European Plant Science Organisation

3rd **Workshop of the *Nutritional Security Working Group***

Session: Improving the nutritional value of major and minor (neglected) crops

27-28 May 2022, Lecce, Italy

Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

Giacomo Mele
giacomo.mele@cnr.it

Department of Biology, Agriculture and Food Sciences
National Research Council of Italy

ISAFOM – Institute for Agricultural and Forest Systems in the Mediterranean

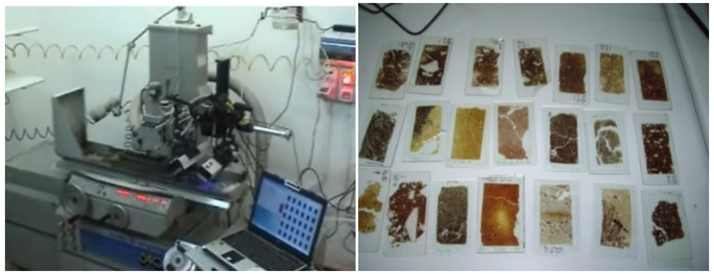
The AGRIFOOD IMAGING LAB team at ISAFOM

Dr. Giacomo Mele

Dr. Laura Gargiulo

Mr. Bruno Di Matteo

Dr. Antonio G. Napolitano



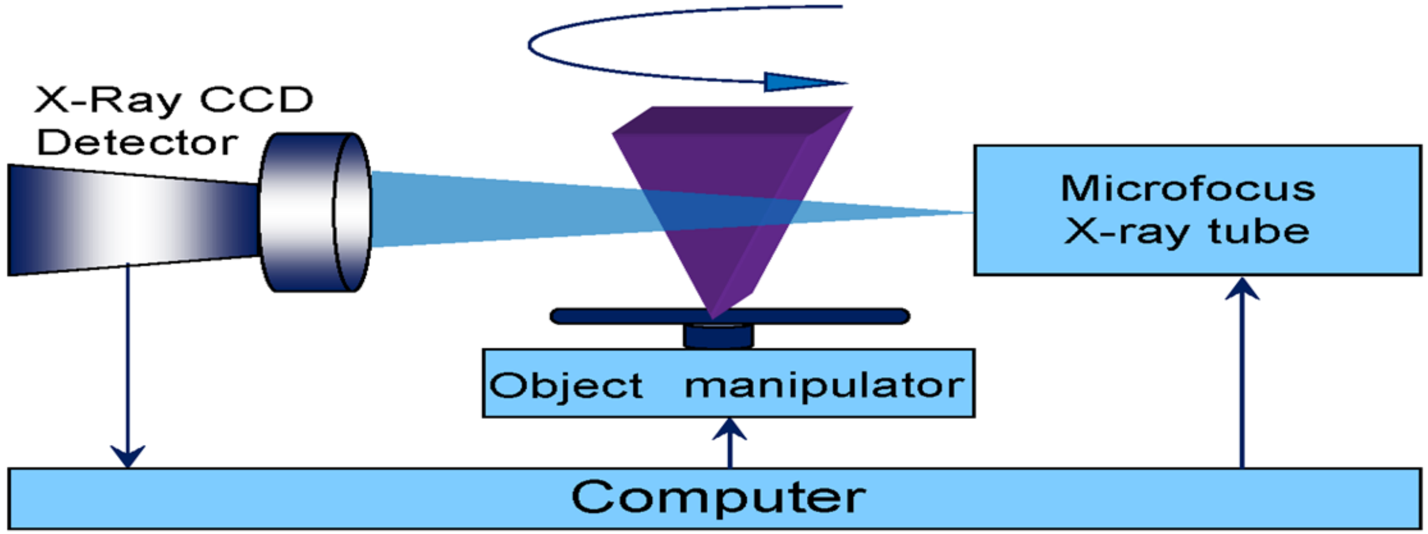
Thin sections

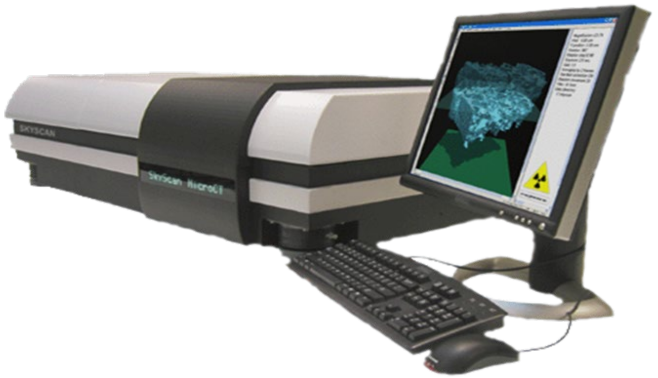
Optical Microscopy

2x

X-ray microCT

Microtomography allows to look inside small opaque objects/samples





Photoelectric absorption of x-rays is very sensitively dependent on Z, the atomic number of the absorbing material

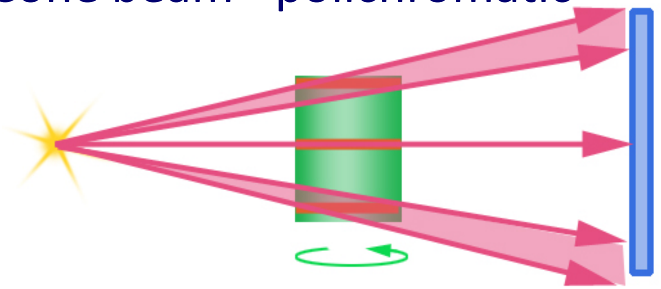
Requirements:
•***partial absorption****:* imaging internal microstructure because some x-rays are stopped by the object, and others go right through.
•***differential absorption****:* different parts of the object having significantly different x-ray absorption.

How does it work ?

1)

Cone beam - polichromatic

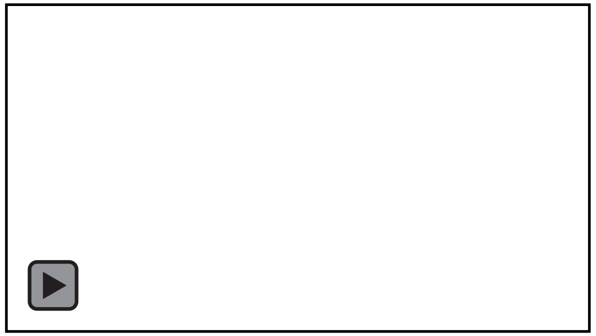
X-ray source



detector

Sample rotation

radiographs

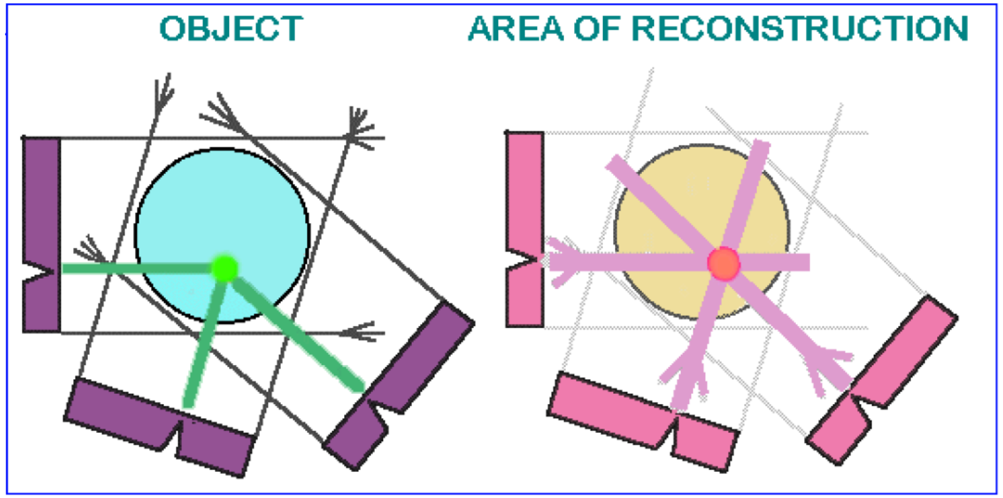


Radiograph = Map of X-ray absorption of the object, cumulated according to the direction of the beam (projection).

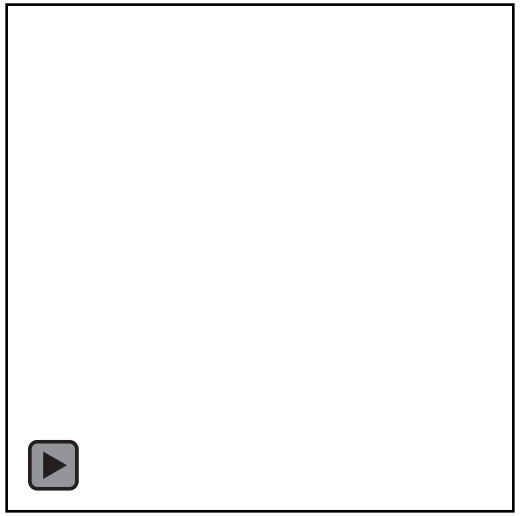
---

2)

Feldkamp backprojection procedure



cross sections



Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

We recently had a role in Project No.: 635727 - H2020-SFS-2014-2015/H2020-SFS-2014-2

PROTEIN 2FOOD

18 partners from Europe, Peru and Uganda

Plant based protein-rich foods throughout the entire food value chain

- CROP PRODUCTION
- Protein ingredients,
- Food prototypes
- Market research,
- Consumer choice,
- Environmental footprint

https://www.protein2food.eu/

We had in charge a Deliverable in the framework of the WP1

D1.3 - Protocols for 3D imaging and analysis of internal structure of seeds for the enhancement of phenotypic characterization

https://www.protein2food.eu/wp-content/uploads/Deliverable-1.3-CNR_Final.pdf

Partnership arisen for this study

SLU - Department of Plant Breeding, Swedish University of Agricultural Sciences, Sundsvägen 10, 23052, Alnarp, Sweden

UNALM - Centro de Investigación e Innovación en Productos Derivados de Cultivos Andinos, CIINCA, Universidad Nacional Agraria La Molina, Avenida La Molina S/n Lima 12, Peru

Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

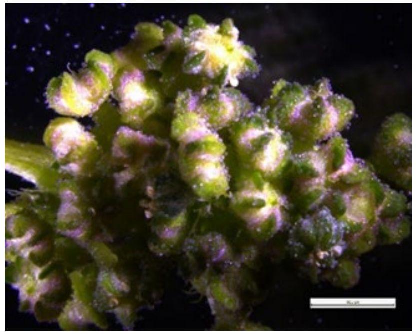
Quinoa ?

- herbaceous plant belonging to Chenopodiaceae family
- native of Andean highlands, South America

- high quality protein seed grain (good amino acid profile)
- gluten free, low glycemic index, vitamins, minerals…
- great adaptability

- Not impressive yield (2.1 - 2.2 t/ha
- Saponin content
- Lower protein content (15-20%) than other protein-rich seeds (e.g. faba beans)

Overall, quite an interesting niche crop predicted to spread fast across the globe (FAO & CIRAD, 2015).



Photos Å. Grimberg.

Main reasons that led to the study

1. Localization of the main seed storage compounds inside the seeds of quinoa is known to have a marked compartmentation:
   - Proteins are located mostly in the embryo.
   - Almost all the total saponin amount in seeds is located in the pericarp.
2. Breeding programs till now have mainly focused on the development of better environmentally adapted varieties rather than to increase seed nutritional properties.

AIMS

- Testing of the assumption 1 by means of two different X-ray microCT approches considering quinoa varieties with contrasting nutritional properties .
- Providing novel morphological traits to help breeders in enhancing quinoa nutrient profile.

3rd **Workshop** of the ***Nutritional Security Working Group*** ***27-28 May 2022, Lecce, Italy***

Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

Material

Plant 1 Plant 2 Plant 3

Titicaca (Denmark)

UNALM

Regalona (Chile)

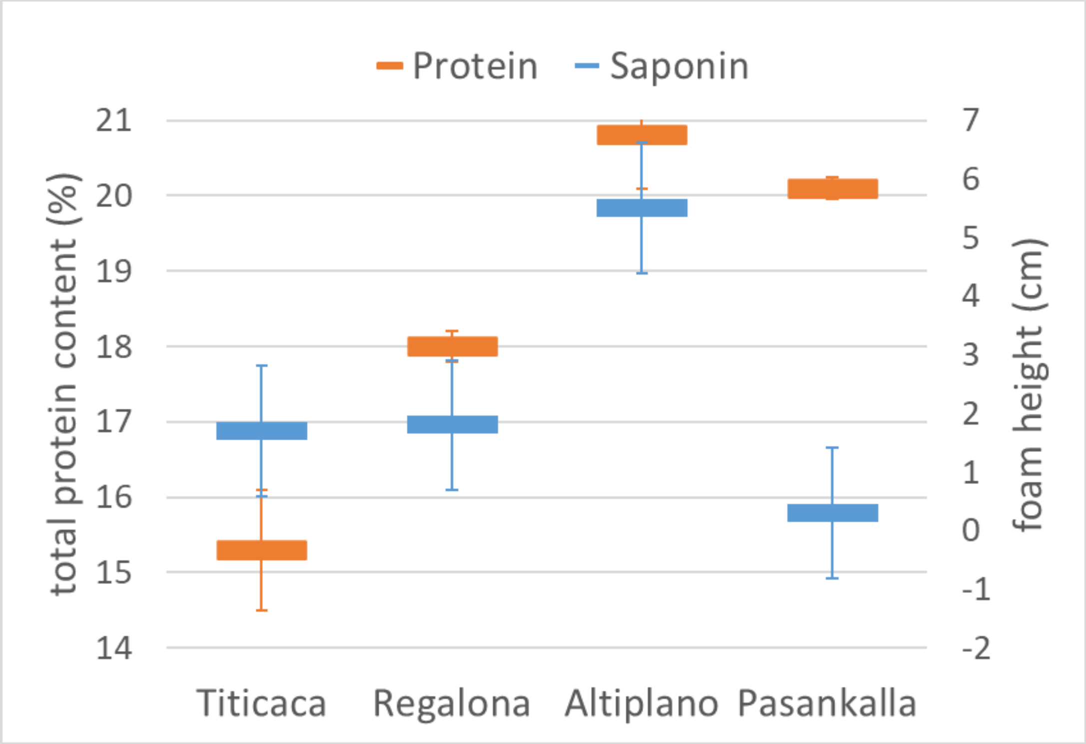
Altiplano (Peru)

Pasancalla (Peru)

Same controlled growth conditions at SLU

Protein content (% DW)

Saponin (foam height, cm) (Koziol, 1991)



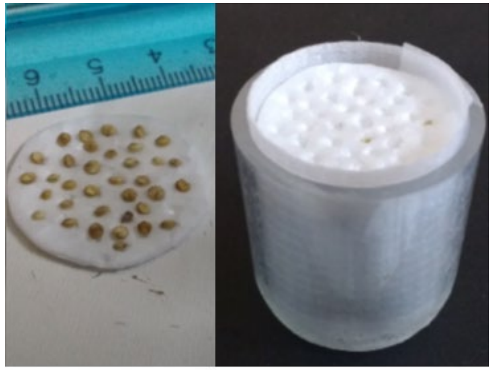
Representative combinations of the two nutritional parameters in their typical variation range

weights

Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

2 scanning approaches

a) Bulk



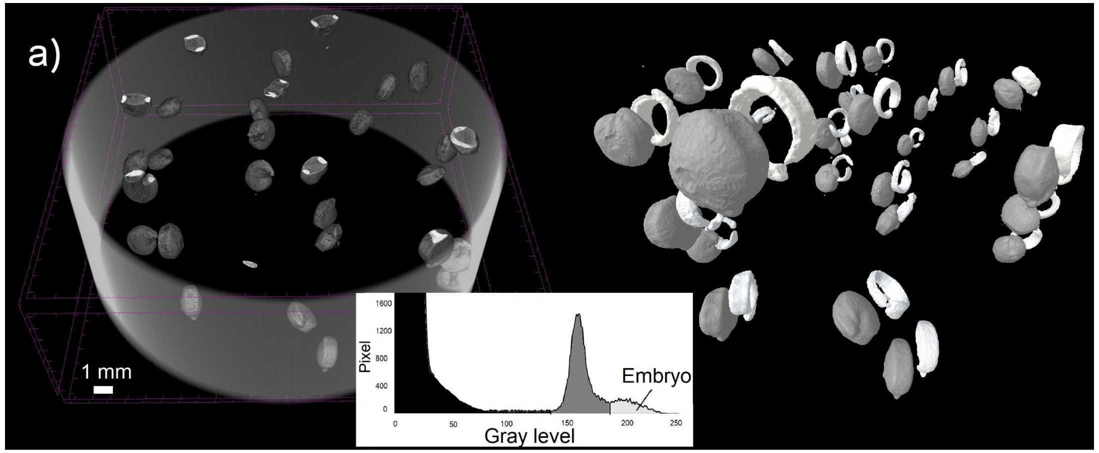
b) Single seed

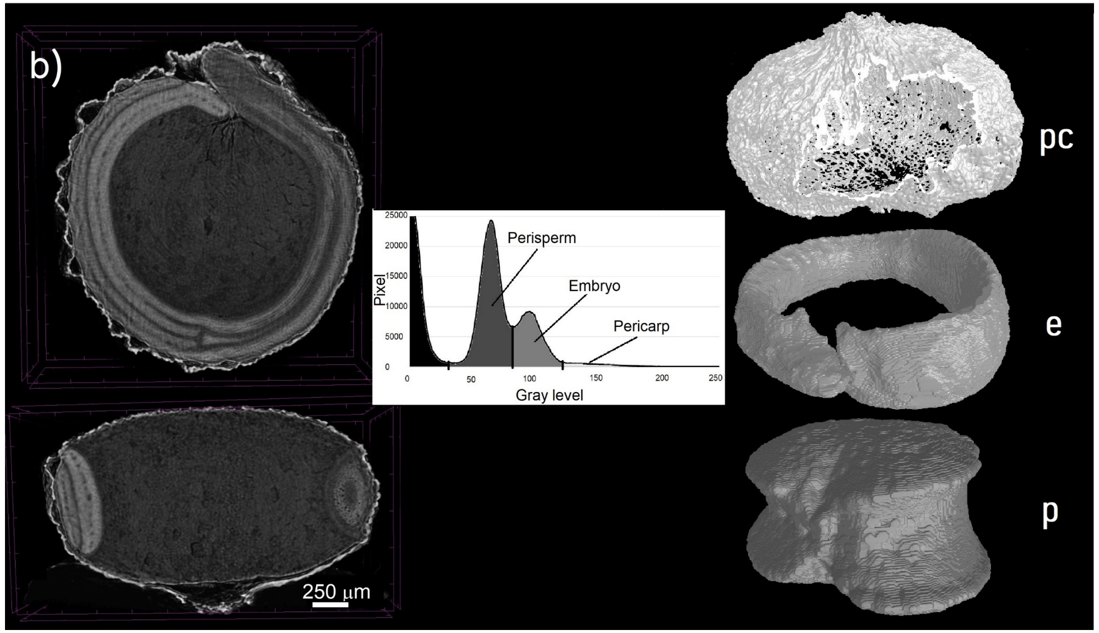
2 protocols

Main parameters	Value
Source voltage (kV)	50
Source current (µA)	200
Filter	No
Rotation step (deg)	0.6
Pixel size (µm)	15

Main parameters	Value
Source voltage (kV)	40
Source current (µA)	250
Filter	No
Rotation step (deg)	0.2
Pixel size (µm)	1

2 information levels



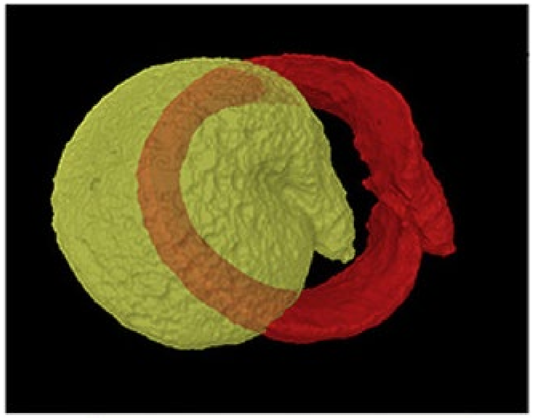


Results

a)

Till 250

Embryo bulk segmentation

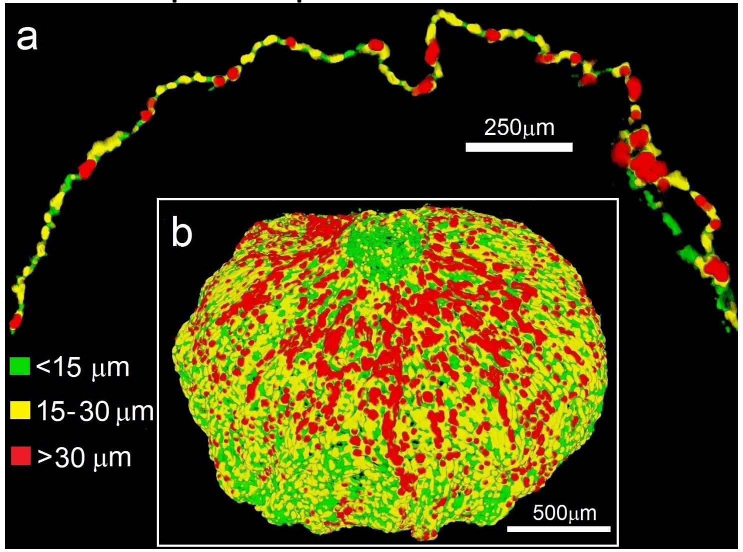


Morphometric parameters scored

4

b)

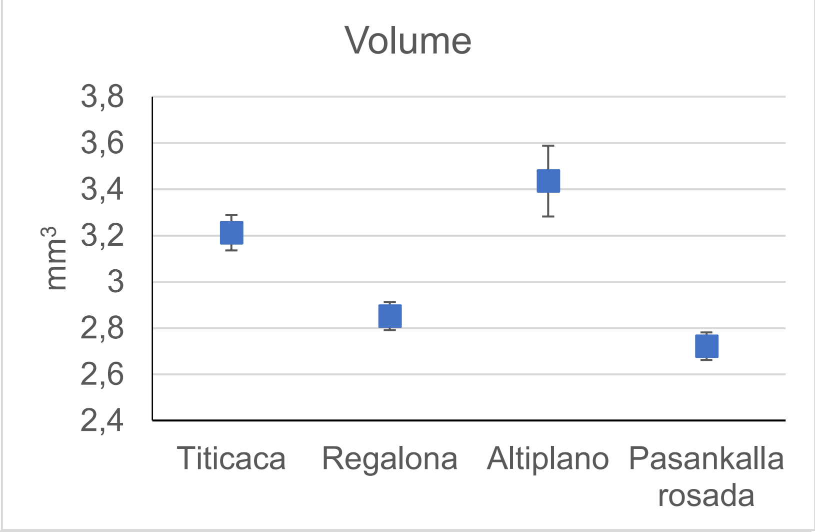
Accurate percarp thickness distribution

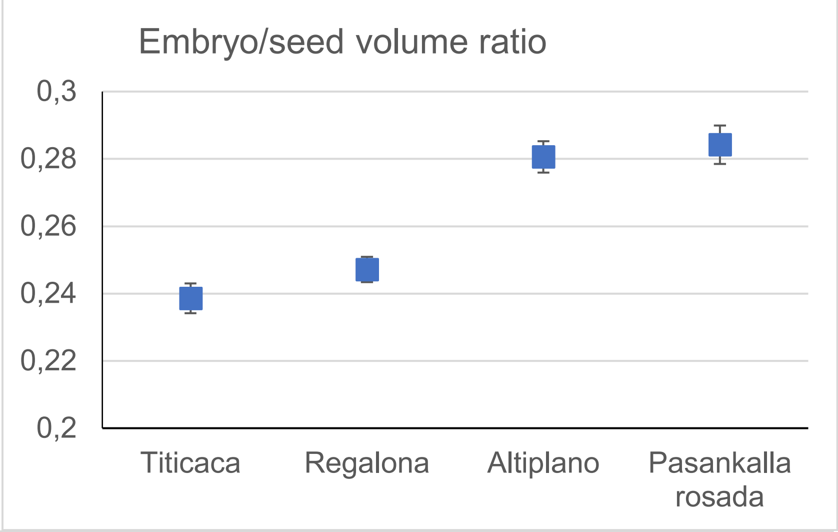


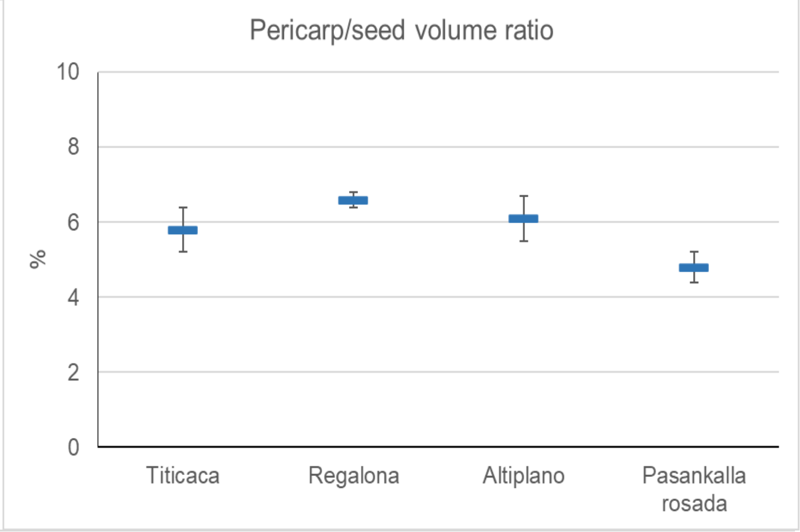
7

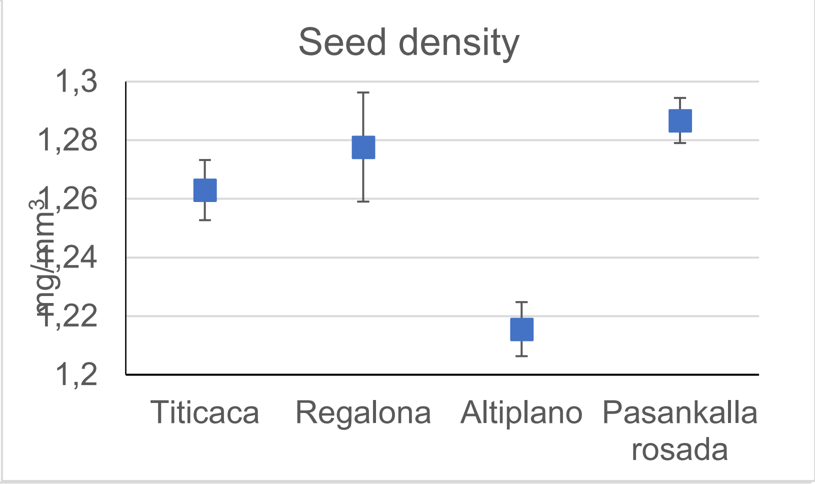
Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

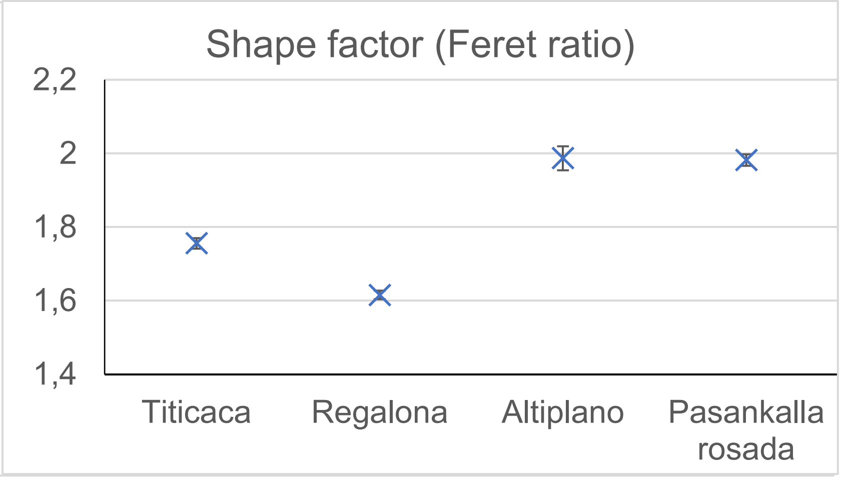
Results

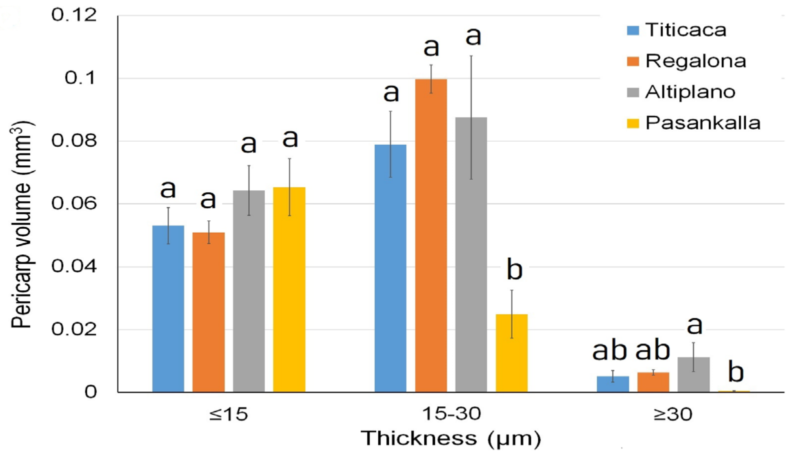












Results (multivariate analysis)

Seed weight (mg) + density ratios of seed parts

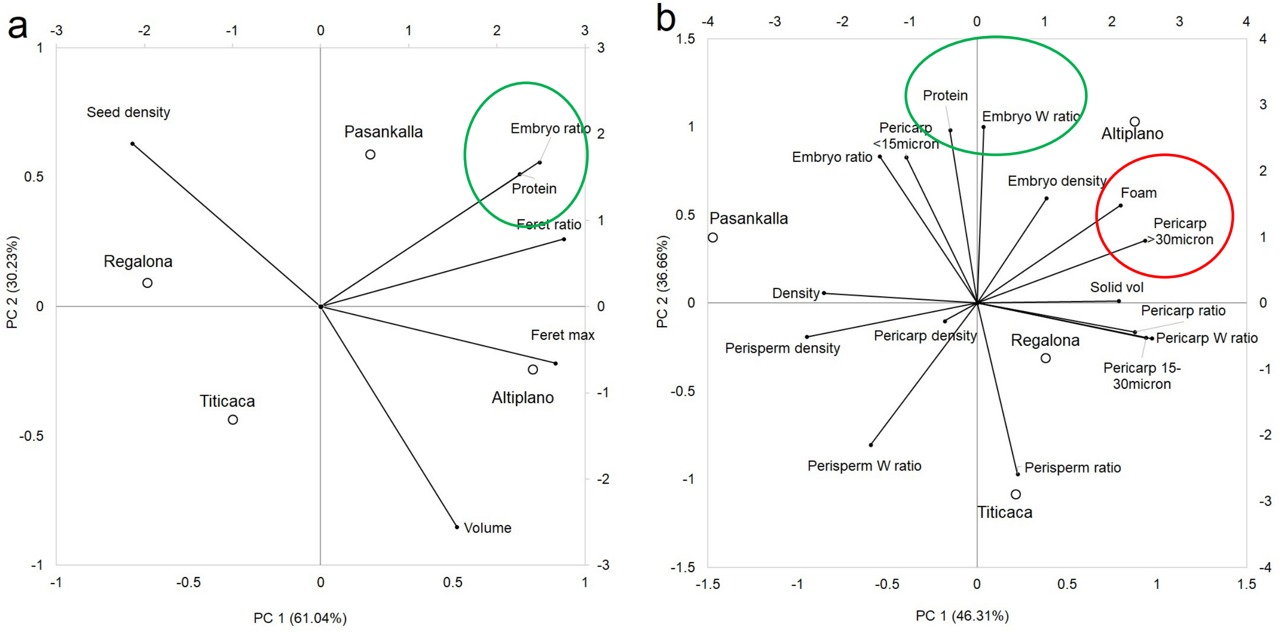
a) 5 morpho-densitometric traits d= *Wtot/Vtot*

Pearson correlation coefficients	Volume (mm^3)	Feret max (mm)	Feret ratio	Seed density (g/cm^3)	Embryo ratio
Protein (% by DW[a])	−0.015	0.389	0.703	−0.341	**0.934****

b) 14 morpho-densitometric traits

Wtot = *Wpc* + *We* + *Wp*
de/dpc = *We*/*Ve* * *Vpc*/*Wpc*
dpc/dp = *Wpc*/*Vpc* * *Vp*/*Wp*

Principal Component Analysis

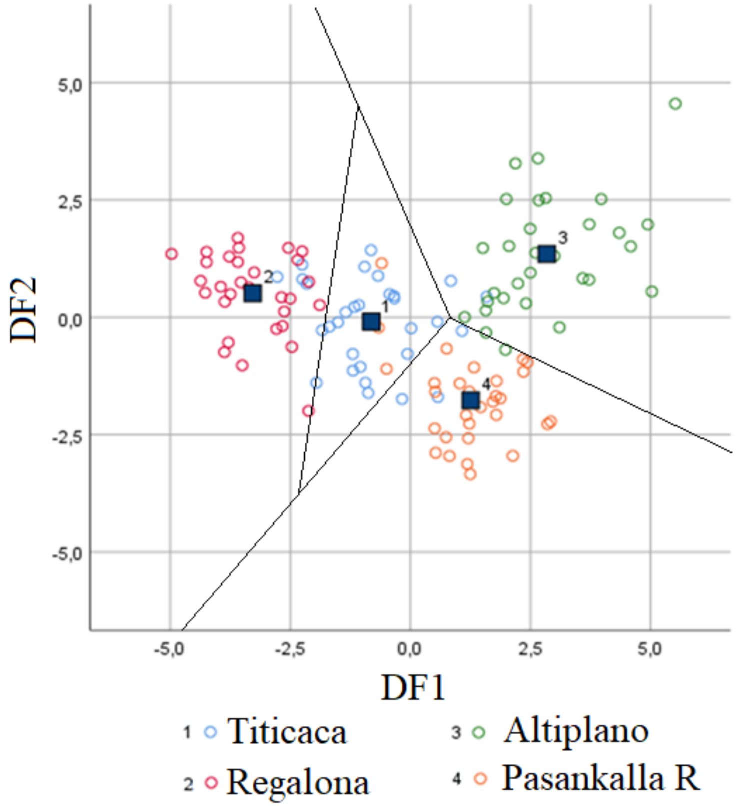


Pearson correlation coefficients	Seed solid phase volume (mm^3)	Seed density (g/cm^3)	Pericarp density (g/cm^3)	Embryo density (g/cm^3)	Perisperm density (g/cm^3)	Pericarp ratio	Embryo ratio	Perisperm ratio	Pericarp W[b] ratio	Embryo W[b] ratio	Perisperm W[b] ratio	Volume of pericarp <15 μm (%)	Volume of pericarp 15–30 μm (%)	Volume of pericarp >30 μm (%)
Protein content (% by DW[a])	−0.184	0.248	−0.193	0.611	−0.013	−0.240	0.885	−0.997**	−0.332	**0.973****	−0.704	0.822	−0.302	0.196
Saponin (foam height cm)	0.782	−0.775	0.035	0.465	0.923*	0.499	0.055	−0.335	0.634	0.585	0.904	0.241	0.570	**0.957****

[a]Dry Weight [b]weight ratio * = P ≤ 0.1, ** = P ≤ 0.05.

(side) Results

Discriminant Analysis
(only bulk scan parameters)



Classification[a,c]							
Group			Predicted Group membership				
			Titicaca	Titicaca	Altiplano	Pasankalla	Total
All cases	Count	Titicaca	26	2	1	1	30
		Regalona	2	28	0	0	30
		Altiplano	0	0	27	3	30
		Pasankalla	3	0	0	27	30
	%	Titicaca	**86,7**	6,7	3,3	3,3	100,0
		Regalona	6,7	**93,3**	0,0	0,0	100,0
		Altiplano	0,0	0,0	**90,0**	10,0	100,0
		Pasankalla	10,0	0,0	0,0	**90,0**	100,0
Cross Validation[b]	Count	Titicaca	24	3	1	2	30
		Regalona	2	28	0	0	30
		Altiplano	0	0	27	3	30
		Pasankalla	4	0	0	26	30
	%	Titicaca	**80,0**	10,0	3,3	6,7	100,0
		Regalona	6,7	**93,3**	0,0	0,0	100,0
		Altiplano	0,0	0,0	**90,0**	10,0	100,0
		Pasankalla	13,3	0,0	0,0	**86,7**	100,0

a. **90,0% of original group cases correctly classified**.
b. Leave-one-out approach.
c. **87,5%**. of original group cases correctly classified with cross validation.

Summary

- X-ray micro-CT on quinoa grains can provide a large amount of novel morpho-densitometric traits.
- Bulk scan approach is more suitable for representativeness and statistical significance of the results.
- Single seed scan approach allows very accurate internal structure grain characterization.
- Results from both scan approaches confirm the localization of the proteins mainly in the embryo.
- Traits from bulk seed scan are sufficient for a very good discrimination among the varieties.
- Single seed scanning results agree with hypothesis that the saponins are predominantly present in the papillose cells of the pericarp.

Conclusions

An increased embryo size is a target trait of interest in quinoa breeding programs for the development of improved quinoa varieties with higher protein content.

Ongoing research

SLU → Comparative gene analyses at transcriptome level

↓

Why do Altiplano and Pasankalla varieties have larger embryo?
Which genes are responsible for this phenotype difference?

First (partial) results:

One such TF-gene 'LEC1-Like' is downregulated in Altiplano and Pasankalla
Closely related to LEC1 (Leafy cotyledon1), a regulator of embryo development

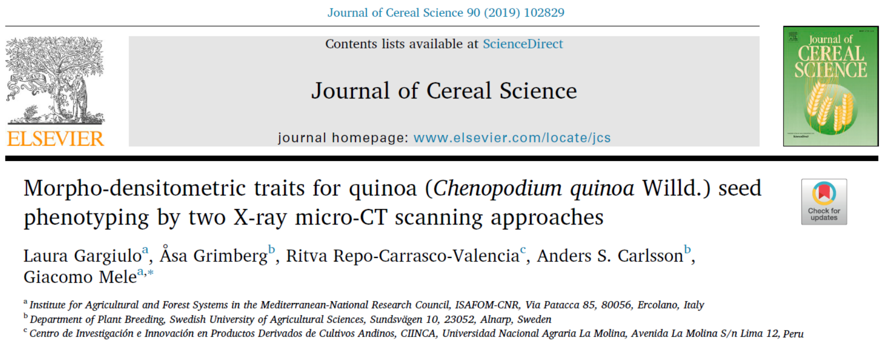
3rd Workshop of the *Nutritional Security Working Group*

27-28 May 2022, Lecce, Italy

Morphological traits of quinoa (Chenopodium quinoa) grains as indicators of nutritional quality

Thanks for your attention

https://doi.org/10.1016/j.jcs.2019.102829



Journal of Cereal Science 90 (2019) 102829

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Morpho-densitometric traits for quinoa (*Chenopodium quinoa* Willd.) seed phenotyping by two X-ray micro-CT scanning approaches

Laura Gargiulo[a], Åsa Grimberg[b], Ritva Repo-Carrasco-Valencia[c], Anders S. Carlsson[b], Giacomo Mele[a,*]

[a] Institute for Agricultural and Forest Systems in the Mediterranean-National Research Council, ISAFOM-CNR, Via Patacca 85, 80056, Ercolano, Italy
[b] Department of Plant Breeding, Swedish University of Agricultural Sciences, Sundsvägen 10, 23052, Alnarp, Sweden
[c] Centro de Investigación e Innovación en Productos Derivados de Cultivos Andinos, CIINCA, Universidad Nacional Agraria La Molina, Avenida La Molina S/n Lima 12, Peru