Vietnam J. Agri. Sci. 2016, Vol. 14, No. 3: 321-327
Tạp chí KH Nông nghiệp Việt Nam 2016, tập 14, số 3: 321-327
www.vnua.edu.vn

EFFECTS OF SALINITY STRESS ON GROWTH AND YIELD OF QUINOA (*Chenopodium quinoa* Willd.) AT FLOWER INITIATION STAGES

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Received date: 08.01.2016 Accepted date: 12.04.2016

ABSTRACT

The objective of this study was to evaluate the growth and yield characteristics of quinoa genotypes grown under salinity stresses at different stages of development. The experiment was conducted in Vietnam National University of Agriculture. Two quinoa genotypes and four NaCl salt concentrations (0, 50, 150 and 300 mM) were laid out in factorial experiment in RCBD with three replications. Salinity stress was induced by irrigation with nutrient solution containing NaCl with corresponding concentrations to quinoa plants grown in sands for two weeks during flowering initiation (35 days after sowing). The results showed that salinity reduced plant height, number of leaves on main stem, number of branches on plant, root length, root dry weight, shoot dry weight, SPAD Chlorophyll Meter Reading (SCMR), panicle length, seed amount, the number of branches per panicle and 1000-seed weight. Quinoa genotypes with high potential for the number of leaves on main stem, the number of branches on plant, root length, root dry weight, SCMR and shoot dry weight under non-stress conditions performed well under salinity conditions.

Keywords: Quinoa, salt tolerance, salinity, Viet Nam

Ảnh hưởng của điều kiện mặn đến sinh trưởng và năng suất của cây diêm mạch ở giai đoạn ra hoa

TÓM TẮT

Nghiên cứu được tiến hành nhằm đánh giá ảnh hưởng của độ mặn tới sinh trưởng và năng suất của một số giống diêm mạch nhập nội. Thí nghiệm tại Học viện Nông nghiệp Việt Nam tiến hành trên 2 giống diêm mạch, ở 4 độ mặn có nồng độ muối (NaCl) khác nhau từ 0, 50, 150 đến 300 mM. Thí nghiệm được bố trí theo thí nghiệm hai nhân tố trên mô hình khối ngẫu nhiên hoàn chỉnh (RCBD), với 3 lần nhắc lại. Mặn nhân tạo được xử lý trong 2 tuần vào thời điểm bắt đầu ra hoa (35 ngày sau gieo) bằng cách sử dụng dung dịch nước muối với độ mặn tương ứng được thêm vào dung dịch dinh dưỡng để tưới cho cây thí nghiệm trồng trên cát sạch. Kết quả thí nghiệm cho thấy độ mặn tăng gây giảm chiều cao thân chính, tổng số lá/thân chính, tổng số cành/cây, chiều dài và khối lượng rễ khô, khối lượng thân lá khô, chỉ số SPAD, chiều dài bông, tổng số hạt/bông, tổng số nhánh/bông và khối lượng 1000 hạt. Nghiên cứu xác định, trong điều kiện bình thường, nếu giống diêm mạch có tổng số lá/thân chính, tổng số cành/cây, chiều dài và khối lượng rễ, khối lượng thân lá khô và chỉ số SPAD đat cao sẽ sinh trưởng tốt trong điều kiện mặn.

Từ khóa: Cây diêm mạch, kháng mặn, mặn, Việt Nam.

1. INTRODUCTION

Salinity is the most severe abiotic stress perceived by plants and affecting 800 million hectares of land worldwide, including 30% of the world's highly productive irrigated land. Salinization is increasing because of poor irrigation management and climate change. Viet Nam is considered one of the five countries most vulnerable to the impacts of climate change and associated phenomenon such as sea level rise, salt-water intrusion and drought. More than 1 million hectares of cultivated land along the coast of Viet Nam (in Mekong delta and the middle part of Viet Nam) are affected by different degrees of salinity. Very low yield and variable growth of rice, peanut or corn cultivation in these lands were observed. People living in these areas are, therefore, under food insecurity as well as malnutrition. Exploiting salt tolerance in crops is for these reasons an important target for plant production in the near future. Most of food and cash crops are "glycophytes" which perform very poor under saline conditions. Meanwhile salinity tolerance is not easy to breed for as it interacts in plants with many physiological processes that are controlled by many genes (Nguyen *et al.*, 2013b). One of important approaches to cope with salinity problems is to directly utilize "halophytes" which are naturally salt tolerant species (Jacobsen *et al.*, 2012).

Quinoa is a multipurpose nutritious crop, a natural halophyte plant which can be grown in soil conditions with various salinity levels from non-saline soil to extremely saline soil (salt concentration in soil solution is as high as 1/2 salt concentration in the sea water) (Bosque-Sanchez *et al.*, 2003; Adolf *et al.,* 2012). No clear seed yield reduction in quinoa grown under soil condition with 40 - 50 dS m^{-1} (400-600 mM NaCl) was observed. Interestingly, a small seed yield increase was found when quinoa plant grown in saline soil with salinity concentration of 5-15 dS m^{-1} (50-200 mM NaCl) (Jacobsen *et al.*, 2003). Quinoa can grow in high saline soil (350-400 mM), whereas yield of other food crops reduced seriously under mild saline condition (40 mM of salinity levels) (Munns and Tester, 2008; Shabala *et al.*, 2013). Because of good adaptation, quinoa has been grown directly under saline conditions (FAO, 2013) and used to elucidate the mechanism of its salt tolerance as well (Shabala *et al.*, 2012). This study aimed at understanding the agronomical and physiological changes in quinoa plant grown under non-salinity stress condition in comparison with different level of salinity stresses. The stressed quinoa plants were grown in sand and irrigated with nutrient solution containing salt under the net-house conditions.

2. MATERIALS AND METHODS

2.1. Materials and experimental design

The experiment was conducted under the net-house condition at the Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi, Viet Nam (latitude 21° 00' N and longitude 105° 56' E, and 7 meters above sea level).

The 2 x 4 factorial experiment was designed in a randomized complete block design (RCBD) with three replications. The experimental factors included: i) two quinoa genotypes (Green and Red) were provided by the Chilean National Agriculture Research Institute (INIA), and ii) four salinity levels: 0 mM NaCl (fresh water - control), 50 mM NaCl (mild stress, popular in salt affected areas in Viet Nam and many other saline soils in the world), 150 mM NaCl (moderate stress) and 300 mM NaCl (extreme stress, comparable to the salt concentration present in seawater).

Clean sand dried until constant weight was used as the substrate to uniformly fill in pots 25 x 20 x 20 cm (5 kg pot^{-1}). Five germinated seeds were sown in each pot. At 12 day after sowing (DAS), the seedlings were thinned to two plants per pot. Yoshida nutrient solution (0.48 g L^{-1} $(NH_4)_2SO_4$, 0.25 g L^{-1} KH_2PO_4, 0.19 g L^{-1} KNO_3, 0.60 g L^{-1} K_2SO_4, 0.60 g L^{-1} $Ca(NO_3)_2$, 0.66 g L^{-1} $MgSO_4$, 0.59 g L^{-1} $FeCl_3$), was used to apply daily to quinoa plants. During two weeks from 35 DAS to 49 DAS, sodium chloride was added 50 mM day^{-1} gradually to the corresponding nutrition containers and irrigated to the pots (to prevent quinoa plants in the higher salt treatments from shock with too severe salt stress treatment at beginning). When the nutrition containers reached required salt concentration of each experimental treatments, salt addition was stopped and irrigation with salt in the nutrition solutions were kept for two weeks. The salinity of drainage water and saturated soil extract was monitored to determine the salinity of the substrate, which was adjusted to maintain salinity at predetermined levels (Jacobsen *et al.*, 2001;

Nguyen *et al.*, 2013a). No salt was added to the nutrient solution used in the control pots. After 49 DAS, normal nutrient solution (without sodium chloride) was applied until the harvest.

2.2. Data collection

Data was collected five times at 35 DAS, 45 DAS (5 days before stopping stress period) and 55 DAS (recovery - 5 days after finishing all stressed treatments) for plant height, the number of leaves/stem, the number of branches/plant, and root length. At the same time, SPAD Chlorophyll Meter Reading (SCMR) was recorded by a SPAD chlorophyll meter (Minolta SPAD 502, Tokyo, Japan) on the second fully expanded leaf from the top of main stem between 10.00 and 12.00 am. Shoot and root samples were separated and dried in hot-air oven at 80°C for 48 hours or until constant weight. Shoot and root dry weights were determined separately.

At harvest, main panicle length, the number of seed/panicle, the number branches/ panicle, 1000-seed weight, and shoot and root dry weight were determined.

Salt tolerance index (ST) for shoot and root dry weight was calculated as the percent of the dry biomass produced in salinity stress conditions over the control condition (Nguyen *et al.*, 2013b).

2.3. Data analysis

The data were subjected to analysis of variance according to a randomized complete block design for factorial experiment using CROPSTAT 7.0 package. Least significant difference (LSD) was used to compare means.

3. RESULTS AND DISCUSSION

3.1. Effects of salinity stress on growth parameters of quinoa plant

3.1.1. Plant height

Salinity stress significantly reduced plant height of quinoa genotypes (Table 1). In fact, significant decrease in plant height was observed when salinity levels increased during salinity stress period from 35 to 45 DAS. After stress period at 55 DAS, there was a recovery in plant height of quinoa genotypes at 50 mM of salinity concentration. However, the recovery in plant height was not clear when higher salt concentrations were added to the irrigated solution. Wilson *et al.* (2002) observed no significant reduction in plant height until the electrical conductivity exceeded 11 dS m^{-1}, even increase in plant height was observed when irrigated with solution not exceeding 25 dS m^{-1} saline water in several genotypes (Gómez-Pando *et al.*, 2010). This suggested that quinoa might utilize salt inclusion mechanism of halophyte

Table 1. Effect of salinity stress on plant height and the number of leaves on main stem of quinoa genotypes

Treatments	Plant height (cm)			No. leaves/stem			No. branches	
	35 DAS	45 DAS	55 DAS	35 DAS	45 DAS	55 DAS	45 DAS	55 DAS
Genotypes								
Red	9.25^{a}	14.80	25.28	5.94	16.24	23.69^{a}	4.97^{a}	14.01^{a}
Green	9.22^{b}	14.47	24.93	5.92	16.19	22.11^{b}	4.67^{b}	13.43^{b}
Salinity levels								
0 mM	9.23	15.41^{a}	27.41^{a}	5.94	17.25^{a}	26.36^{a}	5.33^{a}	15.75^{a}
50 mM	9.24	14.83^{b}	26.15^{a}	5.94	16.81^{a}	24.50^{b}	5.08^{b}	14.25^{b}
150 mM	9.23	14.38^{bc}	23.75^{b}	5.97	15.92^{b}	21.58^{c}	4.58^{c}	13.14^{c}
300 mM	9.24	13.92^{c}	23.09^{b}	5.86	14.89^{c}	19.17^{d}	4.28^{d}	11.75^{d}

Note: Means followed by a lower case letter in a column are not significant different at 5% level by LSD.

plants and under mild stress condition salinity could enhance plant growth (Munns and Tester, 2008; Nguyen *et al.*, 2013b). However, halophytes could keep growing up to 300-400 mM NaCl salt concentration (equivalent to 25-35 dS m^{-1}) in the growing media but not with our quinoa genotypes in the present experiment. This showed that when salinity increases over optimum levels, quinoa plant height will be inhibited. This indicates that quinoa is not as salt tolerant as halophyte and might utilize various strategies to tolerate salinity stresses as found in barley (Nguyen *et al.,* 2013b). This makes quinoa an excellent candidate for salt tolerance study.

3.1.2. Number of leaves, number of branches, root length, and root and shoot weight

Salinity stress significantly reduced the number of leaves on main stem, the number of branches on plant (Table 1), root length, root dry weight (Table 2), and shoot dry weight (Table 3). At 45 DAS, there was no clear effect of mild salinity stress (50 mM) on the number of leaves on main stem, but significant effects were observed at moderate and severe stresses (150 and 300 mM). Meanwhile, significant effects were found at all levels of salinity stress on the number of branches on plant, root length, root dry weight, and shoot dry weight. At 55 DAS, the number of leaves on main stem, the number of branches on plant, root length, root dry weight and shoot dry weight did not recover until harvest at all levels of salinity stress. In previous findings (Ruiz-Carrasco *et al.*, 2008; Panuccio *et al.*, 2014), shoot length and root length all significantly reduced in the presence of salinity. Previous studied showed that shoot and root weight and total dry matter also decreased under saline stress conditions for glycophyte crops (Jacobsen *et al.*, 2001; Ruiz-Carrasco *et al.*, 2008; Gómez-Pando *et al.*, 2010; Eisa *et al.*, 2012; Razzaghi *et al.*, 2012; Panuccio *et al.*, 2014) and in quinoa and others halophyte plant (Koyro, 2006; Geissler *et al.*, 2009).

3.1.3. SPAD Chlorophyll Meter Reading

Salinity significantly reduced SCMR at moderate and severe salinity levels (Table 3). Reduction of SCMR due to salinity stress might be caused by reduction in chlorophyll content (Eisa *et al.*, 2012) which brought about reduction in photosynthesis (Morales *et al.*, 2011; Eisa *et al.*, 2012) in quinoa plant. As the result, growth and yield of quinoa plant also reduced under salinity stress conditions.

3.2. Effects of salinity stress on yield components of quinoa plant

The results also showed that salinity stress significantly reduced panicle length and yield

Table 2. Effect of salinity stress on root length and number of branches on plant of quinoa genotypes

Treatments	Root length (cm)				Root dry weight (mg plant^{-1})		
	35 DAS	45 DAS	55 DAS	Harvest	45 DAS	55 DAS	Harvest
Genotypes							
Red	3.12	6.79	8.77	16.80^{a}	33.33^{a}	76.94^{a}	631.67^{a}
Green	3.12	6.53	8.31	15.96^{b}	31.53^{b}	71.25^{b}	590.83^{b}
Salinity levels							
0 mM	3.13	7.40^{a}	9.74^{a}	17.97^{a}	39.44^{a}	88.61^{a}	681.67^{a}
50 mM	3.12	7.08^{b}	9.15^{b}	16.54^{b}	34.44^{b}	81.11^{b}	633.33^{b}
150 mM	3.13	6.44^{c}	8.06^{c}	16.07^{c}	27.50^{c}	69.17^{c}	596.67^{c}
300 mM	3.12	5.73^{d}	7.21^{d}	14.95^{d}	28.33^{c}	57.50^{d}	533.33^{d}

Note: Means followed by a lower case letter in a column are not significant different at 5% level by LSD.

Table 3. Effect of salinity stress on SCMR and shoot dry weight of quinoa genotypes

Treatments	SCMR			Shoot dry weight (g/plant)			
	35 DAS	45 DAS	55 DAS	35 DAS	45 DAS	55 DAS	Harvest
Genotypes							
Red	30.37	35.14^{a}	43.17^{a}	0.03	0.27^{a}	0.92^{a}	6.20^{a}
Green	30.36	34.52^{b}	42.03^{b}	0.03	0.25^{b}	0.83^{b}	6.08^{b}
Salinity levels							
0 mM	30.38	36.25^{a}	44.43^{a}	0.03	0.31^{a}	1.00^{a}	6.62^{a}
50 mM	30.37	35.30^{b}	43.05^{a}	0.03	0.27^{b}	0.90^{b}	6.22^{bc}
150 mM	30.36	34.23^{c}	42.44^{b}	0.03	0.25^{c}	0.85^{c}	6.04^{c}
300 mM	30.36	33.54^{d}	40.49^{c}	0.03	0.21^{d}	0.75^{d}	5.67^{d}

Note: Means followed by a lower case letter in a column are not significant different at 5% level by LSD.

Table 4. Effects of salinity stress on yield components of quinoa

Treatments	Panicle length (cm)	Seed amount (Mark)*	No. branches/panicle	1000-seed weight (g)
Genotypes				
Red	27.21	3.33	20.33	1.95
Green	26.49	2.98	19.23	1.72
Salinity levels				
0 mM	29.11^{a}	4.20^{a}	22.71^{a}	2.39^{a}
50 mM	27.75^{b}	3.60^{b}	20.56^{b}	2.01^{b}
150 mM	26.22^{c}	2.77^{c}	18.77^{c}	1.63^{c}
300 mM	24.32^{d}	2.05^{d}	17.08^{d}	1.31^{d}

*Note: Means followed by a lower case letter in a column are not significant different at 5% level by LSD. * Mark: 1- Very little, 5- Very plenty.*

components including seed amount, the number of branches on each panicle and 1000-seed weight of both quinoa genotypes. In previous findings, shoot length, root length (Ruiz-Carrasco *et al.*, 2008; Panuccio *et al.*, 2014), number of seeds, dry weight of seeds and seed yield (Jacobsen *et al.*, 2001; Koyro and Eisa, 2008; Razzaghi *et al.*, 2012; Bonales-Alatorre *et al.*, 2013; Peterson and Murphy, 2015) were all significantly reduced in the presence of salinity

3.3. Response of quinoa genotypes to salinity stress

The interactions between genotypes and salinity levels were non-significant for all traits (data not shown). The results indicated that genotypes with good growth under non-stress condition performed well under salinity stress conditions. In fact, Red quinoa genotype showed higher values for all traits as compared to the Green quinoa genotype. However, there were clear differences in the number of branches on plant, root dry weight, SCMR, and shoot dry weight between two quinoa genotypes at 45 and 55 DAS. Meanwhile, the significant differences were found in plant height at 35 DAS, the number of leaves on main stem at 55 DAS and root length at harvest. The differences between quinoa genotypes were not significant in panicle length and yield components (Table 4).

The present study found significant differences among quinoa genotypes for the number of leaves on main stem, the number of branches on plant, root length, root dry weight, SCMR and shoot dry weight. Moreover, non-significant interaction between genotype and salinity level implies that Red genotype performing better under normal condition might adapt better to stress condition when compared with the Green genotypes. This is somewhat

Table 5. Salt tolerance index of quinoa genotypes

Genotypes	Shoot dry weight			Root dry weight			Total biomass		
	ST_{-1}	ST_{-2}	ST_{-3}	ST_{-1}	ST_{-2}	ST_{-3}	ST_{-1}	ST_{-2}	ST_{-3}
Red	0.95	0.92	0.87	0.94	0.89	0.79	0.95	0.92	0.86
Green	0.93	0.90	0.84	0.90	0.85	0.76	0.93	0.90	0.84

Note: $ST_{-1, 2, 3}$: Salt tolerance index at NaCl concentration 50 mM, 150 mM and 300 mM, respectively.

different from other finding in other crops that the small statue plants might tolerate better with abiotic stresses (Munns and Tester, 2008). In fact, Red genotype had higher STs than Green genotype. Therefore, higher SCMR, number of leaves on main stem, number of branches on plant, root length, root dry weight, root and shoot dry weight under normal and stress conditions could be useful traits for selection to salt tolerance.

The salt tolerance indexes (STs) of quinoa genotypes for final shoot and root dry weight were computed (Nguyen *et al.*, 2013b), and STs had downward trend in relation to the increased NaCl concentrations. STs showed that root growth was more affected by saline conditions than shoot growth. Red quinoa genotype had higher STs than Green quinoa genotype in all studied traits (Table 5). ST value for root dry weight was lower than that for shoot dry weight. Higher negative effects of salt stresses on the root system than on the shoots were also found in barley treated with 200mM NaCl in hydroponics (Nguyen *et al.*, 2013a) and the reasons for this might be due to direct damage of saline solutions to the root system. However, in comparison with other crops such as rice, soybean or wheat plant growthof quinoa plants at 40 mM reduced by 60 to 90% compared to normal condition and at 200 mM plant growth was suspended (Wilson *et al.*, 2002; Munns and Tester, 2008). However, at 300 mM plant growth of quinoa reduced only 13 to 24%. Plants with better root system could uptake more nutrient and enhanced stress tolerance (Dinh *et al.*, 2014). In our study, Red genotype with deeper roots and higher root weight could uptake more nutrients to contribute to higher number of branches (panicles), more seed, seed weight and total biomass than Green genotype. Therefore, the Red genotype also had higher ST than the Green genotype.

4. CONCLUSIONS

Salinity reduced growth and yield of two quinoa genotypes. Quinoa genotype with high potential for the number of leaves on main stem, the number of branches on plant, root length, root dry weight, SCMR and shoot dry weight under non-stress condition performed better under salinity conditions as well. Red quinoa tolerates salinity stress better than the Green genotype.

ACKNOWLEDGEMENT

This study was financially funded by Vietnamese - Belgium small project of Vietnam National University of Agriculture (VNUA) and assistance from the Securing Water for Food Award on " Salt Tolerant Quinoa in China, Chile and Viet Nam" funded by USAID. The quinoa seeds were provided by Dr. Ivan Matus of Chilean National Agriculture Research Institute (INIA) with the help of Dr. Ton That Son, VNUA.

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