THE EFFECT OF A RELATIVELY LOW DOSE OF NANO-ZnO PARTICLES ON GROWTH, CHLOROPHYLL CONTENT, GRAIN YIELD, AND YIELD COMPONENTS IN A MEXICAN LANDRACE OF RED MAIZE

J. ESTRADA-URBINA[a], V. VILLALOBOS-GARCÍA[b], A. MÉNDEZ-ALBORES[c*], A. VÁZQUEZ-DURÁN[c*]
[a]*Facultad de Ciencias Agropecuarias y Ambientales, Universidad Autónoma de Guerrero, Iguala de la Independencia, Guerrero 40010, Mexico*
[b]*Centro de Enseñanza Agropecuaria, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, State of Mexico 54714, Mexico*
[c]*Unidad de Investigación Multidisciplinaria L14-A1 (Ciencia y Tecnología de Materiales), Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, State of Mexico 54714, Mexico*

A field-scale experiment was conducted to evaluate the effect of a relatively low dose of nano-ZnO particles (0.16 mg nano-ZnO per seed) on growth parameters, biomass production, photosynthetic pigments, cob components, grain yield, and yield attributes in a Mexican pigmented maize landrace. Seeds were coated with a starch paste containing nano-ZnO and controls comprised both uncoated and starch-coated seeds free of nano-ZnO. The highest plant height, plant stalk diameter, root length, number of secondary roots, and fresh and dry weights of shoots and roots were recorded at 60 d after sowing with the application of nano-ZnO. Also, a significant improvement in leaf chlorophyll concentration occurred as a result of applying nano-ZnO. Cob components, grain yield, and yield attributes were significantly improved by the nano-ZnO application. Furthermore, a significant increment in the FTIR primary active vibrations associated with the peptide-protein, lipids, and carbohydrate, and a high degree of organization at a short-range scale was observed on the outer regions of the starch granules in the F1 progeny of the nano-ZnO treatment. From these results, it can be concluded that seed treatment with a low dose of nano-ZnO particles is a cost-effective method for improving native maize production under rural conditions.

(Received Oct 2, 2019; Accepted December 11, 2019)

Keywords: Nano zinc oxide particles, Native maize seeds, Growth and yield components

1. Introduction

Maize (*Zea mays* L.) is one of the most versatile crops ranking third after wheat and rice in the globe [1]. In Mexico, there are at least 59 races of maize that can be undoubtedly recognized based on their biochemical and morphological characteristics [2]. From the nutritional, political-economic, and social perspectives, maize is the most important crop in the country, covering more than 7.6 million hectares, of which approximately 6 million hectares are cultivated with native landraces [3]. Several previous reports have indicated that native landraces of maize possess adaptive advantages over improved varieties (hybrids) when sown in limited edaphological lands. Such is the case of zinc (Zn), whose deficit in soils has been reported worldwide [4]. In Mexico, Zn deficiency is also a problem in more than 50% of the cultivable land, which leads to poor crop nutrition, reduced crop productivity, and a markedly deficiency of Zn in the Mexican population [5]. To ameliorate these negative effects, zinc sulfate ($ZnSO_4$) is commonly used as a fertilizer; however, this practice has an adverse impact upon the environment due to its irrational use.

Zn is one of the most important essential micronutrient required by plants. It plays a fundamental role in different metabolic processes, such as chlorophyll production, auxin synthesis, enzyme activation, and for cell membrane integrity maintenance [6]. Zn has also influence on biomass production, pollen functionalization, and seed germination because of its participation as

* Corresponding authors: albores@unam.mx

a cofactor on several different classes of enzymes [7]. It is well recognized that the main disadvantage of Zn compounds is their poor solubility; however, as the particle size is decreased —to the nanoscale level— the surface/volume ratio increases considerably, making it more soluble and bioavailable. In recent years, remarkable progresses in agriculture have been obtained by means of nanotechnology. Nowadays, there is an increasing research on the biological effects of zinc nanoparticles on maize under laboratory or greenhouse conditions [4, 7-12]. Currently, the available literature includes only one field experiment regarding the effects of zinc nanoparticles on growth, yield, and zinc content of maize kernels [12]. In the experiment, the authors used maize seeds of the hybrid variety DHM-117 and ZnO-nanoparticulates were applied by foliar spraying using concentrations from 50 to 2000 ppm. To the best of our knowledge, the use of relatively low doses of nano-ZnO to improve the growth, chlorophyll content, and yield components of pigmented native maize seeds has not yet been reported. To date, the unique study is from our research group in which the application of nano-ZnO improved the physiological and sanitary quality of a Mexican landrace of red maize under laboratory conditions [13]. Consequently, the aim of the present study was to evaluate the effect of a relatively low dose of nano-ZnO on growth parameters, chlorophyll content, and yield components of a Mexican landrace of red maize under field conditions.

2. Materials and Methods

2.1. Nano-ZnO particles

Following our previously reported methodology, ZnO nanoparticles were synthesized by an aqueous precipitation route using $Zn(NO_3)_2 \cdot 6H_2O$ and NaOH as precursors [13]. The main properties of the synthesized ZnO particles were: (i) nano-ZnO presented the characteristic absorption peak at 376 nm, (ii) the accurate value of the band gap was 3.17 eV, calculated by applying the Kubelka–Munk function, (iii) ZnO nanoparticles also showed all the characteristic fluorescence emission peaks at 422, 445, 485, and 527 nm, when exited at a wavelength of 325 nm, (iv) transmission electron microscopy images revealed that ZnO particles were quasi-spherical in shape, with diameters in the range of 30-125 nm, (v) the mean and mode particle sizes calculated from the nanoparticle tracking analysis (NTA) were 180 nm and 124 nm, respectively, and (vi) a distinctive band at around 391 cm^{-1} related to the Zn-O vibration mode confirmed the presence of pure nano-ZnO by means of Fourier transform infrared spectroscopy studies [13].

2.2. Maize seeds and seed conditioning with nano-ZnO

Seeds of native red maize (Tlalnepantla-0917) provided by the Peasant Producers of Seeds of the State of Mexico were utilized. The seed was mealy type, with a thousand-kernel weight of 311.5 ± 0.27 g and a test weight of 73.81 ± 0.51 kg/hL. The moisture content was around 14.3 ± 0.14% with a pH value of 6.03 ± 0.02. The seeds have an average germination rate of 97%, as shown by a preliminary standard germination study following the recommendations of the Association of Official Seed Analysts [14]. As described by Estrada-Urbina et al. [13], the seeds were coated with a starch paste containing nano-ZnO (0.16 mg ZnO nanoparticles per seed equivalent to 7.7×10^9 ZnO particles per seed). Controls included both uncoated and starch-coated maize seeds free of ZnO nanoparticles.

2.3. Site description

The field-scale experiment was carried out at the Experimental Agricultural Research Station (EARS) of the National Autonomous University of Mexico (19° 41’ 23.1” N, 99° 11’ 22.9” O) during the 2018 spring/summer season. The EARS has an elevation of 2256 m above the sea level, the climate is sub humid, the average temperature is 15.2 °C, and the annual rainfall is 612.1 mm. The main soil physicochemical properties were: clay-loam texture, 2.3% organic matter, pH 7.37 (1:2.5 soil/water), 0.16 dS/m electrical conductivity, 38.9 mg/kg total Zn, and 1.27 mg/kg DTPA-extractable Zn. The field was plowed by a four-wheel tractor (Ford 6600, 77 hp).

2.4. Crop husbandry

Three seeds were evenly planted every 0.5 m in 0.85-m rows under a randomized complete block design with three replicates. Sowing was done manually on April 19. The seeding density used in this experiment was 60,000 plants per hectare. A standard fertilization protocol for our local maize cropping practice was used. The fertilizers applied in the agricultural station were ammonium sulfate (21-00-00), and diammonium phosphate (18-46-00). No irrigation was used, and weeds were removed manually at days 40 and 60 after sowing.

2.5. Growth parameters and biomass production

Biomass was determined by sampling small plots consisting of 10 consecutive plants from the 3 central rows at 2 times (30 and 60 days after sowing). The plants were weighted (fresh weight), and 9 plants per treatment were selected and dissected into different sections according to the stage (leaves, stem, and ear). Dry matter was determined after drying at 80 °C for 72 h. Plant height, plant stalk diameter, root length, and number of secondary roots were also evaluated.

2.6. Determination of chlorophylls

At 60 d after sowing, chlorophylls were determined by measuring the absorbance of plant extracts in 96% ethanol, as described by Wintermans and De Mots [15]. Spectral analysis was done using a Cary 8454 UV-Vis Diode Array System spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll (Chl $a+b$) concentrations were determined using the following equations:

$$\text{Chl } a = 13.70\ (A_{665}) - 5.76\ (A_{649}) \tag{1}$$

$$\text{Chl } b = 25.80\ (A_{649}) - 7.60\ (A_{665}) \tag{2}$$

$$\text{Chl } a + b = 6.10\ (A_{665}) + 20.04\ (A_{649}) \tag{3}$$

2.7. Harvest (cob and yield characteristics)

At grain maturity, harvest was conducted manually for the 3 central rows by harvesting 10 m per row. Samples of each treatment were collected to register some cob characteristics such as: cob length, cob diameter, number of kernel rows, number of kernels per row, number of kernels per cob, and the grain-cob mass ratio (shelling factor). Yield characteristics were also evaluated including cob weight, test weight (weight of 100 kernels), and rachis weight. Finally, grain yield (Ton/ha) was calculated using the following equation.

$$Yd = MW \times HC \times \frac{100-GM}{86} \times SF \times \left(\frac{1000}{FW}\right) \tag{4}$$

where: Yd= Yield of grain (Kg/ha) standardized to 14% of grain moisture content; MW= Mean weight of harvested cobs (Kg); HC= Harvested cobs; GM= Grain moisture content (%); SF= Shelling factor (grain-cob mass ratio); FW= Furrow width.

2.8. Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR) studies

Maize samples (F1 progeny, 14% moisture content) were powdered in an electric plate-style mill type C-11-1 (Glen Mills, Clifton, NJ, USA) and sieved (60 mesh) to provide ground material with a particle size of < 250 µm. FTIR spectra of maize flours were acquired in a Frontier SP8000 spectrophotometer (Perkin Elmer, Waltham, MA, USA) accessorized with an in-compartment diamond ATR accessory (DuraSamplIR II, Smiths Detection, Warrington, UK). Samples of 25 mg were placed and measured in transmittance mode after pressing them on the ATR crystal. The spectra were recorded from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. The peak areas of the main bands were computed using Spectrum 10.4.2 software. Moreover, a section of the spectra (1060—960 cm^{-1}) was baseline corrected and the resultant FTIR spectrum was deconvoluted with three Gaussian functions. The ratio of areas corresponding to the 1047 cm^{-1} and 1022 cm^{-1} bands ($R_{1047/1022}$) was computed as a useful indicator of starch crystallinity [16].

2.9. Experimental design and statistical analysis

The experiment was arranged in a randomized complete block design with three replicates having a plot size of 8 × 20 m. Analysis of variance (ANOVA) was used to analyze the data with the Statistical Analysis System Software [17]. Tukey's multiple-range test ($p < 0.05$) was used to compare the significance among treatments. The experiment comprised 3 treatments: uncoated seeds (Uncoated), starch-coated seeds (Starch-coated), and seeds coated with a starch paste containing ZnO nanoparticles (Nano-ZnO).

3. Results and discussion

3.1. Growth parameters

Some growth parameters such as plant height, plant stalk diameter, root length, and number of secondary roots were measured at two growth stages of the maize crop (30 d and 60 d after sowing). In general, there were significant differences in growth parameters due to ZnO nanoparticles in both sampling periods. The highest plant height (218 cm), plant stalk diameter (32.4 cm), root length (29.6 cm), and number of secondary roots (40) was recorded at 60 d after sowing with the application of nano-ZnO (Fig. 1). In general, these plants were more vigorous when compared to Uncoated and Starch-coated treatments. Interestingly, Starch-coated treatment significantly improved plant height (33%) and root length (10%) at 60 d after sowing in comparison to Uncoated treatment (Fig. 1). In this context, Subbaiah et al. [12] reported that foliar application of 400 ppm of ZnO nanoparticles significantly increased plant height (35%) in the maize hybrid variety DHM-117 at 60 d after sowing. Moreover, in a pot culture experiment with the maize variety Zhengdan 958, Liu et al. [9] reported that after 8 weeks of growth, plant height increased significantly when used 100 or 200 mg ZnO nanoparticles/kg soil. Munir et al. [18] reported a significant increase in growth attributes in wheat using 100 mg/L ZnO nanoparticles in the priming solution. Data shown by these researchers are consistent with our results.

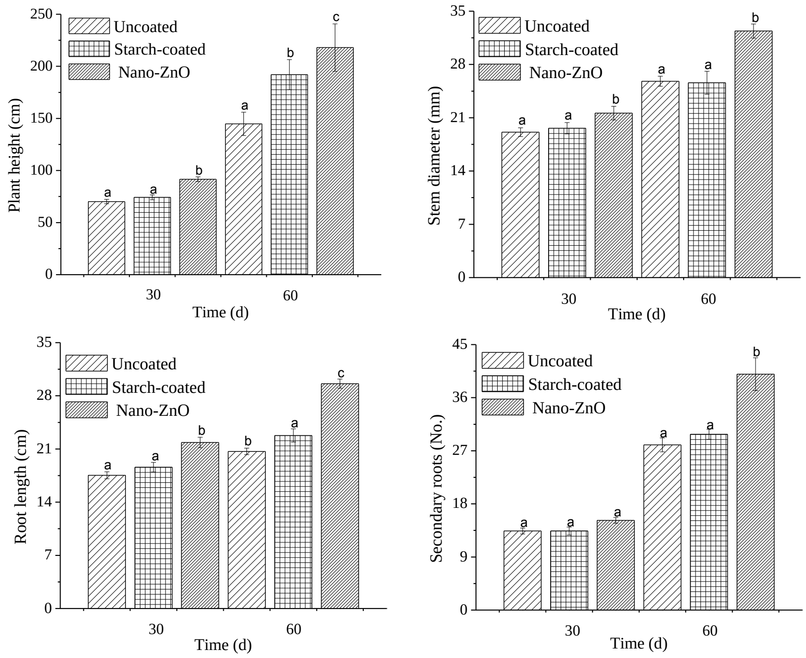

Fig. 1. Effect of a relatively low dose of nano ZnO particles on growth parameters in maize at 60 d after sowing. Mean of thirty plants per treatment ± standard error. In the same sampling date, means not sharing a common superscript differ significantly (Tukey test p < 0.05).

3.2. Plant biomass production

At day 30 after sowing, there were no significant differences in the fresh and dry weights of shoots and roots among treatments (Fig. 2). However, at day 60 after sowing, the fresh and dry weights of shoots of the Nano-ZnO treatment increased by 51% and 40%, respectively. The same trend was observed for fresh and dry weights of roots. At day 60 after sowing, the fresh weight of roots of the Nano-ZnO treatment increased by 54% in comparison to Uncoated treatment. Similarly, the dry weight of roots of the Nano-ZnO increased 71%. Interestingly, Starch coated treatment significantly improved fresh and dry weights of roots in comparison to Uncoated treatment (Fig. 2). In this context, Adhikari et al. [4] developed a protocol to coat seeds of maize (variety NMH-51) with nano scale ZnO at 25 and 50 mg Zn/g seed using ethyl alcohol and crude pine oleoresin as a binding agent. At 45 d after sowing (under greenhouse conditions), authors reported that the highest dry matter weight of shoots was recorded when seeds were coated with 50 mg Zn/g seed of nano ZnO (< 100 nm). Sharifi et al. [11] evaluated the effect of seed priming and foliar application of nano-zinc on the quality of forage maize (cultivar SC704, single cross) under field and greenhouse conditions. The authors found significant improvements in total dry matter as a result of spraying with the nano-zinc treatment at 2 g/L. These results are in accordance with those found in this research.

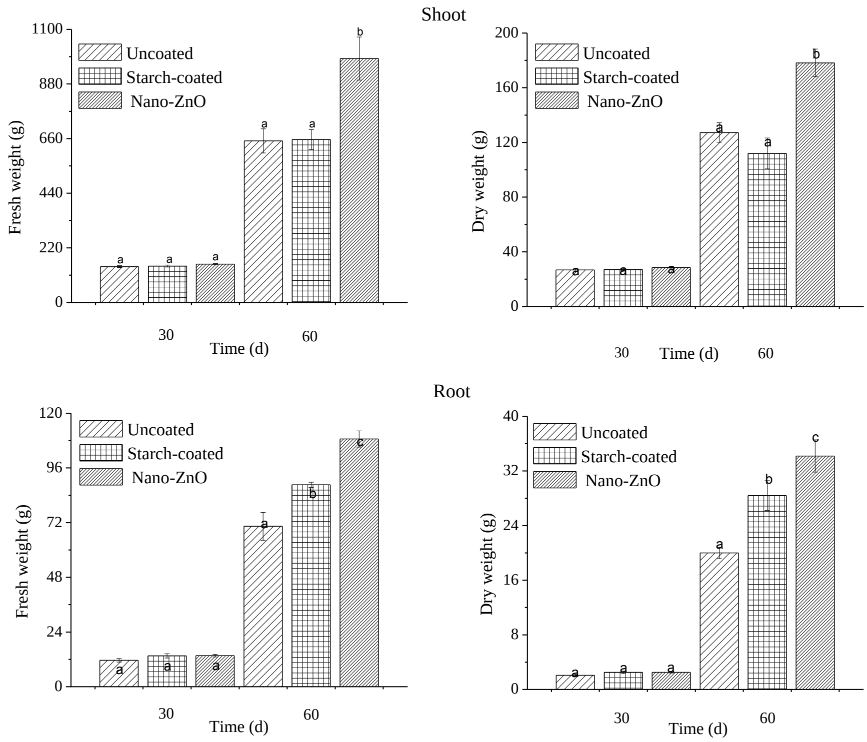

Fig. 2. Effect of a relatively low dose of nano ZnO particles on fresh and dry weights of shoots and roots in maize. Mean of nine plants per treatment ± standard error. In the same sampling date, means not sharing a common superscript differ significantly (Tukey test $p < 0.05$).

3.3. Photosynthetic pigments (chlorophylls)

At day 60 after sowing, the concentrations of chlorophylls in leaves changed significantly among treatments (Table 1). Nano-ZnO treatment resulted in higher leaf Chl *a* concentration (247.5 ± 7.7 mg/L) than that for Uncoated (209.9 ± 7.4 mg/L) and Starch-coated treatments (213.2 ± 11.4 mg/L). Leaf Chl *b* concentration was also significantly affected by treatments. In general, when compared to Uncoated treatment, Starch-coated and Nano-ZnO treatments increased Chl *b* concentrations in 23% and 39%, respectively. Starch-coated and Nano-ZnO treatments resulted in higher Chl *a+b* concentration in comparison to Uncoated treatment. These samples presented average Chl *a+b* concentrations of 302.1 ± 14.3 mg/L and 323.8 ± 3.8 mg/L, respectively. Although there was no statistically significant effect on Chl *a+b* concentration, a slight increment (7%) in the Nano-ZnO treatment was observed (Table 1). In this context, Morteza et al. [19] evaluated the effects of nano titanium dioxide (TiO_2) spray on maize. According to the authors, the highest amounts of Chl *a*, Chl *b*, and Chl *a+b*, were attained by spraying 0.03% nano TiO_2 at the stage of the appearance of male and female flowers. These results are consistent with our findings.

Table 1. Effect of a relatively low dose of nano ZnO particles on leaf chlorophyll concentration in maize at 60 d after sowing.

Treatment	Chlorophyll content (mg/L)		
	Chl *a*	Chl *b*	Chl *a+b*
Uncoated	209.9 ± 7.4 ^{a}	62.9 ± 2.1 ^{a}	273.1 ± 6.8 ^{a}
Starch-coated	213.2 ± 11.4 ^{a}	77.5 ± 4.5 ^{b}	302.1 ± 14.3 ^{b}
Nano-ZnO	247.5 ± 7.7 ^{b}	87.3 ± 1.8 ^{c}	323.8 ± 3.8 ^{b}

Mean of ten replicates per treatment ± standard error. Means, within the same column, not sharing a common superscript differ significantly (Tukey test $p < 0.05$).

3.4. Cob components and yield attributes

In general, cob components (cob length, cob diameter, number of kernel rows, number of kernels per row, and number of kernels per cob) were significantly affected by the nano-ZnO application (Table 2). The maximum cob length (15.6 ± 0.5 cm) was observed in the Nano-ZnO treatment, followed by Starch-coated (14.1 ± 0.6 cm) treatment, and Uncoated (13.5 ± 0.4 cm) treatment. According to the findings of this research, it is clear that Nano-ZnO treatment significantly improved cob diameter, presenting values up to 5.5 ± 0.1 cm. Uncoated treatment showed 13 rows per cob, whereas Starch-coated and Nano-ZnO treatments showed 15 rows per cob. Similarly, Starch-coated and Nano-ZnO treatments showed significantly higher number of kernels per row (28 kernels per row) compared to Uncoated treatment (20 kernels per row). Moreover, compared to Uncoated treatment, Nano-ZnO and Starch-coated treatments showed higher number of kernels per cob, presenting values of 430 ± 22 and 412 ± 31 grains per cob, respectively (Table 2).

Furthermore, the highest cob weight was recorded with the Nano-ZnO treatment, being significantly superior to Starch-coated (8%) treatment and Uncoated (52%) treatment. Also, the highest test weight (weight of 100 kernels) was observed with the application of Nano-ZnO, which was significantly higher than Starch-coated and Uncoated treatments. On the other hand, there were no significant differences in rachis weight and the grain-cob mass ratio (shelling factor) among treatments. Finally, the highest grain yield (1.70 Ton/ha) was attained with the Nano-ZnO treatment, which was significantly higher (7%) compared to Starch-coated (1.58 Ton/ha) and 42% higher compared to Uncoated (1.19 Ton/ha) treatments. These results clearly demonstrate that Nano-ZnO exhibited better cob components and yield attributes, which is in accordance with the other field experiment with maize [12].

Table 2. Effect of a relatively low dose of nano ZnO particles on cob components and yield attributes in a Mexican landrace of red maize.

Treatment	Cob components				
	CL	CD	NR	NKR	NKC
Uncoated	13.5 ± 0.4 [a]	4.7 ± 0.1 [a]	13 ± 0.5 [a]	20 ± 0.8 [a]	259 ± 10.8 [a]
Starch-coated	14.1 ± 0.6 [ab]	4.9 ± 0.1 [a]	15 ± 0.8 [ab]	28 ± 1.3 [b]	412 ± 31.1 [b]
Nano-ZnO	15.6 ± 0.5 [b]	5.5 ± 0.1 [b]	15 ± 0.3 [b]	28 ± 1.1 [b]	430 ± 22.1 [b]
	Yield attributes				
	CW	TW	RW [NS]	SF [NS]	GY
Uncoated	106.9 ± 3.3 [a]	33.4 ± 0.24 [a]	14.3 ± 0.6	0.90 ± 0.001	1.19 ± 0.02 [a]
Starch-coated	150.9 ± 3.4 [b]	33.6 ± 0.04 [a]	14.9 ± 0.6	0.91 ± 0.004	1.58 ± 0.02 [b]
Nano-ZnO	162.8 ± 2.6 [c]	34.1 ± 0.14 [b]	16.7 ± 0.6	0.91 ± 0.003	1.70 ± 0.04 [c]

Mean of thirty cobs per treatment ± standard error. Means, within the same column, not sharing a common superscript differ significantly (Tukey test $p < 0.05$). CL =cob length (cm), CD = cob diameter (cm), NR = number of kernel rows, NKR = number of kernels per row, NKC= number of kernels per cob, CW = cob weight (g), TW = test weight (g), RW = rachis weight (g), SF = shelling factor, GY = grain yield (Ton/ha). NS= no significant (Tukey test $p > 0.05$).

3.5. FTIR-ATR studies of the F1 progeny

To identify the specific functional groups in the maize grains of the F1 progeny, FTIR-ATR studies were conducted. A representative FTIR spectra comparison is showed in Fig. 3. In general, significant increments in the primary active vibrations associated with the peptide-protein (3279, 1643, and 1537 cm^{-1}), lipids (2924, 2853, and 1745 cm^{-1}), and carbohydrate (1150 and 998 cm^{-1}) bands were noted in the Nano-ZnO treatment. Table 3 summarizes the main active vibrations and their corresponding biochemical component.

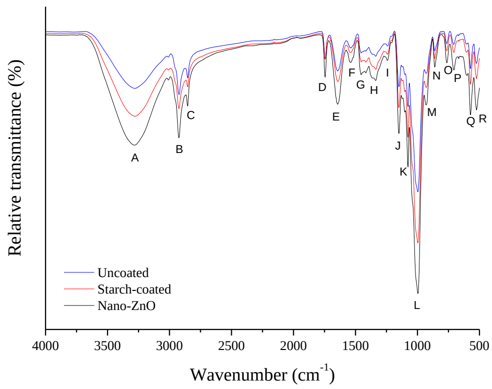

Fig. 3. Comparative Fourier transform infrared spectra of maize grains (F1 progeny).

Table 3. Band assignments of the main active FTIR vibrations of maize grains (F1 progeny).

Band	Wavenumber (cm^{-1})			Functional group and typically assigned biochemical component
	Uncoated	Starch-coated	Nano-ZnO	
A	3279 $_{(br)}$	3279 $_{(br)}$	3283 $_{(br)}$	N–H stretching vibrations (peptide and protein). Amide I.
B	2924 $_{(m)}$	2924 $_{(m)}$	2924 $_{(m)}$	$–(CH_2)_n–$ antisymmetric stretching (lipids).
C	2853 $_{(w)}$	2854 $_{(m)}$	2854 $_{(m)}$	$C–CH_3$ symmetric stretching (lipids).
D	1745 $_{(m)}$	1745 $_{(m)}$	1745 $_{(m)}$	$–CH_2–COOR$ stretching (phospholipid esters).
E	1643 $_{(m)}$	1643 $_{(m)}$	1644 $_{(m)}$	O=C–N–H (80% C=O stretching, 20% C–N stretching) (amide I, peptide, and protein).
F	1537 $_{(w)}$	1538 $_{(w)}$	1539 $_{(m)}$	NH_3^+ deformation (amino acid).
G	1455 $_{(vw)}$	1454 $_{(w)}$	1455 $_{(w)}$	$–(CH_3)_n–$; $–(CH_2)_n–$ antisymmetric bending (lipid and protein).
H	1337 $_{(w)}$	1336 $_{(m)}$	1337 $_{(m)}$	–C=O stretching (carboxylic acids).
I	1241 $_{(vw)}$	1242 $_{(w)}$	1243 $_{(w)}$	$RO–PO_2^-–OR$ antisymmetric stretching (DNA, RNA, phospholipid, and phosphorylated protein).
J	1150 $_{(m)}$	1150 $_{(m)}$	1150 $_{(m)}$	C–O stretching, C–O–H wagging, twisting and rocking (carbohydrates)
K	1076 $_{(m)}$	1076 $_{(m)}$	1076 $_{(m)}$	$RO–PO_2^-–OR$ symmetric stretching (DNA, RNA, phospholipid, and phosphorylated protein).
L	998 $_{(s)}$	997 $_{(s)}$	997 $_{(vs)}$	C–O stretching (carbohydrates), hydrogen bonding of the OH group at C_6 (glucose).
M	930 $_{(m)}$	930 $_{(m)}$	930 $_{(m)}$	C–H bending (aldehyde)
N	860 $_{(m)}$	861 $_{(m)}$	861 $_{(m)}$	CH out-of-plane deformation, NH_2 wag (primary amides).
O	764 $_{(vw)}$	764 $_{(w)}$	763 $_{(w)}$	CH out-of-plane deformation.
P	707 $_{(vw)}$	707 $_{(vw)}$	706 $_{(vw)}$	$–NH_2$ wag (primary amines).
Q	573 $_{(m)}$	573 $_{(m)}$	572 $_{(m)}$	In-plane and out-of-plane ring deformations.
R	525 $_{(w)}$	524 $_{(m)}$	523 $_{(m)}$	In plane and out-of-plane ring deformations.

s = strong, m = medium, w = weak; v = very, br = broad.

Moreover, a marked increment in the peptide-protein (2.1-fold), lipids (1.7-fold), and carbohydrates (1.9-fold) band areas was recorded in the Nano-ZnO treatment when compared to Uncoated treatment. In addition, significant increments in band areas of these main active vibrations were also observed in the Starch-coated treatment, reaching values up to 1.4, 1.2, and 1.5-fold, respectively. The strong band centered at 998 cm^{-1} was used for the quantitative determination of starch short-range structure (crystallinity). The FTIR bands at 1047 cm^{-1} and 1022 cm^{-1} are commonly associated to the crystalline and amorphous structures of starch, respectively. However, the band ratio at 1047/1022 cm^{-1} ($R_{1047/1022}$) is frequently used to quantify the degree of order in starch samples [16, 20-21]. In this research, to estimate this ratio, the distinctive band at 998 cm^{-1} was deconvoluted with three Gaussian functions and the ratio of areas corresponding to the peaks at 1047 and 1022 cm^{-1} was estimated (Fig. 4, profile a). In general, there were significant differences in the $R_{1047/1022}$ among the three treatment groups (Fig. 4, profile b). The maximum $R_{1047/1022}$ was observed in the Nano-ZnO treatment (0.96), followed by Starch-coated (0.88) and Uncoated (0.75) treatments. These results are in close agreement with Sevenou et al. [21] who reported $R_{1047/1022}$ values of 0.75 and 0.95 for maize starch and amylomaize (high amylose starch), respectively. Our results confirmed that a high degree of organization at a short-range scale was observed on their outer regions of the starch granules of the Nano-ZnO treatment. This effect could be associated to higher resistance of this starch to enzymatic hydrolysis [22]. To the best of our knowledge, this is the first field-scale study conducted to evaluate the effects of relatively low contents of nano ZnO particles (0.16 mg ZnO nanoparticles per seed) on the growth, productivity, chlorophyll content, and yield components of a Mexican landrace of red maize.

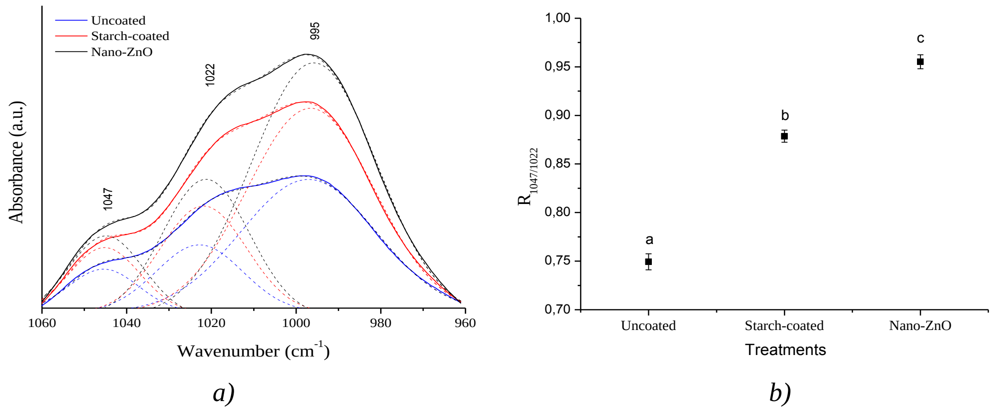

Fig. 4. (a) Deconvolution of the distinctive band at 998 cm^{-1} (starch fingerprint) with three Gaussian functions, and (b) degree of organization at a short-range scale of the starch granules as a function of FTIR ratio ($R_{1047/1022}$).

4. Conclusions

Taken together, these data indicate that a relatively low dose of nano-ZnO particles (0.16 mg nano-ZnO per seed) significantly improved growth parameters, biomass production, photosynthetic pigments, cob components, grain yield, and yield attributes in a Mexican pigmented maize landrace. Further studies regarding gene expression in the native maize seeds treated with nano-ZnO are currently being evaluated.

Acknowledgments

This research was partially supported by UNAM-DGAPA-PAPIME grant number PE104319. For assistance in the field experiment we are grateful to A. Cruz-Alonso, A. Pérez-de León, A. Zavala-Franco, B. Monroy-Torres, D. Hernández-Meléndez, F. Salinas-García, K. Sánchez-Carrillo, and W. Zavala-Toledo.

References

[1] M. Payak, R. Sharma, International Journal of Pest Management **31**(4), 302 (1985).
[2] J. -P. Vielle-Calzada, J. Padilla, in. J. L. Bennetzen, S. C. Hake (eds.), Handbook of maize: Its biology (Springer Science+Business Media, LLC), pp. 543-561 (2009).
[3] I. Vega-Alvarez, A. Santacruz-Varela, M. Rocandio-Rodríguez, L. Córdova-Téllez, H. López-Sánchez, A. Muñoz-Orozco, A. Hernández-Bautista, Pesquisa Agropecuária Brasileira **52**(11), 1023 (2017).
[4] T. Adhikari, S. Kundu, A. S. Rao, Journal of Plant Nutrition **39**(1), 136 (2016).
[5] E. C. Muñoz, J. L. Rosado, P. López, H. C. Furr, L. H. Allen, American Journal of Clinical Nutrition **71**(3), 789 (2000).
[6] A. C. Pandey, S. S. Sanjay, R. S. Yadav, Journal of Experimental Nanoscience **5**(6), 488 (2010).
[7] T. Prasad, P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munaswamy, K.R. Reddy, T. Sreeprasad, P. Sajanlal, T. Pradeep, Journal of Plant Nutrition **35**(6), 905 (2012).
[8] T. Adhikari, S. Kundu, A. Biswas, J. Tarafdar, A. Subba Rao, Journal of Plant Nutrition **38**(10), 1505 (2015).
[9] X. Liu, F. Wang, Z. Shi, R. Tong, X. Shi, Journal of Nanoparticle Research **17**(4),

175 (2015).
[10] J. Potarzycki, W. Grzebisz. Plant Soil and Environment **55**(12), 519 (2009).
[11] R. Sharifi, K. Mohammadi, A. Rokhzadi, Environmental and Experimental Biology **14**, 151 (2016).
[12] L. V. Subbaiah, T. N. V. K. V. Prasad, T. G. Krishna, P. Sudhakar, B. R. Reddy, T. Pradeep, Journal of Agricultural and Food Chemistry **64**(19), 3778 (2016).
[13] J. Estrada-Urbina, A. Cruz-Alonso, M. Santander-González, A. Méndez-Albores, A. Vázquez-Durán, Nanomaterials **8**, 247 (2018).
[14] Association of Official Seed Analysts (AOSA), Rules for Testing Seeds. Journal of Seed Technology **16**(3), 1 (1993).
[15] J. Wintermans, A. De Mots, Biochimica et Biophysica Acta. **109**(2), 448 (1965).
[16] J. J. van Soest, H. Tournois, D. de Wit, J. F. Vliegenthart, Carbohydrate Research **279**, 201 (1995).
[17] SAS/STAT User’s Guide. Version 8. Available online: http://www.okstate.edu/sas/v8/saspdf/stat/pdfidx.htm (accessed on 25 September).
[18] T. Munir, M. Rizwan, M, Kashif, A. Shahzad, S. Ali, N. Amin, R. Zahid, M. F. E. Alam, M. Imran, Digest Journal of Nanomaterials and Biostructures **13**(1), 315 (2018).
[19] E. Morteza, P. Moaveni, H.A. Farahani, M. Kiyani, SpringerPlus **2**(1), 247 (2013).
[20] I. Capron, P. Robert, P. Colonna, M. Brogly, V. Planchot, Carbohydrate Polymers **68**(2), 249 (2007).
[21] O. Sevenou, S. Hill, I. Farhat, J. Mitchell, International Journal of Biological Macromolecules **31**(1-3), 79 (2002).
[22] V. Planchot, P. Colonna, D. Gallant, B. Bouchet. Journal of Cereal Science **21**(2), 163 (1995).