Development of mobile sensors for estimation of grain qualities and contaminants to enhance nutrition and safety of grain-products in developing countries; current status

Jana Kholová[1], Keerthi Chandalavada[1], Vincent Vadez[1], Sunita Choudhary[1], Govindaraj M.[1], Prasanth Bagade[2] Millicent Smith[3], Regan Lindsay[4], Stefan Gerth[4], Joelle Claussen[4], William Palmer[5], Athanas Matheka[6], Joshua N. Cobb[7], Enghwa NG[7]

1 International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Telangana, India

2 The University of Sydney, Sydney NSW, Australia

3 Texas A&M University, 400 Bizzell St, College Station, TX 77843, Texas, USA

4 Fraunhofer IIS, Development Center for X-ray Technology EZRT, Flugplatzstraße 75, 90768 Fürth, Germany

5 **HoneAg,** DSA-18 Hub, 1 Technology Place, Williamtown, Australia

6 Greenforest Foods Limited, MVCW+6, Nairobi, Kenya

7 International Rice Research Institute, Los Baños, 4031 Laguna, Philippines

Introduction

The governments of several developing countries responded to their population “malnutrition crisis” (P.Webb et al., 2018), among others, by promotion of crops of high nutritional value and their enhanced usage for food products formulations (e.g. “Millet Mission” in India or “Blending Policy” in Kenya). Simultaneously, several CGIAR crop improvement programs-initiated the development of nutritionally enhanced crop cultivars (e.g. ICRISAT, CIAT, IIT). In developing countries, the promotion of novel cultivars is generally a slow and tedious process, especially if the improved grain (quantity or quality) doesn’t ultimately result in the economic incentives (e.g. the market price). Thus far, there is no economic advantage directly linked to the trade of bio-fortified crop cultivars in developing countries which does prevents their accelerated adoption. This may change if/once the necessary information on a crops value is enabled.

The creation of regulated markets for trading commodities is fundamental for creating livelihoods for millions of smallholder farmers of the developing world. Most grain trade in developing countries is highly-localized, usually unstandardized and heavily reliant on “middlemen” for price setting, aggregation and transportation. Under such conditions, regulated standards for premium quality crop commodities for domestic markets exclude small-holder farmers as they don’t possess the ability to aggregate produce into the quantities and quality demanded by the commercial market. The local subjective evaluation of grain quality standards necessarily result in bias and a lack of trading opportunities further prevents local farmers from trading their produce to the wider domestic/international market. This situation subsequently prevents the small-holder farmers from reaping the benefits of value-added traits that are often characteristic of new improved varieties and deters them from investing in improved and certified seeds.

Importantly, adulterations and contaminations of food are common in developing countries in general and represent a considerable food safety risk. Apart from health risks, grain contaminations restrain millions of smallholders from the broader/premium/international markets since contamination levels are the main factor determining broader produce trade (especially international trade). The general lack of control over the traded food-crops commodities, particularly the levels of food contaminants in the developing world, do adversely affect human health. Since grain qualities and contaminants do tremendously vary within crop species and with crop cultivation practices, there is no guarantee that one has access to high quality and safe dietary sources.

At present, most **grain qualities and contaminants' estimations depend on wet-lab analytical services which are costly, time consuming, produce a lot of hazardous waste, and are mostly inaccessible** for the under-privileged sections of society in developing countries. Currently, there are only few techniques available for quick, reliable and cost-effective field measurements of agricultural produce qualities which do fit the context of trading systems in developing countries and allow small-holder farming communities to reap the benefits linked to the quality of their produce (De Alencar Figueiredo et al., 2006). Similar situation is observed in the crop improvement programs; in this case, the laboratory facilities may be available but these are usually not rapid enough to facilitate the efficient selection within the breeding populations. **For this purpose, we have initiated development of the relevant sensors for testing multiple grain qualities (NIRs, CT, XRF, visible-spectra imaging based)**. The resulting technology product will be **mobile, rapid, robust, cost-effective yet environment-friendly. The mobility of the proposed technology** solutions is, therefore, a **critical attribute** to cater for the needs of the grain related value chain players in the de-centralized market context of developing countries as well as suit the needs of crop improvement programs which should be primarily guided by these market-demanded traits.

We have initiated a global network of multidisciplinary collaboration to enable such development and in this part, we will briefly describe the stakeholders needs and the current status of the sensor development and testing;

Particularly;

1. The examples of product qualities important for trade, human nutrition and safety, development of nutritionally enhanced cultivars in CGIAR
2. Initial examples sensor-testing, calibration and validation to enable the traits identified in #1

Materials and Methods

Prioritization of qualitative traits

Out of all the qualitative traits which could be possibly estimated using the sensor-based techniques (NIRs, CT, XRF, visible-spectra imaging based) targeting the efforts will be critical. To begin with the traits of general importance which are common across the grain crops species, a series of meetings involving the crop improvement teams across CGIAR and the sensor-development experts has been organized (proceedings available at http://excellenceinbreeding.org/module4). Table 1 shows the initial outcomes of these meetings; i.e. the prioritized traits of global importance across various crops and the sensor technology which could be used to detect these with high throughput and precision. The efforts are continuing to further specify these traits ranges and accuracies required by particular value-chains actors and will provide an end-to-end solution; i.e. to develop the crops with enhanced market value from one hand while enabling estimation of this value and provide the incentives for the quality produce at the market-systems from the other hand.

Plant material used

For cereals calibrations, grain samples from different crop species were collected from trials conducted in the lysimetric system (http://gems.icrisat.org/lysimetric-facility/) or field in ICRISAT, India, Philippines and Kenya. The genotypes were grown in replicated manner and in some experiments under factorial treatments of irrigation schemes. All the samples were collected at the crops maturity, cleaned and dried. For NIRs analysis, all the replications of each genotype were mixed to make a single sample and ground to fine powder using Cemotech sample mill (FOSS technologies). Ground samples were stored in sealed plastic bags and stored at 4°C until the analysis. For CT and XRF analysis, the dried samples of whole-grains were used directly. Each of the crops encompassed 20-250 genotypes; these were:

1) Grains evaluated without shell (sorghum, pearl millet, pigeonpea, chickpea, foxtail millet, finger millet)
2) Grains evaluated with shell (rice, groundnut)

Laboratory analysis of the grains organic and inorganic components was done using standard AOAC (Association of Official Analytical Chemists) protocols (Official methods of Analysis, AOAC, 2000); these were: total protein (AOAC; 2001.11); total fat (AOAC 920.39); Fe, Zn (Inductively coupled plasma mass spectrometry, P.Masson et al., 2010)

Types of sensors used and the current status of their suitability testing

1) Near Infra-Red spectroscopy (NIRs)

The bench-top standard NIRs equipment FOSS XDS NIR spectrometer (FOSS North America, Eden Prairie, MN, USA) was used to predict organic composition of single/multiple species grains and several mathematical models were tested 1) modified partial least square regression method (MPLS) (in winISI software, version 4.3), 2) generalized linear model (GLM) (in HoneAg software, (www.honeag.com)), 3) artificial neural network (ANN), 4) GPR (gaussian process regression). The goodness of calibrations and validations were compared based on coefficient of determination (R^2), slope of the regression; standard error (SEC) and root mean square error (RMSE). The results achieved by FOSS XDS NIR spectrometer will be further compared with the mobile NIRs version (https://www.honeag.com/#products).

2) X-Ray Fluorescence (XRF)

The bench-top standard XRF equipment (Bruker-S2 Puma; EDXRF) was used to predict inorganic composition of cereals species grains. The standard models were used to construct the calibrations the The calibrations achieved by Bruker-S2 Puma; EDXRF were compared with the mobile XRF version (https://alloytester.com/handheld-xrf-analyzer?gclid=Cj0KCQjwgLLoBRDyARIsACRAZe4trYK5fd8DmnKoYPZwX6eWTKfu4Ci8W3MQHyUxsTtivCIbUPHjQpwaAj6XEALw_wcB) coupled with modified algorithms for fluorescence signal analysis. The goodness of calibrations from both these systems were compared based on coefficient of determination (R^2), slope of the regression and root mean square error (RMSE).

3) Computer Tomography (CT)

CT applications appears invaluable to gain the time advantage in evaluation basic grain properties (grain size, shape, damage) of the crops species which have grains covered with the shell which is not easily removable (e.g. groundnut, rice). To access these grain qualities, the portable CT technology was tested (https://www.iis.fraunhofer.de/en/ff/zfp/tech/mobile-computertomographie.html).

Results and discussion

1) Near Infra-Red spectroscopy (NIRs)

NIRs can be a powerful tool to measure traits related to organic grain composition, provided the calibrations are rigorously build and thoroughly tested. Generally, for the industrial usage the NIR calibrations for single species are preferred as they are more accurate and precise compared to multi-species calibrations (De Alencar Figueiredo et al., 2006, E.Elfadl et al., 2012, Kim K. S. et al., 2007). However, in some cases it might difficult to produce single species calibrations, e.g. in the case of calibrations for “millet” grains (pearl-, finger-, codo-, proso-, foxtail-, little-millet, sorghum, teff etc.) or “orphan” legumes (horsegram, Dolichos lablab, cowpea, mothbean, etc), where availability of adequate sample size covering the minimum range of variation required for relevant calibration might be difficult. Thus, here we tested and compared the single and multi-species calibrations as a possibility to overcome the above limitations (Table 3&4). Here, we showed the example of single-cereal (sorghum) and multiple-cereal (sorghum (62), pearl millet (26), finger millet (20), foxtail millet (20)) calibrations using various calibration methods. Principally, the sorghum protein and fat were predicted more accurately with sorghum-specific calibration (Table 2) which was built with sufficient sample size (96) and range of variation (protein: 6.74-14.15, fat:1.5-5.41 w/w%). However, even the multi-species prediction could be considered for sorghum (calibration -R^2: 0.8, 0.92; RMSE: 0.78, 0.66 for protein and fat respectively; validation: R^2: 0.85, 0.78; RMSEP: 0.4, 0.22, slope-1.3, 0.88 for protein and fat respectively) (Table4&5) (Figure 1) and would be better for the crops where sample size and range of variation was not sufficient. The best prediction models will be further tested using HoneAg technology (https://www.honeag.com/#products) for the field applications.

2) X-Ray Fluorescence (XRF)

Since far, XRF-based technologies found their usage in the range of heavy-industrial applications (https://www.iis.fraunhofer.de/en/ff/zfp.html) while their utilization in crop-research is relatively marginal. Here we present the calibrations for estimation of Fe, Zn in finger millet grains content using bench-top standard and hand-held XRF technology. Both the technologies resulted in the comparable and reliable-levels of Fe, Zn calibrations and prediction, however, the hand-held sensor signal had to be analyzed with different algorithms to achieve the similar level of prediction accuracy (Fig. 3 a, b). These sensors will be systematically tested on broader range of materials, however, the presented case of pearl millet clearly shows the mobile XRF application is possible provided the technology is rigorously tested and possibly further developed for estimation of elemental composition of the crops grain material.

3) Computer Tomography (CT)

CT has been widely used in the medical industry as well as in heavy-industry applications (Fuchs T et al., 2013, De Chiffre etal., 2014). Again, the applications for phenotyping of plant-related material are a relatively recent development (https://www.iis.fraunhofer.de/en/ff/zfp.html, Walter A et al., 2015, FE Gomez et al., 2018, Wu D et al., 2018). Despite this, CT-enabled information could quickly become a game-changing factor which can enable critical information for crop improvement programs as well as for estimation of crop-value in the market-systems (rapid in-shell estimates of e.g. grain weight, size and shape, damages and shelling ratio, example on Fig. 4 a, b). In our case, portable CT has been tested to address such demand from rice and groundnut crop improvement programs where the time needed for grain threshing becomes significant barrier in the grain evaluation during the selection process. Similarly, the groundnut processing industries (e.g. Greenforest Foods Ltd., Kenya) indicated the rapid access to this information could overcome the market-barrier between the small-scale producers and the large groundnut processing industries. This technology is currently being systematically tested to address these particular needs.

Conclusion

Despite the booming development of sensor-based phenotyping technologies in developed countries and their rapidly growing applications in food-grain related value-chains, these become largely inaccessible in the context of developing countries. The presented work provides the initial evidence that the context-driven development and design of several sensor-based technologies (hereby discussed NIRs, XRF, CT) could rapidly enable end-to-end solutions to improve the qualities of produced food-grains as well as enabling incentives for producing quality grain to farmers and thus drive the adoption of nutritionally enhanced cultivars. Importantly, some of these technologies are also being tested for detection of the range of contaminants (e.g. aflatoxin, As, Cd) in the market-intended sensor applications. The CGIAR-public-private partnership has initiated these efforts to enable the essential technologies to the key process-drivers in crop improvement programs and value-chains in developing countries in order to contribute to the eradication of malnutrition and enhancement of food safety in support of global sustainable developmental goals (especially #2, 3).

References

1. De Alencar Figueiredo, L. F., Davrieux, F., Fliedel, G., Rami, J. F., Chantereau, J., Deu, M., ... & Mestres, C. (2006). Development of NIRS equations for food grain quality traits through exploitation of a core collection of cultivated sorghum. *Journal of agricultural and food chemistry*, *54*(22), 8501-8509.

2. De Chiffre, L., Carmignato, S., Kruth, J. P., Schmitt, R., & Weckenmann, A. (2014). Industrial applications of computed tomography. *CIRP annals*, *63*(2), 655-677.
3. Elfadl, E., Reinbrecht, C., & Claupein, W. (2012). Development of near infrared reflectance spectroscopy (NIRS) calibration model for estimation of oil content in a worldwide safflower germplasm collection. *International Journal of Plant Production*, *4*(4), 259-270.
4. Fuchs, T., Keßling, P., Firsching, M., Nachtrab, F., & Scholz, G. (2013). Industrial applications of dual X-ray energy computed tomography (2X-CT). In *Nondestructive Testing of Materials and Structures* (pp. 97-103). Springer, Dordrecht.
5. Gomez, F. E., Carvalho, G., Shi, F., Muliana, A. H., & Rooney, W. L. (2018). High throughput phenotyping of morpho-anatomical stem properties using X-ray computed tomography in sorghum. *Plant methods*, *14*(1), 59.
6. Kim, K. S., Park, S. H., Shim, K. B., & Ryu, S. N. (2007). Use of Near-Infrared Spectroscopy for Estimating Lignan Glucosides Contents in Intact Sesame Seeds. *J Crop Sci Biotechnol*, *10*, 185-92.
7. Official Methods of Analysis, 17th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2000.
8. P. Masson , T. Dalix & S. Bussière (2010) Determination of Major and Trace Elements in Plant Samples by Inductively Coupled Plasma–Mass Spectrometry, Communications in Soil Science and Plant Analysis, 41:3, 231-243, DOI: 10.1080/00103620903460757
9. Walter, A., Liebisch, F., & Hund, A. (2015). Plant phenotyping: from bean weighing to image analysis. *Plant methods*, *11*(1), 14.
10. Webb, P., Stordalen, G. A., Singh, S., Wijesinha-Bettoni, R., Shetty, P., & Lartey, A. (2018). Hunger and malnutrition in the 21st century. *bmj*, *361*, k2238.
11. Wu, D., Guo, Z., Ye, J., Feng, H., Liu, J., Chen, G., ... & Liu, Q. (2018). Combining high-throughput micro-CT-RGB phenotyping and genome-wide association study to dissect the genetic architecture of tiller growth in rice. *Journal of experimental botany*, *70*(2), 545-561.

Tables

Table 1. Outcomes of the meeting of the experts; Excellence in Breeding; Phenotyping for crop qualities; October 2018. Examples of required crop qualities defining 1) market classes, 2) processing and storing qualities 3) importance for human nutrition and health 4) crop residues qualities 5) contaminants. The sensors being tested for estimation of the crop quality indicators

	market classess			processing			storing		crop resid	human nutrition						contaminants	
crop/quality	seed size	seed colo	seed shap	cooking	malting	shelling%	rancidity	moisture	IVOD	protein	oleic acid	oil conten	Fe	Zn	Ca	aflatoxin	As
chickpea	*																
cowpea	*																
lentil	*		*														
pigeonpea	*																
soybean	*																
finger millet																	
sorghum			*														
pearl millet	*																
maize	*																
beans	*		*														
rice	*		*														
groundnut	*		*														
sensor techn	seed size	seed colo	seed shap	cooking	malting	shelling%	rancidity	moisture	IVOD	protein	oleic acid	oil conten	Fe	Zn	Ca	aflatoxin	As
NIRs #																	
XRF																	
CT	*		*														
visible-spectra																	

* possible in-shell/in-panicle estimates

sensitive to high moisture content (>~15%w/w)

Table 2: descriptive statistics (mean, standard deviation (SD) and standard error of laboratory (SEL)) of cereals (pearl millet, sorghum, finger millet, foxtail millet) used in the multi species and sorghum calibrations development for protein[w/w%] and fat[w/w%]. Wet lab analysis was done using AOAC methods; 2001.11 and 920.39 for protein and fat respectively)

		Protein [w/w%]			fat[w/w%]		
Crop species	N	mean	SD	SEL	mean	SD	SEL
pearl millet	26	12.55	1.3	0.25	8.44	1.26	0.24
sorghum	62	10.74	1.03	0.13	3.12	1.08	0.13
foxtail millet	20	11.58	1.18	0.26	4.54	5.91	1.32
finger millet	20	7.92	0.97	0.21	2.17	5.91	1.32
multi cereals	142	10.63	1.78	0.14	4.35	2.32	0.19
Sorghum (for sorghum calibration)	96	10.4	1.16	0.012	3.4	0.65	0.07

Table 3: calibration statistics of protein[w/w%] and fat[w/w%] equations developed by MPLS (modified partial least square regression) in winISI software, version 4.3 for sorghum samples

Property	N_{cal}	Treatment	Outliers	R^2_{cal}	SEC	$RMSE_{cal}$	1-VR	SECV	R^2_{val}	$RMSE_{val}$	slope
Protein [w/w%]	96	2^{ND} der-SNV	8	0.94	0.26	0.78	0.88	0.39	0.93	0.43	0.91
Fat [w/w%]	100	2^{ND} der-SNV	8	0.94	0.15	0.15	0.83	0.26	0.92	0.15	0.95

Table 4: calibration statistics of protein [w/w%] and fat [w/w%] equations developed by MPLS (winISI software, version 4.3) and GLM (generalized Linear model-Honeag software (www.honeag.com)) models for multi species (cereals: pearl millet, sorghum, foxtail millet and finger millet)

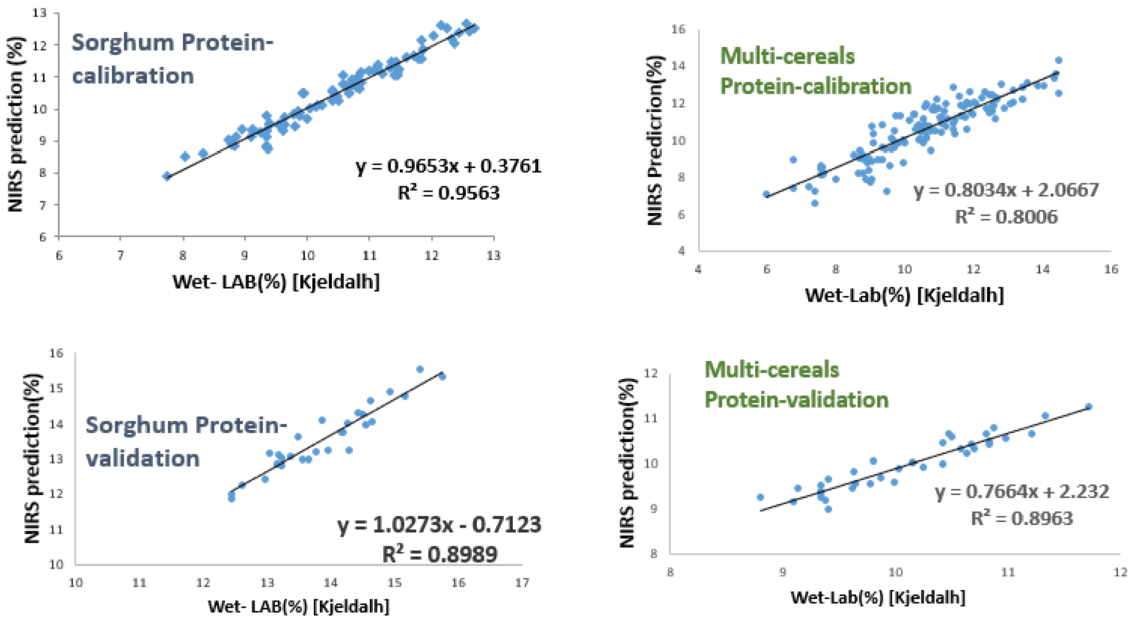
	MPLS								GLM		
Property	N_{cal}	Treatment	Outliers	R^2	SEC	RMSE	1-VR	SECV	Treatment	R^2	RMSE
Protein[w/w%]	MPLS-142 GLM-105	2^{ND} der-SNV	6	0.8	0.75	0.78	0.76	0.81	baseline offset, Savitzky-Golay gap	0.69	1.04
Fat[w/w%]	MPLS-142 GLM-105	2^{ND} der-SNV	8	0.92	0.66	0.78	0.86	0.85	baseline offset, Savitzky-Golay gap	0.88	0.86

Table 5 Validation statistics of protein[w/w%] and fat[w/w%] equations developed by developed by MPLS (winISI software, version 4.3) and GLM (generalized Linear model-Honeag software (www.honeag.com)) models for multi species (cereals: pearl millet, sorghum, foxtail millet and finger millet)

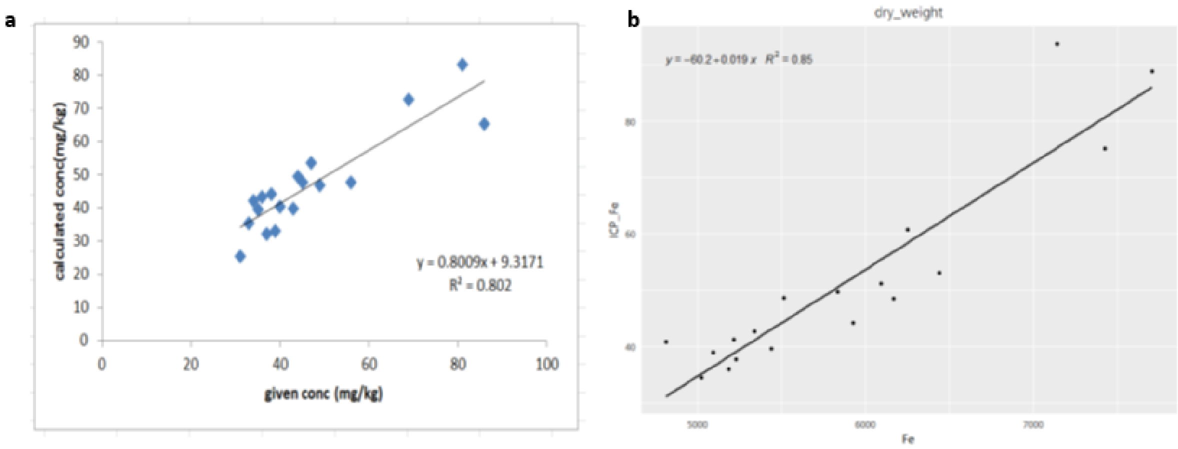
	MPLS			GLM		
Property	N	R^2_{val}	$RMSE_{val}$	N	R^2_{val}	$RMSE_{val}$
protein[w/w%]	46	0.85	0.4	37	0.71	0.98
Fat[w/w %]	45	0.78	0.22	37	0.75	0.9

Figures

Figs. 1 Example of single (sorghum) and multiple (sorghum, pearl millet, finger millet, foxtail millet) species calibrations and validations for protein content by using Modified Partial Least square regression (MPLS) method in WinISI software, version 4.3)



Figs 2. Comparison of calibrations for elemental grain composition (Zn; foxtail millet) using bench-top standard XRF-facility and hand-held XRF sensors.



Figs 3. CT scan of grains with shell (groundnut, rice) enabling the information on grain size, shape, grain weight estimates, grain damages, grain filling success (~shelling ratio).