

# **TOLERANCE OF SOME QUINOA GENOTYPES TO WATER STRESS**

**By**

**MAI MOGHAZI ABUL-FETOUH EL-MOGHAZI**

**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006**

**THESIS**

**Submitted in Partial Fulfillment of the  
Requirements for the Degree of**

**MASTER OF SCIENCE**

**In**

**Agricultural Sciences  
(Agronomy)**

**Department of Agronomy  
Faculty of Agriculture  
Cairo University  
EGYPT**

**2018**

**APPROVAL SHEET**

**TOLERANCE OF SOME QUINOA GENOTYPES  
TO WATER STRESS**

**M.Sc. Thesis  
In  
Agric. Sci. (Agronomy)**

**By**

**MAI MOGHAZI ABUL-FETOUH EL-MOGHAZI**  
**B.Sc. Agric. Sci. (Biotechnology-English), Fac. Agric., Cairo Univ., 2006**

**APPROVAL COMMITTEE**

**Dr. MOHAMED SOLIMAN M. SOLIMAN .....**  
**Head Researcher of Agronomy, Field Crops Res. Inst., ARC**

**Dr. MOHAMED REDA A. SHABANA .....**  
**Prof. of Agronomy, Fac. Agric., Cairo University**

**Dr. AHMED MEDHAT M. AL-NAGGAR.....**  
**Prof. of Agronomy, Fac. Agric., Cairo University**

**Date: 19 / 3 / 2018**

**SUPERVISION SHEET**

**TOLERANCE OF SOME QUINOA GENOTYPES  
TO WATER STRESS**

**M.Sc. Thesis  
In  
Agricultural Sci. (Agronomy)**

**By**

**MAI MOGHAZI ABUL-FETOUH EL-MOGHAZI**  
**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006**

**SUPERVISION COMMITTEE**

**Dr. AHMED MEDHAT MOHAMED AL-NAGGAR**  
**Professor of Agronomy, Fac. Agric., Cairo University**

**Dr. RABIA MOHAMED ABD EL-SALAM**  
**Assistant Professor of Agronomy, Fac. Agric., Cairo University**

**Dr. AYMAN EBRHIM EL-SAYED BADRAN**  
**Assistant Researcher Professor of Plant Breeding, D.R.C.**

**Name of Candidate:** Mai Moghazi Abul-Fetouh El-Moghazi **Degree:** M.Sc.

**Title of Thesis:** Tolerance of Some Quinoa Genotypes to Water Stress

**Supervisors:** Dr. Ahmed Medhat Mohamed Al-Naggar

Dr. Rabia Mohamed Abd El-Salam

Dr. Ayman Ebrahim El-Sayed Badran

**Department:** Agronomy

**Approval:** 19 / 3 / 2018

### ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal crop has recently gained worldwide attention because of its ability to grow in various stress conditions like soil salinity, drought, etc. The objectives were: (1) studying the variability among quinoa genotypes for different traits under different water stress environments, (2) analyzing correlations, heritability and selection gain for studied traits and (3) characterizing quinoa genotypes using ISSR markers. Field experiments were carried out in two seasons at New Salhiya (sandy soil), Sharqiya Governorate and laboratorial work was carried out at laboratories of Desert Research Center, Cairo. A split plot design with 5 replications was used, where 3 irrigation regimes (achieving 95, 65 and 35% FC) were allotted to main plots and 5 quinoa genotypes to sub plots. Data indicated that irrigation regime and quinoa genotype had significant effects on all studied traits. The quinoa variety CICA-17 proved to be the highest yielder under severe water stress (35% FC) and the most drought tolerant, followed by CO-407 and Chipaya. Water stress caused significant increases in root length and water use efficiency (WUE), and significant reductions in the rest of studied traits. Results on thickness of leaf, upper and lower epidermis, palisade and spongy layers and amino acid results concluded that variety CICA-17 is drought tolerant. The best secondary traits for high seed yield, drought tolerance and high WUE of quinoa were inflorescence traits, root length, seed yield/plant and /fed, since they showed high correlation coefficient ( $r$ ), high heritability ( $h^2_b$ ) and high genetic advance (GA) estimates. Analysis of ISSR revealed that 10 ISSR primers produced 53 amplicons, out of which 33 were polymorphic with an average polymorphism of 61.83 %. Data showed a total number of unique ISSR markers of 24; eleven of them were positive and 13 were negative. The genetic similarity ranged from 49 % between Ollague and each of QL-3 and Chipaya to 76 % between CICA-17 and CO-407.

**Key words:** Quinoa, Drought tolerance index, Water use efficiency, Leaf anatomy, ISSR, Amino acids, Unique markers, Genetic diversity, Heritability, Genetic advance.

## **DEDICATION**

*I dedicate this work to whom my heart felt  
thanks TO MY MOTHER AND MY FATHER,  
TO MY BROTHERS AND MY FRIENDS for all  
the support they lovely offered along the period of my  
post-graduation.*

## **ACKNOWLEDGEMENTS**

Thanks to **ALLAH** the most Merciful and the most Beneficial. I wish to express my deepest gratitude and appreciation to **Dr. Ahmed Medhat Mohamed Al-Naggar** Professor of Crop Breeding, Agronomy Dept., Fac. Agric., Cairo University. and the Chairman of the supervision committee for valuable guidance, great help, devoted efforts and sincere concern for supervising the study and constructive guidance throughout the experimental work and the preparation of this manuscript.

Sincere thanks and grateful appreciation are extended to **Dr. Rabie Mohamed Abd El-Salam** Associate Professor, Agronomy Dept., Fac. Agric., Cairo University, for his supervision, valuable guidance, great help, devoted efforts and sincere concern for preparation of this manuscript.

Sincere thanks and grateful appreciation are extended to **Dr. Ayman Ebrahim**, Assistant Researcher Professor of plant Breeding, Desert Research Center, Egypt, for his supervision, valuable guidance, great help, devoted efforts and sincere concern for preparation of this manuscript.

Sincere thanks and grateful appreciation are extended to **Dr. Sedkey Tawfik Bolous**, Researcher Professor of plant anatomy, Desert Research Center, Egypt, for his valuable guidance, great help, devoted efforts and sincere concern for preparation of this manuscript.

Grateful appreciation is also extended to all staff members of Agronomy Department, Faculty of Agriculture, Cairo University, and Staff members of Desert Research Center.

# CONTENTS

	Page
<b>Introduction</b> .....	1
<b>REVIEW OF LITERATURE</b> .....	7
1. Quinoa- an underutilized crop.....	7
2. Definition of drought.....	15
3. Mechanisms of drought tolerance in quinoa.....	16
4. Effects of drought on quinoa traits.....	24
5. Genotypic variability in quinoa traits.....	26
6. Trait interrelationships in quinoa.....	29
7. Heritability in quinoa traits.....	33
8. Quinoa's genetic markers.....	35
<b>MATERIALS AND METHODS</b> .....	45
<b>RESULTS AND DISCUSSION</b> .....	64
<b>1. Morphological, physiological and yield traits of quinoa genotypes</b> .....	64
a. Analysis of variance.....	64
b. Mean performance.....	70
(1). Effect of water stress .....	70
(2). Effect of quinoa genotype.....	74
(3). Effect of quinoa genotype $\times$ water stress.....	81
c. Drought tolerance of quinoa genotypes.....	83
d. Superiority of tolerant (T) over sensitive quinoa genotype .....	87
e. Trait interrelationships.....	90
f. Phenotypic and genotypic coefficients of variation.....	97
g. Variance components .....	98
h. Heritability and expected selection gain.....	101
<b>2. Free amino acid contents in quinoa leaves</b> .....	103
a. Analysis of variance .....	103
b. Mean performance.....	106
(1). Effect of water stress on quinoa free amino acids.....	106
(2). Effect of genotype on quinoa free amino acids.....	109
c. Heritability and genetic advance for free amino acids in quinoa.....	116
<b>3. Leaf anatomical traits of quinoa</b> .....	119
a. Analysis of variance.....	119
b. Mean performance.....	121

## **Contents (continued)**

c. Effect of water stress on quinoa free amino acids.....	<b>121</b>
d. Effect of genotype on leaf anatomical traits .....	<b>122</b>
e. Description of leaf transverse sections of quinoa genotypes	<b>125</b>
f. Heritability and genetic advance for leaf anatomical traits..	<b>134</b>
<b>4. Molecular differentiation and relationships of five quinoa genotype.....</b>	<b>135</b>
a. Genetic diversity among quinoa genotypes.....	<b>136</b>
b. Genotype identification by unique ISSR marker.....	<b>138</b>
c. Genetic relationships among the five quinoa genotypes...	<b>142</b>
d. Cluster analysis as revealed by ISSR .....	<b>143</b>
<b>CONCLUSION.....</b>	<b>147</b>
<b>SUMMARY.....</b>	<b>149</b>
<b>REFERENCES.....</b>	<b>157</b>
<b>ARABIC SUMMARY.....</b>	



## LIST OF TABLES

No.	Title	Page
1.	Name, origin and seed color of quinoa genotypes under investigation.....	45
2.	Soil and water analysis for the experimental site.....	51
3.	Meteorological data during the two growing seasons of the experiment.....	52
4.	Analysis of variance and expected mean squares (EMS) of separate and across the three treatments.....	56
5.	Description of the ISSR loci used in this study.....	60
6.	Combined analysis of variance of split plot for studied traits of quinoa genotypes under three irrigation regimes (treatments) across two seasons.....	65
7.	Combined analysis of variance across seasons of randomized complete blocks design for studied traits of five quinoa genotypes under well watering (95% FC), water stress (65% FC) and severe water stress (35% FC).	68
8.	Summary of means $\pm$ SE (standard error), reduction (Red%) from well watering (WW) to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum (Max) values for all studied traits across all quinoa genotypes across seasons.....	71
9.	Mean performance of studied traits of each quinoa genotype under well watering (WW), water stress (WS) and severe water stress (SWS) across two seasons.....	75
10.	Percentages of reduction in seed yield/fed and increase in water use efficiency (WUE) for each studied quinoa genotype across two seasons.....	83

11.	Drought tolerance index (DTI) and reduction (Red%) in SYPF from WW to WS and SWS for each genotype under WS (65% FC) and SWS (35% FC) conditions.....	<b>84</b>
12.	Superiority (%) of the most tolerant (T) (CICA-17) over the most sensitive (S) quinoa genotype (Ollague) for selected traits under well watering (WW), water stress (WS) and severe water stress (SWS) .....	<b>88</b>
13.	Correlation coefficients between seed yield/plant (SYPP) and other studied traits under well watering (WW), water stress (WS), severe water stress (SWS) and combined across environments and across two seasons.....	<b>90</b>
14.	Correlation coefficients between each of water use efficiency (WUE) and drought tolerance index (DTI) and other studied traits under well watering (WW), water stress (WS), severe water stress (SWS) and combined across environments and across two seasons.....	<b>93</b>
15.	Correlation coefficients for pairs of selected traits combined across all irrigation treatments and seasons.....	<b>95</b>
16.	Phenotypic (PCV %) and genotypic (GCV %) coefficient variation (%) for studied traits of quinoa genotypes under WW, WS and SWS environments across seasons.....	<b>98</b>
17.	Phenotypic ( $\sigma^2_{ph}$ ), genotypic ( $\sigma^2_g$ ), environmental ( $\sigma^2_e$ ) and genotype x environment ( $\sigma^2_{ge}$ ) variance, heritability ( $H^2$ ) and genetic advance (GA%) for studied traits of quinoa genotypes under WW, WS and SWS.....	<b>99</b>
18.	Analysis of variance of split plot for leaf free amino acids of five quinoa genotypes (G) under three irrigation treatments (T) in 2014/2015 season.....	<b>104</b>

19.	Analysis of variance of randomized complete blocks design for leaf free amino acids of five quinoa genotypes under well watering (95% FC), water stress (65% FC) and severe water stress (35% FC).....	<b>105</b>
20.	Summary of means $\pm$ SE (standard error), reduction (Red%) from well watering (WW) to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum (Max) values for all studied traits across all quinoa genotypes across seasons.....	<b>108</b>
21.	Mean performance of studied traits of each quinoa genotype under well watering (WW), water stress (WS) and severe water stress (SWS) in 2015/2016 seasons.....	<b>110</b>
22.	Total leaf free amino acid contents (mg/g dry matter) of five quinoa genotypes evaluated in the field under well watering (WW), water stress (WS), severe water stress (SWS) and combined across genotypes in season 2015/2016.....	<b>112</b>
23.	Percentage of increase (%) of free amino acid contents in the leaf of each quinoa genotype and across genotypes from well watering (WW) to water stress (WS) and severe water stress (SWS) in season 2015/2016.....	<b>114</b>
24.	Heritability in broad sense ( $h^2_b$ ) and genetic advance from selection (GA) for free amino acids of quinoa under WW, WS and SWS environments in 2015/2016 season....	<b>118</b>
25	Analysis of variance of split plot for leaf anatomical traits of five quinoa genotypes (G) under three irrigation treatments (T) in 2014/2015 season.....	<b>120</b>
26.	Analysis of variance of randomized complete blocks design for leaf free amino acids of five quinoa genotypes under well watering (95% FC), water stress (65% FC) and severe water stress (35% FC) .....	<b>120</b>

27.	Summary of means $\pm$ SE (standard error), reduction (Red%) from well watering (WW) to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum (Max) values for thickness of leaf and studied layers across all quinoa genotypes.....	<b>122</b>
28.	Thickness ( $\mu$ ) of leaf studied quinoa genotypes as affected by water stress (WS) and severe water stress (SWS) compared to well watering (WW) .....	<b>124</b>
29.	Heritability in broad sense (H <sup>2</sup> ) and genetic advance from selection (GA) for leaf anatomical traits of quinoa under WW, WS and SWS environments in 2015/2016 season.....	<b>135</b>
30.	Number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by ISSR primers for five quinoa genotype.....	<b>137</b>
31.	Unique positive and negative ISSR markers generated for five quinoa genotypes, marker size (bp) and total number of markers identifying each genotype.....	<b>142</b>
32.	Genetic similarity (GS) matrices among the five quinoa genotypes.....	<b>143</b>

## LIST OF FIGURES

No.	Title	Page
1.	Means and standard errors (bars) for studied traits of each quinoa genotype under well watering (WW), water stress (WS) and severe water stress (SWS) across seasons.....	79
2.	Leaf transverse section for quinoa genotype QL-3 under the soil moisture 95% FC showing that the air spaces are large, chloroplasts are less and there is a rapture in the lower epidermis, under soil moisture 65% FC showing that the air spaces are large, chloroplasts are less and there is a rapture in the lower epidermis and under soil moisture of 35% FC showing that the air spaces were small, and there is a rapture in the upper epidermis and it was swollen (X. 80) .....	126
3.	Leaf transverse section for quinoa genotype Chipaya under the soil moisture 95% FC showing that the air spaces are large, upper and lower epidermis are not normal, under soil moisture of 65% FC showing that the air spaces are less and under soil moisture of 35% FC showing that the air spaces are less (X. 80) .....	127
4.	Leaf transverse section for quinoa genotype CICA-17 at the soil moisture of 95% FC showing that the air spaces are large, upper and lower epidermis are normal, moderate soil moisture stress (65% FC) showing the three layers of palisade cells are well organized in the upper epidermis; upper and lower epidermis are normal and soil moisture of 35% FC showing that the air spaces are large. Upper and lower epidermis are normal (X. 80). .....	129

5.	Leaf transverse section for quinoa genotype CO-407 at the soil moisture of 95% FC showing that the air spaces are small size; upper and lower epidermis are not normal, soil moisture of 65% FC showing upper and lower epidermis are normal and soil moisture of 35% FC showing upper and lower epidermis are normal (X. 80)...	<b>130</b>
6.	Leaf transverse section for quinoa genotype Ollague at the moisture 95% F.C showing that the air spaces are small size, upper and lower epidermis are normal, moisture 65% F.C showing that the air spaces are small size, upper and lower epidermis are not exist and moisture 35% F.C showing that the air spaces are large in size, upper and lower epidermis are not normal (X. 80). .....	<b>131</b>
7.	Banding patterns of five quinoa genotypes amplified with the ISSR primers HB-15, HB-14, HB-13, HB-12, HB-11, HB-10, HB-09, HB-08, 17898A and 14A M: 100bp DNA ladder, Lane 1: QL-3, Lane 2: Chipaya, Lane 3: CICA-17, Lane 4: C0-407, Lane 5: Ollague.....	<b>140</b>
9.	A dendrogram illustrates the genetic distance among quinoa genotypes based on ISSR data.....	<b>144</b>

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal and is one of the 250 species included in the genus *Chenopodium*, commonly known as ‘goosefoot’ genus (Giusti, 1970). It is a dicotyledonous annual species belonging to the family Amaranthaceae (formerly Chenopodiaceae). It is an allotetraploid ( $2n = 4x = 36$ ) annual, crop as it shows disomic inheritance for most of the traits (Ward, 2000 and Maughan et al., 2004). Quinoa is a predominantly autogamous (self-pollinated) species with varying rates of natural hybridization (10–17 %) depending upon the coincidence of flowering with the presence of pollen vectors (Mastebroek et al., 2002 and Spehar and Santos, 2005).

Quinoa has an exceptional balance between oil (4–9%), protein (averaging 16 %, with high nutritional relevance due to the ideal balance of its essential amino acid content) and carbohydrates (64 %) (Bhargava *et al.*, 2006a and Vega-Galvez *et al.*, 2010). Due to its high starch content (51–61 %) it can be used in the same way as cereals for flour production (Bhargava *et al.*, 2006a and Stikic *et al.*, 2012). In addition, quinoa is a good source of vitamins, oil with high linoleate and linolenate content (55–66 % of the lipid fraction), natural antioxidants such as  $\alpha$ - and  $\gamma$ -tocopherol, and a wide range of minerals (Repo-Carrasco *et al.*, 2003; Vega-Galvez *et al.*, 2010; Fuentes and Bhargava 2011 and Stikic *et al.*, 2012).

Quinoa grain also lacks gluten, which has allowed the development of various foods for consumers with celiac disease (*i.e.* people allergic to gluten) (Jacobsen, 2003). Because of its nutritional