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# (54) SALTS, AQUEOUS LIQUID COMPOSITIONS CONTAINING SALTS OF ABSCISIC ACID ANALOGS AND METHODS OF THEIR PREPARATION

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# (57) **ABSTRACT**

The present invention generally relates to salts of abscisic acid analogs, aqueous liquid compositions containing salts of analogs of abscisic acid and methods of their preparation for agricultural use.

# SALTS, AQUEOUS LIQUID COMPOSITIONS CONTAINING SALTS OF ABSCISIC ACID ANALOGS AND METHODS OF THEIR PREPARATION

# FIELD OF THE INVENTION

**[0001]** The present invention generally relates to salts of abscisic acid analogs, aqueous liquid compositions containing salts of abscisic acid analogs and methods of their preparation for agricultural use.

# BACKGROUND OF THE INVENTION

**[0002]** Abscisic acid is a naturally occurring plant hormone which acts primarily to inhibit growth of plants, maintain dormancy of buds, inhibit fruit-ripening, activate the pathogen resistance response defense, induce senescence in already-damaged cells and their proximate neighbors, and help the plant tolerate stressful conditions. See Arteca, R. (1996), *Plant Growth Substances. Principles and Applications*. New York: Chapman & Hall; Mauseth, J. D. (1991), *Botany: An Introduction to Plant Biology.* Philadelphia: Saunders. pp. 348-415; Raven, P. H., Evert, R. F., and Eichhorn, S. E. (1992), *Biology of Plants.* New York: Worth. pp. 545-572.

**[0003]** Abscisic acid owes its name to the belief that this plant growth regulator causes the abscission of leaves from deciduous trees in the fall. Abscin II and dormin are names previously used for this plant hormone. The chemistry and physiology of abscisic acid and its analogs is described by Milborrow, Ann. Rev. Plant Physiol. 1974, 25, 259-307.

**[0004]** Abscisic acid analogs are structural derivatives of 2-cis-,4-trans-(S)-(+)-abscisic acid. An extensive series of analogs of abscisic acid has been prepared by researchers at the Plant Biotechnology Institute of the National Research Council of Canada, Saskatoon, Saskatchewan. Some of these abscisic acid analogs are disclosed in U.S. Pat. Nos. 5,201, 931, 5,518,995 and 6,004,905, which are incorporated herein by reference. Presently preferred abscisic acid analogs include PBI-376, PBI-524, PBI-697 and PBI-410.

**[0005]** Prior art (U.K. Pat. No. 1251867 and Railton and Wareing, Planta 112, 65-69, 1973) taught, inter alia, preparation of amine salts of racemic abscisic acid. A salt of racemic (R,S)- $(\pm)$ -2-trans-,4-trans-abscisic acid with the chiral alkaloid brucine was prepared as a means of resolving a small quantity of the racemate in order to study the physical properties of its enantiomers (J. C. Bonnafous, et al., Tetrahedron Letters, 1119-1122, 1973). Salts of (S)-(+)-abscisic acid are disclosed in a co-pending patent application Ser. No.

entitled SALTS, AQUEOUS LIQUID COMPOSITIONS CONTAINING SALTS OF S-(+)-ABSCISIC ACID, filed on the same day as this application. However, the prior art does not disclose salts of abscisic acid analogs with amines nor does it disclose alkali metal or alkaline earth salts of abscisic acid analogs.

**[0006]** As noted above, abscisic acid analogs are carboxylic acids, and thus in a medium having an acidic pH, they are protonated and in their neutral undissociated form. This uncharged, undissociated form is more lipophilic than a salt of an abscisic acid analog s, and penetration of the uncharged acid form into the plant cuticle would be favored relative to the charged, dissociated form of the abscisic acid analog present at higher pH (Blumenfeld and Bukovac 1972, Planta 107: 261-268). The uncharged, undissociated form of the abscisic acid analog would be expected to cross cell membranes from the apoplast into the cytosol more easily than a salt form would. In spite of this, we have surprisingly found

that treatments comprising the salts of abscisic acid analogs disclosed in this invention perform equally well or even slightly better in biological activity when compared with similar treatments comprising the acid form of the same abscisic acid analog at the same concentration.

**[0007]** Abscisic acid was first defined in the early 1960s as a growth inhibitor accumulating in abscising cotton fruit and in leaves of sycamore trees photoperiodically induced to become dormant. See, Finkelstein R R, Rock CD (2002), *Abscisic Acid Biosynthesis and Response*, The Arabidopsis Book Vol. 45, No. 1 pp. 1-48. Since then, abscisic acid has been shown to regulate many aspects of plant growth and development, including embryo maturation, seed dormancy, germination, cell division and elongation, etc. Although abscisic acid has historically been thought of as a growth inhibitor, young tissues have high abscisic acid levels, and abscisic acid-deficient mutant plants are severely stunted because their ability to reduce transpiration and establish turgor is impaired. Exogenous abscisic acid treatment of mutants restores normal cell expansion and growth.

**[0008]** Abscisic acid is thought to initiate its effects on cells through binding to receptor proteins, although their identities and locations are still largely unknown. Activation of the putative receptor(s) causes a chain of events that results in rapid changes in ion channels and slower changes in the pattern of gene transcription. While many individual components of this chain of events have been identified, a complete picture has not yet been obtained.

**[0009]** Commercial formulations comprising abscisic acid are used in agriculture for various purposes, such as improving stress tolerance, slowing the growth rate, adjusting flowering phase, and other purposes. Abscisic acid has also been reported to possess insect inhibition qualities. See U.S. Pat. Nos. 4,434,180 and 4,209,530 to Visscher. Contents of these patents are herein incorporated by reference. Abscisic acid in a powdered form is currently commercially available from Lomon Biotechnology Company, Ltd., a Chinese company, which markets it as a substance that, among other uses, improves the yield and quality of crops.

[0010] However, one of the problems associated with preparation of formulations is abscisic acid analogs is their relatively poor solubility in water: The solubilities of these compounds in water are even lower than that of (S)-(+)abscisic acid itself, and for (S)-(+)-abscisic acid not more than about 3 grams per liter or alternatively, less than 0.3% by weight will dissolve at ordinary temperatures. Stated differently, a concentration of about 3000 parts per million (ppm) is the highest concentration of (S)-(+)-abscisic acid that can be achieved in pure water at room temperature, and the maximum for abscisic acid analogs is even lower. While abscisic acid analogs have better solubility in some organic solvents, liquid formulations of abscisic acid analogs in organic solvents are unacceptable in some contexts because of flammability, toxicity, or pollution considerations. For example, the Environmental Protection Agency of the U.S. state of California is currently requiring that liquid formulations of agricultural products contain no volatile organic solvent, and several other U.S. states are considering similar regulations. Nonvolatile organic solvents have the detriment that, since they do not evaporate, they remain in the agricultural product as it impinges upon and is absorbed into the target plant, with a probability of causing phytotoxicity and contaminating food products, since the amount of the solvent greatly exceeds the amount of active ingredient applied.

**[0011]** A further problem observed with concentrated solutions of abscisic acid analogs in organic solvents is that it is difficult to prepare more dilute solutions by dilution into

water without having a portion of the abscisic acid analog precipitate out in a gummy form that redissolves only very slowly and with great difficulty. This is of practical importance because a major use of abscisic acid analogs in agriculture or horticulture is for the reduction of transpiration in nursery plants being prepared for transplantation or for sale to consumers, for which purpose the abscisic acid analog may be applied by means of an injection system and automatic or hand applicators. The solution for use in such an applicator must be a concentrate between about 50 and 100 times more concentrated than the dose rate that is actually reaching the plants when they are treated by foliar spray or drench. Thus for a typical application to the nursery plants of 60 to 600 ppm, the concentrate must contain between 3000 and 60,000 ppm of (S)-(+)-abscisic acid in a solution that will mix instantly and completely with the water flowing through the hose, in such a way that there is no possibility of formation of a precipitate that would clog the nozzle through which the water containing active ingredient is applied to the plants or the growing media of the plants. As explained above, the solubility of abscisic acid analogs in water is less than 3000 ppm at ordinary ambient temperature, so such an intermediate solution cannot practically be prepared in water. A solution of an abscisic acid analog in an organic solvent cannot be used in such an injection applicator, because precipitation of the active ingredient will occur during the mixing into the water flowing in the system, and the spray nozzle will be clogged. Because of the solubility limitation, it is also not possible to provide a liquid formulation of the abscisic acid analog in organic solvent at a higher concentration (e.g. 10%) and then at the time of application to prepare an intermediate dilution in water to achieve the desired concentration of 3,000 to 60,000 ppm in the reservoir of the injection applicator.

**[0012]** An identical problem arises in the case of application of an abscisic acid analog to a vineyard, orchard, or agricultural field through an irrigation system, a practice commonly known as chemigation. Again, such a system requires a concentrated solution of the active ingredient in a liquid solvent in such a form such that that solution is instantly and completely miscible with a stream of water flowing through the irrigation system. If any precipitation were to occur, it would block the nozzles (known as emitters) through which the water and dissolved active ingredient reach the target plants. Again in this situation a formulation consisting of an organic solution of the abscisic acid analog would not be acceptable because of the problem of the low water solubility.

**[0013]** While powdered formulations of abscisic acid analogs could be prepared, it is often more convenient to use concentrated liquid solutions instead of powders. Therefore, there is an unmet need in the art for abscisic acid analog formulations comprising salts of abscisic acid analogs, which are much more soluble in water than the acids themselves are.

#### SUMMARY OF THE INVENTION

**[0014]** The present invention is generally directed to salts of abscisic acid analogs.

**[0015]** In a further embodiment, the present invention is generally directed to aqueous compositions comprising an effective amount of a salt of an analog of abscisic acid wherein the concentration of the salt is at least about 0.5% by weight of the aqueous composition. Applicants have unexpectedly discovered that salts of abscisic acid analogs allow for dramatic increases in solubility of abscisic acid analogs in water so that concentrated solutions can be obtained. As a result of Applicants' invention, solutions can be obtained with abscisic acid analog concentration as high as about 50% by

weight. The present invention allows for the creation of concentrated formulations of abscisic acid analogs that are convenient for packaging, storage, transport and handling, but must be diluted prior to use and specifically allows any arbitrary intermediate dilution of these formulations to be made into water without the risk of precipitation of the active ingredient.

**[0016]** Compositions of the present invention generally comprise the salt, an antimicrobial and a surfactant. Other components which enhance the long-term storage stability of the composition or the biological activity of the abscisic acid analog may optionally be included.

**[0017]** Some of the suitable salts of the invention include, but are not limited to, the ammonium salt, the lithium, sodium, potassium, magnesium or calcium salt, organic amine salts or mixtures comprising any number of these. In one embodiment, the organic amine salt is the triethanolamine salt. In another embodiment, the organic amine salt is the dimethylethanolamine salt. In yet another embodiment, the organic amine salt is the ethanolamine salt. These examples of salts are not limiting as other salts may also be suitable for use the present invention. One presently preferred salt is the ammonium salt.

[0018] The present invention is also directed to methods of preparation of the aqueous compositions comprising salts of abscisic acid analogs. In one embodiment, the invention is directed to a method of preparation of the ammonium salt of an abscisic acid analog comprising reacting the abscisic acid analog with a chemically equivalent amount of ammonia in aqueous solution. In another embodiment, the invention is directed to a method of preparation of the lithium salt comprising reacting the abscisic acid analog with lithium hydroxide, lithium bicarbonate or lithium carbonate in aqueous solution. In another embodiment, the invention is directed to a method of preparation of the sodium salt comprising reacting the abscisic acid analog with a chemically equivalent amount of sodium hydroxide, sodium bicarbonate or sodium carbonate in aqueous solution. In another embodiment, the invention is directed to a method of preparation of the potassium salt comprising reacting the abscisic acid analog with a chemically equivalent amount of potassium hydroxide, potassium bicarbonate or potassium carbonate in aqueous solution. In another embodiment, the invention is directed to a method of preparation of the magnesium salt comprising reacting the abscisic acid analog with one-half to one chemical equivalent of magnesium hydroxide, magnesium oxide, or magnesium carbonate or a hydrate thereof in aqueous solution or suspension. In another embodiment, the invention is directed to a method of preparation of the calcium salt comprising reacting the abscisic acid analog with one-half to one chemical equivalent of calcium hydroxide, calcium oxide or calcium carbonate in aqueous solution or suspension. In yet another embodiment, the invention is directed to a method of preparation of an organic amine salt comprising reacting the abscisic acid analog with one chemical equivalent of an organic amine in the presence or, if the amine is a liquid, in the absence of water.

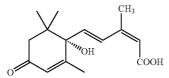
**[0019]** A further embodiment of the invention includes mixtures comprising combinations of salts of the abscisic acid analog with an effective amount of a component or multiple components that enhance the long-term chemical stability of the analog and the mixture as a whole. These include but are not limited to citric acid or one of its water-soluble salts, sulfur dioxide or a water soluble bisulfite or sulfite salt.

**[0020]** A further embodiment of the invention includes mixtures comprising combinations of salts of abscisic acid

analogs with a substantial amount of a component or multiple components which enhance the biological activity of the abscisic acid analog, including but not limited to urea, ammonium nitrate, ammonium acetate, calcium chloride, magnesium nitrate, or a surfactant. Preferred surfactants are gelforming constituents, such as members of the Brij family. [0021] The disclosed embodiments are simply exemplary embodiments of the inventive concepts disclosed herein and should not be considered as limiting, unless the claims expressly state otherwise.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0022]** The present invention relates to aqueous liquid compositions of salts of abscisic acid analogs. Abscisic acid analogs are structural derivatives of 2-cis-,4-trans-(S)-(+)-abscisic acid, whose structure is set forth below:



**[0023]** The tertiary alcohol in these compounds typically defines a chiral center for the molecule so that (R)-, and (S)-structural variants or enantiomers as well as (R,S)-racemic mixtures are possible. The liquid compositions of the present invention may utilize either the (R)-, or (S)-enantiomer or the (R,S)-racemic mixture of any selected abscisic acid analog.

**[0024]** For the purposes of this Application, abscisic acid analogs are defined by Structures 1, 2 and 3, wherein for Structure 1:

**[0025]** the bond at the 2-position of the side chain is a cisor trans-double bond,

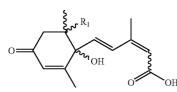
**[0026]** the bond at the 4-position of the side chain is a trans-double bond or a triple bond,

**[0027]** the stereochemistry of the alcoholic hydroxyl group is S-, R- or an R,S-mixture,

[0028] the stereochemistry of the  $R_1$  group is in a cis-relationship to the alcoholic hydroxyl group,

Structure 1

R<sub>1</sub>=ethynyl, ethenyl, cyclopropyl or trifluoromethyl, and



[0029] For PBI-376,  $R_1$  is ethenyl.

- [0030] For PBI-524,  $R_1$  is ethynyl.
- [0031] For PBI-697,  $R_1$  is cyclopropyl.

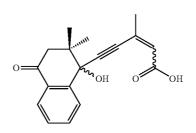
For Structure 2:

**[0032]** the bond at the 2-position of the side chain is a cisor trans-double bond,

**[0033]** the bond at the 4-position of the side chain is a triple bond,

Structure 2

**[0034]** the stereochemistry of the alcoholic hydroxyl group is S-, R- or an R,S-mixture,



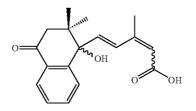
For Structure 3:

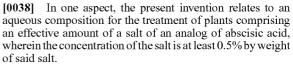
**[0035]** the bond at the 2-position of the side chain is a cisor trans-double bond,

**[0036]** the bond at the 4-position of the side chain is a trans-double bond,

**[0037]** the stereochemistry of the alcoholic hydroxyl group is S-, R- or an R,S-mixture,







**[0039]** As used herein, all numerical values relating to amounts, weight percentages and the like, are defined as "about" or "approximately" each particular value, namely, plus or minus 10%. For example, the phrase "at least 5% by weight" is to be understood as "at least 4.5% to 5.5% by weight." Therefore, amounts within 10% of the claimed values are encompassed by the scope of the claims.

**[0040]** The phrase "effective amount" of a salt means a sufficient amount of the salt to provide the desired biological or chemical effect without at the same time causing additional toxic effects. The amount of salt or other formulation component that is "effective" will vary from composition to composition, depending on the particular agricultural use, the particular salt or salts, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

**[0041]** Liquid compositions of the present invention can be prepared as either ready-to-use dilutions or dilutable concentrates. According to the present invention, a solution containing from 0.5% to as much as 50% by weight of abscisic acid analog can be obtained. The dilutable concentrates can be diluted into water directly to a final application concentration or to any intermediate dilution, without risk of precipitation

of the active ingredient. The aqueous formulations according to the present invention are inexpensive to manufacture, safe to handle and use, and the abscisic acid analog active ingredient is stable under storage and shipping conditions. With the compositions of the present invention there is no risk of fire as there might be with liquid formulations containing a flammable or combustible organic solvent. There is no risk of contributing to the formation of atmospheric pollution or smog as there is with formulations containing a volatile organic solvent. The aqueous formulations of the present invention are less toxic to humans or animals than similar formulations containing an organic solvent. A person having ordinary skill in the art would be able to determine how to prepare the final aqueous solution concentration for direct application to plants, or how to prepare any intermediate dilutions for use in chemigation equipment or injection diluters or similar equipment, without undue experimentation, without any chance of causing precipitation of the active ingredient, and without long and laborious stirring to bring the active ingredient into solution.

**[0042]** The aqueous solution formulations of the present invention may also optionally include an effective amount of an additional ingredient or several additional ingredients in order to enhance the long-term chemical stability of the abscisic acid analog or of the formulation as a whole. Such enhancing ingredients include but are not limited to citric acid or one of its water-soluble salts, sulfur dioxide or a water soluble bisulfite or sulfite salt. The use of water as the solvent allows for a combined liquid formulation comprising any or several of these inorganic components that may comprise a level of the enhancing ingredient equal to the concentration of the abscisic acid analog salt or higher, if desired.

**[0043]** The aqueous solution formulations of the present invention may also optionally include a substantial amount of an additional ingredient or several additional ingredients in order to enhance the biological activity of the abscisic acid analog. Such enhancing ingredients include but are not limited to urea, ammonium nitrate, ammonium acetate, calcium chloride and magnesium nitrate. The use of water as the solvent allows for a combined liquid formulation comprising any or several of these inorganic components or urea that may comprise a level of the enhancing ingredient equal to the concentration of the abscisic acid analog salt or even up to 10 times the amount of abscisic acid analog by weight or more. Again, this provides an advantage over the use of an organic solvent, in which these inorganic components or urea have little if any solubility.

[0044] Additionally, the aqueous solution formulations of the present invention may optionally include a substantial amount of a surfactant, in an amount equal by weight to the content of abscisic acid analog salt or even several times greater. Examples of surfactants that may be incorporated into the compositions of the present invention include, but are not limited to products of the Brij family of polyoxyethylene fatty alcohol ethers (available from Uniquema, Castle Del.), products of the Tween family of polyoxyethylene sorbitan esters (available from Uniquema, Castle Del.), products of the Silwet family of organosilicones (available from Union Carbide, Lisle Ill.), products of the Triton family of alkylphenol ethoxylates (available from Dow Chemical Company, Midland Mich.), products of the Tomadol family of ethoxylated linear alcohols (available from Tomah3 Products, Inc., Milton Wis.), products of the Myrj family of polyoxyethylene fatty acid esters (available from Uniquema Castle Del.), products of the Trylox family of ethoxylated sorbitol and ethoxylated sorbitol esters (available from Cognis Corporation, Cincinnati Ohio), or any of the specific commercial products Latron B-1956 (available from Rohm & Haas, Philadelphia Pa.), Capsil (available from Aquatrols, Paulsboro N.J.), Agral 90 (available from Norac Concepts, Inc., Orleans ON, Canada), Kinetic (available from Setre, Memphis Tenn.), or Regulaid (available from KALO, Overland Park Kans.). The presently preferred surfactants are those of the Brij or Tween families. The most preferred surfactants for inclusion in compositions of the present invention are Brij 98, Brij 78, Tween 20 and Tween 40. The concentration of surfactant in the compositions of the invention may range from about 0.02% up to about 40%. The preferred range of concentrations for the surfactant in the compositions of the invention is from about 0.1% to 30%. The most preferred range of concentrations for the surfactant in the compositions of the invention is from 0.5% to 25%. The surfactant may be included in the compositions of the present invention either together with any one or more of the inorganic salt or urea activity enhancing ingredients or in the absence of any of them.

[0045] The end user can apply compositions of the present invention to plants for various purposes, such as improving stress tolerance, reducing their water utilization, slowing their growth rate, adjusting flowering phase, for seed treatment, preventing preharvest fruit and flower drop and improving the quality and color of fruits. The possible uses may also include, for example, distribution and sale of various concentrated solutions of abscisic acid analogs. Utilizing such high concentrations for shipping and handling allows the use of smaller volumes of water, thus simplifying shipping and handling procedures and decreasing costs. The end user could then dilute the product to a 1% concentration (or other percentage depending on the end user's needs) and fill the supply reservoir of mixing equipment for spray or drench application to ornamental bedding plants ready for shipment. Alternatively, another end user could prepare a diluted solution for injection into the drip irrigation system for a vineyard at the appropriate time to enhance the color or phenolic content of a wine or table grape crop.

[0046] Organic amine compounds that may be employed in the salts useful in the compositions of the present invention are those containing one or two nitrogen atoms. If the amine compound contains one nitrogen atom, it may include from one to six carbon atoms, from zero to three oxygen atoms and zero to four degrees of unsaturation, where a degree of unsaturation is defined as a carbon-carbon multiple bond or a ring in a cyclic structure. If the amine compound contains two nitrogen atoms, it may include from two to ten carbons, zero to four oxygen atoms and zero to four degrees of unsaturation. These organic amine compounds include, but are not limited to methylamine, ethylamine, propylamine, isopropylamine, dimethylamine, diethylamine, trimethylamine, triethylamime, ethanolamine, N-methylethanolamine, N,N-dimethylethanolamine, diethanolamine, triethanolamine, ethylenediamine, tetramethylethylenediamine, and other similar compounds.

**[0047]** In another aspect, the present invention relates to methods of preparation of the aqueous compositions comprising salts of abscisic acid analogs. For example, in one embodiment, the invention is directed to a method of preparation of the ammonium salt of abscisic acid comprising reacting the abscisic acid analog with ammonia. In another embodiment, the invention is directed to a method of prepa-

**[0048]** In a preferred embodiment, at least about 0.25% by weight of Tween-20, a detergent polysorbate, is added to the reaction mixture and resulting formulation when preparing the abscisic acid analog salts.

**[0049]** In another preferred embodiment, the aqueous solution comprises an antimicrobial agent to prevent microbial growth during long-term storage. The presently most preferred antimicrobial agent is potassium sorbate. When the aqueous solution of an abscisic acid analog salt of the present invention is intended for long term storage or for distribution and commercial sale to the user, it is advantageous to incorporate the antimicrobial agent at a concentration of from 0.01% to 1.0% by weight.

**[0050]** In another preferred embodiment, the aqueous solution comprises an agent to prevent undesirable development of coloration or appearance of precipitate during long-term storage. The presently most preferred agents for this purpose are sodium or potassium citrate and sodium or potassium sulfite or bisulfite.

**[0051]** In the preferred embodiments, the pH of the concentrated compositions of the invention and any aqueous solutions at final use dilution prepared from the concentrates are both approximately neutral (near pH 7).

**[0052]** Preferred compositions of the present invention comprise from 0.5 to 50 weight % of abscisic acid analog in the form of a salt, from 0.01 to 1.0 weight % of an antimicrobial agent, optionally from 0.01 to 5% of a stability enhancing agent, optionally from 0.25 to 35 weight % of a surfactant, optionally from 1 to 50 weight % of another activity enhancing component, with the balance being water.

**[0053]** The following examples are intended to illustrate the present invention and to teach one of ordinary skill in the art how to make and use the invention. They are not intended to limit the invention or its protection in any way.

#### EXAMPLES

#### Example 1

# Preparation of an Aqueous Solution Composition of the Ammonium Salt of (S)-(+)-abscisic acid comprising potassium sorbate

**[0054]** In a 600 mL beaker, 55 g of (S)-(+)-abscisic acid of 95% purity was added, followed by 500  $\mu$ L of Tween 20 and 200 mL of water. Then, 10 mL of concentrated aqueous ammonia were added while stirring until the mixture came to equilibrium. Then, additional concentrated ammonia was added dropwise until all solid was dissolved. A homogenous solution was achieved when a total of about 13.5 mL of ammonia has been added. At this point, potassium sorbate (1.25 g) was added to the composition; it quickly dissolved. The mixture was transferred to a 500 ml volumetric flask and was brought up to 500 mL with deionized water. The mixture was stored in a brown glass bottle. The pH was measured to be 6.50.

**[0055]** Thus an aqueous solution composition comprising 10% abscisic acid as the ammonium salt by weight, and further comprising a naturally-occurring antimicrobial preservative, was prepared.

### Example 2

# Preparation of an Aqueous Solution Composition of the Ammonium Salt of Abscisic Acid Analog PBI-524 Comprising Potassium Sorbate

**[0056]** A weight of 1.09 g of abscisic acid analog PBI-524 was combined with 20 mg of Tween-20 and 50 mg of potassium sorbate in 10 mL of water and stirred for a few minutes to achieve a smooth suspension. Ammonia was added in the form of a 1+9 dilution of commercial concentrated product as a titration to bring all of the solid into solution. The mixture was made up on a balance to a total weight of 20 g by adding water, and it was transferred to a brown bottle for storage.

**[0057]** Thus, an aqueous solution composition was prepared comprising 5% by weight abscisic acid analog PBI-524 as the ammonium salt, and further comprising a naturallyoccurring antimicrobial preservative.

#### Example 3

Preparation of an Aqueous Solution Composition of the Ammonium Salt of Abscisic Acid Analog PBI-524 Comprising Potassium Sorbate, Sodium Citrate and Sodium Metabisulfite

**[0058]** A solution was prepared comprising 50 mg potassium sorbate, 20 mg of Tween-20, 100 mg of trisodium citrate dihydrate and 50 mg of sodium metabisulfite in 10 mL of water. After adding 1.09 g of abscisic acid analog PBI-524 the mixture was stirred for a few minutes to achieve a homogeneous suspension. Ammonia was added in the form of a 1+9 dilution of commercial concentrated product as a titration to bring all of the solid into solution. The mixture was made up on a balance to a total weight of 20 g by adding water, and it was transferred to a brown bottle for storage.

**[0059]** Thus, an aqueous solution composition was prepared comprising 5% by weight abscisic acid analog PBI-524 as the ammonium salt, and further comprising sodium citrate, sodium bisulfite and a naturally-occurring antimicrobial preservative.

**[0060]** Preparation of plant specimens for use in the treatment studies of Example 4 that follows was carried out as follows. Tomato (variety: Rutgers) seeds were sown in an 18-cell flat filled with Promix PGX (available from Premier Horticulture Inc., Quakertown Pa.) and grown for 3 weeks to allow for germination and initial growth. Plants were then transplanted into pots (18 cm in diameter and 18 cm in height), filled with Promix BX (available from Premier Horticulture Inc., Quakertown Pa.), and grown for one or two more weeks before treatment, depending on temperature and available light. Plants received daily irrigation and weekly fertilizer (1 g/L all purpose fertilizer 20-20-20, available from The Scotts Company, Marysville, Ohio).

**[0061]** All treatment solutions were made up with distilled water. The (S)-(+)-abscisic acid (95% active ingredient) is available from Lomon BioTechnology Co., Ltd. (Shichuan, China). Twenty L of a 250 ppm solution of (S)-(+)-abscisic acid was prepared and stored in the dark at  $20-25^{\circ}$  C. This

same 250-ppm (S)-(+)-abscisic acid solution was used as a control for all studies to eliminate the possibility of applying an incorrect concentration.

[0062] The experiment was conducted using a randomized complete block experimental design. Solutions of (S)-(+)abscisic acid and blank treatments (plain water) were applied by spray to the aerial parts of the tomato plants at the rate of 20 mL per 6 plants. Plants were then placed in a transparent chamber with humidity controlled within the range of 40 to 60% relative humidity. Leaf transpiration rates were measured at 1, 2, 3, 4 and 7 days after treatment. Measurements were conducted using a LI-1600 Steady State Porometer (LI-Cor, Lincoln, Nebr.). Each day the transpiration rate of the plants of each treatment group was normalized to a percentage of the transpiration rate of untreated plants (plants sprayed with water only) in order to control for day-to-day variability in plant status caused by changes of environmental conditions such as light intensity and temperature. Data of each plant was also averaged over a 3-day period to balance the short term and long term effect of (S)-(+)-abscisic acid on tomato leaf transpiration as well as to reduce experimental variability.

#### Example 4

**[0063]** The effect of abscisic acid analog PBI-524, in comparison with the effects of the abscisic acid analog ammonium salts of the present invention, as prepared in the Examples 2 and 3 was studied in an assay measuring tomato leaf transpiration rate (Table 1). The aqueous solution compositions of the Examples were each diluted with water to a final application concentration of 25 ppm (based on abscisic acid analog content) to match the abscisic acid analog (nonsalt) standard treatment. **[0064]** Thus it has been demonstrated that the ammonium salt compositions of abscisic acid analog BPI-524 of the present invention are at least as efficacious biologically as the abscisic acid analog PBI-524 itself is.

1. A salt or mixture of salts of an analog of abscisic acid.

**2**. The salt of claim **1**, wherein said salt is the ammonium salt.

3. The salt of claim 1, wherein said salt is the sodium salt.4. The salt of claim 1, wherein said salt is the potassium

salt.5. The salt of claim 1, wherein said salt is the lithium,

magnesium or calcium salt.6. The salt of claim 1, wherein said salt comprises ammonium and potassium salts in a nitrogen to potassium weight ratio between 1:20 and 20:1.

7. The salt of claim 1, wherein said salt is an organic amine salt.

**8**. The salt of claim **7**, wherein said organic amine salt contains one or two nitrogen atoms.

9. The salt of claim 7, wherein said organic amine salt is the triethanolamine salt.

**10**. The salt of claim **7**, wherein said organic amine salt is the dimethylethanolamine salt.

11. The salt of claim 7, wherein said organic amine salt is the ethanolamine salt.

12. An aqueous composition for treatment of plants comprising an effective amount of at least one salt of an abscisic acid analog, wherein the concentration of the salt is at least 0.5% by weight of said composition, an effective amount of an antimicrobial agent, an effective amount of a surfactant, optionally one or more performance enhancing additives and optionally one or more additives to stabilize the color.

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Effect of abscisic acid analog ammonium salt with or without Brij 98 on tomato leaf transpiration compared to the effect of the abscisic acid analog itself.

	Transpiration rate (% of control) Days after treatment					
Treatment	1	2	3	4	7	Average
Untreated control (water only)	100.00	100.00	100.00	100.00	100.00	100.00
Positive control (250 ppm (S)-(+)- abscisic acid salt composition of Example 1 = 1 mg/plant)	48.47	75.50	78.06	82.73	97.51	76.46
Surfactant control (250 ppm (S)- (+)-abscisic acid salt composition of Example 1 + 0.05% Brij 98)	23.63	39.53	56.96	63.95	85.07	53.83
25 ppm PBI-524	93.31	84.64	88.95	71.44	91.50	85.97
25 ppm PBI-524 + 0.05% Brij 98	48.41	52.45	54.63	51.55	84.86	58.38
25 ppm abscisic acid analog salt composition of Example 2	87.34	75.59	81.30	70.98	91.55	81.35
25 ppm abscisic acid analog salt composition of Example 2 + 0.05% Brij 98	39.03	52.77	56.28	51.10	82.74	56.39
25 ppm abscisic acid analog salt composition of Example 3	82.25	84.35	80.69	71.09	92.73	82.22
25 ppm abscisic acid analog salt composition of Example 3 + 0.05% Brij 98	49.73	51.98	51.94	53.66	81.66	57.79

**13**. The composition of claim **12**, wherein the concentration of the salt is at least 2% by weight of said composition.

14. The composition of claim 12 that further comprises an antimicrobial agent.

**15**. The composition of claim **12**, wherein said antimicrobial agent is potassium sorbate.

**16**. The composition of claim **12** that further comprises a performance-enhancing additive.

17. The composition of claim 16 wherein said performance-enhancing additive is selected from the group consisting of ammonium nitrate; calcium chloride; and magnesium nitrate. 18. The composition of claim 12 that further comprises one or more additives to stabilize the color.

**19**. The composition of claim **18** wherein said color-stabilizing additive is a sodium or potassium salt of citric acid or sodium or potassium sulfite, bisulfite or metabisulfite.

**20**. An aqueous composition that comprises from about 5 to about 45 weight % of (S)-(+)-abscisic acid as the ammonium salt and from about 0.1 to about 0.5 weight % potassium sorbate.

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