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Effects of Short and Long-Term Hydro priming on Germination Stage and Growth of Some Varieties of Chickpea (*Cicer Arietinum L.*) Under Drought Stress

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Abstract

Water stress is one of the major stresses for the production of different crops in arid and semi-arid areas. Our study aims to study the effect of short-term (3h and 6h) and long-term (16h and 24h) hydro priming on seeds of four Kabuli chickpea cultivars. During germination, three levels of osmotic stress were used: 0, -0.2MPa and -0.5MPa PEG6000. The results showed that the seeds of Farihane and Zahor have a PG of 100% for the seeds treated with "6h hydro priming" and under osmotic stress -0.5MPa. Under the same conditions, seeds of Arifi and Bouchra treated with "24h hydro priming" also have a PG of 100%. Seeds of Arifi treated with '' hydro priming 6h '' and seeds of Zahor treated with '' hydro priming 24h '' showed a decrease in mean germination time (10% and 22.5%, respectively) below -0.5MPa. A reduction in T50 was noted in seeds of Arifi treated with "hydro priming 6h" and seeds of Zahor treated with "hydro priming 24h" (67% and 6%, respectively) at -0.5MPa. The "3h hydro priming" pretreatment of seeds of the Zahor variety increased the stem / root ratio by 64% under osmotic stress -0.5MPa. Under the same conditions, the "24h hydro priming" and "16h hydro priming" pretreatments of Farihane and Arifi seeds improved the S \ R ratio by 37% and 38%, respectively. In conclusion, it is necessary to adapt the time of the Hydro priming pre-treatment according to the cultivated variety for a better germination and a good growth under water stress.

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Introduction

Chickpea (*Cicer arietinum L.*) is one of the most nutritionally, agronomically and economically important seed legumes in the world. The total area of chickpea cultivation in the world was estimated to have exceeded 14.56 million hectares and a production of about 11.5 million tons [1], most of the production is concentrated in India. Osmotic stress is one of the environmental factors limiting plant growth and productivity in the Mediterranean, especially in North Africa, inducing a reduction in germination rate and seedling growth [2]. In the case of osmotic stress, the plant proceeds to maintain turgor through osmotic adjustment [3,4]. The decrease in osmotic potential is obtained by osmolyte accumulation in response to osmotic stress, and is considered to be a key mechanism of drought tolerance in some crop species [5,6].

The germination of seeds is a determining stage in the establishment of the culture and the density of the plants and consequently the yield. However, this stage of development is sensitive to abiotic stress even in the most tolerant species. Thus, the reinforcement of seed germination and the growth of young seedlings, by pre-germination treatments would be of great interest for the subsequent success of the culture under osmotic stress. Priming has been shown in several works to improve seed germination and seedling growth in alfalfa, chickpea and bean [7-10]. It can be a useful tool to overcome the effects of stress through damage repair of old seeds [11] or those exposed to abiotic stress [12,13].

Selection of the priming medium is one of the important challenges faced by seed physiologists. Seed priming involves immersing seeds in an osmoticum such as mannitol, sodium chloride, Polyethylene Glycol (PEG) (osmopriming) or alternatively water (hydro priming) to control the amount of water provided to the seeds and thus increase their ability to adapt and grow even under stressful conditions [14,15]. Indeed, this pre-treatment allows influencing the development of seedlings, by modulating the metabolic and biochemical activities during the reversible phase of germination, the result is that the seed acquires a significant germination potential and thus it can grow under stressful conditions [7,8]. Hydro priming involves using only water, which shortens the hydration time afterwards. It is an economical, safe, and simple technique to increase the ability of seeds to withstand osmotic stress and improve crop production even in harsh environments [16].

Materials and methods

Plant materials

The seeds of chickpea (*Cicer arietinum L.*): Were provided by the National Institute of Agronomic Research (INRA, Settat). The seeds of these varieties are widely used in many Moroccan fields.

Seed priming treatment

Seeds were surface sterilized with sodium hypochlorite 6% for 5 min, and then rinsed several times with sterile distilled water and fully immersed in water for 3, 6, 16 and 24h for 24h at 25°C in dark aseptic conditions. After priming, three levels of water deficit 0, -0.2 and -0.5MPa PEG6000 were used. Unprimed and primed seeds were immediately used for germination tests.

Seed germination

Primed 3,6,16, 24h and Unprimed (UP) seeds of the four chickpea varieties, were germinated in three levels of drought (0, -0.2 and -0.5 MPa) according to [17]. Seed incubated after that for seven days at 25°C in total obscurity. Three replicates of 20 seeds for each petri dish and each treatment were used. Germination Percentage (GP), Mean Germination Time (MGT), Time to 50% germination (T50) and Stem Root Ratio (S / R) were calculated to estimate the effect of Hydro priming on germination parameters and growth. Number of germinated seeds was counted every day. The germination percentage was calculated using: GP= (n /N) × 100, where, n is the number of germinated seeds, N is the total number of seeds. MGT was calculated according to [18] using the formula: MGT = ∑D x n / ∑n, Where n represents the number of seeds germinated on day D, and D the number of days counted from the start of germination. The time to 50% germination (T50) was calculated according to the following formula of [19] modified by [7].

$$T50 = t_i + \frac{\{(\frac{N}{2}) - n_i\}(t_i - t_j)}{n_i - n_j}$$

Where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and $_{tj}$ when $n_i<N / 2< n_j$.

Stem length was measured from the point of cotyledon attachment to the end of the seedling. Likewise, root length (RL) was measured from the point of attachment to the end of the root. Thus, the S / R ratio was calculated [20].

Statistical analysis

Results were expressed as the mean ± standard error. Statistical comparisons were made using SPSS software (version 21) with Tukey's test. The differences were considered significant at $p < 0.05$. It concerned a two-way analysis of variance (ANOVA II). Three replicates per combinations per treatment were analyzed.

Results

Effect of hydro priming on the germination percentage (GP)

The germination percentage varied significantly in most of the varieties studied compared to their respective controls under water stress (Table 1 and Table 2, $p < 0.05$). The seed priming treatment significantly affected the germination percentage. The seeds of the varieties Farihane, Zahor and Arifi treated with Hydro priming (6h) and subjected to stress (-0.5MPa) have a germination percentage of 100% (Table 1). The effect of Hydro priming (16h) had a positive effect on almost all of the seeds studied under stress (-0.5 MPa), with a germination percentage of 100% (Table 2). The germination percentage was improved after priming; this can be explained by an increased rate of cell division in the primed seed [21] and by the stimulation of many metabolic activities involved in the early stages of germination seeds [7,8].

Effect of hydro priming on mean germination time (GMT)

The treated seeds showed a significant variation (Table 1 and Table 2, $p < 0.001$) for this parameter in most of the treated chickpea varieties compared to the Unprimed Seeds (UP). For the Farihane variety, the germination rate increased by 20% in the seeds treated with "Hydro priming 6h" compared to the untreated under osmotic stress -0.5MPa. Under the same conditions, for the Zahor variety, the MGT increased by 10.2% in the seeds treated with "Hydro priming 3h" compared to the untreated ones. In the two Hydro priming treatments (3h and 6h), for the Arifi and Bouchra varieties, the MGT was reduced for the treated seeds compared to the Unprimed Seeds (UP) (Table 1). The "Hydro priming 16h and 24h" treatments improved the germination speed of the seeds of the Farihane variety under stress (-0.5MPa) by 35 and 43% respectively (Table 2). For Arifi variety, the "Hydro priming 16h" treatment improved the MGT by 37% in the treated seeds compared to seeds not primed under stress (-0.5MPa). An improvement in the mean germination time was noted in stressed seeds compared to Unprimed Seeds (UP), this was confirmed by the work of [7-10,22]. They have shown that osmotic stress delays the average germination time compared to unprimed seeds in susceptible species and on the contrary accelerates GMT for tolerant varieties.

Effect of hydro priming on T50

The Hydro priming treatment induced a significant variation (Table 1 and Table 2, $p < 0.001$), for this parameter in most of the chickpea varieties treated compared to their respective controls. For the Farihane variety, a 10% improvement in T50 was observed in seeds treated with "6h hydro priming" compared to those untreated under osmotic stress of -0.5MPa. Under the same conditions, the T50 was improved by 17% and 6% respectively in the seeds of the Zahor and the Arifi varieties treated with "Hydro priming 3h" compared to the untreated ones. For the two hydro-priming treatments (3h and 6h), the T50 was increased in the treated seeds of the Bouchra variety compared to the untreated seeds (Table 1). The "Hydro priming 16h and 24h" treatments improved the T50 of seeds of the Farihane variety under stress (-0.5MPa) by 38 and 41% respectively (Table 2). The "Hydro priming 16h" treatment improved the T50 by 28% in the treated seeds of Arifi variety compared to the unprimed seeds under osmotic stress (-0.5MPa). An improvement in T50 was generally observed in treated seed compared to unprimed seed; this was confirmed by the work of [23,24].

Effect of hydro priming on the stem root ratio

In Table 1 and Table 2, a significant variation ($p < 0.001$) in the S / R ratio was noted in the treated seeds of the chickpea varieties compared to the unprimed seeds (UP). The S / R ratio was increased by 60% in the seeds of the Farihane variety treated with "6h hydro priming" compared to Unprimed Seeds (UP) under osmotic stress -0.5MPa. Under the same conditions, the S / R of the Zahor variety was improved by 83% for seeds treated with "Hydro priming 3h" compared to unprimed seeds. In the two Hydro priming treatments (3h and 6h), for the Arifi and Bouchra varieties, the MGT increased for the treated seeds compared to the non-primed seeds (Table 1). The "Hydro priming 16h and 24h" treatments improved the seed germination rate of the Farihane variety under stress (-0.5MPa) by 73 and 88% respectively (Table 2). For Arifi variety, the "Hydro priming 16h" treatment increased the S / R by 55% in the treated seeds compared to the untreated seeds under stress (-0.5MPa). Seed Hydro priming has a positive effect on the tolerance of plants under abiotic stress by improving seed germination and seedling growth. Indeed, Hydro priming improves the antioxidant system and the expression of genes and proteins [25,26].

Table 1: Measured traits mean in germination stage and growth with short-time hydro priming (3 and 6h) on Germination Percentage (GP), Mean Time to Germination (MGT), T50 and stem / root ratio of some varieties of chickpea (Farihane, Zahor, Arifi and Bouchra) under water stress (-0.2 and - 0.5 MPa PEG6000).

Varieties	Hydro priming	GP %			MGT day			T50 day			S / R ratio		
		0	-0.2	-0.5	0	-0.2	-0.5	0	-0.2	-0.5	0	-0.2	-0.5
		MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa	MPa
FARIHANE	UP	100	100	90 ± 1	0.483 ± 0.001	0.549 ± 0.001	0.591 ± 0.001	0.625 ± 0.002	0.750 ± 0.003	0.800 ± 0.002	0.56 ± 0.001	0.463 ± 0.035	0.420 ± 0.033
	3h	97 ± 3	100	97 ± 4	0.507 ± 0.032	0.620 ± 0.042	0.525 ± 0.015	1.351 ± 0.019	2.416 ± 0.007	0.944 ± 0.006	0.63 ± 0.020	0.593 ± 0.055	0.593 ± 0.005
	6h	97 ± 2	100	100	0.519 ± 0.010	0.614 ± 0.027	0.467 ± 0.027	1.306 ± 0.037	1.042 ± 0.002	0.722 ± 0.002	1.6 ± 0.003	0.48 ± 0.008	1.033 ± 0.007
ZAHOR	UP	100	80 ± 3	70 ± 2	0.467 ± 0.001	0.651 ± 0.001	0.609 ± 0.001	0.500 ± 0.001	0.667 ± 0.001	0.838 ± 0.001	0.66 ± 0.040	0.54 ± 0.027	0.24 ± 0.001
	3h	100	90 ± 4	93 ± 1	0.526 ± 0.025	0.624 ± 0.076	0.547 ± 0.032	1.028 ± 0.021	1.511 ± 0.003	0.693 ± 0.007	0.5 ± 0.020	0.56 ± 0.006	1.403 ± 0.004
	6h	100	87 ± 1	100	0.494 ± 0.005	0.645 ± 0.059	0.565 ± 0.034	0.684 ± 0.0515	1.319 ± 0.008	1.597 ± 0.009	0.5 ± 0.001	0.74 ± 0.018	0.36 ± 0.001
ARIFI	UP	100	100	100	0.491 ± 0.001	0.467 ± 0.001	0.467 ± 0.001	0.714 ± 0.001	0.500 ± 0.001	0.900 ± 0.001	0.76 ± 0.002	0.573 ± 0.015	0.19 ± 0.009
	3h	100	100	80 ± 1	0.526 ± 0.025	0.564 ± 0.013	0.637 ± 0.014	1.050 ± 0.005	0.980 ± 0.001	0.844 ± 0.006	0.42 ± 0.003	0.303 ± 0.025	0.74 ± 0.001
	6h	100	83 ± 4	100	0.535 ± 0.006	0.614 ± 0.021	0.480 ± 0.005	1.806 ± 0.036	1.206 ± 0.005	1.102 ± 0.040	0.79 ± 0.010	0.76 ± 0.01	0.59 ± 0.002
BOUCHRA	UP	90 ± 3	90 ± 2	70 ± 1	0.538 ± 0.001	0.549 ± 0.001	0.651 ± 0.001	0.5625 ± 0.002	0.5625 ± 0.002	0.5 ± 0.001	0.45 ± 0.030	0.25 ± 0.025	0.23 ± 0.001
	3h	100	80 ± 1	70 ± 2	0.520 ± 0.029	0.644 ± 0.009	0.750 ± 0.011	2.321 ± 0.035	0.741 ± 0.031	1.556 ± 0.037	0.56 ± 0.040	0.63 ± 0.01	0.353 ± 0.002
	6h	100	70 ± 3	70 ± 1	0.564 ± 0.013	0.834 ± 0.050	0.812 ± 0.002	2.672 ± 0.143	2.122 ± 0.003	1.656 ± 0.007	0.5 ± 0.001	0.413 ± 0.011	0.23 ± 0.001
Varieties		df	F		df	F		df	F		df	F	
hydro priming		3	64.5***		3	29.062***		3	2.259*		3	9.487***	
stress		2	0.167*		2	20.517***		2	34.584***		2	6.053***	
Varieties *		2	55.042***		2	42.844***		2	9.109***		2	2.517*	
hydro priming		6	8.5***		6	5.917***		6	6.668***		6	8.11***	
Error		72			72			72			72		

*: Significance at 0.05 Probability Level; **: Significance at 0.01 Probability Level; ***: Significance at 0.001 Probability Level; NS: Not Significant at 0.05.

Table 2: Measured traits mean in germination stage and growth with long-term hydro priming (16 and 24h) on Germination Percentage (GP), Mean Time to Germination (MGT), T50 and stem / root ratio of some varieties of chickpea (Farihane, Zahor, Arifi and Bouchra) under water stress (-0.2 and - 0.5 MPa PEG6000).

Varieties	Hydro priming	GP %			MGT day			T50 day			S / R ratio		
		0 MPa	-0.2 MPa	-0.5 MPa	0 MPa	-0.2 MPa	-0.5 MPa	0 MPa	-0.2 MPa	-0.5 MPa	0 MPa	-0.2 MPa	-0.5 MPa
FARIHANE	UP	100	97 ± 1	90 ± 3	0.512 ± 0.014	0.5164 ± 0.021	0.686 ± 0.012	0.849 ± 0.043	0.6964 ± 0.064	0.913 ± 0.044	0.48 ± 0.02	0.73 ± 0.01	0.31 ± 0.01
	16h	100	100	100	0.488 ± 0.012	0.471 ± 0.004	0.444 ± 0.008	0.578 ± 0.040	0.537 ± 0.032	0.560 ± 0.032	0.47 ± 0.03	0.38 ± 0.02	1.16 ± 0.03
	24h	100	100	100	0.482 ± 0.001	0.488 ± 0.012	0.388 ± 0.004	0.578 ± 0.040	0.631 ± 0.079	0.501 ± 0.040	1.63 ± 0.08	0.48 ± 0.01	2.62 ± 0.06
ZAHOR	UP	100	85 ± 4	80 ± 2	0.494 ± 0.005	0.506 ± 0.010	0.697 ± 0.018	0.694 ± 0.020	0.654 ± 0.051	0.631 ± 0.029	0.63 ± 0.01	0.28 ± 0.01	0.28 ± 0.02
	16h	100	100	100	0.512 ± 0.014	0.500 ± 0.008	0.480 ± 0.004	0.631 ± 0.010	0.654 ± 0.051	0.578 ± 0.002	0.44 ± 0.01	0.28 ± 0.01	0.58 ± 0.02
	24h	97 ± 3	97 ± 2	99 ± 1	0.503 ± 0.021	0.509 ± 0.009	0.390 ± 0.060	0.613 ± 0.027	0.633 ± 0.026	0.578 ± 0.040	0.42 ± 0.03	0.51 ± 0.02	0.67 ± 0.08
ARIFI	UP	97 ± 1	90 ± 4	90 ± 2	0.503 ± 0.015	0.505 ± 0.009	0.656 ± 0.014	0.769 ± 0.09	0.631 ± 0.029	0.809 ± 0.064	0.86 ± 0.05	0.89 ± 0.001	0.50 ± 0.03
	16h	100	100	97 ± 3	0.497 ± 0.013	0.482 ± 0.008	0.415 ± 0.005	0.763 ± 0.020	0.631 ± 0.019	0.585 ± 0.018	0.70 ± 0.03	1.01 ± 0.05	1.12 ± 0.06
	24h	97 ± 3	100	100	0.564 ± 0.017	0.512 ± 0.019	0.480 ± 0.004	0.861 ± 0.027	0.654 ± 0.051	0.601 ± 0.040	1.10 ± 0.06	0.77 ± 0.06	0.68 ± 0.02
BOUCHRA	UP	97 ± 2	90 ± 1	80 ± 1	0.542 ± 0.011	0.485 ± 0.012	0.638 ± 0.010	0.703 ± 0.035	0.608 ± 0.001	0.849 ± 0.043	0.38 ± 0.03	0.32 ± 0.08	0.27 ± 0.04
	16h	100	100	100	0.528 ± 0.009	0.500 ± 0.008	0.491 ± 0.008	0.819 ± 0.087	0.631 ± 0.019	0.631 ± 0.011	0.27 ± 0.02	0.33 ± 0.01	0.60 ± 0.03
	24h	97 ± 5	100	100	0.571 ± 0.01	0.497 ± 0.01	0.488 ± 0.009	0.916 ± 0.044	0.694 ± 0.020	0.631 ± 0.019	0.47 ± 0.04	0.51 ± 0.02	0.65 ± 0.02
Varieties		df	F		dF	F		df	F		Df	F	
hydro priming		3	3.395*		3	7.022***		3	7.140***		3	7.627***	
stress		2	2.78*		2	3.946***		2	3.871***		2	7.441***	
Varieties *		2	19.444*		2	10.080***		2	8.320***		2	5.408***	
hydro priming		6	7.55***		6	6.15***		6	7.118***		6	7.89***	
Error		72			72			72			72		

*: Significance at 0.05 Probability Level; **: Significance at 0.01 Probability Level; ***: Significance at 0.001 Probability Level; NS: Not Significant at 0.05.

Conclusion

Hydro priming increased germination and growth of seedlings under water stress in chickpeas, which is consistent with several studies on the effect of priming in stressed plants. Seeds treated with water for 24 h usually have the highest germination rate. Hydro priming treatments (16h and 24h) generally gave the highest germination percentages and S / R under water stress. Therefore, Hydro priming for 16 or 24 h should be recommended for optimal germination and better growth of chickpea under abiotic stress conditions.

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