

PHENOTYPIC FLEXIBILITY IN EXOTIC QUINOA (*Chenopodium quinoa* Willd.) GERMPLASM FOR SEEDLING VIGOR AND VIABILITY

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Exotic quinoa genotypes were evaluated for seedling vigor, viability, heritability, and genotypic and phenotypic variations under lab. conditions. A total of 25 genotypes of quinoa underwent comparison. Distinct demarcation among these genotypes was observed for final germination percentage, mean germination time, time to 50% germination, germination index, root/shoot length and root:shoot ratio. Viable, vigorous genotypes and poorly performing genotypes were identified and grouped from the available exotic germplasm. This selection was made on the assumption that resistance to climatic adversities and better sustainability under poor storage conditions are issues of immense importance in postharvest handling of quinoa seed. For selection of vigorous quinoa genotypes, final germination percentage (more than 90%) and root (more than 3.5 cm) or shoot lengths (more than 4.0 cm) presented themselves as important associated traits, in addition to germination index (22 to 33) and seedling survival percentage (between 90-100%). However, final germination percentage and root length were demonstrated to be reliable and environment-proof traits for the crop.

Keywords: Quinoa (*Chenopodium quinoa* Willd.), germination, germplasm, seedling vigor

INTRODUCTION

Testing of seed vigor is a common practice before field cultivation (Styer *et al.*, 1980). Potential assessment for speed and spread of germination of any seed lot facilitates achievement of superior crop productivity (Basra *et al.*, 2002). This not only saves time and energy but also capital (AOSA, 1990). Seedling vigor is well established as the ability of plants to emerge through the soil surface and grow vigorously under diverse field conditions. This aspect becomes more significant when addressed for seed of alien crops in a particular region. In general, seed vigor and viability changes over the length of storage with a deteriorating trend and in most of the species this deterioration often leads to sterility (Gregg *et al.*, 1994). For such deterioration, vigor is the first indicator resulting in reduced germination (Trawatha *et al.*, 1995). However, slow and non-uniform germination or emergence are proven attributes of low seed vigor and viability (Khan, 1992; Basra *et al.*, 2002), providing enough information to decide the fate of any seed lot for sowing under field conditions.

Quinoa (*Chenopodium quinoa* Willd.) is an ancient grain of people belonging to the Andean region of Latin America. It is presently under cultivation in a number of countries of the Americas, Europe and Asia (Jensen *et al.*, 2000; Jacobsen, 2003). As a pseudo-cereal and a member of *Chenopodeaceae* family, quinoa grain is of high economic, nutritive and edible significance (Jacobsen, 2003; Bhargava

et al., 2006); however, the size of the grain is very small with an approximate 1000 grain weight ranging between 2-5 g and seed diameter of less than 3 mm (Valencia-Chamorro, 2003). Moreover, its potential to yield abundant seed in spite of adverse climatic conditions such as frost, chilling, drought, freezing, salinity and nutritional stress has made it attractive for introduction in arid, semiarid, saline, and highland regions throughout the world (Choukr-Allah, 1996; Jacobsen, 2003; Schabes and Sigstad, 2005; Erley *et al.*, 2005; Bonifacio, 2006). Quinoa is considered among the crops selected for future food security during the 21st century (FAO, 1998a). The significance of quinoa has therefore been increasingly recognized as severe reductions in productivity are being faced due to salinity and water-logging on badly managed irrigated croplands (FAO, 1998b).

A number of external factors affect seed vigor in quinoa with storage conditions as the most critical issue (Bertero and Ruiz, 2010; Abbas *et al.*, 2011). During introduction phase of any crop species, large number of accessions is introduced as potential candidate for their cultivation in target environment or to utilize in breeding and selection programs. However, all the accessions cannot be tested in the target environment due to limited resources and financial constraints. Suitable candidate accession may be chosen from the elite germplasm before cultivation and testing in such target environment. For the purpose, seedling vigor traits have been found reliable, cheaper and rapid way for screening large germplasm (Rauf, 2008). Seedling vigor

traits describe the physiological and biochemical condition of the seed. As seed development takes place on the plant, therefore physiological and biochemical properties are greatly affect the plant performance in the field. Therefore, it has been found that genotypic performance on the basis of seedling trait often corroborate to the field performance of genotype.

On the basis of these grounds, quinoa germplasm was assessed before planting. Selection of seed on the basis of seedling vigor traits under controlled conditions will help in the selection of genotypes having superior seedling vigor in field experiments and under field production conditions.

MATERIALS AND METHODS

Twenty-five quinoa genotypes obtained from United States Department of Agriculture, Plant Introduction Station, Iowa State University, Iowa, USA (Table 1) were analyzed for their vigor by sowing in washed and sterilized 100 mm glass Petri dishes on double-layered Whatman No. 41 autoclaved

filter papers using three replications and standard sowing parameters at $20\pm 2^{\circ}\text{C}$ temperature in a germinator as described by Munir and Basra (2010).

Vigor analyses: Germination and seedling vigor was quantified by daily germination counts at 24 h intervals and was further scrutinized in order to calculate final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T_{50}) and germination index (GI). A seed was considered germinated when radical protrusion was measured to 3 mm or more (Basra *et al.*, 2005). Seedling vigor was assessed by determining seedling fresh and dry weights, and by measuring root and shoot lengths. Final germination was counted and percentage was calculated out of total seed sown.

Time to 50% germination (T_{50}) was determined using the following formula (Coolbear *et al.*, 1984) where 'N' stands for number of germinated seeds, ' n_i ' or ' n_j ' refers to cumulative number of germinated seed by adjacent counts at times ' t_i ' and ' t_j ' with the assumption if $n_i < N/2 < n_j$.

Table 1. Details of quinoa germplasm under test obtained from United States Department of Agriculture

Sr.	Code ¹	G. Line*	Plant name*	Origin
1.	Ames30	Ames-13730	IESP	New Mexico, USA
2.	Ames37	Ames 13737	2WANT	New Mexico, USA
3.	Ames37	Ames-13739	29TES	New Mexico, USA
4.	Ames60	Ames 13760	22GR	New Mexico, USA
5.	Ames62	Ames 13762	47TES	New Mexico, USA
6.	P32	PI 510532	Quinoa de Quiaca.	Peru
7.	P33	PI 510533	K'ello quinoa (Quechua)	Peru
8.	P37	PI 510537	Koito Juira (Aymara)	Peru
9.	P40	PI 510540	Grande (Spain.)	Peru
10.	P42	PI 510542	Villa Juira (Aymara)	Peru
11.	P79	PI 643079	Pasankalla	Peru
12.	P18	PI 634918	Baer	Chile
13.	P19	PI 634919	Pichaman	Chile
14.	P21	PI 634921	UDEC-2	Chile
15.	P22b	PI 634922	UDEC-4	Chile
16.	P93	PI 596293	Colorado 407D	Colorado, USA
17.	P98	PI 596498	Rosa de Junin	Peru
18.	P22a	PI 614922	Sayana	Bolivia
19.	P10	PI 478410	R-66	Bolivia
20.	P24	PI 584524	QQ056	Chile
21.	P05	PI 614905	CQ105	Bolivia (Oruro)
22.	P06	PI 614906	CQ106	Bolivia (Oruro)
23.	P07	PI 614907	CQ107	Bolivia (Oruro)
24.	P08	PI 614908	CQ108	Bolivia (Oruro)
25.	P09	PI 614909	CQ109	Bolivia (Oruro)

(* as per the germplasm database; ¹coding of genotypes made for local identification)

$$T_{50} = t_i + \left(\frac{N - n_i}{2} \right) \frac{(t_j - t_i)}{n_j - n_i}$$

However, mean germination time (MGT) was calculated according to the following equation of Ellis and Roberts (1981). Here ‘n’ refers to total germinated seed counted on day ‘D’, i.e. the number of days counted from the day on which germination started.

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

The standard procedure for calculating germination index (GI) was adopted as described by the Association of Official Seed Analysts (AOSA, 1990):

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Seedlings were analyzed for vigor by first removing them carefully from the filter papers. Five seedlings were randomly selected from each replicate and values were averaged. Root and shoot fresh weights were determined at harvest, whereas root and shoot dry weights were taken following four days drying in oven at 70°C on achievement of concordant weight values.

The following formulae were used for calculation of genotypic and phenotypic coefficients of variation (Singh and Chaudhary, 1985):

$$\text{Phenotypic coefficient of variation (PCV)} = 100 \sqrt{\sigma_p^2} / X$$

$$\text{Genotypic coefficient of variation (GCV)} = 100 \sqrt{\sigma_g^2} / X$$

Where:

$$\sigma_g^2 = (\text{genotypic mean square} - \text{error mean square}) / \text{number of replications}$$

$$\sigma_p^2 = \text{genotype mean square} / \text{number of replications}$$

X = The mean

Heritability, by definition, is the extent to which individual genetic differences contribute to individual differences in observed behavior (the phenotype) in offspring as described by Akinwale *et al.* (2011).

$$H^2 = \frac{\text{Var}(G)}{\text{Var}(P)}$$

Where:

H² = Heritability

Var(G)= Genotypic variance

Var(P)= Phenotypic Variance

Results obtained were compared at the 5% probability level using Fisher’s analysis of variance technique (Steel *et al.*, 1997) as summarized in Table 2.

RESULTS

Seedling vigor traits: Analyses of variance exposed significant variation (P>0.05) among the genotypes for all germination and seedling vigor traits. Seedling vigor traits are represented in Figure 1a,b,c,d,e,f and Figure 2. Genotypes Ames30, Ames37, P37, P40, P42, P79, P19, P93, P22b and P10 were found to have more than 90% final germination. However, the rest of the genotypes including Ames39, Ames60, Ames62, P33, P18, P21, P22a, P98, P12, P05, P06, P07, P08 and P09 had less than 50% seed germination. The highest germination was observed in P37, whereas P05 was recorded with least seed germination. Moreover, genotypes Ames30, Ames37, Ames39, P37, P40, P42, P19, P93, P98, P22b and P10 completed their 50% germination between 1.00 and 1.50 days (Fig. 1b). The lowest T₅₀ was observed in P37, whereas P09 exhibited the maximum T₅₀ with statistically similar duration observed in genotypes P18 and P07. However, genotypes Ames30, Ames37, Ames62, P37, P40, P42, P19, P22a and P22b showed a maximum of 2.5 days mean germination time, while the rest of the genotypes took longer time to germinate.

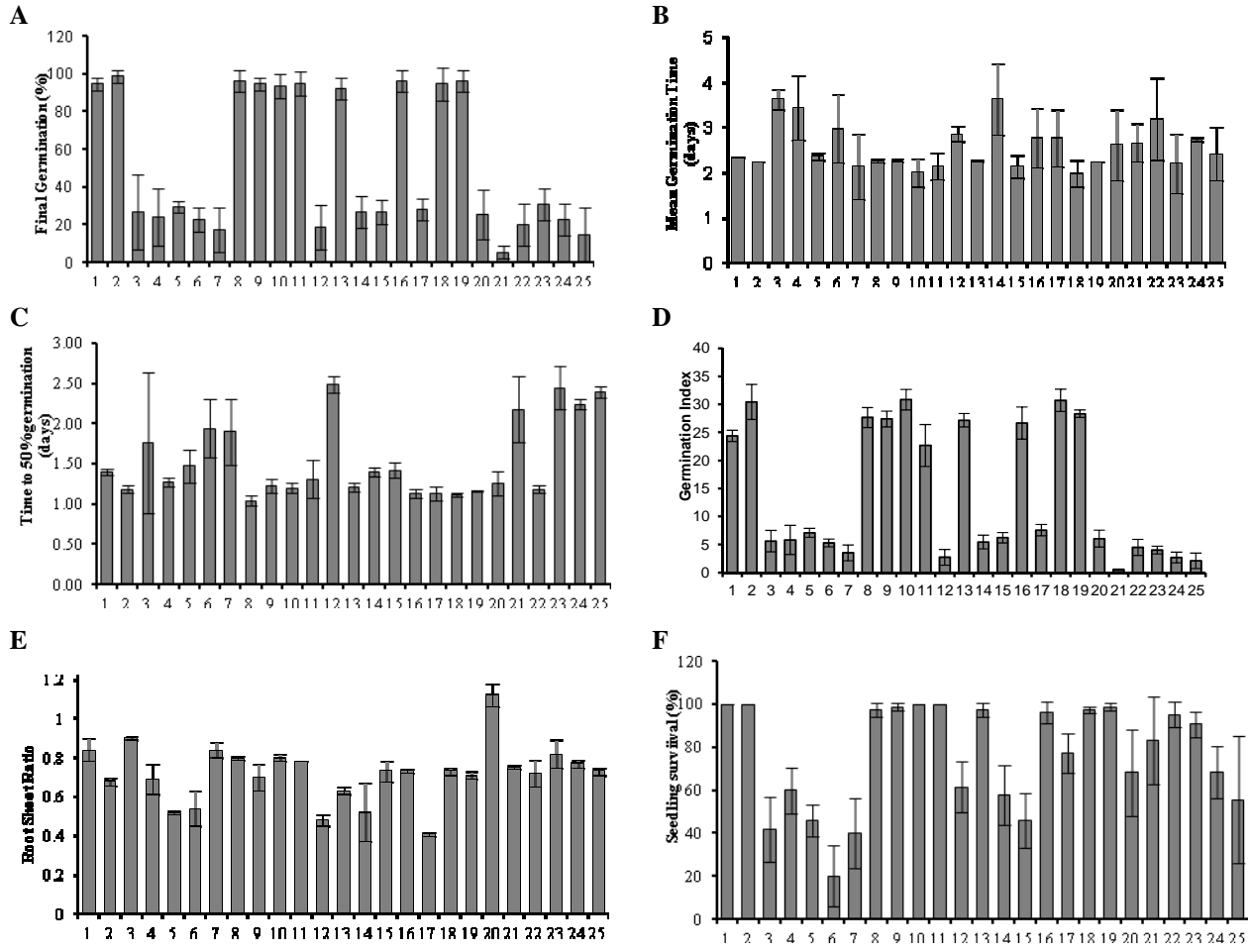
For root length, Ames30, P37, P40, P42, P79, P90, P93, P22b and P10 had significantly longer root lengths, with maximum length observed in P42. Accessions P18 and Ames60 were statistically similar for the shortest root length. Figure 2 explains that genotypes Ames30, Ames37, P37, P40, P42, P79, P19, P93, P22b and P10 had shoot length ranging 4.0 to 5.05 cm. All other genotypes had lesser seedling length, while the lowest value of 0.70 cm was observed in genotype P22a. For root:shoot, genotype P24 had the highest value followed by Ames39 and P07. This ratio was the lowest (0.38) for P98. Genotypes Ames30, Ames37, P37, P40, P42, P93 and P19 had root:shoot between 0.3 and 0.6.

For seedling survival percentage Ames30, Ames37, P42 and P79 exhibited values of one hundred percent, while P19,

Table 2. Summary of the analyses of variance (ANOVA) in quinoa

	DF	FGP (%)	50G	MGT	RL	SL	R/S	SS%
Accessions	24	4009.56**	0.68**	0.71 ^{NS}	5.43**	9.63**	0.07**	1812.29**
Error	50	41.60	0.12	0.47	0.19	0.21	0.00	273.77
Total	74							

Final germination percentage (FGP), Time to 50% germination (50G), Time to mean germination (MGT), Root length (RL), Shoot length (SL), Root shoot ratio (R/S), Seedling survival (SS%)

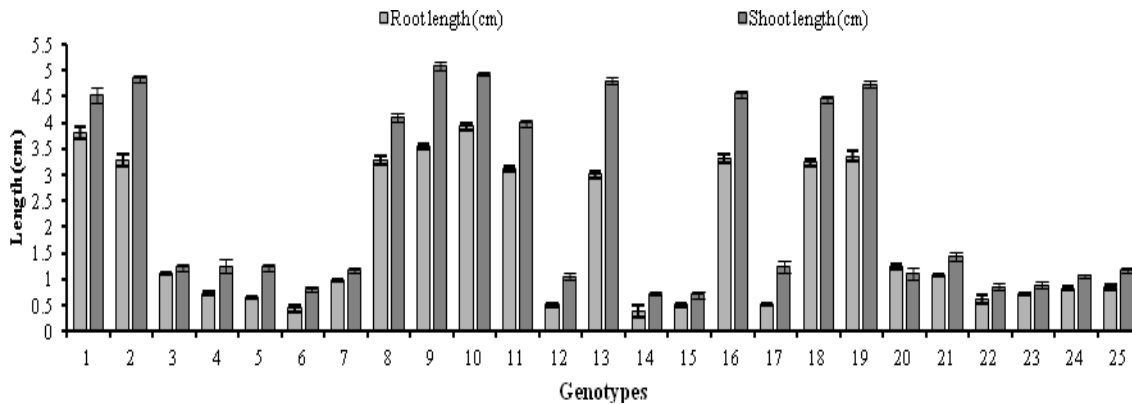


Genotypes

Genotypes

Genotypes: 1. Ames13730; 2. Ames13737; 3. Ames-13739; 4. Ames13760; 5. Ames-13762; 6. PI510532; 7. PI510533; 8. PI510537; 9. PI510540; 10. PI510542; 11. PI643079; 12. PI634918; 13. PI634919; 14. PI634921; 15. PI634922; 16. PI596293; 17. PI596498; 18. PI614922; 19. PI478410; 20. PI452512; 21. PI614905; 22. PI614906; 23. PI614907; 24. PI614908; 25. PI614909

Figure1. Seedling vigor traits of quinoa



*legends for genotypes on x-axis as described in the Fig. 1.

Figure 2. Seedling root and shoot lengths of the exotic quinoa genotypes

P93, P22b and P10 had greater than ninety percent survival. In contrast, P32 showed the lowest seedling survival percentage at 20%.

Heritability, genotypic and phenotypic coefficients: In addition to seedling vigor traits, variation among the genotypes was also estimated through genotypic and phenotypic coefficients of variation. For seedling vigor traits, the highest genotypic variation was observed for root length, shoot length and final germination percentage (Table 3). In addition to the highest genotypic and phenotypic variation among the traits, they also showed the highest heritability estimates. Final germination percentage and root or shoot length was with the highest estimates on the basis of genotypic coefficient of variation (GCV) and heritability (H); therefore, these traits appeared to be the bases for selection of genotypes.

Pearson correlations for seedling vigor traits: Among seedling vigor traits correlations were also estimated (Table 3). These showed that final germination percentage positively and significantly correlated with all the seedling vigor traits. Similarly, mean root length and mean shoot length traits also showed significant positive correlations with all the traits. On basis of these findings it may be inferred that the selection of quinoa genotypes on the basis of final germination percentage, mean germination time and means of root or shoot length would also result in positive impacts on other seedling traits such as germination index and seedling survival percentage.

DISCUSSION

Presence of adequate genetic variation, high heritability and ease with which germplasm may be screened are the basic

criteria for rapid evaluation of germplasm (Rauf, 2008). In this regard, better germination and robust seedling vigor have been found as the basic parameters for selecting suitable genotypes from among introduced germplasm (Dodig and Jovic, 2008; Rauf, 2008; Rau *et al.*, 2008). Therefore, seedling traits have been extensively used for screening germplasm in various species under diverse environmental conditions (Munir, 2004). However, a few studies have been reported in quinoa, thus there was a need to determine the magnitude of genetic variation and scope of seedling traits as basic parameter for screening germplasm. Under the present study, 25 exotic genotypes were studied. Significant variation regarding germination, seedling vigor and survival of the seedlings was observed. Quinoa seeds are orthodox seeds (Ellis *et al.*, 1988). Although orthodox seeds have usually longer storage longevity, the storage life depends upon proper seed drying and storage techniques. Quinoa seeds showed better shelf life if they were dried to the nail dented stage, otherwise the seeds would deteriorate and show poor germination (Ng *et al.*, 2007).

Variation among the genotypes for final germination percentage generated information relevant to the final status of the seed of different genotypes prior to its cultivation in the field. Poor germination seems due to dormancy of the seed. The issue may be due to the incompatibility of genotypes with the storage environment, whereby some of the accessions could not give optimum germination after a two-month storage period. This sort of storage hazard is more pronounced when seed like quinoa undergo cultivation in new lands because of unpredictable storage conditions during transit (Ceccato *et al.*, 2011), and no germplasm dissemination facility can guarantee viability of seed upon arrival at the doorstep of the stakeholder (Ceccato *et al.*,

Table 3. Genotypic and phenotypic variation parameters in quinoa

TRAIT	RANGE	AVERAGE	GCV (%)	PCV (%)	H ²
Root Length	0.38-3.93 cm	1.81	74	78	0.97
Seedling survival	20-100%	75.84	30	37	0.61
Shoot length	0.70-5.09 cm	2.48	72	75	0.15
Final Germination	5.33-98.67%	51.63	70	72	0.91
T50	1.04-2.49 days	1.54	28	56	0.94
Mean Germination	1.99-3.63days	2.57	11	29	0.65

Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Broad Sense Heritability (H²)

Table 4. Correlation matrix among the examined traits of quinoa

PLANT TRAITS	FGP	MGT	GI	MRL	MSL
Mean germination time	-0.53				
Germination index	0.99	-0.53			
Mean root length	0.96	-0.55	0.96		
Mean shoot length	0.97	-0.54	0.98	0.98	
Seedling survival	0.75	-0.44	0.73	0.77	0.77

Final germination percentage (FGP), Mean germination time (MGT), Germination index (GI), Mean root length (MRL), Mean shoot length (MSL)

2011). Germination index and time to 50% germination, in addition to mean germination time, are sufficient to predict performance of any particular seed lot under field conditions (Coolbear *et al.*, 1984; Basra *et al.*, 2005). Vigor and root length are important markers for assessment of performance of any seed lot in the field, even under stressful conditions. Genotypic ability to emerge lengthy roots and shoots upon germination of the seed in the absence of exogenous nutrition predicts the potential of any crop for better field performance (Kamoshita *et al.*, 2006).

Low heritability values in the presence of high PCV values show high environmental influence (Khan *et al.*, 1992). High heritability values for root length, final germination percentage and time to 50% germination show dominant effect of genes. Furthermore, root length also showed high genotypic variation, thus showing higher contribution of genes in the development of phenotype which is in agreement with Malik *et al.* (2000). Similarly, the final germination percentage and root length had high heritability and PCV values which proved significant for determining genetic variability and basic screening of the genotypes as proposed by Ibrahim and Hussain (2006).

According to the correlation matrix of seedling germination and vigor traits; root length, shoot length, seedling survival percentage, germination index, and mean germination time exhibited positive correlations with the final germination percentage, proving dependence of these traits on final seedling count and affirming the above inferences.

In general, two distinct groups of genotypes emerged as a result of their seedling vigor assessment. Ames30, Ames37, P37, P40, P42, P79, P19, P93, P22b and P10 were members of the highly vigorous group due to their uniformity in speed and spread of germination. These quinoa genotypes had higher survival percentage and produced superior seedling stand in less time than that taken by the rest of the genotypes and with negligible fluctuations. Consequently, this group can be selected for further assessment as the better performing germplasm.

CONCLUSIONS

The selection of quinoa genotypes on the basis of final germination percentage and root or shoot length would also result in positive selection for other seedling traits such as germination index and seedling survival percentage. In addition, final germination percentage and root length are identified as reliable and environment-proof (stable) traits for selection in quinoa germplasm. Pre-sowing assessment of these parameters for quinoa seed lots can help to avoid poor field performance of the crop especially while assessing its adaptability in new regions.

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