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Implications of direct and indirect selection parameters for improvement of grain yield and quality components in *Chenopodium quinoa* Willd.

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Abstract

The present study was undertaken to evaluate direct and indirect selection criteria for the improvement of grain yield and quality components in *Chenopodium quinoa* Willd. Correlated response (CR) and relative selection efficiency (RSE) were estimated for grain yield and three quality components, grain protein, grain carotenoid and leaf carotenoid based on different contributing traits. Stem diameter, chlorophyll a, total chlorophyll and leaf carotenoid had high CR and RSE values for grain yield indicating the effectiveness of these traits in increasing grain yield. Grain protein and grain carotenoid exhibited negative CR and RSE values for grain yield indicating that direct selection for grain yield would lead to a slight decrease in these quality characters. Grain size was not of much significance either in increasing grain yield or any of the quality components. Grain yield can also be increased through indirect selection for stem diameter, while leaf pigments are likely to play a major role in enhancement of quality traits like leaf and grain carotenoid.

Keywords: *C. quinoa*; Direct selection; Indirect selection; Grain yield

Introduction

Chenopodium quinoa Willd., a crop of the Andean region, has recently gained worldwide attention due to its rich grain quality comprising large amount of protein ranging from 14.8 to 15.7% (Wright et al., 2002), oil with essential fatty acids (linoleic acid and γ-linolenic acids ranging from 55 to 66% of lipid fraction) (Koziol, 1992) and natural antioxidants (α tocopherol and γ tocopherol) (Ruales and Nair, 1992), along with a wide range of minerals and vitamins (Repo-Carrasco et al., 2003). Quinoa is an allotetraploid (2n=4x=36) and exhibits disomic inheritance for most qualitative traits (Simmonds, 1971; Ward, 2000; Bhargava et al., 2006a). The crop also has the potential to grow in various

stress conditions like soil salinity, soil acidity, drought, frost etc. (Jensen et al., 2000, Jacobsen et al., 2003; Bhargava et al., 2003a, 2006b). Different cultivars of quinoa are known for their adaptability to different agro-ecological zones, ranging from sea level to an altitude of over 4000 meters (Jacobsen, 2003) and offer great scope for agricultural diversification. Its nutritional superiority and wide adaptability encourages its introduction and establishment as a prospective cereal crop in newer areas like the Indo-Gangetic Plains of India (Bhargava et al., 2006b).

Breeding efforts in quinoa have centered on introduction and acclimatization of the crop to newer areas, but reports on a proper breeding methodology to increase grain yield are rare (Bhargava et al., 2003a). Genetic improvement in a crop requires indepth knowledge of variability along with information on interrelationship among various traits so that an efficient selection strategy can be formulated. High heritability estimates along with a high genetic advance are the most important criteria for direct selection, while genotypic correlation between the selected trait and other traits forms the basis of indirect selection. However, correlated response has an added edge in selection of suitable characters over others since correlated response is a resultant effect of heritability, genetic advance, genotypic correlation and selection intensity. The studies on indirect selection response are, therefore, necessary for improvement of component traits contributing towards yield. Although literature on correlated selection response in vegetable chenopods (*C. album*) is available (Bhargava et al., 2003b), but such reports on grain chenopods are totally lacking. Therefore, the present study was undertaken to test the suitability of various traits for carrying out direct and indirect selection.

Materials and methods

Experimental site and experimental material

Field experiments were conducted in the crop years 2002-03 and 2003-04 on sandy loam soil at the experimental field of National Botanical Research Institute, Lucknow (26.5^{o}N latitude and 80.5^{o}E longitude), situated at an altitude of 120 m above sea level. The crop was sown in winter season, during which the minimum and maximum temperature ranges from 2.5^{o}C-19^{o}C and 14^{o}C-29^{o}C respectively. 27 exotic germplasm lines of *C. quinoa* and 2 lines of its distant relative, *C. berlandieri* subsp. *nuttalliae* (Table 1) were sown in mid November in both the years and evaluated for various morphological and quality traits. All the lines selected were tetraploid (2n=36), and had been obtained from USDA and IPK, Gatersleben, Germany.

Experiment

The experimental design was a randomized block with three replications. The plot size was 4 m^2 (2m x 2m). Each plot had 6 rows spaced 30 cm apart and each row had 10 plants separated at 20 cm from each other. For the whole crop season, weeding followed by hoeing was done at an interval of 15 days. Irrigation was provided when needed. No chemical fertilizer was applied either before or during the experiment. These was primarily done to ascertain the potential of the crop for subsistence agriculture since a large chunk of

the farmers in the region are financially weak and seek crops with fewer inputs. We tried to emulate the same type of cultivation practice as followed in the Peruvian altiplano, where farmers do not use any kind of fertilizers for quinoa and the plant survives mainly on residues of manure or fertilizer from the previous crop (Aguilar and Jacobsen, 2003). Each germplasm line was sown in a separate plot and thinning was done to maintain plant density within rows. No fungicide or insecticide was used during the experiment.

Table 1. Germplasm lines, their source, origin and grain color.

Germplasm line	Source	Status	Origin	Altitude (m)	Grain colour
C. quinoa Willd. CHEN 58/77	[a]IPK, Germany	-	-	4000	Light
C. quinoa Willd. CHEN 67/78	IPK, Germany	-	Puno, Peru	-	Dark
C. quinoa Willd. CHEN 71/78	IPK, Germany	-	Bolivia	-	Light
C. quinoa Willd. CHEN 33/84	IPK, Germany	-	-	-	Light
C. quinoa Willd. CHEN 84/79	IPK, Germany	-	Cuzco, Peru	3200	Light
C. quinoa Willd. CHEN 92/91	IPK, Germany	-	Columbia	-	Light
C. quinoa Willd. CHEN 7/81	IPK, Germany	-	-	-	Light
C. quinoa Willd. PI 614938	[b]USDA	-	Oruro, Bolivia	-	Light
C. quinoa Willd. PI 478408	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 478414	USDA	Cultivar	La Paz, Bolivia	3800	Dark
C. quinoa Willd. PI 596498	USDA	Landrace	Cuzco, Peru	3030	Light
C. quinoa Willd. Ames 13219	USDA	-	La Paz, Bolivia	3700	Light
C. quinoa Willd. Ames 13719	USDA	-	New Mexico, USA	-	Light
C. quinoa Willd. PI 587173	USDA	Cultivated	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 510532	USDA	Cultivated	Peru	3000	Light
C. quinoa Willd. PI 614883	USDA	-	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 584524	USDA	Cultivated	Chile	-	Light
C. quinoa Willd. Ames 22156	USDA	Cultivar	Chile	-	Light
C. quinoa Willd. Ames 13762	USDA	-	New Mexico, USA	-	Light
C. quinoa Willd. PI 614881	USDA	-	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 510537	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. PI 510547	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. Ames 22158	USDA	Landrace	Chile	-	Light
C. quinoa Willd. PI 510536	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. PI 478410	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 433232	USDA	-	Chile	-	Light
C. quinoa Willd. Ames 21909	USDA	Landrace	Oruro, Bolivia	3870	Light
C. berlandieri subsp. *nuttalliae* PI 568155 (Saff.) Wilson and Heiser	USDA	Landrace	Mexico	1680	Dark
C. berlandieri subsp. *nuttalliae* PI 568156 (Saff.) Wilson and Heiser	USDA	Landrace	Mexico	2700	Dark

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[b]United States Department of Agriculture.

Ten plants of each germplasm line in each replication were randomly tagged and data were recorded on these plants for 12 morphological traits namely days to flowering, days to maturity, plant height (cm), leaf size (cm^2), stem diameter (cm), primary branches/plant, inflorescence length (cm), number of inflorescence/plant, grain size (mm), 1000 grain

weight (g), dry weight/plant (g) and grain yield/plant (g). Leaf area was measured using the direct method (Akram-Ghaderi and Soltani, 2007) with a leaf area meter of Delta T Devices Ltd. for 3 positions *viz.* top, middle and lower, when the plant was in full bloom. The grain size was measured following the method suggested by Bertero et al, (2004). Apart from this, leaf pigments *viz.* chlorophyll a (mg/g), chlorophyll b (mg/g), total chlorophyll (mg/g) and leaf carotenoid (mg/kg) were determined from fresh leaves collected from the same-tagged plants at the 9-week-old stage. Grain carotenoid (mg/kg) and grain protein (%) was estimated from the bulked seed of each germplasm line of each replication. Chlorophyll and carotenoids were estimated as per the method described by Jensen (1978). Total grain protein was determined following the method suggested by Peterson (1977).

Statistical Analysis

The year x genotype interaction was non-significant for all the traits which might be due to similar environmental conditions in both the years. Thus, data of both the years was pooled and analyzed for simple statistical parameters. Broad sense heritability (H_b) and genetic advance (GA) as percent of mean were calculated as per Singh and Chaudhary (1985). Genotypic correlation analysis was performed according to Dewey and Lu (1959) to determine the relationships between yield and among the component traits using variance and covariance components. Correlated response (CR) and relative selection efficiency (RSE) were estimated as per procedure suggested by Falconer (1989) and Searle (1965), respectively. The estimates were calculated by the following formulas:

$$r_{(x,y)}=Cov(x,y)/\sqrt{\sigma x^2 \sigma y^2}$$

where,

$r_{(x,y)}$ is genotypic correlation between variables x and y;
$Cov_{(x,y)}$ is the genotypic covariance between the two variables;
σx^2 is the genotypic variance of the variable x; and
σy^2 is the genotypic variance of the variable y.

Phenotypic variance ($\sigma^2 p$) = Genotypic variance + Error variance

where,

Genotypic variance ($\sigma^2 g$) = M.S.S. treatment – M.S.S. error/replication
Error variance ($\sigma^2 e$) = M.S.S. error
Heritability (H_b) = $\sigma 2g/\sigma^2 p$
Heritability (%) = $\sigma^2 g/\sigma^2 p$ x 100
Genetic advance (GA %) = $i.H_b.\sigma p$

where,

i = Selection intensity (2.06)
H_b = heritability
σp = Phenotypic standard deviation ($\sqrt{\sigma^2 p}$)
GA (%) = Genetic advance/Mean x 100
Correlated response (CR) = $i_{5\%.}\sqrt{H_{bx.}}\sqrt{H_{by.}}.r_{g..}\ \sigma p_y$

where,

$i_{5\%}$ = standardized selection intensity (2.06)
H_{bx} = heritability in broad sense of x
H_{by} = heritability in broad sense of y

r_g = genotypic correlation between x and y
σP_y = phenotypic standard deviation of y
Relative selection efficiency (RSE) = CR/GA_y (%)

Results and discussion

Mean and range for various traits are presented in Table 2. Days to flowering ranged from 70.78-101.55 days, and days to maturity from 109.33-163.33 days, with an average of 81.73 and 129.51 days, respectively. Dry weight/plant showed almost 50 times variation (1.11-52.89 g) while number of inflorescence/plant ranged from 11.67-141.55. Grain protein among the lines ranged from 12.55-21.02% with an average of 16.22, while grain carotenoid was in the range of 1.69-5.52 mg/kg with a mean of 2.83 mg/kg. Initial field trials of quinoa in India have shown promising results which shows that quinoa could serve as an alternative winter crop for the North Indian Plains and other subtropical regions having similar agro-climatic and edaphic conditions. Seeing its tremendous potential there is an urgent need for its genetic improvement that surprisingly has received very little attention (McElhinny et al., 2007).

Table 2. Mean and range for 12 morphological and 7 quality traits in grain *Chenopodium.*

Traits	Mean ± SE	Range
Days to flowering	81.76 ± 1.18	70.78-101.55
Days to maturity	129.51± 2.51	109.33-163.33
Plant height (cm)	83.76 ± 6.79	11.27-144.03
Leaf size (cm^2)	18.15 ± 1.44	4.42-30.91
Stem diameter (cm)	0.86 ± 0.05	0.32-1.32
Primary branches/plant	20.62 ± 1.08	8.55-35.74
Dry weight/plant (g)	16.37 ± 2.24	1.11-52.89
Inflorescence length (cm)	2.64 ± 0.24	0.84-6.47
Inflorescence/plant	88.59 ± 7.81	11.67-141.55
Grain yield/plant (g)	16.27 ± 2.06	1.29-39.39
Grain size (mm)	1.84 ± 0.03	1.34-2.21
1000 seed weight (g)	2.69 ± 0.15	0.78-4.09
Chlorophyll a (mg/g)	1.26 ± 0.05	0.48-1.82
Chlorophyll b (mg/g)	0.17 ± 0.007	0.07-0.25
Total chlorophyll (mg/g)	1.43 ± 0.06	0.55-2.04
Leaf carotenoid (mg/kg)	484.09 ± 18.37	230.23-669.56
Grain carotenoid (mg/kg)	2.83 ± 0.16	1.69-5.52
Grain protein (%)	16.22 ± 0.47	12.55-21.02

Plant breeders generally improve populations by selection among the genotypes on the basis of their phenotypic performance. Knowledge of heritability is important as it indicates the possibility and extent to which improvement can be brought about through selection. Since heritability (H_b) is the ratio of genotypic variance to phenotypic variance, it can be termed as the heritable portion of phenotypic variance. High heritability indicates more importance of genetic factors in controlling a trait and possibility of its improvement by

appropriate selection programs (Saeidi, 2008). In the present study, high heritability (>90%) was observed for all the traits under study, the highest heritability being shown by grain protein (99.23%) (Table 3). Earlier Bhargava et al. (2003a) have also reported high heritability (H_b) estimates for *C. quinoa*, on normal as well as sodic soil. High heritability alone, however, does not guarantee large gain from selection unless sufficient genetic advance attributable to additive gene action is present. Genetic advance in a trait is a product of heritability and selection differential and expressed in unit of standard deviation, has an added advantage over heritability as a guiding factor in selection programme, where improvement in a trait is desired. Genetic gain as percent of mean was found maximum for dry weight/plant (147.68%), followed by grain yield/plant (132.81%) and inflorescence length (102.08%) (Table 3). All the quality traits had moderate genetic gain, of which grain carotenoid showed the highest value (64.84%). Surprisingly, both the phenological traits *viz.* days to flowering and days to maturity showed low values of genetic gain (15.65 and 21.27% respectively) which suggested that the selection based on these parameters could not be effective. The genetic advance for some traits were high because of extreme genetic variability in the material investigated, and smaller values for genetic advance are expected in further selection cycles in a more improved material.

Table 3. Genetic gain, heritability and genotypic correlation values for various traits in grain *Chenopodium*.

Traits	GA[a] (%)	H_b[b] (%)	Genotypic correlation			
			Grain yield	Leaf carotenoid	Grain carotenoid	Grain protein
Days to flowering	15.65	95.45	0.20	0.10	0.13	-0.24
Days to maturity	21.27	98.14	0.17	0.31	0.37	-0.30
Plant height (cm)	89.35	98.97	0.51**	0.67**	0.30	-0.38*
Leaf size (cm^2)	84.94	94.69	0.45*	0.62**	0.14	-0.38*
Stem diameter (cm)	63.68	94.59	0.67**	0.63**	0.17	-0.40*
Branches/plant	56.56	95.42	0.41*	0.51**	0.44*	-0.22
Inflorescence length (cm)	102.08	98.92	0.15	0.30	0.39*	-0.27
Inflorescence/plant	94.98	95.63	0.61**	0.72**	0.24	-0.16
Grain size (mm)	21.28	84.90	0.27	-0.04	-0.17	-0.01
1000 grain weight (g)	64.34	96.44	0.50**	0.20	-0.23	-0.11
Dry weight/plant (g)	147.68	95.62	0.48*	0.50**	-0.01	-0.43*
Chlorophyll a (mg/g)	44.26	88.30	0.52**	0.87**	0.24	-0.09
Chlorophyll b (mg/g)	49.47	83.33	0.40*	0.77**	0.26	0.01
Total chlorophyll (mg/g)	44.95	89.34	0.51**	0.86**	0.24	-0.08
Leaf carotenoid (mg/kg)	39.29	90.33	0.51**	-	0.51**	-0.27
Grain carotenoid (mg/kg)	64.84	97.60	-0.01	0.51	-	0.07
Grain protein (%)	32.69	99.23	-0.02	-0.23	0.07	-
Grain yield/plant (g)	132.81	91.44	-	0.51	-0.01	-0.02

[a]Genetic advance (%), [b]Heritability in broad sense (%).
*,** Significant at P=0.05 and 0.01 respectively.

The genotypic correlation values of grain yield, leaf carotenoid, grain carotenoid and grain protein are presented in Table 3. Grain yield/plant showed highest positive correlation with all the morphological and quality traits, except for two grain quality traits i.e. grain carotenoid (-0.013) and grain protein (-0.017). Bhargava et al. (2003a) have also reported positive genotypic association of seed yield with most morphological traits in grain chenopods. Grain size showed positive association with grain yield/plant (0.272) but was

negatively correlated with leaf carotenoid (-0.043), grain carotenoid (-0.169) and grain protein (-0.011). Grain protein showed moderate to low genotypic correlation with all the morphological traits, including grain yield/plant (-0.017) indicating that all these traits negatively influence protein content in the seed.

A simple selection strategy to improve any component trait including yield entails selection based on those traits that show high heritability, high genetic advance and positive direct effect on the component trait. Following these criteria, most of the traits were found to be positively contributing towards grain yield, especially inflorescence/plant, dry weight/plant, leaf size and stem diameter. Leaf pigments also contributed towards seed yield while grain quality components act as an impediment in increasing seed yield. This is understandable since yield is inversely proportional to quality.

Leaves of *C. quinoa* are being used as food since pre-Columbian times (Risi and Galwey, 1984; Popenoe et al., 1989). Quinoa leaves contain high amounts of carotenoids ranging from 230.23 mg/kg to 669.56 mg/kg, and was comparatively higher than that found in the grain. Therefore, increase of carotenoid content of leaves should also be a major objective in quinoa breeding. The present study shows that most of the morphological as well as quality traits contribute towards increasing leaf carotenoid content. It is also true for grain carotenoid, where only four traits *viz.* grain size, 1000 grain weight, dry weight/plant and grain yield are negatively influencing carotenoid content. Improvement in grain protein seems possible only by selection through grain carotenoid and chlorophyll b.

Table 4 presents the correlated response (CR) and relative selection efficiency (RSE) for grain yield and three quality traits. Stem diameter, chlorophyll a, total chlorophyll and leaf carotenoid had high CR and RSE values for grain yield indicating the effectiveness of these traits in increasing grain yield. These results are different from those discussed earlier as per Table 3. Leaf size and inflorescence length do not seem to be of much significance in increasing grain yield through indirect selection, while they were important for direct selection. The negative CR and RSE values of grain carotenoid and grain protein for grain yield indicate that direct selection for protein would lead to a slight decrease in these quality characters. This is also supported by negative CR and RSE value for grain protein (-0.086 and –0.006 respectively) (Table 4) *vis-à-vis* grain yield.

All the traits had high CR and RSE values for leaf carotenoid, which exceeded 100% in many cases (Table 4). This was due to high phenotypic variance of leaf carotenoid (10452.156) due to the presence of a large amount of variability (Data not shown). The expected response of leaf carotenoid was maximum on selection of chlorophyll a, chlorophyll b and total chlorophyll. The corresponding RSE values for these traits were also high which points towards a highly positive interaction among the leaf pigments. Grain carotenoid also exhibited high CR and moderate RSE for leaf carotenoid and vice versa, which indicates that selection for either pigment, would lead to high-expected gain in the other.

The prediction of expected response to selection seems to be quite different for two-grain quality traits *viz.* grain carotenoid and grain protein. The negative correlation between grain protein with all the other traits, except chlorophyll b and grain carotenoid, would lead to a negative response of grain protein when selection for these traits is practiced. Also, the CR and RSE values of grain protein *vis-à-vis* chlorophyll b and grain carotenoid were less, which makes indirect selection for grain protein an extremely difficult task. On the other hand, increase in carotenoid content of seeds could be achieved by indirect selection

through days to flowering, branches/plant and the leaf pigments. Interestingly, grain size was not of much significance either in increasing grain yield or any of the quality components.

Table 4. Correlated response (CR %) and relative selection efficiency (RSE %) for grain yield and three qualitative traits in *Chenopodium*

Traits	Grain yield		Leaf carotenoid		Grain carotenoid		Grain protein	
	CR[a]	RSE[b]	CR[a]	RSE[b]	CR[a]	RSE[b]	CR[a]	RSE[b]
Days to flowering	4.43	0.28	19.53	1.25	0.23	0.01	-1.22	-0.08
Days to maturity	3.91	0.18	61.20	2.88	0.68	0.03	-1.55	-0.07
Plant height (cm)	11.45	0.13	132.84	1.49	0.55	0.01	-2.01	-0.02
Leaf size (cm^2)	9.79	0.11	119.98	1.41	0.26	0.01	-1.96	-0.02
Stem diameter (cm)	14.62	0.23	122.12	1.92	0.31	0.01	-2.09	-0.03
Branches/plant	9.08	0.16	100.36	1.77	0.80	0.01	-1.15	-0.02
Inflorescence length (cm)	3.32	0.03	60.45	0.59	0.71	0.01	-1.40	-0.01
Inflorescence/plant	13.44	0.14	140.15	1.47	0.44	0.01	-0.82	-0.01
Grain size (mm)	5.66	0.27	-7.92	-0.37	-0.29	-0.01	-0.05	-0.01
1000 grain weight (g)	11.01	0.17	40.04	0.62	-0.42	-0.01	-0.59	-0.01
Dry weight/plant (g)	10.66	0.07	97.37	0.66	-0.03	-0.01	-2.25	-0.02
Chlorophyll a (mg/g)	11.07	0.25	163.63	3.70	0.41	0.01	-0.45	-0.01
Chlorophyll b (mg/g)	8.22	0.17	140.13	2.83	0.44	0.01	0.06	0.01
Total chlorophyll (mg/g)	10.90	0.24	163.36	3.63	0.42	0.01	-0.39	-0.01
Leaf carotenoid (mg/kg)	10.88	0.28	-	-	0.90	0.02	-1.40	-0.03
Grain carotenoid (mg/kg)	-0.29	-0.01	100.31	1.55	-	-	0.35	0.01
Grain protein (%)	-0.38	-0.01	-45.19	-1.38	0.12	0.01	-	-
Grain yield/plant (g)	-	-	96.97	0.73	-0.02	-0.01	-0.09	-0.01

Conclusions

The results clearly bring forth the importance of leaf pigments viz. chlorophyll and carotenoids for enhancing seed yield in *C. quinoa*. Seed yield can also be increased through indirect selection for stem diameter, while the two phenological traits viz. days to flowering and days to maturity are of little significance. Leaf pigments are likely to play a major role in enhancement of quality traits like leaf carotenoid and seed carotenoid, while indirect selection for high seed protein content in quinoa seems difficult.

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