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In vivo Stem pH can Testify the Acidification of the Maize Treated by Mycorrhizal and Microbial Consortium

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Abstract: The *in vivo* acidic status of the crops treated by a microbial fertilization is unknown. This study aims to clarify if a significant effect of the H^+ asset will appear on the maize plants at juvenile and at waxy stages. Two experiments were conducted. In trial-I, control (C) juvenile unearthed plants were compared with plants treated with a commercial mycorrhizal and microbial mixture (Micosat F: Myc) in root and stalk positions. Importantly, the H^+ concentration in the root was ten-fold less acidic than in the stems, but the H^+ concentration in the Myc treated plants was increased by 216% ($P < 0.0004$). In trial-II, 14 control (C) plots were compared at waxy stage to 14 Myc plots; each plant was cut at the internode below the ear, and the *in vivo* pH measurement was executed at high height (pH1); a second cut was then made at the 2nd internode and the *in vivo* pH was registered at low eight (pH2). The pH2 in the Myc treated plants significantly dropped by -1.6% to –4.3%, consequently H^+ concentration increased by +17% to +35%. A significant rise in the green mass yields of the stalk was observed in the Myc groups by +14% ($P < 0.02$). A Myc treatment caused a kind of botanical modification in the state of hydrogen ions of green maize, reduced with the plant height from roots to ears, which could explain the improved stalk yield.

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1. Introduction

Mycorrhizal associations between a fungus and a plant root are ubiquitous in the natural environment (Hodge, 2000). Arbuscular Mycorrhizal (AM) symbiosis on the crop plants resulted an average yield increase of about 26% (Beauregard et al., 2008). According to Celebi et al. (2010), AM inoculation increases maize silage yield, not only under well watered condition, but also under water deficient conditions. A great deal of attention has been paid to the so called helper bacteria (Kannan et al., 2011; Zhang et al., 2014), which are usually combined with AM in commercial applications, and the effects on the final quality of maize grain have been corroborated. As well as noting significant maize grain yield increments, Berta et al. (2013) also discerned the relative action exercised by *Pseudomonad* bacteria, which increase labile starch proportion, due to the relative action exercised by AM fungi, which in turn promote a more complicated protein structure, with an increase in the undegradable prolamine (zein); interestingly, the combination of AM and bacteria has been shown to be able to improve starch digestibility by 7% in spite of a rise of 22% in the zein content, which is concentrated in the endosperm reticulum. In fact, dry corn with a greater percentage of vitreous endosperm has been demonstrated to affect negatively the starch digestibility and the milk yield of lactating dairy cows (Owens et al., 1986).

In modern wheat, the modification determined by Micosat F treatment has sublimated the sensorial and functional properties of whole bread, which has appeared similar to some ancient types of bread (Torri et al., 2013). In a pioneer effort, Mulder et al. (2013) analyzed the green and brown worlds, paying particular attention to the carbon, nitrogen and phosphorus related traits; in their model, AM are the driver of a conservative mode, which is characterized by low-acidic soil pH, and low inputs, and not of an exploitative mode, which is driven by plant control and features high-mesic soil pH and high inputs. A new soil ecology problem in agroecosystems could involve the interactions between Bt transgenic crops and AM (Liu and Lianfeng, 2008; Flores et al., 2005), which could lead to an unwelcome surprise with regard the specific abundance and diversity of AM when Bt transgenic crops are planted year after year. Cheeke et al. (2012) found that *Bt* maize had lower levels of AMF colonization in their heavier roots than

the non- *Bt* P parental lines. However, reductions in AMF colonization surprisingly increased the number of ears, and showed parasitic relationships instead of a symbiotic one.

The drought resistance of crops treated with AM and/or microbial consortia is a prominent feature of such sustainable *blue technology* (Beltrano and Ronco, 2008; Malusà et al., 2013).

Mycorrhizal symbiosis was often been related to soil pH (Laheurte et al., 1990; Rousk et al., 2009); some studies have been conducted on roots and/or seedlings using microelectrodes (Plassard et al., 1999; Gollany and Schumacher 1993), NMR (Philip et al., 2004), on detached maize leaves (Bogoslavsky and Neumann, 1998), or cowpea and rose leaves (Green et al., 1998).

However, no study involved the *in vivo* pH in maize stems in field crops. The aim of this work was to assess whether a specific type of botanical modification could be promoted in the protonic anabolism of green maize through mycorrhizal and microbial fertilization, and whether this result could be testified by a simple in field operation, such as an *in vivo* pH measurement. In particular the study aims to clarify if a significant modifying effect of the H^+ asset will appear on the maize plants at juvenile and at waxy stages, featuring an axial degradient.

2. Material and Methods

2.1 Microbial treatment

At sowing the treated maize groups received, a commercial mycorrhizal and microbial consortium Micosat F by CCS s.r.l. Aosta (www.micosat.it) composed of: symbiotic fungi, 32 g of milled mychorrized sorghum roots, spores and ifae of *Glomus coronatus* GO01 and GU53, *Glomus caledonium* GM24, *Glomus intraradices* GB67 and GG32, *Glomus mosseae* GP11 and GC11, *Glomus viscosum* GC41, all of which are able to penetrate the host roots in minimal 30 %; Saprophytic fungi: *Beauveria spp.* BB48, *Trichoderma harzianum* TH01, *Trichoderma atroviride* TA28; bacteria of the rhizosphere: *Bacillus subtilis* BR48; minimal $4.25x10^8$ C.F.U. g^{-1} (of which 0.8% *Thricoderma spp.* with minimum $3x10^6$ C.F.U.g^{-1}). The fertilizer dosage 10 kg ha $^{-1}$ was equivalent to 1 g m^{-2}, and was distributed using a microgranulator which positioned the granules close to the seeds. A central control (C) zone was left untreated in each field, and the crop was compared with the Micosat F (Myc) treated plot.

2.2 Field Trials

Four fields were used. Three of the varieties were cultivated in full cycle, sown in April 2013 examined at the end of September and harvested in early October, while one (FAO 550- 127 days) was cultivated as a second cycle crop, after the harvesting of *Lolium multiflorum* in field #2; in this field, after 44 days from sowing, when the 8-9 leaf stage was reached the Trial-I (Table 1) was carried out involving 15 C plants and 15 Myc plants extracted, together with their roots.

The Trial-II (Table 1) featured four fields, examined at the waxy maturity stage, some days before harvesting for silage purposes. Four random replicates of 5 m were examined for each field comparison C vs. Myc group; the intercrop was 75 cm thus each replicate plot included 3.75 m^2.

Table 1. Experimental field setting and results on plots of five meter long in Trial-I [A] and Trial-II.

Field	Treatment	Maize Hybrid	Crop Cycle FAO Class	Trial I [A]	Days at test	Plants N m^{-2}	Replicates (Total plots 28)	Ears kg m^{-2}	Stalks kg m^{-2}	Ears / Stalk Ratio	High Height pH1	Low Height pH2
1-P	C-control	DK	1th cycle	.	128	6.8	2	11.5	11.5	1.0	4.96	5.11
	M-Myc	C6815	600	.	128	6.8	2	13.9	9.4	1.5	4.83	5.12
2-C [B]	C-control	KWS [B]	2nd cycle	15 [B]	113	6.8	4	7.8	7.0	1.1	4.75	4.68
	M-Myc	KONSES	550 - 127 d	15 [B]	113	6.8	4	9.3	8.3	1.1	4.63	4.48
3-M	C-control	LG	1th cycle	.	130	8.3	4	17.4	9.2	1.9	4.87	4.74
	M-Myc	30.681	600	.	130	8.3	4	19.4	9.5	2.0	4.90	4.53
4-G	C-control	Pioneer	1th cycle	.	138	7.2	4	13.1	7.4	1.8	4.81	5.11
	M-Myc	R31A34	700	.	138	7.2	4	15.5	8.5	1.8	4.51	5.11
Mean						.		13.0	8.2	1.6	4.84	4.92

[A] Trial I, days at test = 44; [B] N of plants from second cycle crops, after harvesting *Lolium multiflorum*.

The green mass yields of the epigeal parts were established by separating the stalk (leaves and stem) from the raw intact ears. Each plant was cut at 2/3 of the internode below the ear, and the *in vivo* stem pH1 measurement was made at height high (H1). A second cut was then made at ½ of the 2nd internode and the *in vivo* stem pH2 at eight low (H2) was recorded.

2.3 *In vivo* stem pH

The pH measurements were conducted using a BORMAC "XSpH 70" pH meter (www.giorgiobormac.com), provided with a combined plastic-glass electrode PEEK Double-PoreF, dimensions (LxØ) mm 35×6, specific for meat, cheese and penetration measurements, range pH 0÷14, two decimals. All the *in vivo* measurements were conducted at the center of the cut sections. In Trial-I, the root pH was measured in the center and also at the crown of the collar, perpendicular to the cut that separated the epigeal and the hypogeal parts, while the pH of the stems was measured at internodes 1 and 5, by gently piercing the exterior of the soft stem.

2.4 Statistical analysis

A series of MIXED models (SAS/STAT® 9.2. SAS Inst. Inc., Cary, NC) was used to test the Myc, Fields (F) at the two height conditions, considering the block within field (B) replicate as random. The mixed model [1] considered the pH and H^+ concentrations, and their differences, as separate variables of the two height levels:

$$Y_{ijk} = M + Myc_i + B(F)_{ij} + E_{ijk} \quad [1]$$

where: Y = the *in vivo* pH, or the H^+ concentration, at H1 or at H2 height, or the within plant pH excursion from low to height high s [Δ(pH1-pH2)] at the j^{th} Myc condition, of the k^{th} Block within the field; M = common average; Myc = effect of the Micosat F treatment; B = random effect of the Block (1-4) in the Field replicate; E_{ijk} = error term. A similar GLM fixed effect model was used to analyze the results of the 30 plants from Trial-I.

2.5 Green yields

The results of the weight measurements of the green epigeal parts over each five meter replicate, pertinent to experimental groups C and Myc (No. 28) were expressed as kg m^{-2}. A paired Friedman test was then executed using StaBox v. 1.5, Grimmer Logiciels, Paris, which considered pairing between the fields and the progressive replicates.

3. Results

3.2 *In vivo* stem pH and H^+ concentration

In Trial-I (Table 2), at a first examination of immature plants the pH values were both more acidic in the Myc group, at H2 (pH2 -3%) and at H1 (-4%). Importantly, the pH of the root was much less acidic than that of the stems, but appeared strongly acidified in the Myc plants with -7% and -6% *vs.* the control plants, in the center and in the crown, respectively. As can be seen in Figure 1, at an H^+ scale, the acidic prevalence of the Myc plants at the 9th leaf stage was maximum at the root (+216%), but reduced at the internode levels (+54% and +69%). A similar pattern at the two heights will be revealed at the waxy stage, but with a ten-fold increased acidity level (Table 3).

In the Trial-II main result showed that at the base of the plant the pH2 was particularly significant, because of a concordant response to the Myc treatment in all four fields; at that baseline, the Myc treated plants were highly significantly acidified in all fields, with pH drops from -1.6% to –4.3%. As a consequence the H^+ concentration was increased from 17% to 35%, a highly significant increase in 3 fields.

Table 2. Fixed effect model analysis of the *in vivo* root and stem pH measurements, for the Control (C) and the Mycorrhizal and microbial consortium Micosat F treated (Myc) young maize plants from Field #2, in high (H1) and low (H2) stem height positions. Trial-I (N =30 plants).

			R^2	RMSE	C-Control	M-Myc	*Prob*	M/C (%)
Stem	pH	pH1-(high)	0.36	5.49	5.61	5.38	*0.0005*	-4%
		pH2-(low)	0.17	5.63	5.72	5.53	*0.0217*	-3%
		Δ(pH1 – pH2)	0.01	-0.13	-0.11	-0.15	*0.5536*	35%
	H^+	$H1^+$ High	0.29	3.57	2.54	4.60	*0.002*	81%
	mol/l	$H2^+$ Low	0.17	2.72	2.06	3.38	*0.0234*	64%
		Δ($H1^+$ - $H2^+$)	0.05	-0.85	-0.48	-1.22	*0.2158*	153%
Root	pH	pH Center	0.46	5.88	6.10	5.67	*<.0001*	-7%
		pH Crown	0.38	5.78	5.97	5.59	*0.0003*	-6%
	H^+	H^+ Center	0.36	1.76	0.85	2.67	*0.0004*	216%
	mol/l	H^+ Crown	0.31	2.19	1.17	3.20	*0.0013*	173%

At the higher level of the plant, the pH1 significantly decreased in one field (-1.7%), where the H^+ increased by 23%. In general two different patterns of the axial acidity gradient were found; in fact it was positive in two field (2, 3) either negative in other two fields (1, 4), and the Myc factor appeared as significantly interacting in the fields 3 *vs.* 4. The pH3 and H^+3 showed significant acidification effects of the Myc treatment in 75% of the fields.

3.2 Green yields

A significant growth effect was apparent for the Myc factor on the stalk weight (Table 4); the improvement reached 14% ($P< 0.02$). The observed 12% increase in the ear was not significant ($P<0.20$), while the total green mass yield (+ 12%) was nearly significant ($P < 0.07$).

4. Discussion

About 40 years ago, hydrogen ion was proposed as a wall-loosening factor. This idea, and the subsequent supporting data, gave rise to the Acid Growth Theory, which states that when exposed to auxin, susceptible cells excrete protons into the wall (apoplast) at an enhanced rate, and this results in a decrease in apoplastic pH (David, Rayle and Cleland, 1992). McCue et al. (2000) have hypothesized that acid-induced cell growth and elongation is regulated through the pentose-phosphate pathway, which produces the critical precursors for the synthesis of phenolic secondary metabolites that are important for plant growth and lignification.

Table 3. Mixed model [1] analysis of the *in vivo* stem pH and H^+ measurements, for the Control (C) and the Mycorrhizal and microbial consortium Micosat F treated (M-Myc) maize plants, examined at a height high (H1) (under spike internode) and at a low height (H2) (2^{nd} internode) position, for each field in Trial-II.

Variable	Field	N	RMSE	C- Control	M-Myc	*Prob*	Myc/C %
pH1	1	123	0.043	4.85	4.74	*0.0643*	-2.4%
	2	203	0.041	4.74	4.66	*0.0144*	-1.7%
	3	242	0.023	4.87	4.90	*0.1001*	0.7%
	4	188	0.047	4.83	4.86	*0.3577*	0.6%
. pH2	1	123	0.043	5.07	4.88	*0.0026*	-3.7%
	2	203	0.081	4.67	4.53	*0.0046*	-2.8%
	3	242	0.080	4.74	4.53	*<.0001*	-4.3%
	4	188	0.024	5.14	5.06	*0.0006*	-1.6%
. pH1-pH2	1	123	0.069	-0.22	-0.15	*0.3423*	-33.7%
Δ pH	2	203	0.098	0.07	0.12	*0.314*	69.7%
	3	242	0.093	0.13	0.37	*<.0001*	179.2%
	4	188	0.062	-0.31	-0.20	*0.0035*	-35.8%
H^+1	1	123	60.82	16.30	19.87	*0.1232*	21.9%
mol/l	2	203	137.56	20.05	24.74	*0.0138*	23.4%
	3	242	24.78	14.40	13.23	*0.0692*	-8.1%
	4	188	103.83	16.94	15.60	*0.3783*	-7.9%
H^+2	1	123	27.28	10.94	13.43	*0.1098*	22.7%
mol/l	2	203	357.14	26.05	35.26	*0.0028*	35.4%
	3	242	305.38	22.95	34.93	*<.0001*	52.2%
	4	188	6.64	7.74	9.05	*0.0008*	16.9%
H^+1-H^+2	1	123	83.58	5.37	6.45	*0.6899*	20.1%
Δ H	2	203	439.37	-6.01	-10.53	*0.1821*	75.2%
mol/l	3	242	313.39	-8.55	-21.69	*<.0001*	153.7%
	4	188	109.30	9.19	6.53	*0.0907*	-28.9%
pH3 = pH1+pH2	1	123	0.103	9.92	9.62	*0.0018*	-3.1%
. pH sum	2	203	0.145	9.40	9.19	*0.0007*	-2.3%
	3	242	0.114	9.61	9.44	*0.0001*	-1.8%
	4	188	0.079	9.97	9.92	*0.2329*	-0.5%
$H^+3=H^+1 + H^+2$	1	123	92.68	27.25	33.31	*0.0353*	22.2%
H^+ sum	2	203	550.34	46.12	60.02	*0.0003*	30.1%
mol/l	3	242	347.17	37.37	48.17	*<.0001*	28.9%
	4	188	111.68	24.70	24.68	*0.9872*	-0.1%

Table 4. Trial-II (N =28 plots). Average plot weights of the epigeal yields and significance established according to a Friedman's paired test, comparing the control (C) groups with the Mycorrhizal and microbial consortium Micosat F treated (M-Myc) groups.

N plots	Unit	Part of the plant	C-Control	M-Myc	M/C	*Prob*
		Ear	2.59±0.13	2.84±0.14	10%	*0.20*
28	Kg m^{-2}	Stalk	4.33±0.34	4.93±0.38	14%	*0.02*
		Yield	6.91±0.45	7.77±0.48	12%	*0.07*

A maize stem functions alternately as a net importing and net exporting organ during ontogeny, depending on the whole plant photosynthetic source and sink status. According to Setter and Meller (1984), the [^{l4}C]sucrose and [^{l4}C]glucose uptake capacity of stem tissues is sensitive to inhibitors and temperature, and is increased slightly by acidic pH. Low pH is known to promote root elongation and high pH to inhibit it, and the results of Evans et al. (1980) showed that hormone-induced modifications of cell-wall pH play a role in the modulation of stems and coleoptiles. An enhancement in activity of H^+-ATPase, measured in microsomal fractions of mycorrhizal plants, has been reported by Benabdellah et al. (1999) and by Gianinazzi-Pearson et al. (2000), and this enhancement might be related to energizing transport processes at the periarbuscular membrane (Rosewarne et al., 2007). In a recent study on strawberry (Bona et al., 2015), PGP *Pseudomonad* and AM have revealed additive patterns for fruit weight (+21%), fruit dry matter (+54%) and sucrose content (+54%), while acidity was only increased through their co-inoculation, namely -6% as pH, which corresponded to +59% as H^+, a value which collimates, in a different mature organ, with the Acid Growth Theory.

According to Bogoslavsky and Neumann (1998), a more acidic internal condition (pH 4.5 *vs.* 5.5) is beneficial, in water stressed maize leaves, for an increase in wall extensibility (i.e. wall loosening), which can then facilitate the acceleration of leaf growth; conversely, pH 5.5 buffers appear to inhibit increases in wall acidification and loosening, thus preventing the usual water induced acceleration of leaf growth.

The changes in the pH of xylem sap that are commonly observed under drought stress can be an important component of root to shoot signaling, and may act synergistically with ABA (Wilkinson and Davies, 2002; Schachtman and Goodger, 2008). In the present in-field experiments, an accurate supply of water has been adopted against water stress, and the treated and untreated plants have grown next to each other during a good season, with careful irrigation management.

Thus, the significant increases in green stalk weight of 14%, a value in the ranges signaled by cited references (Bauregard et al., 2008; Celebi et al., 2010), depended significantly on the Myc factor, and can only be explained by an overabundance of protons and H^+ in the whole body of the stem reservoir system. This relative abundance of protons appears even greater in the roots of the treated Myc and remains to the base of the plant, as a characteristic acidic pH. The difference in acidity between the Myc and the untreated plants in the upper part can disappear, and this aspect seems to decrease in the 2nd cycle of the crops. In the present test the effect appeared at the more juvenile 9th leaf stage (Trial-I), and was confirmed, in the same field with a 2nd cycle maize, at the waxy stage during the *in vivo* pH performance recording (Trial-II).

On the basis of these preliminary results, it could be hypothesized that the Myc factor in maize ontogeny could interact with the intrinsic aptitudes, but also with the nutrient availability. According Hodge and Storer (2014) AMF contribution to plant N, and to the mass increase, varies widely, but the reasons for this variability are unclear. In low N systems even small amounts of 'extra' N may confer the plant with a competitive advantage, but it is also likely that competition for N between symbionts occurs. In real conditions of the present in-field Trials, the N levels were not low, but the yield response may be more depending on the yearly-soil rhizosphere conditions.

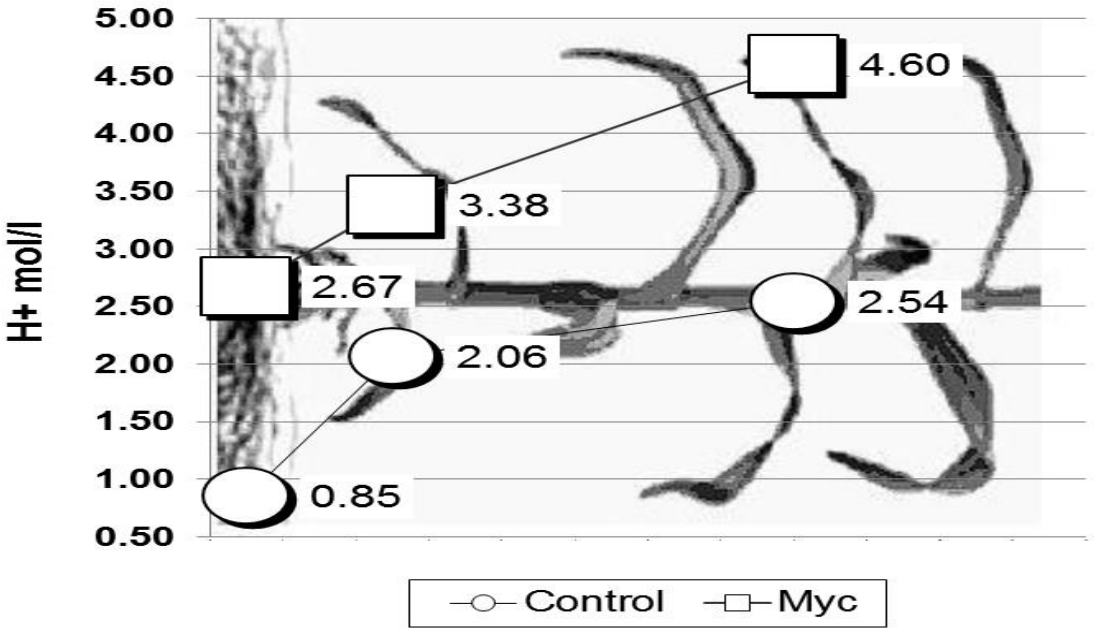

Figure 1. Pattern of the H^+ concentration (in mol/l) in Trial-I (Field 3), for the control (○) and for the Myc (□) at the 9th leaf stage, with prevalence of Myc / control, respectively at root (+214%), at low height (+64%) and at high height (+85%).

Electrolyte leakage, according to Beltrano and Ronco (2008), is negatively associated with pH levels; pH and electrical conductivity are non-linearly correlated dimensions. Electrophysiological studies in root cells have shown an H^+ pump activity in mycorrhizal *Allium porrum* (Fieschi et al., 1992); the electrical potential of the cells was much more negative in mycorrhizal roots than in non-mycorrhizal ones, thus indicating an increased H^+ pump activity in the mycorrhizal roots. Cell wall ontogeny in mycorrhizal symbiosis has been upgraded through genomic and transcriptomic approaches by Balestrini and Bonfante (2014), thus opening a scenario characterized by a broad adaptation of the Primary Cell Wall (PCW) related genes.

In an evolutionary scenario Sabia et al. (2015) have clearly shown that Micosat F treatment is a delaying agents of barley ontogeny as regard to cell wall constituents and digestibility properties. But as regard to the protein complexity increase and the lipid maturity trend, the ontogeny process may be accelerated.

In the wake of the results achieved in maize, giving evidence about variation of the *in vivo* pH, recently Masoero et al. (2015) studied the acidity effects from mycorrhization of vine plants. The results fully confirm the lowering of pH petiole, from 4.55 in the witness to 4.35 in the Mycorrhized (-4%) which corresponded to a strong increase of hydrogen ions, around +81%. From this first research emerges a profound significance of pH in relation to the defense of the plants by bacterial attacks. In fact a positive link was observed between the *in vivo* pH and the susceptibility of different cultivars to the golden Flavescence. Moreover the affected plants showed a petiole pH increase, offering improved livability conditions for the phytoplasmas.

5. Conclusion

It has been concluded that Myc treatment leads to a kind of botanical modification of the protonic anabolism of green maize, which could explain the improved mass yield and clarify the basis of its proven resistance to drought. Great care must be taken in the comparison of yields at different maturity degrees. Moreover, the co-evolution of the starch and of the protein of cereal grains may be complicated by different effects induced by the Mycorrhizal and by the associated microbial bio-fertilizers. The assessment of the acidic status, by means of two *in vivo* pH measurements, may be highly indicative of the effective realization of a Mycorrhizal and microbial fertilization disclosing quantitative and qualitative modifications of the maize crops.

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Competing Interests

The Authors declare that they have no competing interests regarding contents of this paper.

References

Balestrini, R. and P. Bonfante. 2014. Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. Front. Plant Sci. 5:237:1-10.

Beauregard, M.S., C. Hameland and M.S.T. Arnaud. 2008. Arbuscular mycorrhizal fungi communities in major intensive North American grain productions. In 'Mycorrhizae: sustainable agriculture and forestry' (Eds Z.A. Siddiqui, M.S. Akhtar, K. Futai) Springer-Verlag: Berlin, Heidelberg. p. 135-139.

Beltrano, J. and M.G. Ronco. 2008. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and re-watering by the arbuscular mycorrhizal fungus *Glomus claroideum*: Effect on growth and cell membrane stability. Brazilian J. Plant Physiol. 20:29-37.

Benabdellah, K., C. Azcón-Aguilar and N. Ferrol. 1999. Plasma membrane ATPase and H^+ transport activities in microsomal membranes from mycorrhizal tomato roots. J. Exp. Bot. 50:1343-1349.

Berta, G., A. Copetta, E. Gamalero, E. Bona, P. Cesaro, A. Scarafoni, G. D'Agostino. 2013. Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. Mycorrhiza. 24:161-170.

Bogoslavsky, L. and P.M. Neumann. 1998. Rapid regulation by acid pH of cell wall adjustment and leaf growth in maize plants responding to reversal of water stress. Plant Physiol. 118:701-709.

Bona E., G. Lingua, P. Manassero, S. Cantamessa, F. Marsano, V. Todeschini, A. Copetta, G. D'Agostino, N. Massa, L. Avidano, et al. 2015. AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. Mycorrhiza. 25(3):181-193.

Celebi, S.Z., S. Demir, R. Celebi, E.D. Durak, I.H. Yilmaz. 2010. The effect of Arbuscular Mycorrhizal Fungi (AMF) applications on the silage maize (*Zea mays* L) yield in different irrigation regimes. Eur. J. Soil Biol. 46:302-305.

Cheeke, T.E., T.N. Rosenstiel and M.B. Cruzen. 2012. Evidence of reduced Arbuscular Mycorrhizal fungal colonization in multiple lines of *bt* maize. Am. J. Bot. 99:700-707.

David L., D.L. Rayle and R.E. Cleland R.E. 1992. The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol. 99:1271-1274

Evans, M.L., T.J. Mulkey and V.J. Vesper. 1980. Auxin action on proton influx in corn roots and its correlation with growth. Planta. 148:510-512.

Fieschi, M., G. Alloati, S. Sacco and G. Berta. 1992. Potential hyperpolarization in vesicular arbuscular mycorrhizae of *Allium porrum* L: a non-nutritional long-distance effect of the fungus. Protoplasma. 168:136-140.

Flores, S., D. Saxena and G. Stotzky. 2005. Transgenic *Bt* plants decompose less in soil than non-Bt plants. Soil Biol. Biochem. 37:1073-1082.

Gianinazzi-Pearson, V., S.E. Smith, S. Gianinazzi and F.A. Smith. 1991. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces? New Phytol. 117:61-74.

Gollany, H.T. and T.E. Schumacher.1993. Combined use of colorimetric and microelectrode methods for evaluating rhizosphere pH. Plant Soil. 154:151-159.

Green, C.D., A. Stodola and R.M. Augé. 1998. Transpiration of detached leaves from mycorrhizal and non-mycorrhizal cowpea and rose plants given varying abscisic acid, pH, calcium, and phosphorus. Mycorrhiza. 8:93-99.

Hodge, A. 2000. Microbial ecology of the arbuscular mycorrhiza, FEMS Microbiol. Ecol. 32:91-96

Hodge, A. and K. Storer, 2014. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant Soil. 386:1-19.

Kannan, V.R., S. Suganya, E.K. Solomon, V. Balasubramanian, N. Ramesh and P. Rajesh. 2011. Analysis of interaction on between arbuscular mycorrhizal fungi and their helper bacteria by MILPA model. Res. Plant Biol. 1:48-62.

Laheurte, F., C. Leyval and J. Berthelin. 1990. Root exudates of maize, pine and beech seedlings influenced by mycorrhizal and bacterial inoculation. Symbiosis. 9:111-116.

Liu, W. and D. Lianfeng. 2008. Interactions between *Bt* transgenic crops and arbuscular mycorrhizal fungi: A new urgent issue of soil ecology in agroecosystems. Acta Agric. Scandinavica, Section B-Soil Plant Sci. 58:187-192.

Malusà, E., G. Sala, W. Chitarra and L. Bardi. 2013. Improvement of response to low water availability in maize plants inoculated with selected rhizospheric microbial consortia under different irrigation regimes. Int. J. Environ. Qual. 12: 13-21.

Masoero, G., G. Giovannetti, E. Bertero and A. Cugnetto. 2015. The *in vivo* pH of vine decreased with artificial mycorrhization and increased in golden Flavescence: preliminary results in Piedmont. OICCE Times OT Rivista di Enologia. 14(62):19-22 (In Italian with English abstract)

McCue, P., Z. Zheng, J.L. Pinkham and K. Shetty. 2000. A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. Process Biochem. 35(6): 603-613.

Mulder C., F.S. Ahrestani, M. Bahn, D.A. Bohan, M. Bonkowski, B.S. Griffiths, R.A. Guicharnaud, J. Kattge, P.H. Krogh, S. Lavorel, et al. 2013. Connecting the green and brown worlds: allometric and stoichiometric predictability of above- and below-ground network. Adv. Ecol. Res. 49: 69-175.

Owens, F.N., R.A. Zinn and Y.K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634-1648.

Philip, E., P.E. Pfeffer, Y. Shachar-Hill, S.I. Tu and D. Brauer. 2004. Measurement of intracellular pH in maize root tissue with a-methyl fluorinated alanines and *in vivo* 19F NMR spectroscopy. Physiol. Plantarum. 122:373-379.

Plassard, C., M. Meslem and G. Souche. 1999. Localization and quantification of net fluxes of H^+ along maize roots by combined use of pH-indicator dye video densitometry and H^+-selective microelectrodes. Plant Soil. 211:29-39.

Rosewarne, G.M., F.A. Smith, D.P. Schachtman and S.E. Smith. 2007. Localization of proton-ATPase genes

expressed in arbuscular mycorrhizal tomato plants. Mycorrhiza. 17:249-258.

Rousk, J., P.C. Brookes and E. Baath. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. App. Environ. Microbiol. 75:1589-1596.

Sabia, E., S. Claps, F. Napolitano, G. Annicchiarico, A. Bruno, R. Francaviglia, L. Sepe and R. Aleandri. 2015. *In vivo* digestibility of two different forage species inoculated with arbuscular mycorrhiza in Mediterranean red goats. Small Ruminant Res. 123:83-87.

Schachtman, D.P. and J.Q. Goodger. 2008. Chemical root to shoot signaling under drought. Trends Plant Sci. 13:281-287.

Setter, T.L. and V.H. Meller. 1984. Reserve carbohydrate in maize stem. [^{14}C]glucose and [^{14}C]sucrose uptake characteristics. Plant Physiol. 75:617-622.

Torri, T., P. Migliorini and G. Masoero. 2013. Sensory test vs. electronic nose and/or image analysis of whole bread produced with old and modern wheat varieties adjuvanted by means of the mycorrhizal factor. Food Res. Int. 54:1400-1408.

Wilkinson, S. and W.J. Davies. 2002 ABA-based chemical signaling: the co-ordination of responses to stress in plants. Plant, Cell Environ. 25:195-210.

Zhang, L., J. Fan, X. Ding, X. He, F. Zhang and G. Feng. 2014. Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. Soil Biol. Biochem. 74:177-183.