AMERICAN-EURASIAN JOURNAL OF SUSTAINABLE AGRICULTURE

ISSN: 1995-0748, EISSN: 1998-1074
2020, Volume (14), Issue(1):pages (14-21)
DOI: 10.22587/aejsa.2020.14.1.2
Published Online in http://www.aensiweb.com/AEJSA/

Differential analysis of five quinoa (*Chenopodium quinoa W.*) genotypes under different salt stresses in a controlled environment

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Received date: 12 December 2019, Accepted date: 1 25 February 2020, Online date: 2 March 2020

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ABSTRACT

Soil salinization is a global problem which restricts the choice of crops for cultivation. Management and reclamation of saline soils using costly and time consuming methods such as installation of drainage systems is beyond the reach of poor farmers. Therefore, it is important to look for alternate crops which are more salt-tolerant. One such crop is quinoa (*Chenopodium quinoa W.*), which has high nutritious value and capacity to grow in marginal conditions. As crops vary in their tolerance to salinity, they need to be evaluated for different salinity conditions. This study was conducted to evaluate 5 quinoa genotypes (ICBA-Q1, ICBA-Q2), ICBA-Q3, ICBA-Q4 and ICBA-Q5) for their salinity tolerance under four artificially induced salinity (5, 10, 15, 20 dSm^{-1}) levels. The parameters studied were rate of seed germination, plant height, dry biomass, nutritional content and grain yield. The results indicate that salinity had an inhibitory effect on all parameters. Out of 5 quinoa genotypes, ICBA-Q3 and ICBA-Q4 proved more salt-tolerant under saline conditions with regard to dry biomass and grain yield and nutritional contents. Therefore these two genotypes are recommended to farmers for large-scale adaptation in the salt-affected areas of Ethiopia.

KEYWORDS

grain yield, soil salinity, Ethiopia, biomass yield, nutritional contents

INTRODUCTION

Ethiopia has an estimated 11 million ha of land exposed to salinity, which is equivalent to 13% of the total irrigated area of the country [1,2]. These soils are mainly located in the Rift Valley, Wabi Shebelle River Basin, the Denakil Plains and other lowlands of the country, which is home to 10% of the total population [3]. The increasing prevalence of these soils have reduced the production potential of soils, which is directly affecting the food security and livelihood of the rural communities in the country. Due to increasing demand for food, farmers are making attempts to increase irrigated area to increase agricultural productivity. However, this has been done without the provision of effective drainage network. Under these conditions, the problems of soil salinity are likely to increase in future. Therefore, it is importance to develop an integrated strategy for the reclamation of saline soils to boost agricultural production to ensure future food security [3].

In the Rift and lower Awash valley agricultural system of Ethiopia, development of large irrigation schemes without effective drainage systems and poor irrigation management practices have resulted in the rapid rise of saline groundwater. Due to high temperatures, water evaporates from the soil surface leaving the salt behind causing secondary salinization [2]. It is generally believed that in a *business as usual* scenario, salinity problems will further exacerbate with serious concerns about the food security of the country.

For future food security of the country, reclamation of existing saline soils and prevention of other areas from salinity development is of principal importance. For this purpose, there is a need to develop an integrated approach considering the salinity status of soils in different regions. The areas with low to moderate salinity, reclamation can be achieved through effective leaching of salts and proper management of soil and water resources. The highly saline soils can only be reclaimed by the use of chemical amendments and installation of effective drainage systems. However, these strategies are costly, time consuming and difficult to implement by farmers due to lack of financial and technical resources. These soils can be reclaimed by the adoption of *biosaline* approach. This approach involves introduction of salt-tolerant food and forage crops. These integrated food and forage systems can help smallholder farmers to diversify their cropping systems to feed their families and livestock. For this purpose, selection of diverse food and forage species with the capacity to tolerate salt and water stress is of vital importance.

Quinoa (*Chenopodium quinoa Willd*) has emerged as an ideal crop for drought prone and salinized agricultural areas due to its high nutritious value and ability to grow in marginal conditions [4, 5, 6]. Quinoa has long been grown in the Andes region however, its farming is other regions is still in experimental phase [7]. Quinoa can be grown from non-saline to highly saline soils where other plants either fail to grow or grow very poorly [8]. Quinoa is gluten free and rich in proteins and essential amino acids such as lysine, threonine and methionine. It also has high contents of much needed unsaturated fatty acids (i.e. linoleic, oleic and linolenic), of minerals (Ca, Fe, Cu, Zn) and vitamins (A, B2, C and E) [9]. In addition, quinoa is a good source of vitamins, oil (high in omega 3, linoleic and linolenic acids, 55–66% of the lipid fraction), and natural antioxidants such as a and g tocopherol, and it has more minerals such as Ca, Fe, K, Mg, Cu, and Mn than other cereals [19]. Quinoa is suitable for lactose-tolerant consumers and those allergic to gluten. It can also be used as a highly nutritious feed for animals.

Quinoa is heat sensitive and can sustain temperatures up to 35^{o}C. Above this temperature, plant dormancy or pollen sterility may occur [10]. The quinoa may encounter poor seed germination and crop establishment problems in salt-affected areas [6]. The yield differences are huge ranging from 0.6 to 3.9 tha^{-1} depending on soil, water and climatic conditions [10]. This clearly indicates the need for further research to develop varieties that can produce consistent yields under different agro-climatic conditions. In this study, response of five quinoa genotypes to 5 salinity levels (control, 5, 10, 15 and 20 dSm^{-1}) is evaluated for the Afar region of Ethiopia. The outcome of this study is expected to help farmers in the selection of appropriate quinoa varieties for saline areas in different parts of Ethiopia and other countries of the region having similar agro-climatic conditions.

MATERIALS AND METHODS

Study area

The experiments were conducted at the Werer Agricultural Research Center (WARC), Amibara, Ethiopia, which is located at 278 km to the east of Addis Ababa. The area is relatively flat with slope gradients of 1-2% (Fig. 1). The mean annual rainfall is 570mm with a minimum and maximum temperatures of 19°C and 34°C, respectively. Higher soil evaporation due to extreme temperatures causes the creation of saline soils and nutrient disparity in the soils causing poor plant growth. The Vertisols soil type of the area varies from silty clay to clay whereas the texture of the Fluvisols soils sandy loam to silty loam [11].

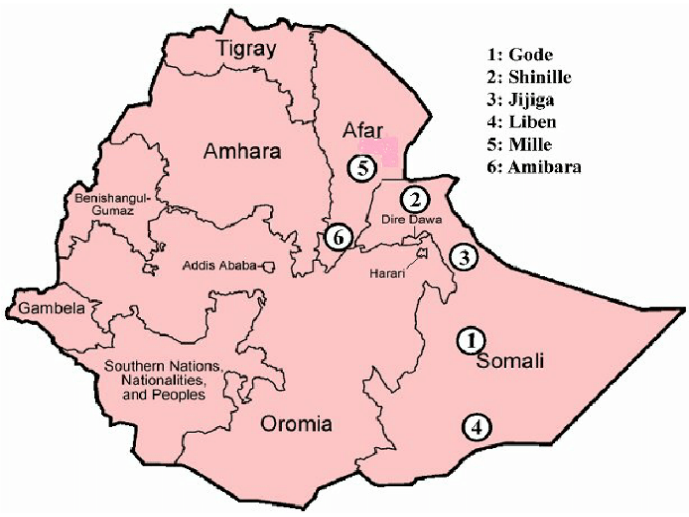

Fig. 1: Location map of the study area.

Observations and measurements

For pot trials under controlled conditions, four salt stress treatments were prepared by mixing 7.3 14.6, 21.4 and 29.1 g of NaCl into 6.0 kg of soil packed per pot to produce salinity levels of 5, 10, 15 and 20 dSm^{-1}. Five quinoa genotypes (ICBA-Q1, ICBA-Q2, ICBA-Q3, ICBA-Q4, ICBA-Q5) taken from International Center for Biosaline Agriculture (ICBA) were evaluated to test their performance under different soil salinity conditions. The treatments were organized in a completely randomized design with three replications.

Ten seeds of each genotype were sown in each pot. The seeds were surface sterilized using 70% ethanol (exposure for 10 sec) followed by immersion for 10 minutes in sodium hypochlorite solution (NaOCl; 5% active chloride). The treated seeds were washed thoroughly with distilled water and were placed on moist filter paper in petri dishes. Uniformity of seed size and quality was ensured before germination test. Since soils of the study area are good in nutrients, no fertilizer was used for these experiments. Irrigations were done with fresh canal water (EC = 0.3 dSm^{-1}).

Irrigations were applied according to crop evapotranspiration ($ET_c = ET_o \times K_c$), which was calculated by multiplying reference evapotranspiration (ET_o) with the crop coefficient (Kc). The reference evapotranspiration (ET_o) was calculated using modified Penman-Monteith equation whereas the Kc values were taken from FAO-56 publication. In addition to total irrigation requirements (ET_c), an additional 10% of the total irrigation amount was applied to leach down salts from the rootzone. Mean germination time (MGT), germination percentage (GP), plant height, biomass and grain yields, shoot and root dry matter yields and other related data was measured. Dry biomass yield was attained by oven-drying fresh biomass at 65°C to constant weight. Seeds with full radicle were considered as germinated. Germination count was done on 5th, 10th and 15th day after plantation. GP was calculated according to [12] whereas MGT was determined using equation of [13].

$$GP = \frac{Total\ germinated\ seeds}{Total\ number\ of\ seeds}$$

$$MGT = \frac{\sum Dn}{\sum n}$$

Where
n = Number of germinated seeds on day D, and
D = Number of days from the start of germination.

Chlorophyll content (SPAD units) of leaves was measured using Minolta Soil-Plant-Analysis Development (SPAD) meter. Plant height was measured with a standard ruler (*i.e.*, stem length from soil level to the top of the flower head). The dried and grounded plant samples were used to analyze nutritional contents of the seeds using standard methods as described in Van Siest et al. [14].

Statistical analysis

Two year experiments were conducted (2017-18) and the data was subjected to analysis of variance (ANOVA) technique [15] for factorial CRD using SAS 9.3 software (SAS Institute, Cary, NC). The significance of differences between the mean values at $p<0.05$ was determined using Least Significance Difference (LCD) test. The comparison between all data obtained was made by using Duncan's Multiple Range Test (DMRT).

RESULTS

Germination percentage (GP), mean germination time (MGT) and germination index (GI)

For all genotypes, increasing salinity affected seed germination. The GP was highest in ICBA-Q3, ICBA-Q4 and ICBA-Q5 in control and gradually decreased with the increasing salt concentration. The lowest GP was recorded in ICBA-Q1 and ICBA-Q2 genotypes at all salinity levels whereas ICBA-Q4 gave highest GP at salinity level of 20 dSm^{-1}. Similarly, mean germination time (MGT) increased with growing salinity levels. The highest MGT was recorded in ICBA-Q1 at 20 dSm^{-1}, followed by ICBA-Q2 (Table 1). MGT for all genotypes was comparable up to salinity level of 10 dSm^{-1}. However at higher salinity levels (15-20 dSm^{-1}), MGT for ICBA-Q3, ICBA-Q4 and ICBA-Q5 were lower than ICBA-Q1 and ICBA-Q2. The lowest MGT was found in ICBA-Q3 in control. GI also followed the trends of GP for all genotypes. The maximum GI was observed in ICBA-Q5 followed by ICBA-Q3 and ICBA-Q4 at control. Lower GI values were observed at the highest salt concentration levels for all genotypes.

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Table 1: Effects of salinity on GP, MGT and GI of five quinoa genotypes.

Parameters	Genotypes	NaCl salt level (dS m^{-1})					LSD ($p \leq 0.05$)	CV (%)
		0	5	10	15	20		
Germination Percentage (%)	ICBA-Q1	36.67	23.33	10.00	16.67	10.00	6.00	17.93
	ICBA-Q2	16.67	16.67	13.33	20.00	16.67		
	ICBA-Q3	83.33	80.00	63.33	53.33	33.33		
	ICBA-Q4	83.33	83.33	56.67	60.00	40.00		
	ICBA-Q5	83.33	86.67	63.33	53.33	36.67		
Mean Germination Time (days)	ICBA-Q1	2.67	3.27	5.88	8.33	13.05	0.52	11.28
	ICBA-Q2	3.11	3.33	5.66	8.50	12.33		
	ICBA-Q3	2.61	3.83	5.27	5.77	10.27		
	ICBA-Q4	3.00	4.33	5.61	7.22	10.94		
	ICBA-Q5	3.16	4.33	5.67	8.07	10.27		
Germination Index (GI)	ICBA-Q1	1.01	0.45	0.23	0.24	0.10	0.22	26.84
	ICBA-Q2	0.31	0.21	0.19	0.23	0.24		
	ICBA-Q3	2.38	2.17	1.57	0.95	0.72		
	ICBA-Q4	2.02	2.32	2.01	1.10	0.76		
	ICBA-Q5	2.59	2.51	1.92	1.25	0.94		

Plant height

For all quinoa genotypes, a declining trend in plant height was noted with the increasing salinity. The maximum plant height was observed for ICBA-Q3 followed by ICBA-Q4 at 0 dSm^{-1}. However, at higher salinity level (20 dSm^{-1}), plant height of ICBA-Q3 and ICBA-Q4 were reduced by 41% and 44%, respectively. Table 2 shows that plant height of all quinoa genotypes reduced significantly after 10 dSm^{-1}. These results agree with those of Jacobsen [16] and Al-Dakheel et al. [17] who found significant reduction in plant height with the increasing salinity levels in Phaseolus species and Lentils.

Table 2: Effects of salinity on plant height of five quinoa genotypes.

Parameters	Genotypes	NaCl salt level (dS m^{-1})					LSD ($p \leq 0.05$)	CV (%)
		0	5	10	15	20		
Plant height (cm)	ICBA-Q1	68.67	67.00	67.67	61.67	42.47	4.01	8.05
	ICBA-Q2	68.33	67.67	67.33	63.67	45.67		
	ICBA-Q3	92. 67	84.00	74.67	63.00	54.66		
	ICBA-Q4	92. 33	85.00	69.47	59.33	51.00		
	ICBA-Q5	70.00	65.33	60.67	56.00	48.33		

Dry biomass yield

In all quinoa genotypes, dry biomass yield was reduced due to increased salt stress (Fig. 2). The highest dry biomass yield was obtained in ICBA-Q3 at 0-5 dSm^{-1} whereas the lowest was obtained in ICBA-Q1 and ICBA-Q2. ICBA-Q4 performed better at higher salinity levels (15-20 dSm^{-1}). The dry biomass yield decreased with the increasing soil salinity in the growth medium, although the response of all five genotypes to different salinity levels was heterogeneous. At 0 dSm^{-1}, dry biomass yield of ICBA-Q1, ICBA-Q2, ICBA-Q3, ICBA-Q4, and ICBA-Q5 was 15.5, 12.5, 29.6, 25.0 and 21.0 g/plant, respectively. However, the dry biomass yield at 20 dSm^{-1} was noted as 5.1, 8.6, 17.1, 18.1, and 13.5 g/plant, registering a drop of 67%, 30%, 42%, 28%, 36% for ICBA-Q1, ICBA-Q2, ICBA-Q3, ICBA-Q4, and ICBA-Q5, respectively.

The highest reduction in dry biomass yield per unit increase of salinity (1 dSm^{-1}) was observed in ICBA-Q3 (0.56 g/plant) followed by ICBA-Q1 (0.48 g/plant) and ICBA-Q2 (0.46 g/plant). The lowest reduction in dry biomass per unit of salinity increase was found in ICBA-Q4 (0.38 g/plant) and ICBA-Q5 (0.22 g/plant). These two genotypes showed more stable dry biomass yields under all salinity levels. The dry biomass yield for ICBA-Q3 declined significantly after 5 dSm^{-1} whereas this was not the case for ICBA-Q4 and ICBA-Q5. This suggests that for higher salinity levels (10-20 dSm^{-1}), ICBA-Q4 and ICBA-Q5 are more suitable due to their higher salt tolerance capacity.

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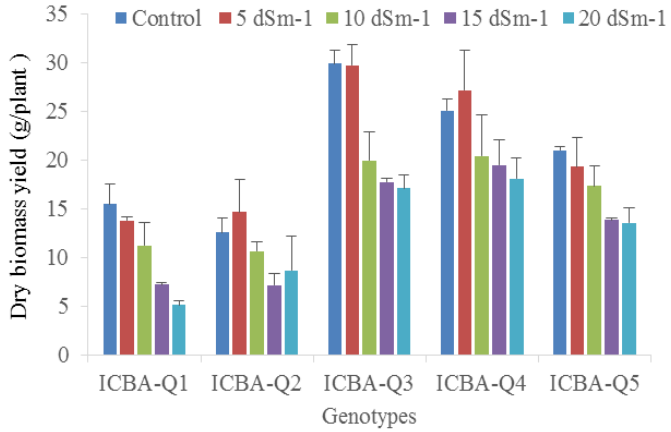

Fig. 2: Dry biomass yield of five quinoa genotypes as affected by different salinity levels.

Grain yield

The grain yield was also negatively affected by increasing salinity levels (Figure 3) however effect was more pronounced at higher salinity levels. ICBA-Q1 and ICBA-Q2 produced lowest grain yield at all salinity levels whereas the highest grain yield at all salinity levels was obtained for ICBA-Q3 followed by ICBA-Q4 and ICBA-Q5. The differences in grain yields under ICBA-Q3 and ICBA-Q4 were non-significant. The grain yield of ICBA-Q4 and ICBA-Q5 was 10% and 42.5% less than the grain yield of ICBA-Q3 at 0 dSm^{-1}. However, the reductions in grain yields at the higher salinity levels were relatively lower than the control i.e., grain yield of ICBA-Q4 and ICBA-Q5 was 4.8% and 38% less than ICBA-Q3. The grain yields of ICBA-Q3 and ICBA-Q4 were comparable at 10-15 dSm^{-1}. However, a significant reduction in grain yields was observed at higher salinity level.

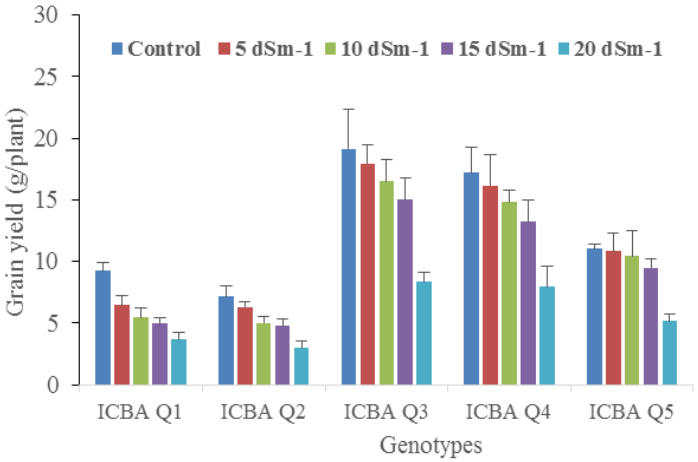

Fig. 3: Grain yield of five quinoa genotypes as effected by different salinity levels.

Nutritional analysis

Due to the distinct variation among quinoa genotypes, their responses to different salinity regimes for nutritional parameters were also different. Table 3 shows that the protein content of quinoa compares well to barley (10.5) and maize (10.2) whereas it is 30% higher than rice (7.2). Similarly, Ash content of quinoa are comparable or higher than wheat and barley (2.2), maize (1.7) and rice (3.4) [18]. The highest average protein content was found in ICBA-Q3 whereas the lowest was recorded in ICBA-Q5. The starch percentage in all genotypes is comparable and is much lower than other competitive crops such as wheat, rice and finger millet. Fe content os quinoa is also higher than the traditional crops such as wheat, rice and maize. Other nutritional parameters also showed similar trends. This makes it a healthy food for humans and can be used in cooking and baking of various products, industrial use of starch, protein, and saponin. It can also be used as a high nutritious feed for animals [19, 20]. This clearly shows that due to its unique nutritional balance, quinoa can be a valuable crop for marginal lands of Ethiopia and other countries of the region.

Table 3: Nutrient composition of 5 quinoa genotypes under different salinity conditions

Genotypes	EC (dSm^{-1})	Amylose (%)	Amylopectin (%)	Ash (%)	Starch (%)	Moisture (%)	Fe (ppm)	Zn (ppm)	Protein (%)
ICBA-Q1	0	20.10	33.93	3.30	54.06	10.31	56.32	77.89	9.02
ICBA-Q1	5	19.45	32.41	3.45	55.12	10.78	55.98	74.97	8.69
ICBA-Q1	10	19.72	34.45	3.31	54.17	10.21	56.10	73.29	9.05
ICBA-Q1	15	18.98	35.01	3.08	50.12	9.70	54.87	73.45	10.06
ICBA-Q1	20	18.72	35.21	2.98	53.42	9.54	52.31	69.87	9.88
Average		19.39	34.20	3.22	53.38	10.11	55.12	73.89	9.34
ICBA-Q2	0	19.47	35.48	3.97	55.18	9.96	57.01	76.31	9.04
ICBA-Q2	5	18.98	34.86	3.54	54.45	10.01	56.98	71.98	8.88
ICBA-Q2	10	19.82	34.96	3.12	54.78	9.81	55.86	72.34	9.91
ICBA-Q2	15	18.65	34.08	2.99	53.98	9.14	56.01	69.87	9.87
ICBA-Q2	20	17.97	33.94	2.67	53.21	9.58	55.23	69.80	10.02
Average		18.98	34.66	3.26	54.32	9.70	56.22	72.06	9.54
ICBA-Q3	0	20.01	33.90	3.21	55.23	14.32	56.21	72.57	9.71
ICBA-Q3	5	19.86	34.08	3.07	53.98	13.98	56.41	68.05	9.01
ICBA-Q3	10	19.74	34.97	2.96	54.97	14.38	55.15	69.08	9.54
ICBA-Q3	15	19.78	34.87	3.01	52.98	13.67	55.04	67.34	11.02
ICBA-Q3	20	18.95	35.07	2.91	52.34	10.56	53.75	64.66	10.68
Average		19.67	34.58	3.03	53.90	13.38	55.31	68.34	9.99
ICBA-Q4	0	19.86	34.00	3.92	56.03	10.95	56.31	69.75	7.93
ICBA-Q4	5	19.45	34.00	3.68	55.90	10.05	55.83	70.12	8.01
ICBA-Q4	10	19.96	33.80	3.43	53.76	9.01	55.98	68.58	7.74
ICBA-Q4	15	18.23	32.98	3.14	54.01	9.31	55.87	69.14	8.56
ICBA-Q4	20	18.00	33.01	2.54	52.69	9.02	53.41	68.51	9.87
Average		19.10	33.56	3.34	54.48	9.67	55.48	69.22	8.42
ICBA-Q5	0	19.52	34.98	4.03	54.56	9.98	55.76	69.45	7.06
ICBA-Q5	5	19.75	33.45	3.86	54.00	9.61	54.98	69.03	7.88
ICBA-Q5	10	19.97	33.01	3.50	52.98	9.00	55.65	67.03	7.72
ICBA-Q5	15	18.72	33.12	3.12	53.02	8.79	55.54	65.86	8.79
ICBA-Q5	20	17.98	32.98	3.06	53.23	8.31	53.91	64.87	8.49
Average		19.19	33.51	3.51	53.56	9.14	55.17	67.25	7.99

DISCUSSION

The rising global demand for nutritious and healthy food has stressed the need to look for alternate crops especially for the marginal areas where agricultural production is low due to unfavorable climatic conditions, low soil fertility and lack of good quality irrigation water. In many countries, scientists are experimenting quinoa production because it is rich in nutrients, tolerant to salinity and uses much less water than other crops. This study was focuses on assessing the feasibility of 5 quinoa genotypes for saline soil conditions of Ethiopia. The results indicate that the seed germination was negatively affected by the increasing salinity. The salinity impedes seed germination either without loss of viability at higher salinities and/or by inducing stress to seeds [21]. Gómez-Pando et al. [22] did a study on 15 salt-tolerant Peruvian accessions of quinoa and found that some genotypes showed decline in germination and plant height under high saline conditions, while others did not or even register an increase.

The results indicate reduction in dry biomass yield with increasing salinity, which might be due to lack of water availability and hydrolysis of reserved foods and their translocation to the growing shoots. Other factors responsible for lower dry biomass yield may include panicle length, chlorophyll concentrations, number of productive tillers, number of primary branches per panicle, and fertility percentage [23]. The reduction in plant growth and dry-matter yield under saline conditions has also been reported in several grain legumes including *P. vulgaris* that can be ascribed by decrease in cell elongation [22].

Gómez-Pando et al. [22] have also found a remarkable influence of quinoa genotypes on root dry mass per plant under saline conditions. This was probably due to the stunted growth of plants caused by high salt concentration in the nutrient medium. The higher salt stress causes reduction in the rate of leaf surface expansion, which results in considerable decrease in the dry weights of shoot, leaves, and roots [24]. This can be linked to the limited supply of metabolites to young growing tissues. Metabolic production usually occur within the leaves and can be affected significantly at high salt stress conditions either due to the low water uptake or toxic effect of NaCl concentration [25].

Quinoa is rich in nutritional parameters needed for a healthy food for humans as well as animals. It can be cooked alone as well by mixing with other cereals such as rice to develop the local taste. Quinoa is a new crop for marginal environments however, many African countries have made significant advances in introducing this crop in the local production systems. There is also evidence that extensive research trials are underway in the countries of the MENA region including Egypt, Yemen, Jordan, Iran, Algeria and Tunisia [6]. This shows that quinoa has a great potential as a food, feed and forage to diversify agricultural production systems in the salt-affected areas. Despite these nutritional qualities, some antinutritional factors (triterpenoid glycoside) are present in quinoa. Saponins, when present in the seeds, confer bitterness. Natural occurrence of saponins in quinoa grain is usually higher but some native varieties have low saponin as well. Even though saponins can be removed by repeated washing or dehulling, this consumes additional resources on postharvest processing. Therefore, more research is needed to evaluate the feasibility of growing quinoa under different environmental conditions [26].

CONCLUSIONS

There are considerable differences on various plant growth parameters with the increasing salinity on five quinoa genotypes. Results clearly revealed that nearly all parameters measured decreased with increasing levels of salinity stress. The performance of ICBA-Q3 was superior under low to moderate salinity conditions whereas ICBA-Q4 and ICBA-Q5 showed more consistent dray matter and grain yields under higher salinity conditions. The nutritional parameters of all genotypes are less affected by increasing salinity levels. However, further optimization of these genotypes is recommended to enhance their productivity under local conditions.

ACKNOWLEDGEMENTS

The authors are thankful to the International Fund for Agriculture Development (IFAD) to provide financial support for this research work. We are also thankful to the Ministry of Agriculture and the staff of the Werer research center of the Ethiopian Institute of Agriculture (EIAR) for their support in implementing these trials.

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