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Simultaneous Selection of Proline Contents and Panicle Plant^{-1} for Evolution of High Yielding Quinoa (*Chenopodium quinoa* wild.) Progenies in Pakistan

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Abstract
Quinoa, a halophyte native of South America is famous for its acclimatization in new environments and adverse climatic conditions. Inadequate native quinoa production has encouraged its cultivation in new regions. One hundred and fifty quinoa genotypes belonging to a variety of origins were sown in the field under Faisalabad conditions to study relationship among morphological and physiological traits. Panicle Plant^{-1} had the highest direct effect on yield. Harvest index also emerged as the key parameter for seed yield determination perhaps being a yield parameter and laborious trait some other parameters are needed for quick assessment of high yielding quinoa under indigenous Pakistani conditions. Among biochemical traits, proline contents showed high positive direct on grain yield. On the basis of this information, a comprehensive quinoa improvement program was initiated for the selection of high yielding quinoa genotypes under shuttle breeding program carried out at two locations for the rapid segregation generation advancement of quinoa population. Simultaneous single plant selection in segregating generation ($F_2 – F_6$) was done for proline contents initially during vegetative phase and subsequent selection of panicle plant^{-1} to constitute high yielding progenies. High yielding progenies were evaluated at three locations which showed significant improvement in economic yield over unselected accessions.

Keywords: Sub-tropical, multiple regression analysis, yield appraisal, introduction.

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Introduction

Quinoa (Chenopodium quinoa Willd.) is highly nutritious chenopod and a pseudocereal with status of staple food in a number of Latin American Andean countries. It is one of the potential climate proof grains for ensuring food security in the current millennium (FAO 1998). Quinoa has also been indicated to show resistance to various abiotic stresses (Valencia-Chamorro, 2003; Bonifacio, 2006) such as salinity of high gradients (Jacobsen, 2003; Jacobsen et al. 2003; Schabes & Sigstad, 2005) and under poor soil nutrition (Erley et al., 2005). In addition, quinoa grain quality meets dietary standard and is gluten free with sufficient fiber, essential amino acids, high concentration of protein, and ash content with exuberant character of least sodium content (Tapia, 1979; Valencia-Chamorro, 2003; Bhargava et al., 2006). Moreover, dependence on few grains has brought inaccessibility of food to 35 million human out of a 155 million population (FAO, 2011). On the basis of these reasons, Quinoa species has been introduced in few countries with motive to diversify the sources of grain species. Crops like quinoa with climate proof potential can be the prospective candidates for achievement of food security of the rising populace especially under salinity, drought, frost and nutrition stresses (Hughes & Stachowicz, 2004; Hajjar et al., 2008). Moreover, high dependency of field crops on the input had significantly increased the production which had reduced the profitability of the farmers. Quinoas being low input requiring respond well under low nutrient and even give acceptable yield and harvest index. On the basis of these grounds, the adaptability of quinoa in central Pakistani Punjab as a new cereal by means of its morphological, physiological and agronomic response assessed through biometric techniques which will help to evaluate its performance under proposed climatic conditions.

Materials and methods

All experiments were carried out (2008-13) at various locations in the province of Punjab, Pakistan.

Evaluation of Quinoa Germplasm

Experiment was conducted with objective to determine correlations among plant traits and to partition these correlations into direct and indirect effect using 150 introduced Quinoa accessions during the year 2008-09, October-March on the Research Farm, department of Agronomy, University of Agriculture Faisalabad, situated between 31.41°N and 73.07°E; Chakwal situated between (32.93° N, 72.86° E) and Bhawalpur 29.40° N, 71.68° E, Pakistan. Field experiments were conducted on a well pulverized and cultivated sandy loam soil to provide optimum seed bed for testing of available accessions under local conditions by employing Augmented Randomized Complete Block Design.

Seeds of accessions were sown on five meter long ridge per accession with 15cm × 75cm plant to plant and row to row distance, respectively through hand placement in a fallow field with six month old cropping history which was kept cultivated to avoid weeds. No artificial fertilizer was applied so that true seed potential may be evaluated for being a low input requiring crop whereas; occasional irrigation was applied on basis of visual observation of need. Weeding was done by hand to avoid any other plant intervention in growth and yield phases of the crop.

Development of Plant Populations

Promising accessions (P-643079, P-510542, P-510540 and P-614922) selected on the basis of initial screening of germplasm having extreme contrasting traits for panicle plant-1 a crossed (P-643079 × P-510542; P-643079 × P-510540; P-614922 × P-510542) to develop trangressive segregants. Single panicle was selected plant^{-1} and remaining was removed with the scissor to facilitate pollination. In order to emasculate, panicles prior to their anthesis were immersed in wide mouth open kettle with temperature adjusted to 48°C for five minutes. Emasculated panicles were covered and pollinated with pollen of other accession. F_1 seed was harvested from emasculated panicles and germinated in green house. F_1 population was self pollinated to develop segregating populations such as F_2 population. F_2 seed was sown in field and promising plants from different crosses were self pollinated and selected.

Single Plant Selections Within and Between Plant Progenies

High yielding plant progenies (F_3) were evaluated on two diverse locations (Faisalabad 31.41°N and 73.07°E; Chakwal (32.93° N, 72.86° E), for three years to acclimatize progenies on these locations in the province of Punjab, Pakistan. Shuttle breeding was used to select promising plant within and between progenies on the basis of yield components. Selected plants were self pollinated by covering their panicle with bags to grow next generation. Initial screening was carried out on proline content and final selection was made on panicle plant^{-1}. Selected progenies were further evaluated for proline contents and panicle plant^{-1} on next locations. Finally, highly acclimatized six progenies were evolved and evaluated.

Evaluation of Selected Progenies over Standard Accessions

Selected progenies were evaluated over three locations (Faisalabad, Chakwal and Bhawalpur) for two years along with standard check accessions for yield, yield contributing traits and adaptability trait in randomized complete block design. Seeds of accessions were sown on five meter long ridge per accession with 15cm × 75cm plant to plant and row to row distance, respectively through hand. Weeding manually done and no pesticide was applied to the crop.

Measurement of Plant Traits

Five plants were randomly tagged for data collection at the time of maturity for various morphological traits. Leaf area (cm^2) was measured with CI-202 portable leaf area meter (CID, Camas, Washington, USA). Plant height (cm) was measured with meter rod from base to the meristematic tip. Number of leaves plant^{-1}, number of panicles plant^{-1}, number of branches plant^{-1} were counted manually. Thousand grain weight and economic yield (seed yield) masses were measured on digital balance. Harvest index was the ratio of seed yield to biological yield. Main panicle length was measured with measuring tape. Stem diameter was measured with vernier caliper. Days to maturity was measured from days to emergence to the reproductive maturity (turned yellow and leaves drop). Seedling survival percentage was the ratio of survived seedling to the total germinated seedling and converted to % age

Physiological and some morphological traits were measured at the time of anthesis. Number of leaves plant^{-1}, number of panicles plant^{-1}, number of branches plant^{-1} were counted manually. Total chlorophyll, proline and soluble phenolic contents were measures according to the procedures described by Nagata & Yamashita (1992), Bates et al., (1973) and Waterhouse (2001), respectively. Leaf water potential was measured in second fully expanded leaf from top using water potential apparatus as described by Scholander et al. (1965).

Biometrical Procedures

Analyses of data were carried out under randomized block design with three factors i.e. accessions or progenies, years and locations. Correlations were computed through statistical software, MiniTab (Minitab 15, 2010, State College, USA). Genotypic and phenotypic coefficient of variation and heritability estimates were computed according to the methods narrated in Chahal & Gossal (2002).

Results

Evaluation of Quinoa Germplasm

150 accessions of quinoa were evaluated for two years. Various evaluation parameters and biometrical measurements have been given in Table 1. Evaluation of quinoa germplasm showed significant variation within quinoa germplasm. The highest magnitude of phenotypic variation in germplasm was observed for grain yield, followed by harvest index and proline contents. However, environmental variation such as location and year contributed significantly to the phenotypic variation. Therefore presence of high phenotypic variation was not indicative of high genotypic variation especially for grain yield (Table 1). Proline contents showed the highest genotypic variation followed by harvest index. These two traits appeared to be good selection criteria as both phenotypic and genotypic variation was high for both traits and genotypic variation was reflected in the phenotypic variation. Other traits such as leaves plant-1, branches plant-1 and panicles plant-1 also showed high genotypic variation as Genotypic coefficient of variation was higher than 50%. Among these traits, panicle plant-1 was less laborious and could be regarded as good selection criterion.

Evaluation of Segregating Generations and Plant Progenies

Plant were selected on the basis of proline contents at the time of anthesis and final selection was carried out on the basis of panicle plant-1 to select best progenies and best plants within progeny while the inferior plants were discarded. Progenies were evaluated along with check in replicated yield trials in F_3-F_5 generation to evaluate their yield potential (Table 2). Over all means showed significant improvement in the seed yield over various generations. The heritability of seed yield per see was lower in earlier generation and moderate in later generation (Table 2).

Table 1. Mean, Ranges, Genotypic and Phenotypic Variability for Various Morphological and Physiological Traits in 150 Quinoa Accessions

Traits	Average	Range	σ^2Genotype	σ^2Phenotype	GCV%	PCV%
Plant Height (cm)	131.51± 7.14	54.18 -165.78	1451.29	1753.31	28.97	31.84
Leaf area (cm^2)	28.26± 1.43	13.06-36.61	45.61	65.61	23.90	28.66
No. of branches $plant^{-1}$	16.99± 2.70	5.45-32.54	81.41	113.68	53.10	62.75
No. of leaves $plant^{-1}$	142.39± 11.72	41.82-281.87	6060.54	8061.25	54.68	63.06
Panicle $plant^{-1}$	16.44± 2.66	4.93-23.90	163.67	229.29	52.36	61.02
Stem diameter	1.42± 0.11	1.20-2.37	0.16	0.26	23.33	29.65
1000-grain weight	1.41± 0.17	1.21-2.92	0.65	0.78	34.83	38.25
Grain yield (kg ha^{-1})	972.55± 47.02	531.21-2102.70	1060421.12	640421.10	47.85	83.82
Harvest index	0.21± 0.01	0.14-0.47	0.03	0.05	57.41	74.12
Seedling survival%	40.60± 1.77	34.53-68.43	441.90	591.46	29.77	34.45
Days to maturity	111.78± 2.59	99.16-131.32	1349.59	1658.91	32.86	36.44
Proline contents ug g^{-1}	0.31± 0.01	0.13-0.61	0.06	0.09	57.64	71.42
Total chlorophyll contents	13.79± 4.20	11.42-21.09	16.52	21.07	22.84	25.77
Leaf water potential	-0.62± 0.03	-0.85 to -0.49	0.01	0.05	16.00	35.77

Evaluation of F6 Plant Progenies

Finally progenies were reduced to six in F6 generation and were evaluated at three locations along with the standard check. Analysis of variance showed significant variation due to progenies and standard checks (Table 3). Plant progenies also showed significant interaction with locations and years ($P \leq 0.01$). However, mean sum of square due to interactions were lower than progenies sum of square.

Therefore, progenies means were averaged over the location and years (Table 3). Mean performance of various progenies along with standard checks have been given in the Table 4. The results showed significant increase of overall plant progenies performance for traits such as biological yield, economical yield, harvest index, proline contents and panicles plant-1 (Table 4). Among the plant progenies, “UAF-CP-7” showed the highest biological yield, while “UAF-CP-9” showed the highest economical yield and panicle plant-1 and proline contents. “UAF-CP-5” also showed comparable performance for these traits. “UAF-CP-12” had the highest harvest index with good yield potential but with lowest biological yield. These advanced lines could be candidate for the varietal approval in Pakistan.

Table 2. Mean performance of various quinoa progenies (F_3 - F_5) for grain yield (kg ha-1) in replicated trials.

Pop*	**NoP****	**h^2**	**Grain Yield (Kg ha^{-1})**	
			Average	**Range**
F_2	130	0.3	1584^{d}±156	1123-2912
F_3	96	0.4	1951^{c}±95	1421-2884
F_4	48	0.4	2254^{b}±46	2154-2987
F_5	19	0.5	2426.^{a}±58	2274-31875

*Pop= population, NoP= number of progenies

Discussion

Quinoa is a highly nutritious grain with high rate of adaptability to a variety of climates round the world including mountain range of Himalaya of indo-Pak subcontinent, central African mountains, Kenya, Vietnam, Mediterranean countries like turkey, North Africa and southern Europe (Risi & Galwey, 1989; Jacobsen, 2003; Jacobsen et al., 2007). Growing demand, high pricing in international market and restricted yields in the native regions have highlighted the need of this crop to be domesticated in nonnative areas of the world in order to fulfill this supply demand gap (Jacobsen, 2003; Jacobsen & Risi 2001, Bhargava et al., 2007). This effort was further triggered by the FAO test of quinoa for Europe, Americas and Asia, and preliminary successes of field research in Greece, Demark, England, Kenya, Turkey, China and Italy has emerged this crop to be a new winter substitute with high worth and climatic resilience (Jacobsen, 2003; Bhargava et al., 2006; 2007).

For being a native to Andes Mountains in the Latin America, quinoa introduction under Faisalabad, Punjab province of Pakistan conditions is acclimatization needed issue. As results indicate, plant progenies with high yield potential and good harvest index (Table 4) were developed which proves quinoa is found to be producing appreciable yields when compared with other new sites. A variety of analyses were done for the study revealed parameters of importance in the evolution of high yield varieties. These analyses revealed that the harvest index is major yield contributing trait but this trait does not help assess the crop appraisal for economic yield at booting or anthesis level (Bhargava et al., 2007). To avoid this laborious trait which is derived from economic and biological yields, an easier reference trait is felt needed to be explored and seems logical to select any genotype for general cultivation under new regional conditions. A number of traits were found to be good selection criteria when judged over several selection criteria such as impact on economic yield of quinoa, heritability and genotypic variation within germplasm. The traits such as harvest index, number of panicles per plant, proline content and biological yield were good selection criteria. Earlier reporting exhibits that increase in biological yield and reproductive partitioning are found to be of immense importance in estimating economic yield (Andrade et al., 1999). This may also be referred to the photosynthetic efficiency and translocation of photosynthates termed as source sink relationship which better be exhibited in the form of harvest index which is ranging between zero to 0.47 according to present data and is a bit contrary to Rojas et al. (2003) with range of 0.06 to 0.87 and 0.26 to 1.43 (Bhargava et al., 2007). Hence, the parametric effect may be combined to contribute yield by increasing harvest index (Khokhar et al., 2010).

Therefore, for yield appraisal during the vegetative phase and on negating the harvest index for being a laborious trait, panicle plant-1 and proline contents can be a reference trait for yield appraisal of quinoa.

Table 3. Analyses of variance for traits (ey= economical yield), yield related (by=biological yield, hi= harvest index), adaptability (pc=proline contents) and panicle plant^{-1} related traits.

S.O.V.	**DF**	**Mean sum of square**				
		BY	**EY**	**HI**	**PC**	**PPP**
Rep	2	10001.62^{NS}	40094.27^{NS}	0.00^{NS}	0.00^{NS}	61.68**
Progenies	9	33442735.06**	11917047.88**	0.27**	0.86**	954.33**
Locations	2	34871631.49**	739467.34**	0.12**	0.02**	50.04**
Year	1	1013439.28**	188215.80**	0.00^{NS}	0.07**	53.71*
Progenies × Locations	18	1129111.64**	154009.74**	0.02**	0.01**	20.26**
Progenies × Years	9	171506.55**	100468.44**	0.01**	0.01**	1.38^{NS}
Progenies × Years	2	1078931.99**	17466.25^{NS}	0.00^{*}	0.01**	0.99^{NS}
Progenies × Locations × Years	18	71968.13^{NS}	77091.24**	0.00**	0.01*	2.36^{NS}
Residual	118	51242.74	20371.61	0.00	0.00	7.85
Total	179					

Where * is significant at $P \leq 0.05$ and ** is highly significant at $P \leq 0.05$

Table 4. Mean Values For Yield (Ey= Economical Yield), Yield Related (By=Biological Yield, Hi= Harvest Index), Adaptability (Pc=Proline Contents) and Panicles Plant-1 (Ppp) Traits In F6 Progenies And Check Varieties

Populations	**BY (Kg ha^{-1})**	**EY (Kg ha^{-1})**	**HI**	**PC ug g^{-1}**	**PPP**
UAF-CP-3	6643.16	2719.91	0.41	0.76	36.53
UAF-CP-7	7415.85	2308.40	0.31	0.69	30.29
UAF-CP-11	5825.42	2441.36	0.42	0.41	32.35
UAF-CP-12	5092.81	2648.64	0.52	0.71	34.41
UAF-CP-9	6116.29	2931.25	0.33	0.82	41.08
UAF-CP-5	7346.1	2835.64	0.42	0.80	38.61
Average	**6406.61^{a}**	**2647.53^{a}**	**0.40^{a}**	**0.70^{a}**	**35.55^{a}**
P-510540	5157.10	1223.56	0.24	0.52	14.27
P-614922	3377.95	1114.54	0.33	0.31	14.58
P-643079	3107.92	1176.84	0.38	0.67	16.63
P-510542	4209.41	767.06	0.18	0.22	11.61
Average	**3963.10^{b}**	**1070.50^{b}**	**0.28^{b}**	**0.43^{b}**	**14.27^{b}**

Proline content was well reported to be regulated and controlled by the environment even in the native region (Aguilar et al., 2003) and varied with the variation in origin of the understudy germplasm either belong to valleys or the altiplano of Bolivia. Moreover, the low temperatures features like chilling and freezing did not affect quinoa proline accumulation much (Jacobsen et al., 2005). On comparing the performance of cultivars with the temperature of the sowing station, higher temperature was the driving force for plant survival to get better yields. Interestingly, the plant progenies with the highest seed yielding differ with each other for proline content but also proved to be higher proline accumulator when compared with the poor yielder group of genotypes.

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