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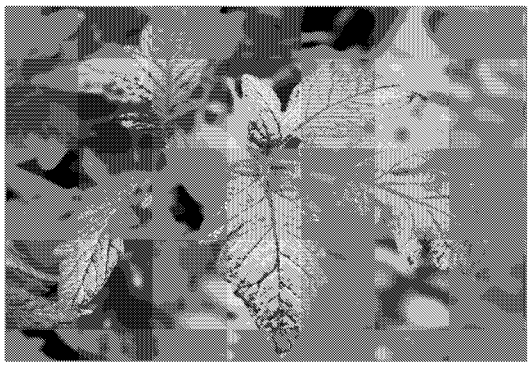
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(54) **Title**: NATURAL PLANT PIGMENTS FOR SUNSCREEN PROTECTION IN SPECIALTY CROPS

FIGURE 1A



FIGURE 1B



(57) **Abstract**: The subject invention pertains to methods of protecting seeds, seed pieces, seedlings, plants, or plant parts against damage from harmful light, for example, high-light or ultraviolet light. The methods of the current invention comprise treating the seeds, the seed pieces, the seedlings, the plants, or the plant parts with compositions comprising antioxidants, for example, anthocyanins, anthocyanidins, flavones, flavonols, carotenoids, or combinations thereof. The methods of the current invention comprise protecting the seeds, the seed pieces, the seedlings, the plants, or the plant parts against damage from the harmful light by administering the antioxidant containing extracts from antioxidant containing plants, or antioxidant containing plant parts.

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DESCRIPTION

NATURAL PLANT PIGMENTS FOR SUNSCREEN PROTECTION IN SPECIALTY CROPS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Serial No. 62/091,875, filed December 15, 2014, the disclosure of which is hereby incorproated by reference in its entirey.

BACKGROUND OF THE INVENTION

Plant pigments provide photo-protection and general anti-oxidant functions. Plant species have a remarkable ability to rapidly increase the concentrations of carotenoids, anthocyanins, anthocyanidins, flavones, and/or flavonols to protect tissues against high-light conditions. Anthocyanins and flavones are also elevated in leaf tissues after exposure to ultraviolet light (UV-light). Exposure to high-light and UV-light can damage leaf tissues and fruits and can result in sunscald and sunburn. Products currently being marketed to protect against sunscald or other type of light damage are based on calcium carbonate, kaolin clays, or titanium dioxide/zinc oxide. In essence, these products form a barrier to light penetration by covering the surface of plant tissues with a thick coating of reflective material.

The current invention proposes the novel use of extracted plant pigments rich in anti-oxidants to provide selective filtering of harmful light wavelengths and provide sun protection for plants.

BRIEF SUMMARY OF THE INVENTION

The current invention provides methods of protecting seeds, seed pieces, seedlings, plants, or plant parts against damage from harmful light, for example, high-light or UV-light. The method comprises treating the seeds, seed pieces, seedlings, plants, or plant parts with compositions comprising antioxidants. Non-limiting examples of antioxidants that can be used according to the current invention include anthocyanidins, anthocyanins, carotenoids, flavones, flavonols, or combinations thereof. The compositions comprising antioxidants can be produced by preparing extracts from antioxidant containing plants or antioxidant containing plant parts.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication, with color drawing(s), will be provided by the Office upon request and payment of the necessary fee.

Figures 1A-1B. A. Example of a current method of protecting plants from harmful light. Protecting material forms undesirable residue on the plant which may be toxic for further use of the plants, for example, for food, and is aesthetically unpleasant. B. Protecting plants from harmful light by using extracts containing antioxidants. Extracts containing antioxidants do not form aesthetically unpleasant residue on the treated plants and since the extract are prepared from antioxidants containing plants they are not toxic for subsequent use of the treated plants, for example, for food.

Figures 2A-2B. Treatment Bilberry extracts protects tomato plants from harmful UV-light. A. Untreated tomato foliage exposed to UV-light. B. Tomato plants treated with Bilberry extract before exposure to UV-light.

Figure 3. Measuring chlorophyll fluorescence using Open-FluorCam FC 800, Photon Systems Instruments. Fluorimeter shines high intensity light on to a leaf and measures light fluorescence coming from the leaf. This method measures the efficiency of light usage in photosynthetic pathways.

Figure 4. Decreases in Fv/Fm (indication of photosynthetic efficiency) in leaf treated with Bilberry extract showing the ability of Bilberry extract to limit light penetration deep into the leaf mesophyll layers.

Figure 5. Bilberry extract protected tomato plants from very harsh UV-B treatment (2x ambient); whereas, water treated plants were heavily damaged and photosynthesis was deactivated upon exposure to UV-B treatment.

DETAILED DISCLOSURE OF THE INVENTION

The terms “about”, “approximately”, “approximate”, and “around” are used in this patent application to describe some quantitative aspects of the invention, for example, the concentration of polyamines. It should be understood that absolute accuracy is not required with respect to those aspects for the invention to operate. When these terms are used to describe a quantitative aspect of the invention the relevant aspect may be varied by up to ±10%. Thus, the terms “about”, “approximately”, “approximate”, and “around” allow for variation of the various disclosed quantitative aspects of the invention by ±1%, ±2%, ±3%,

±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or up to ±10%. For example, 10% plant extract can contain 9% to 11% of the plant extract.

Natural pigments rich in antioxidants, for example, anthocyanins, anthocyanidins, carotenoids, flavones, and flavonols can protect seeds, seedlings, plants, or plant parts against harmful light. Non-limiting examples of damage from harmful light include sunscald or sunburn.

Protecting seeds, seedlings, plants, or plant parts from harmful light is intended to convey that the treated seeds, seedlings, plants, or plant parts exhibit less damage as compared to untreated seeds, seedlings, plants, or plant parts. The treated seeds, seedlings, plants, or plant parts can exhibit some damage from harmful light even after treatment with the compositions of the current invention.

The current invention provides a novel use of extracts from plants containing antioxidants as protectant against damage from harmful light to seeds, seed pieces, seedlings, plants, and plant parts. Non-limiting examples of antioxidants that can provide protection to plants from harmful light include anthocyanins, anthocyanidins, carotenoids, flavones, and flavonols.

For the purpose of this invention “a plant” refers to a living organism of the kind exemplified by trees, shrubs, herbs, grasses, ferns, and mosses, typically growing in a permanent site, absorbing water and inorganic substances through its roots, and synthesizing nutrients in its leaves by photosynthesis using the green pigment chlorophyll.

Extracts containing antioxidants can be administered to seeds, seedlings, plants or plant parts of agronomic important and/or specialty crops to protect them from harmful light. Foliar applications of plant extracts comprising antioxidants can provide protection from harmful light to seedlings and plants. Alternatively, soil drenching of seedlings and plants with antioxidants will result in plant uptake and translocation to leaf tissues and can provide protection from harmful light to the seedlings and plants grown in the treated soil.

The current invention provides methods of protecting seeds, seedlings, plants, or plant parts from the damage from harmful light, the method comprising treating the seeds, seedlings, plants, or plant parts with a composition comprising an antioxidant. Damage as defined in the current invention refers to reduction in photosynthetic efficiency, reductions in plant biomass accumulations, photo-oxidation of plant pigments, sunscald to leaf, fruit or seed tissues, and/or reductions in light absorption. The method of current invention can also be used to protect germinating seeds from damage from harmful light.

Harmful light as used in the current invention refers to light that is harmful to the plants because it has high intensity or wavelengths that can damage plant tissues. Whether specific wavelengths of light are harmful to a given plant depends on genetics, length of exposure, or other interacting environmental factors. For example, light of a particular intensity or a particular wavelength may be harmful for a plant which is highly sensitive to high intensity light or the particular wavelength light; whereas, the same intensity or same wavelength light may not be harmful to another plant which is tolerant to high intensity light or the particular wavelength light. Light having intensity which is harmful to the plants is referred to as "high-light". High-light can have intensity from about 500 $\mu mol/m^2/s$ to about 2000 $\mu mol/m^2/s$, about 750 $\mu mol/m^2/s$ to about 1500 $\mu mol/m^2/s$, about 1000 $\mu mol/m^2/s$ to about 1200 $\mu mol/m^2/s$. Typically, a light of wavelengths closer to the wavelength of UV-light, i.e., wavelengths closer to about 220 nm to about 400 nm is also harmful to plants. Light having wavelengths between about 220 nm to about 400 nm is referred to herein as "UV-light", and is composed of UVC light from 220 nm to 290 nm, UVB light from 290 nm to 320, and UVA light from 320 nm to 400 nm.

Seeds, seed pieces, seedlings, plants, or plant parts that can be protected according to the methods of current invention include but are not limited to, agriculturally and/or commercially important crop plants or ornamental plants. Examples of agriculturally and/or commercially important crop plants include, but are not limited to, to alfalfa, barley, corn (maize), popcorn, sweet corn, sorghum, cotton, soybean, sugar beets, sunflower, sugarcane, rape, canola, peanuts, rice, oats, triticale, rye, agave, wheat, potato, tomato, fruits (*i.e.*, apple, apricot, avocado, breadfruit, banana, blackberry, blackcurrant, blueberry, cherimoya, cherry, clementine, coconut, cranberry, durian, fig, grapefruit, grape, guava, jackfruit, kiwi, lemon, lime, loganberry, mandarin, mango, mangosteen, melon, nectarine, orange, papaya, peach, pear, persimmon, pineapple, plum, pomegranate, quince, satsuma, strawberry, tamarillo, ugli fruit, watermelon etc.), nuts (*i.e.*, almond, beech, butternut, brazil nut, candlenut, cashew, chestnuts, colocynth, filbert, hickory, pecan, shagbark hickory, kola nut, macadamia, mamoncillo, maya nut, oak acorns, ogbono nut, paradise nut, pili nut, pistachio, walnut, etc.), vegetables (*i.e.*, asparagus, artichoke, leafy greens, melons, snapbean, lima bean, cabbage, pea, spinach, pumpkin, onions, garlic, squash, eggplant, carrots, broccoli, sweet potato, zucchini, etc.), turfgrass (*i.e.*, bahiagrass, bluegrass, buffalograss, fescue, bentgrass, bermudagrass, ryegrass, St. Augustinegrass, zoysiagrass, etc.), forage grasses, switchgrass,

forage legumes (*i.e.*, clover, lesepedeza, etc.), ornamental plants, forest plants (*i.e.*, hardwood trees, pines, shrubs, vines, or wild flowers), herbs or other flavor enhancing crops (*i.e.*, peppermint, spearmint, thyme, basil, coriander, dill, rosemary, Irish moss, arrowroot, sesame, etc.), and plantation crops (*i.e.*, oil palm, cocoa, coffee, hops, pineapple, eucalyptus, etc.).

The antioxidant present in the compositions of the current invention can be selected from anthocyanins, anthocyanidins, carotenoids, flavones, flavonols, or a combination thereof. The combinations of antioxidants can contain two, three, four, or five different types of antioxidants. The numbers of various combinations that can be formed from certain antioxidants are summarized in the table below:

Table 1. The numbers of various combinations possible with anthocyanin, anthocyanidin, carotenoids, flavones, or flavonols.

Number of antioxidants in a combination	Number of various combinations
2	10
3	10
4	5
5	1

Thus, various combinations of antioxidants can be selected from anthocyanins, anthocyanidins, carotenoids, flavones, flavonols as follows: Two antioxidant combinations: [anthocyanin, anthocyanidin], [anthocyanin, carotenoids], [anthocyanin, flavones], [anthocyanin, flavonols], [anthocyanidin, carotenoids], [anthocyanidin, flavones], [anthocyanidin, flavonols], [carotenoids, flavones], [carotenoids, flavonols] and [flavones, flavonols]; Three antioxidant combinations: [anthocyanin, anthocyanidin, carotenoids], [anthocyanin, anthocyanidin, flavones], [anthocyanin, anthocyanidin, flavonols], [anthocyanin, carotenoids, flavones], [anthocyanin, carotenoids, flavonols], [anthocyanin, flavones, flavonols], [anthocyanidin, carotenoids, flavones], [anthocyanidin, carotenoids, flavonols], [anthocyanidin, flavones, flavonols] and [carotenoids, flavones, flavonols]; Four antioxidant combinations: [anthocyanin, anthocyanidin, carotenoids, flavones], [anthocyanin, anthocyanidin, carotenoids, flavonols], [anthocyanin, anthocyanidin, flavones, flavonols], [anthocyanin, carotenoids, flavones, flavonols] and [anthocyanidin, carotenoids, flavones, flavonols]; and five antioxidant combinations: [anthocyanin, anthocyanidin, carotenoids,

flavones, flavonols]. Accordingly, the compositions used in the methods of the current invention comprise of extracts of an antioxidant containing plant or an antioxidant containing plant part wherein the antioxidant comprises anthocyanin, anthocyanidin, carotenoid, flavone, flavonol, or a combination thereof. In certain embodiments, the extracts are powdered extracts. Non-limiting examples of antioxidant containing plants include plants of *Vaccinium spp.* Non-limiting examples of plants from *Vaccinium spp.* include blueberry, cranberry, bilberry, Rubus berries, black raspberry, red raspberry, blackberry, blackcurrant, cherry, eggplant peel, black rice, Concord grape, muscadine grape, red cabbage, violet petals, black soybeans, black chokeberry, Amazonian palmberry, Acai, Aronia, pomegranate, and Red-fleshed peaches. In certain embodiments extracts from Bilberry, Acai, Aronia, Red Cabbage, or combinations thereof can be used in the methods of the current invention.

Non-limiting examples of the plant parts rich in one or more of antioxidants include roots, stems, leaves, fruits, and flowers.

In some embodiments, the compositions used in the methods of current invention comprise about 0.01% (w/v) to about 10% (w/v), about 0.05% (w/v) to about 5% (w/v) or about 0.1% (w/v) to about 1% (w/v) of the extract from the antioxidant containing plant or the antioxidant containing plant part.

Other embodiments provide methods of coating seeds, seed pieces, seedlings, plants, or plant parts with a paste comprising extracts of the antioxidant containing plants, or the antioxidant containing plant parts. The paste comprising extracts of the antioxidant containing plants can be allowed to dry and the seeds, the seedlings, or the plants treated in this manner can be, subsequently, planted.

The treatment of the seeds, seedlings, plants, or plant parts according to the methods of the current invention comprises administering the compositions of the current invention to the seeds, seedlings, plants, or plant parts. When the compositions are administered to seedlings or plants they can be applied to foliage, stems, flowers, or fruits of the seedlings or plants.

Alternately, the treatment of the seeds, seedlings, or plants can comprise administering the compositions of the current invention “in-furrow.” In-furrow treatment comprises administering the compositions to the soil in which seeds are germinating, or in which the seedlings or plants are growing.

The compositions used in the methods of current invention may be administered to the seeds, seedlings, or plants in-furrow daily, weekly, monthly, semi-annually, or annually.

The frequency of administration depends upon the nature and type of seeds, seedlings or plants to be protected from harmful light as well as severity of the exposure to the harmful light. For example, the compositions can be administered more frequently to plants in environments of severe exposure to harmful light and less frequently in environments of less severe exposure. Also, the compositions can be administered more frequently for seeds, seedlings, or plants that are highly sensitive to harmful light and less frequently for seeds, seedlings, or plants that are less sensitive to harmful light.

The terms "applying", "application", "treating", "treated", "administering", "administer", or "administered" relate to the application of the compositions disclosed herein to a seed, a seedling, a plant, or a plant part. The compositions may be applied to a seed, a seedling, a plant, or a plant part by spray application, drenching, watering/sprinkler systems, or soaking. For example, seeds can be soaked, sprayed, or washed with compositions as disclosed herein prior to packaging or planting. Alternatively, seeds, seedlings, or plants can be sprayed or have compositions comprising antioxidants applied via other known techniques to increase protection from harmful light of the seedlings or plants.

The compositions disclosed herein can be mixed with other treatments, for example, fertilizers, fungicides, or pesticides. Nutrients can also be administered to the seeds, the seedlings, or the plants along with the compositions of the current invention.

Extracts containing anthocyanin, anthocyanidins, carotenoids, flavones, flavonols, or combinations thereof suitable for protecting seeds, seed pieces, seedlings, plants, or plant parts can be obtained from commercial sources or by the preparation of extracts from natural sources, *i.e.* raw materials. For example, extracts from plants containing antioxidants for use in the disclosed invention can be obtained from a commercial source, such as NutriCargo, LLC (Belleville, NJ).

Alternately, the extracts can be prepared from raw materials. The raw material can be dried and converted into a powder before any extraction is performed. Alternately, raw material can be directly processed to prepare the extracts. The raw materials can be cultivated or harvested from its natural habitat.

In an embodiment of the invention, the raw materials are wild-crafted. Wild-crafting refers to obtaining uncultivated raw materials from their natural habitat or the wilderness. For example, Acai, Aronia, Bilberry, Pomegranate, Black currant, Purple corn, and Gogi berry can be cultivated or wild-crafted.

A number of methods of preparing extracts rich in anthocyanin, anthocyanidins, carotenoids, flavones, flavonols, or combinations thereof from plants are known to a person of ordinary skill in the art, for example, solvent extraction. Dai *et al.* provide a review of known methods to extract anthocyanins, anthocyanidins, carotenoids, flavones, and/or flavonols from antioxidant containing plants (see, Dai *et al.*, "Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties", *Molecules*, *15*, 7313-7352 (2010). The methods described in Dai *et al.* are only examples of the methods of producing anthocyanins, anthocyanidins, carotenoids, flavones, and/or flavonols rich extracts from plants. Additional methods of producing antioxidant rich extracts from plants are well known to a person of ordinary skill in the art. Such methods for producing antioxidant rich extracts useful in the context of the disclosed invention are also within the purview of the current invention.

Accordingly, the following non-limiting embodiments are provided:

1. A method of protecting a seed, a seed piece, a seedling, a plant, or a plant part, from damage from harmful light, the method comprising treating the seed, the seed piece, the seedling, the plant, or the plant part with a composition comprising an antioxidant.

2. The method of embodiment 1, wherein the seed is a crop seed, fruit seed, nut, vegetable seed, turfgrass seed, forage grass seed, legume, or a forage legume.

3. The method of embodiment 1, wherein the seed is a germinating seed.

4. The method of embodiment 1, wherein the plant is a vegetable, a vine, a row crop, or an ornamental plant.

5. The method of embodiment 1, wherein the plant part is foliage, a stem, a flower, or a fruit.

6. The method of embodiment 1, wherein the seed or the plant is selected from the group consisting of, alfalfa, barley, corn (maize), popcorn, sweet corn, sorghum, cotton, soybean, sugar beets, sunflower, rape, canola, peanuts, rice, oats, triticale, rye, wheat, potato, tomato, apple, apricot, avocado, breadfruit, banana, blackberry, blackcurrant, blueberry,

cherimoya, cherry, clementine, coconut, cranberry, durian, fig, grapefruit, grape, guava, jackfruit, kiwi, lemon, lime, loganberry, mandarin, mango, mangosteen, melon, nectarine, orange, papaya, peach, pear, persimmon, pineapple, plum, pomegranate, quince, satsuma, strawberry, tamarillo, ugli fruit, watermelon, almond, beech, butternut, brazil nut, candlenut, cashew, chestnuts, colocynth, filbert, hickory, pecan, shagbark hickory, kola nut, macadamia, mamoncillo, maya nut, oak acorns, ogbono nut, paradise nut, pili nut, pistachio, walnut, artichoke, a leafy green, melon, snapbean, lima bean, cabbage, pea, spinach, pumpkin, onions, garlic, squash, eggplant, carrot, broccoli, sweet potato, zucchini, bahiagrass, bluegrass, buffalograss, fescue, bentgrass, bermudagrass, ryegrass, St. Augustinegrass, zoysiagrass, switchgrass, clover, lesepedeza, wild flower seed, peppermint, spearmint, thyme, basil, coriander, dill, rosemary, Irish moss, arrowroot, sesame oil palm, cocoa, coffee, hops and, eucalyptus.

7. The method of embodiment 1, wherein the antioxidant is selected from anthocyanin, anthocyanidin, flavone, flavonol, carotenoid, or a combination thereof.

8. The method of any of preceding embodiments, wherein treating the seed, the seed piece, the seedling, the plant, or the plant part comprises applying thereto the composition comprising the antioxidant.

9. The method of embodiments 1-8, wherein treating the seed, the seedling, the plant, or the plant part comprises applying the composition comprising the antioxidant as a soil drench to the seed, the seedling, or the plant for uptake and translocation of the antioxidant.

10. The method of embodiment 9, wherein said method comprises soaking the roots or shoots of the seedling or the plant in the composition comprising the antioxidant.

11. The method of embodiments 9, wherein treating the seed, the seedling, the plant, or the plant part comprises soaking the seed, the seedling, the plant, or the plant part in the composition comprising the antioxidant.

12. The method of any of the preceding embodiments, said method further comprising planting the seed, the seedling, or the plant and germinating the seed, or growing the seedling or the plant.

13. The method of embodiment 1, wherein the composition comprises an extract of an antioxidant containing plant or an antioxidant containing plant part.

14. The method of embodiment 13, wherein the antioxidant containing plant is selected from Bilberry, Acai, Gogi, Aronia, or Red Cabbage.

15. The method of embodiment 13, wherein the antioxidant containing plant part or the antioxidant containing plant part is a fruit from Bilberry, Acai, Gogi, or Aronia.

16. The method of embodiment 13, wherein the composition comprises about 0.01% to about 10%, about 0.05% (w/v) to about 5% (w/v), or about 0.1% (w/v) to about 1% (w/v) of the extract from the antioxidant containing plant or the antioxidant containing plant part.

17. The method of any of embodiments 1-17, the method further comprising administering a second agent to the seed, the seed piece, the seedling, the plant, or the plant part.

18. The method of embodiment 19, wherein the second agent is a pesticide, a nutrient, a fungicide, a fertilizer, or a combination thereof.

19. The method of any of the embodiments 1-19, further comprising exposing the treated seed, the treated seed piece, the treated seedling, the treated plant, or the treated plant part to the harmful light.

20. The method of any of embodiment 19, wherein the harmful light comprises high-light and/or ultraviolet light.

21. The method of embodiment 20, wherein the high-light comprises intensity of about 500 μmol/m^2/s to about 2000 μmol/m^2/s, about 750 μmol/m^2/s to about 1500 μmol/m^2/s, or about 1000 μmol/m^2/s to about 1200 μmol/m^2/s.

22. The method of embodiment 20, wherein the UV-light has wavelengths of about 300 nm to about 450 nm.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1 – USE OF BILBERRY FRUIT EXTRACT FOR PROTECTION FROM HARMFUL LIGHT

Bilberry extracts performed the best out of several plant extracts tested according to the current invention. Bilberry extracts provided the most protection to chlorophyll pigments from harmful light. It also provided protection from high intensity UV-B radiation. Bilberry (*Vaccinium myrtilus L.*) has the highest anti-oxidant concentration of all the berries tested.

EXAMPLE 2 – DARK COLORED PIGMENTS PROVIDE THE MOST PROTECTION FROM HARMFUL LIGHT

Dark colored pigments can mitigate photo-oxidative injury in leaves by shielding chloroplasts from high-light. Dark colored pigments can also protect leaves by scavenging reactive oxygen species (ROS). Just like sunscreens, dark pigments can block harmful UV-light and protect leaves. Therefore, dark colored pigments are naturally produced in plants in response to high-light & UV-light exposure. However, these pigments are usually located deep in leaf mesophyll and hence may not protect the leaves to the full potential of these pigments. Therefore, dark pigments can provide better protection when applied to leaf surface even at lower concentrations than naturally present in the leaf mesophyll and treating leaves with dark pigments can increase recovery of the leaves from damage from harmful light for better growth and higher yield.

EXAMPLE 3 – PROTECTION OF FLORIDA BROADLEAF MUSTARD MICROGREENS FROM HARMFUL HIGH-LIGHT

Florida Broadleaf Mustard microgreen was grown under low intensity light (275 μmol/m^2/s). Various extracts containing antioxidants were sprayed on different groups of seedlings (3 applications of the extracts/group); whereas, water was sprayed on a control group of seedlings. Both water and antioxidant extract treated seedlings were exposed to high intensity light (550 μmol/m^2/s) to produce stress inducing conditions. Seedlings from various treatment groups were harvested 5 days later and essential chlorophyll leaf tissue pigment was measured.

Table 2 provides chlorophyll-*a* and chlorophyll-*b* contents of microgreen seedlings from various treatment groups. The results indicate that extracts containing antioxidants from Bilberry fruits provided maximum protection to microgreen seedlings from high-light damage as indicated by highest amount of chlorophyll-*a* present in the group of microgreen seedlings treated with extracts from Bilberry fruits.

Table 2. Chlorophyll-*a* and Chlorophyll-*b* values in microgreen seedlings treated with extracts from different antioxidant containing extracts followed by exposure to high-light.

Treatment	**Chl-*a***	**Chl-*b***	**Total Chl**	***a*/*b* ratio**
	mg per 100 g Fresh Weight			
Water	**19.29**	**13.54**	**32.83**	**1.42**
Bilberry Extract	**30.52**	**15.69**	**46.20**	**1.95**
Gogi Extract	**16.79**	**10.66**	**27.45**	**1.58**
Pomegranate Extract	**15.15**	**11.08**	**26.23**	**1.37**
Aronia Extract	**17.39**	**15.75**	**33.14**	**1.10**

EXAMPLE 4 – PROTECTION OF TOMATO PLANTLETS FROM UV-LIGHT BY BILBERRY EXTRACT

7 weeks old Tomato plantlets were sprayed with either water or Bilberry extracts. Bilberry extract was prepared by mixing Bilberry powder in water at a concentration of about 10% (w/v). Tomato plants from each treatment group were exposed to UV-light at the intensity of 14.0 $\mu mol/m^2/s$ for 72 hours in otherwise greenhouse conditions. This level represents twice the ambient UV-B light level which amounts to a strong exposure to UV-light. Glass glazing filtered out all other UV-light.

The stress caused in the tomato plantlets by the exposure to UV-light was measured by estimating Fv/Fm values. Chlorophyll fluorescence parameter Fv/Fm is the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) after dark-adaptation. Fv/Fm represents maximum quantum yield of PSII. Fv/Fm values indicate whether plant stress affects photosystem II in a dark adapted state. Light that is absorbed by a leaf follows three competitive pathways. It may be used in photochemistry to produce ATP and NADPH used in photosynthesis, it can be re-emitted as fluorescence, or it can be dissipated as heat. The Fv/Fm test is designed to allow the maximum amount of the light energy to take the fluorescence pathway. It compares the dark-adapted leaf pre-photosynthetic fluorescent state, called minimum fluorescence, or Fo, to maximum fluorescence called Fm. The difference between maximum fluorescence and minimum fluorescence is Fv, or variable fluorescence.

In maximum fluorescence, the maximum number of reaction centers have been reduced or closed by a saturating light source. In general, the greater the plant stress, the fewer open reaction centers available, and the Fv/Fm ratio is lowered. Fv/Fm is a measuring protocol that works for many types of plant stress. **Figure 3** represents an example of an apparatus used for calculation of Fv/Fm in plant leaves.

In Fv/Fm measurements, minimum fluorescence is measured after dark adaption using a modulated light source. This is a measurement of antennae fluorescence using a modulated light intensity that is too low to drive photosynthesis. Next, an intense light flash, or saturation pulse, of a limited duration, is used, to expose the sample, and close all available reaction centers. With all available reaction centers closed, or chemically reduced, maximum fluorescence is measured. Fv/Fm is a normalize ratio created by dividing variable fluorescence by maximum fluorescence.

It is a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. An Fv/Fm value in the range of

0.79 to 0.84 is the approximate optimal value for many plant species, with lowered values indicating plant stress. Fv/Fm is a fast test that usually takes a few seconds. Dark adaptation times vary from about fifteen minutes to overnight.

Non-photochemical quenching (NPQ) is a mechanism employed by plants and algae to protect themselves from the adverse effects of high-light intensity. It involves the quenching of singlet excited state chlorophylls (Chl) via enhanced internal conversion to the ground state (non-radiative decay), thus harmlessly dissipating excess excitation energy as heat through molecular vibrations. NPQ occurs in almost all photosynthetic eukaryotes (algae and plants), and helps to regulate and protect photosynthesis in environments where light energy absorption exceeds the capacity for light utilization in photosynthesis.

Non-photochemical quenching is measured by the quenching of chlorophyll fluorescence and is distinguished from photochemical quenching by applying a bright light pulse to transiently saturate photochemical quenching thus removing its contribution from the observed quenching. Non-photochemical quenching is not affected if the pulse of light is short. During the pulse, the fluorescence reaches the level reached in the absence of any photochemical quenching, known as maximum fluorescence.

Table 3 provides Fv/Fm and NPQ measurements of tomato plantlets from various treatment groups. The results indicate that extracts containing antioxidants from Bilberry fruits protect tomato plantlets plants from UV-light damage as indicated by a measurable amount of Fv/Fm value in tomato plantlets treated with Bilberry extract; whereas, in water treated plantlets Fv/Fm value was undetectable and indicated extreme tissue damage.

Table 3. NPQ and Fv/Fm values in tomato plantlets treated with Bilberry extract followed by exposure to UV-light.

Treatment	Light Stress	NPQ	Fv/Fm
Bilberry	No UV-B	**0.48**	**0.65**
Bilberry	UV-B	**0.21**	**0.31**
Water	No UV-B	**0.66**	**0.78**
Water	UV-B	**Non-detected**	**Non-detected**

Note that treatment with Bilberry extract causes reduction in Fv/Fm even in the absence of UV-light stress. This can be because Bilberry pigments absorb light and reduce

the light penetration into the leaf. More light penetrates into the leaf when the pigment is washed off as indicated in **Table 4** below.

Table 4. Effect of the treatment with Bilberry extract on Fv/FM values

Treatment	UV-Light Stress	Wash	NPQ	Fv/Fm
Bilberry	No	No	0.48	0.65
Bilberry	No	Yes	0.54	0.75
Bilberry	Yes	No	0.21	0.31
Bilberry	Yes	Yes	0.41	0.55

EXAMPLE 5 – PROTECTION OF TOMATO PLANTS FROM UV-LIGHT

Fluorimeter shines light into leaf tissues and then measures light fluorescence (Figure 3). Decreases in Fv/Fm from bilberry extract clearly show ability of Bilberry extracts to limit light penetration deep into the leaf mesophyll layers (Figure 4). Treatment with Bilberry extracts protected tomato plantlets from very harsh UV-B treatment (2x ambient) as shown in Figure 5. The tomato plantlets that received water treatment were heavily damaged and photosystem II was deactivated. Bilberry extract treated tomato plantlets fared significantly better after exposure to very harsh UV-B treatment.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light quality and quantity thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

CLAIMS

We claim:

1. A method of protecting a seed, a seed piece, a seedling, a plant, or a plant part, from damage from harmful light, the method comprising treating the seed, the seed piece, the seedling, the plant, or the plant part with a composition comprising an antioxidant.

2. The method of claim 1, wherein the seed is a crop seed, fruit seed, nut, vegetable seed, turfgrass seed, forage grass seed, legume, or a forage legume.

3. The method of claim 1, wherein the seed is a germinating seed.

4. The method of claim 1, wherein the plant is a vegetable, a vine, a row crop, or an ornamental plant.

5. The method of claim 1, wherein the plant part is foliage, a stem, a flower, or a fruit.

6. The method of claim 1, wherein the seed or the plant is selected from the group consisting of, alfalfa, barley, corn (maize), popcorn, sweet corn, sorghum, cotton, soybean, sugar beets, sunflower, rape, canola, peanuts, rice, oats, triticale, rye, wheat, potato, tomato, apple, apricot, avocado, breadfruit, banana, blackberry, blackcurrant, blueberry, cherimoya, cherry, clementine, coconut, cranberry, durian, fig, grapefruit, grape, guava, jackfruit, kiwi, lemon, lime, loganberry, mandarin, mango, mangosteen, melon, nectarine, orange, papaya, peach, pear, persimmon, pineapple, plum, pomegranate, quince, satsuma, strawberry, tamarillo, ugli fruit, watermelon, almond, beech, butternut, brazil nut, candlenut, cashew, chestnuts, colocynth, filbert, hickory, pecan, shagbark hickory, kola nut, macadamia, mamoncillo, maya nut, oak acorns, ogbono nut, paradise nut, pili nut, pistachio, walnut, artichoke, a leafy green, melon, snapbean, lima bean, cabbage, pea, spinach, pumpkin, onions, garlic, squash, eggplant, carrot, broccoli, sweet potato, zucchini, bahiagrass, bluegrass, buffalograss, fescue, bentgrass, bermudagrass, ryegrass, St. Augustinegrass, zoysiagrass, switchgrass, clover, lesepedeza, wild flower seed, peppermint, spearmint,

thyme, basil, coriander, dill, rosemary, Irish moss, arrowroot, sesame oil palm, cocoa, coffee, hops and, eucalyptus.

7. The method of claim 1, wherein the antioxidant is selected from anthocyanin, anthocyanidin, flavone, flavonol, carotenoid, or a combination thereof.

8. The method of any of preceding claims, wherein treating the seed, the seed piece, the seedling, the plant, or the plant part comprises applying thereto the composition comprising the antioxidant.

9. The method of claims 1-7, wherein treating the seed, the seedling, the plant, or the plant part comprises applying the composition comprising the antioxidant as a soil drench to the seed, the seedling, or the plant for uptake and translocation of the antioxidant.

10. The method of claim 9, wherein said method comprises soaking the roots or shoots of the seedling or the plant in the composition comprising the antioxidant.

11. The method of claims 9, wherein treating the seed, the seedling, the plant, or the plant part comprises soaking the seed, the seedling, the plant, or the plant part in the composition comprising the antioxidant.

12. The method of claim 1, said method further comprising planting the seed, the seedling, or the plant and germinating the seed, or growing the seedling or the plant.

13. The method of claim 1, wherein the composition comprises an extract of an antioxidant containing plant or an antioxidant containing plant part.

14. The method of claim 13, wherein the antioxidant containing plant is selected from Bilberry, Acai, Gogi, Aronia, or Red Cabbage.

15. The method of claim 13, wherein the antioxidant containing plant part or the antioxidant containing plant part is a fruit from Bilberry, Acai, Gogi, or Aronia.

16. The method of claim 13, wherein the composition comprises about 0.01% to about 10%, about 0.05% (w/v) to about 5% (w/v), or about 0.1% (w/v) to about 1% (w/v) of the extract from the antioxidant containing plant or the antioxidant containing plant part.

17. The method of claims 1-7 or 12-16, the method further comprising administering a second agent to the seed, the seed piece, the seedling, the plant, or the plant part.

18. The method of claim 17, wherein the second agent is a pesticide, a nutrient, a fungicide, a fertilizer, or a combination thereof.

19. The method of claim 1, further comprising exposing the treated seed, the treated seed piece, the treated seedling, the treated plant, or the treated plant part to harmful light.

20. The method of any of claim 19, wherein the harmful light comprises high-light and/or ultraviolet light.

21. The method of claim 20, wherein the high-light comprises intensity of about 500 $\mu mol/m^2/s$ to about 2000 $\mu mol/m^2/s$, about 750 $\mu mol/m^2/s$ to about 1500 $\mu mol/m^2/s$, or about 1000 $\mu mol/m^2/s$ to about 1200 $\mu mol/m^2/s$.

22. The method of claim 20, wherein the UV-light has wavelengths of about 300 nm to about 450 nm.

FIGURE 1A



FIGURE 1B





FIGURE 2B



FIGURE 2A

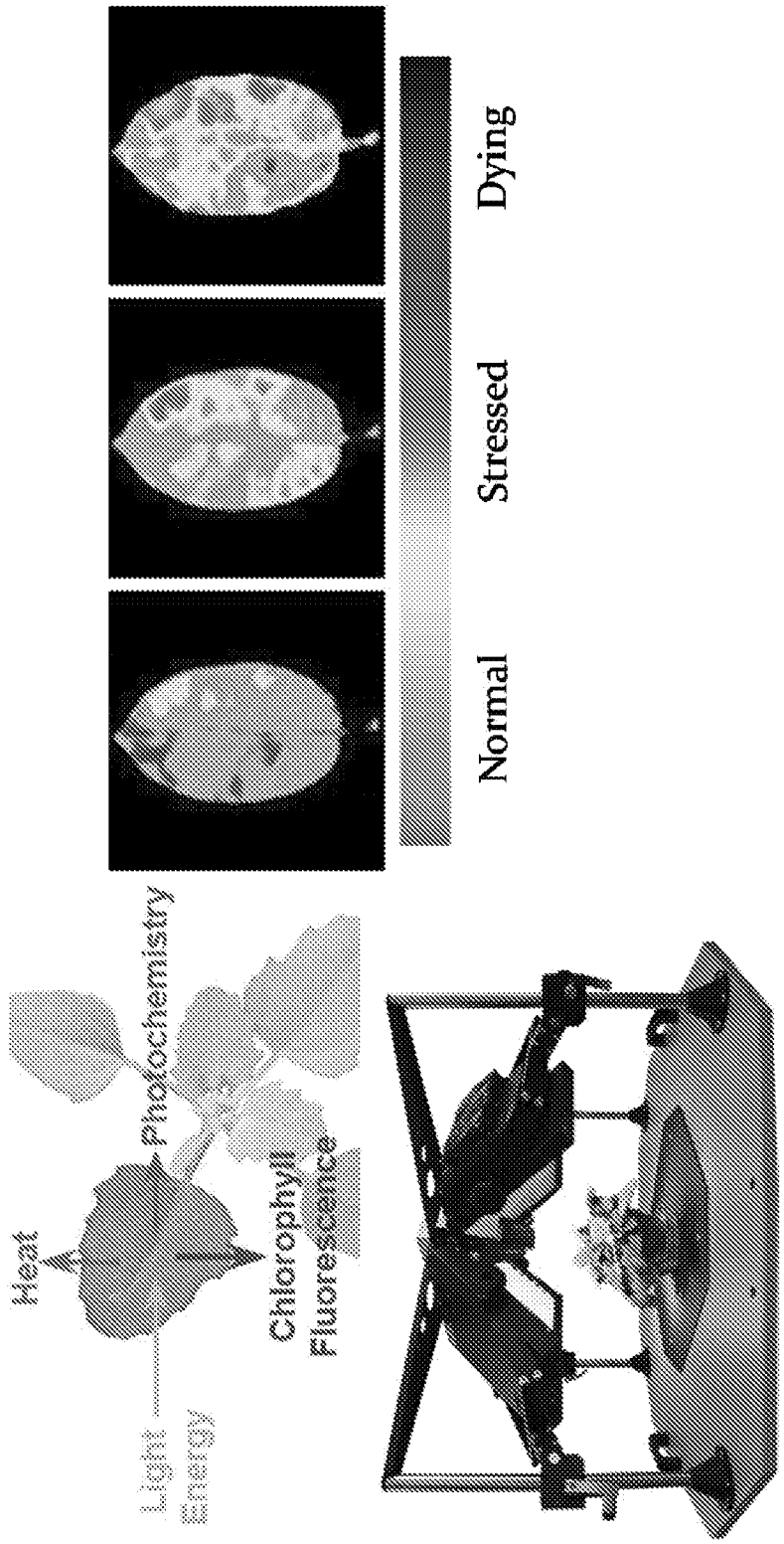

Normal
Stressed
Dying
Light Energy
Heat
Photochemistry
Chlorophyll Fluorescence

FIGURE 3

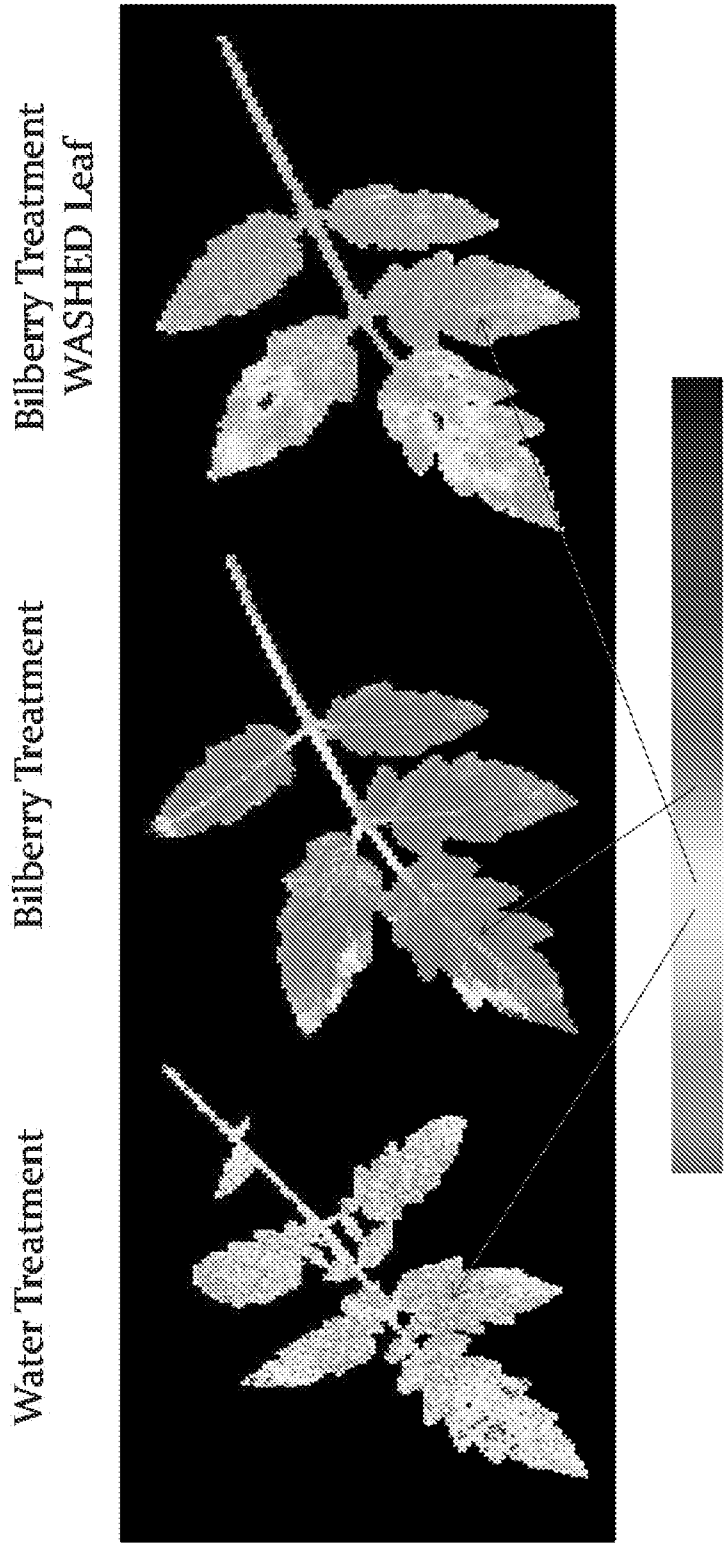

Water Treatment
Bilberry Treatment
Bilberry Treatment
WASHED Leaf
Normal
Stressed
Dying

FIGURE 4

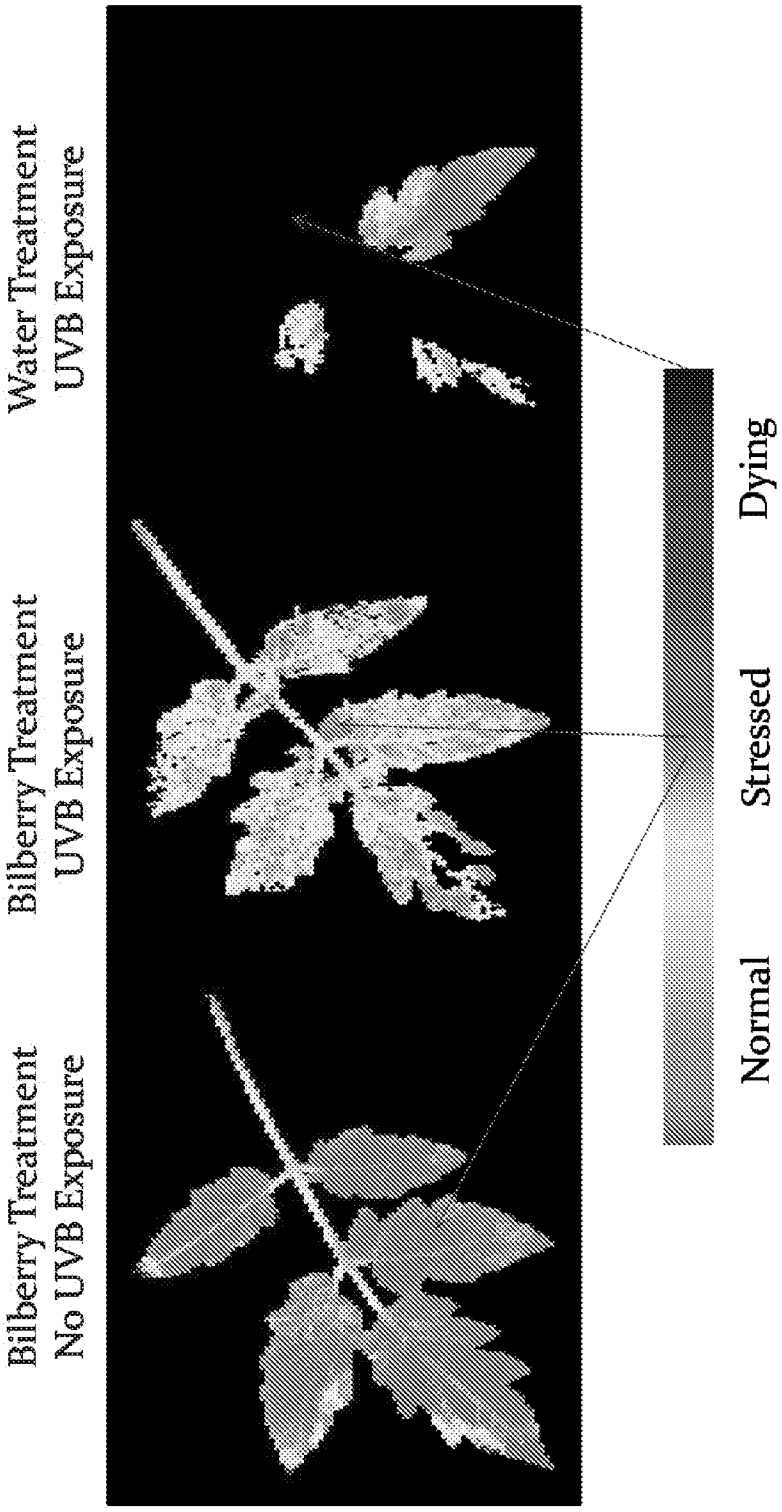

Bilberry Treatment
No UVB Exposure
Bilberry Treatment
UVB Exposure
Water Treatment
UVB Exposure
Normal
Stressed
Dying

FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/065782

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. A01G13/00(2006.01)i

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)

Int.Cl. A01G13/00, A01P21/00, A01N 3/00-57/00

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2016
Registered utility model specifications of Japan 1996-2016
Published registered utility model applications of Japan 1994-2016

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	JP 2014-8 A (YAMASAKI,Seiji) 2014.01.09, 【0024】-【0025】, 【0031】-【0032】, 【0036】, 【0041】 (No Family)	1-8,12-22 9-11
A	WO 2007/110801 A2 (LESCHOT SANHUEZA,Andres Antonio) 2007.10.04, whole document & JP 2009-531415 A & US 2007/0232495 A1 & EP 2003968 A & CA 2647216 A & AU 2007231051 A & MX 2008012253 A & AR 59270 A	1-22

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07.03.2016	22.03.2016

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