The Response of Some Quinoa (*Chenopodium quinoa* Willd.) Genotypes to Water Stress

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Abstract

This experiment was conducted at Homs Agriculture Research Center Lab, General Commission for Scientific Agricultural Research (GCSAR), Syria, during 2019, to assess the impact of osmotic stress on germination process and initial seedling growth of five introduced quinoa varieties. The osmotic stress was generated artificially using PEG_{6000}. completely randomized design was applied with six replicates. Results showed that, with decreasing osmotic potentials, germination percentage, germination index, shoot and radicle length were decreased, while the average of germination time increased. Results also indicated that there were significant differences among quinoa varieties in response to water stress, and the two varieties Giza1 and NSL-1.6389 were the most tolerant. Seed germination recovery indicated that PEG had an osmotic inhibition on quinoa seeds.

Key words: Water stress, Germination process, Seedling, Quinoa varieties.

Introduction:

Quinoa (*Chenopodium quinoa* Willd.) is a traditional Andean seed crop increasingly attracting attention because of its healthy nutritional attributes, and its high yield potential under adverse soil and climate conditions such as drought (Cocozza *et al*., 2012; Sun *et al*., 2014).

The grain of quinoa is a pseudo-cereal with attractive nutritional proprieties, and this attribute has greatly increased its consumption in recent years. It contains unsaturated fatty acids, antioxidants, and essential amino acids, and it is rich in Fe, Mg, fibers, and vitamins while containing high levels of gluten-free protein (Abugoch, 2009; Gordillo-Bastidas *et al*., 2016).

The Incan people treated the quinoa grain as sacred and referred to it as the “mother grain” (Koyro and Eisa, 2008). And because the quinoa plant shows high phenotypic and genetic variability, interest in this crop has also increased globally (Gamez *et al*., 2019).

The United Nations has announced 2013 as the International Year of Quinoa. General manager of the FAO declared that quinoa can become main ally in the war on hunger and malnutrition in developing countries (FAO 2013).

Abiotic stress is the primary cause of crop losses, decreasing yields by more than 50% Worldwide (Mittler, 2006). Water shortage is a serious problem affecting plant growth and yield in the Mediterranean region (Souza *et al.,* 2004). Due to the need of developing and identifying drought tolerant crop lines, understanding the functioning capacity of drought tolerant plants under water deficit conditions is inevitable (Bhardwaj and Yadav, 2012).

Syrian economic depends to a great degree on cultivation which 70% of it is un-irrigated, low yielded, oscillation among years, doesn't get sufficiency resources important for life. In the last year's drought appears to be a true problem which force the Syrian government to import wheat for the first time, as a result to successive years of drought, and stopping the cultivation of some crops as cotton and sugar beet in some areas (Abbas, 2009).

Germination can be considered as composed by two main part-processes: imbibition (water uptake) and metabolic reactions conducting the poorly defined embryo to a new plant (Sigstad and Prado, 1999). This process is a critical stage in plant life cycle, depending on the genetics of each species and environmental conditions that seeds are exposed. In arid and semi-arid regions with recurrent adversities such as salinity and water deficiency, the water absorption by the seed during the germination process is hampered by the negativity of the soil matrix potential (Santos *et al.,* 2016). In this sense, evaluation of the germination process and seedling development under conditions of water deficiency are substantial since they may be related to crop sensitivity or tolerance at subsequent development stages.

In the seedling stage selection for drought tolerance genotypes can be made by using polyethylene glycol (PEG 6000) for inducing osmotic stress, due to its high molecular weight this osmotic substance has been used in many studies on drought stress (Jatoi *et al*., 2014).

In Syria, quinoa had entered since 2014 by General Commission for Scientific Agricultural Research (GCSAR), but it is assumed that its cultivation areas will suffer from water scarcity. A recent study in the Syrian conditions found the possibility to follow the deficit irrigation (60% of total consumption) which saved approximately 31.5% of irrigation water without affecting the productivity of quinoa crop (Al-Jbawi *et al*., 2018). Thus, aiming to identify quinoa tolerance to cropping in water deficient environments, the objective of this research was to evaluate the effect of water stress on the germination process and initial growth of quinoa seedlings.

Materials and Methods:

The research was carried out at Homs research center laboratory, General Commission for Scientific Agricultural Research (GCSAR) in Syria, during 2019. A completely randomized design was laid with six replicates.

Seeds were surface-sterilized for 20 min in 20 % (v/v) sodium hypochlorite, then rinsed and soaked in distilled water. This sterilization procedure is required to eliminate saponine from seeds and to avoid contamination by microorganisms during the germination process. The entire sterilization procedure, including soaking, took 1 h and did not affect the germination process (Panuccio *et al*., 2014).

Five varieties of quinoa (Table 1) were germinated on Whatman filter paper in Petri dishes of 10 cm in diameter, embedded in 8 mL of aqueous solutions containing polyethylene glycol (PEG_{6000}) concentrations required to obtain osmotic potentials corresponding to -2, -4, -6, -8, and -10 bar. The control treatment (0 bar) corresponded to the solution containing only distilled water.

Table 1. Quinoa varieties and their sources

No.	Var.	Source
1	**Giza1**	Seed and Plant Improvement Institute (Iran)
2	**Titicaca**	
3	**Red Carina**	
4	**Q26**	
5	**NSL-106398**	International Center for Biosaline Agriculture (ICBA)

For the evaluation of water stress, the standard germination test was carried out with 6 replicates of 50 seeds in each dish. After sowing, the seeds were incubated in a growth chamber (VS-91G09M-800, vision Scientific Co. LTD), for five days at constant temperature of 23±1 °C in darkness with a relative humidity of 70 %.

Germination percentage (GP), which showed the percentage of normal seedlings as recommended by the rules of seed germination. The germinated seeds were counted daily from the second day, and the experiment was considered finished when no further radical protrusions were observed after an average of five days. The germinated seeds were those that developed a normal radicle and had extended at least 2 mm and had a normal shoot.

Germination index (GI), which was calculated as described in the association of official seed analysis (AOSA, 1983) by the following equation:

$GI = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn)$,

where: GI: the germination index; G1, G2, G3, ..., Gn: the number of seeds that germinated on the first, second, third day, etc. until the n^{th} day of counting; N1, N2, N3, ..., Nn: the first, second, third, etc. to the n^{th} day after sowing.

GI gives an indication of the rapidity of germination; and it increases when the number of germinated seeds increases and the time required for germination decreases.

Mean germination time (MGT), a speed index as quicker germination corresponds to lower values of MGT, and it was computed according to (Kader, 2005),

$MGT\ (days) = \sum fi.ni / N$

Where, fi: is a day during germination period, ni: is the number of germinated seeds on day fi. N: is the total number of germinated seeds.

Germination recovery (GR), after 5 days non germinated seeds from all different PEG solutions treatments were transferred to distilled water to study germination recovery. The germination recovery was determined by counting the number of recovered seeds from total number of seeds using the following formula:

Recovery (%) = (a –b) c * 100 (Hajri *et al*., 2018).

where, *a*: is the total number of seeds germinated after being transferred to distilled water, *b*: is the number of seeds germinated in from osmotic treatments of PEG and *c*: is the total number of seeds.

Seedling length, at six days after sowing, the length of shoot and primary root of ten normal seedlings of each replicate were measured by means of a millimeter ruler.

Results:

For germination percent (GP) and germination index (GI), there was a significant difference (p<0.01) between the simple effects of the factors and the interaction Var*Op (Table 2). In terms of mean germination time (MGT), there was no significant interaction between the varieties and osmotic potentials, therefore, the factors acted independently on MGT. For germination recovery (GR) there was no significant of the factors and their interaction Var*Op (Table 2).

Table 2. Analysis of variance for germination percent (GP) %, germination index (GI), mean germination time (MGT) and germination recovery (GR) % of five quinoa varieties exposed to different concentrations of PEG_{6000}

Source of variation	**DF**	**Mean square**			
		GP	**GI**	**MGT**	**GR**
Variety (Var)	4	2289.81**	69.06**	0.193**	28.61
Osmotic Potential (Op)	5	41104.74**	1169.97**	4.074**	20.73
Var*Op	20	704.89**	18.23**	0.018	13.23
Residual	240	8.181	0.345	0.016	31.64

***, ** Significant differences at level of probability 5% and 0.01 respectively.**

Mean comparisons for the germination variables and interactions are presented in Table (3). Measurements regarding different osmotic potentials and varieties were significant at 1% level. As PEG concentration was increased (osmotic potentials decreased), GP and GI were decreased. The highest values were noticed at control and the lowest at Op -10 bar.

The GP mean of all varieties under control reached 74.50 %, but decreased to (73.56, 66.56, 63.11, 34.83 and 8.67%) at the osmotic potentials -2, -4, -6, -8 and -10 bar respectively. The varieties Giza1 and NSL-1.6389 were verified the superior to the others in Gp (%) (Table 3).

The GI mean of all varieties decreased significantly with decreasing OP solution level, and the two varieties Giza1 and NSL-1.6389 were superior to the others, so they considered the higher vigor and faster in germination compare to the other varieties.

The MGT mean increased significantly with decreasing OP solution level, and the two varieties Giza1 and NSL-1.6389 had the lowest values attained to 3.416, 3.318 day respectively (Table 3). This indicates that both varieties have the faster population of seeds that germinated. Also, here were no significant differences between the independent factors and their interaction regarding GR (Table 3).

Table 3. Means of GP (%), GI, MGT (day) and GR (%) of five quinoa varieties exposed to different concentrations of PEG_{6000}

Variety	**Osmotic Potential (bar)**						
	0	**-2**	**-4**	**-6**	**-8**	**-10**	**mean**
Gp (%)							
Giza1	76.94	73.61	68.06	63.06	55.83	15.83	58.89a
Titicaca	73.33	72.22	66.39	62.78	20.00	4.44	49.68b
Red Carina	70.83	70.28	65.28	63.06	18.89	4.17	48.75b
Q26	72.78	71.39	64.17	62.50	20.28	4.17	49.21b
NSL-106398	78.61	75.28	68.89	64.17	59.17	14.72	60.14a
mean	74.50a	73.56b	66.56c	63.11d	34.83e	8.67f	-
$LSD_{0.01}$var= 1.238, $LSD_{0.01}$op= 1.356, $LSD_{0.01}$var*op= 6.064, CV=5.4%							
GI							
Giza1	25.89	24.65	22.08	20.30	17.71	4.32	19.14a
Titicaca	24.17	23.81	21.45	20.24	6.24	1.18	16.18b
Red Carina	23.17	22.83	20.81	20.03	5.56	1.11	15.58c
Q26	23.92	23.36	20.36	20.07	6.17	1.11	15.83bc
NSL-106398	29.72	25.31	22.53	20.33	19.14	3.96	19.67a
mean	24.77a	23.97b	21.45c	20.20d	10.96e	2.34f	-
$LSD_{0.01}$var= 0.521, $LSD_{0.01}$op= 0.570, $LSD_{0.01}$var*op= 1.275, CV=3.4%							
MGT (day)							
Giza1	2.920	3.057	3.277	3.390	3.517	4.317	3.416b
Titicaca	3.067	3.110	3.343	3.400	3.747	4.557	3.537a
Red Carina	3.167	3.187	3.390	3.477	3.940	4.473	3.606a
Q26	3.090	3.143	3.433	3.473	3.910	4.530	3.597a
NSL-1063980	2.857	2.990	3.243	3.433	3.433	4.343	3.383b
mean	3.020d	3.097d	3.337c	3.435c	3.709b	4.444a	-
$LSD_{0.01}$var= 0.113, $LSD_{0.01}$op= 0.124, $LSD_{0.01}$var*op= 0.278, CV=3.6%							
GR (%)							
Giza1	-	-	-	-	91.7	94.8	93.2
Titicaca	-	-	-	-	96.6	92.5	94.5
Red Carina	-	-	-	-	98.2	95.0	96.6
Q26	-	-	-	-	98.2	97.5	97.9
NSL-106398	-	-	-	-	100.0	96.7	98.3
mean	-	-	-	-	96.9	95.3	-
$LSD_{0.01}$var= 9.24, $LSD_{0.01}$op= 5.84, $LSD_{0.01}$var*op= 13.07, CV=5.9%							

There was a significant interaction of Var* Op, on shoot length, and there was no significant interaction on root length (Table 4). There was a significant difference ($p < 0.01$) between the varieties and the osmotic potentials of the solution containing PEG_{6000} (Table 4).

Table 4. Analysis of variance for shoot length SL (mm) and root length RL (mm) of five quinoa varieties exposed to different concentrations of PEG_{6000}

Source of variation	DF	Mean square	
		SL (mm)	RL (mm)
Variety (Var)	4	254.79**	7.722**
Osmotic Potential (Op)	5	1671.67**	368.624**
Var*Op	20	16.70**	1.699
Residual	60	4.16	2.333

***, ** Significant differences at level of probability 5% and 0.01 respectively.**

Mean comparisons for the seedling variables and interactions are presented in Table (5). The independent factors regarding different osmotic potentials and varieties were significant at 1% level. Shoot length mean achieved 36.60 mm at the control treatment, and increased significantly at Op -2 bar, then it decreased with the decrement of osmotic potentials, but the lowest value was at Op -10 bar (Table 5).

Root length mean over all varieties under control treatment reached 22.07 mm, and decreased to (20.87, 18.67, 14.87, 14.87, 12.27 and 9.60 mm) at the osmotic potentials -2, -4, -6, -8 and -10 bar respectively.

Results also showed that the two varieties Giza1 and NSL-1.6389 were superior to the others in SL and RL (Table 5).

Table 5. Means of shoot length SL (mm) and root length RL (mm) of five quinoa varieties exposed to different concentrations of PEG_{6000}

Variety	Osmotic Potential (bar)						
	0	-2	-4	-6	-8	-10	mean
SL (mm)							
Giza1	37.33	48.33	45.67	38.33	32.33	23.00	37.50a
Titicaca	37.33	47.00	42.00	36.67	22.67	14.00	33.28b
Red Carina	35.33	41.67	38.33	31.00	20.33	13.33	30.00c
Q26	37.00	42.00	40.00	31.33	22.00	12.67	30.83c
NSL-106398	36.00	48.67	47.00	41.00	33.00	23.67	38.22a
mean	36.60c	45.53a	42.60b	35.67c	26.07d	17.33e	-
$LSD_{0.01}$var= 1.808, $LSD_{0.01}$op= 1.980, $LSD_{0.01}$var*op= 4.428, CV=6.0%							
RL (mm)							
Giza1	22.00	21.00	18.33	15.67	13.00	10.67	16.78ab
Titicaca	23.00	19.67	17.00	13.67	11.00	8.67	15.50b
Red Carina	21.00	20.67	19.67	14.67	12.00	8.67	16.11ab
Q26	21.67	20.67	19.67	14.67	12.00	9.33	16.33ab
NSL-106398	22.67	22.33	18.67	15.67	13.33	10.67	17.22a
mean	22.07a	20.87a	18.67b	14.87c	12.27d	9.60e	-
$LSD_{0.01}$var= 1.355, $LSD_{0.01}$op= 1.484, $LSD_{0.01}$var*op= 3.318, CV=9.3%							

Discussion:

The effect of water stress on germination has been addressed by several authors and in different species (kaya *et al.*, 2006; Abbas and Al-Jbawi, 2013; Barbieri *et al.*, 2019). This stage is very important for the development of the plants, particularly those that live in environments exposed to drought.

Our results revealed that low osmotic potentials notably affected germination percentage, germination speed and mean germination time negatively in all tested varieties of Quinoa, but it varied in its response to drought. So, the results showed a significant interspecific variation in drought tolerance during germination, and the study concluded that both varieties Giza1 and NSL-1.6389 were the most tolerant to osmotic stress. However, slow germination under water stress is obviously due to decreased water potential of the germination medium, which restricts the water availability to the seeds. In this regard, Pratap and Sharma, (2010) mentioned that germination at higher level of water stress may be attributed to the water deficit in the seeds below the threshold, which may lead to degradation and inactivation of the essential hydrolytic enzymes.

When the osmotic potential of growth medium decreased, some toxic effects in seeds undergoing the germination process, in addition to the water absorption restriction, these effects may lead to cell metabolism alterations, reduction in germination percentage and speed, and changes in the development and growth of seedlings. These findings are consistent with the studies done by Dodig *et al*., (2008) and Santos *et al*., (2016).

The high potential of quinoa varieties to continue in growing after transportation from the osmotic potentials to the control medium (distilled water) means that the embryo is not affected, and it kept its vitality during germination later. The results also indicating that PEG_{6000} had no toxic effects on seed germination. Merah (2001) and Khajeh-Hosseini *et al.,* (2003) indicated that PEG molecules did enter the seeds and found that there was no PEG toxicity during seed germination.

Significant reduction in shoot and root length at higher concentrations of PEG_{6000} was observed as compare to control. Similar trend of seedling length reduction was observed in many researches and in different species, in maize (Farsiani and Ghobadi, 2009), in sugar beet (Abbas and Al-Jbawi, 2013), in wheat (Spanic *et al*., 2017) and in barely (Helal *et al*., 2018). The reason for low shoot and root length may be due to increase in osmotic potential by increasing drought, which leads to dehydration, ionic imbalance in transpiring leaves that caused reduction in meristem activity and cell elongation, consequently inhibit the growth.

Conclusion:

The present investigation results indicated that water stress significantly reduced seed germination, shoot and root length in all varieties, but there were a clear variations between quinoa varieties in response to water stress, and the two varieties Giza1 and NSL-1.6389 were the most tolerant. Seed germination recovery indicated that PEG had an osmotic inhibition of quinoa seeds. Further field trials are needed for potential use of these varieties in arid environments.

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