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Spectroscopic Kernel Quality from a Symbiotic Corn Production

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Abstract

The management of the ainoculation of a plant's roots, by means of biofertilizers (BF) containing arbuscular mycorrhizal (AM) fungi, is aimed at inducing modifications of the quality of the seeds. It is here shown that a seed-soil treatment can be elicited in the fingerprints of a symbiotic treatment using Near Infra Red (NIR)-SCiO NIR-SCiO spectra collections of single kernels: overall, a sensitivity of 73% and a specificity of 73% have been achieved, thus suggesting that it may be possible to assign the symbiotic origin of corn from just twenty kernels, provided that the dataset is adequately representative of the cultivar and AM. A global correlation study has shown a positive general trend (R^2 0.45) of quality *vs.* quantity, in the sense that an increase in yield corresponded to an increase in the spectral differences between the symbiotic spectra and the control ones, but the inverse was also true, as a result of the parasitic behaviour of the BF treatments. The efficacy of the symbiosis can be back predicted from the NIR spectra; in fact, around 90% of the positive yield outcome results were discriminated from the negative ones. A reduction in the foliar pH (R^2 0.37) and an increase in the foliar protein (R^2 0.43) were observed as immediate phenotypic signs of a productive symbiosis. The commercial raw composition of the kernels appeared to only be affected slightly by the BF treatments; thus, till now uncharted secondary compounds of the maize kernels are involved, as supported by animal performances.

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Introduction

Symbiotic Agriculture (SA) is a cultivation method that systematically integrates the use of biofertilizers in the management of all rotating crops. Biofertilizers with Arbuscolar Mycorrhizal fungi (AM) have important biological effects on colonized plants, as they improve the nutrient absorption, particularly as regard the phosphorus bound in agrarian soils[1], with a consequent stimulating effect on plant growth, and an enhancement of their resistance (a)biotic stresses[2]. The resilience capacity of crops are improved under slightly unfavorable conditions, where the saprotrophyc or parasitic tendencies of the AM can be mitigated. Klironomos[3] compared local and exotic species with both local and exotic mycorrhizal fungi and noted how plant growth responses to inoculation can range from highly mutualistic to parasitic: no single species of plants did better with all the tested AM fungi.

Thanks to a favorable alignment of agronomical inputs, SA can enhance productions, up to luxuriance. In the first part of this maize study[4], we examined some critical points necessary to achieve successful yields using complex biofertilizers. Further knowledge was obtained concerning potato[5]. In short, it is necessary that soil has a hospitable attitude toward new microbial agents: if the organic substance is deficient, or the microflora is too aggressive, it becomes difficult for the minimal doses of bio-fertilizer, which are precisely inoculated near the roots, to be hosted, multiplied and spread. A mycelial network may arise and expand, thereby connecting a high density corn plantation -up to 6 m^{-2}- whose roots prevent any mutual contact rsulting from allelopathy.

Previous biofertilizer studies have shown that any qualitative modifications of seeds will have a effect on the primary[6, 7] and secondary compounds, that is, there will be a rise in antioxidants[8,9,10] with beneficial consequences on animal feeding [11, 12].

The aims of the present study have been to increase knowledge on symbiotic corn production, with emphasis on the quality results, from tests in real fields using rapid analysis methods.

Experimental Procedure

Three recently published rapid methods, namely the NIRS-litter-bag technique[13], the raw foliar pH[14,15,16] and foliar NIR-Tomoscopy[17], have been used together, in a holistic model, for a symbiotic corn yield production[4]. The aim of this study has been to investigate the quality of symbiotic corn. First, the spectral NIR signature of the single kernels was obtained and elaborated in order to assess whether this method could fingerprint the source of the corn (Control *vs.* Symbiotic), as induced by a biofertilizer in different corn cultivars. Moreover, some commercial qualities of the integer grain were determined, by means of a bench NIRS instrument, and elaborated as univariate. Finally, by chaining the results of this second part to the previous results, a correlation has emerged which has highlighted the relationships among the phenotype variables that affect the quantity of the corn as well as their consequences on the variation in the quality traits.

Materials and Methods

Experiment Set-up

Twenty-six pairwise comparisons, namely Symbiotic inoculated (S) *vs.* Control non-inoculated (C) were obtained from a total of 44 plots in 4 centers, with four cultivars and six AM types, over a period of 2 years.

In 2018, three centers collaborated in the set up and the realization of the calibration experiments (Table 1). The inoculation was performed using a Micosat F ® bio-fertilizer, as coating (1 kg ha^{-1}) or granulate (10 kg^{-1}). In 2019 validation experiments were performed using a Micosat F ® bio-fertilizer and four other AFM types as coatings (1 kg ha^{-1}) at a University Center in soil where cation exchange capacity appears to be average, while the phosphorus supply assimilable is low, as is that of exchangeable potassium (Table 2).

[1]Biota composition: finely ground cultivated *Sorghum sudanensis* roots, containing spores and ifae of *Funneliformis coronatus* GO01 and GU53, *F. caledonium* GM24, F. *intraradices* GB67 and GG32, *F. mosseae* GP11 and GC11, *F. viscosum* GC41; saprotrophic fungi: *Streptomyces spp*. ST60, *Streptomyces spp.* SB14, *Streptomyces spp.* SA51, *Beauveria spp.* BB48, *Trichoderma viride*, *Trichoderma harzianum* TH01, *Trichoderma atroviride* TA28, *Trichoderma spp.*; rhizosphere bacteria: *Bacillus subtilis* BA41, *Pseudomonas fluorescens* PN53, *Pseudomonas spp.*

Table 1. Plan of the experiments, 2018 calibration and 2019 validation.

Experiments	Pairwise comparison	Cultivars	Yield	Bio-fertilizer	Type / dose
2018 calibration, N. 52 plots					
2018-1 CREA-IC (BG, Italy)	1	Pioneer P1547.	Corn 14.5% DM	Micosat F MF[1]	Granular 10kg/ha
	2		Corn 14.5% DM		
	3		Corn 14.5% DM		
	4		Corn 14.5% DM		
	5		Corn 14.5% DM		
	6		Corn 14.5% DM		
	7		Corn 14.5% DM		
	8		Corn 14.5% DM		
2018-2 DISAFA-1 (TO, Italy)	9		DM waxy spikes		Tan 1kg/ha
	10		DM waxy spikes		
	11		Corn 14.5% DM		Granular 10kg/ha
	12		Corn 14.5% DM		
2018-3 Maïsadour	13	MAS 68K	Corn 14.5% DM		
2019 validation, N. 50 Plots					
2019-1	14	DK4316	Corn 14.5% DM	MF[1]	Tan 1kg/ha
2019-2 DISAFA-2 (TO, Italy)	15-18	MAS DM6318	Corn 14.5% DM	AM_09[2]	
	19-22		Corn 14.5% DM	AM_ 07[3]	
	23-26		Corn 14.5% DM	AM_ 05[4]	
	27-30		Corn 14.5% DM	AM_ 12[5]	
	31-34		Corn 14.5% DM	MF[1]	
	35-38	MAS Shaniya	Corn 14.5% DM	AM_ 09[2]	
	39-42		Corn 14.5% DM	AM_ 07[3]	
	43-46		Corn 14.5% DM	AM_ 05[4]	
	47-50		Corn 14.5% DM	AM_ 12[5]	
	51-55		Corn 14.5% DM	MF[1]	

Table 2. Characteristics of the soil in the University Centre, Carignano, IT [GPS: 44°53'07.5"N; 7°41'01.8"E;]

Sand	28.10%
Silt	67.20%
Clay	4.80%
pH	8.1
Organic substance	1.70%
Organic carbon	0.99%
Total nitrogen	0.10%
C / N ratio	10.1
Equivalent phosphorus	10 p.p.m.
Cation exchange capacity	10.1 meq 100 g^{-1}

PT65 and *Pochonia chlamidosporia*, in a relative percentage of 40% crude inoculum and 21.6% bacteria and saprotrophic fungi; [2]*Rhizophagus intraradices* CA502; [3]*Gigaspora rosea* NY328A; [4]*Sclerocystis sinuosa* MD126; [5]*Claroideoglomus claroideum* ON393.

Kernel Scanning

An NIR-SCiO mod. 2 (Consumer Physics inc, Herzliya, Tel Aviv, Israel) (Figure 1), was used to scan about twenty-five kernels selected from corn samples from each plot. Each grain was put in the center of a reverberant pill sample holder, with the embryo facing downward (Figure 2), and then scanned from 740 to 1070 nm (331 points at a 2 nm interval).

Fingerprinting of the Symbiotic Treatment in the NIR-SCiO Spectra of the Kernels

Chemometric elaborations were carried out, by means of "*The SCiO-Lab*" software, which operates by means of AKA (As Known As) matrices and provides a percentage recognition of the matrix cells. The method used for the calibrative classification was the Random Forest algorithm. The percentages of fingerprinting for the Kernel Control (K_CC) and the Kernel Symbiotic (K_SS) classes were analysed according to the free MedCalc online software. The interaction Cultivar* AM type was tested, by means of a Friedman test for paired comparisons (StatBox V6.5, Grimmersoft, Paris). The predictivity of the models was established, by means of a leave-one-out validation procedure, within the four cultivars and considering the different AM types.

Connecting the NIR Spectra of the Symbiotic Kernels to the Yield Response

A wide range of Yield responses to the Symbiotic treatments was observed during the experiments developed for the present paper. Is it possible that the NIR spectra of the kernels produced in plants treated with a BF can contain some information on the degree of the Yield result? For this purpose, the PLS procedure of the *SCiO-Lab* software was applied to the collection, for over 1338 spectra.

NIRS Bench Analyses

Duplicate spectra of the whole grain plots were obtained using a DA1650 NIRS-FOSS™ instrument, provided with a calibration model to predict five components on a DM basis: Ash, Protein, Starch, Fiber, Fat and the NIRS undetermined Residual.

Univariate Analyses

Kernel quality data from the Control and paired Symbiotic plots were analysed using the Friedman test for paired comparisons.

Correlation and Regression Analyses

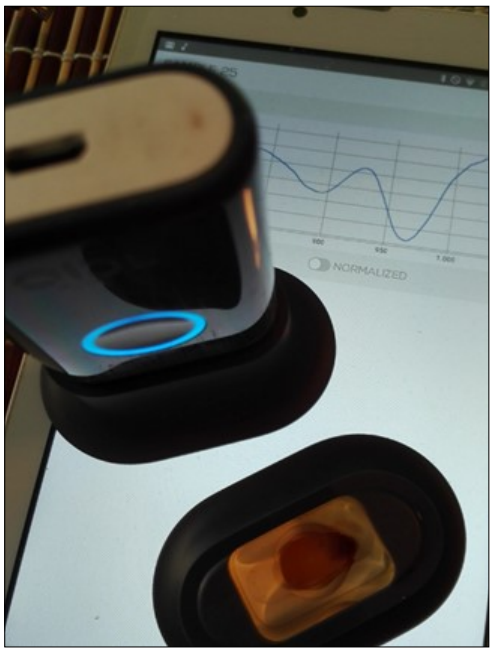

Figure 1. NIRS-SCiO™ (Consumer Physics, Tel Aviv) device ready to scan a kernel.

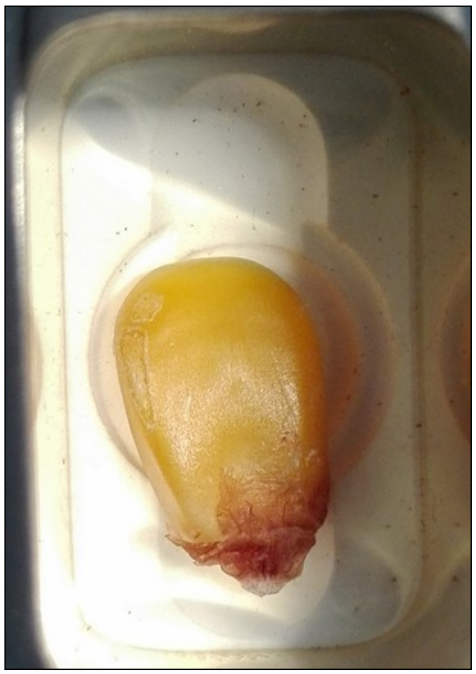

Figure 2. A kernel in the center of the reverberant pill sample holder, with the embryo facing downward.

In order to explore the relationships between the quality traits and the quantity variations in yield, the regressions of the main variables, namely the Kernel spectral fingerprint (K_CC, K_SS and their average K_), were computed on the results of measurements previously obtained from the phenotype of the plants. The independent variables were expressed in terms of a plot effect-size, computed as the Ln of the response ratio (S/C), where the mean of the inoculated treatment (S) was divided by the mean of the non-inoculated control (C), namely d_Y = Ln(S/C). This mode of expression is arithmetically equivalent to calculating the relative prevalence of S over C (d_Y= S/C -1). Only the selected co-variables that were significant at a Pearson test were included in the regression study of the main variables.

Results

NIR-Tomoscopy of the kernels

The average reflectance spectra of the Symbiotic kernels were close to the Control ones (Figure 3). The brilliant values obtained in the reverberant chamber should be noted, especially compared to the average NIR spectra of the leaves.

Spectral Fingerprint of the Cultivars

The spectral fingerprinting of the Kernel collection was able to perfectly discriminate the four cultivars (Table 3).

Spectral Fingerprinting of the Symbiotic Treatment and the Interactions

The fingerprinting of the Control and Symbiotic types produced the same value of 73% for the whole collection of kernel spectra (Table 4, r. 2), but only when Micosat F was used. The fingerprint values otherwise increased for the S (88%), but decreased to 55% for the C, when the other five types of AM were included in the Symbiotic treatment: these two values are biased by the different numbers in the two classes. As far as the cultivars are concerned, higher values characterized DK4316, which was only tested in one field, with respect to P1547, which was tested in 16 plots. When different AMs were combined with two different cultivars, the results showed variable fingerprint responses to the five types (Figures 4 and 5), and an interaction cultivar * AM appeared for Friedman's test (P 0.05, Figures 4 and 5 combined).

Validation of the Spectral Fingerprinting of the Symbiotic

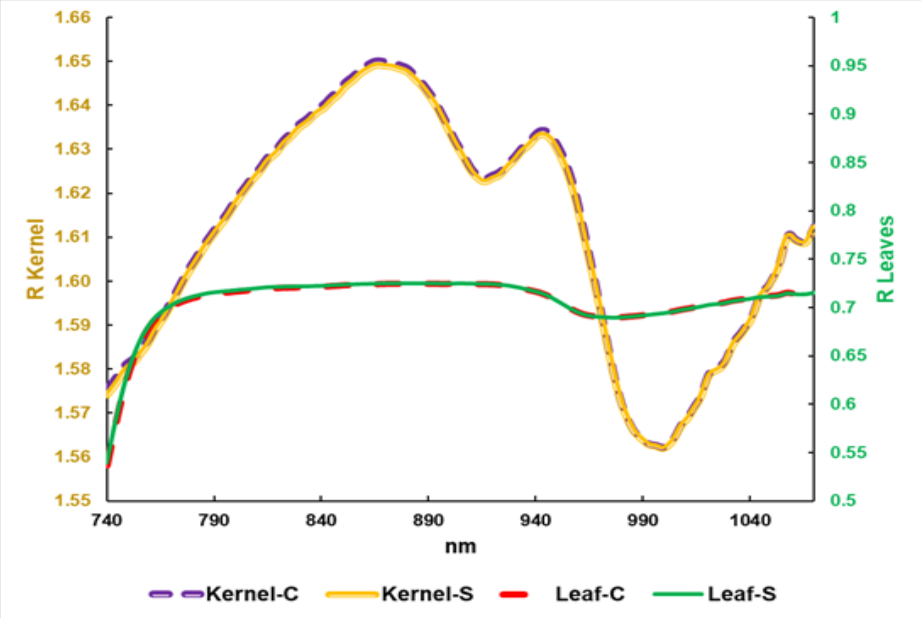

Figure 3. Average NIRS-SCiO spectra of the kernels: Control (C) and Biofertilized (S-Symbiotic) (No. 2024), and of the C and S leaves (No.1316).

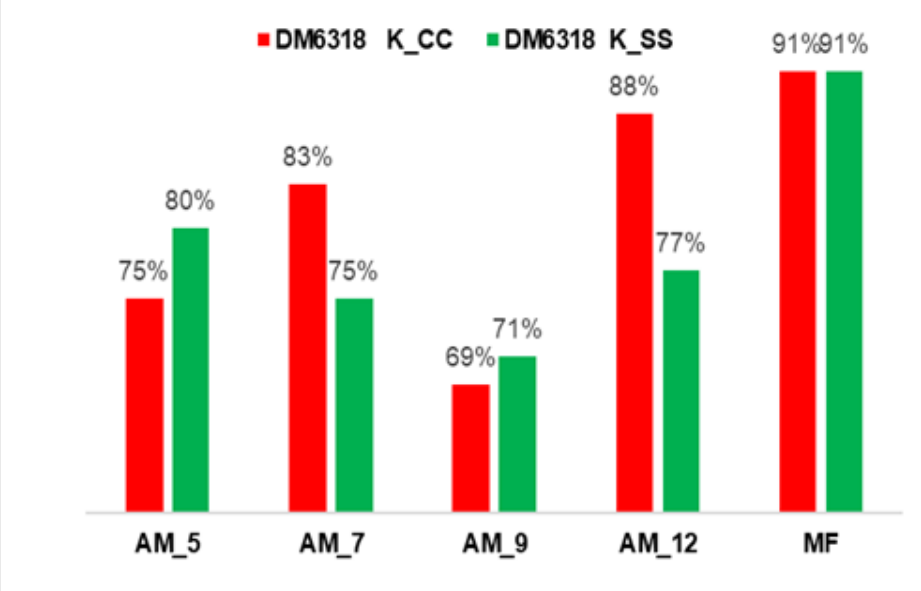

Figure 4. Different responses in the NIR kernel fingerprint from the AM[£] types in the DM6318 cultivar.

£ AM_5: Sclerocystis sinuosa MD126; AM_7: Gigaspora rosea NY328A; AM_9:Rhizophagus intraradicesCA502; AM_12: Claroideoglomus claroideum ON393.

Table 3. Classification of the cultivars (Cv.) from the NIR spectra of the kernels.

Predicted Cv.				
Shaniya	0%	10%	0%	90%
P1547	0%	0%	100%	0%
DM6318	0%	90%	0%	10%
DK4316	100%	0%	0%	0%
Observed Cv. ---->	DK4316	DM6318	P1547	Shaniya
No. in the observed Cv.	121	477	951	476

Table 4. Calibration models of the classification of the Control and Symbiotic kernels from four different maize Cultivars (Cv.) and five Arbuscular Mycorrhizal (AM) types from the NIR spectra.

Cultivars (Cv.)	Biofertilizer type	No.	No. Obs.	K_CC	No. Obs.	K_SS	Note
All Cv.	All types of AM	2024	687	55%	1338	88%	
All Cv.	Only Micosat F	1389	687	73%	702	73%	
DK4316	Only Micosat F	121	61	100%	60	98%	
P1547	Only Micosat F	951	469	70%	482	69%	
DM6318	All types AM	477	80	81%	397	79%	Fig. 4
DM6318	Only Micosat F	157	80	92%	77	89%	
Shaniya	All types of AM	476	77	76%	399	77%	Fig. 5
Shaniya	Only Micosat F	157	77	70%	80	69%	

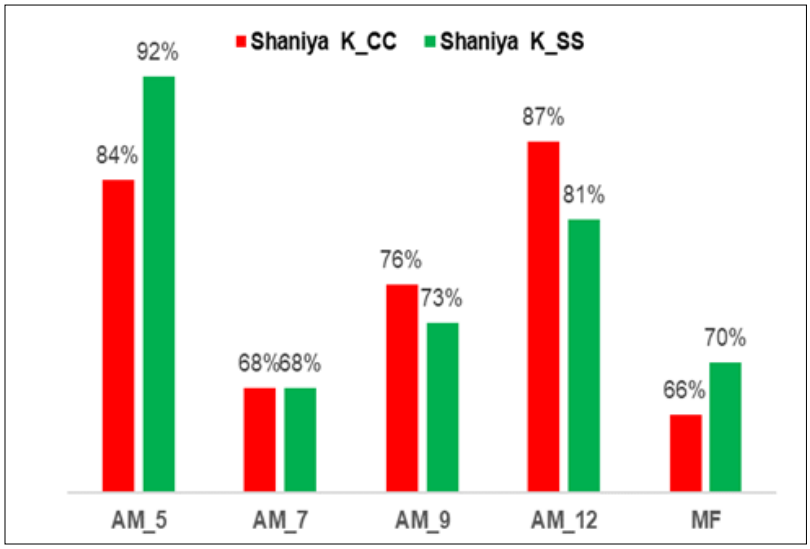

Figure 5. Different responses in the NIR kernel fingerprint from the AM[£] types in cultivar Shaniya.

[£]AM_5: *Sclerocystis sinuosa* MD126; AM_7: *Gigaspora rosea* NY328A; AM_9: *Rhizophagus intraradices*CA502; AM_12: *Claroideoglomus claroideum* ON393.

Treatments

In general, Kernel symbiotic models built for one cultivar cannot be extrapolated to a different cultivar. In fact, although the calibration models that excluded each cultivar were apparently satisfactory (Table 5, left), the leave-one-out validation (Table 5, right) showed high inaccuracies, especially in the K_CC values, which were systematically underestimated in the DK4316 and P1547 cultivars, while the K_SS values over-performed in the previous cultivars, but were inefficient (three cases) or underestimated (one case).

Raw Composition of the Kernels and Correlations

Only the fat content was slightly increased in the Symbiotic kernel (P 0.05; Table 6). On average, the starch was increased (P 0.12) together with the fiber, while the protein, ash and undetermined residuals were decreased. The within constituent correlations of the C and S values were all significant, except for the fiber and the residual undetermined by NIRS. The two spectral fingerprints of the kernels were highly correlated (r 0.83).

The between-trait printout (Table 5) showed significant correlation coefficients between the three main kernel variables and fourteen co-variables, out of a total of sixty, obtained from different sources in the growth phase [4].

The induced respiration (SIR) from the soil source variables was negatively correlated with the Control kernel fingerprint level (r -0.46) and with the yield results.

As far as the plant sources are concerned, all the foliar pH records (S, C, S/C) were negatively correlated with the NIRS C and S fingerprints of the kernels, clearly showing that a higher (protonic) energy charge in the leaves promoted the kernel diversification.

Among the kernel components, the fat and the starch contents were positively related to the high NIRS fingerprinting and characterization, while the protein and the fiber levels reduced the spectral originality of the C and the S kernels.

In short, the quantitative results, in terms of yield from the BF management were significantly and positively correlated with a higher diversification of the kernel, thereby permitting a higher fingerprinting in the

Table 5. Leave-one-out validation of the models for the classification of the Control and Symbiotic kernels from four different maize Cultivars (Cv.) and Arbuscular Mycorrhizal (AM) types from the NIR spectra.

	Calibration					Leave-one-out validation				
AM types	Cv. excluded	No. Obs.	K_CC	K_SS	No. Obs.	K_CC	P	K_SS		Cv. validated
MF-Micosat F	DK4316	1268	71%	71%	121	5%	**-	100%	**	DK4316
MF-Micosat F	P1547	438	79%	79%	951	0%	**-	100%	**	P1547
MF-Micosat F	DM6318	1232	73%	74%	477	68%	**	51%		DM6318 all AMs
"	DM6318	1232	73%	74%	157	68%	**	43%		DM6318 only MF
MF-Micosat F	Shaniya	1229	68%	73%	476	79%	**	35%	**-	Shaniya all AMs
"	Shaniya	1229	68%	73%	157	79%	**	58%		Shaniya only MF

P Threshold 50%: ** P<0.01; **- underestimated P<0.01.

Table 6. NIRS estimated composition of the kernels from the 26 pairwise comparisons, Symbiotic effect-size-and correlation.

Constituent	Control		Symbiotic		Effect-size	P (C<>S)	r (C,S)	P(r)
	C	Std. Dev	S	Std. Dev	Ln (S/C)			
Protein %	9.26	0.51	9.19	0.54	-0.7%	0.95	0.75	**
Fat %	3.74	0.07	3.76	0.09	0.4%	0.05	0.90	**
Fibre %	2.73	0.33	2.80	0.33	2.5%	0.84	0.23	
Ash %	1.91	0.04	1.90	0.05	-0.3%	0.30	0.59	**
Starch %	74.77	0.63	74.96	0.83	0.3%	0.12	0.53	**
Residual %	7.59	0.68	7.39	0.85	-2.6%	0.24	0.33	
Spectral Fingerprint	84.4%	12.6%	82.5%	11.2%	-2.3%	0.20	0.83	**

P: ** <0.01

Control groups (r 0.69) as well as in the Symbiotic one (r 0.55), where a higher productive level was available for the plants (Table 7).

Regression of the NIR Spectral Fingerprint of the Kernels on the Size-effect of Yield, Foliar pH and Foliar Protein

A few relationships can be considered to highlight how the NIRS kernel fingerprint – the average of the two K_CC and K_SS values - depended on the main plant variables. The main regression was the one on Yield dressing (Figure 6).

By removing the two outliers and reversing the variables, a plausible parabolic model was obtained to estimate the yield size-effect from the NIRS fingerprint of the Control group, as shown in Figure 7 (R^2 0.70).

As shown in Figure 8, despite the presence of two outliers, the variation in foliar pH, due to the increase in acidity of the leaves of the plants treated with BF, was responsible for a progressive increase in yield as well as for a higher spectral level of characterization.

Moreover, the foliar protein may be considered a sign of AM symbiosis or parasitism. A close parabolic relationship (Figure 9) linked the increase in foliar protein - from a BF treatment - to the NIRS fingerprint (R^2 0.43) rather than to the Yield performances (R^2 0.29), and a sparse response dispersed the points, with two presumed outliers in the negative outcome zone.

Connecting the NIR Spectra of the Symbiotic Kernels to the yield Response

The calibration of the symbiotic response in yield was successful (Figure 10). The R^2 0.56 did not represent the more instructive relationships that emerged from a quadrant analysis (Table 8).

In fact, a cut-off around the zero-crossing point showed that 90% of the kernels could be correctly classified, correlating the productive outcomes from the field (Table 8).

Discussion

In the first part of the study, it was shown that a Bio-fertilizer can be positive, null or even negative for yield. A symbiotic corn yield model was formulated and validated by fitting the data from the plant phenotype variables, in particular pertaining to the foliar pH and the protein level of the leaves – as issued from NIRS tomoscopy with data from a soil litter-bags test. In the present part, we will only be able to formulate a model for quality if the term "quality" is clearly identified. As far as the commercial composition of the corn is concerned, the results showed that the centesimal composition of primary compounds was not affected or just slightly affected by the BF management. However, these NIRS analyses were obtained for a small number of samples per plot. Moreover, the kernel spectra provided for many samples per plot were more informative of the organic compounds embedded in the cortical region of the seed observed with the embryo facing downward. The position of the embryo had a significant effect on quantitative calibrations[18,19]. Reliable models were developed to predict protein and starch contents with the embryo facing upward (scanned from below), whereas the oil content required the embryo to be facing downward. As can be seen in Figure 1, the position (scanned from above) was more favorable for protein and starch than for variations in the fat content. In fact, the complexity of protein is expected to change more than its quantity. In general, mycorrhizal fungi intervene in the modification of seed proteins, thereby benefiting their complexation in favor of the less soluble fractions. Corn seed proteins are classified into groups according to their solubility, starting with albumin (35%, soluble in water), globulins (8%, soluble in salt water), prolamine-zeine (32%, soluble in alcoholic solution) and gluteline (20%, soluble in diluted alkali). As maturation progresses, the soluble albumin yields quotas in favor of insoluble zeine and gluteline. The contribution of mycorrhizal fungi is that of anticipating the maturation of the seed with an increase in insoluble zeins (+30% compared to the control) and a reduction in soluble albumin (-32%) [6, 7]). The zeins and glutelins in the endosperm form the connective tissue of the starch granules.

A trial connected to the present paper[12] showed that Symbiotic corn in a total mixed ration was very appreciated by dairy cows and buffered the first critical part of lactation, thereby leading to healthy milk (-16% in milk amyloid A; P 0.049) with better coagulation and cheesemaking properties. The effect of mycorrhized maize on the milk quality and properties reflected a

Table 7. Pearson correlations of the Kernel fingerprint for the Control (K_CC) and Symbiotic (K_SS) types and of the size-effect on Yield [Ln (Yield_S / Yield_C)] with the phenotypic measurements on the soil, plant and kernel, from the 26 pairwise comparisons.

	Main variables					
	Kernel				Yield	
Co-variables	K_CC	P	K_SS	P	Ln (Yield_S / Yield_C)	P
Soil Induced Respiration	-0.46	*	-0.37		-0.53	**
Foliar pH C	-0.54	**	-0.50	**	-0.38	
Foliar pH S	-0.77	**	-0.71	**	-0.75	**
Foliar pH ln(S/C)	-0.47	*	-0.44	*	-0.64	**
NIRS foliar fingerprint ln(S/C)	0.35		0.42	*	0.27	
Foliar protein ln(S/C)	0.60	**	0.57	**	0.45	*
Yield ln(S/C)	0.69	**	0.55	**	1.00	
Kernel protein C	-0.62	**	-0.57	**	-0.62	**
Kernel protein S	-0.54	**	-0.49	**	-0.58	**
Kernel fat C	0.58	**	0.59	**	0.44	*
Kernel fat S	0.61	**	0.61	**	0.54	**
Kernel fiber C	-0.42	*	-0.25		-0.29	
Kernel fiber S	-0.45	*	-0.43	*	-0.38	
Kernel starch S	0.41	*	0.48	*	0.39	*
NIRS Kernel fingerprint C (K_CC)	1.00		1.00		0.69	**
NIRS Kernel fingerprint S (K_SS)	1.00		1.00		0.55	**

P: * <0.05; ** <0.01

Table 8. Classification of the response in yield from 1388 BF treated kernel spectra.

Predicted	Positive	10%	89%
	Negative	90%	11%
		Negative	Positive
		Measured	

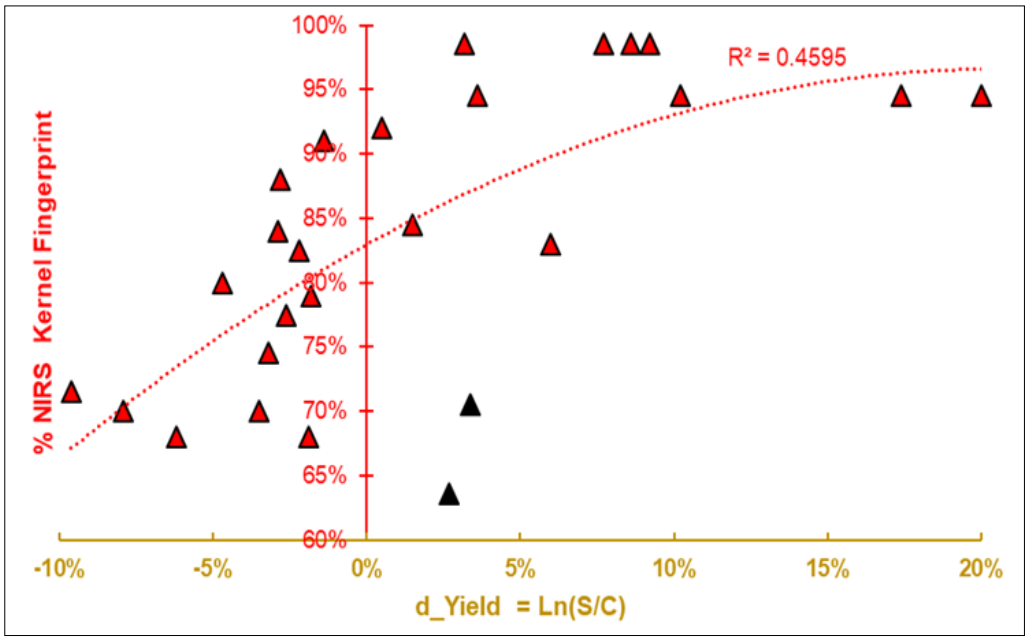

Figure 6. Regression of the average NIR spectral fingerprint of the Kernels on the size-effect of yield [d_Yield = Ln(S/C)]. Two presumed outliers are in black.

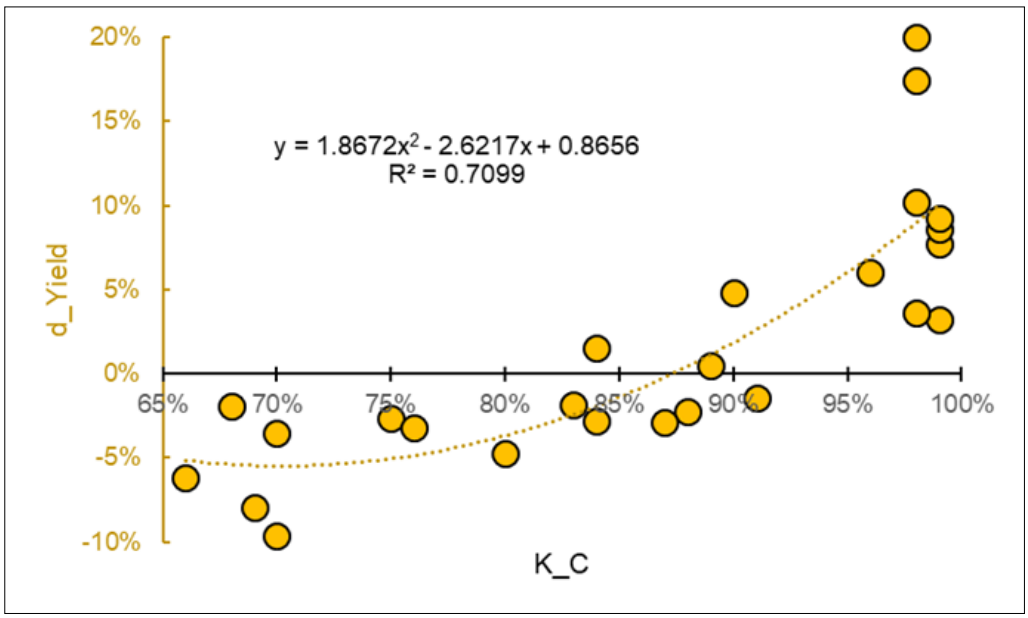

Figure 7. Prediction of the size-effect of yield on [d_Yield = Ln(S/C)] from the average NIR spectral fingerprint of the Control Kernels. Two outliers have been removed.

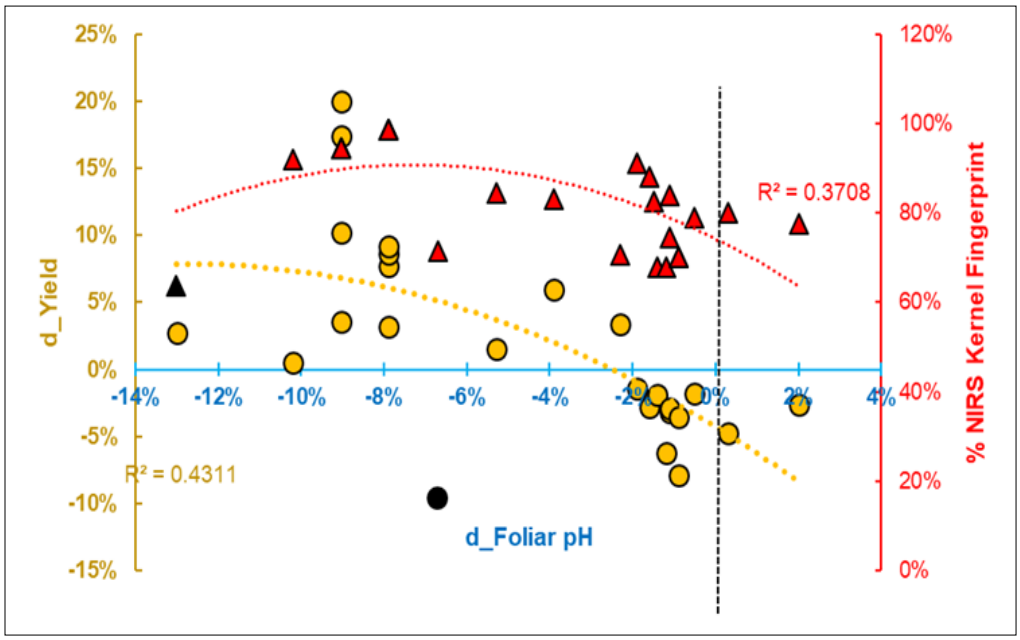

Figure 8. Regression of the NIR spectral fingerprint of the Kernels and the size-effect of yield [d_Yield = Ln(S/C)] on the foliar pH. Two presumed outliers are in black.

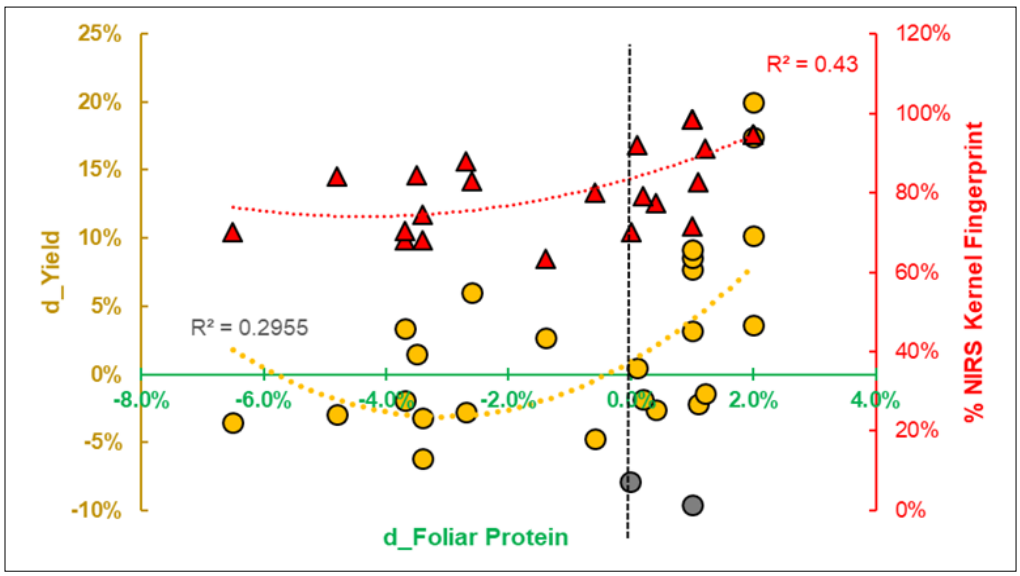

Figure 9. Regression of the NIR spectral fingerprint of the Kernels and the size-effect of yield [(d_Yield = Ln(S/C)] on the foliar protein. Two presumed outliers are in gray.

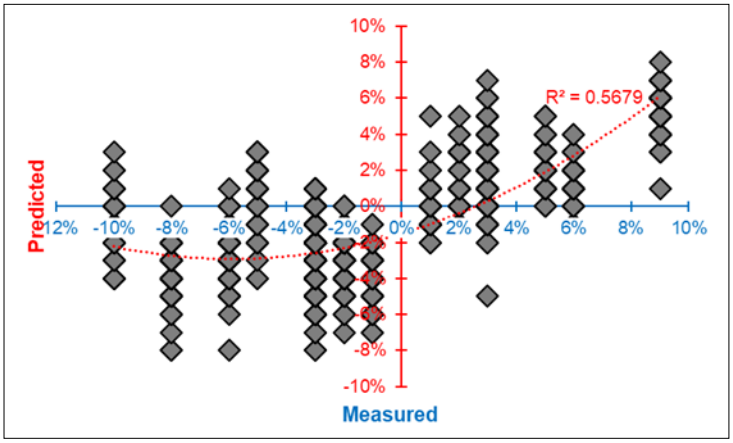

Figure 10. Plot of the measured yield response [d_Yield = ln(S/C)] *vs.* the one predicted from the NIR spectra (No. 1388).

positive effect on the overall condition of the animals, as confirmed by a higher dry matter intake (+11% P 0.003). The AA. suggest that the treatment could affect some intrinsic characteristics of the maize and ration, such as palatability and degradability.

In a previous analogous trial [20], the milk yield was the same in the two groups, but the Symbiotic group showed an increased milk protein content. This was likely due to a higher dry matter intake (22.35 *vs.* 21.11 kg d^{-1}, +6%, P 0.015) for the S group, which also showed a tendency to have a higher ADG (272.21 vs. 124.72 g d^{-1}). Further investigations are needed in order to clarify the degradability of the S diet. The protozoa count in the rumen were significantly higher in the S diet (+15.6%, P <0.05), and the total bacteria behaved accordingly (6.91 vs. 6.19 log10 g^{-1} DM, P <0.01).

The shelf life of corn mainly depends on the kernel shell, and after eighteen months storage for broilers, bio-fertilized corn was found to have totally preserved its properties [11], Symbiotic corn had maintained its nutritive properties, while the Control had lost about the 26% of its feeding value.

Single kernel NIR reflectance and transmittance technologies have been developed over the last two decades to establish the physical quality and chemical traits of a range of cereal grains as well as to detect and predict the levels of toxins produced by fungi [21, 22].

The handheld NIR instrument used in this experiment has furnished an excellent kernel discrimination of the conventional (Control) *vs.* Symbiotic sources. In fact, 20 seeds were enough to have a 95% chance of appropriately assigning the category, thus confirming previous results [23] pertaining to the detection of falsified medicines. The instrument is also suitable for practical applications, as shown by its capacity to discriminate frozen milk samples originated from either grass-fed or from conventional fed cows [24] as well as to assess oxidative stress from a simple NIR-tomoscopy of the ear of rabbit does [25].

Conclusion

The main conclusion concerns the advances in knowledges from testing the responses to Biofertilizers with different Arbuscular Mycorrhiza sources in several specific maize cultivars. For this purpose, from a simple NIR SCiO scan of 20 treated and 20 untreated kernels, it was be plausible to testify the symbiotic origin of a corn from specific cultivar at 95% certainty and to argue about its agronomic traceability and sustainability.

A corollary conclusion of this work is that further studies are needed to establish the nature of symbiotic modifications in kernels. Designing the ideotype mycorrhizal symbionts to produce healthy food[26] capitalizing AM effects on the biosynthesis of plant secondary metabolites with health-promoting activity, may boost sustainable biotechnological tools to produce safe and healthy plant foods. Secondary compounds of

maize kernel have beneficial effects on animal feeding, and who knows how many other hitherto unknown quality properties?

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