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Full Length Research Paper

Effects of temperature and water stresses on germination of some varieties of chickpea (*Cicer arietinum*)

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Chickpea production did not progress, in spite, of intensification of agricultural practices. Drought and thermal stresses were the major factors that decreased yield when the crop is generally sown in spring. Nevertheless, winter sowing has opened new opportunities for increasing chickpea production in arid areas. This culture is based on two essential factors: (i) preclude rainfall during the flowering and fruiting period (April/May) after a beneficial one in January and February for a good crop development and (ii) reducing the risk of infection by *Ascochyta rabiei* (considered as the main pathogen of chickpea) whose development is limited in arid area. The influence of temperature on seeds germination of the four varieties (Ghab4, Neyra, Kasseb and Chetoui) has been evaluated with seven temperatures (5, 10, 15, 20, 25, 30 and 35°C) and the effect of drought stress has been determined with seven water stresses induced by different solution of PEG 4000 (0, 1, 2, 3, 4, 5 and 10 g/L). During germination, the tested varieties presented differential sensitivity to thermal stress. The Chetoui and Ghab4 varieties were more tolerant, to cold temperature, than the other varieties. Osmotic potential induced by PEG 4000 had significant effects on seeds germination. Chetoui and Ghab4 were the best tolerant to drought stress. Therefore, we opted for the Chetoui variety that better meets the conditions of stresses induced by low temperatures and water deficit. This best performing variety must have, throughout their development cycle, been tolerant to environmental stresses; which allows us to obtain early tools for discriminative selection between chickpea genotypes.

Key words: Chickpea, thermal stress, drought stress, germination.

INTRODUCTION

Chickpea is an important food legume that can be grown under a wide range of environments. It is well known as a drought tolerant crop that performs well in low input agriculture and is considered as the second important leguminous plant in the world. It characterizes at least 33 countries where it covers about 15% (10 millions of ha) of cultivated areas and represents 14% of the production of leguminous seeds (FAO, 1994). Chickpea are developed in tropical, subtropical and dry Mediterranean regions. Nevertheless, it needs wet soil within semi-arid to subhumid bioclimate, and requires a low relative humidity for seeds maturity. The culture of this leguminous plant is encountered in temperate regions for the Kabuli type, whereas, the Desi type is characteristic of semi-arid tropics and India (Malhotra et al., 1987). The agri-food importance of chickpeas is related to its great richness in proteins: 25.3 to 28.9% (Hulse, 1991). This leguminous plant is considered as an alternative source of proteins for human nutrition (Tejera et al., 2006).

Germination is considered as a critical step in plants

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Table 1. Phenological characteristics of the studied varieties.

Variety	Type	Origin	Country	Weight of 100 seeds (g)	Sowing season
Chetoui	Kabuli	INRAT	Tunisia	30.80	Winter
Kasseb	Kabuli	INRAT	Tunisia	32.25	Winter
Neyra	Kabuli	INRAT	Tunisia	36.80	Winter
Ghab4	Kabuli	ICARDA	Syria	28.60	Winter

development cycle. In fact, it controls the onset of seedling, its connection to the environment and probably its subsequent productivity. One of the environmental constraints for the germination is the temperature that negatively affects the seed germination (Verma et al., 2010). In fact, the optimum temperature for maximum final germination is between 10 and 15°C (Ellis et al., 1986). Clarke et al. (2004) showed that low temperatures represent a major constraint for improving the yield of chickpea in numerous regions of the world.

Drought plays an important role not only in determining germination rates, but also affects seedling development (Van den Berg and Zeng, 2006). Establishment of seedling is the most critical life stage in dry environment, and the lack of soil moisture is often the main reason for seedling mortality (Schütz et al., 2002). With increasing drought stress, water availability decreases, adversely changes the percentage of germination and growth of seedling. The germination in nine genotypes stopped completely at -0.8 MPa. All factors were significantly affected by osmotic potential; results of decreasing the osmotic potential led to significant reduction in germination percentage (Yücel et al., 2010).

Osmotic solutions are used to impose water stress under "*in vitro*" conditions (Kaya et al., 2006). For example, Bibi et al. (2009) used the PEG 4000 to induce water stress of germination from seedling of chickpea genotypes. Verslues et al. (1998) reported that PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low-Mr osmotica, which infiltrate the cell wall with solute. Moreover, Smita and Nayyar (2005) also observed reduction in root length of *Cicer arietinum* under water stress and detrimental effects could be due to reduction in root-hair diameter as well as distortion and plasmolysis, thus rendering the uptake of available water by roots (Zahran and Sprent, 1986).

The comprehension of physiological mechanisms involved in the resistance to water and thermal stresses, is essential for the selection of resistant varieties of chickpea. This work aimed to undertake a behavioral study of some varieties of chickpea confronted with osmotic and thermal stresses at the germination stage.

MATERIALS AND METHODS

The plant materials consist of four varieties of chickpea seeds from Syria (01 variety) and Tunisia (03 varieties). Table 1 shows the main genotypic and phenotypic characteristics of the used varieties. The seeds of different varieties were disinfected in saturated solution of calcium hypochlorite for 5 min, and then rinsed thoroughly with sterile distilled water. After that, they were placed in aerated distilled water for 1 h. Finally, the seeds were germinated in Petri dishes. The germination percentage and the T_{50} (time required for 50% of seeds germination) were determined using radicle protrusion (3 mm) as a criterion for germination.

The influence of temperature on seeds germination of the four varieties (Ghab4, Neyra, Kasseb and Chetoui) was analyzed by placing the seeds at temperature ranging from 5 to 35°C. The germination tests were conducted in four replications, each with 25 seeds placed in Petri dishes (25 seeds per dish totalizing 100 seeds per treatment and per variety). The Petri dishes were lined by two layers of filter papers, at the top and base, and placed in germination chamber under controlled conditions. The germination lasted from 7 to 14 days.

The water potential influence on germination medium was studied at 20 ± 5°C. The germination tests were applied in three replications of 30 seeds placed in Petri dishes (10 seeds per dish totalizing 90 seeds per treatment and per variety). The Petri dishes were lined by two layers of filter papers, at the top and base, soaked by Polyethylene Glycol solution (PEG 4000) with increasing concentrations; 0, 1, 2, 3, 4, 5 and 10 g/L. All were placed in germination chamber under controlled conditions. The germination percentage measurements were taken every 2 h.

Statistical analysis

The analysis of variance (ANOVA 1) was used to determine whether a given factor, such as the variety, has a significant effect on seed germination. For comparison of means, the Tukey HSD test was used, and gave a significant differences of these data at $p<0.05$.

RESULTS AND DISCUSSION

Temperature effect on germination

Whatever the concerned variety was, all the seeds were able to germinate on a wide thermal range (15 to 35°C). The thermal optimum was about 20°C (Ghab4, Chetoui) and 25°C (Neyra, Kasseb) (Figure 1). At optimum temperature, the latency time was about 24 h and the T_{50} varied from 40.0 h (Ghab4) to 90.9 h (Neyra) (Figure 2). Except the seeds lot of Kasseb variety, 80 to 100% of this variety seeds germinated in 7 days between 10 and 30°C (Figure 1). Our results agree with many other research works. In two populations, final percentage germination was affected by temperature. The optimum constant temperature for maximum final germination was between

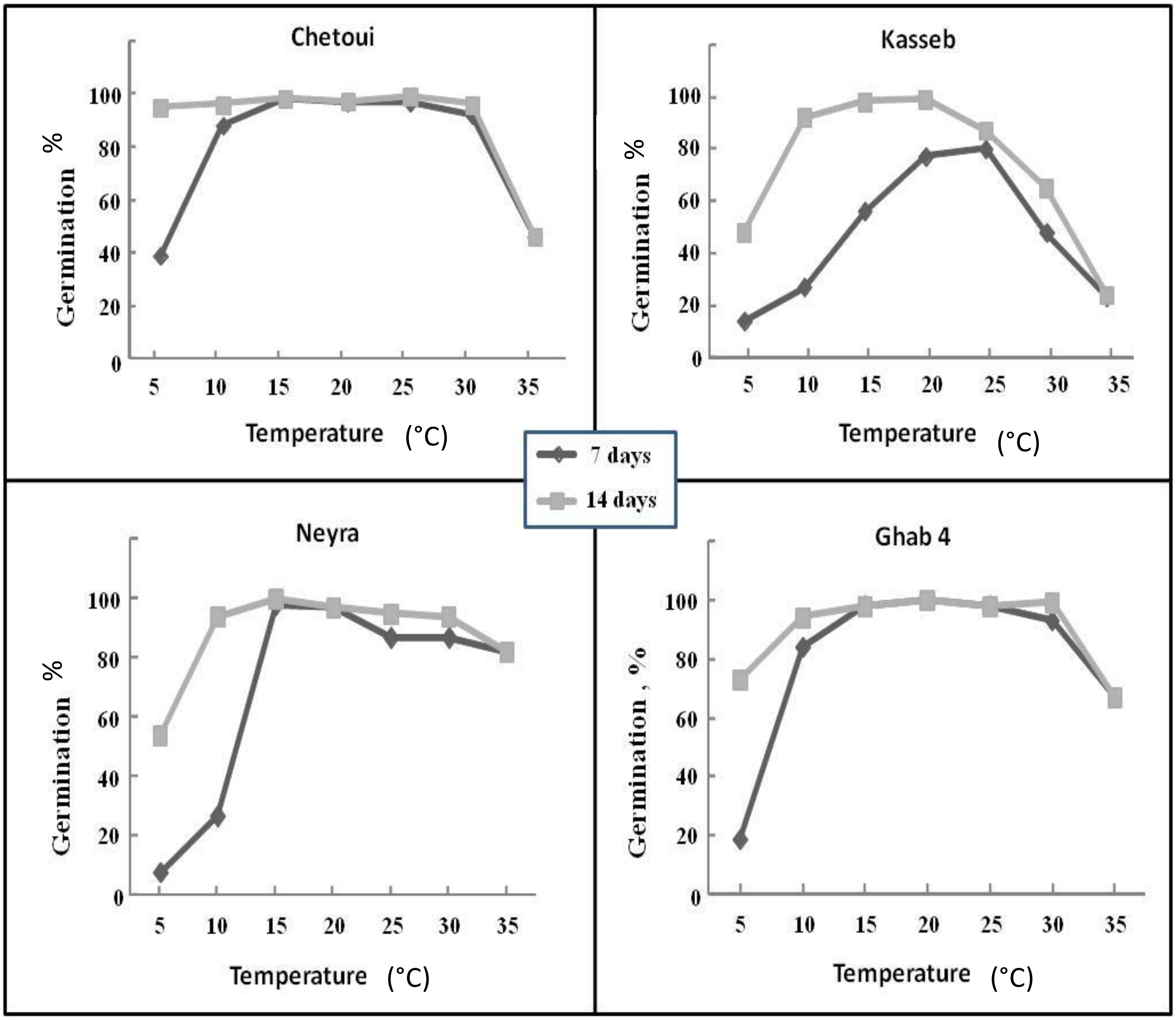

Figure 1. Percentages of seed germination obtained after 7 and 14 days for the 4 varieties placed at different temperatures. Means of 100 replications.

10 and 15°C, cooler than the optimum temperature for the rate of germination (Ellis et al., 1986). Therefore, Singh and Diwakar (1995) recommended an optimal temperature of 28 to 33°C for the germination of chickpea seeds. The Chetoui and Ghab4 varieties germinated faster than those of Neyra and Kasseb (Figure 2). In addition, the seeds of variety Chetoui seemed to be less sensitive to cold temperature than those of the other varieties (Figures 1 and 2).

Water stress effect on germination

Several works showed that polyethylene glycol (PEG) is an indicator of water stress for *in vitro* culture (Turhan and Baser, 2004; Bibi et al., 2009). The medium water potential impact on the germination was studied at 20°C (germination optimal temperature). Figure 3 shows the germination percentage obtained after 168 h of sowing in presence of PEG 4000 solution. Seeds placed on distilled water served as a control.

The germination percentage varied from 70 to 97% on control medium, respectively, for the Kasseb and Ghab4 varieties. In presence of low amount of PEG (1, 2, 3, 4 g/L), the germination rate of Chetoui and Ghab4 varieties was not significantly affected (Figure 3). This pleads in favor of a resistance of varieties studied to moderate water stress. On the other hand, this germination rate of Kasseb and Neyra varieties was significantly affected at first hour of germination.

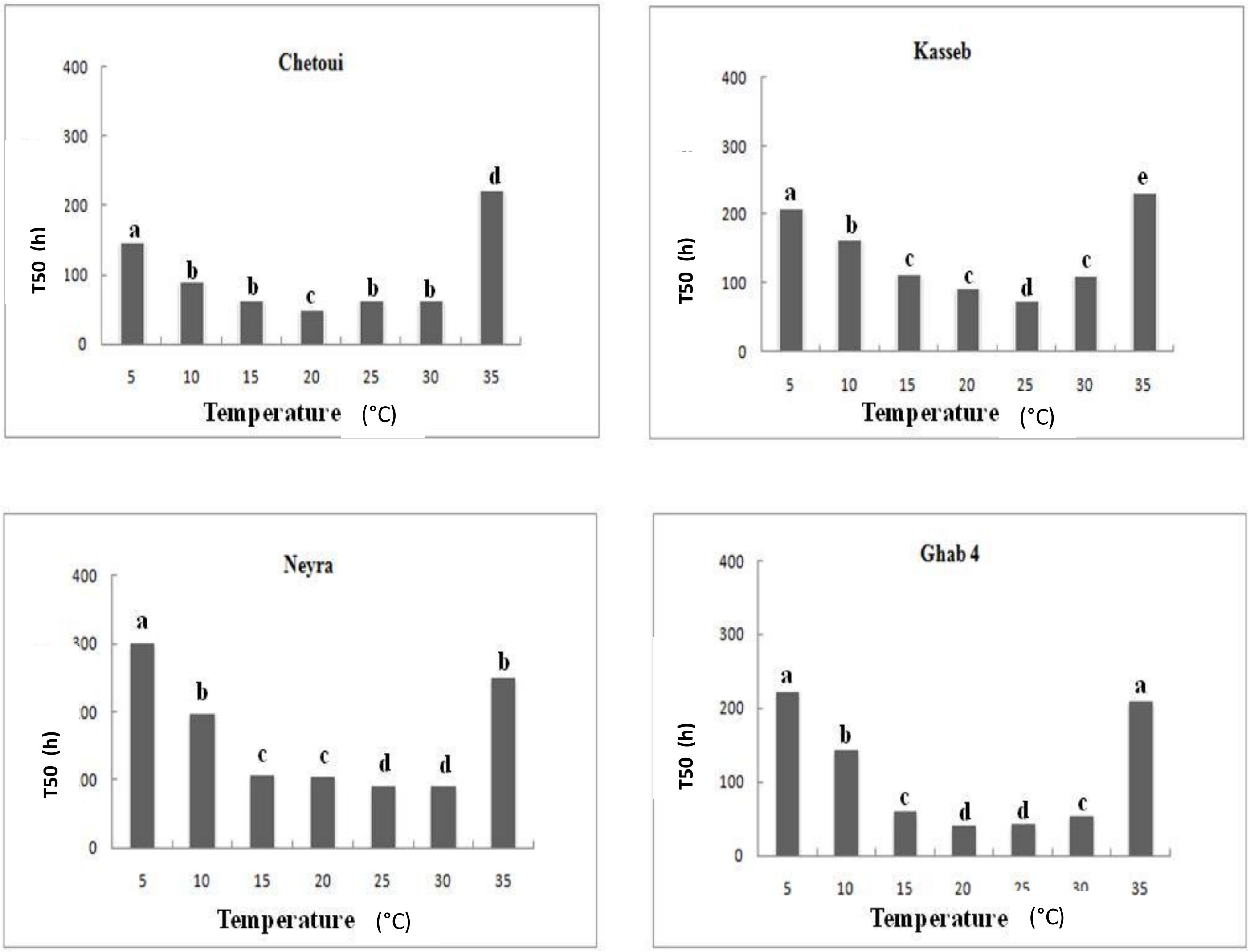

Figure 2. Influence of the temperature on the time to obtain 50% of germination (T_{50}) with the seeds of the 4 varieties. Means of 100 replications. For the comparison of means, the Tukey HSD test was used. Differences of values were significant at $p<0.05$.

At the high PEG concentration (10 g/L), the inhibition of seeds germination was highly significant for all the varieties studied. This decrease of germination percentage was more pronounced for the Kasseb variety (51%). Similar results was obtained by Kouakou et al. (2008) who showed that the germination rates varied between 92.57% for control plants and 15.33% for the medium which was mostly concentrated in PEG (50 g/L) among cotton plant. In general, the germination rate decreased considerably with the increase of osmotic substrate water stress (Djibril et al., 2005).

The results show that the germination of seeds of Kasseb and Neyra varieties took place quickly. As well, the variation of PEG medium concentration did not significantly affects latency time than the Ghab4 variety treated with PEG 10 g/L (Figure 3). For lower PEG dose (1, 2, 3, 4 and 5 g/L), the variation of T_{50} varied from significant to highly significant by comparison of the treated seeds to control. However, the T_{50} highly increased in the presence of PEG (10 g/L); this variation is highly significant (Figure 4). The observed differences could be explained by the fact that PEG acts as an osmotic factor by dehydrating the seeds (Almansouri et al., 2001). This may inhibit the hydrolysis of the seeds nutrient reserves and thus the germination (Dirik, 2000). It is worth noting that germination and seedling emergence were strictly affected by PEG.

Conclusion

This work is useful for plant breeders by proposing early test for screening new or existing genotypes for their tolerance to drought and thermal stresses. Consequently,

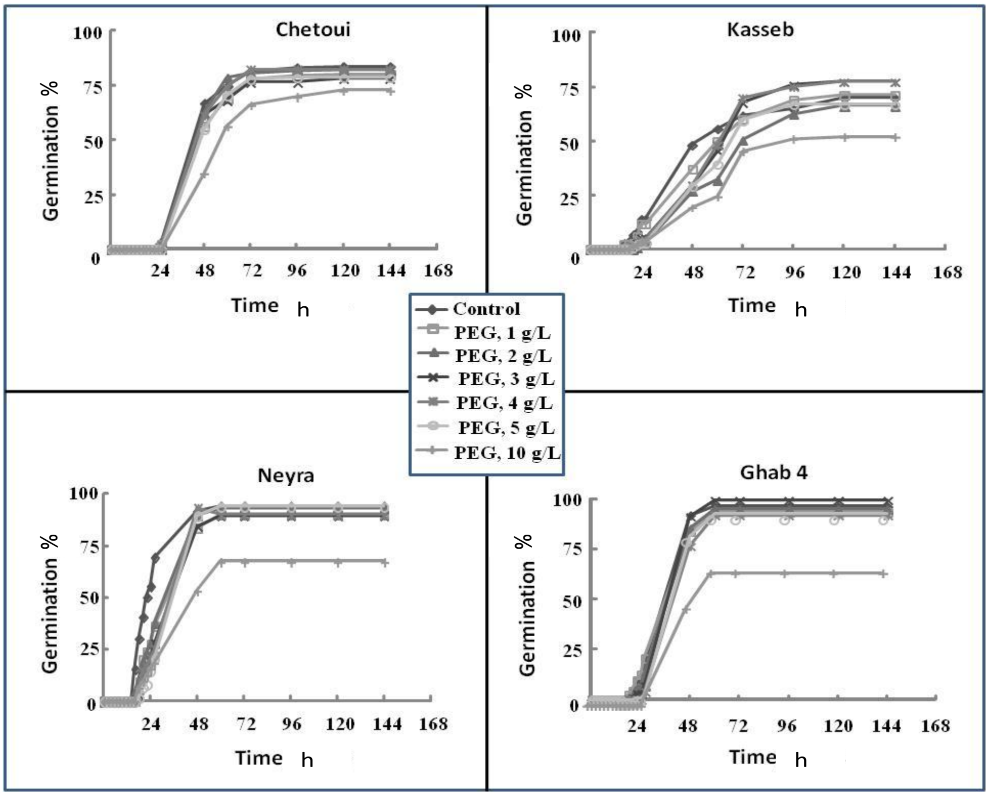

Figure 3. Variation of the percentage of germination for the 4 varieties of chickpea (Chetoui, Kasseb, Neyra et Ghab4) in function of different concentrations of PEG. Means of 90 replications.

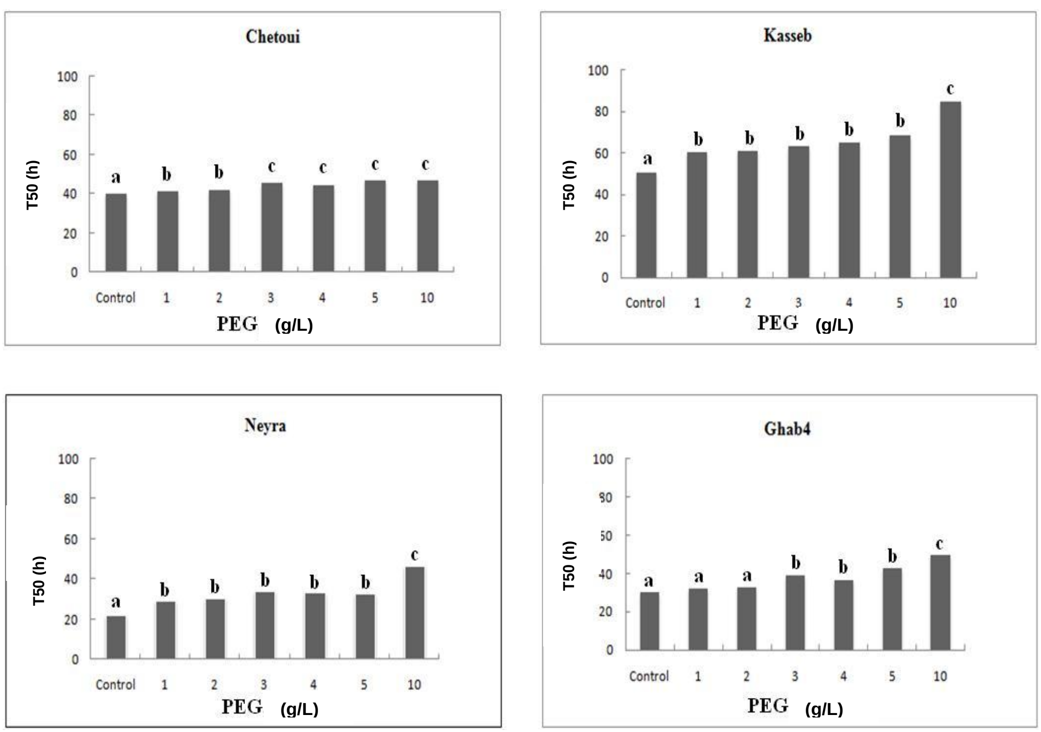

Figure 4. Variation of necessary time for the germination of 50% of seeds (T_{50}) for the 4 varieties of chickpea in function of different concentrations of PEG. Means of 90 replications. For the comparison of means, the Tukey HSD test was used. Differences from control values were significant at $p<0.05$.

in germination, there were differences among genotypes. The assessment of the effect of these stresses on the germination parameters in four chickpea varieties allowed us to conclude that the considered parameters were affected by drought and thermal stresses. The strong variation of temperature had significant effects on seeds germination. From this perspective, Chetoui and Ghab4 varieties can be evaluated as the most tolerant varieties to temperature stress than the others at germination stage. Osmotic potential with PEG 4000 affected significantly seed germination. Chetoui and Ghab4 showed that these varieties are the most tolerant to drought stress. The idea was to search for winter varieties tolerant to water stress in order to introduce them in arid areas. Therefore, we opted for the Chetoui varieties which meet well the conditions of stresses induced by low temperatures and drought. This variety should be observed in fields of drought conditions; it is recommended as a tolerant variety.

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