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Determination of an optimal priming duration and concentration protocol for pepper seeds (Capsicum annuum L.)

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ABSTRACT

Seed priming is a simple pre-germination method to improve seed performance and to attenuate the effects of stress exposure. The objective of this study was to determinate an optimal priming protocol for three pepper cultivars (Capsicum annuum L.): 'Beldi', 'Baklouti' and 'Anaheim Chili'. Seeds were primed with three solutions of NaCl, KCl and CaCl₂ (0, 10, 20 and 50 mM) for three different durations (12, 24 and 36h). Control seeds were soaked in distilled water for the same durations. After that, all seeds were kept to germinate in laboratory under normal light and controlled temperature. Results indicated that priming depends on concentration, duration and cultivar. The best combinations that we obtained were: KCl priming (10 mM, 36h) for 'Beldi' cultivar, CaCl₂ priming (10 mM, 36h) for 'Baklouti' cultivar and finally NaCl priming (50 mM, 24h) for 'Anaheim Chili' cultivar. Generally, priming had an effect on total germination percentage, mean germination time, germination index and the coefficient of velocity compared to control seeds. The beneficial effect of seed priming could be used for improving salt tolerance on germination and early seedling growth for pepper cultivar.

Key words: priming protocol, mean germination time, germination index, coefficient of velocity, 'Beldi', 'Baklouti', 'Anaheim Chili'

IZVLEČEK

DOLOČITEV OPTIMALNEGA ČASA PREDOBDELAVE SEMEN IN KONCENTRACIJSKI PROTOKOL ZA IZBOLJŠANJE KALITVE SEMEN PAPRIKE (*Capsicum annuum* L.)

Predobdelava semen pred kalitvijo je preprosta metoda za izboljšanje kalitve semen in zmanjšanje učinkov izpostavljenosti stresu. Predmet raziskave je bil izdelava optimalnega protokola predobdelave semen za tri sorte paprike (Capsicum annuum L.): 'Beldi', 'Baklouti' and 'Anaheim Chili'. Semena so bila pred kalitvijo obdelana z raztopinami NaCl, KCl in CaCl₂ (0, 10, 20 in 50 mM) v treh različnih trajanjih (12, 24 in 36h). Kontrolna semena so bila za enak čas namočena v destilirano vodo. Po predobdelavi je bil z vsemi semeni narejen kalitveni test v laboratoriju pri sobni svetlobi in nadzorovani temperaturi. Rezultati so pokazali, da je učinek predobdelave odvisen od koncentracije in vrste raztopin, trajanja predobdelave in sorte. Najboljša kalitev je bila dosežena pri predobdelavi s KCl (10 mM, 36h) za sorto 'Beldi', s CaCl₂ (10 mM, 36h) za sorto'Baklouti' in z NaCl (50 mM, 24h) za sorto 'Anaheim Chili'. V splošnem je imela predobdelva v primerjavi s kontrolo učinek na odstotek kalitye, povprečen čas kalitye, kalityeni indeks in koeficient hitrosti kalitve. Blagodejni učinek predobdelave semen bi lahko uporabljali za izboljšanje tolerance kalitve na slanost in hitrejšo zgodnjo rast sadik različnih sort paprike.

Ključne besede: protokol predobdelave, povprečni čas kalitve, kalitveni indeks, koeficient hitrosti kalitve, *Capsicum annuum* 'Beldi', 'Baklouti', 'Anaheim Chili'

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1 INTRODUCTION

Priming involves exposing seeds to an external water potential low enough to restrict germination and yet permit pre-germinative physiological and biochemical activities (Bradford, 1986).

Priming treatment such as NaCl, KCl and CaCl₂ can increase and accelerate seed germination and seedling emergence under salt stress for many species. It was effective in alleviating the effect of salt stress on wheat plants through altering the levels of different plant phytohormones (Igbal et al., 2006). Generally, primed seeds germinate faster and more uniformly than unprimed seeds of the same lot (Soughir et al., 2013; Elouaer and Hannachi, 2012). The difference between primed and control seeds are greater in arid and semi-arid conditions when there are more exposure to difficult environmental conditions such as salinity or water deficit. According to Levitt (1980), plants must be exposed to salt stress to develop salt tolerance. This means that seeds should germinate in saline conditions. For example, it has been shown that NaCl priming could be used as an adaptation method to improve salt tolerance of some vegetable seeds (Cayuela et al., 1996; Cano et al., 1991). Also, Khan et al. (2009) observed that NaCl priming of seeds improved seedling vigor of pepper under salt stress conditions. Similarly, Soughir et al. (2013) demonstrated that seed priming could be used successfully to ameliorate the emergence of fenugreek plants cultivated in pots under salt stress. So, the optimization of priming technique is important to determinate the optimal soaking duration and the optimal concentration of priming agent.

Hot pepper (Capsicum annuum L.) is an important vegetable in Tunisia. It is an excellent source of vitamin but the production of this crop is affected by environmental stress such as drought, salinity, chilling and heat (Almansouri et al., 2001). In fact, salinity can affect pepper germination and seedling growth either by creating osmotic pressure that prevent water uptake or by toxic effects of sodium and chloride ions (Hopper et al., 1979). So, the need to develop new cultivars with higher salt tolerance has increased strongly. Certainly, priming is one of the cheapest methods which improve seeds performance but seeds priming response depends on many factors such as cultivar, osmotic potential, temperature and duration of the treatment.

This is why the mean objective of this study is to evaluate the effect of soaking pepper seeds in three different solutions (NaCl, KCl and CaCl₂) with three different concentrations and durations on germination parameters.

2 MATERIAL AND METHODS

This study was conducted in the research unit "conservation and valorization of vegetal resources by creation of botanical garden", of the High Institute of Agriculture (Chott Mariem, Tunisia).

Plant material was composed of three cultivars ('Beldi', 'Baklouti' and 'Anaheim Chili') of pepper seeds (Capsicum annuum L.). These cultivars are the most cultivated in Tunisia. Seeds were initially sterilized with a 1 % solution of sodium hypochlorite for 20 min and then rinsed 3 times with distilled water.

Three solutions (NaCl, KCl and CaCl₂) were used as priming agents with 3 concentrations for each solution (10, 20 and 50 mM). Seeds from each cultivar were soaked in every solution for 3

different durations (12, 24 and 36 hours) at 25°C.Control seeds were soaked in distilled water for the same durations. At the end of these durations, all seeds were removed and dried. Following this, every 20 seeds were placed between two filter papers and set to germinate in 90 mm diameter Petri dishes. Seeds were kept to germinate in laboratory under normal light and controlled temperature (25 °C). Each treatment includes 10 Petri dishes in a completely randomized design.

For the next 7 days, filter papers were moistened with 10 ml of distilled water and the number of germinated seeds was counted daily. Based on the result of the higher germination percentage that we have obtained, we fixed the best priming

concentration for each cultivar and we repeated the germination experience for 7 other days (seeds of each cultivar were primed with the concentration which gave the best germination percentage) to choose the best combination between priming duration and concentration for each cultivar. Parameters measured in this stage are given below:

Total germination (GT) was measured on the final days using the formula GT (%) = (total number of germinated seeds/ total seed) x 100.

Mean germination time (MGT) was calculated according to the equation: MGT = Σ Dn / Σ n (Moradi *et al.*, 2008), where (n) is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination Index (GI) was calculated as described in the Association of Official Seed Analyst (1983)by following $GI = \sum (Gt / Tt)$ where Gt is the number of seeds germinated on day t and Tt is the number of days. The coefficient of velocity (CV) was calculated using the following formula (Scott et al., 1984): CV= 100 [\sum Ni / \sum Ni Ti]. All the data were subjected to an analysis of variance using SPSS 13.0 software and the difference between means were compared by a Duncan multiple range test at 5% level of probability (Table 1). For each cultivar, every studied parameter (MGT, GI, and CV) is compared alone (Duration and priming solution are the variables). Means of the same column, followed by the same letter, are not significantly different.

3 RESULTS AND DISCUSSIONS

According to the results, all studied traits were affected by the experimental factors. In fact, there was difference between control seeds (soaked in distilled water) and primed seeds. The highest final germination percentage obtained for 'Beldi' cultivar (95 %) belonged to KCl priming (10 mM) at 36 hours (Figure 1). Concerning 'Baklouti' cultivar, the highest final germination percentage (91 %) belonged to CaCl₂ priming (10 mM) at 36 hours (Figure 5) and finally the highest final germination percentage for 'Anaheim Chilli' cultivar (97 %) belonged to NaCl priming (50 mM) at 24 hours (Figure 9). In general, priming with KCl, CaCl2 and NaCl proved superiority over control but it depends on variety, duration and also concentration of priming agent (Figure 1-9). For example, in 'Beldi' cultivar, we obtained 95 % of germination for KCl primed seeds with 10 mM at 36 hours but germination regressed to 65 % when osmotic potential increased to 20 mM (for the same duration). The three cultivars responded differently to priming agent but also to duration and concentration. The same result was obtained by using CaCl₂ priming in 'Baklouti' cultivar; the germination percentage of primed seeds decreased from 91 % to 60 % by increasing solution concentration from 10 mM to 20 mM when seeds were soaked for 36 hours.

Priming with KCl, CaCl₂ or NaCl in pepper seeds had better effects on seed germination compared with control seeds. These ameliorations in primed seeds might be due to pre-germinative metabolic activities which prepare seeds for radicle protrusion. It can also be the results of metabolic repair processes or osmotic adjustments during priming (Bray *et al.*, 1989).

This good response of seed priming is coherent with the findings of Coolbear and Grierson (1979) who declared that higher germination rate in primed seeds was a result of higher levels of nucleic acid found in tomato cultivars.

According to table 1, the maximum value of coefficient of velocity (CV) was obtained for 'Anaheim Chilli' cultivar (26.8) after soaking seeds for 24 hours in NaCl solution (50 mM). For 'Beldi' cultivar, the maximum value of coefficient of velocity (21, 5) was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). Finally, for 'Baklouti' cultivar, the highest coefficient of velocity (20, 8) was obtained after soaking seeds for 36 hours in CaCl₂ solution (10 mM). Priming affects the lag phase in germination and causes early DNA replication (Bray *et al.*, 1989), it increases RNA and protein synthesis (Fu *et al.*, 1988), makes greater ATP availability (Mazor *et al.*, 1984) and repairs deteriorated seed parts

(Shaha, 1990). Also, the must ameliorative effect of priming should be the repair of damaged DNA. Primed seeds have more time to complete the process of repair because of water uptake is slower in priming (Varier et al., 2010).

According to table 1, germination index (GI) was the highest for 'Anaheim Chilli' cultivar (4,93) after soaking seeds for 24 hours in NaCl solution (50 mM). Then, germination index was the highest (4, 4) for 'Baklouti' cultivar after soaking seeds for 36 hours in CaCl₂ solution (10 mM).

Concerning 'Beldi' cultivar, the best germination index (4,1) was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). According to Soughir *et al.* (2012), higher value of germination index in fenugreek primed seeds indicate a better vigor of seeds than control. Ruan *et al.* (2002) demonstrated that KCl and CaCl₂ seed priming improved germination index of rice. Also, Sadeghi *et al.* (2011) showed that osmopriming increased total germination percentage, germination index while decreased mean germination time in soybean seeds.

The shortest mean germination time (MGT) was 3,72 days and it was obtained for 'Anaheim Chilli' cultivar (Table 1) after soaking seeds for 24 hours in NaCl solution (50 mM). The best MGT obtained for 'Beldi' cultivar was 4,65 days and that was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). For 'Baklouti' cultivar, the least MGT (4,81) was obtained after soaking seeds for 36 hours in CaCl₂ solution (10 mM). Yamauchi and Winn (1996) found that seeds priming broke

down dormancy which resulted in an earlier emergence. This earlier emergence may be related to a rapid water uptake comparing to the control treatment. Similarly, Kaya *et al.* (2006) signaled that primed seeds had more rapid water uptake than control seeds in sunflower. Priming improves mean germination time by accelerating imbibition, which facilitate the emergence phase and the rapid multiplication of radicle cells. Rapid germination in primed seeds can be due to the increasing activity of the degrading enzymes, such as α -amylase, synthesis of RNA DNA, ATP and the number of mitochondria.

It becomes evident that priming increase free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds (Afzal *et al.*, 2006). The values of total germination mean germination time, coefficient of velocity and germination index were better than in control seeds. These results are consistent with the work of several researchers (Soughir *et al.*, 2012; Elouaer and Hannachi, 2012).

We conclude that the use of priming technique can enhance the germination of pepper seed under normal or under saline conditions. Nevertheless, beneficial effects of priming for later growth and development stages of plants remain unclear (Farhoudi and Sharifzadeh, 2006). In fact, Passam and Kakouriotis (1994) reported that benefits of NaCl priming did not persist beyond the seedling stage in cucumber. So, additional work is needed to evaluate the effect of seeds priming on early seedling growth of these plants under field conditions.

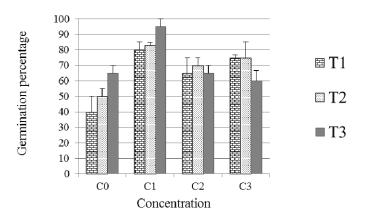


Figure1: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Beldi' cultivar.

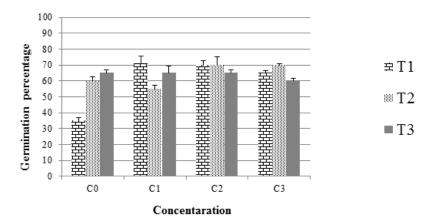


Figure 2: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Baklouti' cultivar.

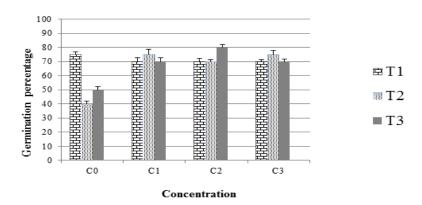


Figure 3: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar.

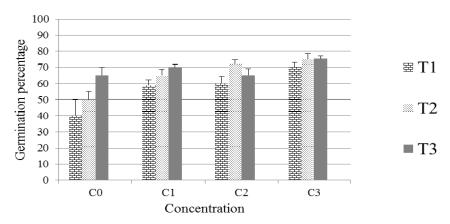


Figure 4: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of CaCl₂ priming agent on the germination percentage (%) of 'Beldi' cultivar.

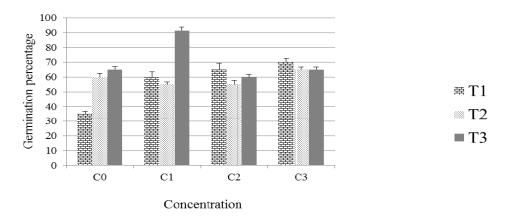


Figure 5: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of CaCl₂ priming agent on the germination percentage (%) of 'Baklouti' cultivar.

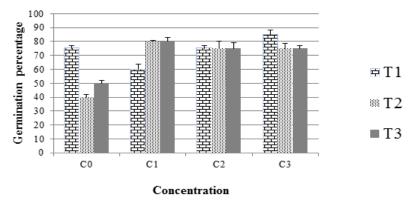


Figure 6: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of CaCl₂ priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar.

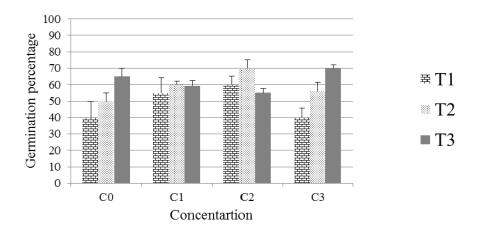


Figure 7: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Beldi' cultivar

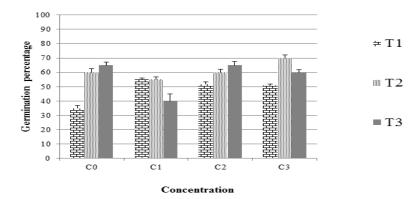


Figure 8: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Baklouti' cultivar

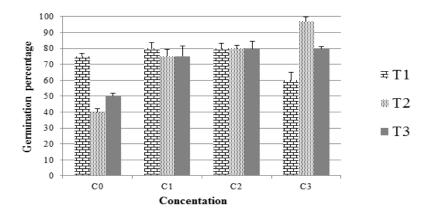


Figure 9: Role of duration (T1= 12h; T2 = 24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar

Table1: Effect of priming treatment with KCl, CaCl₂ and NaCl on the germination index (GI), Coefficient of velocity (CV) and Mean germination time (MGT) of three pepper cultivars 'Beldi', 'Baklouti' and 'Anaheim Chilli'

		KCl (10 mM)			CaCl ₂ (10 mM)			NaCl (50 mM)		
	T(hours)	GI	CV	MGT	GI	CV	MGT	GI	CV	MGT
Beldi	12h	2.5 ^b	13.9°	7.19 ^a	2.6 ^b	14.7 ^b	6.80ª	2.1 ^b	12.5 ^b	8ª
	24h	2.9 ^b	15.6 ^b	6.41ª	2.4 ^b	13.8 ^b	7.24ª	2.2 ^b	12.8 ^b	7.81ª
	36h	4.1ª	21.5ª	4.65°	2.25 ^b	13.1 ^b	7.63ª	2.3 ^b	13.3 ^b	7.51ª
	Control	2.45 ^b	14.58°	6.93 ^a	2.45 ^b	14.58 ^b	6.93 ^b	2.45 ^b	14.58 ^b	6.93ª
Baklouti	12h	2.8 ^b	15.3 ^b	6.53 ^b	3.1 ^b	16.1 ^b	6.21 ^b	1.9 ^c	11.9°	8.40 ^a
	24h	2.58 ^b	13.8 ^c	7.24 ^a	2.9 ^b	15.3 ^b	6.53 ^b	2.6 ^b	13.1 ^b	7.63ª
	36h	2.45 ^b	12.8°	7.81 ^a	4.4ª	20.8ª	4.81°	2.3 ^b	12.8 ^b	7.81 ^a
	Control	2.85 ^b	14.03 ^b	7.13 ^a	2.85 ^b	14.03 ^b	7.13 ^a	2.85 ^b	14.03 ^b	7.13
Anaheim Chilli	12h	3.2 ^b	14.2 ^b	7.04 ^a	3.3 ^b	18.6b	5.37 ^b	3.2 ^b	17.4 ^b	5.74 ^b
	24h	2.85°	14.5 ^b	6.89 ^a	3.2 ^b	17.4 ^b	5.74 ^b	4.93ª	26.8ª	3.72 ^e
	36h	2.9°	15.6 ^b	6.41ª	3.11 ^b	16.3 ^b	6.13ª	3.15 ^b	18.3 ^b	5.46 ^b
	Control	3.16 ^b	18.2 ^b	5.50 ^b	3.16 ^b	18.2 ^b	5.50 ^b	3.16 ^b	18.2 ^b	5.50 ^b

4 CONCLUSIONS

Priming gave better results than control for the three cultivars. This shows that the profit of this method is a general phenomenon. But the best results were obtained for the seeds of "Anaheim Chilli" cultivar which were soaked in a solution of NaCl (50 mM, 24 h). The best combinations that

we chose were: KCl priming (10 mM, 36h) for 'Beldi' cultivar, CaCl₂ priming (10 mM, 36 h) for 'Baklouti' cultivar and finally NaCl priming (50 mM, 24 h) for 'Anaheim Chili' cultivar. Additional molecular research is needed to explore priming advantages and to confirm these results.

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