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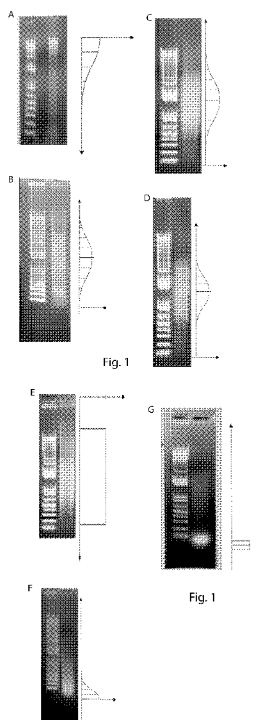
(54) **Title:** USE OF NUCLEIC ACIDS AS FERTILISERS AND STIMULANTS



(57) Abstract: The present invention relates to the use of nucleic acids, such as DNA and RNA, of a species as fertilisers and stimulants for a different species phylogenetically distant from the one from which the nucleic acids used were obtained.

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USE OF NUCLEIC ACIDS AS FERTILISERS AND STIMULANTS

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The present invention relates to the use of nucleic acids as fertilisers and stimulants. In particular, the present invention relates to the use of nucleic acids, such as DNA and RNA, of a species as fertilisers and stimulants for a different and phylogenetically distant species in comparison to the one from which the nucleic acids used were obtained.

It is well known that the human population must deal with a growing demand for food due to the increase in the world population. At the same time, the increase in climatic, biotic and abiotic stresses are causing a crisis in world food production. Furthermore, the price of chemical fertilisers is increasing while their availability is decreasing.

The world population has recently surpassed 8 billion inhabitants, and it is estimated that it will reach 9.7 billion by 2050. FAO estimates of 2009 predicted that the consequent demand for food in the year 2050 would be such as to require a 70% increase in global food production compared to 2009 (FAO (2009) How to Feed the World in 2050). A recent meta-analysis conducted by researchers of Wageningen University (van Dijk, et al. (2021)) estimated that the percentage increase in the demand for food due to the population increase, economic development and urbanisation will fluctuate between 35% and 56%. Therefore, one of the great challenges for the future will be a sufficient food production for a population undergoing marked growth, a challenge that is very complex from various standpoints.

Together with the growing demand for food production, several emerging problems are becoming increasingly felt and serious, such as the stresses due to climate change, biotic and abiotic stresses, the simultaneous increase in the prices of chemical fertilisers and the availability thereof.

The development of synthetic chemical fertilisers, particularly those based on nitrogen and phosphorus, has revolutionized modern agriculture in the last two centuries. Together with the improvement in the variety of crops and in the methods for controlling diseases and parasites, the development of fertilisers has given rise to a considerable increase in harvests. In recent years, the supply and use of fertilisers has becoming increasingly costly due to various geopolitical factors, the depletion of resources and the growing global demand for mineral fertilisers. The

prices of fertilisers have increased by nearly 30% since the beginning of 2022, after an 80% increase last year (April 2022 edition of the World Bank Commodity Markets Outlook (World Bank Commodities Price Data)).

Furthermore, it has been known for some time that the excessive use of fertilisers causes problems of pollution and environmental degradation (Savci, 2012). For this reason, legal regulations limit the use of chemical fertilisers in agriculture. Plant biostimulants (BPs) can play a fundamental role in addressing the challenges of sustainability, because they can reduce dependency on fertilisers, in particular on the use of chemical products from outside the farm (Chiaiese et al., 2018).

In addition to the problem of chemical fertilisers, climate change and abiotic stresses, such as drought, high salinity, increasingly marked thermal stresses, extreme temperatures and a lack of nutrients, are further serious challenges that modern agriculture must face. These stress factors can significantly reduce crop yields and compromise the quantity and quality of products, leading to food insecurity and economic losses for farmers. In addition to abiotic stress, crops are also vulnerable to biotic stress factors, such as parasites and diseases, which can be further exacerbated by climate change.

Conventional farming practices have constantly been based on the use of non-renewable products such as fertilisers and pesticides. Though their introduction has enabled substantial progress for humanity (Cooper and Dobson, 2008), agrochemical products also represent a serious, unresolved threat to human health and the environment (Fenner et al., 2013). Considering global demographic pressure on agricultural production, a more sustainable approach is necessary to satisfy the growing demand for plant biomass intended for human consumption, animal feed and energy production. Furthermore, recent studies have shown that climate change is potentially negatively impacting global crop yields, and it is predicted that its effects will increase further (Ray et al., 2019).

The most promising solution for sustainable crop production for the future is the use of natural biostimulant products, whose function, as also defined by the EBIC (European Biostimulant Industry Council) and the new European Fertilising Product Regulation, is the following: "a product stimulating plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient

use efficiency, tolerance to abiotic stress, quality traits or availability of confined nutrients in soil or rhizosphere". Furthermore, biostimulants are distinguished from fertilisers in that they do not directly provide nutrients to the plant (as in the case of fertilisers), but rather act by improving, stimulating and increasing some physiological and metabolic processes taking place during the life cycle.

The growing demand for food combined with the increase in the risks of failed harvests due to climate changes and the necessity of reducing and optimising the use of chemical fertilisers requires the development of new sustainable approaches.

In recent decades, various methods for improving the sustainability of the agricultural production systems through the reduction of pesticides and synthetic fertilisers have been proposed. A promising, environmentally friendly solution is represented by the use of natural plant biostimulants which have been demonstrated to directly improve crop productivity and efficiency in the use of nutrients, in addition to being capable of improving tolerance towards a wide range of stress factors, both biotic and abiotic (Rouphael and Colla, 2020). In particular, among the various biostimulants, microalgae have been receiving growing attention. Various published scientific papers present the use of microalgae in agricultural production and, in particular, the advantages in the treatment of seeds, and foliar and root applications which provide, for example, better rooting, higher yields, tolerance to drought and salinity and resistance to pathogens (González-Pérez et al., 2021).

Biostimulants are thus natural or synthetic substances that improve the growth and development of a species, such as, for example, a plant species, increase the uptake of nutrients and tolerance to abiotic stress and improve the overall health of the species, for example of a crop. Biostimulants can help plants to cope with abiotic stress by stimulating physiological and biochemical processes which increase plant resilience. For example, some biostimulants can promote root growth and development and thus help plants to access the nutrients and water in soil which, at present, is less fertile and/or subject to water stress. Other biostimulants can increase the production of antioxidants, which help to protect plants from oxidative stress caused by high temperatures or drought (Rouphael and Colla, 2020). Furthermore, biostimulants can improve efficiency in the use of nutrients in the soil, thus reducing the need for synthetic fertilisers. By improving the uptake of nutrients and promoting an efficient use thereof, biostimulants can

contribute to reducing the runoff and washout of fertilisers, which can have negative impacts on the environment and human health (Xu et al., 2022).

In the light of the foregoing, there is an evident need to provide novel biostimulant products and methods and natural fertilisers which overcome the disadvantages of the known products and methods.

The present invention fits into this context; it has the aim of providing novel products and methods which are more efficient in stimulating and enhancing the performances of current biostimulants.

It is known that fragmented DNA produces an inhibitory effect on a species from which the DNA is derived and on a phylogenetically similar species, i.e. one having a similar genome (Mazzoleni et al., 2015; WO2014/020624). In other words, recent results have demonstrated a species-specific inhibitory effect of self-DNA fragments. In particular, the inhibitory effect of self-DNA fragments has been demonstrated in various living organisms belonging to different kingdoms, including plants, algae, bacteria, fungi, protozoa, and insects.

According to the present invention, it has now been found that a mixture of random fragments of nucleic acids (DNA or RNA) of a species, or a composition comprising said mixture, has a biostimulant and fertilising effect for a target species which is different from the species to which the nucleic acid fragments belong. According to the present invention the species (i.e. the species to which the nucleic acid fragments belong, or the target species) can comprise, for example, a plant, an alga, a microorganism such as a bacterium, a microalga, or a cyanobacterium. In particular, according to the present invention, alongside a fertilising effect, a very evident and efficacious biostimulant effect has surprisingly been observed when the target species (for example a plant) is exposed to a mixture of nucleic acid fragments of one or more different species (for example a mixture of DNA fragments originating from microalgae and/or cyanobacteria). The biostimulant effect is even greater and more efficient when the mixture of random nucleic acid fragments comprises fragments of a length from 50 bp to 15000 bp. Even more preferably, greater biostimulant and fertilising effects are observed when the mixture of random nucleic acid fragments has a size distribution of the fragments of nucleic acid (for example DNA) indicated, according to the present invention, as a “broad-range” distribution, as defined further below.

The mixture according to the present invention is capable of increasing

production, of helping a plant to overcome biotic and abiotic stresses, and of stimulating organisms such as plants and microorganisms to use fewer nutrients. Therefore, the present invention provides an important contribution in germination, in germination vigour, in imparting greater vigour to the plant, in increasing production and harvest quality, in obtaining higher efficiency in the use of fertilisers, in reducing the use of chemical fertilisers, in increasing efficiency in the use of the fertilisers, and in increasing resistance and resilience to biotic and abiotic stresses. The object of the present invention thus constitutes an advancement in the known technique and methods as an efficient solution for confronting the abovementioned problems.

The use of random fragments of nucleic acids, such as, for example, total DNA, as a fertiliser and stimulant in agriculture, as opposed to the existing techniques and products, has significant advantages. A first advantage is given by the use of natural products. A further advantage is given by the absence of toxicity from the use of nucleic acids, which, being primary metabolites, ubiquitous in nature, do not cause any type of environmental contamination. A further advantage derives from the fact that nucleic acids such as DNA, for the proposed uses, can be obtained by extraction from natural material or by synthesis based on extraction and synthesis techniques, as well as amplification techniques, by now widely disseminated and well established in molecular biology. Significant but non-limiting examples of possible production techniques include the dissolution of cell tissues, inactivation of cellular nucleases and recovery of nucleic acids from a solution containing a biological lysate. Furthermore, according to non-limiting examples, the production of nucleic acids can be achieved starting from residual biomasses, waste from natural processes, agro-industry waste and residual crop residues, in which to carry out an industrial process of industrial production by extraction of nucleic acids, mixtures of nucleic acid fragments, or mixtures of biomasses with a high nucleic acid content. Furthermore, the nucleic acids can also be synthesised from template molecules or *de novo* with various approaches known to the person skilled in the art. The nucleic acid molecules can be amplified, thus producing multiple copies identical or similar to the template considered, for example by cloning or PCR-based techniques. PCR variants are useful for the purpose of producing the active ingredient to be used, as they ensure the random amplification of nucleic acid fragments from a template sample. For example, an approach of the random

amplified polymorphic DNA (RAPD) type is well suited to our purpose as, unlike classic PCR, it does not require knowledge of the starting template DNA sequence.

Furthermore, as regards the processes for producing microorganisms (such as plants, bacteria, algae, cyanobacteria, and fungi) in bioreactors, photobioreactors and hydroponic and microalgae culture tanks, the addition of solutions of randomly fragmented nucleic acids according to the present invention enables higher production yields.

As mentioned above, the fertilisers and biostimulants of the present invention can allow for a drastic reduction in nitrogen fertilisers (N) of synthetic origin and phosphate fertilisers (P) of synthetic origin. The applicant has in fact carried out stimulation tests with the products of the present invention, in which the stimulation under conditions of deprivation of nitrogen fertilisers (N) and phosphate fertilisers (P) has been verified (N and P deficiency trials). The present invention thus enables a more efficient use of soil nutrients by the target organisms, thereby making possible a drastic reduction in fertilisers, in the range of 30% to 70%, the harvest yield and production being equal.

Furthermore, the applicant has verified that the nucleic acid composition as per the present invention can be mixed with crop protection products of chemical origin, such as pesticides, fungicides, and herbicides, giving a better result in terms of the protection of crops, of the treated seeds, of cultivation, and of the final quality of the harvested product, while also enhancing the efficiency and effects of the crop protection products in question.

Therefore, a specific object of the present invention is the non-medical use of

a nucleic acid of a species, the source species or
of a composition comprising said nucleic acid of a species
as a biostimulant and/or fertiliser for a target species, wherein
the source species is a species that is different from the target species.

More specifically, according to the present invention the nucleic acid can be selected from DNA or RNA or a mixture of DNA and RNA. According to some embodiments of the present invention, the composition according to the present invention does not comprise RNA, for example it does not comprise small RNAs (small RNA molecules), such as siRNA or miRNA, or it comprises a mixture of DNA and RNA.

Therefore, according to the present invention, the fertilising and/or biostimulant effect is provided by the nucleic acid as defined above, preferably by DNA. The nucleic acid can be genomic DNA contained in a cell of the source species, and which can thus be obtained by breaking the cell itself, or it can be a mixture of genomic DNA and DNA secreted by the live cell. Breakage of the cell can be induced from outside or can take place following the natural death of the cell. According to the present invention, no selection is made of portions of DNA or RNA of the source species or of any fragments of secreted DNA or secreted RNA. Therefore, genomic DNA or genomic RNA means the total genomic DNA or total genomic RNA of the source species and secreted DNA or secreted RNA means the set of fragments of DNA or RNA secreted by the source species. Furthermore, according to the present invention it is not necessary to use any specific construct in order to use the nucleic acids.

According to the present invention, the nucleic acid can be whole or fragmented. The whole nucleic acid, or in any case nucleic acid fragmented into large-sized polynucleotide fragments, may be further fragmented due to natural, and hence random, degradation on the site of application. In such a case the fertilising and/or biostimulant effectiveness will not be immediate, since it will be obtained gradually as the nucleic acid is fragmented and will be prolonged over time. The nucleic acid can be fragmented randomly in order to obtain a mixture of fragments of different sizes. The fragments can be natural or synthesised. The fragments can be obtained by means of known methods; the nucleic acid can be amplified and then fragmented by means of procedures of a physicochemical type, for example sonication, a thermal treatment, such as combustion, pyrolysis or enzymatic degradation, and the fragments obtained can optionally be amplified. The biostimulant and/or fertilising effect according to the present invention is not performed by one or more specific small RNAs, such as siRNA or miRNA. Should the nucleic acid fragments used according to the present invention unintentionally comprise some small RNAs, such as siRNA and/or miRNA, these will not be used in percentages sufficient to allow them to perform any biostimulant and/or fertilising effect, whereas the biostimulant and/or fertilising action will be performed by the set of fragments of randomly fragmented nucleic acid.

According to embodiments of the present invention, the nucleic acid can be used in the form of a mixture of fragments of nucleic acid from the source species

having different length, and greater than 10 bp, preferably greater than 50 bp. According to the present invention, use can also be made of fragments of a greater length, for example of a length greater than 1000 bp or 100000 bp or greater than 1000000 bp.

According to some embodiments of the present invention, the mixture of fragments can comprise fragments of a length from 10 bp to 1000000 bp, from 10 bp to 100000 bp, from 10 bp to 15000 bp or from 50 bp to 15000 bp.

According to a further embodiment of the present invention, the mixture of fragments can comprise fragments with a distribution, referred to in the examples as a “broad-range” distribution, of the fragment lengths and of the percentage of fragments of each length selected from:

uniform across the whole range of fragment lengths;

Gaussian across the whole range of fragment lengths;

half-normal across the whole range of fragment lengths. According to a preferred embodiment of the present invention, the mixture of fragments can comprise fragments of a length from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp, with a (broad-range) distribution of fragment lengths and of the percentage of fragments of each length selected from:

uniform in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp;

uniform in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp;

Gaussian in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp;

Gaussian in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp;

half-normal in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp; or

half-normal in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp.

According to a further embodiment of the invention, the mixture of fragments can comprise fragments of a length from 10 bp to 200 bp with a distribution (referred to in the examples as “short-range” distribution) of the fragment lengths and of the percentage of fragments of each length selected from:

uniform in the whole range from 10 bp to 200 bp;

uniform in a range selected from 10 bp and 50 bp or 10 bp and 100 bp;

Gaussian in the whole range from 10 bp to 200 bp;

Gaussian in a range selected from 10 bp to 50 bp or 10 bp to 100 bp;

half-normal in the whole range from 10 bp to 200 bp;

half-normal in a range selected from 10 bp to 50 bp or 10 bp to 100 bp.

According to the present invention, both in the broad-range distribution and in the short-range distribution, the nucleic acid fragments can preferably be DNA fragments. The length of the fragments of nucleic acid, such as, for example, DNA, can be measured, for example, by gel electrophoresis.

The term “uniform” indicates that the fragments of the mixture have a different length from each other in the specified range of lengths and that the fragments of a different length are present in an equal percentage. The term “Gaussian” or “half-normal” indicates that the fragments of the mixture have a different length from each other in the specified range of lengths and that the fragments of a different length are present in a different percentage according to a Gaussian or half-normal distribution.

According to one embodiment of the present invention, when use is made of a mixture of nucleic acid fragments in which the nucleic acid is RNA, the length of the fragments is greater than 200 bp or nucleotides (nt); therefore, the ranges of length described above will have, as the lower limit, a length greater than 200 bp or nucleotides.

According to the present invention, the source species must be different from the target species, i.e. it must be a species that is phylogenetically distant from the target species, such that the nucleic acid (DNA or RNA) of the source species does not have an inhibitory effect on the target species. For example, the source species can belong to a taxonomic order that is different from the taxonomic order of the target species.

According to a preferred embodiment of the present invention, the source species can belong at least to a taxonomic order differing from that of the target species.

For example, according to the present invention, the source species is an alga, a microalga or a cyanobacterium and the target species is a plant.

According to embodiments of the present invention, the source species or the target species can be selected in the group consisting of a plant, an animal organism, for example an insect or fish, a fungus, an alga or a microorganism, such as a microalga, a bacterium, or a cyanobacterium.

5 According to the present invention, when the microorganism is a microalga, the microalga can be selected in the group consisting of *Arthropsira platensis*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Clorella vulgaris* or a mixture of said microalgae. For example, said microalgae can be used as a source species, i.e. as a species whose nucleic acid, such as DNA, RNA or mixtures of DNA and RNA, is 10 used as a biostimulant and/or fertiliser according to the present invention.

Therefore, the present invention can be advantageously used in agriculture either in soil or out of soil, for example in hydroponic culture tanks. Furthermore, the present invention can be advantageously used to increase the yields of processes for the production of microorganisms in bioreactors, photobioreactors or in 15 hydroponic culture tanks.

According to the present invention, the nucleic acid (whole or fragmented), such as, for example, DNA, is in a form isolated from cells of the source species, i.e. it is not a nucleic acid still enclosed within the cell itself, but is rather a nucleic acid outside the cell. Said nucleic acid can be used in a purified form or in a non-purified form. 20 For example, the nucleic acid is in a purified form if obtained by extraction from cells of the source species and purified from these, or through production by synthesis. Alternatively, the nucleic acid can be used in a non-purified form, i.e. in the form of a composition comprising or consisting of a mixture of a nucleic acid, such as DNA, together with other cellular constituents of the source species from which it 25 originates, including dead cells of the source species. For example, a composition of this type can be produced by lyophilisation or drying of the cells of the source species, such as a plant, an animal organism, for example an insect or fish, a fungus, an alga or a microorganism, such as a microalga, a bacterium, or a cyanobacterium.

30 The nucleic acid or the composition according to the present invention can be advantageously used as a source of nucleic acids with broad-range or short-range fragmentation, to decrease soil fatigue in monocultures by increasing the DNA fragments of a species differing from the one cultivated and decreasing the DNA fragments of the same cultivated species which are inhibitory for the crop itself. In

this manner, it improves the balance, in percentage terms, of nucleic acids of the species differing from the one cultivated (stimulants) and nucleic acids of the same species as the one cultivated. The cultivated species is thus made stronger, healthier and more resistant to biotic and abiotic agents. Furthermore, said product or composition according to the present invention can be advantageously used as a source of basic oligonucleotides and polynucleotides ready for use by plants in cell division, quickly and efficiently, thus providing an effective fertiliser as well as a stimulant.

The composition according to the present invention can also comprise, in addition to the nucleic acid, micro elements and/or macro elements, such as, for example, copper, iron, magnesium, sulphur and/or potassium.

Furthermore, the composition according to the present invention can further comprise plant protection products (or crop protection products), such as, for example, pesticides, fungicides, herbicides, nematicides, miticides, arthropocides, and bactericides.

The composition can be formulated in a form selected in the group consisting of dispersion, suspension, wettable or soluble powders, emulsions in water or in other solvents, dispersible micronized powder, suspensions of microcapsules, emulsifiable concentrates, fluid pastes, macro emulsions, or oil dispersions. Therefore, the composition can also comprise excipients and/or adjuvants, other fertiliser and stimulant compounds that are different from the nucleic acids of the invention, chemical compounds with a function as binders, wetting agents, suspending agents, excipients, amendments, solvents, and surfactant agents, as well as other substances useful for the purposes of the application.

The nucleic acid and the composition according to the present invention can be administered to the target species by exposing the target species to the nucleic acid or to the composition according to the invention, for example by surface contact, cytotropic administration, systemic administration, for example by injection, ingestion or inhalation, root contact, leaf contact, fertigation, seed dressing, seed treatment, in bioreactors, in photobioreactors, in culture liquids, hydroponics, in soil, out of soil, or aeroponics.

Based on the foregoing, the present invention further relates to a method for fertilising and stimulating a target species (organism, such as a plant, or a microorganism), said method comprising or consisting of the following steps: a)

extracting a nucleic acid, for example the total DNA, of at least one source species differing from the target species; b) producing random fragments of said nucleic acid, for example total DNA, to obtain a mixture of random nucleic acid fragments; and c) placing the target species in contact with said mixture of random fragments.

5 A specific object of the present invention is also a method for the high-yield production of microorganisms in bioreactors or photobioreactors, or cultivation of plants both out of soil and in soil, characterised by stimulating growth in reactors or in hydroponic systems by adding compositions of nucleic acids with the above-described characteristics to the culture medium or to the plants. According to the 10 process of the invention, the nucleic acids are added to the culture medium or to the growth substrate by using guided or simple controlled addition techniques, in batches, semi-batches, continuous, with direct addition to the medium before the start, or continuously, or semi-continuously, by means of an external container of the mixture of DNA fragments added at the start or during growth. Furthermore, the 15 integration of fertigation systems in open fields or crop protection with systems for adding the nucleic acids constitute a further application of the present invention.

As shown in the experimental examples, compositions such as biomasses and/or solutions with a higher content of nucleic acids are more stimulating. Furthermore, when the nucleic acid fragments have a broad-range distribution, the 20 stimulant effect is greater compared to that obtained with fragments with a short-range distribution.

According to the present invention, the term “species” refers to an abstract concept and a species as such cannot be stimulated. The references to a species must thus be understood as indicating identifiable biological systems, i.e. individual 25 cells or organisms of the species or pluralities of identifiable biological systems of the species, that is to say, a population.

The term “target species” refers to the species on which it is desired to exert a biostimulant and/or fertilising effect.

The term “source species” refers to the species from which the sequences of 30 nucleic acids such as the DNA used as biostimulants and/or fertilisers are derived. As mentioned above, the source species must be a species differing from the target species and other than a species phylogenetically similar to the target species.

The term “phylogenetically similar species” refers to a species having a similar genome. The skilled person will understand that species which are closely

related phylogenetically have a more similar genome than species which are phylogenetically distant. Phylogenetically similar thus means having a phylogenetically close relationship. Phylogenetic similarity can thus be determined based on known phylogenetic relationships. Therefore, phylogenetically similar species can be species within the same taxonomic order. Within a given order, the phylogenetically similar species originate from a same monophyletic group (clade), such as from a same family, a same subfamily, a same tribe, a same subtribe, or a same genus. Furthermore, techniques for determining genomic similarity (or relatedness) are readily available. Genomic similarity can be determined, for example, by determining the renaturation/reassociation kinetics of single-strand DNA (ssDNA) fragments of the genomes from both species. Alternatively, or in addition, one can investigate the denaturation (fusion) of double-stranded DNA (dsDNA) fragments renatured from mixtures of ssDNA fragments of the genomes from both species. The latter technique allows for defining the melting temperature Tm, that is to say, the temperature at which half of the DNA strands are in the ssDNA state and the correlated T50H. Approaches implying renaturation/denaturation kinetics and the evaluation of melting profiles were introduced in the early 1970s (see de Ley et al., Eur J Biochem. January 1970;12(1):133-42) to determine the relatedness of bacteria, but these approaches implying the analysis of melting temperature profiles were also used to determine the relatedness of eukaryotic species (see, for example, Sibley and Ahlquist, J Mol Evol (1984) 20:2-15).

As is further known, since the publication of WO2014/020624, the phylogenetic similarity of species can be determined by observing whether the DNA fragments originating from a species are inhibitory for another species. Therefore, a phylogenetically similar species is a species whose DNA obtained by random fragmentation of the extracted DNA or obtained by random synthesis of fragments from the DNA of that species is inhibiting for the target species. It will be clear to the skilled person that, based on this functional definition, the phylogenetics can be determined with tests similar to the ones presented in WO2014/020624 and in the experiments appended thereto. Within a same taxonomic order, a source species will be phylogenetically similar to a target species when the DNA obtained from the source species by random fragmentation of the extracted total DNA or by random synthesis of fragments from the total DNA is inhibiting for the target species. Preferably, according to the present invention, the source species belongs to a

different order from that of the target species.

The nucleic acids, such as DNA or RNA, used according to the present invention can be natural or synthetic. Furthermore, the DNA used according to the present invention can be the total genomic DNA, i.e. DNA obtained by extraction, 5 by breakage or by lysis of cells of the source species. The DNA used according to the present invention also can be a mixture of genomic DNA and secreted DNA, i.e. DNA (or DNA sequences or fragments) actually secreted by living cells of the source species or sequences of synthetic DNA with the same sequence as the ones actually secreted by cells or tissues of the source species.

10 The term “expose” a target species means that the composition of nucleic acids, such as, for example, DNA, which is the object of the invention is administered to a target species through any suitable means and system, such as surface contact, systemic administration, absorption, injection, for example by application to the seed, leaf spraying, fertigation, drip-irrigation, injection, or administration to the roots 15 or onto/into the soil, and in general through systems for the administration of biostimulants and fertilisers. The composition of nucleic acids, such as, for example, DNA, which is the object of the invention can be formulated in a form for dry or liquid treatments, selected from the group consisting of dispersions, for example in the form of an aerosol, suspension, wettable or soluble powders, dispersible powders, 20 emulsions in water or other solvents and polymers, dispersible granules, suspensions of microcapsules, emulsifiable concentrates, fluid pastes, macro emulsions, oily dispersions, lures, compounds for seed treatments such as coating, pelleting, and dressing, or for foliar application, such as adjuvants, stabilisers, and binders, compounds for application to roots and to the soil, in hydroponic farming 25 and vertical farming systems, and in controlled cultivation systems. Systems with adjuvants, polymers, stabilisers, binders, dissolvents, and/or aggregating agents can be used.

The determination of the ranges of concentration in which the composition of nucleic acids, such as, for example, DNA, of the invention is stimulating and 30 fertilising for the target species falls within the scope of knowledge of the skilled person. The skilled person will understand that the concentration of nucleic acids, such as DNA, which is necessary may depend on factors such as the species from which derives the nucleic acid that can stimulate and nourish the target species or the target cell in a more or less effective manner, the possible administration of an

additional fertiliser or biostimulant, the route of application to the target species, the possible additional administration of other chemical compounds such as, for example, fungicides, nematicides, and herbicides, and additional protection products. For many applications, suitable concentrations of nucleic acids, such as, for example, DNA, can be in the range of 0.5-1500 ppm, such as 2-1300 ppm, 2-1000 ppm, 5-1000 ppm, 10-1000 ppm, 50-1000 ppm, 100-1000 ppm, 200-1000 ppm, or 500-1000 ppm. For other applications lower or higher concentrations may be desired, depending on the conditions of application and the growing conditions.

According to a possible further embodiment of the present invention, the nucleic acid fragments, such as, for example, DNA, can be released by a carrier. Said carrier can be a host species, for example a species selected from a microalga species, cyanobacteria, microorganisms, microbes, bacteria, and a multicellular organism, such as a multicellular plant.

The term “uniform” indicates that the fragments of the mixture have a different length from each other in the specified range of lengths and that the fragments of a different length are present in an equal percentage. The term “Gaussian” or “half-normal” indicates that the fragments of the mixture have a different length from each other in the specified range of lengths and that the fragments of a different length are present in a different percentage according to a Gaussian or half-normal distribution.

According to the present invention, when referring to plants or the rhizosphere, the term “biostimulant” means a product, whose function, as also defined by the EBIC (European Biostimulant Industry Council) and the new European Fertilising Product Regulation, is the following: "a product stimulating plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits or availability of confined nutrients in soil or rhizosphere". It should be carefully noted that biostimulants are distinguished from fertilisers in that they do not directly provide nutrients to organisms such as, for example, a plant, or to microorganisms such as, for example, microalgae, but rather act by improving and increasing some physiological and metabolic processes taking place during the life cycle. In general, the term “biostimulant” means a product that acts on cell metabolism and accelerates the cell’s natural physiological and metabolic processes.

The term “fertiliser” means a source of nutrients for plants, organisms and microorganisms, such as, for example, nitrogen and phosphorus fertilisers, etc., and thus tied to the content of nutrients.

According to the present invention, the nucleic acids are also “direct oligonucleotide and polynucleotide fertilisers”. i.e. they represent a direct source of oligonucleotide and polynucleotide fragments of different sizes that a cell can use “directly” in the cell duplication process to form new DNA helices of daughter cells. Hence, a direct oligonucleotide and polynucleotide fertiliser is a mixture of oligonucleotide and polynucleotide fragments that the cell can use directly in the duplication process for the formation of new helices, with a process that consumes less energy and is faster and more effective inside the cell. The present invention refers to a composition of nucleic acids, for example DNA, with a broad-range distribution, or a broad size range of the fragments, with a concentration of fragments in the solution as previously specified. Furthermore, the present invention refers to compositions of microalgae, cyanobacteria, algae, natural extracts, in solid or liquid form, or natural extracts, in which the content of nucleic acids, for example DNA, contained in the composition, is high and has a broad-range distribution.

An object of the present invention also consists in biostimulant products, in solid, powder, or liquid form, natural extracts, extracts in solid form, hydrolysed extracts, extracts decomposed from natural substrates, natural decomposition of natural substances, wherein the content of nucleic acids contained in the product is maintained high, and the profile of the nucleic acid fragments contained is preferably of the broad-range type.

Such as, for example, in the case of biostimulants based on cyanobacteria or microalgae, wherein the cultivation, production, processing and post-processing process leads to a finished product with a high nucleic acid content and a broad-range profile of the nucleic acid fragments contained therein.

The present invention will now be described by way of non-limiting illustration with particular reference to example embodiments and to the appended figures, wherein:

Figure 1 shows an example of a “broad-range” spectrum of nucleic acid fragments with a half-normal distribution (Fig. 1.A); examples of “broad-range” spectra of nucleic acid fragments with a Gaussian distribution with standard deviation, Gaussian peak and different amplitudes (Figures 1.B, 1.C, 1.D); an

example of a “broad-range” spectrum of nucleic acid fragments with a uniform distribution (Figure 1.E); examples of “short-range” spectra of nucleic acid fragments with a fragment distribution according to a narrow half-normal distribution (10-200 bp) (Fig.1.F) or with a distribution of fragments of a size smaller than 50 bp (Fig.1.G);

5 **Figure 2** shows the stimulation of plant germination, rooting and development in tomato seeds (*Solanum lycopersicum)* by exposure to a solution of DNA fragments extracted from the microalga *Arthropsira platensis* and randomly fragmented with a DNA fragmentation spectrum profile of the broad-range type.

Figure 3 shows the stimulation of plant germination, rooting and development 10 in maize seeds (*Zea Mays*) by exposure to a solution of DNA fragments extracted from the microalga *Arthropsira platensis* and randomly fragmented with a DNA fragmentation spectrum profile of the broad-range type.

Figure 4 shows a comparison of the effects of two biostimulant solutions of DNA fragments extracted from the microalga *Arthrospira platensis,* a DNA 15 fragmentation spectrum profile of the short-range type and of the broad-range type, respectively, applied on tomato seeds *(Soleanum lycopersicum)*, at an equal DNA concentration.

Figure 5 shows a comparison of the effects of two biostimulant solutions of DNA fragments obtained from two different biomasses of *Arthrospira platensis* 20 *(APSCM2M and APCP biomasses)* having different DNA fragmentation profiles, broad-range and short-range, applied on tomato seeds *(Soleanum lycopersicum)*.

Figure 6 shows the stimulatory effect on cell growth in a photobioreactor of the species *Scenedesmus obliquus* with a solution of DNA fragments extracted from tomato plant residues and randomly fragmented.

25 **EXPERIMENTAL EXAMPLES**

In the examples of experiments reported below, the nucleic acid composition used in the various treatments was prepared by carrying out an extraction of DNA from natural substances, for example microalgae and cyanobacteria, with standard extraction procedures, and subsequently the total DNA extracted was treated by 30 (non-limiting examples of the invention) a) random sonication with an immersion sonicator or an external vibration sonicator, until obtaining the production of a composition of random fragments with a broad-range or short-range spectrum as described above, or b) random natural decomposition until obtaining the production of a composition of random broad-range or short-range fragments as described

above. Verification of the level of fragmentation in the cases described was performed by means of standard agarose or polyacrylamide gel electrophoresis procedures and staining techniques of the SYBR safe type, and visualisation with UV.

5 **EXAMPLE 1**

Biostimulant effect of a solution of DNA extracted from the microalga Arthrospira platensis with a broad-range distribution of the DNA fragments, applied on tomato seeds (Solanum lycopersicum)

An experiment was conducted to evaluate the biostimulant effect on tomato
10 seeds of a solution of extracted DNA randomly fragmented with a broad-range distribution of the fragments.

DNA extraction and quantification: DNA extraction from *A. Platensis* was carried out using the standard extraction protocol described in the article by Jagielski, 2017. As indicated in the protocol, in order to eliminate the RNA a
15 treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes. Dry powder of the microalga *A. Platensis* produced by M2M Engineering S.a.s. was used for the extraction. The amount of biomass used was 130 mg of dry powder.

The amount of DNA extracted from the biomass was quantified with a Qubit
20 fluorometer. From 130 mg of dried biomass, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/µl. The total amount of DNA extracted from 130 mg of biomass was a total of 152 µg. The DNA was randomly fragmented by sonication until obtaining a broad-range fragmentation profile (Fig.1.E) with a uniform distribution. Furthermore, based on the results of
25 agarose gel electrophoresis, it was possible to evaluate the size and corresponding amount of fragmented DNA molecules present in the samples and the size distribution of the fragments. The DNA extracted from the biomass of *A. Platensis* was composed of and characterised by a broad-range distribution from 10 bp to 15000 bp with a uniform distribution (see the broad-range distribution in Figure 1.E).
30 The biostimulant solution used for the experiment had a DNA concentration of 5.8 mg/L dissolved in distilled water (corresponding to 5 g/L of solution of the initial microalgae powder) and 0.58 mg/L of DNA dissolved in water (corresponding to 0.5 g/L of solution of the initial microalgae powder).

The assays were conducted in a growth chamber at a constant temperature

(28°C, with a light-dark photoperiod of 12h/12h).

Ten tomato (Solanum lycopersicum) seeds were placed on sterile filter paper wetted with 4 ml of DNA biostimulant solution (solution-1: 5.8 mg/L of DNA and solution-2: 0.58 mg/L of DNA) in a 9 cm Petri dish. Three Petri dishes were used for three replications for each test sample. Every experiment was replicated 3 times in three Petri dishes and distilled water was used as a control in three Petri dishes with 4 ml of solution and 10 seeds as in the DNA biostimulant solutions.

The biostimulant effect of the two solutions comprising DNA at different concentrations was evaluated by determining the percentages of seed germination, root development, and shoot development of the tomato seedlings.

Results (Fig.2):

The results are shown in figure 2, which shows a marked biostimulation effect on the seeds and on the germinated seedlings. In particular, one observes a:

- +10% increase in germinations for the 5.8 mg/L DNA solution, and +3.33% for the 0.58 mg/L DNA solution;
- +34.30% increase in root development for the 5.8 mg/L DNA solution, and +26.79% for the 0.58 mg/L DNA solution;
- +23.04% increase in seedling development for the 5.8 mg/L DNA solution, and +15.91% for the 0.58 mg/L DNA solution.

Therefore, based on the results of example 1, it may be concluded that:

a) the randomly fragmented DNA solution with a fragment distribution of the broad-range type has very evident biostimulant effects with a stimulation of and increase in germinations, root development and seedling development compared to the untreated control;
b) the solution with a DNA concentration of 5.8 mg/L has greater stimulating effects than those obtained with the solution with a DNA concentration of 0.58 mg/L. Therefore, a higher content of broad-range randomly fragmented DNA in the solution has a greater biostimulation effect.

EXAMPLE 2

Biostimulant effect of a solution of DNA extracted from the microalga Arthrospira Platensis with a broad-range distribution profile of the DNA fragments, applied on maize seeds (Zea Mays)

An experiment was conducted to evaluate the biostimulant effect on maize seeds of a solution of extracted DNA randomly fragmented with a broad-range

distribution of the fragments.

<u>DNA extraction and quantification</u>: DNA extraction from *A. Platensis* was carried out using the standard extraction protocol described in the article by Jagielski (2017). As indicated in the protocol, in order to eliminate the RNA a treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes. Dry powder of the microalga *A. Platensis* produced by M2M Engineering S.a.s. was used for the extraction. The amount of biomass used was 130 mg of dry powder.

The amount of DNA extracted from the biomass was quantified with a Qubit fluorometer. From 130 mg of dried biomass, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/µl. The total amount of DNA extracted from 130 mg of biomass was a total of 152 µg. The DNA solution was randomly fragmented by sonication until obtaining a fragmentation profile with a uniform broad-range distribution (Fig.1.E). Furthermore, based on the results of the agarose gel electrophoresis, it was possible to evaluate the size, the corresponding amount of fragmented DNA molecules present in the samples and the distribution of the fragments. The DNA extracted from the biomass of *A. Platensis* was composed of and characterised by a broad-range distribution from 50 bp to 15000 bp with a uniform distribution (Figure 1.E).

The DNA biostimulant solution used for the experiment was a 5.8 mg/L concentration of DNA dissolved in distilled water (corresponding to 5 g/L of solution of microalgae powder) and a 0.58 mg/L concentration of DNA (corresponding to 0.5 g/L of solution of microalgae powder).

The assays were conducted in a growth chamber at a constant temperature (28°C, with a light-dark photoperiod of 12h/12h).

Ten maize seeds (*Zea Mays*) were placed on sterile filter paper wetted with 4 ml of DNA biostimulant solution (solution-1: 5.8 mg/L and solution-2: 0.58 g/L) in a 9 cm Petri dish. Three Petri dishes were used for three replications for each test sample. Every experiment was replicated 3 times in three Petri dishes and distilled water was used as a control in three Petri dishes with 4 ml of solution and 10 seeds as in the DNA biostimulant solutions.

The biostimulant effect of the two biostimulant solutions based on DNA at different concentrations was evaluated by determining the percentages of seed germination, root development, and shoot development of the maize seedlings.

Results (Fig.3):

The results are shown in Figure 3, which shows a marked biostimulation effect on the seeds and on the germinated seedlings. In particular, one observes a:

- +10% increase in germinations for the 5.8 mg/L DNA solution, and +3.3% for the 0.58 mg/L DNA solution;
- +47.3% increase in root development for the 5.8 mg/L DNA solution, and +27.6% for the 0.58 mg/L DNA solution;
- +10.5% increase in seedling development for the 5.8 mg/L DNA solution, and +5.29% for the 0.58 mg/L DNA solution.

Therefore, based on the results of example 2, it may be concluded that:

c) the randomly fragmented DNA solution with a DNA fragment distribution of the broad-range type has very evident biostimulant effects with a stimulation of and increase in germinations, root development and seedling development compared to the untreated control;

d) the solution with a DNA concentration of 5.8 mg/L has greater stimulating effects than those obtained with the solution with a DNA concentration of 0.58 mg/L. Therefore, a higher content of broad-range randomly fragmented DNA in the solution has a greater biostimulation effect.

EXAMPLE 3

Comparison of the effects of two solutions of DNA fragments extracted from the microalga Arthrospira Platensis, with a short-range and broad-range DNA fragmentation profile, respectively, applied on tomato seeds (Soleanum lycopersicum), at an equal DNA concentration.

An experiment was conducted to evaluate the biostimulant effect on tomato seeds (*Solanum lycopersicum*) of two biostimulant solutions of DNA fragments extracted from *Arthrospira platensis*, with a different fragmentation profile: one solution with a “short-range” fragmentation profile and a fragment size distribution concentrated in the range with values of less than 200 bp (FIG. 1.F), and a solution of DNA fragments with a “broad-range” fragmentation profile and a broad-range fragment size distribution of between 10 bp and 15000 bp (FIG. 1.A). The DNA extraction technique, the seeds used, and the DNA concentration used were the same both for the solution with a broad-range distribution and the solution with a short-range distribution.

DNA nucleic acid extraction and quantification

DNA extraction from *A. platensis* was carried out using a standard DNA extraction protocol based on the article by Jagielski (2017). As indicated in the protocol, in order to eliminate the RNA a treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes. The powder of the microalga *A.Platensis* was produced by M2M Engineering S.a.s.. The amount of biomass used was 130 mg of dry powder for both samples.

The amount of DNA extracted from the biomass was quantified with a Qubit fluorometer. From 130 mg of dried biomass, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/µl. The DNA in the solution was then randomly fragmented by sonication until obtaining, in the first case, a broad-range fragmentation profile (Fig.1.A) and, in the second case, a short-range fragmentation profile (Fig.1.F). Agarose gel electrophoresis runs were carried out to verify the distribution profile of the DNA fragmentation of the two solutions, short-range and broad-range. Two DNA solutions were prepared: solution-1 broad-range with a DNA concentration of 5.8 mg/L, and a second solution, solution-2, with a DNA concentration equal to the first, 5.8 mg/L, with a short-range distribution, obtained by dilution in distilled water.

The assays were conducted in a growth chamber at a constant temperature (28°C, with a light-dark photoperiod of 12h/12h).

Ten tomato seeds (*Solanum lycopersicum*) were placed on sterile filter paper wetted with 4 ml of DNA biostimulant solution (solution-1: 5.8 mg/L broad-range and solution-2: 5.8 mg/L short-range) in a 9 cm Petri dish. Three Petri dishes were used for three replications for each test sample. Every experiment was replicated 3 times in three Petri dishes and distilled water was used as a control in three Petri dishes with 4 ml of solution and 10 seeds as in the DNA biostimulant solutions.

The biostimulant effect of the two DNA solutions with an equal concentration (5.8 mg/L) but different fragment distributions – short-range and broad-range – was evaluated by determining the percentages of seed germination, root development, and shoot development of the tomato seedlings.

Results (Fig. 4)

The results are shown in figure 4, which shows an:

- increase in germinations with solution-1, broad-range: +10% (increase in germination in the broad-range solution), solution-2, short-range: +0% (no

increase in germination in the short-range solution)

- increase in root development with solution-1, broad-range: +34.30%, solution-2, short-range: +16.30%
- increase in plant development with solution-1, broad-range: +23%, solution-2, short-range: +13.2%

Conclusions: based on the results of example 3, it may be concluded that:

- the DNA concentration being equal, DNA solutions with a broad-range spectrum showed surprisingly greater stimulating effects than DNA solutions with a short-range spectrum.
- Short-range DNA solutions did not show a stimulating effect on germination, and a much lesser effect on roots and seedlings, whereas, surprisingly, the broad-range solutions showed strongly stimulating effects on germinations, and root and seedling development.

Therefore, the solutions of randomly fragmented DNA with a broad-range spectrum are surprisingly and markedly stimulating compared to the short-range solution.

EXAMPLE 4

Comparison of the effects of two biostimulant solutions of DNA fragments obtained from two different biomasses of *Arthrospira Platensis*, with different fragmentation profiles, broad-range and short-range, applied on tomato seeds *(Soleanum lycopersicum)*.

An experiment was conducted to evaluate the biostimulant effect on tomato seeds (*Solanum lycopersicum*) of two biostimulant solutions of DNA fragments obtained from two different biomasses of *Arthrospira platensis*, and having different fragmentation profiles: a solution with a “short range” fragmentation profile (obtained from the APCP biomass) with a fragment size distribution concentrated in the range with values of less than 50 base pairs (FIG. 1.G), and a solution of DNA fragments with a “broad-range” fragmentation profile (obtained from the APSCM2M biomass) with a Gaussian fragment size distribution of between 10 bp and 15000 bp (FIG. 1.B).

A. platensis biomasses used: APCP biomass, a common commercial product available on the market, in which the short-range profile of the internal content of DNA fragments was verified (Fig.1.G), and APSCM2M biomass produced by M2M Engineering S.a.s., in which the broad-range profile of the internal content of DNA fragments was verified (Fig.1.B).

Verification of DNA content in the two different biomasses, extraction, quantification and verification of DNA fragment distribution by gel electrophoresis:

The extraction of the internal DNA of the two biomasses of *A. platensis was* carried out using a standard extraction protocol based on the article by Jagielski
5 (2017). As indicated in the protocol, in order to eliminate the RNA a treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes.

The amount of dry powder of the microalga *A. platensis* that was used for the two biomasses, APCP and APSCM2M, was the same; more precisely, 130 mg of
10 dry powder were used for both samples.

The amount of DNA extracted from the two different biomasses was quantified with a Qubit fluorometer. In reference to APSCM2M, from 130 mg of dried biomass, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/µl. The total amount of DNA extracted from 130 mg of
15 biomass was a total of 152 µg. In reference to APSC, from 130 mg of dried biomass, 0.6 ml of DNA were extracted and dissolved in milli-Q water, with a DNA concentration of 43 ng/µl. The total amount of DNA extracted from 130 mg of biomass was a total of 25.8 µg. Therefore, the DNA nucleic acid content of the APSCM2M biomass was much higher compared to that of the APCP biomass
20 (nearly six times higher in terms of DNA content in the APSCM2M biomass). Furthermore, in order to verify the distribution of the DNA fragments contained in the two biomasses, gel electrophoresis runs were carried out. Based on the results of the agarose gel electrophoresis, it was possible to evaluate the size and corresponding amount of DNA molecules present in the samples: the DNA extracted
25 from the biomass of *A. platensis* APSCM2M was composed of and characterised by a broad-range distribution from 50 bp to 15000 bp with a Gaussian distribution (1.B); in the case of APCP, by contrast, the size distribution and distribution of the amount of fragments was a short-range distribution (FIG.1.G).

Test on tomato seeds

30 Two biostimulant DNA solutions were prepared from the two different biomasses, APSCM2M and APCP, in the following manner:

Solution-APSCM2M: 5 g of dry powder biomass of *A.platensis APSCM2M* were dissolved in distilled water, with a concentration of 5 g/L of dry powder. The solution was shaken for 5 min with an orbital shaker until complete dissolution of the

dry powder in water. In the solution obtained by dissolving APSCM2M, the corresponding concentration of DNA in the solution released was 5.8 mg/L of DNA.

Solution-APCP: the solution of *A. platensis* APCP was prepared in a similar manner; 5 g/L of APCP powder was dissolved in distilled water. In the solution obtained from APCP powder dissolved at 5 g/L, the DNA concentration in the solution was 0.9 mg/L of DNA.

The two solutions thus obtained from the two biomasses, APCP and APSCM2M, dissolved in distilled water were the following:

Solution-APSCM2M powder dissolved in water, 5 g/L of dissolved powder, 5.8 g/L concentration of DNA contained in the solution, with a broad-range DNA distribution;

Solution-APCP powder dissolved in water, 5 g/L of dissolved powder, 0.9 g/L concentration of DNA contained in the solution, with a short-range DNA distribution.

In order to verify the effect of the two solutions APCP and APSCM2M dissolved in water at 5 g/L, as described, assays were performed on tomato seeds in Petri dishes, as described below, and the biostimulation effect was evaluated.

Ten tomato seeds (*Solanum lycopersicum*) were placed on sterile filter paper wetted with 4 ml of biostimulant solution, Solution-APSCM2M, 5 g/L of APSCM2M powder, and Solution-APCP, 5 g/L of APCP powder, respectively, in 9 cm Petri dishes. Three Petri dishes were used for three replications for each test sample. Every experiment was replicated 3 times in three Petri dishes and distilled water was used as a control in three Petri dishes with 4 ml of solution and 10 seeds as in the DNA biostimulant solutions. The assays were conducted in a growth chamber at a constant temperature (28°C, with a light-dark photoperiod of 12h/12h).

The biostimulant effect of the two solutions, Solution-APSCM2M and Solution-2 APCP, containing DNA in solution with different distributions of the DNA fragments – broad-range (Solution-APSCM2M) and short-range (Solution-APCP) – and with different concentrations, was evaluated by determining the percentages of seed germination, root development, and shoot development of the tomato seedlings.

Results (Fig. 5)

The results are shown in figure 5, which shows a:

- +20.8% increase in germinations with Solution-APSCM2M compared to the untreated control (and greater than +12.5% compared to Solution-APCP)

- +8.33 increase in germination with Solution-APCP compared to the untreated control (but -12.5% compared to Solution APSCM2M)
- +75.9% increase in root development with Solution-APSCM2M compared to the untreated control (and +38.7% greater compared to Solution-APCP)
- +37.1% increase in root development with Solution-APCP compared to the untreated control (but -38.7% less compared to Solution APSCM2M)
- +65.5% increase in seedling development with Solution-APSCM2M compared to the untreated control (and +43.13% greater compared to Solution-APCP)
- +22.4% increase in seedling development with Solution-APCP compared to the untreated control (but -43.13% less compared to Solution APSCM2M)

Therefore, based on the results of example 4, it may be concluded that:

- Natural biomasses containing larger amounts of DNA within them are more stimulating;
- Natural biomasses containing DNA with a broad-range profile are unexpectedly much more stimulating than biomasses with a short-range nucleic acid content.
- Solutions obtained from natural biomasses containing larger amounts of DNA within them are more stimulating;
- Solutions obtained from natural biomasses containing nucleic acids with a broad-range profile are unexpectedly much more stimulating than biomasses with a short-range DNA content.

EXAMPLE 5

Stimulation effect on the growth curve of the microalga *Scenedesmus obliquus* in a photobioreactor (BPR) upon addition of a solution of randomly fragmented nucleic acid fragments, extracted from tomato (*Solanum lycopersicum*) as per the present invention

In order to demonstrate the possible use of a nucleic acid composition as a stimulant product in cultivation photobioreactors, or in cell bioreactors, a growth test was conducted in a photobioreactor on the green microalga *Scenedesmus obliquus* by adding to the culture medium a DNA nucleic acid solution extracted from a different species (tomato, *Solanum lycopersicum*) with two different DNA concentrations in the solution, 0.6 mg/L in the first, and 0.3 mg/L in the second. Figure 6 shows the dynamics of the growth curve of the microalga *Scenedesmus obliquus* in the presence of the two solutions with different DNA concentrations, compared to the control, and shows a significant stimulant effect and an increase in

growth, compared to the control, when a solution of DNA of a different species (tomato in this case) is added into the culture medium.

The experiment was performed in 3 identical cylindrical laboratory photobioreactors with a volume of 2 litres each, with different tomato DNA concentrations. The conditions in the photobioreactor were completely controlled and optimised, with optimised LED light spectra, culture temperature=25°C, lighting 12h Day/12h Night, and irradiation 400 μmol/m2 sec.

The growth media used are basal media (BBM) for *Scenedesmus obliquus*. The growth curve was derived with absorbances using a spectrophotometer and measurements of the dry matter collected by centrifugation in an upper centrifuge followed by lyophilisation.

The results are shown in figure 6 and show that:

- cell growth is stimulated in the presence of nucleic acids;
- higher concentrations of DNA in a solution have a more stimulating effect;
- the productivity and productions of a cell culture in a bioreactor tend to increase.

<u>EXAMPLE 6</u>

Effect of a DNA biostimulant solution of fragments obtained from a biomass of *Arthrospira Platensis* with a DNA fragmentation profile of the broad-range type, applied on field-grown durum wheat *(Triticum durum), with application on seeds (seed treatment application) and with application on leaves (foliar treatment)*.

A field experiment was conducted to evaluate the biostimulant effect on a durum wheat crop (*Triticum durum*) of a DNA biostimulant solution of fragments obtained from a biomass of *Arthrospira platensis*, having a DNA fragmentation profile of the “broad-range” type (obtained from the APSCM2M biomass) with a Gaussian fragment size distribution of between 10 bp and 15000 bp (FIG. 1.B).

<u>Biomass of A. platensis used</u>: APSCM2M biomass produced by M2M Engineering S.a.s., within which the broad-range profile of the internal content of DNA fragments was verified (Fig.1.B).

Verification of DNA content in the <u>A. platensis</u> biomass APSCM2M*, extraction, quantification and verification of DNA fragment distribution by gel electrophoresis:*

The extraction of the internal DNA of the *A. platensis* biomass was carried

out using a standard extraction protocol based on the article by Jagielski (2017). As indicated in the protocol, in order to eliminate the RNA a treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes. For the DNA extraction, use was made of an amount of 130 mg of dry powder of the microalga *A. platensis*, APSCM2M, from which the DNA was extracted, quantified and characterised by gel electrophoresis.

The amount of DNA extracted from the biomass was quantified with a Qubit fluorometer; from 130 mg of APSCM2M dry powder, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/μl. The total amount of DNA extracted from 130 mg of biomass was a total of 152 μg. Furthermore, in order to verify the distribution of the DNA fragments contained in the biomass, a gel electrophoresis run was carried out. Based on the results of the agarose gel electrophoresis, it was possible to evaluate the size and corresponding distribution and amount of DNA molecules present in the sample: the DNA extracted from the *A. platensis* biomass APSCM2M was composed of and characterised by a broad-range distribution from 50 bp to 15000 bp with a Gaussian distribution (1.B).

Test on wheat crop with seed treatment (*application on seeds*) *and foliar treatment (application on leaves)*

Two biostimulant solutions of DNA were prepared from the APSCM2M biomass (with a DNA distribution of the broad-range type), the first for application on seeds (seed treatment), and the second for application on leaves (foliar treatment), in the following manner.

For the seed treatment, Solution-APSCM2M-Seed was prepared for application on seeds: 0.6 g of dry powder biomass of *A.platensis APSCM2M* was dissolved in 0.6 L of water, with a final concentration of 1 g/L of dry powder in water. The solution was shaken for a few minutes until complete dissolution of the dry powder in water. In the solution obtained by dissolving APSCM2M, the corresponding concentration of DNA in the solution released was 1.17 mg/L of DNA. For the seed treatment, Solution APSCM2M-Seed was applied to 100 Kg of seeds of *Triticum durum*, which were then sown in a field for the test.

For the foliar treatment, prior to application Solution-APSCM2M-Foliar was prepared for application on leaves; it was obtained in the following manner: 75 g of dry powder biomass of *A.platensis APSCM2M* (with a broad-range DNA distribution)

was dissolved in 30 L of water, with a final concentration of 2.5 g/L of dry powder dissolved in water. The solution was stirred in the containment tank for a few minutes until complete dissolution of the dry powder. In the solution obtained by dissolving APSCM2M, the corresponding concentration of DNA in the solution released was 2.92 mg/L of broad-range DNA. The solution was then applied to the crop with a system of spraying on leaves in stage BBCH 39-41.

The solution was applied with a distribution per unit of surface area of 750 g/ha, hence 75 mg per square metre of crop surface area.

Summing up, the two solutions obtained were the following:

Solution-APSCM2M-Seed powder dissolved in water, 1 g/L of dissolved powder, concentration 1.17 mg/L of DNA contained in the solution, with a broad-range DNA distribution;

Solution-APSCM2M-Foliar powder dissolved in water, 2.5 g/L of dissolved powder, concentration 2.92 mg/L of DNA contained in the solution, with a broad-range DNA distribution.

In order to verify the stimulant effect on the wheat crop, Solution APSCM2M-Seed was applied to the seeds prior to sowing in the field, and Solution APSCM2M-Foliar was prepared and applied on the crop in the field at the time of foliar application and then sprayed on the crop in the field.

The test plots compared to verify the stimulant effect were:

T0: Untreated control;

T1: Seed application only, i.e. of Solution APSCM2M-Seed on the seeds;

T2: Seed application + one foliar application, i.e. application of Solution APSCM2M-Seed on the seeds prior to sowing and one foliar application Solution APSCM2M-Foliar in phenological stage BBCH 39-41.

Sowing in the field was carried out with a plot seeder having row spacing of 15 cm and the seed dose was calculated so as to favour the development of 350 plants/m^2.

The test was carried out with 4 replications for each test plot, with a plot distribution of the different test plots in randomised blocks.

The seeds used were of the species *Triticum durum*, variety *Antalis* (origin Italy) commonly used for wheat production.

The biostimulant effect of the biostimulant solutions on seeds and leaves was evaluated by determining, for the different test plots, T0, T1, T2, the following

parameters:

- Emergence (plants/m²);
- Vigour (on a scale of 0 – 10);
- Harvest and grain weight
- Grain yield (t/ha 13% moisture)
- Proteins (% dry matter);
- Weight of a thousand seeds
- Analysis of % content of nitrogen;

Finally, the plant nitrogen efficiency indices were calculated:

- Nitrogen uptake of grain (kg/ha)
- Nitrogen uptake of whole plant (kg/ha)
- Harvest index (ratio of grain weight to total plant weight)
- NUE – nitrogen use efficiency (kg of grain/kg of nitrogen available in the soil)

Results

The results obtained are the following:

- +15% increase in grain yield (Tonnes/Ha 13% moisture) with the application of APSCM2M to seeds only;
- +22.5% increase in grain yield (Tonnes/Ha 13% moisture) with the application of APSCM2M to seeds + one foliar application;
- +10% increase in the % of proteins in the grain with the application of APSCM2M to seeds + one foliar application;
- +8.9% increase in emergence (plants/m^2) with the application of APSCM2M to seeds only;
- Increase of +3.9 in the NUE with the application of APSCM2M to seeds only;
- Increase of +5.8 in the NUE with the application of APSCM2M to seeds + one foliar application.

EXAMPLE 7

Effect of a DNA biostimulant solution of fragments obtained from a biomass of *Arthrospira Platensis* with a DNA fragmentation profile of the broad-range type, applied on field-grown industrial tomatoes *(Solanum lycopersicum), with application by fertigation*.

A field experiment was conducted to evaluate the biostimulant effect on a field-grown industrial tomato crop (*Solanum lycopersicum*) of a DNA biostimulant

solution of fragments obtained from a biomass of *Arthrospira platensis* having a broad-range fragmentation profile (obtained from the APSCM2M biomass) with a Gaussian size distribution of the fragments between 10 bp and 15000 bp (FIG. 1.A).

Biomass of A. platensis used: biomass APSCM2M produced by M2M Engineering S.a.s., within which the broad-range profile of the internal content of DNA fragments was verified (Fig.1.A).

Verification of DNA content in the A. platensis biomass APSCM2M*, extraction, quantification and verification of DNA fragment distribution by gel electrophoresis:*

The extraction of the internal DNA of the *A. platensis* biomass was carried out using a standard extraction protocol based on the article by Jagielski (2017). As indicated in the protocol, in order to eliminate the RNA a treatment was performed using an RNAse A enzyme for 30 minutes at 37°C. The enzyme was then subsequently deactivated at 70° C for 15 minutes. For the DNA extraction, use was made of an amount of 130 mg of dry powder of the microalga *A. platensis*, APSCM2M, from which the DNA was extracted, quantified and characterised by gel electrophoresis.

The amount of DNA extracted from the biomass was quantified with a Qubit fluorometer; from 130 mg of APSCM2M dry powder, 1 ml of DNA was extracted and dissolved in milli-Q water, with a DNA concentration of 152 ng/µl. The total amount of DNA extracted from 130 mg of biomass was a total of 152 µg. Furthermore, in order to verify the distribution of the DNA fragments contained in the biomass, a gel electrophoresis run was carried out. Based on the results of the agarose gel electrophoresis, it was possible to evaluate the size and corresponding distribution and amount of DNA molecules present in the sample: the DNA extracted from the *A. platensis* biomass, APSCM2M, was composed of and characterised by a broad-range distribution from 50 bp to 15000 bp with a Gaussian distribution (1.B).

Field test on industrial tomato crop with application by fertigation

For the fertigation treatment, prior to every application Solution-APSCM2M-Ferti was prepared for application by fertigation; it was obtained by dissolving 50 g of dry powder biomass of *A.platensis APSCM2M* in 30 L of water, with a final concentration of 1.6 g/L of dry powder. The solution was stirred in the containment tank for a few minutes until complete dissolution of the dry powder in water. In the solution obtained by dissolving APSCM2M, the corresponding concentration of DNA

in the solution released was 1.88 mg/L of DNA. The solution was then applied to the crop with a plant fertigation system.

The biostimulant solution thus described was prepared and applied to the soil by fertigation a total of 6 times, applied every ten days from phenological stage
5 BBCH 13-15 after transplanting.

The biostimulant solution described was applied with a distribution per unit of surface area of 500 g/ha, hence 50 mg per square metre of crop surface area.

The tomato plants used for the field test were of the species *Solanum lycopersicum*, variety *Eventus (origin Italy)* commonly used for the production of
10 industrial tomatoes.

The biostimulation test was conducted both on a test plot with full nitrogen fertilisation (100% N test plot) and a test plot with a reduction in nitrogen fertilisation (50% N test plot) in order to evaluate the efficiency of nitrogen uptake by the tomato crop.

15 In order to verify the stimulant effect on the tomato crop, Solution APSCM2M-Ferti was prepared and applied on the crop in the field at the time of application by fertigation and applied on the tomato crops every ten days, starting from BBCH 13-15 after transplanting, and for a total of 6 applications over the whole test cycle.

The test plots compared to verify the stimulant effect were:

20 T0: Untreated control, under a nitrogen fertilisation regime of 100% N, full nitrogen fertilisation;

T1: Untreated control, under a nitrogen fertilisation regime of 50% N, nitrogen fertilisation reduced by one half;

T2: 100% N and application of the biostimulant by fertigation, i.e. application
25 of the biostimulant solution APSCM2M-Ferti as described, every ten days, and under a full nitrogen fertilisation regime (100% N);

T3: 50% N and application of the biostimulant in fertigation, i.e. application of the biostimulant solution APSCM2M-Ferti as described, every ten days, and under a regime of nitrogen fertilisation reduced by one half (50% N).

30 The test was carried out with 4 replications for each test plot, with 24 m^2 per plot (12 m long x 2 m wide), and a distribution of test plots in randomised blocks.

The biostimulant effect of the biostimulant solution was evaluated by determining, for the different test plots T0,T1, T2, T3, the following parameters:

- Chlorophyll content (SPAD)

- Plant biomass
- Number of fruits
- Number of inflorescences
- Production

5 *Results*

The results obtained are the following:

Increase in chlorophyll content in the treated test plots:

- +6.6% increase in chlorophyll content in T4 compared to the control T2 with the application of APSCM2M by fertigation and 50% N;
10 - +6.5% increase in chlorophyll content in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N;

Increase in plant biomass in the treated test plots:

- +15.4% increase in plant biomass in T4 compared to the control T2 with the application of APSCM2M by fertigation and 50% N;
15 - +9.4% increase in chlorophyll content in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N.

Increase in inflorescences in the treated test plots:

- +10.2% increase in the formation of inflorescences in T4 compared to the control T2 with the application of APSCM2M by fertigation and 50% N;
20 - +6.5% increase in the formation of inflorescences in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N.

Increase in the formation of fruits in the treated test plots:

- +20.0% increase in the formation of fruits in T4 compared to the control T2 with the application of APSCM2M by fertigation and 50% N.
25 - +16.6% increase in the formation of fruits in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N.

Increase in ripe fruits in the treated test plots (increase in fruit ripening):

- +17.5% increase in the weight of ripe fruits in T4 compared to the control T2 with the application of APSCM2M by fertigation and 50% N;
30 - +12% increase in the weight of ripe fruits in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N.

At the same time, there was a decrease in unripe fruits in the treated test plots:

- 20% decrease in the weight of unripe fruits T4 compared to the control T2 with

the application of APSCM2M by fertigation and 50% N;

- +25% increase in the weight of unripe fruits in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N.

Increase in the sugar content in the treated test samples:

5 - +9.3% increase in the fruit sugar content in T3 compared to the control T1 with the application of APSCM2M by fertigation and 100% N (and hence increase in the degrees Brix in the treated test plots).

Summing up, the test plots treated with the biostimulant solution APSCM2M with a broad-range DNA spectrum surprisingly had very positive biostimulant effects
10 in terms of increase in the formation of inflorescences, increase in the formation of fruits (fruit setting), increase in fruit ripening and increase in the sugar content and hence the degrees Brix, increase in plant growth and biomass, biostimulant effects both in the test plots with full fertilisation and under conditions of limited fertilisation, and stimulation to greater nitrogen uptake by crops.

15 **References**

1) FAO (2009): How to Feed the World in 2050. Rome.
https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf

2) Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis
20 of projected global
food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, 2(7), 494-501.
https://doi.org/10.1038/s43016-021-00322-9

3) Savci, S. (2012). Investigation of effect of chemical fertilisers on
25 environment. *APCBEE Procedia*, 1, 287-292.
https://doi.org/10.1016/j.apcbee.201203047

4) Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M. C., & Rouphael, Y. (2018). Renewable
sources of plant biostimulation: microalgae as a sustainable means to improve crop
30 performance. *Frontiers in Plant Science*, 9, 1782
https://doi.org/10.3389/fpls.201801782

5) Fenner, K., Canonica, S., Wackett, L. P., & Elsner, M. (2013). Evaluating pesticide
degradation in the environment: blind spots and emerging

opportunities. *Science*, 341(6147), 752-758. DOI:10.1126/science.1236281

6) Ray, D. K., West, P. C., Clark, M., Gerber, J. S., Prishchepov, A. V., & Chatterjee, S. (2019).
Climate change has likely already affected global food production. *PLOS ONE*, 14(5),
e0217148. https://doi.org/10.1371/journal.pone0217148

7) Rouphael, Y., & Colla, G. (2020). Biostimulants in agriculture. *Frontiers in Plant Science*, 11, 40.
https://doi.org/10.3389/fpls.202000040

8) González-Pérez, B. K., Rivas-Castillo, A. M., Valdez-Calderón, A., & Gayosso-Morales, M. A.
(2022). Microalgae as biostimulants: A new approach in agriculture. *World Journal of Microbiology and Biotechnology*, 38(1), 4.
https://doi.org/10.1007/s11274-021-03192-2

9) Xu, J., Min, J., Sun, H., Singh, B. P., Wang, H., & Shi, W. (2022). Biostimulants decreased
nitrogen leaching and NH3 volatilization but increased N2O emission from plastic-shed
greenhouse vegetable soil. *Environmental Science and Pollution Research*, 29(4), 6093-
6102. https://doi.org/10.1007/s11356-021-16039-y

10) Jagielski, T., Gawor, J., Bakuła, Z., Zuchniewicz, K., Żak, I., & Gromadka, R. (2017). An
optimized method for high quality DNA extraction from microalga Prototheca wickerhamii
for genome sequencing. *Plant Methods*, *13*, 1-8. https://doi.org/10.1186/s13007-017-0228-9

CLAIMS

1. A non-medical use of

a nucleic acid of a species, the source species, or

of a composition comprising said nucleic acid of a species

5 as a biostimulant and/or fertiliser for a target species, wherein

the source species is a species that is different from the target species.

2. The use according to claim 1, wherein the nucleic acid is selected from DNA or RNA or a mixture of DNA and RNA.

3. The use according to any one of the preceding claims, wherein the nucleic

10 acid is in the form of a mixture of nucleic acid fragments of the source species of a different length from each other and greater than 10 bp, preferably greater than 50 bp.

4. The use according to claim 3, wherein the mixture of fragments comprises fragments of a length from 10 bp to 1000000 bp, from 10 bp to 100000 bp, from 10

15 bp to 15000 bp or from 50 bp to 15000 bp.

5. The use according to any one of the preceding claims, wherein the mixture of fragments comprises fragments with a distribution of fragment length and of percentage of fragments of each length selected from:

uniform across the whole range of fragment lengths;

20 Gaussian across the whole range of fragment lengths; or

half-normal across the whole range of fragment lengths.

6. The use according to any one of claims 1-5, wherein the mixture of fragments comprises fragments of a length from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp, with a distribution of fragment length and of percentage of

25 fragments of each length selected from:

uniform in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp;

uniform in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp;

30 Gaussian in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp;

Gaussian in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp;

half-normal in the whole range from 10 bp to 15000 bp, preferably from 50 bp to 15000 bp; o

half-normal in a range selected from 50 bp-10000 bp, 50 bp-5000 bp or 50-1000 bp.

5 7. The use according to any one of claims 1-5, wherein the mixture of fragments comprises fragments of a length from 10 bp to 200 bp with a distribution of fragment length and of percentage of fragments of each length selected from:

uniform in the whole range from 10 bp to 200 bp;

uniform in a range selected from 10 bp and 50 bp or 10 bp and 100 bp;

10 Gaussian in the whole range from 10 bp to 200 bp;

Gaussian in a range selected from 10 bp and 50 bp or 10 bp and 100 bp;

half-normal in the whole range from 10 bp to 200 bp;

half-normal in a range selected from 10 bp and 50 bp or 10 bp and 100 bp.

8. The use according to any one of the preceding claims, wherein the source 15 species belongs at least to a taxonomic order differing from that of the target species.

9. The use according to any one of the preceding claims, wherein the source species or the target species is selected in the group consisting of a plant, an animal organism, for example an insect or a fish, a fungus, an alga or a microorganism, 20 such as a microalga, a bacterium, or a cyanobacterium.

10. The use according to claim 9, wherein when the microorganism is a microalga, the microalga is selected in the group consisting of *Arthropsira platensis*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Clorella vulgaris* or a mixture of said microalgae.

25

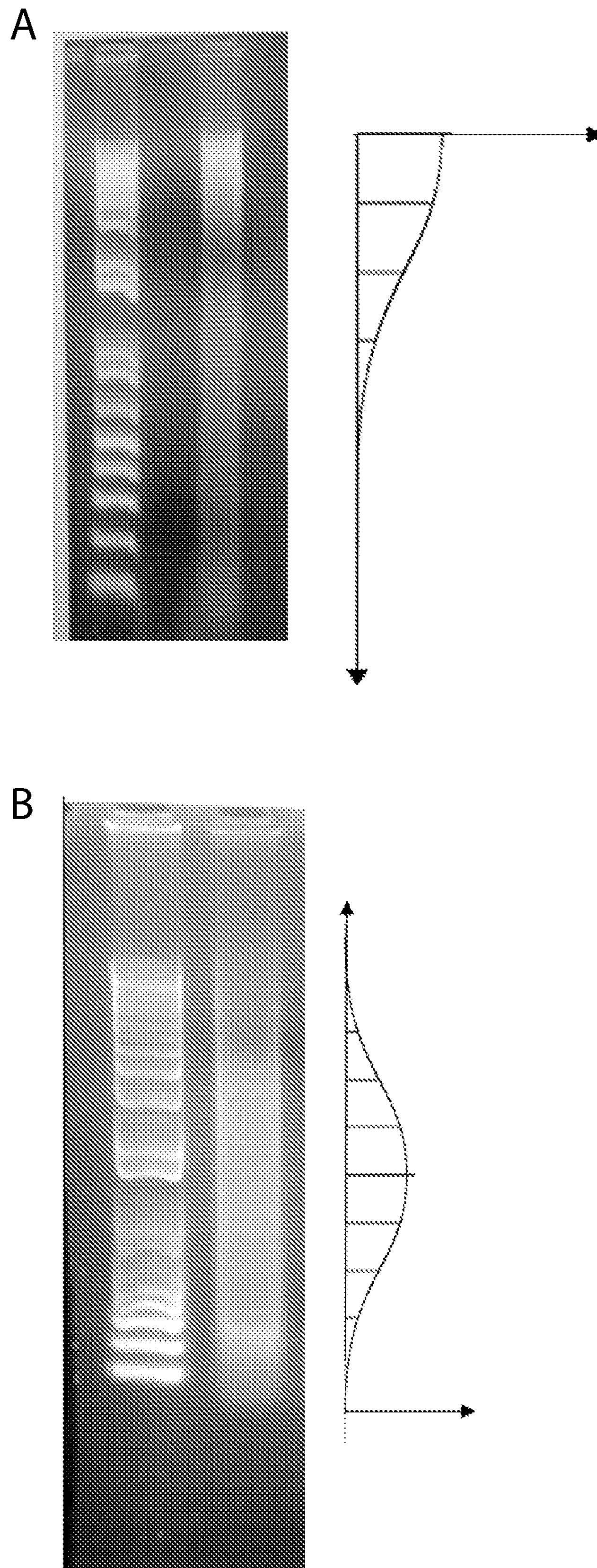

Fig. 1

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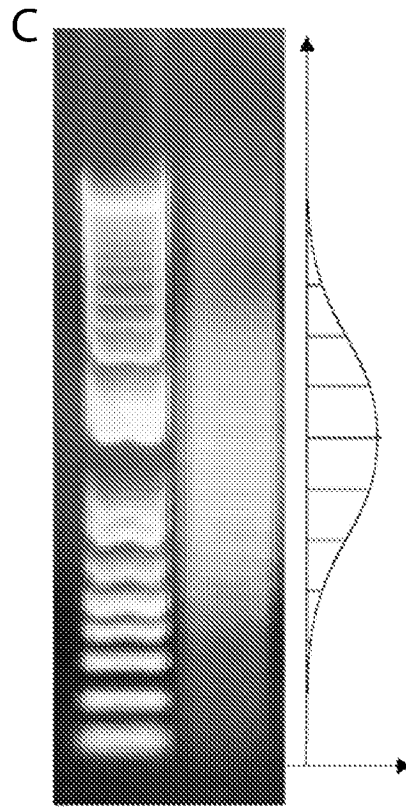

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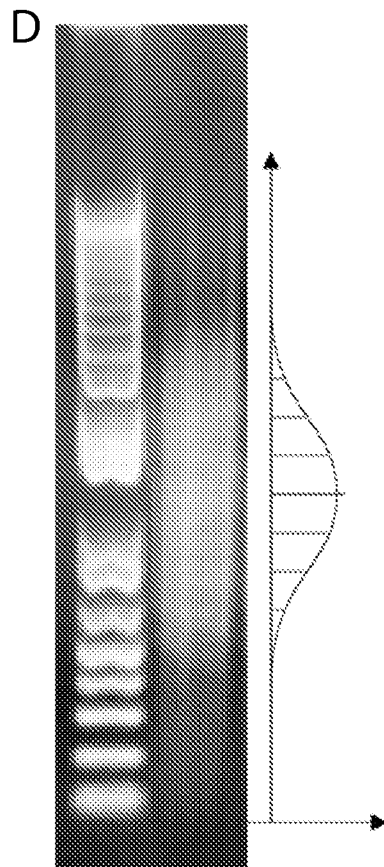


Fig. 1

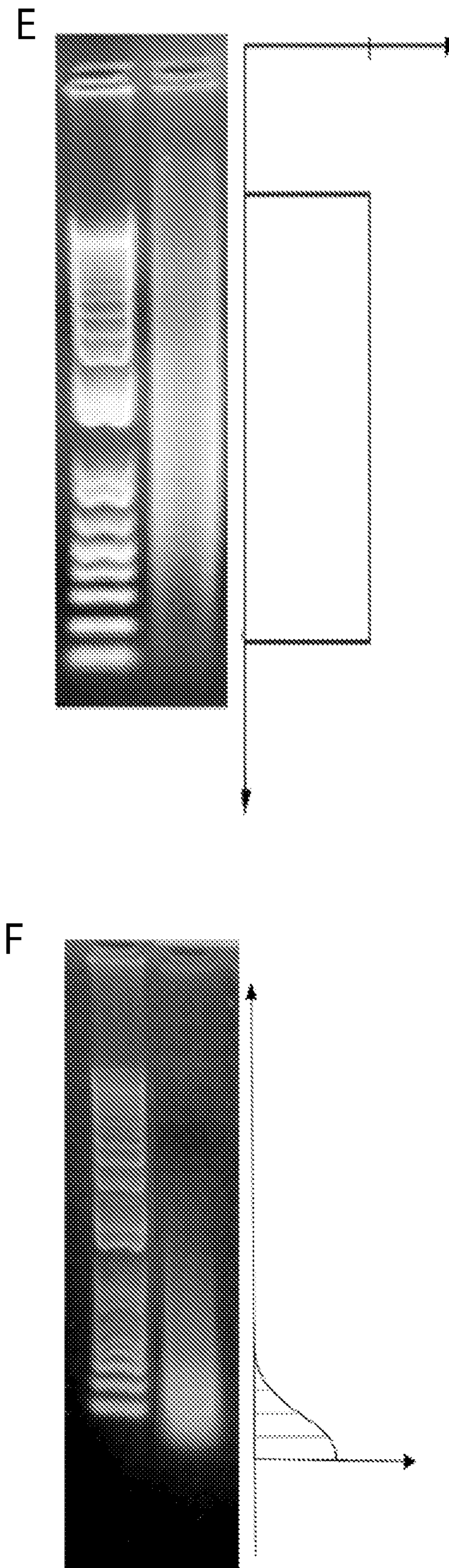


Fig. 1

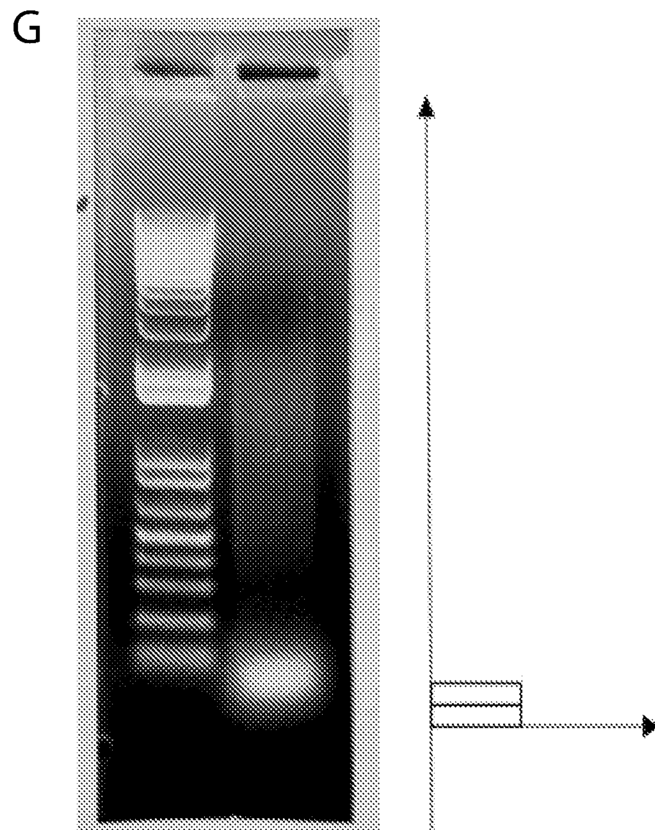
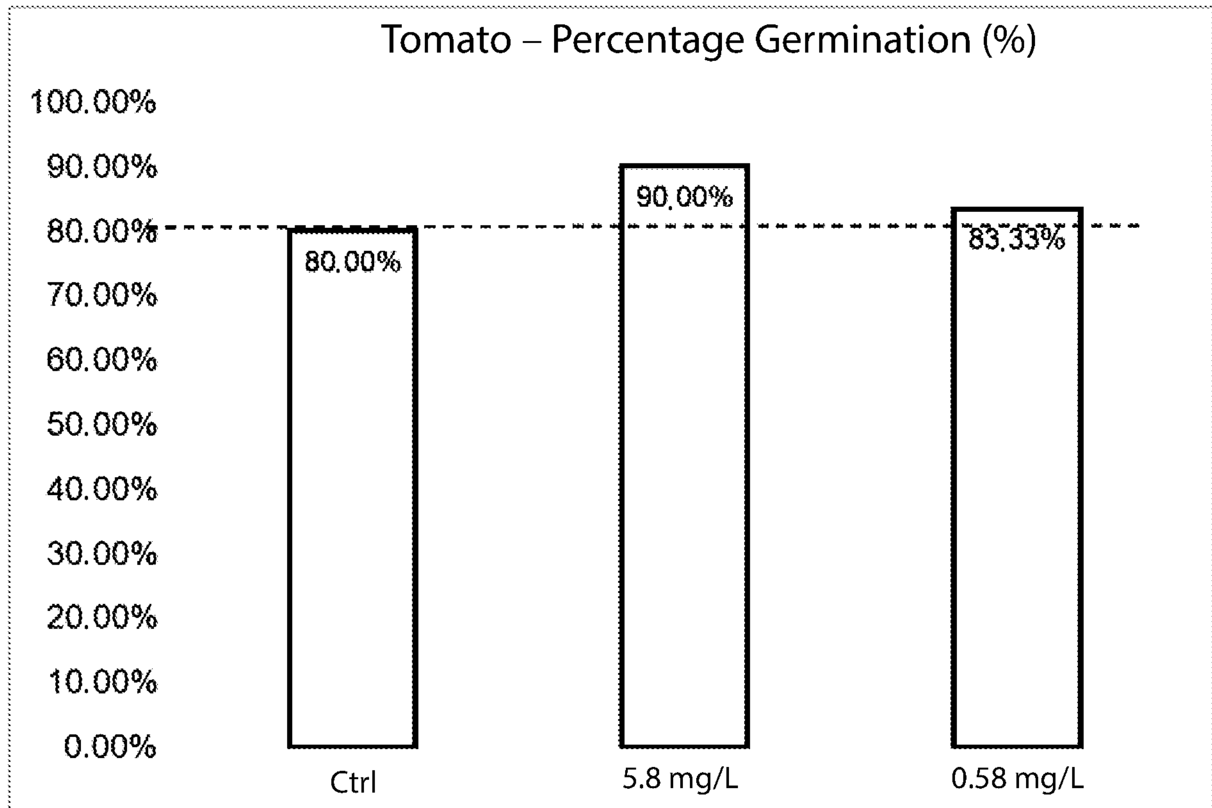


Fig. 1

A



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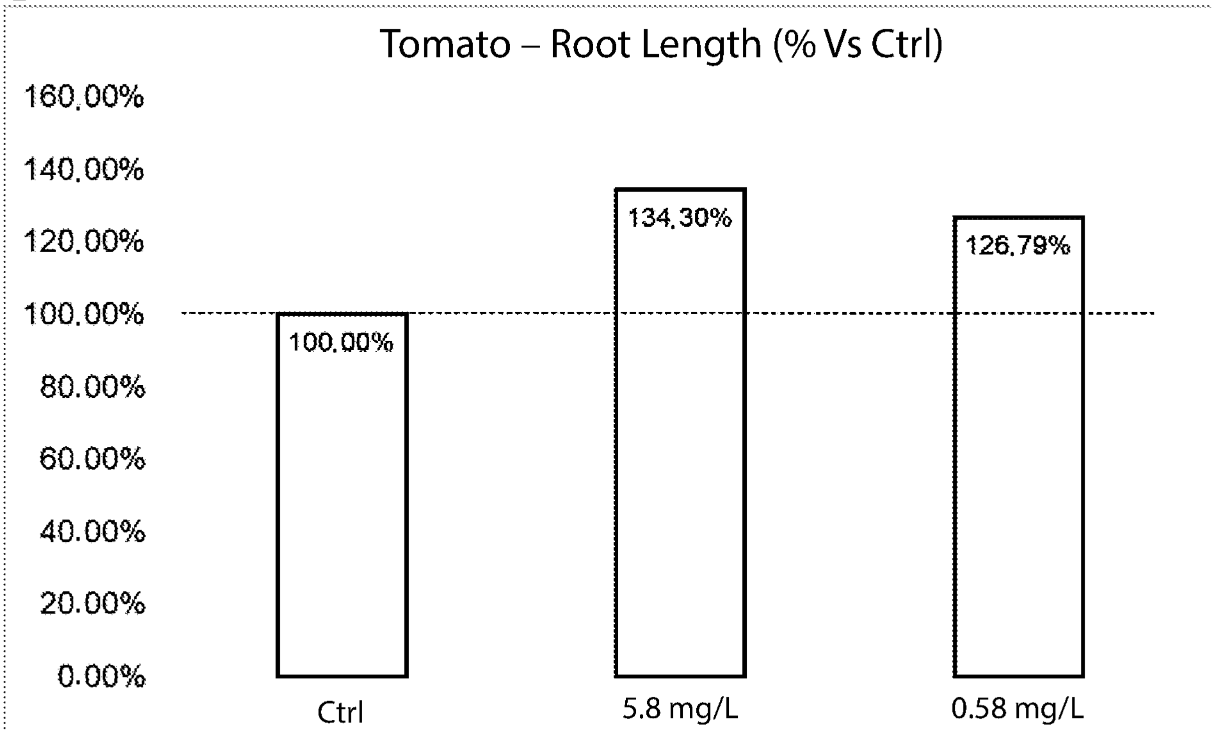

Fig. 2

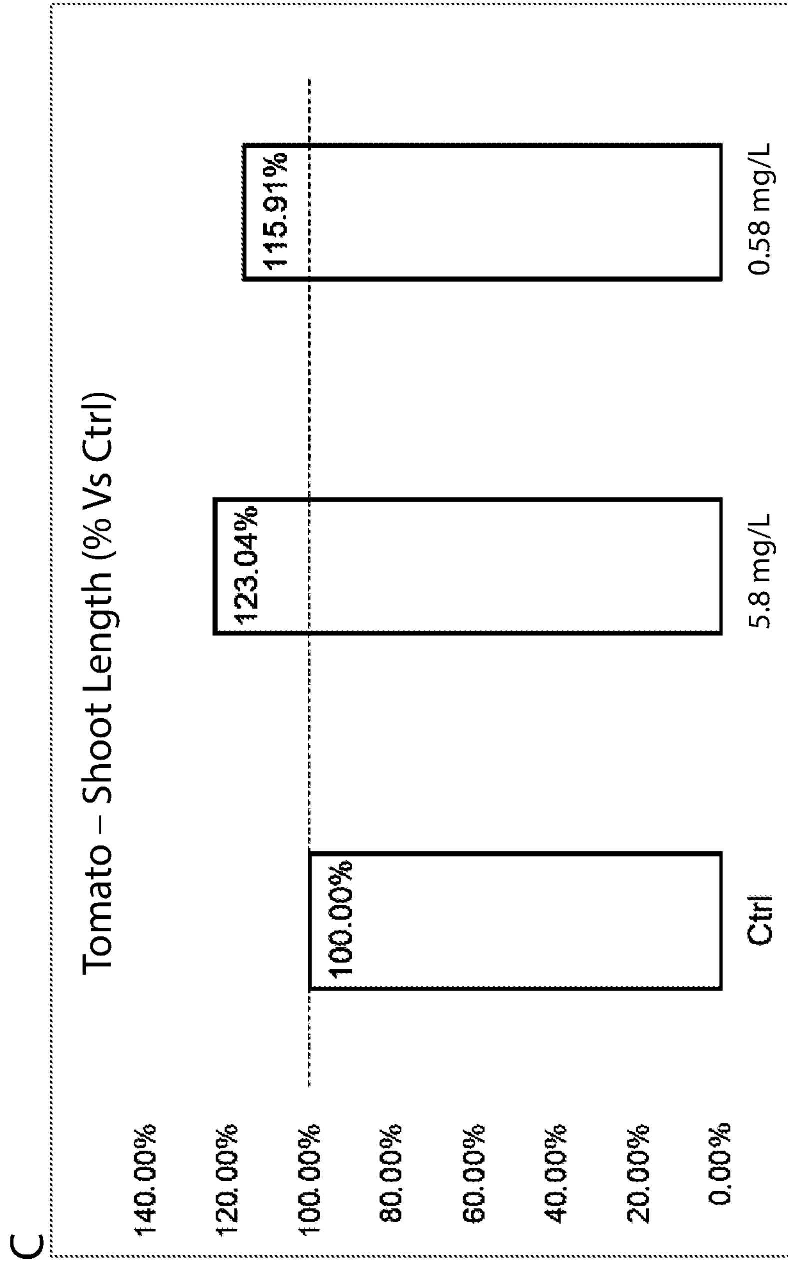

Fig. 2

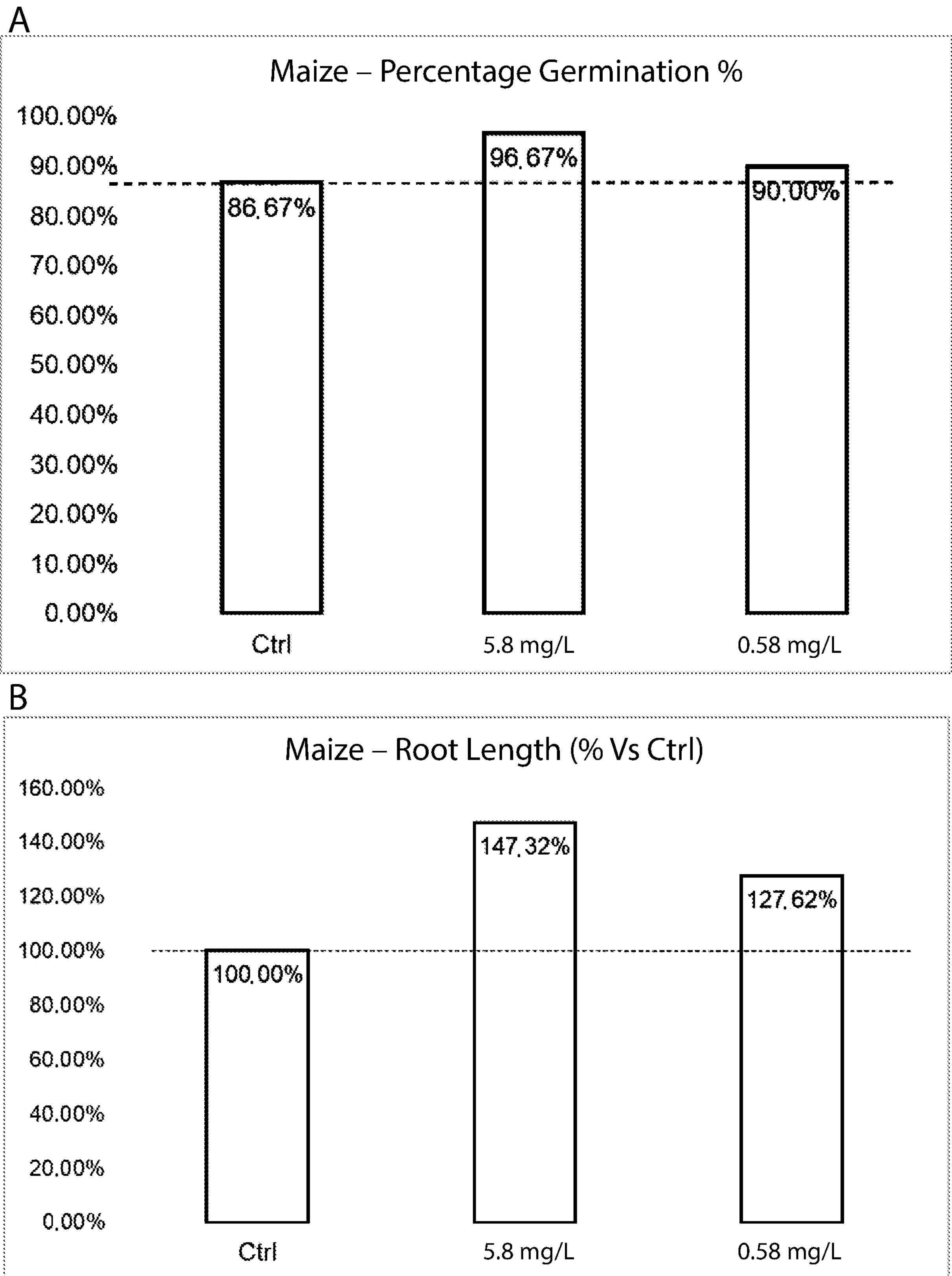

Fig. 3

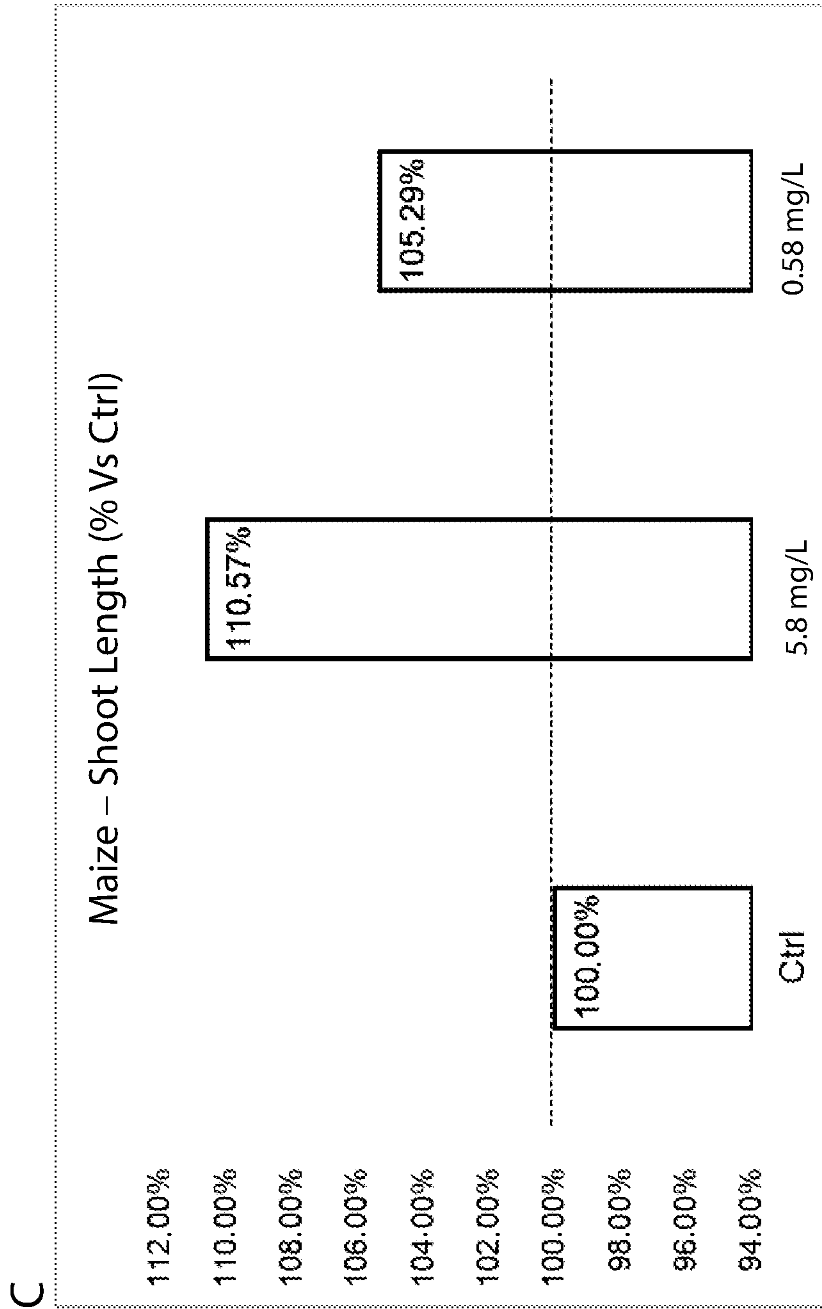

Fig. 3

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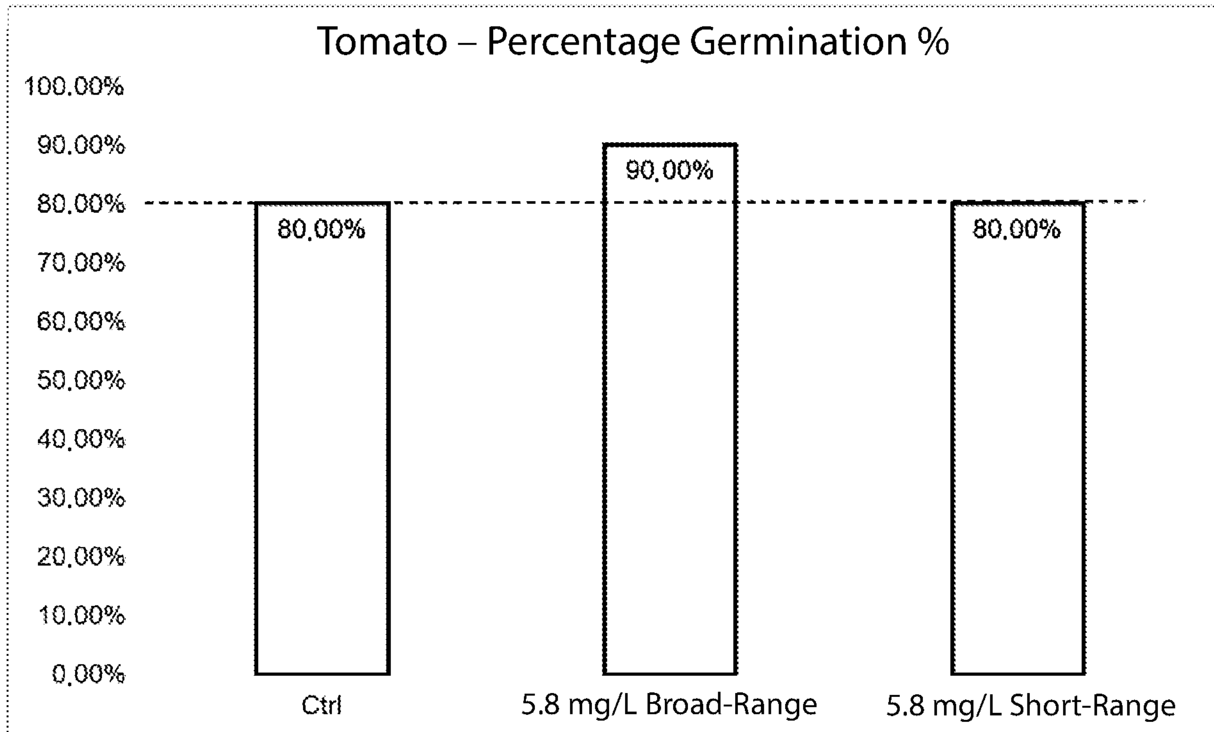

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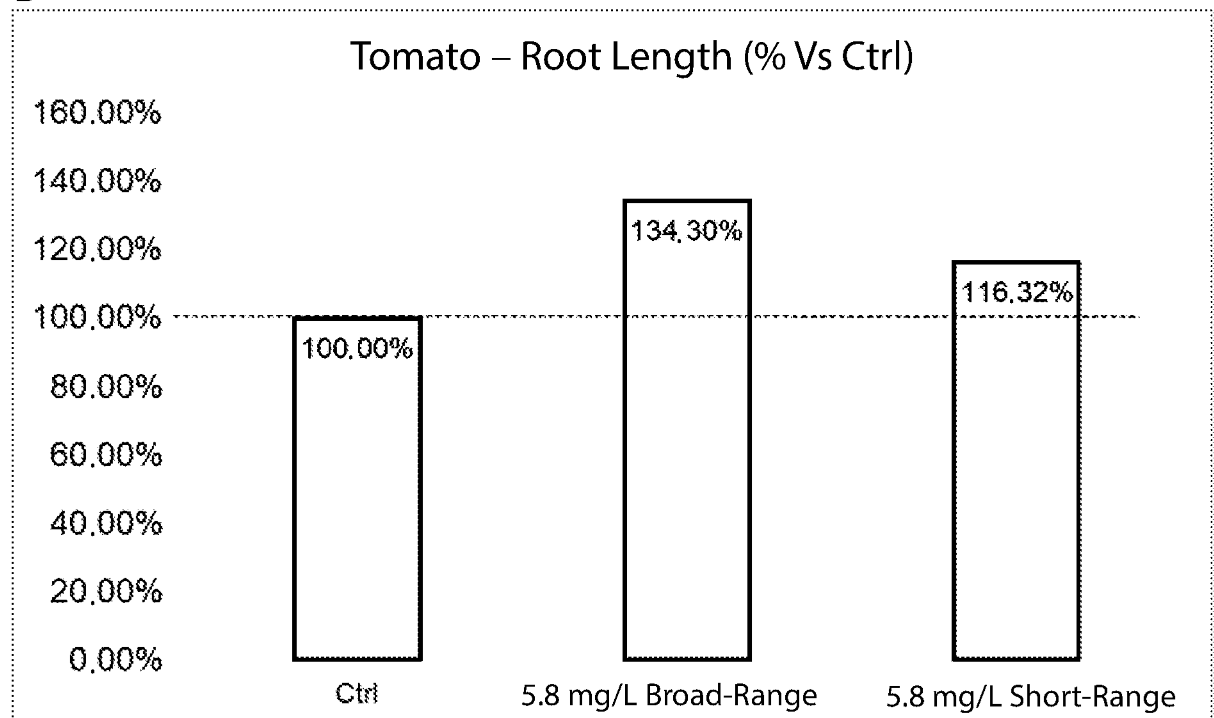

Fig. 4

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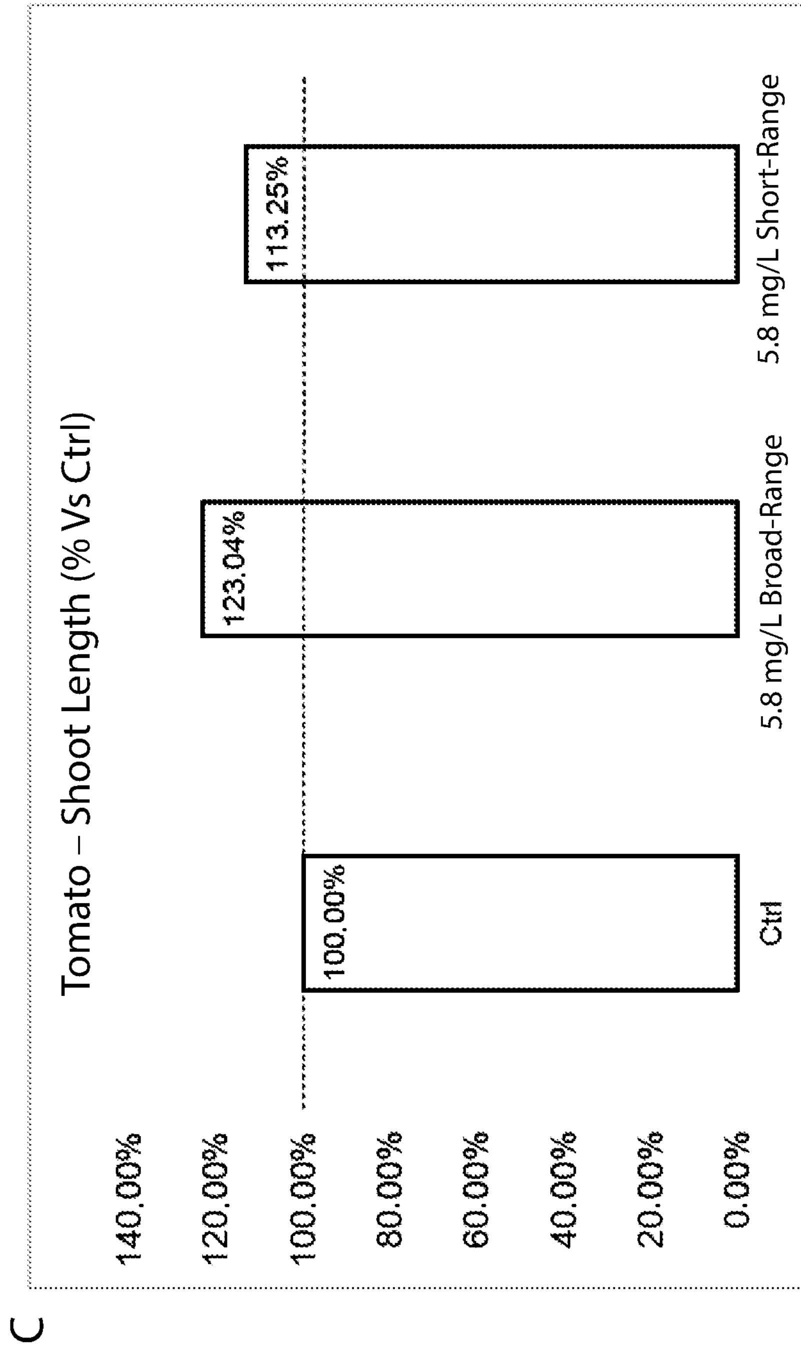

Fig. 4

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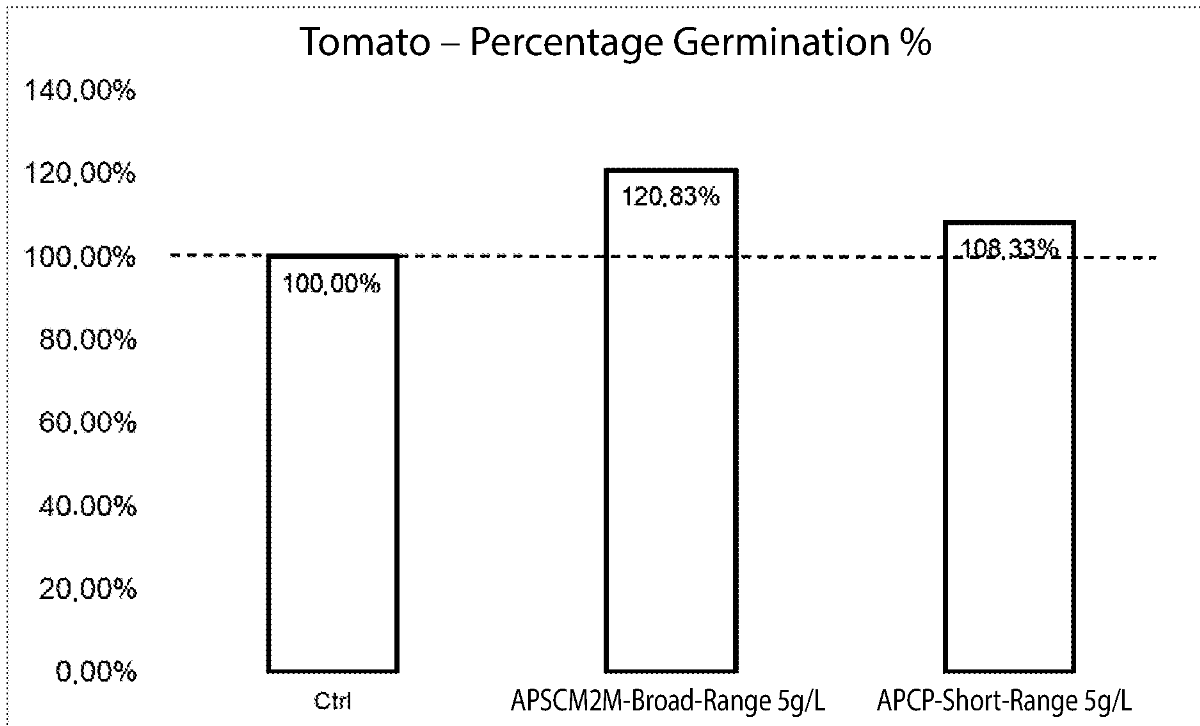

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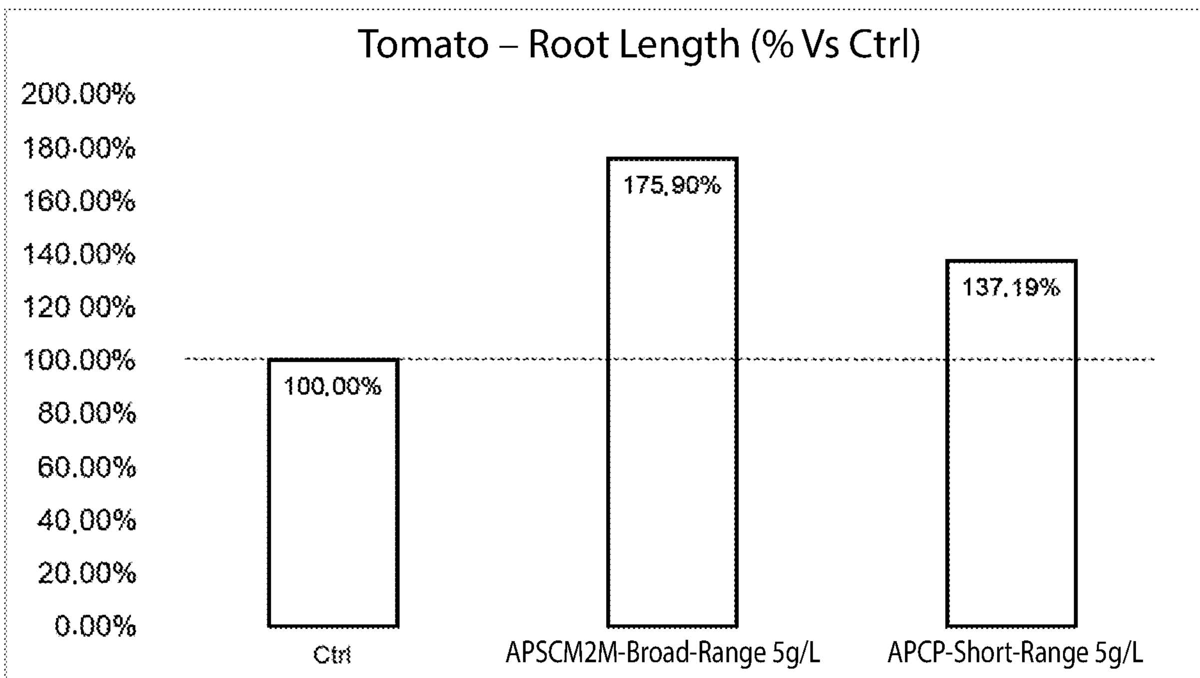

Fig. 5

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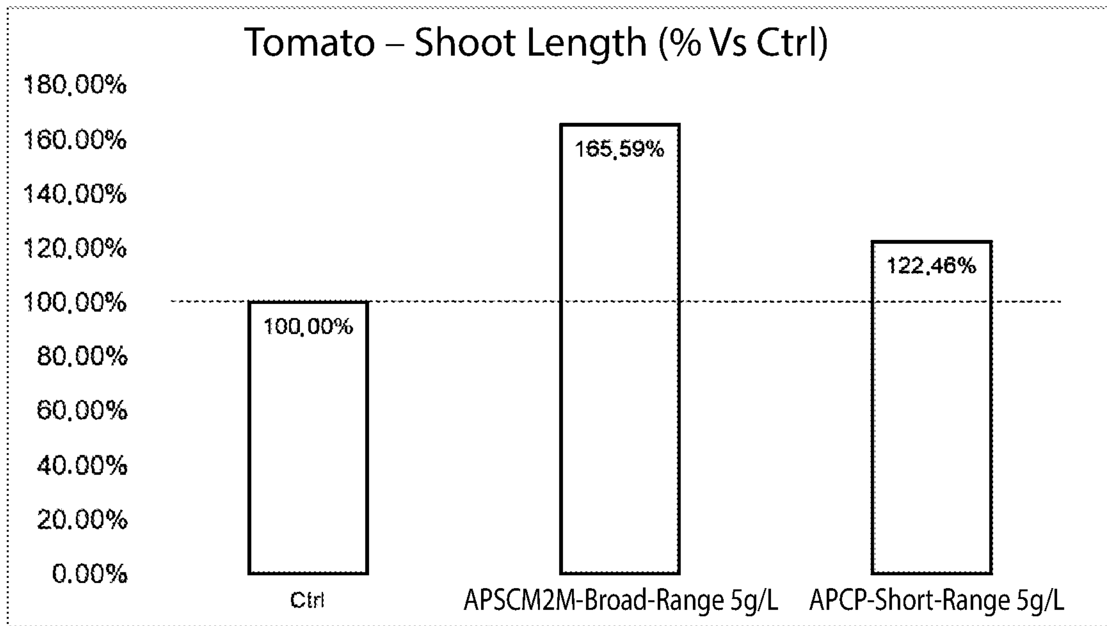

Fig. 5

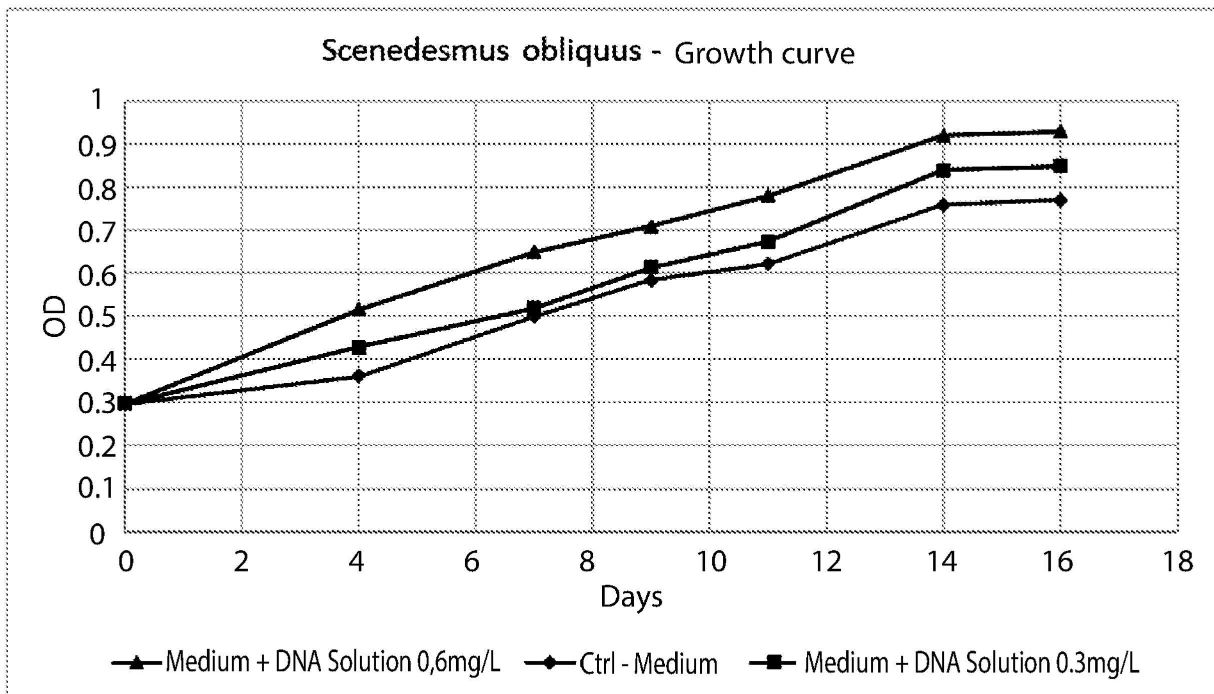

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/061558

A. CLASSIFICATION OF SUBJECT MATTER
INV. C05F7/00 C05F11/10 C12N15/11
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C05F A01N C12N C05D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/017455 A1 (VALAGRO SPA [IT]) 16 February 2023 (2023-02-16) claims page 2, line 24 - line 31 page 3, line 35 - page 4, line 35 page 9, line 25 - page 10, line 11 page 13, line 4 - line 16 -----	1-9
X	WO 2023/017456 A1 (VALAGRO SPA [IT]) 16 February 2023 (2023-02-16) page 2, line 26 - page 3, line 3 page 4, line 4 - page 5, line 5 page 8, line 20 - page 9, line 11 page 9, line 24 - page 10, line 2 page 12, line 33 - page 13, line 9 page 14, line 21 - line 27 claims -----	1-9
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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2025	18/02/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cardin, Aurélie

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/017457 A1 (VALAGRO SPA [IT]) 16 February 2023 (2023-02-16) page 2, line 30 - page 3, line 9 page 4, line 3 - line 26 page 6, line 32 - page 7, line 9 page 8, line 13 - page 9, line 11 page 9, line 18 - line 30 page 12, line 26 - page 13, line 2 claims	1-9
X	WO 2016/020874 A1 (VALAGRO SPA [IT]) 11 February 2016 (2016-02-11) claims	1-10
X	EP 4 134 436 A1 (VALAGRO SPA [IT]) 15 February 2023 (2023-02-15) paragraph [0006] paragraph [0011] - paragraph [0012] paragraph [0027] paragraph [0030] - paragraph [0031] claims	1-9
X	EP 4 134 437 A1 (VALAGRO SPA [IT]) 15 February 2023 (2023-02-15) paragraph [0015] paragraph [0026] paragraph [0035] claims	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/061558

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2023017455 A1	16-02-2023	EP 4384625 A1	19-06-2024
		WO 2023017455 A1	16-02-2023
WO 2023017456 A1	16-02-2023	EP 4134438 A1	15-02-2023
		WO 2023017456 A1	16-02-2023
WO 2023017457 A1	16-02-2023	EP 4134439 A1	15-02-2023
		WO 2023017457 A1	16-02-2023
WO 2016020874 A1	11-02-2016	AU 2015300679 A1	02-03-2017
		AU 2021286356 A1	20-01-2022
		BR 112017002328 A2	16-01-2018
		DK 3055415 T3	08-05-2017
		EP 3055415 A1	17-08-2016
		EP 3202907 A1	09-08-2017
		EP 3722432 A1	14-10-2020
		ES 2626560 T3	25-07-2017
		HU E034851 T2	28-03-2018
		NZ 728986 A	22-12-2023
		PL 3055415 T3	29-09-2017
		US 2017226509 A1	10-08-2017
		US 2024117372 A1	11-04-2024
		WO 2016020874 A1	11-02-2016
EP 4134436 A1	15-02-2023	NONE	
EP 4134437 A1	15-02-2023	NONE	