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RESEARCH ARTICLE

Cultivation of New Emerging Agro-Nutritional Crop of Quinoa at Madinat al-Hikmah Karachi, Sindh, Pakistan

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Abstract:

Aim:

Quinoa is a popular source of protein, minerals and alternative to traditional grains. The objective of this study is to introduce the Quinoa in the semi-arid zone of Sindh province of Pakistan.

Method:

A variety of NARC-9 from the agricultural Punjab province was cultivated and subjected to analyze the growth, morphological characters of the varieties obtained, saponin, protein and the elemental composition *viz*. Cd, Cu, Fe, K, Na, Pb, and Zn.

Result:

The result demonstrated the optimum growth and no disease were found in the experimental area. At least three major varieties of quinoa were obtained. Seed morphological data of these three quinoa cultivars were collected. The average saponin levels were quite reasonable. Overall proteins band pattern revealed very high polymorphism in quinoa cultivars and the results were also in good agreement with earlier studies.

Conclusion:

All quinoa cultivars of Madinat al-Hikmah showed high concentrations of albumin than globulin concentrations (*i.e.* 48-52% and 24-27%, respectively) as compared to control seeds from market that had similar concentrations of the two fractions *i.e.* 35.58% and 37.68%, respectively. Likewise, low concentrations of prolamin 14-16% and glutelin 11-12% compared to control seeds 13% rank our crop much better quality than the imported one in the market. The trend of elemental accumulation was followed as K >Na >Fe >Zn >Cu >Pb >Cd, while for comparison it was Na >K >Zn >Fe >Cu >Pb >Cd >Pb for wheat grown under similar conditions. Traditional grains together make a major contribution to the total nutritional element intake of the average Pakistani citizen through diet, not only because of large amounts consumed, but also in part by suitable levels of their proteins and elemental up take for good health. Thus the successful cultivation of quinoa in the semi-arid zone of Sindh will certainly prove beneficial.

Keywords: Nutritional food, Quinoa, Protein, Saponin, SEC FPLC, SDS PAGE.

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1. INTRODUCTION

Since 1960, there has been a three-fold increase in the estimated population (>170 million) of Pakistan, which is predicted to be as much as double in the coming few years (*i.e.* 2025); ultimately leading to reduced food availability throughout the country [1]. Consequently, this menace leads to the declaration of "Quinoa" as a food for the future security by food and agricultural organization (FAQ) [2]. The major limitation in the increment of food supply is the diffident expansion of cultivated land as well as infrequent resources of water, which can eventually increase the crop production. The current situation thus, is inevitable to extend the land or increase the crop production and therefore conserve the natural resources for food. Cultivation offers the possibility of not only preserving economically important wild plants in their natural habitats but also providing farmers with new healthy food crops.

Food contains nutrients which are substances that promote growth and energy [3]. A number of medicinal plants which also possess significant food value have been investigated such as Coriander, Fennel, Fenugreek, Sesame, Wheat, *etc.* [4 - 7] with success for crop cultivation at Hamdard University, Madinat al-Hikmah, situated on the outskirts of Karachi in Sindh province of Pakistan. The historical name of the place is Deh Bund Murad Khan. Besides these crops, a newly emerging crop in Pakistan *i.e.* Quinoa has been recently introduced for the first time in Sindh, and also cultivated successfully at Hamdard University. Previously, the only cultivation of Quinoa was reported in Pakistan by the Department of Crop Physiology, University of Agriculture Faisalabad in Punjab province of Pakistan.

The nutritional status of food, perhaps can be attained by the quality of its proteins. This ultimately depends on characteristics like digestibility, composition of amino acids, anti-nutritional factors as well as ratio of tryptophan *i.e.* large neutral aromatic amino acid within a protein [8]. Quinoa (*Chenopodium quinoa* Willd) is a food crop found in the Andes of South America with potential as alternative to traditional grains elsewhere [9, 10]. The seeds are famous for their outstanding protein source, and recognized further in the presence of an essential amino acid "lysine", which is absent in many other cereals. Besides this, other amino acids like methionine and histidine ratios are also higher as compared to soy, barley and/or wheat proteins [11]. The reported content of protein in quinoa seeds ranges between 12% to 23%, whereas the comparative analysis indicates the protein values as 16.3% which is perceived to be higher than other cereal grain *e.g.* 7.5% in rice, 11% in barley, 15.4% in wheat and 13.4% in corns [12 - 14]. Along with the high nutritional values as a balanced protein source, evaluations claim Quinoa to be an important source of vitamins, minerals, flavonoids as well as some high-quality oils [15].

There are hundreds of varieties of quinoa, ranging in color from white to red and purple to black, with multiple biological properties such as the reduction of chronic disease risk, anti-inflammatory, anticarcinogenic, antihypertensive, analgesic and also used as a disinfectant of the urinary tract infections [16, 17]. Moreover, the importance has been further recognized for its applications in the nutraceutical industries. Due to its excellent nutritional value and a possible potential for production in various climatic zones, quinoa has been classified as one of the humanity's most promising crops [18]. These reports can also open new avenues for its use as a medicinal crop. Although a lot of work has been done on quinoa in different countries regarding cultivation, nutritional value and industrial uses but with reference to Pakistan a very little work has been carried out related to the cultivation in different ecological zones as well as other aspects of food supplements and potential for herbal industries. Thus in the present study, investigation was conducted to determine the yield of quinoa under the semi-arid zone as well as nutritional values of crop as first step from Hamdard University, Karachi.

2. MATERIALS AND METHODS

2.1. Procurement of Quinoa Seeds

The healthy and mature quinoa seeds (1 kg) of NARC-9 were obtained with the collaboration of Dr. Saddar uddin Siddiqui (PSO/Curator), National Agricultural Research Centre (NARC), Islamabad and Prof. Dr. Shahzad Basra, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan for the cultivation of quinoa at Hamdard University, Madinat al-Hikmah, Karachi during 2014-2015.

2.2. Field Experimental Site and Set Up

A field experiment of quinoa was initiated in mid-November, 2014 to find out the yield of variety NARC-9 at Hamdard University, Madinat al-Hikmah. Topographically, the site of Madinat al-Hikmah and surrounding is a part of generally flat plain which is about 20 m above sea level. It is semi-arid zone area [4]. The conventional method

was used for seeds propagation. The total area of cultivation was used as one hectare (1 ha = 10^4 m^2). Half acre (*i.e.* 0.405 ha) was used as control (with no fertilizers) and the other half was fertilized (Table **1**). Four to six seeds were sown on the ridges during November, 2014 (normal soil, winter weather, no rain, always sunny, temperature 15 - 20°C) in 75 cm spaced rows maintaining 15 cm plant to plant distance using seed rate of 250 g/0.405 ha as well as filled up with soil. Quinoa seeds germinated after 6-8 days. The plots were irrigated after the germination of seeds at the interval time's (*i.e.* 19.12.2014, 19.1.2015) and flowering stage with water reservoir. When the healthy seedlings were sufficiently strong and attained the height of about 30.4 cm, the fertilizers were applied at dose of 1 kg of diammonium phosphate (DAP) + 10 kg of urea/ 0.405 ha to observe the effect of this dose on the growth of quinoa. The plots were cleaned from weeds during the month of mid-January and mid-March. The plants growth was slow till mid-January and enhanced gradually till early February, 2015. As the weather warmed up, there was high activity in plants growth (increase in height and branching). The panicle appeared in March 2015, and the growth was observed under fertilizers dose as compare to control. The growth parameters of Quinoa were plant height, number of branches per plant, number of leaves per plant, leaf length and leaf width. Crop at full blooming and maturity were harvested in April, 2015. Orange, red and white panicles were separated when the main panicle become resistant when pressed and tied into bundles as well as sun dried for a week. They were manually threshed, separately weighed and stored in the jute bags under dry ventilated condition.

Table 1. The effect of fertilizer dose on the growth of *Chenopodium quinoa* Willd at Madinat al-Hikmah, Karachi Sindh, Pakistan.

Plot-A (0.405 ha) 250 g	**Dose**	**Date of Sowing**	**Plant to Plant Distance**	**Mode of Sowing**	**Irrigation**	**Plant Height (cm)**	**No Of Branches**	**No Of Leaves (cm)**	**Leaf Length (cm)**	**Leaf Width (cm)**	**Date of Harvesting**	**Total Yield /0.405 ha**	**Total Yield /0.405 ha**	**Total Yield /0.405 ha**
Control	No treatment	12.11. 2014												
Orange			15cm	Ridges	3 times	91.44 cm	2 0	14	4	2	15.4.2015	3 kg		
Red				Ridges		121 cm	25	10	4	3			23 kg	
White				Ridges		152 cm	28	12	5	4	Same as above			27kg
Plot-B (0.405 ha) 250 g														
Orange	DAP 10 kg + Urea 10 kg/ 0.405 ha	15.11. 2014	15cm	Ridges	3 times	121 cm	24	16	6	4	28.4.2015	46 kg		
Red				Ridges		137.16 cm	28	12	5	3			115 kg	
White				Ridges		152.4 cm	35	28	4	3	Same as above			120 kg
Total Yield/ A+B (1 ha)	-	-	-	-		-	-	-	-	-	-	49 kg	138 kg	147 kg

2.3. Seed Morphology

Mature and healthy seeds of Quinoa were collected. The specimens were deposited in Karachi University Herbarium for authentication. The voucher specimen number assign is 92801. Only 10-20 seeds per variant (*i.e.* orange, red and white panicles) were studied for their morphological characters under stereomicroscope (Nikon XN Model), compound microscope (Nikon type-102) and scanning electron microscope (JSM-6380A). For scanning electron microscopy, dry seeds were directly mounted on metallic stubs using double adhesive tape and coated with gold for a period of 6 min in sputtering chamber and observed under SEM. The terminology used is in accordance to earlier reports [19, 20] with slight modification. The seed characteristics *viz*. shape, size, color, surface, hilum and strophiole were studied.

2.4. Estimation of Total Saponins in Quinoa Seeds

Estimation of total saponins in the three separated quinoa cultivars (*i.e.* orange, red and white panicles, respectively) of quinoa seeds was performed ideally as described in [21].

2.5. Seed Storage Proteins Analysis

The separated seeds were ground manually with mortar pistol. The grounded seeds were defatted and homogenized with 4 volumes of cold acetone (-20ºC) for 15 min. The seed flour was air dried under hood for 24 hours before any further use. For major seed storage protein analysis, micro-extraction method recently described by us [22] was used. Briefly, seed flours (500 µg) successively extracted (triplicate in eppendorf tubes) with (i) 500 µl deionized water, (ii) 500 µl 5.0 M NaCl, (iii) 500 µl 70% ethanol and (iv) 500 µl 0.2 M sodium phosphate buffer, pH8 was used for the extraction of four major seed storage proteins *i.e.*, albumin, globulin, prolamin and glutelin, respectively. Each extraction was performed for 20 min at 350 rpm at 25°C using thermomixer comfort (Eppendorf, Germany). All extracts were centrifuged at 14,000 rpm for 15 min at 4°C (Biofuge Primo R Heraerus, Japan) and after supernatant separated, extraction was repeated twice in order to remove all the remaining protein of each fractions. The supernatants of each fraction were pooled and stored at -20°C until subjected for protein estimation. Total protein concentration of all extracts was measured by modified dye-binding assay of [23] using bovine serum albumin (Merck, Germany) as a standard. Measurements were performed in a microplate reader (Sunrise-Tecan, Austria). Four major seed storage proteins obtained were also subjected to SDS-PAGE and SEC FPLC analysis.

2.6. SDS PAGE Analysis

For protein finger printing, single seed protein analysis was performed as initially described [24, 25] with slight modifications by us [22]. Briefly, single naked seeds were crushed on a glass surface using a watch glass and transferred into eppendorf tubes (1.5 cm^3). The seed flour was directly subjected for protein extraction in buffer under dissociating denaturing conditions [26]. Finally, samples were centrifuged at 14,000 rpm for 10 min and the supernatants (*ca*. 10-15 µl) were subjected to SDS-PAGE analysis using Mini-PROTEAN® 3 Cell (Bio-Rad Lab, UK). The crude proteins were separated in 12% resolving and 5% stacking gels at 140 V for 1 h and at the end of electrophoresis the gel were stained with 0.2% colloidal Coomassie Brilliant Blue G-250 [27]. For further differential complementation, defatted *ca* 100 mg of each seed flour was also extracted in physiological phosphate buffered saline, pH7.5 (PBS, Amresco, USA) and 15-20 µg of protein samples were also subjected for 12% SDS PAGE as aforementioned.

2.7. Size Exclusion Chromatography (SEC FPLC)

The chromatographic behavior of the seed crude proteins (*ca*. 1 mg/cm^3) extracted in physiological phosphate buffered saline, pH7.5 (PBS, Amresco, USA) was also subjected to fast protein liquid chromatography (ÄKTA-basic, GE Healthcare, UK) using a size exclusion column (TSK-2000SW, 7.5 x 300 mm, Tosoh Bioscience, Japan). The column was equilibrated and eluted in the same buffer. The flow rate was maintained at 0.4 cm^3/min and the absorbance was recorded at 280 nm. Column was previously calibrated under identical conditions with known molecular weight standard proteins (Bio-Red Lab, USA) of 670, 158, 41, 17, and 1.3 kDa, respectively. The data were compared and analyzed by automated software UNICORN 5.0 (GE Healthcare, UK).

2.8. Elemental Assay

For elemental analysis, 10 to 20 g of material of each sample was precisely weighed and placed in individual beakers then 10 cm^3 of HNO_3 (Conc.) was added to it, as well as heated on hot plate at 80°C and continuously stirred with the help of glass rod. Later 2-3 cm^3 of H_2O_2 was added until the homogenous and clear solution was obtained. The solutions were filtered through Whatman filter paper (No. 42, England) and further diluted into 50 cm^3 deionizer water [28]. The resulting solutions were used for the elemental analysis. Flame atomic absorption spectrophotometer (AAnalyst-700, Perkin-Elmer USA) was used for the purpose of estimating the contents of Cd, Cu, Fe, K, Na, Pb and Zn. Instrumental setting, calibration, sensitivity for specific elements as laid down in the operational manual were strictly followed as described very recently by us [29].

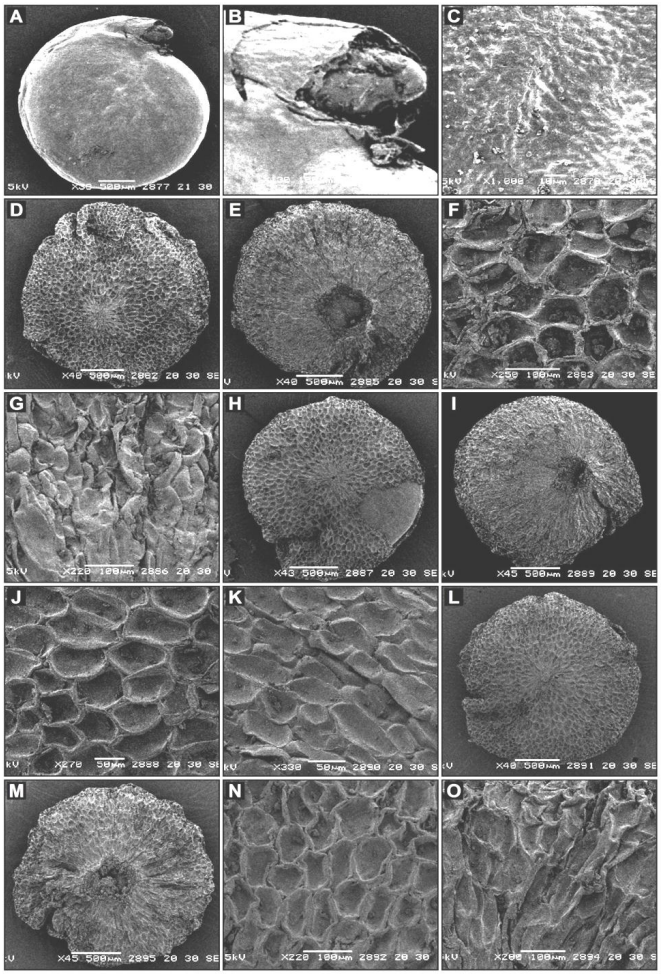

Fig. (1). Scanning electron micrographs of the seeds of quinoa cultivars. Control: A, seed; B, strophiole; C, surface; Orange panicled cultivar.: D, ventral view of seed; E, dorsal view of seed; F, ventral surface; G, dorsal surface, White panicled cultivar.: H, ventral view of seed; I, dorsal view of seed; J, ventral surface; K, dorsal surface, Red panicled cultivar.: L, ventral view of seed; M, dorsal view of seed, N, ventral surface; O, dorsal surface. (Scale bar: A,D,E,H,I,L,M = 500µm; B,F,G,N,O = 100µm; C = 10µm; J,K = 50µm).

3. RESULTS AND DISCUSSION

3.1. Cultivation of Quinoa

Quinoa (*Chenopodium quinoa* Willd) is a very high quality food crop with potential as alternative to traditional grains, thus subjected to field experiment at semi-arid zone of Hamdard University, Madinat al-Hikmah, Karachi. As aforementioned, the conventional method was used for seeds propagation. The effects of fertilizer (plot A) and with no fertilizers (plot B) as control plots were prepared for this experiment (Table **1**). The crop obtained in the experimental area thrived well and any disease occurrence was also observed throughout the experimental periods (six months, Nov-April, 2014-15). The plant height, number of branches, leaves, length and width were recorded and found increased with the application of fertilizers *i.e.* DAP and urea as compared to control (Table **1**). The result revealed that the dose of DAP and urea proved successful for fertilization as compared to cultivation without fertilizer. From only 250 g of seeds, 46 kg, 115 kg, 120 kg/0.405 ha of orange, red and white panicle seeds were obtained, respectively. While from the same amount of seeds harvested without fertilizers yield 3 kg, 23 kg, 27 kg/0.405 ha of orange, red, white panicle seeds, respectively. The result showed that the total yields of grains obtained from A + B plots of Madinat al-Hikmah were 49 kg, 138 kg, and 147 kg/ha as Orange, Red, White panicle seeds, respectively. The cultivated quinoa grain of Hamdard University was also deposited in the National Agricultural Research Centre (NARC), Islamabad, Pakistan for the accession numbers. NARC has assigned the accession numbers of 32786, 32787 and 32788 for white, red and orange panicle seeds, respectively. It is observed that the crop is self-pollinated with low outer crossing rates as also previously reported by Bhargava *et al.* [30] from India. Quinoa has limited cross pollination that is why different color

of plants were found in the experimental field. This result certainly shows the need for further studies concerning the development of new lines of quinoa. Thus, in order to prove, obtained seeds of three different quinoa cultivars (*i.e.* orange, red and white panicle) were further subjected to morphological and biochemical investigations.

3.2. Morphology and Seed Characteristics of Quinoa Cultivars

Seed morphology of three quinoa cultivars may be well correlated with gross morphology established by SEM microscopy. The orange panicle quinoa seeds are characterized by having rugosely tuberculate surface, white panicled quinoa have foveate seed surface and red panicle quinoa remains distinct due to the presence of reniform-orbicular seeds. The remaining cultivars are characterized by having broad elliptic-pyriform seeds. However, imported seeds purchased from the local market and used as control showed the presence of a distinct strophiole on seeds not found in any cultivars which is mainly because of processing effect such as polishing and/or removal of total saponins *etc.* (Fig. **1**). Thus, it is concluded that seed morphological data is quite enough to distinguish the three quinoa cultivars. The key characteristic features of quinoa seeds summarized as: size 2-2.5 x 2-2.5 mm, broad elliptic-pyriform or reniform-orbicular, white, orange or reddish (white, whitish brown or light brown), surface varies on both sides, ruminate, reticulate, sparsely tuberculate, rugosely tuberculate, foveate or rugosely scalariform, with a central depression or scar or sometimes with a tubular like structure (Table **2**). Hilum with a conspicuous or inconspicuous strophiole was observed as illustrated in Fig. (**1**).

Table 2. Key to the obtained quinoa cultivars.

1	+	Seeds 2.5 mm long, strophiole conspicuous……..……..…….Control
	-	Seeds 2 mm long strophiole inconspicuous……..……2
2	+	Seeds broadly elliptic-pyriform…….. 3
	-	Seeds reniform- orbicular…… Red panicle (samp 4)
3	+	Seeds surface rugosely tuberculate…..……Orange panicle (samp 2)
	-	Seeds surface foveate……White panicle (samp 3)

3.3. Total Saponin Analysis of Quinoa Seeds

Saponins belong to a diverse group of chemical compounds widely distributed in the plant kingdom and extensively characterized by their structure, comprising of a triterpene or steroid aglycone (or amphipathic glycosides) and one or more sugar moieties. The presence of varying concentrations of saponins was reported in several edible seeds and often a limitation for a direct use because of bitter taste and unknown side effects [31]. Thus, as quality assessment, the total saponins in the three quinoa cultivars were estimated and found as proximate of 0.63%, 0.5% and 0.38% (mean, *n=3*) for red, orange and white panicles seeds, respectively. The average saponin concentration of quinoa whole seeds obtained in our cultivars is quite less and reasonably corroborated with the previous report which ranges between 2-8% [32].

3.4. Single Seed and Seed Storage Protein Analysis of Quinoa Cultivars

Three obtained quinoa cultivars were subjected for protein finger printing using both single seed analysis by SDS PAGE as well as physiologically extracted proteins by SDS-PAGE and SEC FPLC, while market available imported quinoa seeds were used as control. Despite the varying band intensities which mainly arise because of the variation in seeds size, single seed analysis by SDS PAGE revealed identical band pattern (Fig. **2A**). Several major and minor bands ranging between 15 to 50 kDa polypeptides were resolved well by single seed analysis by gel electrophoresis in dissociating-denaturing (SDS + DTT, pH8.8) under reduced conditions. The overall protein profiling revealed high polymorphism in quinoa cultivars and the results are in good agreement with earlier report [9]. In order to obtain differential insight, total proteins were also extracted from defatted seed flour in PBS at physiological pH7.5 and a known concentration of ~20 µg of proteins was subjected for 12% SDS-PAGE (Fig. **2B**). As expected, results were found to be highly differential, except three major bands of approximate molecular weights of 30, 90 and 97 kDa which are polymorphic. These results were further complemented by subjecting the same set of samples to size-exclusion chromatography using FPLC system. Comparative chromatographic profiles of the three quinoa cultivars clearly revealed differences, a) in the early eluted peaks at the first void volume of the column related to the high molecular weight proteins and b) the peaks eluted after second void volume corresponding to intermediate to low molecular weight polypeptides (Fig. **3**).

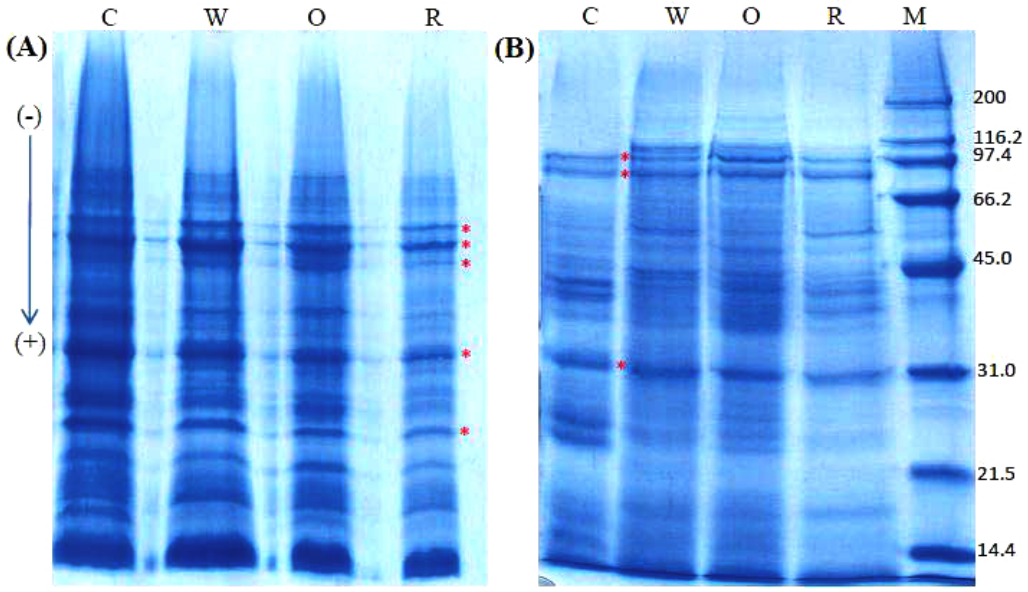

Fig. (2). Protein finger printing of the obtained cultivar of Quinoa (*Chenopodium quinoa* Willd.) in comparison with market available as control. **(A)** Representative gel of single seed protein analysis using 12% polyacrylamide gel electrophoresis under dissociating and denaturing conditions *i.e.* SDS PAGE. (**2B**) 12% SDS PAGE analysis of the total proteins extracted under physiological buffer (PBS pH7.5). See "Materials and Methods" for detail. Lane C, control seed; W, white panicle seed, O, orange panicle seed, R red panicle seed and lane M is the known molecular weight marker (kDa). Asterisk indicates the polypeptides that were highly polymorphic.

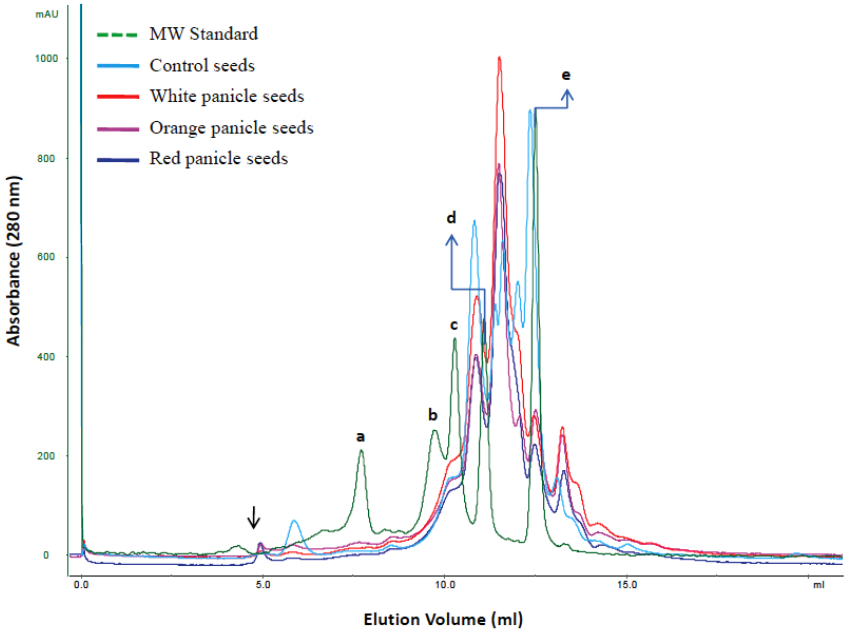

Fig. (3). Comparative size exclusion chromatographic (SEC FPLC) analysis of the three-obtained cultivar of Quinoa (*Chenopodium quinoa* Willd.) with market available as control. The flow rate was maintained at 0.4 cm^3/min and monitored at 280 nm. Column was previously calibrated with known molecular weight marker proteins (green broken line) labeled as a to e (*i.e.* 670, 158, 41, 17, and 1.3 kDa, respectively). Inverted arrow indicates the void volume of the column.

In a parallel approach, total seed storage proteins (SSPs, *i.e.* albumin, globulin, prolamin and glutelin) analysis of the three obtained quinoa cultivars in comparison with market available product was also performed (Table **3**). Though as expected, intra-cultivar variations in concentrations of different SSPs are not significant (*e.g.* albumin 48.5 to 51.6%, globulin 24.4 to 27.5%, prolamin 14.9 to 16.1% and glutelin 11.4% to 11.9%) but quite variable to control

seeds (*i.e.* albumin 35.6%, globulin 38%, prolamin 18% and glutelin 13%). Interestingly, all Quinoa cultivars of Madinat al-Hikmah demonstrated high concentrations of albumin than globulin concentrations (*i.e.*48-52% and 24-27%, respectively) as compared to control seeds from market with very similar concentrations of these two fractions (*i.e.* 35.58% and 37.68%, respectively). Likewise, low concentrations of prolamin (14-16%) and glutelin (11-12%) compared to control seeds (13%) make our crop much better than the imported seeds. In order to complement our results, SDS-PAGE analysis under both dissociating (only SDS, pH 8.8) and dissociating and denaturing conditions (SDS + DTT, pH 8.8) of the extracted seed storage proteins were also performed. Fig. (**4**) demonstrated the comparative gels of red panicle seeds *vs*. the market control seeds (as representative result), clearly revealed better quality of SSPs in quinoa cultivars of Madinat al-Hikmah. Thus, the better quality, with high concentrations of albumin and globulin proteins and low concentrations of prolamin and glutlin are among the several important attributes that make quinoa an attractive and alternative crop [9, 33].

Table 3. Comparison of concentration and total percent yield of major seed storage proteins (SSPs) in the three cultivars of Quinoa (*Chenopodium quinoa* Willd.) and market available control seeds.

SAMPLE	ALBUMIN (Alb)		GLOBULIN (Glo)		PROLAMIN (Pro)		GLUTELIN (Glut)	
	(mg/g) [a]	% yield [b]	mg/g	% yield	mg/g	% yield	mg/g	% yield
Control Seeds	0.46 ± 0.10	35.58%	0.38 ± 0.06	37.68%	0.25 ±0.03	17.83%	0.17 ± 0.01	12.69%
White	0.74 ± 0.06	48.50%	0.44 ± 0.03	27.50%	0.25 ± 0.03	14.92%	0.20 ± 0.01	11.39%
Orange	0.95 ± 0.24	49.41%	0.43 ± 0.01	25.43%	0.31 ± 0.01	16.11%	0.21 ± 0.01	11.70%
Red	0.87 ± 0.14	51.60%	0.41 ± 0.02	24.41%	0.26 ± 0.04	15.41%	0.20 ± 0.01	11.89%

[a] Concentrations in mg/g of seed flour. Values are mean of three independent extractions in triplicates ±SD. [b] % yield of a particular protein in total protein contents of seed flour.

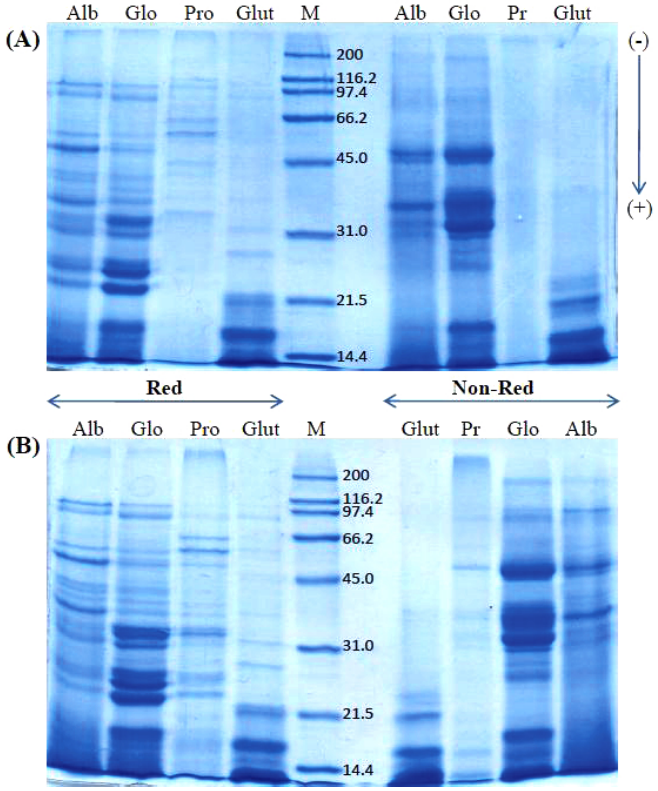

Fig. (4). SDS PAGE analysis of the seed storage proteins extracted from Quinoa (*Chenopodium quinoa* Willd.). 12% polyacrylamide gel electrophoresis under dissociating (non-reduced) and dissociating/denaturing (Red, reduced) conditions were performed. (**A**) Control seeds from market and (**B**) Red cultivar of Madinat al-Hikmah as representative gel.

3.5. Elemental Analysis of Quinoa Cultivars

In an earlier report, Koziol [34] determined the mineral concentration in quinoa and reported that it contained more potassium (9267 mg/kg dry wt.), iron (132 mg/kg) and, copper (51 mg/kg) and zinc (44 mg/kg) than other common cereals. Likewise, Stikic *et al.* [2] detected the elemental composition of quinoa whole seeds and reported as Fe (49.63 mg/kg), Cu (2.89 mg/kg), and Zn (18.70 mg/kg).

In the present investigation, quinoa leaves (QL) and orange, red, white panicle grains were also analyzed for the composition of seven major elements (Table **4**). Among these elements Fe, K and Na were found in large amounts (4.389 - 26.725 mg kg^{-1}), Cu and Zn were present in small quantities (0.288-1.902 mg kg^{-1}), while Cd and Pb were detected in very small amounts (0.063-0.285 mg kg^{-1}). Potassium (K) was present at highest amount in quinoa leaves (49.81 mg kg^{-1}) and the lowest value was found in orange panicle (18.98 mg kg^{-1}). QL contained highest levels of sodium (Na) (25.67 mg kg^{-1}), while the lowest amount was present in red panicle (21.50 mg kg^{-1}). Iron (Fe) present in highest amount in QL (14.940 mg kg^{-1}), while in the lowest value was found in white panicle (1.058 mg kg^{-1}). Zinc (Zn) was found highest in QL (3.560 mg kg^{-1}), while the lowest value was observed in orange panicles (0.900 mg kg^{-1}). Likewise, copper (Cu) was present in highest values in QL (0.611 mg kg^{-1}), while in the lowest concentrations found in red panicles (0.020 mg kg^{-1}). The Pb was also highest in QL (0.891 mg kg^{-1}), while the minimum values were found in red paniccles (0.114 mg kg^{-1}). Cd was present as highest in QL (0.072 mg kg^{-1}), while the lowest amount in orange panicles (0.057 mg kg^{-1}).

Table 4. Elemental composition of quinoa grain (mg kg^{-1}) as compared to wheat grain of Madinat al-Hikmah.

Plant Samples	Cd Mean ± SD	Cu Mean ± SD	Fe Mean ± SD	K Mean ± SD	Na Mean ± SD	Pb Mean ± SD	Zn Mean ± SD
Orange Panicle	0.057±0.0006	0.153±0.0297	1.559±0.1423	18.98±0.008	18.95±0.541	0.138±0.0390	0.900±0.0102
Red Panicle	0.060±0.0004	0.020±0.0502	BDL	19.08±0.033	21.50±0.298	0.114±0.0299	1.649±0.0040
White Panicle	0.065±0.0005	0.371±0.0022	1.058±0.0047	19.03±0.007	20.94±0.412	BDL	1.150±0.0083
Quinoa Leave	0.072±0.0017	0.611±0.0038	14.940±0.1440	49.81±0.002	25.67±0.028	0.891±0.0155	3.560±0.0684
Seed	0.049±0.0012	0.137±0.0059	0.942±0.1703	18.91±0.001	13.09±1.761	0.038±0.0717	0.738±0.0040
Wheat	0.069±0.0014	0.453±0.0138	1.528±0.0182	19.02±0.011	22.53±0.220	BDL	2.586±0.0248
Field Soil	0.104±0.0018	0.317±0.0058	39.580±0.1540	47.44±0.002	24.22±0.226	1.879±0.0248	0.300±0.0010

SD = Standard Deviation, BDL = Below Detection Limits

The trend of elemental accumulation appeared to be K >Na >Fe >Zn >Cu >Pb >Cd, while for comparison with wheat on the same soil, it was Na >K >Zn >Fe >Cu >Pb >Cd >Pb (Table **4**). Crops accumulate elements according to the nature of soil. Thus the elemental composition of Madinat al-Hikmah soil was also determined which revealed the following trend of concentration of elements K >Fe >Na >Pb >Cu >Zn >Cd (Table **4**). The present findings clearly indicated that trace elements will be essential for good grains. More than 70% of the grain samples contained less than 0.05 μg/g of copper [35]. Wheat, rice as well as pulses together provide contribution to the total macro and micro elements intake of the average Pakistani citizen through diet, not only because of large amounts consumed but also in part by suitable levels of their elemental make up. Thus food items studied are an adequate source of nutrient elements [36, 37]. According to a recent report of the food and agricultural organization (FAO, USA), they estimated that there are 805 million starved people in the World. This means, they regularly do not have enough food to live and/or not having active life [38]. Traditional grains together make a major contribution to the total nutritional element intake of the average Pakistani citizen through diet, not only because of large amounts consumed but also in part by suitable levels of their protein, elemental makeup for best health.

CONCLUSION

In conclusion, it is already proved that Quinoa is very good for a well-balanced diet but on the other side it is high-priced. The modern food industry relies for more on technology particularly on mechanization and biochemistry than human and animal labor. In this way, food is raised manipulated, preserved and move around, resulting in food industry that is to a great degree global in nature, with food and related resources travelling great distances. Our presented data from field experiments to laboratory research for quality check nicely demonstrate the success story about the cultivation of quinoa in semi-arid zone at Hamdard University, Madinat al-Hikmah, Karachi. Moreover, the illustration as economical growth also suggests that it must be promoted in agricultural and food industry of Pakistan because of its high nutritional value.

LIST OF ABBREVIATIONS

DAP	=	Diammonium phosphate
DTT	=	Dithiothreitol
FAQ	=	Food and agricultural organization
FPLC	=	Fast protein liquid chromatography
ha	=	Hectare (1 ha = 10^4 m^2)
kDa	=	Kilodalton
PBS	=	Phosphate buffered saline, pH7.5
QL	=	Quinoa leave
SDS-PAGE	=	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	=	Size exclusion chromatography
SSP	=	Seed storage proteins

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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