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Assessment of Variation and Stability Parameters of Five Quinoa Genotypes under Drought Stress Conditions

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ENVIRONMENTAL changes are a phenomena that deserve continuous assessment for their impact on the quality and productivity of crop genotypes worldwide, especially required in the Middle East, whether for the development of the agricultural sector or for economic profits. Many crop plants have diverse adaptive mechanisms to tolerate or resist a biotic stress condition. Thus, this study aims to evaluate differential responses of five quinoa (*Chenopodium quinoa* Willd.) genotypes in twelve environments (three irrigation levels and two locations during the two growing seasons 2016/2017 and 2017/2018. Although drought is a biotic stress that has been studied in quinoa the assessment of drought tolerance indices and environmental variation of quinoa genotypes across environments has not been studied satisfactorily. Therefore, in the present work, evaluation of yield reliability is conducted depending on drought tolerance indices and stability of tested genotypes as estimated by grain yield. Results show that Line 14 of the tested genotypes excel in the grain yield and stability parameters across different environments, whereas Chipaya genotype shows the best performance under high drought stress conditions. Yield reliability of the tested genotypes is estimated to combine relatively high yield under drought stress conditions as well as response to improved agricultural conditions and high production in favorable environments.

Keywords: Genotypes, Quinoa, Reliability, Stability, Stress, Variation.

Introduction

Plant production and drought are strongly linked since water deficit is always a significant risk factor that threatens crop production in arid and semi-arid regions of the world (Forouzani & Karami, 2011). With the increasing demand for food resources worldwide, and in order to insure future food security it is important to evaluate and improve cultivated varieties by plant breeding scientists, depending on the assessment of tested genotypes under different environmental conditions (Steduto et al., 2012; Badran & Moustafa, 2014; Algosaibi et al., 2015, 2017). Quinoa (*Chenopodium quinoa* Willd.) has been planted in the Andean region for thousands of years, providing highly nutritious food to low income farmers (Pearsall, 1992). For centuries *Chenopodium* spp. has been planted as a leafy vegetable (*Chenopodium album*) as well as an important subsidiary grain crop (*Chenopodium quinoa and C. album*) for human and animal food stuff due to the high-protein and essential amino acids (Bhargava et al., 2003), and the wide range of vitamins and minerals (Repo-Carrasco et al., 2003). As a result, the food and agriculture organization (FAO) selected it as one of the crops prepared to offer food security in the 21st century (Jacobsen et al., 2003). The quinoa plant is also reported to be tolerant to salinity (Pulvento et al., 2012; Algosaibi et al., 2015), water deficit (Algosaibi et al., 2017) in addition to a number of other environmental stress conditions (Jacobsen et al., 2003; Razzaghi et al., 2011; Almadini et al., 2019). Defining the indicators that plant breeders may apply in open field to improve quinoa, for its tolerance or adaptation to different environmental stresses, remains a matter that needs much effort. Hence, in plant breeding programs, it is important to focus on assessing genotypes under different environments before recommending certain ones.

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The relative performances of genotypes vary from one environment to the other according to the interaction between the genotype and the environment. Such differential responses of genotypes in different environments make it difficult for plant breeders to determine which genotypes should be selected. Different methods have been proposed to address the problems with different genotypes through environment interactions based on the regression coefficient and mean square deviations from linear regression as stability parameters (Finlay & Wilkinson, 1963; Eberhart, & Russell, 1966; Badran, 2015). There is a lack of information pertaining to quinoa growth under environmental stress conditions in Egypt. Therefore, the aim of this paper is to provide a review of the responses of some quinoa genotypes through the study of genetic diversity under different environmental conditions based on stability coefficients and drought tolerance indices taking high yield into consideration.

Materials and Methods

Plant material and experimental conditions

The experimental work of this study was carried out during the two successive growing seasons of 2016 /2017 and 2017/2018 during the period from December to April in two locations: the first was at Wadi Sanour in the Eastern Desert, about 10 km south of Beni Suef, located between latitude (28˚15′ 38"- 29˚ 17′ 25″) North and longitude (31˚ 02′ 59″ - 32˚ 11′ 54″) East and the second location was at Wadi Elmlook, Kilo 52 Cairo-Alexandria Desert Road, Giza Governorate, Egypt.

Seeds of five quinoa (*Chenopodium quinoa* Willd.) genotypes were used in this study obtained from multiple places according to Table1. The genotypes were evaluated under three levels of water irrigation where the quantity of normal water irrigation was 3030m^3/ha, approximately; abbreviated as T1) applied for each plot according to Algosaibi et al. (2017). Drought stress was applied after two weeks from sowing by controlling water supply through irrigation tapes by 85% (T2= 2575 m^3/ha, approximately) and 65% (T3= 1970m^3/ha, approximately) from the normal irrigation level (T1). Irrigation water from wells was collected and soil samples were assembled before planting in the study sites at depth ranging from 0 to 30cm. Next, chemical analyses were performed for assessments as shown in Table 2 a & b.

TABLE1. Name, institution offered origin and attribute of tested fivequinoa genotypes

No	Genotype	Institution offered	Origin	Attribute
1	*C. quinoa* Willd. Ames (Line 12)	N.B.R.I., Lucknow	India	Light creamy
2	*C. quinoa* Willd. PI (Line 14)	N.B.R.I., Lucknow	India	Very light creamy
3	*C. quinoa* Willd. Q3	International Center for Biosaline Agriculture (ICBA)	United Arab Emirates (UAE)	Light creamy
4	*C. quinoa* Willd. Q5	International Center for Biosaline Agriculture (ICBA)	United Arab Emirates (UAE)	Dark creamy
5	*C. quinoa* Willd.Chipaya	Madison university, Wiscanson, USA	AltiplanoSalares, Bolivia	Mixed (white,yellow and Paige)

TABLE 2-a. Chemical analysis of saturated soil paste extract in two locations

Location	Depth (cm)	pH	EC (dS/m)	Cations (meq/L)				Anions (meq/L)			
				Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	SO_4^{--}	HCO_3^-	CO_3^{--}
L1	0:30	7.9	8.00	26.8	0.75	8.4	40.0	45	30.15	0.8	-
L2	0:30	7.73	5.62	10.4	2.5	2.6	34.5	9.6	37.8	2.9	-

Note, L1= The first location; L2= The second location.

TABLE 2-b. Chemical composition of irrigation water used in experimental sector

Location	pH	EC (dS/m)	Soluble cations (meq/L)				Soluble anions (meq/L)			
			Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	SO_4^{--}	HCO_3^-	CO_3^{--}
L1	7.51	3.15	16.50	0.72	7.00	6.20	26.0	1.42	3.00	-
L2	7.35	0.51	2.5	0.21	0.51	1.10	2.8	1.31	0.20	-

Note, L1= The first location; L2= The second location.

Drought tolerance index

Tolerance or sensitivity parameters were calculated for the grain weight (kg ha^{-1}) of the tested genotypes as follows:

1. Drought tolerance index (DTI): DTI= (Yp) × (Yd)/(Ýp)^2 according to Fernandez (1992).

2. Yield injury % (YI): YI= (Yp-Yd)/Yp × 100 according to Blum et al. (1983).

3. Superiority measure (SM): SM= Yd/Yp (Lin & Binns, 1988).

where, Yp= Grain yield of genotype under normal conditions; Yd= Grain yield of genotype under high drought stress; Ýp= Mean yield of all tested genotypes under normal conditions.

Experimental design and statistical analysis

Five genotypes of quinoa plant were tested at twelve different environments (three irrigation treatments during two seasons in two locations) in a randomized block design with three replications per case. The average number of grains weight ha^{-1}, based on 25 plants per replication was calculated for each environment. The genetic stability parameters were calculated for all the tested traits as suggested by Eberhart and Russell (1966) using the following model in order to evaluate the stability of the tested genotypes under twelve different environments: $Y_{ij} = m + B_i I_j + \delta_{ij}$ with $\sum_j I_j = 0$, b= 1. Two parameters of stability were calculated: a) the regression coefficient (bi) and b) mean square deviations (S^-d^2) from linear regression. The selection was estimated for yield reliability (YR) according to Kang & Pham (1988) using Rank-sum method with modification by introducing drought tolerance indices factor and consequently, genotypes across the different environments. This arrangement was based on a) the average of yield b) drought tolerance indices and c) stability parameters. According to the results, the tested genotypes could be arranged by which the minimum total rank represented the best yield reliability. Statistical analysis was employed using the least significant difference (LSD) at 5% level in order to compare the means performance.

Results

The interaction between genotypes and different environments

Results of grain yield ha^{-1} in Table 3 confirm that there are significant differences at all the studied levels i.e., between tested genotypes and between different environments in addition to the interaction between them. On the other hand, the analysis of variance shows that the differences between the tested environments are higher than the differences between the tested genotypes. Moreover data in Table 4 show the interaction between genotypes and different environments as well as the effect of drought levels on the tested genotypes. Results reveal that the highest average varieties under different environments are scored for Line14 and Chipaya genotype at 1493.27 and 1483.71, respectively.

TABLE 3. Analysis of variance pooled data of grain yield h^{-1}

Source of variance	d.f	Mean square of grain yield
Genotypes (G)	4	332603.2 *
Environments (E)	11	1494989.7 *
Replicates in E	24	453.07278
G * E	44	6211.097 *
Error	96	425.32136

Note. d.f.= Degree of freedom;* = Significant at 5% probability level.

Drought tolerance indices of the studied quinoa genotypes

Different drought tolerance indices were estimated for the grain yield ha^{-1} of quinoa genotypes using normal irrigation conditions and high drought stress conditions during the studied seasons (Table 5).

Selection based on the largest values of drought tolerance index (DTI), and superiority measure (SM) showed more tolerance to stress conditions, while the smallest values of these indices indicated higher sensitivity. Therefore, based on these indices Chipaya was the best genotype followed by Line 14 whereas Line 12 and Q3 at the lowest ranks. According to yield injury data in Table 5, Line 12 recorded the highest value of harm as a sensitive genotype under drought stress during the two growing seasons, while all indices indicated that Q5 genotype was of medium tolerance to drought stress. Hence, the results of tested genotypes could generally be classified into three groups based on drought tolerance indices as follow: a) Chipaya and Line 14 genotypes with the highest tolerance, b) Q5 genotype with medium tolerance and c) Line 12 and Q3 genotypes exhibiting the lowest tolerance (the most sensitive).

TABLE 4. The interaction between genotypes and environments based on grain yield (kgha^{-1})

Environment			Line 12	Line14	Q 3	Q 5	Chipaya
Location 1st	Season 1st	T1	1580.28	1747.09	1592.85	1672.78	1678.42
		T2	1090.37	1267.91	1126.22	1162.31	1233.99
		T3	815.86	1106.42	886.65	1020.46	1124.57
	Season 2nd	T1	1798.32	1940.98	1912.83	1903.82	1919.32
		T2	1270.31	1476.86	1305.99	1343.27	1441.78
		T3	911.70	1101.20	958.38	1006.21	1136.21
Location 2nd	Season 1st	T1	1660.71	1854.11	1620.23	1861.64	1782.78
		T2	1234.69	1540.98	1344.78	1470.13	1534.44
		T3	961.38	1192.46	958.82	1139.05	1264.4
	Season 2nd	T1	1679.52	1840.97	1637.56	1805.97	1820.65
		T2	1381.84	1599.65	1488.66	1560.14	1569.51
		T3	998.79	1250.58	985.96	1157.58	1298.40
Mean			**1281.98**	**1493.27**	**1318.24**	**1425.28**	**1483.71**

Note. T1= Normal irrigation; T2= Medium drought stress; T3= Severe drought stress.

TABLE 5. Tolerance indices of studied quinoa genotypes using weight of grain ha^{-1} understress and non- drought stress condition during the growing two seasons at thetwo study locations

Genotype	Drought tolerance index (DTI)				Yield injury (%) (YI)				Superiority measure (SM)			
	The first location		The second location		The first location		The second location		The first location		The second location	
	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Line 12	0.471	0.457	0.518	0.543	48.37	49.30	42.11	40.53	0.516	0.507	0.579	0.595
Line 14	0.706	0.595	0.717	0.746	36.67	43.27	35.69	32.07	0.633	0.567	0.643	0.679
Q3	0.516	0.511	0.504	0.523	44.34	49.90	40.82	39.79	0.557	0.501	0.592	0.602
Q5	0.625	0.530	0.687	0.677	39.06	47.37	38.81	35.90	0.609	0.526	0.612	0.641
Chipaya	0.689	0.608	0.731	0.766	33.00	40.80	29.08	28.68	0.670	0.592	0.709	0.713
Mean	0.599	0.539	0.628	0.648	40.12	46.08	37.17	35.21	0.599	0.539	0.629	0.648

Parameters of stability analysis

The stability parameters for the performance of the studied genotypes under different environments are important factors in breeding programs intending to determine plant productivity. Data in Tables 6 and 7 indicate that, quinoa genotypes responded differently across several environmental conditions, suggesting the importance of genotype assessment under three levels of drought in two locations during two successive seasons, in order to determine the best genotype for grains yield ha^{-1}. According to variance the analysis of the data in Table 6, and due to the interaction between the genotypes and environments in Table 3 the sum of squares is partitioned into two parameters: 1) Sum of squares due to genotype x environment (linear), which is the sum of squares due to regression, and 2) Sum of squares due to deviation from linearity of response; i.e., sum of squares due to pooled deviation.

TABLE 6. Mean square of pooled data of grains weight for tested five quinoa genotypesat 12 differentenvironments

Source of variance	d.f.	Mean square of grains weight
Genotypes	4	332603.2*
Environments	11	1494989.7*
Rep. in Env.	24	453.07278
Env. x Genotypes	44	6211.0973*
Error	96	425.32136

Note.d.f.= Degree of freedom; * = Significant at 5% probability level.

TABLE 7. Analysis of variance for 5 quinoa genotypes under 12 different environments

Source of variance	d.f.	S.S	M.S
Total	59	6016195.9	
Genotypes (G)	4	443470.93	110867.73*
Env. + (G. x Env.)	55	5572725	101322.27
Env.(linear)	1	5481628.9	5481628.9
G. x Env.(linear)	4	29626.805	7406.7013*
Pooled deviation	50	61469.288	1229.3858
Line 12	10	12046.216	1204.6216
Line 14	10	3368.8109	336.88109
Q3	10	25045.112	2504.5112
Q5	10	11388.622	1138.8622
Chipaya	10	9620.5275	962.05275
Pooled error	120	17234.866	143.62388

Note.d.f.= Degree of freedom; *= Significant at 5% probability level.

According to this model, results in Table 7 indicate that the most important stability parameter with the minimum deviation mean square is Line 14, followed by Chipaya cultivar at 336.881 and 962.053 respectively, for grains yield. Furthermore, regression coefficient (bi) and deviation mean squares (S^2di) were estimated for grain yield (kg h^{-1}) of five tested genotypes, through twelve environments. Standard error of regression coefficient (S.E.b), and standard error of mean (S.E.m) are illustrated in Table 8. The data presented in Table 7 show that Line 14 and Chipaya cultivar could be considered stable genotypes for grains yield, among the various studied environments, due to the lowest values in sum of deviation squares. On the other hand, Q3 genotype exhibited the lowest stability, with the highest deviation square, at 2504.5112. Data in Table 4 show that, the nearest regression coefficient (bi), and the least regression to unity were recorded with Chipaya cultivar followed by Line14 genotype, at 0.8687 and 0.9693, respectively; Line 14 genotype had the lowest deviation mean square (S^2di) at 530.138. The genotype Q3, followed by Line 12 showed the lowest stability according to the values of their regression coefficients, and deviation mean squares, at 1.0545, 4865.399 and 1.0598, 2265.619, respectively. Similarly, results in Table 8 show that, Q5 genotype had medium stability of grain yield of 1425.281 kg ha^{-1}, compared to the mean of all tested genotypes under all environments, at 1400.496 kg ha^{-1}.

General rank of yield reliability

According to the data in Tables 4, 5 and 8, results in Table 9 and Fig 1 are presented in ascending order as follow: a) Averages based on the high grain yield, b) Arrangement of the averages based on drought tolerance indicators, and c) Yield stability of the tested genotypes across different environments. The order of the three aforementioned criteria was then combined for the purpose identifying the superior genotypes under study. By examining the results, data according to the Eberhart and Russell model (Eberhart & Russell, 1966) and the Kang and Pham Rank-sum method (Kang & Pham, 1988), based on the highest average grain yield and the lowest in environmental variation confirm that the best genotype was Line 14, followed by Chipaya variety, this is useful for determining yield reliability. The lowest ranks were classified with Line 12 and Q3 genotypes respectively (Table 9). Based on drought tolerance indicators, Chipaya variety recorded the first rank followed by Line 14. On the other hand, when the three parameters were merged together in one order, Line 14 occupied the first rank, followed by Chipaya variety.

Discussion

The studied quinoa genotypes did not show obvious symptoms under drought stress conditions, as evidenced by water deficit tolerance during growth (Martínez et al., 2009; Razzaghi et al., 2012; Algosaibi et al., 2017; Maliro et al., 2017), and by modifying the root system, accumulating some compounds responsible for adjusting osmotic pressure, and reducing leaf stomatal conductance (Bosque Sanchez et al., 2003; Jacobsen et al., 2009; Gonza´lez et al., 2011; Alvarez-Flores et al., 2018 Ebeed et al., 2019; Radwan et al., 2020). Maintaining the quinoa yield under differential environmental changes, mainly drought, salinity, high temperature and other stresses, depends on the selection and breeding of varieties resistant to such conditions. These efforts are hampered by the lack of appropriate indicators that can be used in breeding programs, in order to resist environmental stresses (Algosaibi et al., 2015, 2017; Morales et al., 2017). Therefore, the aim of this study is to identify and discuss some these indicators as follows:

Drought tolerance indices

Regarding drought or stress tolerance index, resistant genotype of irrigation water deficit can be identified as the species producing higher grain yield under drought stress than the average tested genotypes. Based on identification, and the results in Tables 4 and 5, the studied genotypes can be divided into three groups of high, medium, and low resistances under high water stress conditions, compared to the normal conditions. This conclusion coincides with that of Fernandez (1992) that classified the studied genotypes into four groups according to their performance under well irrigated and drought stress conditions. In the same context, Nasir ud-Din et al. (1992), Pourdad (2008), Pireivatlou et al. (2010), Badran & Moustafa (2014) assert a reliable scientific index for the selection of high yield varieties under environmental stress conditions by assessing the results of the tolerance index (TOL), harmonic mean (HM), stress susceptibility index (SSI), geometric mean productivity (GMP), and stress tolerance index (STI) of the tested varieties.

TABLE 8. Mean values of grains weight (kg h^{-1}) over environments, regression coefficient (bi) and deviation mean squares (S^2di) for five tested genotypes

Genotypes	Mean	bi	S^2di
Line 12	1281.981	1.0598	2265.619
14 Line	1493.268	0.9693	530.138
Q3	1318.244	1.0545	4865.399
Q5	1425.281	1.0477	2134.101
Chipaya	1483.706	0.8687	1780.482
Mean	1400.496	1.0000	-
$L.S.D_{(0.05)}$	9.785	-	-
S.E. (b)	0.033	-	-
S.E. (m)	10.572	-	-

Note. S.E (b)= Standard error of regression coefficient; S.E (m)= Standard error of population mean.

TABLE 9. The rank of studied genotypes according to the average of grains yield (kg h^{-1}), drought tolerance indices and environmental variation a cross environments

Genotype	Rank according to grains yield (kg h^{-1}) (a)	Rank according to drought tolerance indices (b)	Rank according to environmental variation (c)	Sum of ranks (a + b + c)	General rank of yield reliability
Line 12	5	5	4	14	5
Line 14	1	2	1	4	1
Q3	4	4	5	13	4
Q5	3	3	3	9	3
Chipaya	2	1	2	5	2

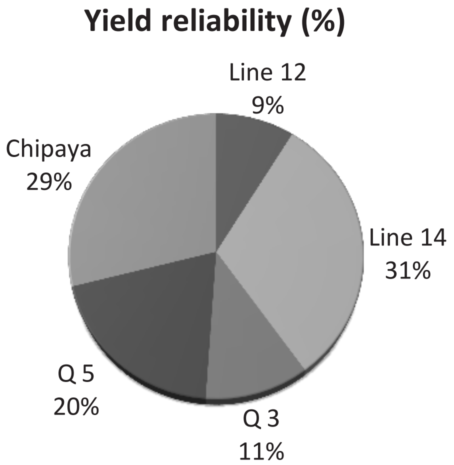

Fig. 1. Yield reliability (%) of tested genotypes

The interaction between genotypes and different environments

The interaction between genotypes and the different environments (G x E) plays an important role in identifying genotypes that combine between high yield and stability. Such environmental interaction is attributed to the impact of the site and the season (Singh et al., 2006). The interaction (G x E) has high impact on the performance of genotypes, leading to the differentiation of genotypes across different environments (Tables 3 and 6). This type of interaction (G x E) is considered the most important type which makes it difficult for the breeder to select the best genotypes because the correlation between the phenotypes and the genotypes under study is weakened. As a result, the efficiency of the selection and the genetic progress required is reduced, especially under the influences of heat and drought stresses (Romagosa & Fox 1993; Annicchiarico, 2002; Trethowan et al., 2005; Singh et al., 2006). Therefore, in this study Eberhart & Russell model (1966) is used to check the reliability by which the genotype with unit regression coefficient (b= 1) and the smallest sd^2 value or not significantly different from zero (Sd^2= 0) is considered the highest stability (Badran, 2015). Yield stability parameters of the tested genotypes across environments in Tables 7 and 8 are estimated according to Eberhart and Russell model, which represents the performance stability of the genotype across environments; referred to as sensitivity; low sensitivity= high stability. This model provides an opportunity to exploit the positive effects of the interaction resulting in better performance in unsuitable environments or seasons. The results would increase the level of security in food production or agricultural income, making this model particularly attractive to the governmental institutions responsible for breeding programs and for the certification of varieties for breeders in private companies. This model is more interesting than other models since the former reduces the interaction effect (G x E) and hence leads to crop response that may express improved agricultural conditions (Lin et al., 1986; Simmonds, 1991; Romagosa & Fox, 1993; Cleveland, 2001; Annicchiarico, 2002; Badran, 2015).

Yield reliability

Indicators identifying genotypes with stress tolerance indices and high stability, and without considering high grain yield are not suitable for plant breeders and companies that prefer selecting distinct high-yielding genotypes at different environments. However, there may be a strong negative correlation between grain yield and stability parameters (Mohammadi et al., 2012). In addition, there are mixed results suggesting a positive relationship between these parameters and grain yield (Kilic et al., 2010). Therefore, the best rank of a genotype can be obtained by estimating the average grain yield (kg h^{-1}), and stress tolerance indices under environmental variation, as shown in Table 9 and Fig. 1.

Conclusion

Quinoa cultivation environments in Egypt are characterized by great variability, including the agricultural seasons, the amounts of irrigation water added during the season as well as the different locations. In such environments, the effect of the interaction is relatively large; thus the breeder cannot ignore the effect of these important interactions on the performance of the genotypes, based on the overall mean of the genotypes across these environments. Therefore, some breeders rely on the order of results according to the yield reliability and based on drought tolerance indices, environmental variation (stability) and the higher grain yield. This is not an alternative rather complementary to the experience of the breeder.

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تقييم مدلولات التباين والثبات لخمسة طرز وراثية من الكينوا تحت ظروف إجهاد الجفاف

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أصبحت التغيرات البيئية ظاهرة تستحق التقييم المستمر لتأثيرها على جودة وإنتاجية الطرز الوراثية للمحاصيل في جميع أنحاء العالم ، وخاصة في منطقة الشرق الأوسط. وذلك مطلوب سواء لتطوير قطاع الزراعة أو الأرباح الاقتصادية. حيث تمتلك العديد من نباتات المحاصيل آليات أقلمة متنوعة لتحمل أو مقاومة ظروف الإجهادات الغير حيوية. وبالتالي ، تهدف هذه الدراسة إلى تقييم الإستجابات المختلفة لخمسة طرز وراثية من الكينوا (*Chenopodium quinoa* Willd.) في إثني عشر بيئة (ثلاثة مستويات للري وموقعين خلال موسمي نمو (2016/2017 و 2017/2018). ويعتبرالجفاف إجهاد غيرحيوي تم دراسته في الكينوا ولكن لم يتم دراسة تقييم مؤشرات تحمل الجفاف والتباين البيئي للتراكيب الوراثية للكينوا عبر البيئات بشكل مرض. لذلك، في العمل الحالي تم إجراء تقييم موثوقية المحصول اعتمادًا على مؤشرات تحمل الجفاف وثبات الطرز الوراثية المختبرة لمحصول الحبوب. وقد أظهرت النتائج تفوق السلالة 14 من الطرز الوراثية المختبرة في محصول الحبوب ومعايير الثبات عبر البيئات المختلفة ، بينما أظهر التركيب الوراثي شيبايا أفضل أداء في ظل ظروف الإجهاد العالي للجفاف. وقد تم تقدير موثوقية المحصول للطرز الوراثية المختبرة للمحصول العالي نسبيا تحت ظروف إجهاد الجفاف والاستجابة لتحسن الظروف الزراعة والإنتاج العالي في البيئات المناسبة.