(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
**24 December 2020 (24.12.2020)**

WIPO | PCT

(10) **International Publication Number**
**WO 2020/254854 A1**

(51) **International Patent Classification:**
*A01N 59/00* (2006.01) *A01P 21/00* (2006.01)

(21) **International Application Number:**
PCT/IB2019/055044

(22) **International Filing Date:**
17 June 2019 (17.06.2019)

(25) **Filing Language:** English

(26) **Publication Language:** English

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published:**
- *with international search report (Art. 21(3))*
- *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** USE OF AT LEAST ONE SILICATE TO INCREASE THE ANTIOXIDANT ACTIVITY OF PLANTS

(57) **Abstract:** The present invention concerns the use of particles of at least one silicate to increase the antioxidant activity, such as in particular the catalase activity, of a plant. The silicate is especially selected from micas, aluminosilicates, tourmalines, serpentines and mixtures thereof. The particles are preferably contained in a composition which further contains a liquid carrier serving as a vehicle for said particles. The present invention further concerns a method for increasing the antioxidant activity of a plant wherein particles of at least one silicate are applied onto at least one part of said plant.

WO 2020/254854 A1

# USE OF AT LEAST ONE SILICATE TO INCREASE THE ANTIOXIDANT ACTIVITY OF PLANTS

The present invention concerns the use of at least one silicate under the form of solid particles to increase the antioxidant activity of a plant.

The present invention further concerns a method for increasing the antioxidant activity of a plant wherein particles of at least one silicate are applied onto or next to at least one part of said plant.

Agrochemical compositions comprising mineral components such as silicates are already known in the prior art.

For example, patent application CN 102726613 A discloses a nutrient solution for plants under the form of an aqueous composition containing chitine as well as different mineral ingredients including tourmaline powder and magnetite powder.

Such a composition is used as seed dressing agent that aims at increasing the germination rate, and further acts as a fertilizer and growth promoter.

The inventors of the present application have now discovered that mineral compounds belonging to the family of silicates have an effect on plants which is totally different from the effects disclosed in the prior art and which consists in increasing the antioxidant activity of plants.

The present invention thus concerns the use of particles of at least one silicate to increase the antioxidant activity of a plant.

In a manner know by one skilled in the art in the phytotherapeutic field, the antioxidant activity of a plant designates its ability to defend and protect itself against oxidative stress by the synthesis and the activation of antioxidant compounds.

An oxidative stress appears when the plant is in contact with oxidant agents such as in particular reactive oxygen species and free radicals.

The term “reactive oxygen species” (also known as ROS) embraces substances containing one or more activated atoms of oxygen and are not necessarily radicals (for example $H_2O_2$ is not a radical).

Free radicals are any chemical species that exist independently and contain unpaired electron(s). Some free radicals do not have oxygen atoms (for example, transition metals or carbon-centered radicals). Both ROS and free radicals promote oxidative stress through oxidation of cell compounds. An oxidative stress corresponds to the physiological state (or condition) when loss of electrons (oxidation) exceeds gain of electrons (reduction) leading to chemical (oxidative) damage of cell compounds. Oxidative stress is therefore associated with severe and long-term redox (reduction/oxidation) imbalance due to the lack of electrons.

A plant performs its antioxidant activity through the synthesis and activation of antioxidant compounds, that is to say compounds which are effective in eliminating reactive oxygen species and free radicals. Such compounds can be of enzymatic and of non-enzymatic nature.

As disclosed by V. Demidchik in Environmental and Experimental Botany 109 (2015) 212–228, the major enzymatic antioxidants which show high affinity to specific ROS include cytosolic Cu–ZnSOD, mitochondrial Mn-SOD, chloroplastal Fe-SOD (all SODs: superoxide + 2H+ $\rightarrow H_2O_2 + O_2$), catalases ($2H_2O_2 \rightarrow 2H_2O+O2$), peroxidases ($R/HOOH + R\text{-}H2 \rightarrow R + 2H_2O/ROH$), peroxiredoxins ($ROOH \rightarrow ROH$), thioredoxins and glutaredoxins (both: $R–S–S–R \rightarrow 2R–SH$). Non-enzymatic antioxidants are non-specific to different ROS and comprise ascorbic acid, glutathione, proline, polyamines, betaine, carotenes, some flavonoids and -tocopherol. Mention shall also be made of enzymes maintaining ROS scavenger function, such as monodehydroascorbate reductase, dehydroascorbate reductase, thioredoxin reductase, glutathione reductase, glutathione S-transferases and of compounds which reduce oxidised antioxidants, such as ascorbic acid and glutathione. Mention shall further be made of compounds that decrease catalytic activity of transition metals, such as metollothioneines (small Cys-rich proteins), phytochelatines (oligomers of glutathione). As further explained by V. Demidchik, oxidative stress has several damaging consequences on plant organisms. Oxidative stress causes reversible or irreversible (causing complete loss of some physiological activities) modifications of bio-molecules such as proteins, polynucleic acids, carbohydrates and lipids.

Among these, oxidation of lipids is particularly dangerous because it propagates free radicals through so-called “chain reactions”. Lipid oxidation (also known as lipid peroxidation) is widely considered as a “hallmark” of oxidative stress. Severe lipid peroxidation leads to the damage of membranes, collapse of their barrier function, followed by disintegration of organelles, oxidation and dysfunction of proteins, DNA and RNA.

ROS can oxidize any proteinogenic amino acid. Such modification to this key organic component of life results in loss of a given protein-mediated function, such as specific metabolic, structural, transport or regulatory activities. Protein oxidation also results in accumulation of toxic protein aggregates, and in the case of severe damage, induces PCD (programmed cell death).

Carbohydrates are the most abundant group of organic molecules in plants. They mechanically support and shape plant cells (cellulose, pectin, etc.), store reduced carbon (starch, sucrose etc.), regulate enzyme activities and osmotic pressure (low molecular weight sugars), provide non-enzymatic antioxidant defense (flavonoids, mannitol etc.) and play other key roles. Oxidation of carbohydrates is potentially harmful for plants.

Oxidative damage of DNA can be reason of aging of seeds stocks and, sometimes, death of crop plants.

A fundamental enzyme which has a key contribution in the antioxidant activity of a plant is catalase. In a manner known per se, catalase is an enzyme which catalyzes the decomposition of hydrogen peroxide into water and oxygen according to the following reaction scheme:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Hydrogen peroxide is known as being a ROS having a toxic effect for plants.

The present invention allows in particular to increase the catalase activity in plants.

According to a preferred embodiment of the invention, the increase of the antioxidant activity is evaluated by assessing the increase of the catalase activity in the plant.

By increasing the antioxidant activity of plants, the use according to the present invention increases the resistance thereof against oxidant agents such as reactive oxygen species and free radicals, and allows avoiding the damaging consequences of oxidative stress as disclosed above.

In a more general manner, the invention promotes the resistance of plants to external conditions (including pollution, climate) and to diseases. The plant is stronger, healthier and the product obtained from such plant such as for example plant extracts, vegetable raw material and foodstuffs are of better quality.

In particular, the inventors have discovered that the plants treated in accordance with the present invention become more resistant to hydric stress.

Other subjects, characteristics, aspects and advantages of the invention will emerge even more clearly on reading the description and the examples that follow.

In the present description, and unless otherwise indicated:

- the expression "at least one" is equivalent to the expression "one or more" and can be replaced therewith;
- the expression "between" is equivalent to the expression "ranging from" and can be replaced therewith and implies that the limits of the range are included;
- the term “compound in CX” designates in a manner known per se a compound having X atoms of carbon in its molecule.

The present invention uses solid particles of one or more silicate.

The silicate is preferably selected from the group consisting of micas, aluminosilicates, tourmalines, serpentines and mixtures thereof.

The aluminosilicates can belong to clays or not.

Among the aluminosilicates, mention can be made of some groups as feldspars, zeolites and kaolinites, and of specific aluminosilicates such as for example petalite, prehnite, delhayelite, bannisterite, allophane, crysocolla, minehillite, wickendurgite, franklinphilite.

Among the aluminosilicates from clay materials, besides the kaolinites, mention can be made of minerals from chlorite group, illite, glauconite, montmorillonite, palygorskite, pyrophyllite, sauconite and vermiculite.

According to a most preferred embodiment the silicate is selected from the group consisting of tourmalines.

The D90 size of the particles of silicate preferably ranges from 0.1 to 30 µm, preferably from 0.5 to 20 µm, more preferably from 1 to 15 µm, even more preferably from 2 to 10 µm and most preferably from 5 to 7 µm.

In a manner known per se in the field of particle size analysis, D90 designates the point in the volume size distribution of a population of particles, up to and including which, 90% of the total population is contained. In other words, D90 is defined by the value in the particle size distribution of a sample where 90% of the particles present in the sample are equal of below that value. For example, a sample of particles having a D90 of 1 µm means that 90% by volume of the sample has a size of 1 µm or smaller.

The size distribution of a sample of particles can be measured using the laser diffraction particle size analysis method (using, for example, Malvern or Cilas particle size analyzers). One advantageous way to carry out the process consists in suspending the particles in water and in determining their particle size by laser diffraction using the method described in standard ISO 13320:2009.

According to a preferred embodiment, the particles of at least one silicate are used in combination of one or more additional mineral compound(s).

The additional mineral compounds usable according to the invention can be chosen in particular from oxides, sulfates, carbonates, and phosphates.

The oxides can advantageously be chosen from titanium dioxide, silicon dioxide and magnesium oxide.

The sulfates are advantageously chosen from alkali metal and alkaline-earth metal sulfates, preferably from barium sulfate, calcium sulfate and strontium sulfate.

According to a particularly preferred embodiment of the invention, use is made of particles of barium sulfate.

The carbonates are advantageously chosen from calcium carbonate and sodium carbonate.

The phosphates can be chosen from zirconium phosphates, cerium phosphate and apatite, and mixtures thereof.

Said one or more additional mineral compounds are preferably used under the form of particles which are distinct from said particles of silicate. In this case, the D90 size of the particles of additional mineral compound(s) preferably ranges from 0.1 to 30 µm, preferably from 0.5 to 20 µm, more preferably from 1 to 15 µm, even more preferably from 2 to 10 µm and most preferably from 3 to 4 µm.

According to a preferred embodiment of the present invention, the particles of at least one silicate are contained in a composition which further contains a carrier serving as a vehicle for said particles of at least one silicate and optionally of said additional mineral compound(s).

The carrier is preferably liquid at ambient temperature (25°C) and atmospheric pressure ($1,013.10^5$ Pa).

According to a preferred embodiment, the composition is under the form of a suspension of said particles within the liquid carrier.

According to a preferred embodiment, the composition contains an aqueous liquid carrier that is to say a carrier containing water. The carrier can be made of water, or of water mixed with one or more organic fluid which can be water-miscible or not.

When the carrier contains water and one or more water-immiscible organic fluids, said carrier is in the form of an emulsion.

The organic fluids can be for example chosen from natural or synthetic oils, in particular mineral oils, vegetable oils, fatty alcohols, fatty acids, esters containing at least one fatty acid and/or at least one fatty alcohol.

The fatty alcohols and fatty acids mentioned above are those which contain from 8 to 32, preferably from 10 to 26 and more preferentially from 12 to 22 carbon atoms.

The organic fluids when used are preferably water-miscible in any proportions. They can thus be chosen from mono-alcohols containing from 2 to 5 carbon atoms, such as ethanol, isopropanol and polyols such as, in particular, glycol, glycerol, saccharides such as sorbitol.

It is of course possible to use mixtures of organic fluids and in particular any mixtures of any of the fluids described above.

According to one particularly preferred embodiment, the carrier of the composition used in the present invention is water.

The composition advantageously contains at least 20% by weight of water, more preferentially at least 30% by weight of water, more preferentially at least 40% by weight of water, relative to the total weight of said composition.

When one or more organic fluids are present, the composition preferably contains from 0.5% to 20% by weight of organic fluid(s), more preferentially from 1% to 10% by weight of organic fluid(s), relative to the total weight of said composition.

The particles of at least one silicate preferably represent from 10 to 80% by weight, more preferably from 15 to 70% by weight, more preferably from 20 to 60% by weight, and even more preferably from 25 to 40% by weight, with regard to the total weight of the composition.

When the one or more additional mineral compounds as disclosed above are present, they preferably represent from 5 to 40%

by weight, more preferably from 10 to 35% by weight, even more preferably from 15 to 30% by weight, with regard to the total weight of the composition.

The above amounts of particles of silicate particles and of additional mineral compound(s) – when present- are disclosed with regard to the ready-to-use composition, that is to say, the composition that is directly applied onto the plant material.

According to an embodiment of the invention, such composition is prepared extemporaneously (that is to say, just before use), by diluting a solid or liquid concentrate comprising said particles of silicate particles and optionally said additional mineral compound(s) with a liquid carrier comprising in particular as water.

The composition containing said particles of silicate may further contain one or more surfactants.

These surfactants can for example be chosen from the group consisting of betaines, amine oxides, ethoxylated fatty amines, fatty amines, ether carboxylates, acid or non acid mono- and di-ester phosphates, optionally polyalkoxylated, alkylmonoglycosides, alkylpolyglycosides, and mixtures thereof.

The betaine surfactants are in particular those described in WO2006/069794. Preferably, the betaine surfactants are chosen from the betaines having formula $R^1R^2R^2N^+\text{-}CH_2COO^-$ (I), the betaines having formula $R^1\text{-CO-NH-}R^4R^2R^2N^+\text{-}CH_2COO^-$ (II), and mixtures thereof, wherein the $R^1$ group is a linear or branched hydrocarbon group, preferably an alkyl group containing 2 to 30 carbon atoms, preferably 2 to 24 carbon atoms, preferably 3 to 20 carbon atoms; the $R^2$ groups which are identical or different, are a C1-C3 alkyl group, preferably a methyl group, and the $R^4$ group is a divalent linear or branched hydrocarbon group containing 1 to 6 carbon atoms, optionally substituted with a hydroxyl group, preferably a group of formula $\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}$ or $\text{-}CH_2\text{-CHOH-}CH_2\text{-}$.

Preferably, in formulae (I) and (II) above, $R^2$ is a methyl group. $R^1$ is preferably an alkyl group. This group is usually actually a mixture of different groups having different numbers of carbon atoms, being linear or branched, and optionally having some insaturations. These mixtures come from the reagents used to prepare them, which are actually distillation cuts and/or have a natural origin. In the present specification the number of carbon atoms in the $R^1$ group refers to the number of carbon atoms of the two most represented species.

The preferred betaine surfactants are those wherein $R^2$ is a methyl group, $R^1$ is a lauryl alkyl group mixture, preferably having more than 50% by weight of C12 and $R^4$ if present is $-CH_2-CH_2-CH_2-$.

Betaines of formula (I) are preferred. They are often referred to as alkyl betaines, and are preferably an alkyldimethyl betaine based surfactant, for example lauryl dimethyl betaine based surfactant ($R^2$ is a methyl group and $R^1$ is a lauryl C12 group).

Betaines of formula (II) are often referred to as alkyl amidoalkyl betaines.

The amine oxide surfactants which may be used in the present invention are in particular those described in WO2006/069794.

Such amine oxides surfactants can be chosen from the amine oxides having formula $R^1R^2R^2N\rightarrow0$ (III), the amine oxides having formula $R^1$-CO-NH-$R^4R^2R^2N\rightarrow0$ (IV), and mixtures thereof, wherein $R^1$, $R^2$ and $R^4$ are as described in formulae (I) and (II) above.

In formulas (III) and (IV) above, the $R^2$ group is preferably a methyl group. $R^1$ is preferably an alkyl group. This group is usually actually a mixture of different groups having different numbers of carbon atoms, being linear or branched, and optionally having some insaturations. These mixtures come from the reagents used to prepare them, which are actually distillation cuts and/or have a natural origin. In the present specification the number of carbon atoms in the $R^1$group refers to the number of carbon atoms of the two most represented species.

The preferred amine oxide surfactants are those wherein $R^2$ is a methyl group, $R^1$ is a lauryl alkyl group mixture, preferably having more than 50% by weight of C12 and $R^4$ if present is $-CH_2-CH_2-CH_2-$.

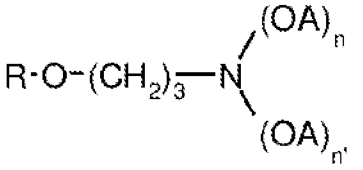
Amine oxides of formula (III) are preferred. They are often referred to as alkyl amine oxides, and are preferably an alkyldimethyl amine oxide based surfactant, for example lauryl dimethyl amine oxide based surfactant ($R^2$ is a methyl group and $R^1$ is a lauryl C12 group).

Amine oxides of formula (IV) are often referred to as alkyl amidoalkyl amine oxides.

The fatty amines or ethoxylated fatty amines useful as surfactants in the present invention may comprise at least one hydrocarbon group containing 2 to 24 carbon atoms, optionally polyalkoxylated.

The fatty amines or ethoxylated fatty amines may more particularly be selected from amines comprising at least one linear or branched, saturated or unsaturated group containing 2 to 24 carbon atoms, preferably 8 to 18 carbon atoms, optionally comprising 2 to 30 oxyethylene groups, or a mixture of a plurality thereof. Examples include ethoxylated tallow amines. The fatty amines or ethoxylated fatty amines may be selected from ethoxylated fatty amines comprising at least one or several, linear or branched, saturated or unsaturated, group(s) containing 6 to 24 carbon atoms, preferably 8 to 20 carbon atoms, comprising 2 to 30 oxyethylene groups, or mixtures thereof.

Examples include the compounds having the following formula (V):



$$R\text{-}O\text{-}(CH_2)_3-N\begin{matrix}(OA)_n\\(OA)_{n'}\end{matrix}$$

wherein R represents a linear or branched, saturated or unsaturated hydrocarbon group containing 6 to 24 carbon atoms, preferably 8 to 20 carbon atoms; OA represents an oxyalkylene group; and n, n', which may or may not be identical, represent a mean number in the range 1 to 30.

Examples of such amines to be cited are amines derived from copra and containing 5 oxyethylene (OE) units, oleic amines containing 5 OE, amines derived from tallow containing 5 to 20 OE, for example 10 OE, compounds corresponding to the above formula in which R is an alkyl group containing 12 to 15 carbon atoms and the total number of OE units is in the range 20 to 30.

The ether carboxylates useful as surfactants in the present invention preferably have the following formula (VI): $R(OCH_2CH_2)_nOCH_2CO_2$, wherein R is a linear or branched alkyl, alkenyl, alkylphenyl or polypropyleneoxy group having from 6 to 20, for example 8 to 14, aliphatic carbon atoms and n is a number ranging of from 1 to 30, preferably of from 2 to 20. The ether carboxylate has preferably a counter ion being ammonium or potassium, or obtained from an amine or alkanolamine having up to 6 carbon atoms.

The optionally polyalkoxylated acid or non acid mono- and di-ester phosphates useful as surfactants in the present invention are selected from acid or non acid phosphate mono- or di-esters, optionally polyalkoxylated, having the following formula (VII):

$(A)_{3-m}P(=O)(OM)_m$

wherein:

- A, identical or different, represents a group $R'^1-O(CH_2-CHR'^2-O)_n$ wherein:

$R'^1$ represents a linear or non linear, saturated or unsaturated C6-C20 hydrocarbon group, preferably C8-C18;

$R'^2$ represents a hydrogen atom or a methyl or ethyl group, preferably a hydrogen atom;

n is a mean number of motifs in the range 0 to 10, preferably in the range 2 to 10;

- M represents a hydrogen atom, an alkali or alkaline- earth metal, a $N(R^3)_4^+$ type radical wherein the $R^3$ groups, identical or different, represents a hydrogen atom or a linear or non linear, saturated or unsaturated C1-C6 hydrocarbon group optionally substituted with a hydroxyl group;

- m is a whole or average number in the range 1 to 2.

The acid or non acid mono- and di-ester phosphate, optionally polyalkoxylated may be in the form of a monoester, a diester, or a mixture of these two esters.

The preferred surfactants are chosen from anionic surfactants such as ether carboxylates, optionally polyalkoxylated acid or non acid mono- and di-ester phosphates, and mixtures thereof.

When the composition used in the present invention comprises one or more surfactants, the total amount of said surfactant(s) preferably ranges from 0.5 to 20 % by weight based on the total weight of the composition.

According to a preferred embodiment, the composition containing said particles of silicate further contains one or more thickening agents.

Suitable thickening agent can be in particular chosen from polysaccharides such as for example xanthan gum, alginates, carboxylated or hydroxylated methylcelluloses, synthetic macromolecules of the polyacrylate, polymaleate, polyvinylpyrrolidone, polyethylene glycol or polyvinyl alcohol type.

When the composition used in the present invention comprises one or more thickening agents, the total amount of thickening agent(s) preferably ranges from 0.05 to 5 % by weigh, preferably from 0.1 to 2 % by weight, based on the total weight of the composition.

The composition containing said particles of silicate may further contain one or more dispersant. Suitable dispersants include in particular polycarboxylate polymers such as sodium polycarboxylate.

When the composition comprises one or more dispersant(s), the total amount thereof preferably ranges from 0.05 to 5%, preferably from 0.1 to 2% by weight, more preferably from 0.3 to 1% by weight, based on the total weight of the composition.

According to a preferred embodiment, the composition containing said particles of silicate further contains one or more biocide.

A biocide is a chemical substance capable of killing different forms of living organisms used in fields such as medicine, forestry, and mosquito control.

Usually biocides are divided into two sub-groups:

- pesticides, which includes herbicides, insecticides and insect repellants, fungicides, rodenticides, algicides, moluscicides and miticides,

- antimicrobials, which includes germicides, antibiotics, antibacterials, antivirals, antifungals, antiprotozoals and antiparasites.

Useful biocides may be chosen, in a non-limitative manner, among the following compounds: triazine herbicides, sulfonylurea herbicides, uracils, urea herbicides, acetanilide herbicides, organophosphonate herbicides, glyphosate salts, glyphosate esters, nitrilo oxime fungicides, imidazole fungicides such as for example carbendazim, triazole fungicides, sulfenamide fungicides, dithio-carbamate fungicides such as for example thiram, chloronated aromatic, dichloro aniline fungicides, strobilurin fungicides such as for example pyraclostrobin, carbamate insecticides, organo thiophosphate insecticides; perchlorinated organic insecticides, phenylpyrazole insecticides such as fipronil, methoxychlor, miticides, propynyl sulfite, triazapentadiene miticides, chlorinated aromatic miticides, tetradifan, dinitrophenol miticides, binapacryl, thiophanate-methyl and mixtures thereof.

Preferably, the biocide used in the present invention is a pesticide, and more preferably a pesticide selected from herbicides, insecticides and fungicides. According to a particularly preferred embodiment, the composition contains at least one fungicide.

The composition containing said particles of silicate may further contain one or more fertilizers, preferably chosen from water-soluble fertilizers such as for example foliar fertilizers (fertilizers which are taken up by the leaves of the plants), such as urea or foliar macro- or microelement fertilizer, including chelates.

The composition containing said particles of silicate may further contain additional ingredients, which can be chosen, from all additives and adjuvants useful in agrochemical compositions such as for example nutrients, anti-foaming agents, colorants such as pigments, etc...

The present invention can be implemented on any type of plant. The plants are preferably chosen among agricultural and horticultural plants, shrubs, trees and grasses.

The plant species include but are not limited to corn (Zea mays), Brassica sp. (e.g., B. napus, B. rapa, B. juncea), alfalfa (Medicago sativa), rice (Oryza sativa), rye (Secale cereale), sorghum (Sorghum bicolor, Sorghum vulgare), millet (e.g., pearl millet (Pennisetum glaucum), proso millet (Panicum miliaceum), foxtail millet (Setaria italica), finger millet (Eleusine coracana)), sunflower (Helianthus annuus), safflower (Carthamus tinctorius), wheat (Triticum aestivum), soybean (Glycine max), tobacco (Nicotiana tabacum), potato (Solanum tuberosum), peanuts (Arachis hypogaea), cotton (Gossypium barbadense, Gossypium hirsutum), sweet potato (Ipomoea batatus), cassava (Manihot esculenta), coffee (Cofea spp.), coconut (Cocos nucifera), pineapple (Ananas comosus), citrus trees (Citrus spp.), cocoa (Theobroma cacao), tea (Camellia sinensis), banana (Musa spp.), avocado (Persea americana), fig (Ficus casica), guava (Psidium guajava), mango (Mangifera indica), olive (Olea europaea), papaya (Carica papaya), cashew (Anacardium occidentale), macadamia (Macadamia integrifolia), almond (Prunus amygdalus), sugar beets (Beta vulgaris), sugarcane (Saccharum spp.), canola, oats, barley, vegetables, ornamentals, woody plants such as conifers and deciduous trees, squash, pumpkin, hemp, zucchini, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, soybean, sorghum, sugarcane, rapeseed, clover, carrot, and Arabidopsis thaliana.

Further examples of plants include tomatoes (Lycopersicon esculentum), lettuce (e.g., Lactuca sativa), green beans (Phaseolus vulgaris), lima beans (Phaseolus limensis), peas (Lathyrus spp.), cauliflower, broccoli, turnip, radish, spinach, cabbage, asparagus, onion, garlic, pepper, celery, and members of the genus Cucumis such as cucumber (C. sativus), cantaloupe (C. cantalupensis), and musk melon (C. melo).

Mention can further be made of ornamentals species including but not limited to hydrangea (Macrophylla hydrangea), hibiscus (Hibiscus rosasanensis), petunias (Petunia hybrida), roses (Rosa spp.), azalea (Rhododendron spp.), tulips (Tulipa spp.), daffodils (Narcissus spp.), carnation (Dianthus caryophyllus), poinsettia (Euphorbia pulcherrima), and chrysanthemum; and of conifer species including but not limited to conifers pines such as loblolly pine (Pinus taeda), slash pine (Pinus elliotii), ponderosa pine (Pinus ponderosa), lodgepole pine (Pinus contorta), and Monterey pine (Pinus radiata), Douglas-fir (Pseudotsuga menziesii); Western hemlock (Tsuga canadensis); Sitka spruce (Picea glauca); redwood (Sequoia sempervirens); true firs such as silver fir (Abies amabilis) and balsam fir (Abies balsamea); and cedars such as Western red cedar (Thuja plicata) and Alaska yellow-cedar (Chamaecyparis nootkatensis).

In one embodiment, the plant is chosen from leguminous plant species including but not limited beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, green pea, moth bean, broad bean, kidney bean, lentil, dry bean, etc. Legumes include, but are not limited to, Arachis, e.g., peanuts, Vicia, e.g., crown vetch, hairy vetch, adzuki bean, mung bean, and chickpea, Lupinus, e.g., lupine, trifolium, Phaseolus, e.g., common bean and lima bean, Pisum, e.g., field bean, Melilotus, e.g., clover, Medicago, e.g., alfalfa, Lotus, e.g., trefoil, lens, e.g., lentil, and false indigo. Typical forage and turf grass for use in the methods described herein include but are not limited to alfalfa, orchard grass, tall fescue, perennial ryegrass, creeping bent grass, lucerne, birdsfoot trefoil, clover, stylosanthes species, lotononis bainessii, sainfoin and redtop. Other grass species include barley, wheat, oat, rye, orchard grass, guinea grass, sorghum or turf grass plant.

According to a preferred embodiment, the plant is selected from the group consisting of soy, cane, cotton, wheat, beans, rice, and preferably the plant is soy.

The particles of at least one silicate can be applied on the plant, or next to the plant, at any stage of its development. It can be applied on the whole plant or on parts thereof.

According to a first preferred embodiment, the particles of silicate are applied onto or next to seeds of the plant. Such application is preferably carried out by coating the seeds with a composition containing said particles as disclosed above.

Suitable known coating techniques may be utilized to coat the seeds or agglomeration of seed, and any suitable equipment or technique may be employed. Equipment that may be utilized for coating can include but are not limited to drum coaters, rotary coaters, tumbling drums, fluidized beds and spouted beds. The seeds may be coated via a batch or a continuous coating process.

According to a second preferred embodiment, the particles of silicate are applied onto the foliar system of the plant. Such application is preferably carried out by spraying a composition containing said particles as disclosed above onto the leaves of the plant. For example, the composition can be sprayed onto a field using appropriate means well known in agriculture.

In a general manner, the invention can be easily carried out using conventional and commercially available application equipment.

The invention can be implemented onto or next to plants which are under hydric stress or not.

According to a preferred embodiment, the plant in under hydric stress. Indeed, in this case the inventors have discovered that the increase of antioxidant activity is surprisingly higher.

Hydric stress conditions hereby means that the target soil area receives a total amount of water - which includes all sources of water including in particular irrigation and natural rainfall - of 50% or less with regard to the amount of water needed by the plant in normal watering conditions.

The amount of water needed by the plant in normal watering conditions depends on several parameters including in particular the type of plant, the climate, the planting conditions. The determination thereof is a routine procedure for one skilled in the art in the field of agriculture.

Another subject of the invention is a method for increasing the antioxidant activity of a plant wherein particles of at least one silicate are applied onto or next to at least one part of said plant.

The detailed description, given above, of the use according to the invention also applies to the method according to the invention.

In particular, in the method of the present invention, the embodiments below are preferred independently of each other:

The silicate can be selected from the group consisting of micas, aluminosilicates, tourmalines, serpentines, and mixtures thereof, preferably from the group consisting of tourmalines.

The particles of at least one silicate can be used in combination with particles of one or more additional mineral compound(s).

Said additional mineral compound can be selected from the group consisting of oxides, sulfates, carbonates and phosphates; preferably from the group consisting of alkali metal and alkaline-earth metal sulfates; more preferably from the group consisting of from barium sulfate, calcium sulfate and strontium sulfate; and most preferably said additional mineral compound is barium sulfate.

The silicate particles have a D90 size ranging from 0.1 to 30 µm, preferably from 2 to 10 µm and more preferably from 5 to 7 µm.

Said particles are contained in a composition which further contains a carrier serving as a vehicle for said particles.

The carrier can be a liquid carrier, preferably an aqueous liquid carrier, and most preferably the carrier is water.

The composition may further comprise one or more dispersant(s) preferably one or more polycarboxylate polymer(s) such as sodium polycarboxylate.

The composition is in the form of a suspension.

Said particles of at least one silicate represent from 10 to 80% by weight, preferably from 15 to 70% by weight, more preferably from 20 to 60% by weight, and even more preferably from 25 to 40% by weight, with regard to the total weight of the composition.

The plant can be selected from the group consisting of soy, cane, cotton, wheat, beans, rice, and preferably the plant is soy.

Said particles of silicate can be applied onto or next to seeds of the plant, preferably by coating the seeds with a composition comprising said particles.

The particles of silicate can be applied onto the foliar system of the plant, preferably by spraying a composition containing said particles as disclosed above onto the leaves of the plant.

The plant may be under hydric stress.

The method of the invention aims in particular at increasing the catalase activity in a plant.

The examples of implementation of the invention below are given purely by way of illustration and shall not be interpreted at limiting the scope thereof.

## EXAMPLES

The experiments described below were done in the city of Botucatu, Sao Paulo state in Brazil, wherein the predominant climate is temperate (mesothermic) with rains in the summer and drought in the winter (Cwa - Köeppen), with average annual temperature of 20.5 °C and annual pluviometric precipitation of 1.533 mm.

Tests were done in green house with the following characteristics: 30m of length, 7m of width and 3m height, covered with polyethylene film of low density and closed in the laterals with shading in 75% of the total area.

The minerals used in the below described experiments are:

- A powder of black tourmaline from Micro Service - Tecnologica em Micronização, Produtos e Processos Industriais with 2 different granulometries D90 = 6 µm (referenced as G2g below) and D90 = 4 µm (referenced as G2 below);
- A powder of barium sulfate from Venator P&A Spain S.L. with a granulometry D90 = 3.5 µm (referenced as G1g below)

The particle size distribution was measured by using the Malvern Mastersizer 2000 particle size analyzer, with the following parameters:

- Material: tourmaline (RI = 1.62) – barium sulfate (RI = 1.643)
- Dispersant media: water
- Pumping: 1250 rpm
- Mixing: 500 rpm
- Ultrasound: 20%
- Obscuration: 10-20%.

The following compounds have been used to prepare the samples:

- Lamegal HS/B = Aqueous solution of sodium polycarboxylate Mw = 2000 g/mol at a concentration of 40 to 42% by weight, from the company Lamberti;
- Geropon T 36 = Aqueous solution of sodium polycarboxylate at a concentration of 90% by weight, from the company Solvay;
- Proxel GXL = Aqueous solution of 1,2-benzisothiazolin-3-one (antifungal agent) from the company Lonza;
- Rhodopol 23 = Aqueous solution of xantan gum at a concentration of 2% by weight from the company Solvay.

<u>Compositions used:</u>

Three compositions under the form of aqueous suspensions of mineral particles were prepared, using the following protocol:

Lamegal HS/B, Geropon T36 and Proxel GXL were added in water and mixed until homogeneous solution. Then, the mineral (G2, G2g or G1g) was added and mixed until homogeneous dispersion. Finally, Rhodopol 23 was added and mixed until homogeneous formula. The mechanical agitator used was a IKA RW20 model with naval propeller stirrer.

The compositions are detailed in the tables below, where all amounts are expressed as percentages by weight of raw material (and not as active matter for the commercial products).

Composition G2g – 60% by weight of active particles:

| Component | Amount (% by weight) |
|---|---|
| G2g particles (tourmaline D90 = 6 µm) | 60.00 |
| Lamegal HS/B | 0.05 |
| Geropon T36 | 0.65 |
| Proxel GXL | 0.50 |
| Rhodopol 23 | 6.50 |
| Demineralized water | 32.30 |

Composition G2 – 40% by weight of active particles:

| Component | Amount (% by weight) |
|---|---|
| G2 particles (tourmaline D90 = 4 µm) | 40.00 |
| Lamegal HS/B | 0.06 |
| Proxel GXL | 0.30 |
| Rhodopol 23 | 11.20 |
| Demineralized water | 48.44 |

Composition G1g – 60% by weight of active particles:

| Component | Amount (% by weight) |
|---|---|
| G1g particles (barium sulfate D90 = 3.5 µm) | 60.00 |
| Lamegal HS/B | 0.10 |
| Geropon T36 | 0.65 |
| Proxel GXL | 0.50 |
| Rhodopol 23 | 6.50 |
| Demineralized water | 32.25 |

Example 1 : Seed coating

The above prepared compositions were applied on seeds of soy according to the treatment schemes detailed in Table 1 below.

| Treatment | Composition | Dose of composition applied (in ml per kg of seeds) |
|---|---|---|
| T1 (blank) | None | - |
| T2 (comparative) | G1g | 3.4 |
| T3 | G2 | 7 |
| T4 | G2g | 3.8 |
| T5 | G1g + G2g | 1.7 ml G1g + 1.9 ml G2g |

Table 1: treatment schemes for seed coating

The seeds were coated using a NoroGard R300 Laboratory Seed Coating device. The compositions indicated in Table 1 above were mixed in the above amounts with two commercial seed treatment products (2ml of Standak 250 FS (fungicide commercialized by BASF) + 2ml Protreat (fungicide commercialized by Novozymes) per kg of seed) and the final mixture was applied onto the seeds (except for the blank T1 where the seeds were not coated at all);

Each treatment by each formulation as shown in Table 1 has been replicated 6 times (ie performed on 6 batches of seeds, and the results below are the average results obtained for the 6 batches).

Each replicate was composed of 1 planter, with 5 plants.

Planters of 35 liters were used, with 44.5 cm height and 36.6 cm of diameter, with sandy soil. No additional fertilizer nor nutrients were added in the soil. The collected soil was collected from the soil layer classified as distroferric red latosol.

The seeds that have been coated with the above described formulations (except for the blank which has not been coated) were added in the planters in 5 furrows containing 3 seeds each, and after 10 days the thinning was performed, leaving only 1 plant per furrow.

Two different conditions have been tested: without hydric stress (Experiment 1) and with hydric stress (Experiment 2).

For Experiment 1, the plants were watered every day. The target soil area received an average amount of water, corresponding to the amount of water needed by the plant in normal watering condition, of 4.16 mm per $m^2$ per period of 2 days.

For experiment 2, the plants were watered every two days. The target soil area received an average amount of water of 2.08mm per $m^2$ per period of 2 days.

The antioxidant activity of the plants was determined using the protocol detailed hereunder.

Three collections of leaves were accomplished for the analyses of catalase, at 10, 20 and 30 days after planting, being collected 4 leaves of each treatment, which were selected and standardized with the limbo totally expanded. These leaves were put in plastic bags that were covered by aluminum foil and immediately frozen in liquid nitrogen to stop any ongoing reaction. After, bags were stored in an ultra freezer at -80ºC.

The activity of the catalase (CAT, EC 1.11.1.6 (CAT = catalase and EC = Enzyme Commission number: each enzyme has a different number, and this one is the reference for catalase)) was measured based on the methodology proposed by KAR and MISHRA in KAR, M.; MISHRA, D. Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiology, v. 57, p. 315-319, 1976 (KAR and MISHRA 1976), with some minor changes as described below.

*Extractor solution*: 17.41g of $K_2HPO_4$ (dibasic) is added in 1000 ml of distilled water (solution A), while 6.8g of $KH_2PO_4$ (monobasic) is added in 500 ml of distilled water (solution B). Both solutions are homogenized, separately. Then, the monobasic solution is added into the dibasic solution until reaching pH 7.8 in order to prepare the solution C. After, 0.372g of ethylenediaminetetraacetic acid (EDTA), 0.462g of DL-dithiothreitol (DTT) and 0.300g of polyvinylpolypyrrolidone (PVPP) are added in 1000 ml of solution C.

*Determination solution:* 17.41g of $K_2HPO_4$ (dibasic) is added in 1000 ml of distilled water (solution A), while 6.8g of $KH_2PO_4$ (monobasic) is added in 500 ml of distilled water (solution B). Both solutions are homogenized, separately. Then, the monobasic solution is added into the dibasic solution until reaching pH 7.5 in order to prepare the determination solution.

*Enzymatic extract*: It was used 0.1g of frozen fresh leaves, which were grinded in liquid nitrogen. Then, it was added 3mL of a solution called extractor solution. Sample is homogenized and centrifuged at 10000 x g (the gravitational force in Earth) for 25 minutes at 4°C. The supernatant obtained from this process is called enzymatic extract.

*Catalase activity determination*: the catalase activity is determined by measuring the decrease of $H_2O_2$ concentration in a given period of time, as described hereunder.

In a quartz cuvette it was added 1950 μL of determination solution, 150 μL of extraction solution, 750 μL of $H_2O_2$ (50 mM) and 150 μL of enzymatic extraction. In parallel, a blank was prepared by combining 1950 μL of determination solution, 300 μL of extraction solution and 750 μL of $H_2O_2$ (50 mM). The absorbance at 240 nm was measured in a spectrophotometer NI-2000 UV Vis, from Novainstruments, at 0 and at 80 seconds, aiming at verifying the absorbance decrease (when the absorbance of the sample reaches the same absorbance as the blank), which happened at 80 seconds in the present assay. The unit of catalase activity used was the amount of enzyme which breaks down 1 µmol of $H_2O_2$/min under the assay conditions described, and it was calculated as follows:

Catalase activity (µmol of $H_2O_2$ $min^{-1}$ $mg^{-1}$ of protein) = [(absorbance at 0 seconds – absorbance at 80 seconds) x A x B] / (C x D x E x F)

Where:

A = cuvette path length (in cm)
B = volume of enzymatic extraction (in µL)
C = weight of frozen fresh leaves (in mg)
D = volume of solution after centrifugation (in µL)
E = final time considered for absorbance reading (in minutes)
F = molar coefficient of $H_2O_2$ at 240nm (39.4 $\mu M^{-1}$ $cm^{-1}$)

The results of Experiment 1 (no hydric stress) and of Experiment 2 (hydric stress) are respectively detailed in Table 2 and 3 below, which indicates the average of results obtained for samples from three different collection times (10, 20 and 30 days after planting).

| Treatment | % increase of catalase activity |
|---|---|
| T1 (blank) | 0 |
| T2 (comparative, G1g 3.4 ml) | 105 |
| T3 (G2, 7 ml) | 95 |
| T4 (G2g, 3.8 ml) | 104 |
| T5 (1.7 ml G1g + 1.9 ml G2g) | 113 |

Table 2: Results of Experiment 1 (seed coating no hydric stress)

| Treatment | % increase of catalase activity |
|---|---|
| T1 (blank) | 0 |
| T2 (comparative, G1g 3.4 ml) | 83 |
| T3 (G2, 7 ml) | 274 |
| T4 (G2g, 3.8 ml) | 189 |
| T5 (1.7 ml G1g + 1.9 ml G2g) | 365 |

Table 3: Results of Experiment 2 (seed coating, hydric stress)

The above results show that the application of particles of tourmaline onto the seeds allow achieving an important increase of the antioxidant activity of the soy plant. The results are even higher in conditions of hydric stress. The best results were achieved with treatment T5 using a combination of particles of tourmaline and of barium sulfate.

## Example 2 : Foliar application

The same compositions as described in example 1 were used.

The compositions were sprayed onto leaves of soy following the treatment schemes detailed in Table 4 below.

| Treatment | Composition | Dose of composition applied expressed in l/ha |
|---|---|---|
| T'1 (blank) | None | - |
| T'2 (comparative) | G1g | 1.2 |
| T'3 | G2 | 2.4 |
| T'4 | G2g | 1.3 |
| T'5 | G1g + G2g | 0.6 l G1g + 0.65 l G2g |

Table 4: treatment schemes for foliar application

Each treatment by each composition as shown in table 4 has been replicated 6 times. Each replicate was composed of 1 planter, with 5 plants.

Planters of 35 liters were used, with 44.5 cm height and 36.6 cm of diameter, with sandy soil. No additional fertilizer nor nutrients were added in the soil. The collected soil was collected from the soil layer classified as distroferric red latosol.

The seeds were added in the planters in 5 furrows containing 3 seeds each, and after 10 days the thinning was performed, leaving only 1 plant per furrow.

The different treatments were applied when plants were in the growth stage R1 (flowering). The application was done by using a manual cone nozzle spray with pressurized $CO_2$, at 0.3 kg/cm$^2$.

Only one condition has been tested in this case: no hydric stress.

The antioxidant activity of the plants was determined using the protocol detailed hereunder.

The collection of leaves was accomplished for the analyses of catalase, at 14 days after sowing, being collected 4 leaves of each treatment, which were selected and standardized with the limbo totally expanded. These leaves were put in plastic bags that were covered by aluminum foil and immediately frozen in liquid nitrogen to stop any ongoing reaction. After, bags were stored in an ultra freezer at -80°C.

The activity of the catalase (CAT, EC 1.11.1.6) was measured using exactly the same method as described in example 1 above (based on the methodology proposed by KAR and MISHRA in KAR, M.; MISHRA, D. Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiology, v. 57, p. 315-319, 1976 with minor changes as described in example 1).

The results are respectively detailed in Table 5 below:

| Treatment | % increase of catalase activity |
|---|---|
| T’1 (blank) | 0 |
| T’2 (comparative, G1g 1.2 l/ha) | 43 |
| T’3 (G2, 2.4 l/ha) | 137 |
| T’4 (G2g, 1.3 l/ha) | 163 |
| T’5 (0.6 l G1g + 0.65 l G2g / ha) | 270 |

Table 5: Results of Example 2 (foliar application, no hydric stress)

The above results show that the application of particles of tourmaline onto the leaves allow achieving an important increase of the antioxidant activity of the soy plant. The best results were achieved with treatment T’5 using a combination of particles of tourmaline and of barium sulfate.

# CLAIMS

1. Use of particles of at least one silicate to increase the antioxidant activity of a plant.

2. Use according to claim 1, wherein the silicate is selected from the group consisting of micas, aluminosilicates, tourmalines, serpentines, and mixtures thereof, preferably from the group consisting of tourmalines.

3. Use according to anyone of claims 1 or 2, wherein the particles of at least one silicate are used in combination with particles of one or more additional mineral compound(s).

4. Use according to claim 3, wherein said additional mineral compound is selected from the group consisting of oxides, sulfates, carbonates and phosphates; preferably from the group consisting of alkali metal and alkaline-earth metal sulfates; more preferably from the group consisting of from barium sulfate, calcium sulfate and strontium sulfate; and most preferably said additional mineral compound is barium sulfate.

5. Use according to anyone of the preceding claims, wherein the silicate particles have a D90 size ranging from 0.1 to 30 µm, preferably from 2 to 10 µm and more preferably from 5 to 7 µm.

6. Use according to anyone of the preceding claims, wherein said particles are contained in a composition which further contains a carrier serving as a vehicle for said particles.

7. Use according to the preceding claim wherein the carrier is a liquid carrier, preferably an aqueous liquid carrier, and most preferably the carrier is water.

8. Use according to anyone of claims 6 and 7, wherein the composition further comprises one or more dispersant(s) preferably one or more polycarboxylate polymer(s) such as sodium polycarboxylate.

9. Use according to anyone of claims 6 to 8, wherein the composition is in the form of a suspension.

10. Use according to anyone of claims 6 to 9, wherein said particles of at least one silicate represent from 10 to 80% by weight, preferably from 15 to 70% by weight, more preferably from 20 to 60% by weight, and even more preferably from 25 to 40% by weight, with regard to the total weight of the composition.

11. Use according to anyone of the preceding claims, wherein the plant is selected from the group consisting of soy, cane, cotton, wheat, beans, rice, and preferably the plant is soy.

12. Use according to anyone of the preceding claims, wherein said particles of silicate are applied onto or next to seeds of the plant, preferably by coating the seeds with a composition comprising said particles.

13. Use according to anyone of the preceding claims, wherein the particles of silicate are applied onto the foliar system of the plant, preferably by spraying a composition containing said particles onto the leaves of the plant.

14. Use according to anyone of the preceding claims to increase the catalase activity in a plant.

15. Use according to anyone of the preceding claims, wherein the plant is under hydric stress.

# INTERNATIONAL SEARCH REPORT

| International application No |
|---|
| PCT/IB2019/055044 |

**A. CLASSIFICATION OF SUBJECT MATTER**
INV. A01N59/00 A01P21/00
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)
A01N

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, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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☒ Further documents are listed in the continuation of Box C.

☒ 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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|---|---|
| Date of the actual completion of the international search<br>8 November 2019 | Date of mailing of the international search report<br>20/11/2019 |
| Name and mailing address of the ISA/<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Authorized officer<br>Hateley, Martin |

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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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. |
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2

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2019/055044

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