Summer 2019

Quality of Wheat Grains (Triticum aestivum) Generationally Exposed to Cerium Oxide Nanoparticles (nCeO2)

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QUALITY OF WHEAT GRAINS (*Triticum aestivum*) GENERATIONALLY EXPOSED TO CERIUM OXIDE NANOPARTICLES (*n*CeO_2)

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Chemistry

By

Oluwasegun M. Abolade

August 2019

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ABSTRACT

The impacts of generational exposure to engineered nanomaterial on grain quality are poorly documented. This study was performed on wheat grains harvested from plants grown in soil amended with cerium oxide nanoparticles (*n*CeO_2) at the 2nd and 3rd generations. Third generation experiment was performed at low and high nitrogen (N) soil levels. The goal was to investigate changes in grain fatty acid and elemental contents due to parental exposure (C_1 vs T_1 in 2nd generation, C_1C_2 vs T_1T_2 in 3rd generation) or current generation exposure (C_2 vs T_2 in 2nd generation, C_3 vs T_3 in 3rd generation); C = control (0 mg *n*CeO_2/kg soil), T = treated (500 mg *n*CeO_2/kg soil); 1 = first generation, 2 = second generation, and 3 = third generation. Fatty acid (FA) analysis was performed in 2nd and 3rd generation grains while elemental analysis was done in third generation grains only. All data were subjected to a two-way ANOVA to determine statistical significance of parental exposure or current generation exposure. The results showed that parental exposure at T_1 increased the concentrations of most FA while generational exposure T_1T_2 at high N only increased linoleic and total fatty acids. Also at high N, T_1T_2 decreased elemental contents (P, Mg, K, Mn, Fe) even without changes in their concentrations. At low N soil, current exposure to *n*CeO_2 at 3rd generation (T_3) affected uptake of few elements (e.g. P, Mn, Fe) while current exposure at 2nd and 3rd generations consistently decreased myristic acid concentration. These findings showed that parent life-history could affect grain quality depending on soil N.

KEYWORDS: grains, generational, soil nitrogen level, fatty acid, elemental nutrient, and content

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ACKNOWLEDGEMENTS

I would like to thank my Advisor, Dr. Cyren Rico for his patience in teaching and mentoring me throughout this study. I sincerely appreciate how he would go to the laboratory with me whenever I encountered a problem in my research and his insistence that I adhere to basic laboratory and scientific etiquettes. His words of encouragement and motivation kept me in progress. Dr. Rico, I will really miss working with you.

I would also like to thank my parents: Mr. Joseph and Mrs. Grace Abolade for their support and encouragement all these years. I also want to express my appreciation to my invaluable heartbeat: Victoria Olubukola Iyanunioluwatiteminikan Abolade for her persistent prayer and encouragement. I would like to thank all my professors, those that taught and helped me all these years. I would like to specially thank the members of my thesis committee Dr. Richard Biagioni, Dr. Adam Wanekaya, and Dr. Babur Mirza for taking their time to read through my thesis and making sure all information was correct. I would like to thank Dr. Breyfogle, Dr. Schick, and Linda Allen for their words of encouragement and guidance all throughout my program.

I would like to thank the Missouri State University Scholarship Foundation, Chemistry Department, and Graduate College for their summer research fellowship, thesis funding, and faculty research grant that made this thesis possible. I also acknowledge support from the National Science Foundation under Grant No. 1828069 for the ICP-MS award to the Chemistry Department.

I dedicate this thesis to God: My source and strength.

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1. INTRODUCTION

1.1. Background to study

Current nanophytotoxicity studies have focused on documenting immediate toxicity responses and engineered nanoparticles (NPs) uptake in plants and neglected the long-term generational implications of NPs exposure.[1,18] The majority of studies, especially recent metabolic investigations, reveal that NPs do not cause acute toxic effects in plants but induce subtle phenological or phenotypic modifications which eventually alter the quality and composition of seeds.[3] When grown in succeeding generations, seed quality affects physiological and biochemical processes that alter growth, survival, and productivity in progeny plants. Therefore, multigenerational exposure to engineered nanoparticles may have long-term environmental and ecological implications that need to be investigated.

While agriculturists plan to restore the non-synthetic genomic diversity of various domesticated crops, environmental engineers need technologies to cut-down on fertilizer consumption without altering agricultural yields, hereby making the planet more sustainable and safe.[62] Studies have shown that plants significantly increase their yield when exposed to engineered NPs such as nano-iron pyrite[62] and nanoceria (nCeO$_2$)[1,63] at levels between 100 µg/ml to 500 mg/ml when used alone to prime grains and / or added to fertilizer. The study hypothesizes that NPs could be used to replace conventional fertilizer in improving plant yield. ENPs (e.g. nCeO$_2$) uptake in plants has been reported to be via several routes such as root surface / uptake, adsorption to leaf surface in agglomerated form, and air exchange into the leaf structure.[32] Studies have also revealed that ENPs (e.g. nCeO$_2$) affect plants through variety of ways such as shoot elongation, reduction in germination rate (e.g., corn, tomato, cucumber, and

others), improved root growth (e.g., cucumber and corn), and reduced root growth (e.g., tomato and alfalfa plants).[32] Cerium oxide nanoparticles or nanoceria (nCeO$_2$) exhibit negligible dissolution in environmental media and are predicted to accumulate and persist in soil, and therefore interact with plants in nanoparticulate form.[18,72] Various studies have shown that nCeO$_2$ do not cause plant mortality (i.e. plants go to full maturity and harvest) but significantly alter macromolecular (e.g. carbohydrates, protein, fatty acids) and nutrient (e.g. Ca, P, K, Mn, Fe) compositions of grains even in the absence of Ce accumulation.[1] Therefore, it is highly possible that repeated exposures to nCeO$_2$ alter grain quality and performance of plants in the terrestrial environment.

Soil nitrogen level has been reported to influence the growth, yield, and grain quality.[1] Report has shown that soil nitrogen level (N) in plants exposed to metal oxide nanoparticles (e.g. nCeO$_2$) improve its productivity and grain quality.[64] Similarly, study has reported that N values of grains produced during the two consecutive generations exposure to nCeO$_2$ were changed.[18] Consequent on these observations, there is need to progress on studying the effect of intergenerational exposure of engineered nanomaterial on plants at varying soil nitrogen level.

1.2. Research overview

Engineered nanomaterials have been reported in previous studies to modify agronomic characteristics including quality of plants grown to maturity, growth, chlorophyll content, and yield biomass, among others.[13,14] Although a number of studies have demonstrated that nCeO$_2$ interacts with plants, its impacts on grains' quality are yet to be extensively explored.

Wheat is a prominent cereal crop with close to 70% of its 600 million tons yearly production consumed as food by humans.[1,14,15] The interaction of nanometal oxides such as

nCeO_2, *n*TiO_2, and *n*ZnO with wheat have been reported,[1,16,17] yet implications on wheat grains' nutritional quality at varying soil nitrogen levels and generational nanoceria exposures are still limited.[1,18] This study hopes to provide understanding on long-term transgenerational responses of wheat to *n*CeO_2 exposure.

This project was performed to investigate the influence of two-generation exposures (C_1C_2 vs T_1T_2 where "C" represents control generation with no exposure to *n*CeO_2, T represents treated generation exposed to 500 mg *n*CeO_2 per kg soil, and subscripts 1 and 2 indicate 1st and 2nd generation) or *n*CeO_2 treatment (C_3 vs T_3) cultivated in low or high nitrogen amended soil on the quality of third generation wheat grains.

1.2.1. Research hypothesis. The hypothesis tested was that generational exposure to nanoceria at varying soil nitrogen levels will increase the fatty acid and elemental concentration of wheat grains. This is because previous study reported that plants exposed to nanoceria for two consecutive generations at normal soil N had improved growth and nutrient uptake.[18]

1.2.2. Research objectives. To determine the effects of cerium oxide nanoparticles, soil nitrogen level, and generational exposure on fatty acid and elemental content of wheat grains.

1.2.3. Evaluated quality parameter. The quality parameters investigated in this study were fatty acids and elemental (current treatment) compositions. Fatty acid concentration was determined by gas chromatography while elemental composition was measured using inductively coupled plasma with mass spectrometry (ICP-MS).[1,19]

1.3. Data analysis

Statistical analysis was performed separately for low N and high N treatments to determine the statistical significance of parental exposure [i.e. parental exposure at first

generation (C_1 vs. T_1) or second generation (C_1C_2 vs. T_1T_2)], current exposure at second or third generation [i.e. second generation (C_2 vs. T_2) or third generation exposure (C_3 vs. T_3)], and their interactions. The data was analyzed following a two-way ANOVA test using General Linear Model in SAS statistical package (SAS Institute, Cary, NC). All values were reported as mean ± standard error (SE), $n = 6$.

2. LITERATURE REVIEW

2.1. Nanotechnology

Nanotechnology is a field that involves manipulation of matter at the atomic, molecular, and supramolecular scale. It is a field that is rapidly developing and the wide-range use of engineered nanoparticles (ENPs) in consumer products and the industry (i.e. electronics, agriculture, and pharmaceuticals) has been of global concern both in their unavoidable release and eventual accumulation in the environment.[20,21]

2.2. Nanoparticles

Irrespective of their dispersal state; gaseous, liquid or solid media, ENPs are defined as particle with at least one-dimension size ranging from 1 to 100 nanometers.[22]

2.2.1. Class of nanoparticles. Currently, USEPA (2017)[72] classifies ENPs into four categories: (I) Dendrimers: These are tree-like structure synthetic polymer used for unique chemical function such as catalysis, drug targeting and delivery.[23] (II) Composites: These are a combination of ENPs with other nanoparticles or larger materials like ceramic and concrete to enhance flame-retardant, mechanical, heat properties. (III) Carbon-based materials: These are primarily carbon and commonly take the form of fullerenes (spherical and ellipsoidal carbon nanomaterials), single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) which have used to improve film coatings and in electronics. (IV) Metal-based materials: These nanomaterials include quantum dots, nanogold, nanosilver and metal oxides, such as titanium dioxide ($n\mathrm{TiO_2}$), cerium dioxide ($n\mathrm{CeO_2}$), zinc oxide (nZnO).[24] A concise information on the various classes of NPs can be found in Figure 1.

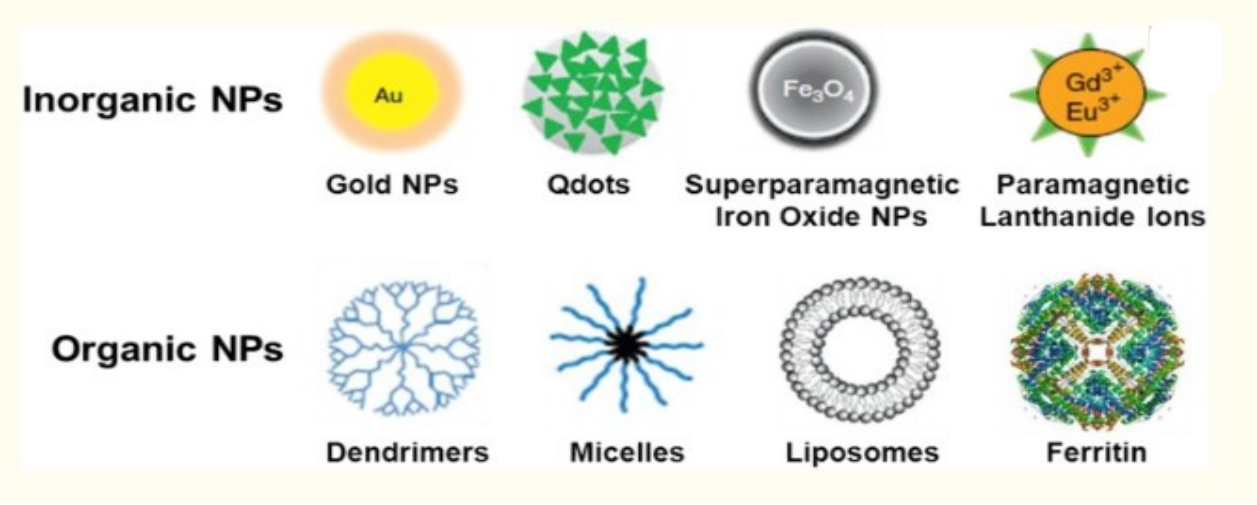

Figure 1**.** Different classes of nanoparticles from organic and inorganic origin.[24]

2.2.2. Properties of nanoparticles. ENPs have unique properties lacking in their bulk equivalent counterpart. These include: large surface area, variable oxidation state, and high surface area to volume ratio.[27] Consequent on these unique properties, they will differ in behavior and environmental fates when compared to 'traditional' bulk (organic and inorganic).[25,26]

2.2.3. Sources and environmental fate of nanoparticles. The speculated growth of the world population (9.8 billion by 2050) will result in exponential demand for food. More than 50% of world daily caloric intake is derived directly from cereal grains consumption but only 34% of cereal production had been consumed by human due to harvest losses and use of cereal-based animal feed.[27]

Having reported that the use of ENPs and developing nanotechnologies in agricultural practice greatly enhance food security via reducing nutrient losses from fertilizer,[30,36] there is need to meticulously assess the accumulative effect of deliberate use of ENPs on ecology and

humans. Although applications of nano-fertilizers in agriculture, water purification, and soil remediation could lead to deliberate release of ENPs into the environment,[33,34] most of it are unintentionally released as a result of industrial and domestic processes.[28,29] Soils are the major 'sink' for ENPs once released leading to their prolonged interaction with terrestrial plants. Consequently, exposed plants exhibit adverse attributes such as irregular photosynthetic rates, alteration in accumulation and translocation of nutrients, as well as 'trophic transfer' of ENPs within food webs which could have harmful impact on ecology and human health. Currently, literature reports have shown that the interaction of ENPs with plants have both positive and negative impacts. ENPs have been proposed to help plants in the uptake as well as translocation of macro and micronutrients.[30] On the contrary, some ENPs [e.g. silver nanoparticles (*n*Ag)] have phytotoxic effects on plants at high exposure concentration leading to inhibition of seed germination and root elongation in some plant species.[30] Figure 2 shows different sources of *n*CeO_2 and how they end-up in the environment.

2.3. Nanoscale metal oxides

Nanoscale metal oxides are found in nature or synthesized for use.[26] They have been utilized in various applications: health, sustainable chemistry, commercial products, and environmental technologies primarily because of their outstanding physiochemical properties such metal-oxygen binding, unique magnetic and electronic properties, as well as variable crystalline structure.[31] Quite a number of these nanometal oxides have been investigated for their potential applications in agriculture including nanoceria (*n*CeO_2), nano zinc oxide (*n*ZnO), and nano titanium oxide (*n*TiO_2), among others.[23,33,34]

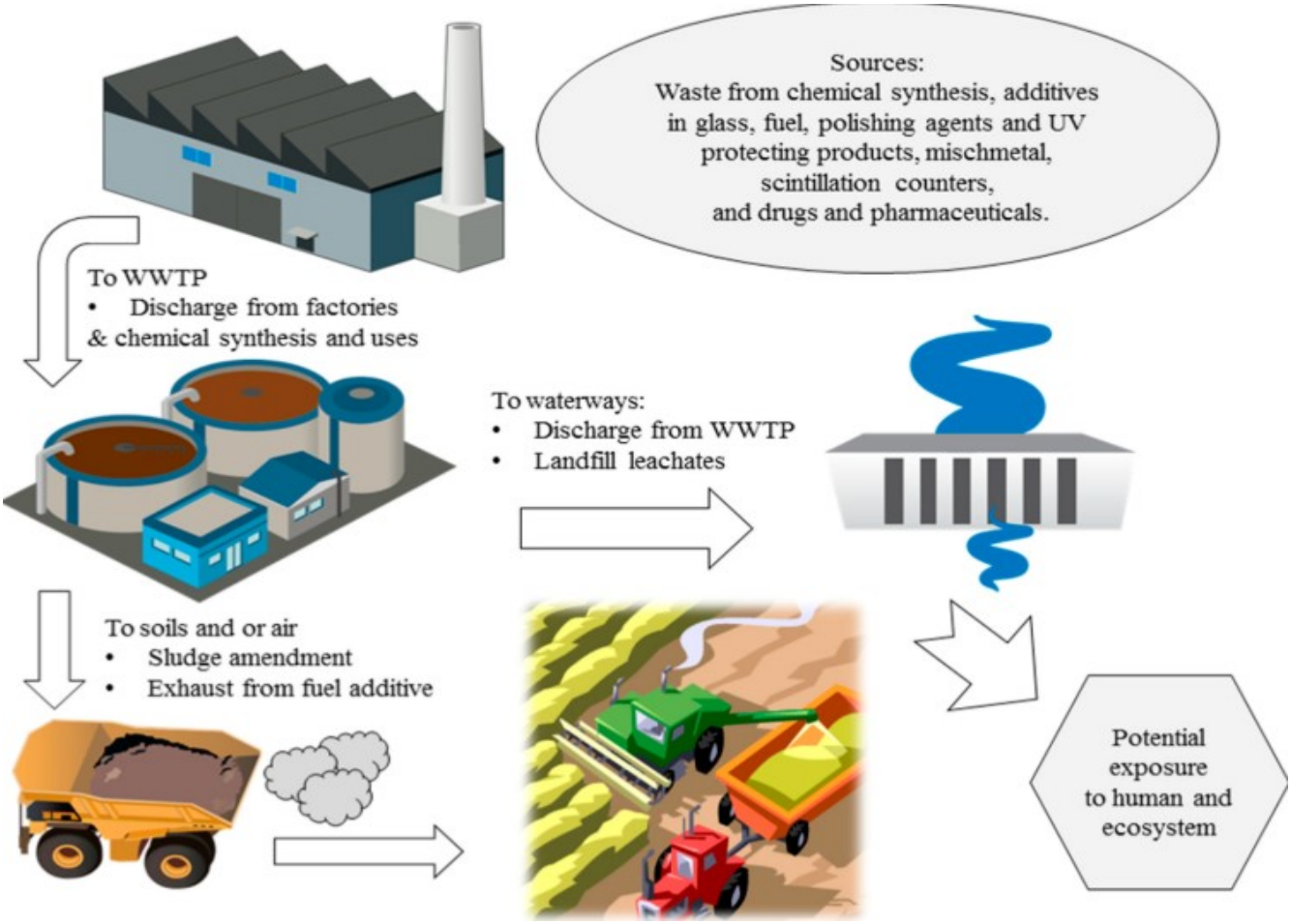

Figure 2. Flowchart showing sources, fate, and exposure routes of nCeO$_2$ in the terrestrial environment.[32]

2.3.1. Nanoceria (nCeO$_2$). nCeO$_2$ is an oxide of the rare-earth metal cerium. It is a pale yellow-white powder. It is highly stable in a range of environmental media allowing it to be found in different food crops.[37,38] Cerium (Ce) is a chemical element with atomic number and weight of 58 and 140 g/mol.[35] It has characteristic soft, ductile, and silvery-white color which tarnishes on exposure to air. It belongs to the lanthanides series in the periodic table with varying oxidation state of +3 and +4 (exceptional stable state). It is one of the most abundant rare earth

elements (0.0046 % by weight of earth crust) having concentration ranging from 2 to 150 mg/kg in soils.[20] Dominant forms of Ce include different ores but not limited to cerianite (CeO_2), allanite [Ce_2 (Al, Fe^{+3})$_3$(SiO_4)$_6$ (SiO_3OH) (OH)$_3$], zircon (CeO_2-ZrO_2), monazite ($CePO_4$), rhabdophane ($CePO_4 \cdot H_2O$).

2.3.2. Uses of nanoceria. Nanoceria is an important commercial product and an intermediate in the purification of the element from their ores. Its unique property is the reversible conversion to a nonstoichiometric oxide.[59] Manufactured *n*CeO_2 had been used in wide-range of products like paint coatings, polishing powder, catalysis, and fuel additives.[21,22] *n*CeO_2 has been speculated to have conservative annual global production of 1000 tonnes. Nanoceria also has wide-range applications in fuel catalysis, UV coatings, chemical-mechanical planarization, and paints.[37,38] Its various uses would perhaps allow it to end-up in soil or landfill.[37]. *n*CeO_2 has found applications in different fields as depicted in Figures 3 and 4.

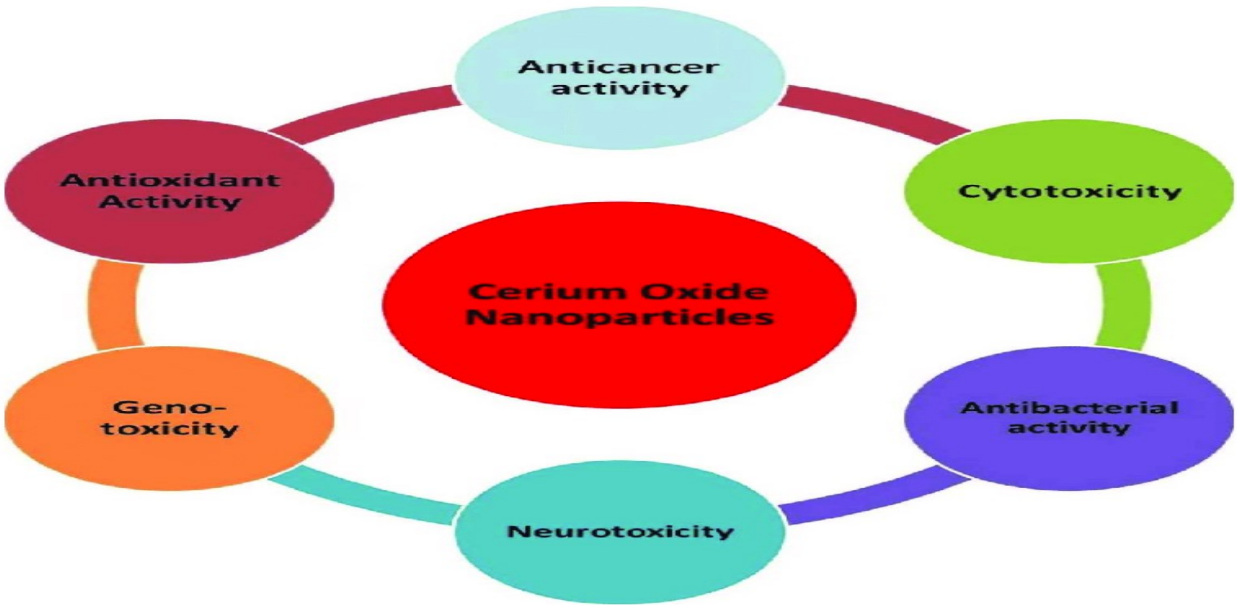

Figure 3. Applications of *n*CeO_2 in the biomedical field.[40] *n*CeO_2 exhibits beneficial activities and prevents toxicities.

Figure 4. Application of nCeO$_2$ in plant to improve growth.[65]

2.4. Wheat

Wheat belongs to the cereal family.[41] It is a grass widely cultivated for its grains which is a staple food globally [42,43,44] It belongs to the genus *Triticum* and the most commonly grown species is *aestivum*. It is cultivated on more land area than any other food crop (220.4 million hectares).[45] It is the second most-produced cereal and has higher global trade than all other crops combined.[45] Figure 5 gives pictorial representation of wheat plant and grains.

2.4.1. Production, consumption, and growth. Over the past six decades, global production of wheat has tripled and speculated to grow further through the middle of the 21st century.[45] Global consumption of wheat is increasing thereby facilitating the production of processed foods. It is a good source of fiber, carbohydrates, vegetal protein[45,46,47] and essential amino acids.[52,53,54] It is an annual grass that grows well in temperate region with maximum and minimum temperatures of 30-32ºC and 3-4ºC, respectively.[48,49] Optimal growth requires

temperature of about 25ºC and adequate source of irrigation. Conversely, excess water can lead to waterlogging predisposing it to diseases that can lead to yield losses. Generally, wheat growth lifecycle has three distinct major phases: (i) The vegetative / tittering phase: commences with sprouting or initiation of leaves, the reproductive phase; (ii) The stem extension phase / continued development of floret, and the grain-filling phase; (iii) The heading and ripening phase / continuous growth to full weight gain.[48] Figures 5, 6, 7 and 8 represent the unique significance of wheat crop in the globe with reference to top ten wheat producing countries, its consumption rate in the U. S. referring to its wide-range of use as food-crops, and its different growth stages respectively.

Figure 5. Wheat plant showing (A) fully grown spike prior to harvesting and (B) grains after harvesting.[39]

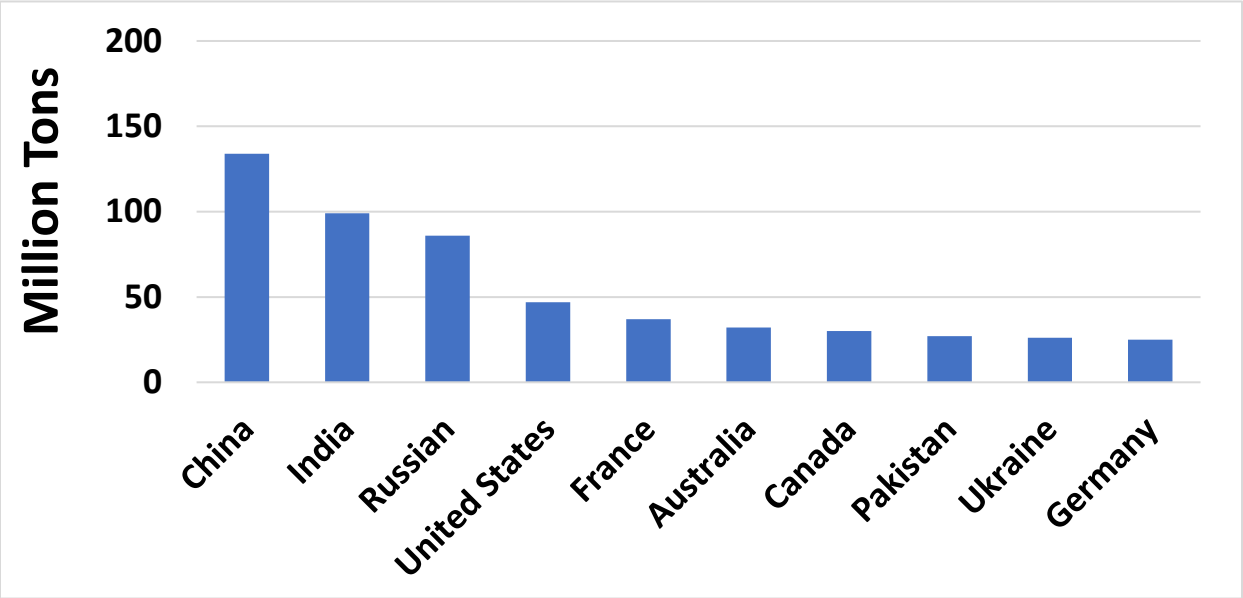

Figure 6. Chart showing statistics of top ten countries in the world leading in wheat production.[50]

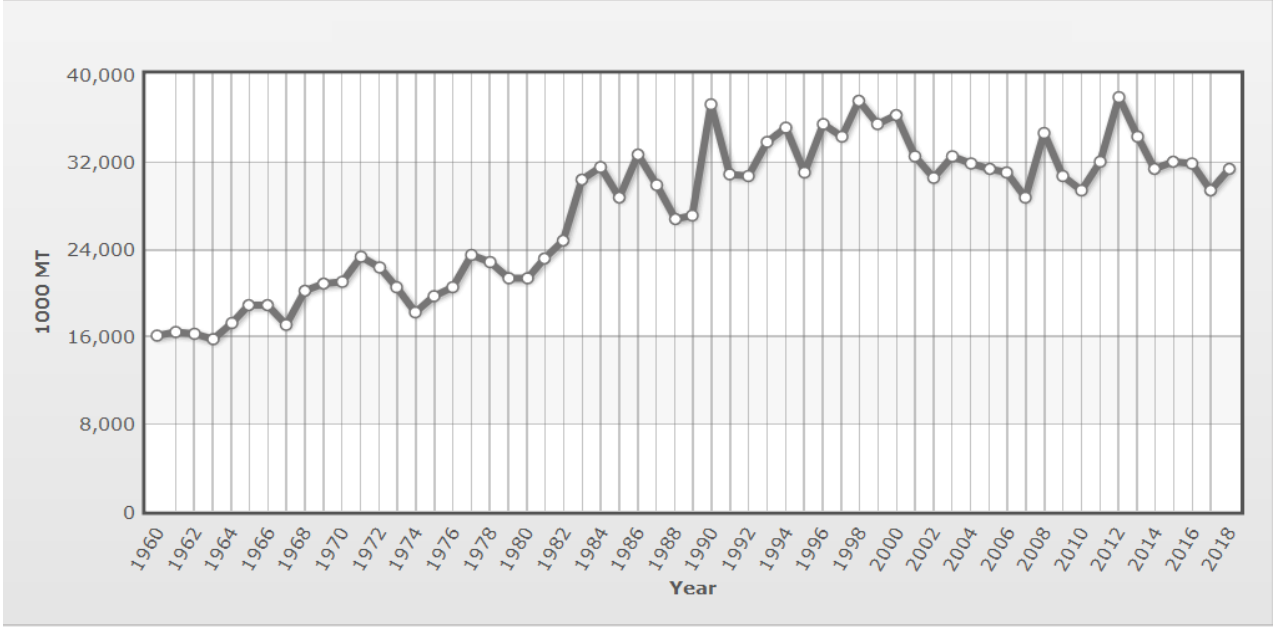

Figure 7. Chart showing statistics of wheat consumption in United States for close to six decades: it underscores the unique importance of wheat as a food crop.[51]

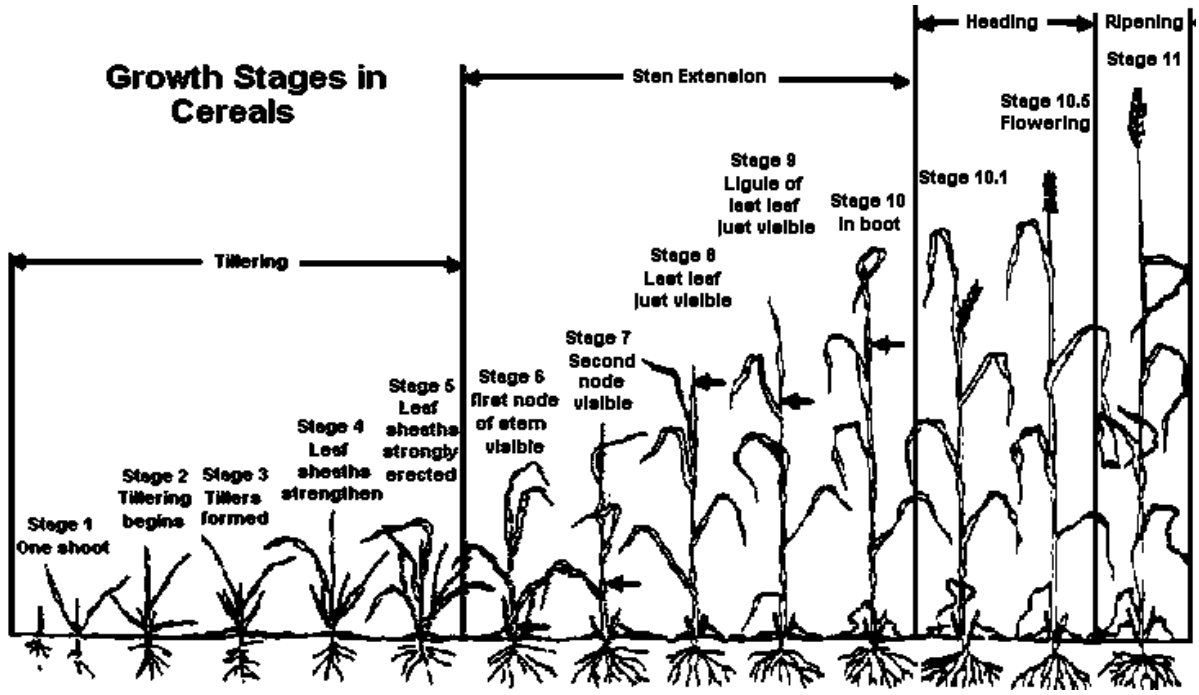

Figure 8. Chart showing information on different stages in wheat growth lifecycle.[48]

2.4.2. Classes of wheat. (i) Hard red spring - It is a hard, brownish, high-protein wheat used for bread and hard baked goods. (ii) Hard white - It is a hard, light-colored, opaque, chalky, medium-protein wheat planted in dry, temperate areas. Used for bread and brewing. (iii) Hard red winter - It is a hard, brownish, mellow high-protein wheat used for bread, hard baked goods. (iv) Soft red winter - It is a soft, low-protein wheat used for cakes, pie crusts, biscuits, and muffins. (v) Soft White – It is a soft, light-colored, very low protein wheat grown in temperate moist areas. Used for pie crusts and pastry.[52,53]

2.5. Generational studies and soil nitrogen level

Generational studies in plants exposed to engineered nanoparticles have been increasingly reported in the literature. Reports have shown that first generation exposure to *n*TiO_2 promoted growth but adversely affected the photosynthetic ability of basil treated again with *n*TiO_2 at second generation.[69] Other studies have shown that *n*CuO modified gene expressions in two-generation exposed *Arabidopsis thaliana*, *n*CeO_2 induced plant retardation in generationally-exposed tomato but enhanced growth and seed maturity in wheat, and *n*ZnO induced minimal generational effects on seed composition of *Phaseolus vulgaris*[4,5,18] For multigenerational studies,[6] reported reduced growth and productivity in *Brassica rapa* exposed to *n*CeO_2 for three generations while germination rates in three-generation treated *Arabidopsis thaliana* was found to be drastically reduced.[2]

Related studies in plants have shown that previous generation exposure to environmental stress improves fitness and tolerance to the same stress in succeeding generations. For example, *Arabidopsis thaliana* that experienced metal stress (i.e. Ni, Cd) for three generations imparted stress tolerance in the offsprings.[7] Progeny generation of salt-stressed *Arabidopsis thaliana* also exhibited improved survival rate and reproductive output when exposed to similar salt stress.[8,9] Soil nutrient conditions experienced by parents had significant effects on size of offspring of *Senecio sp*,[10] or biomass and carbon storage in progeny of *Plantago lanceolate*.[11] Likewise, nitrogen-stressed rice imparted increased tolerance to nitrogen limitation for two progeny generations.[12] A similar repeated generational exposures to engineered NPs may affect the performance of progeny generations.

The influence of nitrogen level on the quality of plants' macromolecular component has been reported in literature. Report showed that high level of nitrogen in soil has the capability of

improving quality as well as increasing plants' resilience to harmful conditions than low N level.[55,57] Similarly, report has shown that that increased soil nitrogen has the potential to improve quality of wheat.[56,58]

3. MATERIAL AND METHODS

3.1. Grains production, generational studies, and nitrogen treatment

The studies on the first and second wheat life cycles have been reported[1,18] and grains (parental grain / exposure) harvested from them were used in this third of a series of long-term complete life cycle studies of wheat exposed cerium oxide nanoparticles (*n*CeO_2). This investigation involved treatment combinations of soil nitrogen (N) level (i.e. low or high), grain type (i.e. generationally exposed grains), and *n*CeO_2 exposure (i.e. 0 mg vs 500 mg *n*CeO_2 per kg soil). For example, the two grain types were cultivated in low and high N soil amended with 0 or 500 mg *n*CeO_2 per kg soil to produce 3rd generation (G3) grains giving four treatment combinations (i.e. $C_1C_2C_3$, $C_1C_2T_3$, $T_1T_2C_3$, and $T_1T_2T_3$). Each treatment combination had six replicates ($n = 6$). High N soil was achieved by amending the soil with Yoshida nutrient solution that contained nitrogen as ammonium nitrate (it is the only component of the nutrient solution modified for this purpose) whereas low N soil was created by adding nutrient solution that contained lower amount of ammonium nitrate (Table 1). Figure 9 presents a schematic diagram of the experimental design described above. Table 2 shows the definition of all terminologies used in this study.

nCeO_2 were purchased from Meliorum Technologies (Rochester, NY) and were rods with primary particle size of 67 ± 8 x 8 ± 1 nm, surface area of 93.8 m^2/g, and 95.14% purity while its hydrodynamic particle size is 231 ± 16 nm in distilled water.[70] Table 1 presents schedule of Yoshida nutrient solution added to wheat. Additional information on the component of Yoshida solution can be found in Appendix B-2.

Table 1. Schedule of Yoshida Nutrient Solution (YNS) addition to the plants and the total amount of N added.

Date	Concentration of N added (mg/L)		Volume of YNS (mL)	mg N added	
	Low N	High N		Low N	High N
July 14, 2016	0	80	200	0	16
August 11, 2016	40	80	100	4	8
August 12, 2016	40	80	150	6	12
August 15, 2016	40	80	100	4	8
August 16, 2016	40	80	100	4	8
August 17, 2016	40	80	100	4	8
August 18, 2016	40	80	100	4	8
August 19, 2016	40	80	150	6	12
August 22, 2016	40	80	100	4	8
August 23, 2016	40	80	100	4	8
August 24, 2016	40	80	100	4	8
August 29, 2016	40	80	100	4	8
Total	**-**	**-**	**-**	**48**	**112**

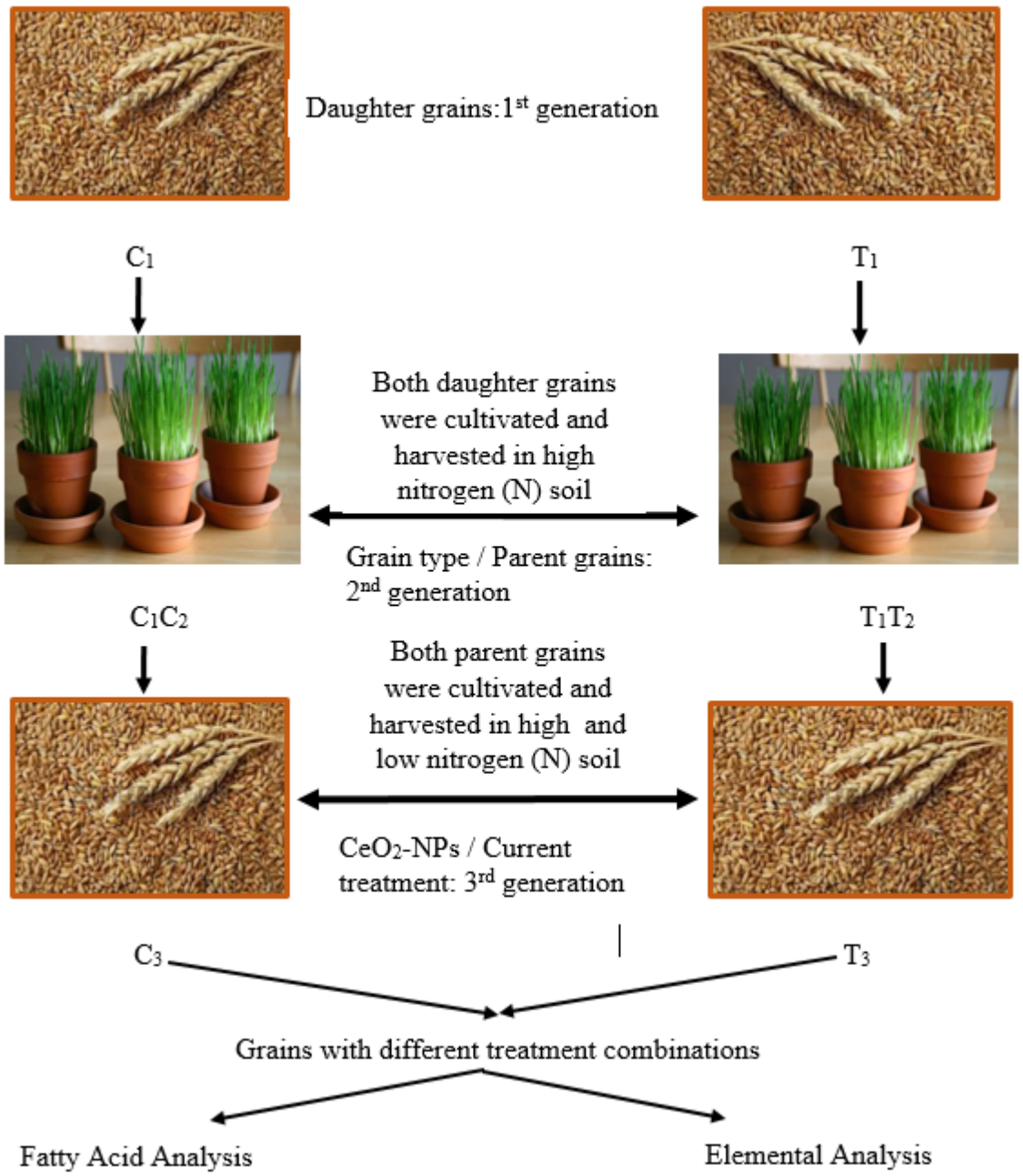

Figure 9. Grain production, intergenerational studies, and varying nitrogen level. C_1C_2, T_1T_2 = generationally exposed seeds, C = control with 0 mg of *n*CeO_2 per kg soil, T = treated with 500 mg of *n*CeO_2 per kg soil, C_3, T_3 = *n*CeO_2 treatment type, 1 = 1st generation, 2 = 2nd generation, 3 = 3rd generation.

Table 2. Definition of used terms.

Terms	Definition
Normal soil N	This describes soil nitrogen level without addition of Yoshida nutrient solution.
High soil N	This describes soil nitrogen level after being enriched with Yoshida nutrient solution which contains ammonium nitrate to supply full amount of N (i.e. 112 mg). The calculation on N concentration can be found in appendix B-3.
Low soil N	This describes soil nitrogen level after being enriched it with Yoshida nutrient solution which contains ammonium nitrate to supply half the amount of high N (i.e. 48 mg)
Parental grains / exposure	This describes grains cultivated and harvested after exposure to *n*CeO_2 for two consecutive generations in 0 mg and 500 mg per *n*CeO_2 per kg soil i.e. C_1C_2 or T_1T_2 respectively.
Current grains / exposure	This describes grains cultivated and harvested after exposure to 0 mg and 500 mg per *n*CeO_2 per kg soil i.e. C_3 or T_3 respectively at the 3rd generation only.
Quality parameter	Contextually, it describes wheat grain composition (protein, carbohydrate, lipid, mineral, fiber, phytic acid) that makes it unique as a food crop. This study investigate fatty acid / lipid and mineral composition of wheat grains.[71]

3.2. Soil preparation and *n*CeO_2 addition in soil

The soil was a 3:1 (v:v) mixture of potting soil (i.e. without added fertilizer, SunGro Horticulture) and sand thoroughly mixed using a cement mixer. The mixture ratio of potting soil and sand was based on preliminary experiment. The *n*CeO_2 suspension was poured evenly into a pot containing 200-gram dry weight equivalent of soil mix to give the necessary 500 mg *n*CeO_2 per kg soil treatment. The pots were prepared and aged in the growth chamber three days before seedlings were transplanted.

3.3. Plant cultivation and management

Wheat seedlings were prepared and grown to full maturity as described previously.[2] Two nine-day-old seedlings were transplanted into each pot (one seedling/100 g dry weight soil) and grown in growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with these conditions: 16-h photoperiod, temperature was 20 / 10˚C, 70% humidity, and light intensity of 300 µmol/m^2-s for the first 40 days, after which the conditions were kept at 16-h photoperiod, 25/15˚C, 70% humidity, 600 µmol/m^2-s until harvest. Yoshida nutrient solution was added during the experiment (Table 1). Ladybugs (family Coccinellidae) were used as a biological control to prevent possible wheat green bug (*Schizaphis graminum*) infestation. At harvest, plant materials were oven-dried and weighed for total biomass. Two soil core samples were collected from each pot in the soil experiment to estimate total root biomass.

3.4. Treatment combinations

This study used two sets of wheat grains which were treated with *n*CeO_2 exposure at different generations and soil nitrogen levels.

3.4.1. Second generation treatment (G2). This is the first set of grains which had been exposed to nanoceria treatment for two generations at normal soil nitrogen (N). This set of grains was only used for fatty acid analysis. Treatment combinations: C_1C_2, T_1T_2; C= 0 mg *n*CeO_2, T= 500 mg *n*CeO_2 / kg soil; 1= 1st, 2= 2nd generations; C= control, T= treated.

3.4.2. Third generation treatment (G3). This is the second set of grains which had been exposed to nanoceria treatment for three generations at low and high soil nitrogen levels. These sets of grains were used for fatty acid and elemental analyses. Treatment combinations: $C_1C_2C_3$,

$C_1C_2T_3$, $T_1T_2C_3$, $T_1T_2T_3$ C= 0 mg *n*CeO_2, T= 500 mg *n*CeO_2 / kg soil 1= 1st, 2= 2nd, 3= 3rd generations; C= control, T= treated.

3.5. Quality control

This was done to ensure consistency and accuracy of the method. This was achieved by preparing repeat and blank samples for extraction and gas chromatography determination as well as to prepare blank samples. Similarly, repeat and blank samples were done for digestion and ICP-MS determination.

3.6. Fatty acid analysis

3.6.1. Methylation and extraction of fatty acids. Fatty acid concentration was determined as described in the literature.[36] The powdered wheat grains (200 mg) were placed in a total of 2 mL methylating solution comprising 1 mL of methanolic sulfuric acid (5% H_2SO_4 in methanol) and 1 mL of 1 mg/mL internal standard (i.e. tridecanoic acid in toluene) resulting in 0.5 mg/mL of tridecanoic acid in the reaction mixture. This mixture was vortexed and then incubated for ninety (90) minutes at 80°C in a water bath in a sealed tube. Extraction of fatty acid methyl ester was done with 1 mL hexane twice after cooling to room temperature. Subsequently, 1000 mg of anhydrous sodium sulfate was added to the organic phase to dry any water in the organic phase from esterification of fatty acids. The organic phase was then collected in amber GC vial and analyzed for fatty acid methyl acid methyl esters in gas chromatograph using flame ionization detector. Figure 10 and 11 give simplified esterification reaction for the methylation of grains fatty acid composition and detailed schematics on fatty acid analysis respectively.

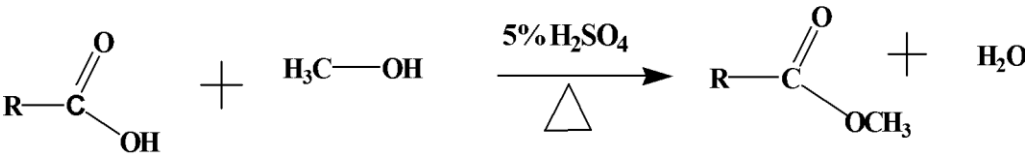

Figure 10. Esterification reaction: methylation of fatty acids in wheat grains.

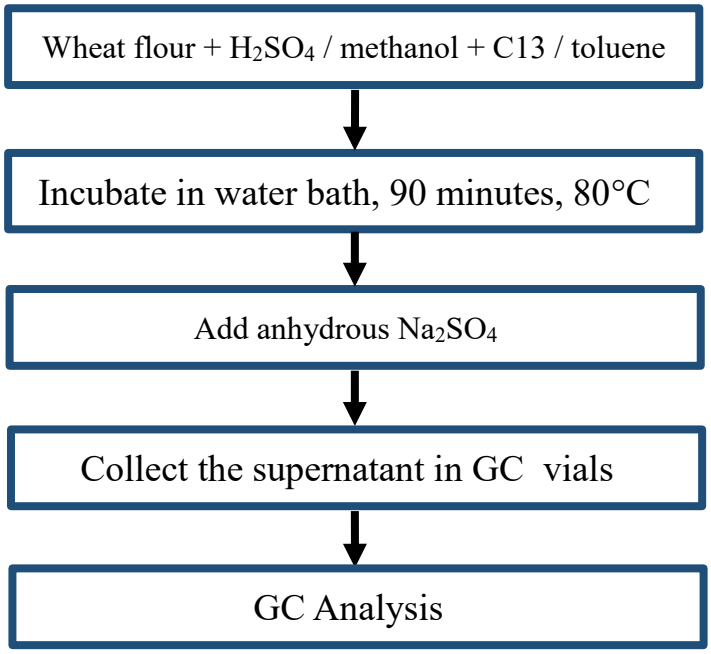

Figure 11. Detailed schematics of fatty acid analysis.

3.6.2. **Gas chromatography analysis.** A Varian 430 GC gas chromatography with a CP-8400 autosampler and flame ionization detector at 220 °C. Information on gas chromatography operating conditions can be found in Table 3.

Table 3. Gas Chromatography operation conditions.

Parameters	Condition
Stationary phase / column	SPTM-2330 (Non-bonded; poly(80 % biscyanopropyl / 20 % cyanopropylphenyl siloxane) phase
Column parameter (L x I.D x film thickness)	30 m x 0.25 mm x 0.2 µm
Carrier	Helium
Oven temperature programming	Initially at 160 °C for 2 minutes, then ramp to 220 °C at 10 °C / minute, hold at 220 °C for 1 minute
Flow rate	1 mL / minute
Injection volume	1µl
Injection temperature	240 ^{0}C
Injection type	Split with ratio 25:1
Acquisition length	9 minutes
Detector	FID
Flow rate	1 mL / min
Pulse duration	0.1 minute
Pulse pressure	10 Psi
Rinse solvent and volume	Cyclohexane, 5 µL / s

3.7. Fatty acid methyl ester (FAME) standards

The FAMEs standards (Sigma-Aldrich Co, St. Louis, MO. USA) were used for the determination of the calibration curve. These standards were used to prepare a stock solution from which a series of working calibration standards with concentrations of 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL were prepared (Table 4). For instance, the highest standard concentration was prepared by addition of 100 µL of FAMEs stock, 100 µL of 10 mg/mL C13 in toluene, and 1800 µL of toluene. The calibration standards were applied to identify the retention time and generate response curve. A total of two replicates of two runs per replicate was used. Table 4 gives detail on the chemical standards and concentrations used.

Table 4. FAMEs standards and concentrations used in the fatty acid analysis.

FAMES	Chemical name	Stock Concentration (mg/mL)	Standard solution concentration (mg/mL)
Methyl dodecanoate	C12:0	10	0.0625, 0.125, 0.25, 0.50
Methyl tetradodecanoate	C14:0	10	0.0625, 0.125, 0.25, 0.50
Methyl hexadecanoate	C16:0	20	0.125, 0.25, 0.50, 1.0
Methyl cis-9- hexadecenoate	C16:1	10	0.0625, 0.125, 0.25, 0.50
Methyl octadecanoate	C18:0	10	0.0625, 0.125, 0.25, 0.50
Methyl cis-9-octadecenoate	C18:1	20	0.125, 0.25, 0.50, 1.0
Methyl cis-9, 12,- octadecadienoate	C18:2	10	0.0625, 0.125, 0.25, 0.50
Methyl cis-9, 12, 15- octadecatrienoate	C18:3	10	0.0625, 0.125, 0.25, 0.50
Methyl tridecanoate	C13:0 (internal standard)	10	0.50

3.8. Elemental analysis

3.8.1. Chemical standards. Macro- and micronutrient ICP standards (Sigma-Aldrich Co, St. Louis, MO. USA) used are presented in Table 4. Using this stock solution, a series of working standard solutions of 50, 100, 500, 1000, 2000, and 4000 µg/L, and internal standard solution of 100 µg/L Indium were used. Peach leaves (NIST 1547; Gaithersburg, MD. USA) was used as the standard reference material. Information on chemical standards can be found in Table 5.

3.8.2. Digestion of samples. Macro- and micronutrient concentrations in wheat were measured according to method described in the literature.[1] A microwave-accelerated reaction system (CEM Mars 6^{TM}, Matthews, NC) was used to digest powdered wheat samples (250 mg) in 5 mL plasma pure HNO_3 for 20 minutes, then the digestates were diluted to 50 mL using

Millipore water. The digestion temperature programming has three stages: Stage 1 was 100% power for 5 minutes with a maximum temperature of 140 °C; Stage 2 was 50 % power for 5 minutes with a maximum temperature of 160 °C and stage 3 was 50 % power for 10 minutes with a maximum temperature of 160 °C.

Table 5. Elemental stock chemical standards.

Element	**Stock concentration (**mg/L)
Calcium	100
Magnesium	100
Phosphorus	100
Potassium	100
Sodium	100
Iron	10
Manganese	10

3.8.3. Inductively coupled plasma-mass spectrometer (ICP-MS). Analysis of elemental concentration were done according to previous literature report[19] using 7900 ICP-MS Agilent Technologies with SPS4 autosampler. ICP-MS operating conditions and elemental analysis schematics can be found in Table 6 and Figure 12, respectively.

Table 6. Inductively coupled plasma-mass spectrometry operating conditions.

Parameters	**Conditions**
Power (W)	1500
Carrier gas (L/min)	0.9
Makeup gas (L/min)	0.15
Auxiliary gas (L/min)	0.9
Plasma gas (L/min)	15
Sample uptake (µL/min)	400
Nebulizer	Gas concentric, micromist
Sample tube internal diameter (mm)	1.02
Internal standard tube diameter	1.52
Spray chamber	Quartz cooled to 2°C
Interface cones	Ni
Octopole reaction system	Standard mode (no gas), He modes
Repetitions	3
Rinse time	2 minutes

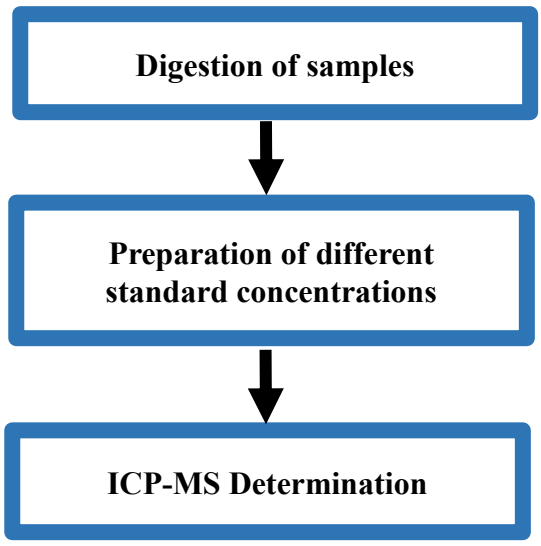

Figure 12. Schematics of elemental analysis.

3.9. Statistical analysis

A two-way analysis of variance (ANOVA) was performed on all quality parameters data to ascertain statistical significance. The ANOVA compared data between parent grains (C_1C_2 vs T_1T_2) and between current treatments (C_3 vs T_3) and their interactions. All data were reported as means ± standard error (SE). The two-way ANOVA testing used General Linear Model in SAS statistical package (SAS Institute, Cary, NC). The results on ANOVA for fatty acids concentrations, and elemental concentration and content can be found in Appendix A-1 to A-4.

4. RESULT AND DISCUSSION

4.1. Standard calibration for fatty acid analysis

The standard calibration was achieved by preparing stock of all fatty acid methyl esters (FAMEs) of common fatty acids in wheat from which different concentrations: 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL were prepared for gas chromatographic determination. The values presented in Figure 13 and Table 7 are for a typical calibration curve and concentration of FAMEs standard.

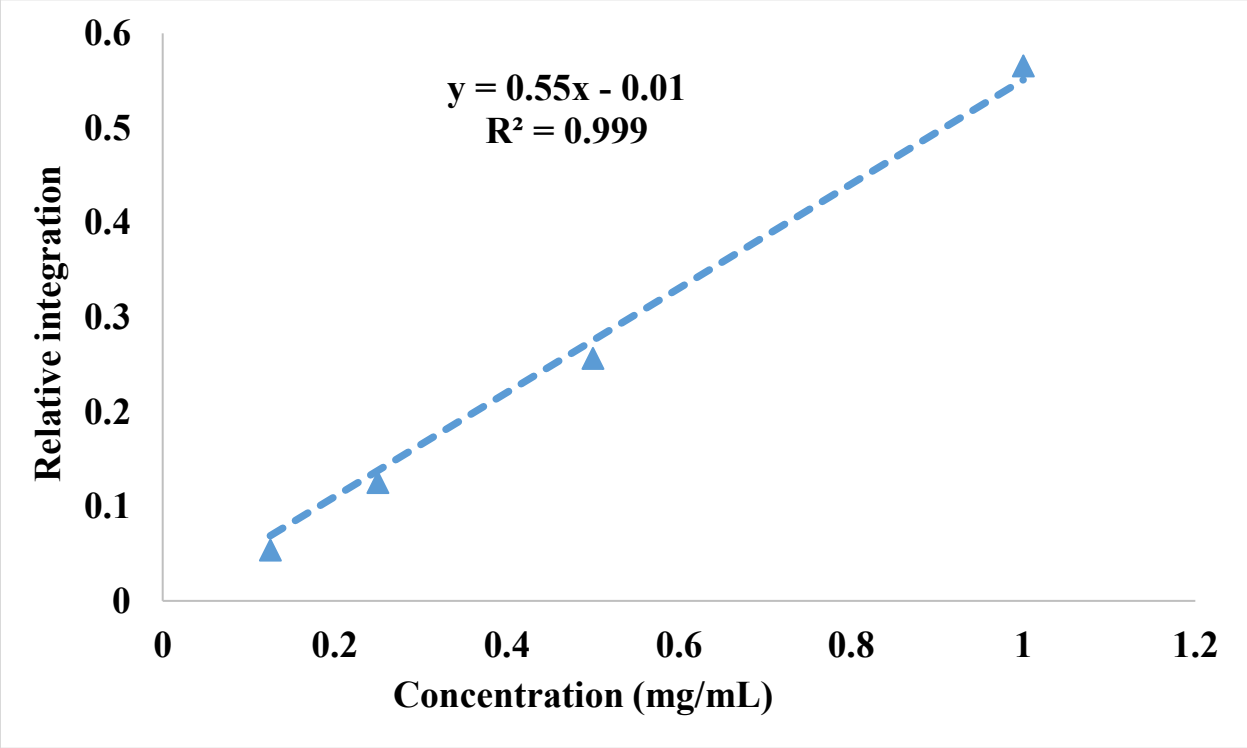

Figure 13. C16:1 standard curve: A typical standard calibration curve used in the study.

Table 7. Relative integration of FAMEs at different standard concentrations. Standard concentration values on the left are for all fatty acids except C16:0 and C18:1 which used values on the right.

Conc. (mg/ml)	(C12:0)	C13:0	(C14:0)	(C16:0)	(C16:1)	(C18:0)	(C18:1)	(C18:2)	(C18:3)
0.0625 / 0.125	0.11	1	0.08	0.12	0.06	0.04	0.10	0.05	0.05
0.125 / 0.25	0.23	1	0.18	0.26	0.13	0.09	0.25	0.12	0.13
0.25 / 0.5	0.46	1	0.36	0.52	0.26	0.19	0.50	0.24	0.26
0.50 / 1.0	0.92	1	0.74	1.10	0.55	0.42	1.11	0.52	0.57
SLOPE	0.93	.	0.74	1.12	0.55	0.43	1.15	0.54	0.59
INTERCEPT	-0.007	.	-0.008	-0.028	-0.010	-0.017	-0.049	-0.021	-0.024
CORREL	0.999	.	0.999	0.999	0.999	0.999	0.999	0.999	0.999

4.2. Relative fatty acid abundance in grains

Wheat grains composition contain only 1-3% fatty acids that small modifications in concentrations may cause significant impacts on chemical and physical properties of grains and possibly the growth and physiology of the daughter plants.[60] Fatty acid analysis was performed in 2nd and 3rd generation grains to better assess the generational effects of exposure to *n*CeO_2. Tables 8 - 19 present the data on modification of fatty acid of grains generationally exposed to engineered nanomaterials (ENMs) at varying soil nitrogen levels (high and low). The fatty acids detected were those commonly found in wheat. Lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and total fatty acid while stearic acid (C18:0) and palmitoleic acid (C16:1) were not detected in the grains.

4.2.1. Fatty acid analysis in second generation grains (G2): The influence of *n*CeO_2 on the relative fatty acid (FA) abundance in these grains were analyzed (Tables 8 and 9). Data from this study revealed that parental exposure (i.e. T_1, treated in the first generation) markedly

increased palmitic (C16:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), and total fatty acids concentrations by 85.6, 8.5 17.1, 8.2, and 11.8% compared to C_1 (i.e. control in the first generation) (Table 8). These findings revealed that previously-exposed plants (T_1) produced more grain fatty acids than plants not previously exposed (C_1) to *n*CeO_2. This observation is quite similar to literature report that exposure at T_1 increased linolenic acid but decreased linoleic acid,[1] while this study increased both. This is significant because grain yield from second generation study as reported did not change between C_1 and T_1 signifying that generational exposure could change seed quality.[18]

Table 8. Effect of first generation exposure on fatty acid concentrations (μg/g) of wheat grains.[a]

Fatty acid	C_1	T_1
Lauric acid (C12:0)	403 ± 13	404 ± 11^{ns}
Myristic acid (C14:0)	880 ± 63	704 ± 49**
Palmitic acid (C16:0)	2352 ± 76	4365 ± 79****
Oleic acid (C18:1)	2226 ± 107	2415 ± 68**
Linoleic acid (C18:2)	10817 ± 275	12672 ± 316****
Linolenic acid (C18:3)	893 ± 26	966 ± 23***
Total Fatty Acid	19286 ± 444	21566 ± 513****

[a]C_1 or T_1 indicate grains were harvested from plants exposed to 0 or 500 mg *n*CeO_2 per kg soil at 1st generation. Values are means ± SE (n = 12). ns represents no significant difference between means. **, ***, **** represent significance at $p \leq 0.05$, 0.01, and 0.001 respectively.

The second generation study also revealed that *n*CeO_2 exposure at second generation (G2) decreased both myristic and linolenic acid by 20 and 5.1%, respectively, compared to control (C_2) (Table 9). Myristic acid notably decreased but in general fatty acid concentrations

were not affected suggesting that current 2nd generation exposure to *n*CeO_2 does not induce severe changes in fatty acid synthesis. In the first generation study, *n*CeO_2 also did not modify fatty acid concentration in first generation grains.[1] Appendix C-1 to C-2 also present bar chart information on the fatty acid changes.

Table 9. Effect of cerium oxide nanoparticles (*n*CeO_2) on the fatty acid concentrations (μg/g) of second generation wheat grains (G2).[a]

Fatty acid	C_2	T_2
Lauric acid (C12:0)	408 ± 10	399 ± 14^{ns}
Myristic acid (C14:0)	880 ± 69	704 ± 43**
Palmitic acid (C16:0)	2617 ± 87	4100 ± 68^{ns}
Oleic acid (C18:1)	2338 ± 127	2303 ± 49^{ns}
Linoleic acid (C18:2)	11636 ± 376	11853 ± 215^{ns}
Linolenic acid (C18:3)	954 ± 31	905 ± 18*
Total Fatty Acid	20548 ± 585	20304 ± 372^{ns}

[a]C_2 or T_2 indicate 0 or 500 mg *n*CeO_2 treatment per kg soil at 2nd generation. Values are means ± SE (n = 12). ns represents no significant difference between means. *, ** represents significance at $p \leq 0.1$ and 0.05 respectively.

4.2.2. Effects of generational exposure on fatty acid concentration in third generation grains (G3):

Data from 3rd generation study showed that G3 grains at low N did not show significance difference in fatty acid concentrations due to parental or current exposure (Tables 10, 12). In the case of high N (Table 11), results revealed that lauric acid (C12:0) decreased by 5.7% while linoleic (C18:2) and total fatty acids increased by 3.4 and 3.0% in T_1T_2 grains compared to C_1C_2. It is highly possible that higher fatty acid concentrations from parent seeds caused the plants to produce more photosynthates which resulted in more fatty acid synthesis. Alternative data presentation as bar chart were presented in Appendices C-3 and C-4.

Table 10. Effect of generational exposures to cerium oxide nanoparticles (*n*CeO_2) on the fatty acid concentrations (μg/g) wheat grains at low N.[a]

Fatty acid	C_1C_2	T_1 T_2
Lauric acid (C12:0)	263 ± 13	278 ± 11[ns]
Myristic acid (C14:0)	983 ± 108	885 ± 71[ns]
Palmitic acid (C16:0)	2655 ± 142	2560 ± 128[ns]
Oleic acid (C18:1)	1845 ± 112	1859 ± 83[ns]
Linoleic acid (C18:2)	8165 ± 514	7871 ± 486[ns]
Linolenic acid (C18:3)	794 ± 39	790 ± 33[ns]
Total Fatty Acid	14730 ± 827	14244 ± 734[ns]

[a]Low N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying half N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg *n*CeO_2 per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means.

Table 11. Effect of generational exposures to cerium oxide nanoparticles (nCeO$_2$) on the fatty acid concentrations (μg/g) wheat grains at high N.[a]

Fatty acid	C_1C_2	T_1T_2
Lauric acid (C12:0)	175 ± 3	165 ± 5**
Myristic acid (C14:0)	976 ± 51	1006 ± 40^{ns}
Palmitic acid (C16:0)	2492 ± 51	2544 ± 28^{ns}
Oleic acid (C18:1)	1719 ± 38	1768 ± 31^{ns}
Linoleic acid (C18:2)	8563 ± 177	8856 ± 95*
Linolenic acid (C18:3)	772 ± 17	792 ± 9 ^{ns}
Total Fatty Acid	14697 ± 287	15131 ± 182***

[a]High N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying full amount of N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg nCeO$_2$ per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means. *, **, ***- represent . P ≤ 0.1, 0.05, and 0.01 respectively.

Table 12. Effect of cerium oxide nanoparticles (nCeO$_2$) on the fatty acid concentrations (μg/g) of third generation wheat grains (G3) at low N.[a]

Fatty acid	C_3	T_3
Lauric acid (C12:0)	265 ± 12	277 ± 12^{ns}
Myristic acid (C14:0)	987 ± 80	881 ± 100^{ns}
Palmitic acid (C16:0)	2601 ± 138	2614 ± 131^{ns}
Oleic acid (C18:1)	1854 ± 107	1850 ± 89^{ns}
Linoleic acid (C18:2)	7957 ± 499	8079 ± 501^{ns}
Linolenic acid (C18:3)	777 ± 39	807 ± 32^{ns}
Total Fatty Acid	14466 ± 798	14508 ± 763^{ns}

[a]Low N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying half amount of N; C_3 or T_3 indicate 500 mg nCeO$_2$ treatment per kg soil at 3rd generation. Values are means ± SE (n = 12). ns represents no significant difference between means.

Clearly, parental exposure and environmental factor (i.e. soil N) affected fatty acid synthesis in grains.[61,62] Results showed that grain fatty acid was only affected at high N but not at low N. The findings also showed that the highly significant change in fatty acid concentrations in parent seeds did not result in similar or even stronger effects in daughter grains. This was demonstrated by the smaller significant increase in fatty acid concentrations recorded in T_1T_2 grains (3.0%) despite the large significant increase in T_1 grains (11.8%).

4.2.3. Effects of cerium oxide nanoparticles on fatty acid concentration in third generation grains (G3): Cerium oxide nanoparticles on current generation (i.e. 3rd generation) did not affect fatty acid concentration of grains in both low and high N soil except for myristic acid at high N wherein *n*CeO_2 exposure at third generation (T_3) markedly reduced myristic acid concentration by 11.1% compared to control (C_3) according to Table 13. This finding is similar to that recorded in 2nd generation grains (G2) which suggest that myristic acid is sensitive to *n*CeO_2 exposure. The data is similar to previous study which reported that application of biofertilizer in seeds reduced saturated fatty acid but increased unsaturated ones (C18:1, C18:2, and C18:3).[65,66,67] This data demonstrates that in general *n*CeO_2 does not induce changes on fatty acid accumulation in grains but generational exposure to *n*CeO_2 could promote modifications in fatty acid concentration.

Table 13. Effect of cerium oxide nanoparticles (*n*CeO2) on the fatty acid concentrations (µg/g) of third generation wheat grains (G3) at high N.[a]

Fatty acid	C_3	T_3
Lauric acid (C12:0)	168 ± 4	173 ± 4^{ns}
Myristic acid (C14:0)	1050 ± 50	933 ± 41**
Palmitic acid (C16:0)	2524 ± 45	2513 ± 34^{ns}
Oleic acid (C18:1)	1746 ± 34	1741 ± 35^{ns}
Linoleic acid (C18:2)	8640 ± 156	8778 ± 117^{ns}
Linolenic acid (C18:3)	777 ± 15	787 ± 11^{ns}
Total Fatty Acid	14905 ± 251	14924 ± 218^{ns}

[a]High N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying full amount of N; C_3 or T_3 indicate 500 mg *n*CeO_2 treatment per kg soil at 3rd generation. Values are means ± SE (n = 12). ns represents no significant difference between means. **- indicate $p \leq 0.05$.

4.3. Mineral accumulation

4.3.1. Cerium uptake in third generation grains: The data presented in Tables 15-20 consist of macro and microelements analyzed. The value for cerium (Ce) concentration in the grains was excluded as it was not detected in the grains. This result was in agreement with the finding in 1st and 2nd generation studies showing the lack of translocation and accumulation of Ce in wheat grains.[18] Table and Figure 14 present percent recovery from reference standard and standard calibration curve respectively. Appendix C-5 to C-7 present these changes in bar chart.

Table 14. Percent element recovered from peach leave (NIST 1547).

Element	% recovery
Mn	100
Fe	101
Ce	93
Mg	110
P	104
K	115
Ca	120

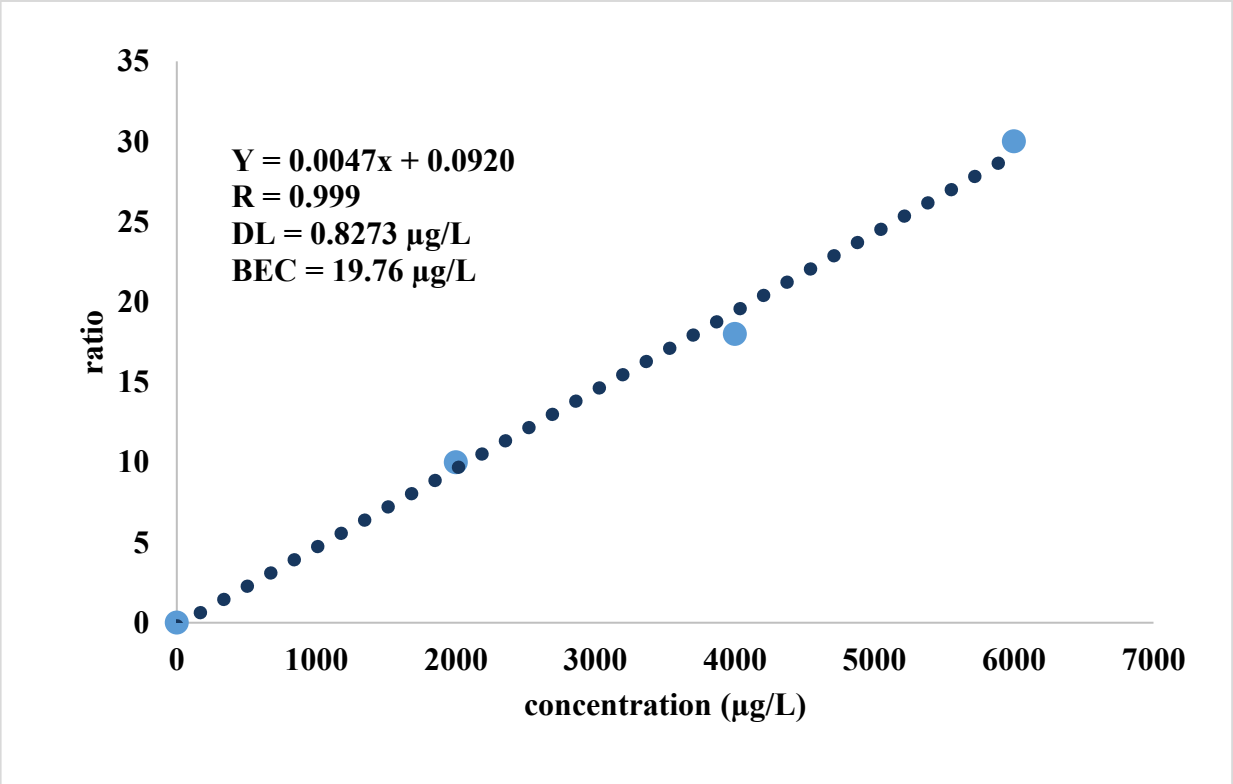

Figure 14. A typical standard calibration curve used in elemental analysis.

4.3.2. Elemental analysis of third generation grains: Results revealed differences in impacts of generational exposure on elemental uptake. Elemental uptake was measured as elemental concentration and elemental content (i.e. concentration × grain yield). Data on grain yield is provided in Appendix B-2.

Results showed that grain elemental concentrations did not change in either low N or high N (Tables 15, 16). Likewise, the elemental contents did not change at low N following the trend of elemental concentrations at low N (Table 17). Surprisingly, T_1T_2 significantly reduced grain nutrients in Table 18 (i.e. P, Mg, K, Mn, and Fe) at high N by 10.7, 11.5, 10.3, 9.5, and 17.2% compared to C_1C_2 despite the lack of change in the elemental concentrations (Table 17).

Table 15. Grain elemental concentrations of wheat previously exposed to *n*CeO_2 for two generations cultivated at low N soil.[a]

Element	C_1C_2	T_1T_2
P (μg/g)	2602 ± 75	2615 ± 61^{ns}
Mg (μg/g)	1195 ± 18	1172 ± 16^{ns}
K (μg/g)	4499 ± 98	4488 ± 83^{ns}
Ca (μg/g)	377 ± 33	364 ± 23^{ns}
Mn (μg/g)	75.1 ± 4.1	75.4 ± 1.4^{ns}
Fe (μg/g)	37.3 ± 2.3	37.7 ± 1.6^{ns}

[a]Low N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying half amount of N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg *n*CeO_2 per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means.

Table 16. Grain elemental concentrations of wheat previously exposed to *n*CeO_2 for two generations cultivated at high N soil.[a]

Element	C_1C_2	T_1T_2
P (μg/g)	2369 ± 52	2280 ± 95[ns]
Mg (μg/g)	1194 ± 22	1133 ± 41[ns]
K (μg/g)	4399 ± 62	4198 ± 95[ns]
Ca (μg/g)	1391 ± 87	1342 ± 46[ns]
Mn (μg/g)	67.1 ± 1.1	65.0 ± 2.2[ns]
Fe (μg/g)	39.9 ± 1.8	35.1 ± 1.6[ns]

[a]High N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying full amount of N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg *n*CeO_2 per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means.

The modifications in grain elemental uptake provided peculiar findings. First, the reductions in nutrient contents were due to decreases in the accumulation or movement of these elements to the grains since there were no differences in total yield. Since there were no differences in the yield parameters (i.e. total yield and grain weight) at low N, the reductions in nutrient contents were due to decreases in the accumulation or movement of these elements to the grains. Second, reductions of grain nutrients (i.e. P, Mg, K, Mn, and Fe) by T_1T_2 at high N (Table 18) were opposite to the observed lack of effects in the second generation study reported previously in literature[18] wherein previous exposure for one generation (i.e. T_1 was exposed to 500 mg *n*CeO_2 per kg soil in second generation) did not alter the grain elemental uptake. The trend is similar to previous work which reported that concentration of elemental nutrient was not significantly different at 500 mg *n*CeO_2 per kg soil.[18] Also, report from previous study have

shown that P, K, Ca, Mg, Mn, and Cu concentration in wheat grain treated with sewage sludge did not significantly different.[68,69] This finding could indicate that continuous generational exposure to *n*CeO_2 decreases seed elemental content. Third, generational exposure (i.e. T_1T_2) affects elemental content more in nitrogen-rich soil. Similar to the results in fatty acid content, T_1T_2 affects grain quality at nitrogen-rich soil.

Table 17. Grain elemental contents of wheat previously exposed to *n*CeO_2 for two generations cultivated at low N soil.[a]

Element	**C_1C_2**	**T_1T_2**
P (mg)	76.9 ± 2.2	78.9 ± 2.5^{ns}
Mg (mg)	35.5 ± 1.0	35.4 ± 0.7^{ns}
K (mg)	133.2 ± 4.0	135.5 ± 4.0^{ns}
Ca (mg)	11.2 ± 1.0	11.0 ± 0.7^{ns}
Mn (μg)	2349 ± 115	2189 ± 45.2^{ns}
Fe (μg)	916.2 ± 54.9	1167 ± 63.2^{ns}

[a]Low N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying half the amount of N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg *n*CeO_2 per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means.

Table 18. Grain elemental contents of wheat previously exposed to *n*CeO_2 for two generations cultivated at high N soil.[a]

Element	C_1C_2	T_1T_2
P (mg)	69.2 ± 2.8	61.8 ± 2.3**
Mg (mg)	34.8 ± 0.9	30.8 ± 0.9***
K (mg)	128.2 ± 2.7	115.0 ± 4.8**
Ca (mg)	40.5 ± 2.7	36.5 ± 1.3^{ns}
Mn (μg)	1957 ± 44	1770 ± 67**
Fe (μg)	1162 ± 54	962 ± 57**

[a]High N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying full amount of N; C_1C_2 or T_1T_2 indicate parental exposure to 0 or 500 mg *n*CeO_2 per kg soil at 1st and 2nd generations. Values are means ± SE (n = 12). ns represents no significant difference between means. **, *** represent $p \leq 0.05$ and 0.01 respectively.

4.3.3. Effects of cerium oxide nanoparticles on elemental contents in third generation grains (G3):

Exposure to cerium oxide nanoparticles at 3rd generation (T_3) significantly altered elemental contents in both low and high N. T_3 decreased P and Mn but increased Fe. contents by 8.8, 9.8, and 22.5 respectively, compared to C_3 (Table 19). However, only Mn and Fe contents changed (7.1 and 14.2% decrease) in T_3 compared to C_3 (Table 20). Considering the current results and those reported in 1st and 2nd generation studies,[1,18] the effects of *n*CeO_2 on grain elemental contents do not show consistent trend. This could probably due to different environmental and soil conditions which could affect nutrient accumulation in seeds. However, it also becomes apparent in these generational studies that Mn and Fe were sensitive to *n*CeO_2 exposures.

Table 19. Effect of cerium oxide nanoparticles (nCeO$_2$) on the elemental contents of third generation wheat grains (G3) at low N soil.[a]

Element	C_3	T_3
P (mg)	81.5 ± 2.1	74.3 ± 2.1**
Mg (mg)	34.7 ±1.0	36.1 ± 0.8^{ns}
K (mg)	136.8 ± 4.0	132.0 ± 4.0^{ns}
Ca (mg)	12.1 ± 0.9	10.1 ± 0.8^{ns}
Mn (μg)	2353 ± 73	2122 ± 43**
Fe (μg)	1011 ± 56	1238 ± 68**

[a]Low N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying half the amount of N; C_3 or T_3 indicate current exposure to 0 or 500 mg nCeO$_2$ per kg soil at 3rd generation. Values are means ± SE (n = 12). ns represents no significant difference between means. ** represent $p \leq 0.05$.

Table 20. Effect of cerium oxide nanoparticles (nCeO$_2$) on the elemental contents of third generation wheat grains (G3) at high N soil.[a]

Element	C_3	T_3
P (mg)	67.8 ± 2.3	63.2 ± 2.5^{ns}
Mg (mg)	34.0 ± 1.4	31.6 ± 0.8^{ns}
K (mg)	123.5 ± 5.4	119.8 ± 3.0^{ns}
Ca (mg)	40.6 ± 2.4	36.4 ± 1.7^{ns}
Mn (μg)	1932 ± 62	1795 ± 58*
Fe (μg)	1143 ± 55	981 ± 61**

[a]High N soil indicates addition of normal amount of nutrient solution with ammonium nitrate supplying full the amount of N; C_3 or T_3 indicate current exposure to 0 or 500 mg nCeO$_2$ per kg soil at 3rd generation. Values are means ± SE (n = 12). ns represents no significant difference between means. *, ** represent $p \leq 0.1$ and 0.05 respectively.

5. CONCLUSION

This study provides evidence that previous generation exposure to *n*CeO_2 affects the grain fatty acid and nutrient profile in progeny plants. However, the offspring environment (i.e. soil nitrogen) also modulates the influence of parental life-history. Data showed that for second generation wheat grains, parental exposure (T_1) relative to current treatment (T_2) increased fatty acid accumulation. Third generation grains were observed to behave differently. It was observed that wheat grains fatty acid and elemental accumulations differ in their response to *n*CeO_2 exposure alongside at soil low and high nitrogen levels. Moreover, this study underscores the significance of generational exposure to *n*CeO_2 at varying soil nitrogen level on the quality of food crops. Although both parental exposure (T_1T_2) and current exposure (T_3) increased fatty acid synthesis relative to control (C_1C_2, C_3) for most of the fatty acids, the former had greater capacity relative to latter to increase FA accumulations in grains at high N soil. Conversely, data showed that relative to control (C_1C_2, C_3), both parental and current exposure did not significantly change fatty acid composition at low N. This observation implies that parental exposure (T_1T_2) at high N will help to increase FAs production of wheat grains. Result showed that T_1T_2 decreased the allocation of all elements (macro and microelements) in grains at high nitrogen soil as well as decreased accumulation of most elements (Mg, K, and Ca) in the grains at low N soil respectively. The observed trend above explains the basis that regardless of the exposure, soil nitrogen level plays no significant role in increasing grains elemental concentration. Furthermore, both parental and current exposure to *n*CeO_2 (i.e. T_1T_2, T_3) reduced most elements content accumulation at low N soil while all elements content allocation

decreased at high N soil respectively. Hence, the difference in nitrogen level changes the effect of generational or current exposures on grain quality.

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APPENDICES

Appendix A: ANOVA of quality parameters analyzed

Appendix A-1. ANOVA of fatty acid concentrations in 2nd generation wheat grains generationally-exposed to *n*CeO_2.[a]

Fatty acids in grain	Parental Exposure (C_1 vs. T_1)	Current Exposure (C_2 vs. T_2)	Interactions
C12:0	0.9517	0.444	0.3668
C14:0	0.0153**	0.0154**	0.0060***
C16:0	< 0.0001***	0.8484	< 0.0001***
C18:1	0.0549*	0.7039	0.1615
C18:2	< 0.0001***	0.4817	0.0004***
CI8:3	0.0093***	0.0631*	0.0036***
TOTFAT	0.0004*	0.6058	0.0001***

Normal N indicated soil nitrogen without the addition of nutrient; *,*,*** - $p \leq 0.10$, 0.05, and 0.01, respectively.

Appendix A-2. ANOVA of fatty acid concentrations in 3rd generation wheat grains.

Fatty acid in grain	Parental Exposure (C_1C_2 vs. T_1T_2)	Current Exposure (C_3 vs. T_3)	Interactions
	Low N		
C12:0	0.2385	0.3268	0.0058*
C14:0	0.3038	0.2615	0.2061
C16:0	0.5210	0.9287	0.2928
C18:1	0.9007	0.9733	0.6675
C18:2	0.5954	0.8254	0.1993
CI8:3	0.9203	0.4729	0.6437
TOTFAT	0.5729	0.9420	0.2252
	High N		
C12:0	0.0237**	0.2172	0.0306**
C14:0	0.5269	0.0195**	0.0428**
C16:0	0.2289	0.8053	0.3871
C18:1	0.1772	0.8768	0.9251
C18:2	0.0594*	0.3603	0.6587
CI8:3	0.1646	0.4981	0.9929
TOTFAT	0.0836*	0.8983	0.9844

[a]High or Low N soil indicates addition of nutrient solution with or without ammonium nitrate; *, **, = $p \leq 0.10$, and 0.05, respectively.

Appendix A-3. ANOVA of elemental concentrations in 3rd generation wheat grains generationally-exposed to *n*CeO_2 at low or high N soil.

Element in grain	Parental Exposure (C_1C_2 vs. T_1T_2)	Current Exposure (C_3 vs. T_3)	Interactions
Low N			
Ca	0.7162	0.0360**	0.0364**
K	0.9211	0.0084***	0.4888
Mg	0.3702	0.8391	0.8935
P	0.8510	<0.0001***	0.4608
Fe	0.8587	0.0168**	01773
Mn	09397	0.0076***	0.0781*
High N			
Ca	0.6092	0.2065	0.1837
K	0.1047	0.7551	0.5799
Mg	0.2031	0.1539	0.8247
P	0.4177	0.2139	0.4703
Fe	0.0324**	0.0213	0.0743*
Mn	0.3840	0.1101	0.3089

[a]High or Low N soil indicates addition of nutrient solution with or without ammonium nitrate; *, **, *** = $p \leq 0.10$, 0.05, and 0.01, respectively.

Appendix A-4. ANOVA of elemental content in 3rd generation wheat grains generationally-exposed to *n*CeO_2 at low or high N soil.[a]

Element in grain	Parental Exposure (C_1C_2 vs. T_1T_2)	Current Exposure (C_3 vs. T_3)	Interactions
Low N			
Ca	0.9068	0.1247	0.1529
K	0.6816	0.3794	0.3942
Mg	0.9378	0.2635	0.1077
P	0.5208	0.0255**	0.3366
Fe	0.7868	0.01400**	0.0613*
Mn	0.4186	0.0144**	0.4857
High N			
Ca	0.1345	0.1232	0.0332**
K	0.0257**	0.5120	0.1886
Mg	0.0091***	0.1013	0.1554
P	0.0285**	0.1595	0.6719
Mn	0.0272**	0.0953*	0.7574
Fe	0.0122**	0.0378**	0.4237

[a]High or Low N soil indicates addition of nutrient solution with or without ammonium nitrate; *, **, *** = $p \leq$ 0.10, 0.05, and 0.01, respectively.

Appendix B: Other assessment and measurement used

Appendix B-1. Grain biomass yield of wheat previously exposed to *n*CeO_2 for two generations cultivated in soil.[a]

Grain yield	C_1C_2	T_1T_2
Low N	29.70 ± 0.85	30.17 ± 0.61
High N	29.17 ± 0.53	27.40 ± 0.98

[a]High or Low N soil indicates addition of nutrient solution with or without ammonium nitrate.

Appendix B-2. Yoshida nutrient solution component. It is the presence or absence of Ammonium nitrate constituent that determines high or low N.

Element	Reagent	Weight (g) 500mL solution
N	NH_4NO_3	45.70
P	$NaH_2PO_4 \cdot 2H_2O$	20.15
K	K_2SO_4	35.70
Ca	$CaCl_2$	44.30
Mg	$MgSO_4 . \cdot 7H_2O$	162.00
Mn	$MnCl_2 \cdot 4H_2O$	0.75
Mo	$(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$	0.04
B	H_3BO_3	0.47
Zn	$ZnSO_4 \cdot 7H_2O$	0.02
Cu	$CuSO_4 \cdot 5H_2O$	0.02
Fe	$FeCl_3 \cdot 6H_2O$	3.85
	citric acid (monohydrate)	5.95

Appendix B-3. Calculation on the concentration of N for high N soil.

Volume of nutrient solution stock = 4 L

Volume of nutrient solution added to soil throughout the cultivation period = 1.2 L

Volume of nutrient solution added to soil from 500 mL bottle = 5 mL

Molar mass of ammonium nitrate / AmN (i.e. form of N added) = 80 g mL^{-1}

Molar mass of 2N component of ammonium nitrate = 28 g moL^{-1}

Mass of ammonium nitrate = 45.70 g

Number of mole of N in ammonium nitrate = 2

Concentration of N added to soil = 96 mg =

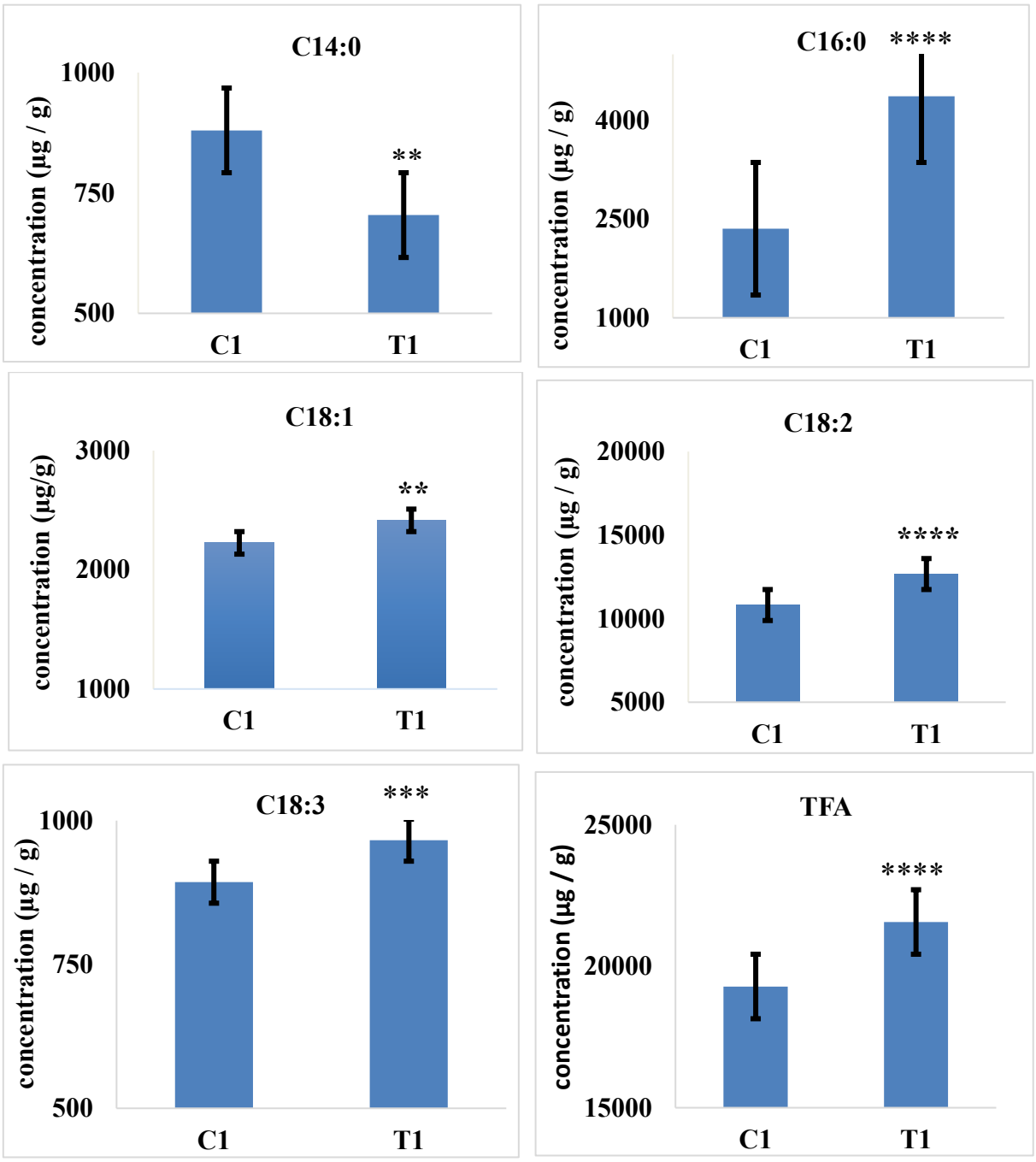
$$\frac{45.7 \text{ g} \times 1 \text{ mol of AmN} \times 5 \text{ mL} \times 2 \text{ mol of N} \times 28 \text{ g} \times 1.2 \text{ L}}{80 \text{ g} \times 500 \text{ mL} \times 1 \text{ mol of AmN} \times 1 \text{ mol of N} \times 4 \text{ L}}$$

High N = 96 mg

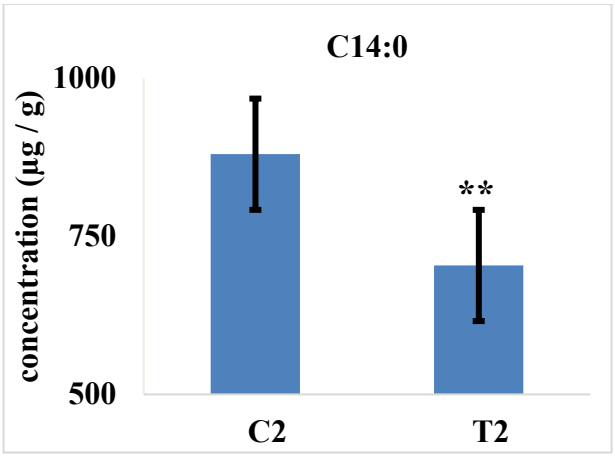
Low N = 48 mg

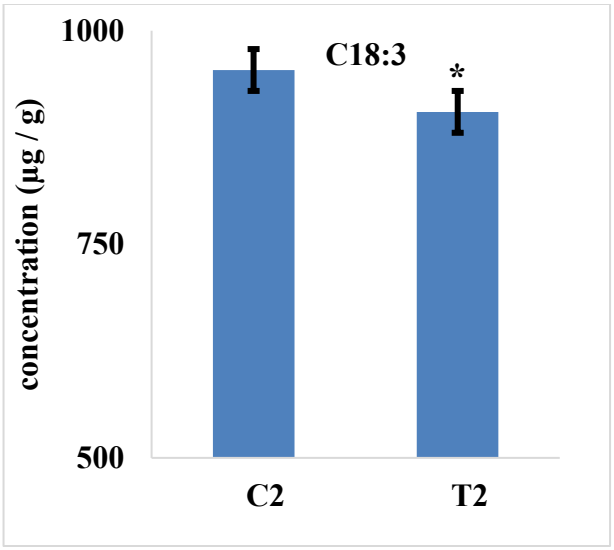
Appendix C: Bar charts of changes in measured quality parameter

Appendix C-1. Changes in fatty acid of second generation wheat grains (parental exposure). Values = means ± SE. *, **, ***,**** - indicate p ≤ 0.1, 0.05, 0.01 and 0.001 respectively.

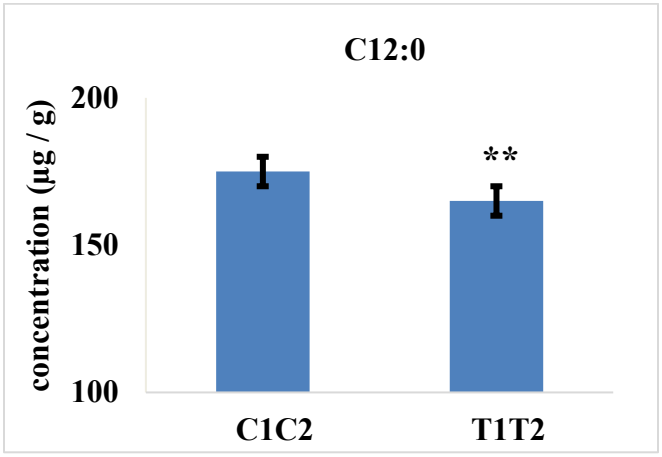


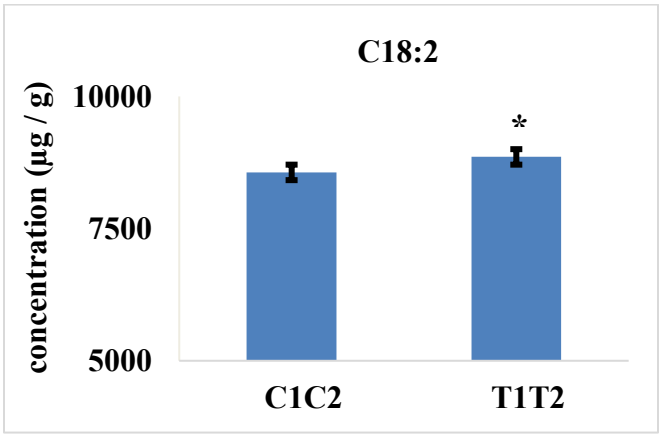
Appendix C-2. Changes in fatty acid of second generation wheat grain (current exposure).

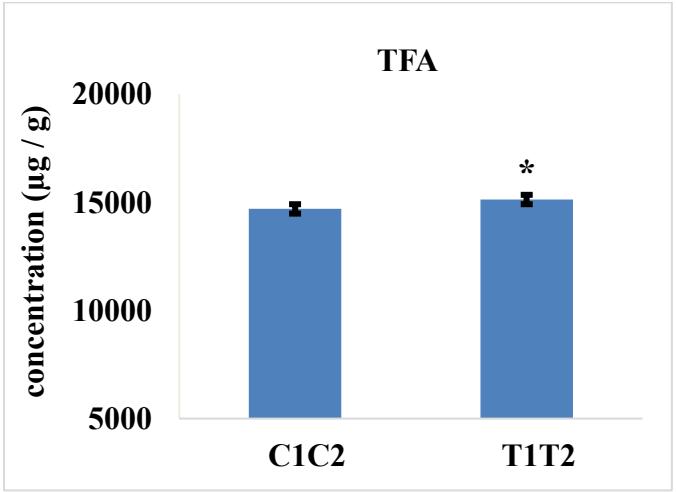

C14:0
1000
750
500
concentration (µg / g)
**
C2
T2


C18:3
1000
750
500
concentration (µg / g)
*
C2
T2

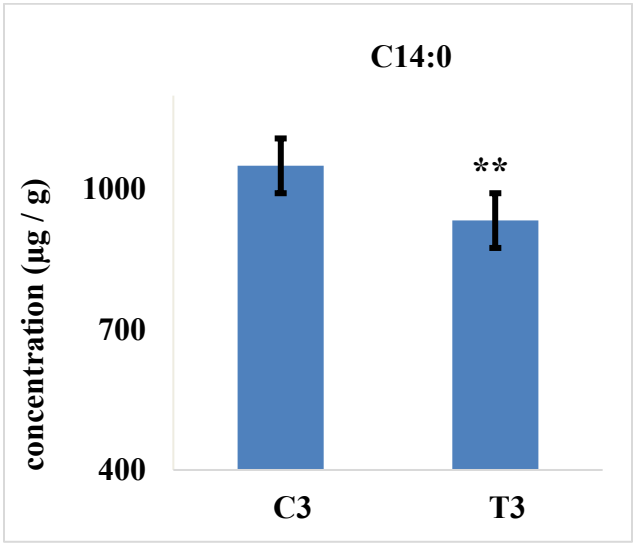
Appendix C-3. Changes in fatty acid of third generation wheat grains (parental exposure at high N).



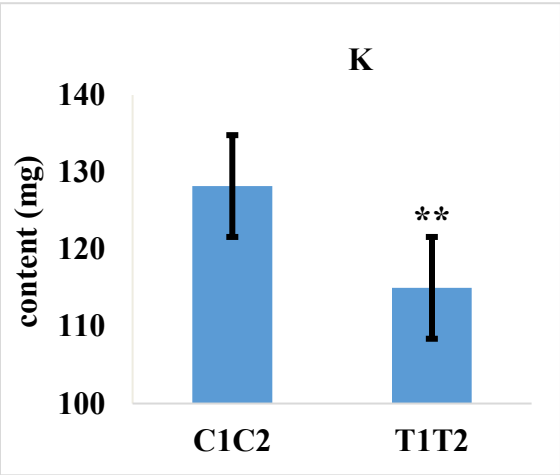


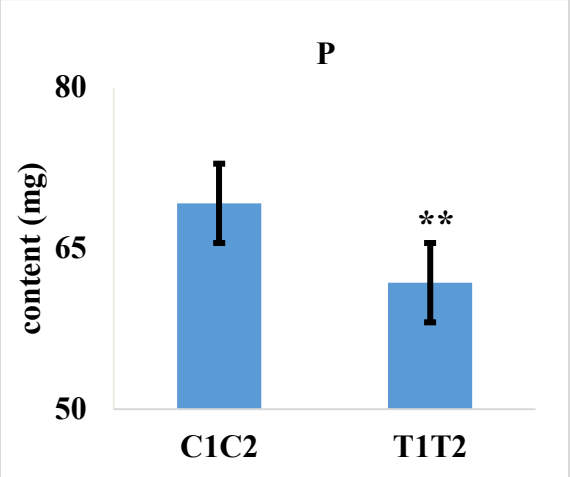


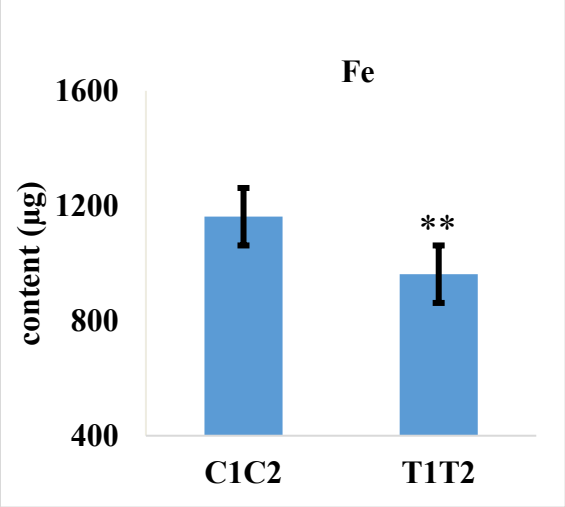
Appendix C-4. Changes in fatty acid of third generation wheat grains (current exposure at high N).

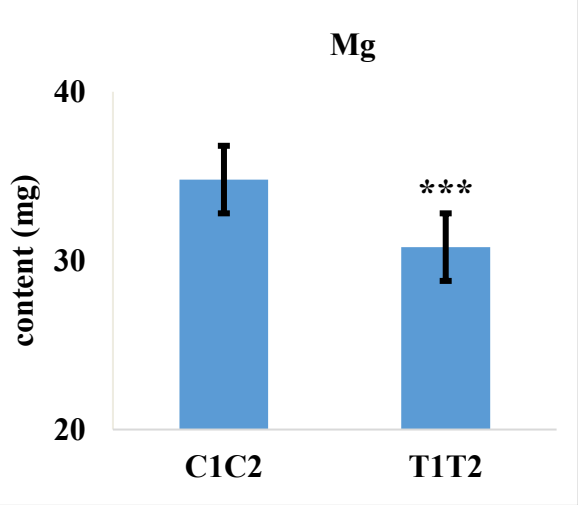


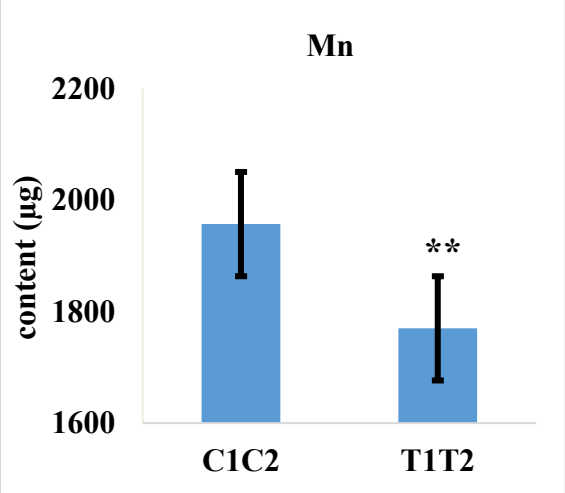
Appendix C-5. Changes in elemental content of third generation wheat grains (parental exposure at high N).



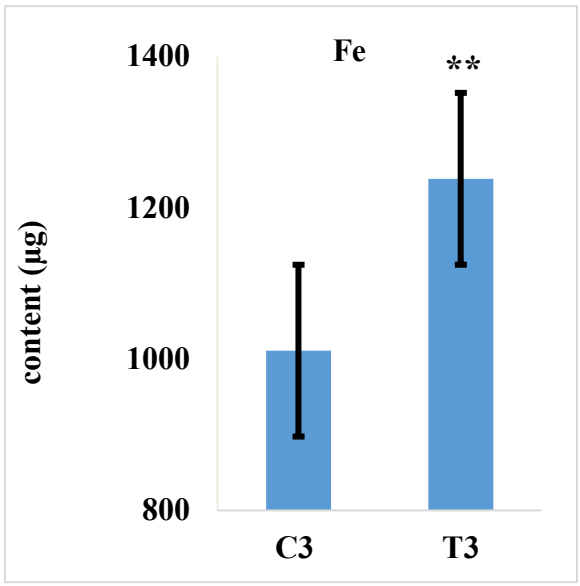


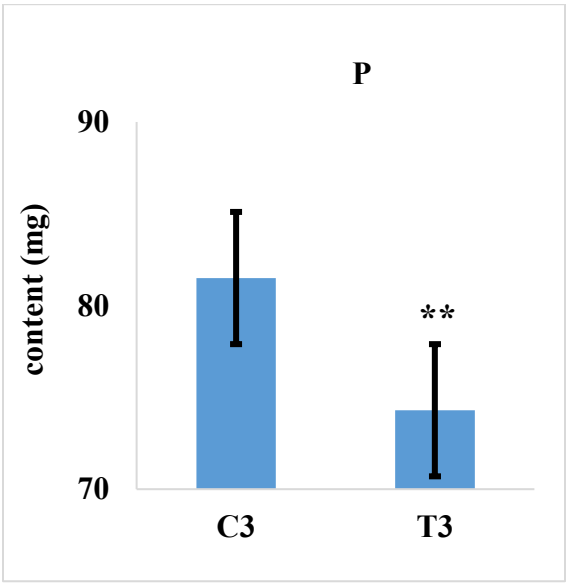


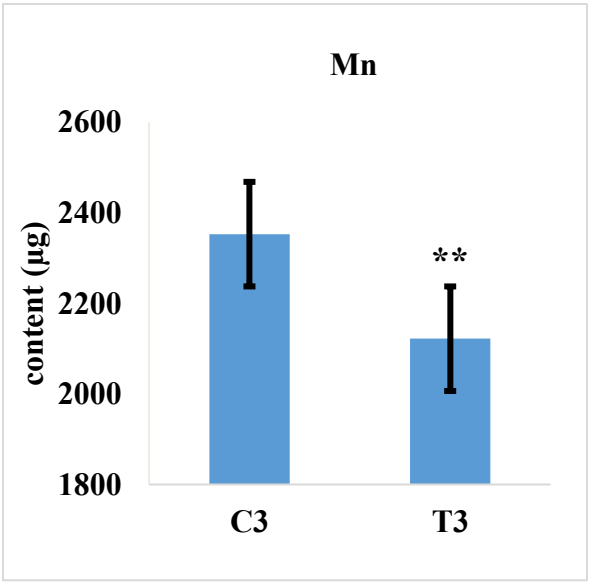




Appendix C-6. Changes in elemental content of third generation wheat grains (current exposure at low N).







Appendix C-7. Changes in elemental content of third generation wheat grains (current exposure at high N).