ZEPTER'S Super Tomato

The results of this study indicates a new scientific contribution to the understanding of light regulation of plant development as well as the effects of 3HFWC substance on tomato developmental processes. The significance of these results is reflected in the potential improvement of the technology of tomato production, ie obtaining fruits with increased lycopene content up to 200%.

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Nanotechnology and biotechnology are the two most promising scientific fields of this century. Biotechnology studies metabolic and physiological processes of biological systems. These two scientific fields are closely related and play a vital role in the development and application of many useful "tools" in the research of biological systems. Nanotechnology is a modern branch of science and economics that studies the creation, manipulation, and use of small molecule materials. The first postulates of nanotechnology were presented by Richard Feynman back in 1959, who presented the idea of the possibilities to manipulate individual molecules and atoms. The term nanotechnology was first defined by Professor Norio Taniguchi of Tokyo Science University as "a production technology to get the extra high accuracy and ultrafine dimensions, i.e. the preciseness and fineness on the order of 1 nm (10⁻⁹ m)."

Since then, the term has been constantly evolving, and it denotes everything from the science of atom and molecule manipulation to the synthesis of new life forms. This branch of science can provide classical, quantum and advanced nanomaterials. The benefits of nanotechnology are connected with the advances in medicine and pharmacology, with the development of new energy and water purification systems, with the development of information technologies, and with food production. Nanotechnology is a relatively new scientific field, and an extensive research is needed to extract from it as much useful knowledge as possible for the benefit of humankind.

Many countries around the world are aware of the potential of nanotechnology in the agricultural sector and are developing programs that would reduce crop production costs and increase yields, protect the environment from overuse of herbicides and/or pesticides, and produce functional foods. The application of nanotechnology in agriculture is very promising, but there is still a huge time distance between discoveries and end products that have commercial significance. One of the most interesting areas of application of nanotechnology in agriculture is the production of nutritionally advanced plants with the improved properties of edible parts. Properties such as the composition of fruits or seeds, their taste, durability, or freshness, constitute output or consumer properties, which are a very interesting and economically important ground for modern nanotechnological manipulations. Nanotechnology, like all other modern technologies, carries many potential risks, problems, controversies, and unknowns.

With regard to biological systems, new insights that nanotechnology offers are expected to cause a shift in relation to the classical approaches to research, primarily in elucidating the phenomena manifested by biomolecules. During evolution, a large number of biological systems, compounds, and processes that function at the molecular and nanometer levels have developed, and their properties still cannot be surpassed by synthetic technologies. Therefore, nanotechnology needs to "learn from nature," that is, to understand the structures and functions of biological systems in order to develop useful nano-quantum-systems ("biomimicry").

Manipulation of matter at the atomic level is an inevitable consequence of continuous progress in the field of research in physics, chemistry, and biology. Biologists are well acquainted with similar mechanisms described by molecular nanotechnology, such as programmed and self-replicating processes, which enable construction with atomic precision. Biology is a definite proof of the existence of nanotechnology, because biological systems, even when macroscopic, are basically nano systems, because the diameter of the basic DNA strand in human chromosomes is only 2 nm. In recent decades, a great deal of research has been devoted to the interaction of plants with nanomaterials (created atom-by-atom) and nanoparticles (shredding existing macroscopic material to nano levels or creating a "bunch of atoms" of the same or different atoms), as well as to the development of a new discipline – phytonanotechnology.

Priorities in this research include the development of intelligent systems that would enable controlled absorption, transport, and accumulation of nanomaterials and/or nanoparticles, an increase in their efficiency in modulating plant growth, development, and metabolism, as well as a reduction of the effects of phytotoxicity. The effects of a large number of nanomaterials and nanoparticles on the development processes in different plant species has been a subject of numerous investigations. Scientific studies have tried to shed light on the ways and pathways of assimilation of nanomaterials and/or nanoparticles, their impact on cell structure, and plant physiology (Figure 1).



Figure 1. Interaction of nanomaterial (NM) and plant.

A plant cell has a cell wall – a specific barrier that regulates the entry of nanoparticles into the plant organism. This mechanism has not yet been elucidated, but it is known that the entry of nanoparticles into plant cells depends on their physicochemical characteristics, size, shape, and surface charge. A plant can absorb nanoparticles through the root system or through the leaves. Their transport to other plant organs is based on very complex mechanisms that require the formation of complexes with transport proteins of the cell wall or membrane, cytoplasm, and nucleus.

Nanoparticles in the plant organism increase the growth parameters in all stages of plant development. These changes take place at the cellular, physiological, and molecular levels through changes in cell structure, plant transpiration, increased photosynthetic activity, and the production of specific biologically active substances. Plant seeds have built-in instructions in their genetic material for manipulating atoms and molecules. Based on the DNA data, ribosomes are involved in protein production and energy collection, collecting atoms from the local environment, and possibly making more proteins.

A lot of research is focused on the development of "intelligent synthesis", which should enable the positioning of atoms in the right places, thus enabling the creation of biologically active substances with improved properties. We should be cautious about the use of nanoparticles in plant breeding, because if nanoparticles are not compatible with and complementary to biomolecules, i.e. safe to use, then the possibility of side effects increases, which can affect the yield and quality of plant products.

Fullerenes are the third allotrope of carbon, the other two being graphite and diamond, and they belong to the family of carbon clusters. They are made of five- and six-membered carbon rings, with 12 pentagons closing the structure. Their general formula is $C_{20} + 2m$, where m represents an arbitrary number of hexagons. Fullerene molecules differ in size, i.e., in the number of carbon atoms.

The most famous is certainly the C_{60} molecule, whose structure is composed of 20 hexagons and 12 pentagons with the shape of a truncated polyhedron. This unique molecule, which is built of only one chemical element in the form of a spherical cage, shows the duality of behavior of waves and particles, i.e., it has the properties of both an electromagnetic wave and a particle. Due to its stability and ability to bind and release electrons easily, as well as to its optical properties, it is widely used in various scientific disciplines.

Of the newer nanomaterials, it is important to mention fullerols, polyhydroxylated soluble

derivatives of fullerene, that have antioxidant, catalytic, toxic, and antitoxic properties. These molecules are very interesting for scientists because of their photoactivity. Fullerols are known as powerful antioxidants due to their ability to "scavenge" free radicals. In the last decade, there have been a number of scientific studies describing the effects of fullerols on the processes of plant growth and development. Some of these studies have shown both negative and positive effects on plant development. In onion plants, fullerol caused cell damage, but in Arabidopsis it stimulated the growth of seedlings by as much as 40%. It is assumed that its beneficial effect on the increase of biomass and metabolites in plants is related to the antioxidant properties of this molecule. There are data that fullerols have a positive effect on the germination of tomato seeds because they stimulate faster water absorption and accelerate the transport of nutrients, which results in an increase in plant biomass. There are currently no data on the possible mechanisms of the influence of water-soluble fullerol on physiological and biochemical processes in plants.

The tomato (*Solanum lycopersicum L.*) is one of the most widespread vegetable crops that is consumed around the world both fresh and processed. This plant species is of great importance in human nutrition and is believed to have positive effects on health. Tomato fruit is rich in dietary fibers, which are mostly found in the epidermal cell walls and include indigestible carbohydrates and lignin. Dietary fibers can be water-soluble, such as pectins, β -glucans and galactomannans; indigestible oligosaccharides, such as inulin; or water-insoluble, such as cellulose, hemicellulose and lignin. All these fibers have different roles in disease prevention.

Insoluble fibers regulate bowel function and water absorption. Soluble fibers, which form solutions, can influence the regulation of blood glucose levels after eating by reducing digestibility and absorption of carbohydrates with a high glycemic index. In addition, soluble fibers have the effect of reducing the levels of total and LDL cholesterol in the blood. Therefore, dietary fibers can reduce the risk of many chronic diseases, including cardiovascular disease, obesity, diabetes, and various types of cancer. Dietary fibers are carriers of other biologically active substances as well, such as vitamins, carotenoids, and polyphenols. Fibers isolated from the tomato skin are rich in antioxidants, of which lycopene is dominant, as well as minerals, such as potassium, magnesium, and calcium, while having a low content of sodium, iron, and zinc. This sodium-to-potassium ratio makes tomato a very important food that has a protective effect in preventing cardiovascular diseases.

Fresh fruits, as well as tomato-based foods are rich in vitamins A, C, and E. In addition to the nutrients mentioned above, the tomato also contains various molecules of primary and secondary metabolism, carotenoids, and phenolic compounds, which are effective natural antioxidants. Lycopene is a plant pigment that gives fruits and vegetables a red color. The most important sources of lycopene are the tomato and watermelon. Since lycopene is a lipophilic compound, a higher percentage of lycopene is found in tomatoes thermally processed in oil (ketchup, tomato sauce) than in fresh fruits. The strong red color of lycopene protects tomatoes from aggressive UV rays. The recommended daily intake of lycopene has not yet been precisely established, but many studies recommend at least 3-6 mg per day in order to achieve optimal positive effects on human health.

It is very difficult, however, for the body to get the required amount of lycopene from food. In modern living conditions, most people do not consume enough fresh fruits and vegetables, so their body lacks necessary antioxidants. Because of this, people who do not take enough lycopene through their diet are recommended to increase their daily intake with dietary products that contain lycopene. Today, there are many clinical studies that have proven that high daily intake of lycopene provides protection against various types of diseases. Due to its lipophilic character, lycopene tends to settle in tissues, primarily in the prostate, liver, and adrenal glands. By increasing lycopene levels in tissues, oxidative damage to biological systems is reduced, including damage to cell membranes and other structures, such as DNA molecules, lipids, and proteins. Free radicals damage these cellular structures and molecules.

Free radicals originate from various pollutants, solar and ionizing radiation, some drugs, tobacco smoke, stress, and/or big physical effort. Also, the body itself produces free radicals in the metabolism of fatty substances, as well as part of the normal immune response. Lycopene and other antioxidants bind free radicals and neutralize their harmful effects, preventing tissue damage. Many studies indicate that lycopene is the most powerful "scavenger" of free radicals among carotenoids. Increased levels of lycopene in adipose tissue cells contribute to the improvement of the overall antioxidant status of the whole body, which is very important for reducing the risk of heart attack, cancer, and other diseases.

Lycopene greatly reduces the harmful effects of UV radiation on the skin because it helps protect the skin from both short-term harmful effects (redness, erythema) and long-term ones (skin cancer). Beta-carotene is often used in combination with lycopene as an oral sunscreen. Vitamins E and C, in synergy with lycopene and beta-carotene, contribute to preserving the vitality, elasticity and natural freshness of the skin. There is a growing body of research worldwide that shows that a diet rich in vegetables, or an increased intake of plant antioxidants, such as carotenoids, reduces many risks of cardiovascular disease, cancer, and other disorders related to aging.

Antioxidants are molecules that can successfully remove prooxidants while creating products that are non-toxic and non-damaging to the cells. Antioxidants protect the body from prooxidative action in several ways: by inhibiting the formation of free radicals, reducing the oxidative capacity of prooxidants, and inhibiting oxidative enzymes. Therefore, the function of antioxidants is to neutralize free radicals and protect cells from their toxic effects, thus preventing the appearance and development of diseases related to oxidative stress. Antioxidants are produced in the cell or are taken into the body through food or supplements. They work by preventing the formation of new free radicals in the body, destroying the radicals already created in the body (as "scavengers"), or repairing damage to the cell caused by their action.

According to their composition, antioxidants are divided into enzymes that repair DNA and protein molecules, proteins (ferritin, albumin, transferrin, lactoferrin), or small molecules (vitamin E, carotenoids, vitamin C, glutathione, alpha-lipoic acid, ubiquinone, flavonoids) damaged by free radicals. According to their mode of action, antioxidants can be divided into primary, secondary, and tertiary antioxidants. Primary antioxidants are also preventive, because they prevent the formation of free radicals (albumin, transferrin). Secondary antioxidants (scavengers) remove the free radicals that have already been formed (superoxide dismutase, catalase, glutathione peroxidase, glutathione, vitamin C, vitamin E, carotenoids, flavonoids). Tertiary antioxidants (enzymes) repair the damage or remove the biomolecules damaged by radicals before their accumulation causes new damage (phospholipases, proteases, peptidases, DNA polymerases).

Antioxidant activity depends on many factors, the most important of which are bioavailability, oxidative potential, the rate of reaction with free radicals, stability, and low reactivity of the resulting antioxidant/free radical derivative. Antioxidants from food are defined as molecules that can "cleanse" free radicals and stop the chain reactions they cause. Due to the fact that the antioxidant components of some plant species are used in human nutrition, there is a growing trend all over the world to produce functional foods. Functional food is any whole, enhanced, enriched, or improved food that, in addition to satisfying energy needs and the intake of necessary nutrients (for example, vitamins and minerals), provides prevention from nutrition-related diseases, also improving physical fitness and mental health of individuals. Given that lycopene is the main ingredient of the tomato fruit that has a functional effect, it would be significant to improve

its synthesis by applying modern methods of nanobiotechnology.

Researchers from the Institute for Biological Research had a great pleasure to conduct a scientific study funded by the "ZEPTER IN-TERNATIONAL LLC" company with the aim of investigating the effects that three types of light in combination with 3HFWC substance based on furellol and aqueous layers strong hydrogens bonds (patent PCT/EP2019/083307) exert on the growth and development of tomato plants (Figure 2).

The results of this study indicate a new scientific contribution to the understanding of light regulation in plant development, as well as the effects of 3HFWC substance on the tomato developmental processes. The significance of these results involves a potential improvement of the tomato production technology, i.e., in obtaining fruits with increased lycopene content up to 200%. The results of our study have shown that the exposure of tomato plants to sources of polarized and hyperpolarized light, individually or in interaction with the 3HFWC substance, led to significant changes in their growth, development, and metabolism. The responses of tomato plants to these treatments differed depending on the ontogenetic phase of the development. In the seedling development phase, biomass increase is successfully stimulated by polarized and hyperpolarized light sources in the presence of 3HFWC substance. There were also physiological changes in seedlings, which included the increase in the synthesis of the photosynthetic pigment chlorophyll. A significant increase in biomass production was achieved by controlled exposure of tomato plants to sources of polarized and hyperpolarized light, either individually or in the presence of 3HFWC substance.

The applied treatments were equally effective in stimulating flower formation. The greatest contribution of this study is that it indicates that the use of hyper-harmonized light and 3HFWC substance can induce an increase in lycopene synthesis in fruits. These results undoubtedly confirm the enormous potential of the newly synthesized 3HFWC nanosubstance produced at TFT Nano Center (ZEPTER GROUP) for improving the nutritional value of tomato fruits and creating a new functional food product.



Figure 2. Effects of three types of light and 3HFWC substance on the tomato growth (38 - 71 %) and fruit development (120-200%)