to screen the $M_2$ segregating material and selected morphological mutants in $M_3$ and $M_4$ generation for identification of mutant(s) having increased level of resistance to Ascochyta blight and cold tolerance. The results of screening of $M_2$ segregating material indicated that mutagenic treatments were effective in inducing variability for Ascochyta blight resistance and cold tolerance. An ILC 482 mutant proved one of two sources of cold tolerance in the world chickpea germplasm screened at ICARDA during 1989-90. This is the first ever report of induction of cold tolerance in chickpea through induced mutations. The mutant will be of great value as a source of cold tolerance and for studying mechanisms of cold tolerance.

## INTRODUCTION

Kabuli chickpea is usually grown in the Mediterranean basin and the crop is normally planted in the spring (February to May), largely raised on residual soil moisture, since in the typical Mediterranean environment rainfall occurs almost exclusively in winter. Limited available moisture may restrict yields and delayed planting can also result in the reproductive phase of the growth (a particularly sensitive stage of phenological development) coinciding with increasing and possibly limited temperature (Hawtin and Singh, 1981).

The possibility that winter chickpea would make better use of available moisture and could be harvested earlier was pointed out by Hawtin (1975). Later research has shown that winter sown chickpea substantially outyield the spring sown crop. However, winter sown chickpea must possess resistance/tolerance to Ascochyta blight and cold.

Ascochyta blight disease caused by the fungus *Ascochyta rabiei* (Pass) Lab. is one of the most important disease of chickpea. The disease has been reported from almost all the major chickpea growing countries of the world. The yield loss caused by this disease can reach up to 100% during epiphytotic years. The development of disease is influenced by climatic factors which include rainfall, temperature and humidity. No suitable chemical control measure have so far been evolved. Foliar application with currently available fungicides has limited scope at present (Nene, 1984). Breeding for disease resistance using either conventional methods or through mutation induction is the best way to control Ascochyta blight. Mutation breeding has been successfully used for the induction of disease resistance. For example, golden mosaic virus in

*Phaseolus vulgaris* (Tulmann *et al.*, 1980); yellow mosaic virus in mungbean (Shakoor *et al.*, 1977; Haq and Shakoor, 1980) and Ascochyta blight in chickpea (Haq and Shakoor, 1980).

Kabuli chickpea genotypes ILC 482 and ILC 3279 along with other genotypes have been released in different countries of Mediterranean areas for winter sowing because they possess good levels of field tolerance to Ascochyta and high yielding potential. However, these lines are susceptible to cold. No single line resistant to both stresses is available in the world germplasm screened so far (ICARDA, 1988). Under these conditions, mutation breeding has a specific role to play, as it is possible to induce a specific improvement in a genotype without altering its otherwise acceptable phenotype. In Finland, mutants of *Brassica compestris oleifer* has been selected, surpassing the mother variety with regard to winter hardiness (Kivi *et al.*, 1974). An increased degree of winter hardiness has been observed in some mutants of *Hordeum vulgare* (Enchev, 1976) and *Triticum aestivum* (Khvostova, 1967).

Studies were undertaken to induce genetic variability in Kabuli chickpea genotypes viz. ILC's 482, 3279 and 6104 and screen the $M_2$ segregating material and selected morphological mutants in $M_3$ and $M_4$ generation for identification of mutant(s) having increased level of resistance to Ascochyta blight and cold tolerance. Genotypes ILC 482 and ILC 3279 have fairly good amount of field tolerance to Ascochyta blight and the induction of cold tolerance may help in combining resistance to both stress conditions.

## MATERIALS AND METHODS

**Screening for Ascochyta blight resistance:** The procedure of raising $M_1$ and $M_2$ generations are given in the papers 1 and 3. For screening against Ascochyta blight resistance, following material was screened at International Centre for Agricultural Research in the Dry Areas (ICARDA) near Aleppo, in the Ascochyta blight screening nurseries raised during 1987-88, 1988-89 and 1989-90 crop seasons, respectively.

**1987-88 season**

A set of $M_1$ seed from all the surviving plants of ILC 482.

**1988-89**

i. A set of $M_1$ seed from all the surviving plants of ILC 6104.
ii. Fifteen promising morphological mutants derived from ILC 482 and ILC 3279 along with parental lines and a susceptible check.

**1989-90 season**

i. Seed obtained from $M_1$ plants of ILC 3279 was not sufficient to make a second set. To make available sufficient seed for screening against stress conditions, an additional cycle of seed treatment, raising of $M_1$ and $M_2$ generations was practiced in case of ILC 3279. The dose levels used were same as used earlier (40, 50 and 60 kR gamma irradiation, and 0.1% and 0.2% EMS). From the $M_2$ material planted during 1988-89, seed were collected from each plant by single seed descent (SSD) method and were bulked dosewise. Two such sets were prepared and one set was planted in the Ascochyta blight screening nursery for the identification of resistant/tolerant mutant(s).
ii. A set of 151 morphological mutants from ILC 482, ILC 3279 and ILC 6104 in $M_4$ generation and the three parent lines.

Each season Ascochyta blight screening nursery was planted in mid-November. The susceptible check ILC 263 was planted after every 9 test rows throughout the field and as a strip all-round the field. The nursery was inoculated with a mixture of 4 races (1, 2, 3, 4) multiplied from the laboratory cultures in mid-February, March and April. The mist irrigation system was used to create the humidity in the field for the development of the disease. Data for blight resistance were recorded using a 9-point scale devised by Singh *et al.* (1981) for scoring the material under field conditions. The scale is described as follows:

1 = No lesions visible on any plant (highly resistant).

3 = Lesions visible on less than 10% plants, no stem girdling (resistant).

5 = Lesions visible on up to 25% plants, stem girdling on less than 10% plants but little damage (tolerant).

7 = Lesions present on most plants, stem girdling on less than 50% plants, resulting in the death of a few plants and causing considerable damage (susceptible).

9 = Lesions profuse on all plants, stem girdling present on more than 50% plants and death of m ost plants (highly susceptible).

**Screening for cold tolerance:** For cold tolerance studies during 1988-89 and 1989-90 growing seasons, the following material was tested in cold tolerance nurseries:

**1989-89 season**

Fifteen important morphological mutants from ILC 482 and ILC 3279 and the two parent lines.

**1989-90 season**

i. 'A set of 151 true breeding morphological mutants from ILC 482, ILC 3279 and ILC 6104 in $M_4$ generation and the three parent lines.

ii. A second set of seed collected by SSD method from $M_2$ generation of ILC 3279.

Each season, cold tolerance nursery was planted in the first week of October in unreplicated, 2 m, 2 row plots with inter- and intra-row spacing of 45 and 10 cm, respectively. The susceptible check (FLIP 81-61 C) was sown after every nine test lines. The first irrigation was given immediately after the planting to ensure rapid emergence and the second after 4 weeks. The crop was protected from Ascochyta blight by periodic spraying of fungicide chlorothalonil (tetrachloroisophthalonitrile) at the rate of 40 kg a.i. $ha^{-1}$. The experimental area was hand weeded. Visual cold tolerance ratings were assigned after the susceptible checks were killed, on a 1-9 scale described by Singh *et al.* (1989). The scale is described as follows:

1 = No visible symptoms of damage.

2 = Highly tolerant, up to 10% leaflets show withering and drying, no killing.

3 = Tolerant, 11 to 20% leaflets show withering and up to 20% branches show withering and drying, no killing.

4 = Moderately tolerant, 21 to 40% leaflets and up to 20% branches show withering and drying, no killing.

5 = Intermediate, 41 to 60% leaflets and 21 to 40% branches show withering and drying, up to 5% plant killing.

6 = Moderately susceptible, 61 to 80% leaflets and from 41 to 60% branches show withering and drying, 6 to 25% plant killing.

7 = Susceptible, 81 to 99% leaflets and 61 to 80% branches show

**Table 1. Field reaction of $M_2$ generation of ILC 482 to Ascochyta blight at ICARDA, Aleppo, Syria during 1987-88**

| Genotype | Treatment | Number of plants studied | Number of plants with a disease rating of | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 |
| ILC 482 | Control | 269 | 0 | 0 | 0 | 0 | 0 |
| | 40 kR | 13440 | 0 | 0 | 7 | 15 | 29 |
| | 50 kR | 7900 | 0 | 0 | 2 | 8 | 17 |
| | 60 kR | 9288 | 0 | 0 | 3 | 10 | 22 |
| | 0.1% EMS | 5936 | 0 | 0 | 2 | 5 | 14 |
| | 0.2% EMS | 3564 | 0 | 0 | 4 | 11 | 26 |
| Total | | 40378 | 0 | 0 | 18 | 49 | 108 |

withering and drying, 26 to 50% plant killing.

8 = Highly susceptible, 100% leaflets and 81 to 99% branches show withering and drying, 51 to 99% plant killing.

9 = 100% plant killing.

## RESULTS AND DISCUSSION

### Screening for Ascochyta blight resistance

**1987-88 season:** The results of field reaction of $M_2$ generation of ILC 482 to Ascochyta blight are presented in Table 1. Out of a

**Table 2. Field reaction of $M_2$ generation of ILC 6104 to Ascochyta blight at ICARDA, Aleppo, Syria during 1987-88**

| Genotype | Treatment | Number of plants studied | Number of plants with a disease rating of | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 |
| ILC 6104 | Control | 285 | 0 | 0 | 0 | 0 | 0 |
| | 40 kR | 1790 | 0 | 0 | 0 | 0 | 2 |
| | 50 kR | 1205 | 0 | 0 | 0 | 0 | 1 |
| | 60 kR | 1042 | 0 | 0 | 0 | 0 | 4 |
| | 0.1% EMS | 994 | 0 | 0 | 0 | 0 | 5 |
| | 0.2% EMS | 1530 | 0 | 0 | 0 | 0 | 4 |
| Total | | 6846 | 0 | 0 | 0 | 0 | 16 |

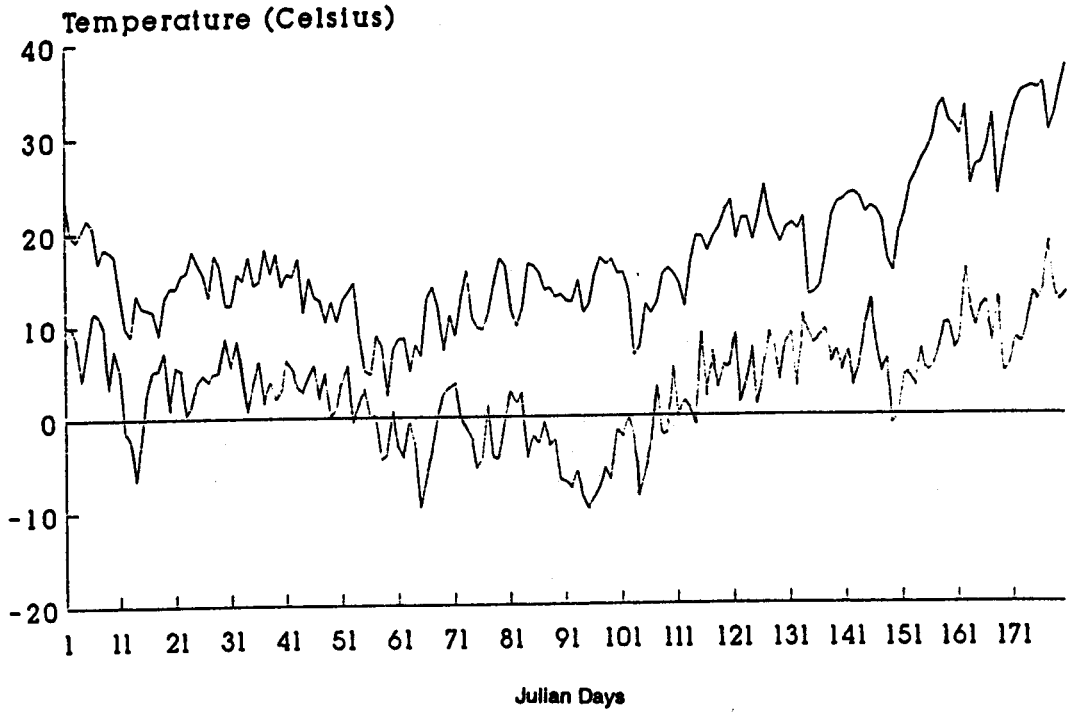


Fig. 1. Minimum and maximum daily temperatures at Tel Hadya: 1.11.88-29.4.89.

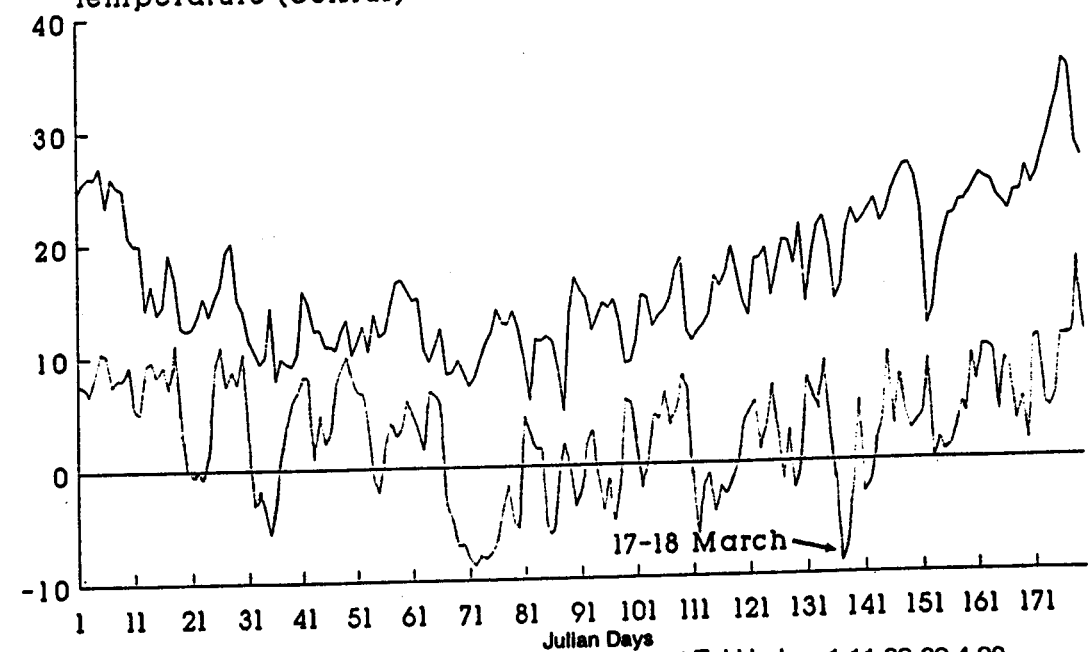


Fig. 2. Minimum and maximum daily temperatures at Tel Hadya: 1.11.89-29.4.90.

**Table 5. Cold tolerance of $M_3$ generation of ILC 3279 at ICARDA, Aleppo, Syria during 1987-88**

| Genotype | Treatment | Number of plants studied | Number of plants with a cold rating of | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| ILC 3279 | Control | 92 | 0 | 0 | 0 | 0 | 0 | 0 | 92 |
| | 40 kR | 5234 | 0 | 7 | 98 | 181 | 144 | 28 | 4774 |
| | 50 kR | 5424 | 1 | 5 | 105 | 278 | 208 | 31 | 4796 |
| | 60 kR | 3695 | 0 | 0 | 16 | 114 | 39 | 3 | 3523 |
| | 0.1 EMS | 2572 | 0 | 0 | 7 | 12 | 16 | 3 | 2534 |
| | 0.2% EMS | 2885 | 0 | 0 | 2 | 13 | 14 | 6 | 2850 |
| Total | | 19902 | 1 | 12 | 228 | 598 | 421 | 71 | 18569 |

maturing material at Tel Hadya. Late planted genotypes were less affected. In the cold tolerance nursery, all plants showing varying levels of tolerance were killed. The only surviving entry was mutant line No. 16119, a derivative of ILC 482. The mutant gave 3 rating. The results confirmed the cold tolerant nature of this mutant exhibited during 1988-89 (Table 3). It may be mentioned here that from the world germplasm of Kabuli chickpea screened in the cold tolerance nursery during 1989-90, the only surviving entry was ILC 3470 and was rated as 5. The mutant No. 16119 and the germplasm line ILC 3470 will be of a great value as a source of cold tolerance and for studying the mechanisms of cold tolerance. This is the first ever report of induction of cold tolerance in chickpea through induced mutations.

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