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(54) Title: ABIOTIC STRESS TOLERANCE

(57) Abstract: The present invention relates to methods for improving the tolerance of crop plants to abiotic stress. In particular, the invention relates to methods for improving the tolerance of rice plants to heat stress.

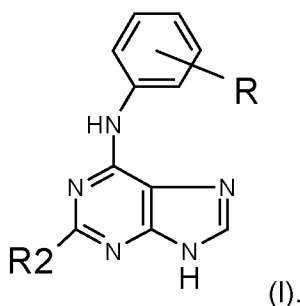


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ABIOTIC STRESS TOLERANCE

The present invention relates to methods for improving the tolerance of crop plants to abiotic stress. In particular, the invention relates to methods for improving the tolerance of rice plants to heat stress.

Certain substituted 6-anilinopurine derivatives are known in the art to inhibit cytokinin oxidase, and may therefore be useful in controlling the levels of cytokinin in plants. WO2009/003428 describes substituted 6-anilinopurine derivatives of formula (I)



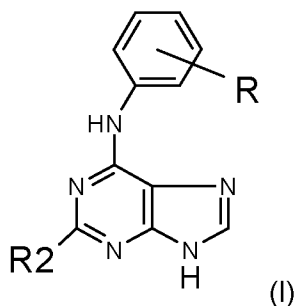
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Rice is one of the most important human food crops in the world. Rice is particularly susceptible to heat stress; extremely high temperatures can lead to poor rice yields. Many of the world's main rice producing areas experience frequent heat stress events, in particular in Asia and western Africa. Therefore there is a need for technology to improve the tolerance of rice to heat stress, in order to prevent or minimise yield loss.

15

According to the present invention, there is provided a method for improving the tolerance of rice plants to heat stress, comprising treating rice plants or the locus at which rice plants are growing, with a compound of formula (I)

20



wherein R denotes one to five substituents independently selected from the group consisting of hydrogen, halogen, hydroxyl, amino, alkyloxy and alkyl; and R2 is selected from the group consisting of amino, halogen, nitro, thio, alkylthio and alkyl;

25

wherein at the time of treatment the rice plants are at reproductive growth phase, and heat stress is present or will be present within 10 days after treatment.

According to the present invention, there is provided a method for improving the tolerance of rice plants to heat stress, comprising (a) growing rice plants, (b) monitoring the air temperature at or near the locus at which said rice plants are growing, and (c) applying a compound of formula (I) to rice plants that are at a reproductive growth phase or the locus at which said rice plants are growing, when heat stress is present or will be present within 10 days after treatment.

There are 3 main growth stages of rice, namely the vegetative phase, reproductive phase, and ripening phase. Although different varieties develop at different rates, and environmental factors strongly affect the duration of the different growth stages, the growth stages are well characterised, and defined on the rice "BBCH" (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale, a system for a uniform coding of phenologically similar growth stages of plants.

"Reproductive growth phase" refers to the period during which the panicle forms and emerges from the base of the tiller and flowering begins (BBCH 30 to 51). The reproductive stage typically starts about 60 days after sowing / germination (typically 45 days after transplanting), and lasts for approximately 25 to 30 days. It includes panicle initiation (BBCH 30), panicle formation (BBCH 32), internode elongation (BBCH 34), spikelet differentiation (BBCH 35), meiosis (BBCH 39), booting (BBCH 41-49), and heading (BBCH 51). Critical growth stages are shown in the table below.

<b>Critical growth stages</b>	<b>BBCH</b>	<b>Visual characteristic</b>	<b>Indicative time (Days After Sowing or Transplanting, DAS or DAT)</b>
Transplanting	13.2	3 <sup>rd</sup> leaf developed and 4 <sup>th</sup> leaf emerging	14 DAS
Active tillering	23.0	3 tillers on main stem	20 DAT
Maximum tillering	26.0	Broad diamond stem base with 6 tillers or more	40 DAT
Panicle initiation	30.0	Oval stem base & leaf 3 at same height as leaf 4	45 DAT
Panicle formation	32.0	Circular stem base & leaf 2 at <50% height of leaf 3	50 DAT

Spikelet differentiation	35.0	Hollow stem base & leaf 2 at >50% height of leaf 3	55 DAT
Meiosis	39.0	Auricles of flag leaf and leaf 2 at 0mm	55-60 DAT
Heading	51.0	Panicles visible from sheath	66 DAT
Active ripening	75.0	Panicles bent to 2-3 o'clock (semi-circles)	70 DAT
Harvesting	88.0	About 80% grains in straw colour	At least 10 days after active ripening

In one embodiment, the rice plants are at a BBCH growth stage from 30 to 51 at the time of treatment. In a further embodiment, the rice plants are at a BBCH growth stage from 30 to 40 at the time of treatment.

5 In one embodiment, the rice plants are treated after the start of the reproductive growth stage.

In one embodiment, the rice plants are treated before the heading growth stage, corresponding to BBCH growth stage 51. Heading typically takes place from 75 to 95 days after germination.

10 In one embodiment, the rice plants are at panicle initiation, panicle formation or spikelet differentiation growth stage at the time of treatment, corresponding to BBCH growth stage 30 to 35. In a further embodiment, the rice plants are at panicle initiation or panicle formation growth stage at the time of treatment, corresponding to BBCH growth stage 30 to 32. Typically, panicle initiation and formation takes place from 50 to 75 days (especially  
15 from 60 to 70, or from 60 to 65 days) after germination.

In a further embodiment, the rice plants are at panicle formation or spikelet differentiation growth stage at the time of treatment, corresponding to BBCH growth stage 32 to 35. Typically, panicle formation and spikelet differentiation takes place from 50 to 75 days (especially from 60 to 75, or from 65 to 70 days) after germination.

20 In a further embodiment, the rice plants are at panicle formation growth stage at the time of treatment, corresponding to BBCH growth stage 32. Typically, panicle formation takes place from 55 to 75 days (especially from 60 to 70, from 60 to 65 days) after germination.

In one embodiment, the compound of formula (I) is applied to rice plants from  
25 55 to 80 days after germination, from 60 to 75 days after germination, from 60 to 70 days after germination, or from 55 to 65 days after germination.

In one embodiment, the compound of formula (I) is applied to rice plants from 40 to 70 days after transplantation, from 45 to 65 days after transplantation, from 45 to 60 days after transplantation, from 45 to 55 days after transplantation, or from 45 to 50 days after transplantation.

In one embodiment, the compound of formula (I) is applied to an entire rice crop, or a locus where a rice crop is growing, wherein at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the plants in the crop or that locus are at the desired growth stage.

“Heat stress” refers to temperature conditions that negatively affect plant metabolism and development, and can also limit plant growth or reduce yield potential. The temperature threshold varies by plant species and variety. Heat stress may occur during the daytime or night time.

Heat stress includes conditions in which the daily minimum (nighttime) temperature is at least 22°C, and/or the daily maximum (daytime) temperature is at least 32°C. In one embodiment, the daily minimum temperature is at least 22°C, at least 23°C, at least 24°C, at least 25°C, at least 26°C, at least 27°C, at least 28°C, at least 29°C, or at least 30°C. The daily minimum temperature may be measured over a given day, or over several consecutive days, for example 3, 4, 5, 6 or 7 days. In a further embodiment, the daily maximum temperature is at least 32°C, at least 33°C, at least 34°C, at least 35°C, at least 36°C, at least 37°C, at least 38°C, at least 39°C, or at least 40°C. The daily maximum temperature may be measured over a given day, or over several days, for example 2, 3, 4, 5, 6 or 7 days within a time span of 7 days.

In one embodiment, the minimum nighttime temperature is at least 22°C, and the maximum daytime temperature is at least 36°C.

Heat stress includes conditions in which the average daily minimum temperature is at least 25°C, and/or the average daily maximum temperature is at least 34°C. In one embodiment, the average daily minimum temperature is at least 25°C, at least 26°C, at least 27°C, at least 28°C, at least 29°C, or at least 30°C. The average daily minimum temperature may be measured over a given day, or over several consecutive days, for example 3, 4, 5, 6 or 7 days. In a further embodiment, the average daily maximum temperature is at least 34°C, at least 35°C, at least 36°C, at least 37°C, at least 38°C, at least 39°C, or at least 40°C. The average daily maximum temperature may be measured over a given day, or over several days, for example 2, 3, 4, 5, 6 or 7 days within a time span of 7 days.

The present invention is particularly useful when heat stress occurs both during the night and day, so that the rice plants are subjected to continuous heat stress with no recovery period. In one embodiment, the average daily minimum temperature is at least 24°C, and the average daily maximum temperature is at least 35°C.

5 In one embodiment, the average daily minimum temperature is at least 25°C, and the average daily maximum temperature is at least 34°C.

In one embodiment, the average daily minimum temperature is at least 25°C, and the average daily maximum temperature is at least 37°C.

10 In a further embodiment, the average daily minimum temperature is at least 27°C, and the average daily maximum temperature is at least 37°C.

In a further embodiment, the average daily minimum temperature is at least 27°C, and the average daily maximum temperature is at least 39°C.

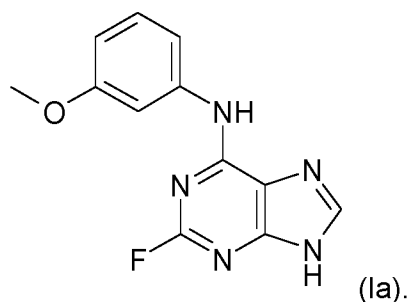
15 Heat stress can be influenced by other conditions than air temperature. For example heat stress may be more severe where there is high air moisture, high radiation and/or high wind speed.

In one aspect of the present invention, environmental conditions are monitored so that heat stress can be forecast several days or weeks in advance.

20 In one embodiment, treatment of rice plants is made within 5 days, 4 days, 3 days, 2 days, or 1 day after the onset of heat stress. In one embodiment, rice plants are treated when heat stress has been present for less than 5 days, less than 4 days, less than 3 days, less than 2 days or less than 1 day. In a further embodiment, treatment of rice plants according to the present invention is made before the onset of heat stress, for example at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days or at least 10 days before heat stress  
25 conditions are present. Preferably treatment is made at least 1 day before the onset of heat stress, and heat stress occurs within 5 days after treatment.

30 The compound of formula (I) includes several variants. In one embodiment R is methoxy. In a further embodiment R2 is halogen. In a still further embodiment R is methoxy and R2 is halogen. In one aspect of the invention R2 is fluorine.

Preferably, the compound of formula (I) has the formula (1a):



In one embodiment, the compound of formula (I) or (Ia) is applied in the form of a composition, which composition further comprises one or more formulation adjuvants.

5 The compound of formula (I) is applied at a rate from 0.1 to 1000g ai/ha. For example, the compound is applied at a rate from 1 to 100g ai/ha, from 5 to 50g ai/ha, from 5 to 40 g ai/ha, from 5 to 30 g ai/ha, from 5 to 25 g ai/ha, from 5 to 20 g ai/ha, from 5 to 15 g ai/ha, from 5 to 10 g ai/ha. In one embodiment, the compound of formula (I) is applied at a rate of 10 g ai/ha.

10 In one embodiment, the compound of formula (I) is applied to the plants more than once before or during the period of heat stress. For example, successive applications of the compound of formula (I) may be made at regular intervals, for example every 1, 2, 3, 4 or 5 days. Preferably at least the first application of the compound of formula (I) is made before the onset of heat stress.

15 In one embodiment, the compound of formula (I) is applied to the plants twice.

In a further embodiment, the compound of formula (I) is applied twice at a rate of 10 g ai/ha, with an interval of 4 to 10 days between applications.

In one embodiment, the method of the present invention does not result in any adverse effects to the rice plants, such as phytotoxicity or lodging.

20 The compound or composition of the present invention may be applied via any suitable method, including foliar spray, soil drench, soil irrigation, drip irrigation, application through sprinklers or central pivot, and incorporation into soil. In one embodiment, the compound of formula (I) is applied via foliar spray.

The present invention may be used to improve tolerance to heat stress for any rice species and variety. Common cultivated rice species include *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). There are many different rice varieties within each species; for example, major varieties of *Oryza sativa* include *indica*, *japonica*, *aromatic* and *glutinous*. Although heat stress may conditions vary between rice varieties, the rice growers are skilled in determining the specific conditions that constitute heat stress for a given rice variety.

30 Common rice varieties include *Oryza sativa* variety ADT43, ADT45, NK5251, NK3325 and TN11.

Tolerance to heat stress may be measured by counting the number of rice plants that do not exhibit yellowing or burned leaf tips. For example these parameters can be assessed by making a visual assessment of damage, or by measuring NDVI (Normalized Difference  
5 Vegetation Index) using a remote sensing device such as Green Seeker™.

Tolerance to heat stress may also be monitored by measuring many different aspects of the rice plant, for example the number of empty spikelets, percentage ripening, thousand grain weight, grain yield, plant stand, photosynthetic activity, transpiration rate or canopy  
10 temperature.

The following effects may be indicative of an improvement in tolerance to heat stress, for example a reduction in the number of empty spikelets, increase in percentage ripening, increase in thousand grain weight, increase in yield, increase in plant stand, increase in photosynthesis, increase in leaf transpiration, or a reduction in canopy temperature. The present invention also leads to more uniform timing of ripening within a rice crop, which  
15 results in improved grain homogeneity and quality of the rice harvest. A more uniform timing of ripening is important because over-ripened rice grains are more easily damaged during milling, leading to lower quality of the harvest.

In one embodiment, there is provided the use of a compound of formula (I) as  
20 described herein to improve the tolerance of rice plants to heat stress.

In one embodiment, the compound of formula (I) is applied in combination with at least one further active ingredient or product that improves tolerance to heat stress. The further active ingredient or product may act synergistically with the compound of formula (I) such that the rates of application of each component is lowered, and/or the heat stress  
25 tolerance is improved.

A synergistic effect exists whenever the action of an active ingredient combination is greater than the sum of the actions of the individual components. The action to be expected E for a given active ingredient combination obeys the so-called COLBY formula and can be calculated as follows (COLBY, S.R. "Calculating synergistic and antagonistic responses of  
30 herbicide combination". Weeds, Vol. 15, pages 20-22):

ppm = milligrams of active ingredient (a.i.) per liter

X = % action by first active ingredient using p ppm of the active ingredient

Y = % action by second active ingredient using q ppm of the active ingredient.

According to Colby, the expected (additive) action of active ingredients A + B using p + q  
35 ppm of active ingredient is

$$E = X + Y - \frac{X \cdot Y}{100}$$



If the action actually observed O is greater than the expected action E, then the action of the combination is super-additive, i.e. there is a synergistic effect. In mathematical terms, synergism corresponds to a positive value for the difference of (O-E). In the case of purely complementary addition of activities (expected activity), said difference (O-E) is zero.  
5 A negative value of said difference (O-E) signals a loss of activity compared to the expected activity.

The further active ingredient may be any active ingredient that enhances tolerance to heat stress. In particular, there may be mentioned a strobilurin fungicide, for example selected from the group consisting of azoxystrobin, pyraclostrobin, trifloxystrobin, and  
10 fluoxastrobin. In one embodiment, the further active ingredient is azoxystrobin or pyraclostrobin. In particular, the further active ingredient is azoxystrobin.

The further product may be any product that enhances tolerance to heat stress. In particular there may be mentioned biostimulant products that contain one or more micronutrients, macronutrients, plant hormones, or amino acids. Examples of biostimulant  
15 products include seaweed extracts, Quantis™, Isabion™, Vitazyme™, Megafol™, Releaseed™, Biozyme™, TerraSorb™, Aminocore™, Radical™, Proplex™, Bio-forge™, Terrabiogen™, Folicist™, Cytozyme™, Cytoplant™, and Greenstim™.

In one embodiment, the further active ingredient is selected from the group consisting of azoxystrobin, Quantis™ and Isabion™.

20 The further active ingredient or product that enhances tolerance to heat stress may be applied at the same time as the compound of formula (I) or sequentially before or after application of formula (I). In one aspect, the further active ingredient is applied as a tank mix partner with the compound of formula (I).

In one embodiment of the invention, the method for improving the tolerance of rice  
25 plants to heat stress further includes the step of applying azoxystrobin.

In one aspect of the invention, there is provided a mixture, composition, or spray solution comprising a compound of formula (Ia) and azoxystrobin.

30 The term "plants" refers to all physical parts of a plant, including seeds, seedlings, saplings, roots, tubers, stems, stalks, foliage, and fruits.

The term "locus" as used herein means fields in or on which plants are growing, or where seeds of cultivated plants are sown, or where seed will be placed into the soil. It includes soil, seeds, and seedlings, as well as established vegetation.

35 Where a range of numbers is disclosed herein (for example, 1 to 10), this is intended to include all numbers and intervening values within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any sub-range of numbers and intervening values within

that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7). Additionally, it is intended that the both the upper and lower limits specified are included within the range.

Where ranges or values used herein are preceded by the term "about", this term is intended to provide support for both the exact number that it precedes, and also a number that is near to or approximately the number that it precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating number may be a number, which would be rounded to or be substantially equivalent to the specifically recited number. For example, the term "about 5" includes 5.0, 4.5, 5.4, 4.92, 5.01, and so on.

The compound of formula (I) may be applied in the form of a composition. The composition can be in the form of concentrates which are diluted prior to use, although ready-to-use compositions can also be made. The final dilution is usually made with water, but can be made instead of, or in addition to, water, with, for example, liquid fertilisers, micronutrients, biological organisms, oil or solvents.

The compositions according to the invention are generally formulated in various ways using formulation adjuvants, such as carriers, solvents and surface-active substances. The formulations can be in various physical forms, e.g. in the form of dusting powders, gels, wettable powders, water-dispersible granules, water-dispersible tablets, effervescent pellets, emulsifiable concentrates, micro-emulsifiable concentrates, oil-in-water emulsions, oil-flowables, aqueous dispersions, oily dispersions, suspo-emulsions, capsule suspensions, emulsifiable granules, soluble liquids, water-soluble concentrates (with water or a water-miscible organic solvent as carrier), impregnated polymer films or in other forms known e.g. from the Manual on Development and Use of FAO and WHO Specifications for Pesticides, United Nations, First Edition, Second Revision (2010). Such formulations can either be used directly or diluted prior to use. The dilutions can be made, for example, with water, liquid fertilisers, micronutrients, biological organisms, oil or solvents.

The formulations can be prepared e.g. by mixing the active ingredient with the formulation adjuvants in order to obtain compositions in the form of finely divided solids, granules, solutions, dispersions or emulsions. The active ingredients can also be formulated with other adjuvants, such as finely divided solids, mineral oils, oils of vegetable or animal origin, modified oils of vegetable or animal origin, organic solvents, water, surface-active substances or combinations thereof.

The active ingredients can also be contained in very fine microcapsules. Microcapsules contain the active ingredients in a porous carrier. This enables the active ingredients to be released into the environment in controlled amounts (e.g. slow-release). Microcapsules usually have a diameter of from 0.1 to 500 microns. They contain active

ingredients in an amount of about from 25 to 95 % by weight of the capsule weight. The active ingredients can be in the form of a monolithic solid, in the form of fine particles in solid or liquid dispersion or in the form of a suitable solution. The encapsulating membranes can comprise, for example, natural or synthetic rubbers, cellulose, styrene/butadiene copolymers, polyacrylonitrile, polyacrylate, polyesters, polyamides, polyureas, polyurethane or chemically modified polymers and starch xanthates or other polymers that are known to the person skilled in the art. Alternatively, very fine microcapsules can be formed in which the active ingredient is contained in the form of finely divided particles in a solid matrix of base substance, but the microcapsules are not themselves encapsulated.

10 The formulation adjuvants that are suitable for the preparation of the compositions according to the invention are known per se. As liquid carriers there may be used: water, toluene, xylene, petroleum ether, vegetable oils, acetone, methyl ethyl ketone, cyclohexanone, acid anhydrides, acetonitrile, acetophenone, amyl acetate, 2-butanone, butylene carbonate, chlorobenzene, cyclohexane, cyclohexanol, alkyl esters of acetic acid, 15 diacetone alcohol, 1,2-dichloropropane, diethanolamine, p-diethylbenzene, diethylene glycol, diethylene glycol abietate, diethylene glycol butyl ether, diethylene glycol ethyl ether, diethylene glycol methyl ether, N,N-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, dipropylene glycol, dipropylene glycol methyl ether, dipropylene glycol dibenzoate, diproxitol, alkylpyrrolidone, ethyl acetate, 2-ethylhexanol, ethylene carbonate, 1,1,1-trichloroethane, 2- 20 heptanone, alpha-pinene, d-limonene, ethyl lactate, ethylene glycol, ethylene glycol butyl ether, ethylene glycol methyl ether, gamma-butyrolactone, glycerol, glycerol acetate, glycerol diacetate, glycerol triacetate, hexadecane, hexylene glycol, isoamyl acetate, isobornyl acetate, isooctane, isophorone, isopropylbenzene, isopropyl myristate, lactic acid, laurylamine, mesityl oxide, methoxypropanol, methyl isoamyl ketone, methyl isobutyl ketone, 25 methyl laurate, methyl octanoate, methyl oleate, methylene chloride, m-xylene, n-hexane, n-octylamine, octadecanoic acid, octylamine acetate, oleic acid, oleylamine, o-xylene, phenol, polyethylene glycol, propionic acid, propyl lactate, propylene carbonate, propylene glycol, propylene glycol methyl ether, p-xylene, toluene, triethyl phosphate, triethylene glycol, xylenesulfonic acid, paraffin, mineral oil, trichloroethylene, perchloroethylene, ethyl acetate, 30 amyl acetate, butyl acetate, propylene glycol methyl ether, diethylene glycol methyl ether, methanol, ethanol, isopropanol, and alcohols of higher molecular weight, such as amyl alcohol, tetrahydro-furfuryl alcohol, hexanol, octanol, ethylene glycol, propylene glycol, glycerol, N-methyl-2-pyrrolidone and the like.

Suitable solid carriers are, for example, talc, titanium dioxide, pyrophyllite clay, silica, 35 attapulgite clay, kieselguhr, limestone, calcium carbonate, bentonite, calcium montmorillonite, cottonseed husks, wheat flour, soybean flour, pumice, wood flour, ground walnut shells, lignin and similar substances.

A large number of surface-active substances can advantageously be used in both solid and liquid formulations, especially in those formulations which can be diluted with a carrier prior to use. Surface-active substances may be anionic, cationic, non-ionic or polymeric and they can be used as emulsifiers, wetting agents or suspending agents or for other purposes. Typical surface-active substances include, for example, salts of alkyl sulfates, such as diethanolammonium lauryl sulfate; salts of alkylarylsulfonates, such as calcium dodecylbenzenesulfonate; alkylphenol/alkylene oxide addition products, such as nonylphenol ethoxylate; alcohol/alkylene oxide addition products, such as tridecylalcohol ethoxylate; soaps, such as sodium stearate; salts of alkyl-naphthalenesulfonates, such as sodium dibutyl-naphthalenesulfonate; dialkyl esters of sulfosuccinate salts, such as sodium di(2-ethylhexyl)sulfosuccinate; sorbitol esters, such as sorbitol oleate; quaternary amines, such as lauryltrimethylammonium chloride, polyethylene glycol esters of fatty acids, such as polyethylene glycol stearate; block copolymers of ethylene oxide and propylene oxide; and salts of mono and di-alkylphosphate esters; and also further substances described e.g. in McCutcheon's Detergents and Emulsifiers Annual, MC Publishing Corp., Ridgewood New Jersey (1981).

Further adjuvants that can be used in pesticidal formulations include crystallisation inhibitors, viscosity modifiers, suspending agents, dyes, anti-oxidants, foaming agents, light absorbers, mixing auxiliaries, antifoams, complexing agents, neutralising or pH-modifying substances and buffers, corrosion inhibitors, fragrances, wetting agents, take-up enhancers, micro-nutrients, plasticisers, glidants, lubricants, dispersants, thickeners, antifreezes, microbicides, and liquid and solid fertilisers.

The compositions according to the invention can include an additive comprising an oil of vegetable or animal origin, a mineral oil, alkyl esters of such oils or mixtures of such oils and oil derivatives. The amount of oil additive in the composition according to the invention is generally from 0.01 to 10 %, based on the mixture to be applied. For example, the oil additive can be added to a spray tank in the desired concentration after a spray mixture has been prepared. Preferred oil additives comprise mineral oils or an oil of vegetable origin, for example rapeseed oil, olive oil or sunflower oil, emulsified vegetable oil, alkyl esters of oils of vegetable origin, for example the methyl derivatives, or an oil of animal origin, such as fish oil or beef tallow. Preferred oil additives comprise alkyl esters of C8-C22 fatty acids, especially the methyl derivatives of C12-C18 fatty acids, for example the methyl esters of lauric acid, palmitic acid and oleic acid (methyl laurate, methyl palmitate and methyl oleate, respectively). Many oil derivatives are known from the Compendium of Herbicide Adjuvants, 10th Edition, Southern Illinois University, 2010.

The inventive compositions generally comprise from 0.1 to 99 % by weight, especially from 0.1 to 95 % by weight, of compounds of the present invention and from 1 to

99.9 % by weight of a formulation adjuvant which preferably includes from 0 to 25 % by weight of a surface-active substance. Whereas commercial products may preferably be formulated as concentrates, the end user will normally employ dilute formulations.

5 The rates of application vary within wide limits and depend on the nature of the soil, the method of application, the crop plant, the pest to be controlled, the prevailing climatic conditions, and other factors governed by the method of application, the time of application and the target crop. As a general guideline compounds may be applied at a rate of from 1 to 2000 l/ha, especially from 10 to 1000 l/ha.

Preferred formulations can have the following compositions (weight %):

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Emulsifiable concentrates:

active ingredient: 1 to 95 %, preferably 60 to 90 %  
 surface-active agent: 1 to 30 %, preferably 5 to 20 %  
 liquid carrier: 1 to 80 %, preferably 1 to 35 %

15

Dusts:

active ingredient: 0.1 to 10 %, preferably 0.1 to 5 %  
 solid carrier: 99.9 to 90 %, preferably 99.9 to 99 %

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Suspension concentrates:

active ingredient: 5 to 75 %, preferably 10 to 50 %  
 water: 94 to 24 %, preferably 88 to 30 %  
 surface-active agent: 1 to 40 %, preferably 2 to 30 %

25

Wettable powders:

active ingredient: 0.5 to 90 %, preferably 1 to 80 %  
 surface-active agent: 0.5 to 20 %, preferably 1 to 15 %  
 solid carrier: 5 to 95 %, preferably 15 to 90 %

30

Granules:

active ingredient: 0.1 to 30 %, preferably 0.1 to 15 %  
 solid carrier: 99.5 to 70 %, preferably 97 to 85 %

The following Examples further illustrate, but do not limit, the invention.

<u>Wettable powders</u>	a)	b)	c)
active ingredients	25 %	50 %	75 %

sodium lignosulfonate	5 %	5 %	-
sodium lauryl sulfate	3 %	-	5 %
sodium diisobutyl naphthalenesulfonate	-	6 %	10 %
phenol polyethylene glycol ether (7-8 mol of ethylene oxide)	-	2 %	-
highly dispersed silicic acid	5 %	10 %	10 %
Kaolin	62 %	27 %	-

The combination is thoroughly mixed with the adjuvants and the mixture is thoroughly ground in a suitable mill, affording wettable powders that can be diluted with water to give suspensions of the desired concentration.

<u>Powders for dry seed treatment</u>	a)	b)	c)
active ingredients	25 %	50 %	75 %
light mineral oil	5 %	5 %	5 %
highly dispersed silicic acid	5 %	5 %	-
Kaolin	65 %	40 %	-
Talcum	-		20

- 5 The combination is thoroughly mixed with the adjuvants and the mixture is thoroughly ground in a suitable mill, affording powders that can be used directly for seed treatment.

<u>Emulsifiable concentrate</u>	
active ingredients	10 %
octylphenol polyethylene glycol ether (4-5 mol of ethylene oxide)	3 %
calcium dodecylbenzenesulfonate	3 %
castor oil polyglycol ether (35 mol of ethylene oxide)	4 %
Cyclohexanone	30 %
xylene mixture	50 %

Emulsions of any required dilution, which can be used in plant protection, can be obtained from this concentrate by dilution with water.

<u>Dusts</u>	a)	b)	c)
Active ingredients	5 %	6 %	4 %
Talcum	95 %	-	-
Kaolin	-	94 %	-
mineral filler	-	-	96 %

Ready-for-use dusts are obtained by mixing the combination with the carrier and grinding the mixture in a suitable mill. Such powders can also be used for dry dressings for seed.

<u>Extruder granules</u>	
Active ingredients	15 %
sodium lignosulfonate	2 %
carboxymethylcellulose	1 %
Kaolin	82 %

- 5 The combination is mixed and ground with the adjuvants, and the mixture is moistened with water. The mixture is extruded and then dried in a stream of air.

<u>Coated granules</u>	
Active ingredients	8 %
polyethylene glycol (mol. wt. 200)	3 %
Kaolin	89 %

The finely ground combination is uniformly applied, in a mixer, to the kaolin moistened with polyethylene glycol. Non-dusty coated granules are obtained in this manner.

10

#### Suspension concentrate

active ingredients	40 %
propylene glycol	10 %
nonylphenol polyethylene glycol ether (15 mol of ethylene oxide)	6 %
Sodium lignosulfonate	10 %
carboxymethylcellulose	1 %
silicone oil (in the form of a 75 % emulsion in water)	1 %
Water	32 %

The finely ground combination is intimately mixed with the adjuvants, giving a suspension concentrate from which suspensions of any desired dilution can be obtained by dilution with water. Using such dilutions, living plants as well as plant propagation material

can be treated and protected against infestation by microorganisms, by spraying, pouring or immersion.

Flowable concentrate for seed treatment

active ingredients	40 %
propylene glycol	5 %
copolymer butanol PO/EO	2 %
Tristyrenephenole with 10-20 moles EO	2 %
1,2-benzisothiazolin-3-one (in the form of a 20% solution in water)	0.5 %
monoazo-pigment calcium salt	5 %
Silicone oil (in the form of a 75 % emulsion in water)	0.2 %
Water	45.3 %

5 The finely ground combination is intimately mixed with the adjuvants, giving a suspension concentrate from which suspensions of any desired dilution can be obtained by dilution with water. Using such dilutions, living plants as well as plant propagation material can be treated and protected against infestation by microorganisms, by spraying, pouring or immersion.

10

Slow Release Capsule Suspension

28 parts of the combination are mixed with 2 parts of an aromatic solvent and 7 parts of toluene diisocyanate/polymethylene-polyphenylisocyanate-mixture (8:1). This mixture is emulsified in a mixture of 1.2 parts of polyvinylalcohol, 0.05 parts of a defoamer and 51.6 parts of water until the desired particle size is achieved. To this emulsion a mixture of 2.8 parts 1,6-diaminohexane in 5.3 parts of water is added. The mixture is agitated until the polymerization reaction is completed. The obtained capsule suspension is stabilized by adding 0.25 parts of a thickener and 3 parts of a dispersing agent. The capsule suspension formulation contains 28% of the active ingredients. The medium capsule diameter is 8-15 microns. The resulting formulation is applied to seeds as an aqueous suspension in an apparatus suitable for that purpose.

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The composition of the present invention may be applied to a plant, part of the plant, plant organ, plant propagation material or a plant growing locus.

25

The application is generally made by spraying the composition, typically by tractor mounted sprayer for large areas, but other methods such as dusting (for powders), drip or



drench can also be used. Alternatively the composition may be applied in furrow or directly to a seed before or at the time of planting.

The composition of the present invention may be applied pre-emergence or post-emergence. Where the composition is used to regulate the growth of crop plants or enhance the tolerance to abiotic stress, it may be applied post-emergence of the crop. Where the composition is used to inhibit or delay the germination of seeds, it may be applied pre-emergence. Where the composition is used to control pests, it may be applied as a preventative (before pest establishment) or curative (after pest establishment) treatment.

The present invention envisages application of the compositions of the invention to plant propagation material prior to, during, or after planting, or any combination of these. Although active ingredients can be applied to plant propagation material in any physiological state, a common approach is to use seeds in a sufficiently durable state to incur no damage during the treatment process. Typically, seed would have been harvested from the field; removed from the plant; and separated from any cob, stalk, outer husk, and surrounding pulp or other non-seed plant material. Seed would preferably also be biologically stable to the extent that treatment would not cause biological damage to the seed. It is believed that treatment can be applied to seed at any time between seed harvest and sowing of seed including during the sowing process.

Methods for applying or treating active ingredients on to plant propagation material or to the locus of planting are known in the art and include dressing, coating, pelleting and soaking as well as nursery tray application, in furrow application, soil drenching, soil injection, drip irrigation, application through sprinklers or central pivot, or incorporation into soil (broad cast or in band). Alternatively or in addition active ingredients may be applied on a suitable substrate sown together with the plant propagation material.

The rates of application of compositions of the present invention may vary within wide limits and depend on the nature of the soil, the method of application (pre- or post-emergence; seed dressing; application to the seed furrow; no tillage application etc.), the crop plant, the prevailing climatic conditions, and other factors governed by the method of application, the time of application and the target crop. For foliar or drench application, the compositions of the present invention are generally applied at a rate of from 1 to 2000 g/ha, especially from 5 to 1000 g/ha. For seed treatment the rate of application is generally between 0.0005 and 150g per 100kg of seed.

Crops are to be understood as being those which are naturally occurring, obtained by conventional methods of breeding, or obtained by genetic engineering. They include crops which contain so-called output traits (e.g. improved storage stability, higher nutritional value and improved flavour).

Crops are to be understood as also including those crops which have been rendered tolerant to herbicides like bromoxynil or classes of herbicides such as ALS-, EPSPS-, GS-, HPPD- and PPO-inhibitors.

5 Crops are also to be understood as being those which naturally are or have been rendered resistant to harmful insects. This includes plants transformed by the use of recombinant DNA techniques, for example, to be capable of synthesising one or more selectively acting toxins, such as are known, for example, from toxin-producing bacteria. Examples of toxins which can be expressed include d-endotoxins, vegetative insecticidal proteins (Vip), insecticidal proteins of bacteria colonising nematodes, and toxins produced by  
10 scorpions, arachnids, wasps and fungi.

Normally, in the management of a crop a grower would use one or more other agronomic chemicals or biologicals in addition to the composition of the present invention.

## EXAMPLES

Example A. Field trials

Field trials were setup during the hot season in India to test the effect of treatment with the compound of formula (Ia) on tolerance of rice plants to heat stress.

Trials were conducted in 2012, 2013, 2014, and 2015 in the Tamil Nadu rice production area (India) at the following sites: Kelamoor (lat. 12.42, long. 79.81), Kokkur (lat. 11.02, long. 79.32), Nachinargudi (lat. 11.01, long. 79.3), Velamoor (lat. 12.41, long. 79.8), Kumbakonam (lat. 10.97, long. 79.42) and Ramapuram (lat. 12.43, long. 79.83). The test sites had similar soil chemical properties, with an average soil pH of 6.6.

Rice (*Oryza sativa* L.) of the local varieties ADT43, ADT45 and NK5251 were mechanically transplanted at four plants per hill with a plant spacing of 16 cm and a row spacing of 30 cm. All trials were performed in good level of water management. Fertilizer was applied in accordance with the best local practice across the trial area.

Commercial treated seeds (no neonicotinoids as seed treatments) were used. Weed control was according to the best local practice in order to provide excellent weed control. Foliar fungicides and insecticides were applied according to common practice (no products that contain neonicotinoids or strobilurins).

The trial design was a randomized bloc design. Plot size was 40 m<sup>2</sup> (4 m width [12 rows] by 10 m row length). Each trial had four replicates. In total, eleven trials were conducted.

The compound of formula (Ia) was applied at a rate of 10 g ai / ha with small-plot hand-held boom sprayer. Application of the compound was as broadcast foliar spray in close proximity to leaf/canopy surface (25-40 cm distance from the crop). The used spray volumes were between 250 L/ha and 500 L/ha. Application was done either at panicle initiation (BBCH 30), at panicle formation (BBCH 32), at spikelet differentiation (BBCH 35), at meiosis (BBCH 39) or at heading (BBCH 51). In some experiments, the compound was applied twice (2 × 10 g ai / ha) at consecutive growth stages (i.e., BBCH 30 + 32, BBCH 32 + 35, BBCH 35 + 39, and BBCH 39 + 51).

Temperature was recorded nearby the trial sites. During and up to 14 days after the critical growth stages (i.e. from panicle initiation until heading) the mean daily minimum temperature was 25.1°C (in the range of 22.6°C to 27.1°C) and the mean daily maximum temperature was 35.5°C (in the range of 29.0 to 38.9°C).

Tolerance to heat stress was assessed by measuring the following yield components: number of panicles per area, number of grains per panicle, percentage of ripened (filled) grains, number of tillers per area, percentage of productive tillers, grain yield per area at 14% moisture and thousand grain weight (TGW).

The data in Tables 1 to 3 represent averages of the results from all eleven independent field trials, expressed as a percentage compared to the untreated control. The percentage increase or decrease was calculated for each trial relative to the untreated control for that trial, prior to calculating the mean percentage change across all 11 trials.

5

TABLE 1: The effect of formula (Ia) treatment in dependence of the developmental stage (BBCH) at the time of application (*in the case of double application, the BBCH stage of the last application was taken*). The mean daily minimum temperature was 25.1°C (22.6°C to 27.1°C) and the mean daily maximum temperature was 35.5°C (29.0°C to 38.9°C) during the 14 days after application.

10

BBCH at application	Grain yield (% of UTC)	Empty spikelets (% of UTC)	TGW (% of UTC)
30	+6.0	-7.5	+2.5
32	+7.4	-9.3	+2.2
35	+4.7	-3.8	+1.8
39	+4.9	-6.6	+1.6
51	+2.8	-3.5	+2.0

TGW: Thousand grain weight

UTC: Untreated control

Table 1 shows that an increase of grain yield was achieved when formula (Ia) was applied between BBCH 30 (panicle initiation) and BBCH 51 (heading) (Table 1). Thereby, the highest efficacy was observed when application was done at panicle initiation and in particular at panicle formation. The increase of grain yield was mainly due to a reduction of empty spikelets but also due to an increase of the thousand grain weight (TGW).

20

TABLE 2: The efficacy of formula (Ia) treatment in dependence of the maximum air temperature during a time span of five days after application (*in the case of double application, the maximum temperature after the last application was taken*). Application was made between panicle initiation (BBCH 30) and heading (BBCH 51) growth stages.

Maximum temperature (°C)	Grain yield (% of UTC)	TGW (% of UTC)
≤ 35	+0.9	+1.4
35.1 – 36	+1.1	+2.3
36.1 – 37	+5.5	+2.2
> 37	+6.6	+1.8

The efficacy of foliar treatment with formula (Ia) on rice grain yield depended also on the temperature conditions after the application. The results in Table 2 show that the efficacy of treatment with formula (Ia) is highest when daily maximum temperatures are high (above 36°C). A clear beneficial effect of treatment with formula (Ia) was observed when the daily maximum temperature was above 36°C at least one day after application but within 5 days after application. The efficacy of formula (Ia) in improving tolerance to heat stress was best when daily maximum temperatures were above 37°C.

10

TABLE 3: The effect of formula (Ia) treatment on grain yield (as percent change compared to untreated controls) in dependence of the developmental stage at application and the maximum air temperature during a time span of five days after application (*in the case of double application, the developmental stage at and the maximum temperature after the last application was taken*).

15

BBCH at application	≤ 36°C	> 36°C
30	+1.6	+7.5
32	+0.7	+9.0
35	+1.8	+5.4
39	+2.6	+5.1
51	-0.7	+3.6

Table 3 shows that heat stress protection was achieved by treatment with formula (Ia) of plants at growth stages from panicle initiation (BBCH 30) to heading (BBCH 51) at the time of treatment. However, heat stress mitigation was highest when the daily maximum temperature was above 36°C during the five days after application, and rice plants were at panicle formation (BBCH 32) or panicle initiation (BBCH 30) at the time of treatment with formula (Ia).

Further trials were conducted in the Tamil Nadu rice production area (India) in 2016 using the protocol as described above using rice variety ADT45, with treatment timings of panicle initiation (BBCH 30), panicle formation (BBCH 32), spikelet differentiation (BBCH 35) and meiosis (BBCH 39) only. The compound of formula (Ia) was applied at a rate of 10g AI/ha. Grain yield was measured at 14% moisture content (MC). The results are shown in Table 4.

TABLE 4: The effect of formula (Ia) treatment applied at different growth stages on grain yield (as percent change compared to untreated controls) across 7 trials each with 4 replicates, conducted in 2016. The mean daily minimum temperature was 25.13°C (22.90°C to 27.8°C) and the mean daily maximum temperature was 34.13°C (31.40°C to 37.30°C) during the 14 days after application

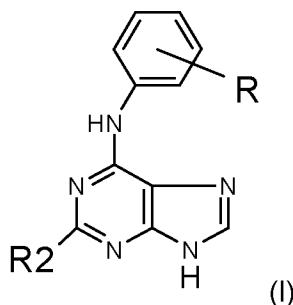
BBCH at application	Yield at 14% MC (kg/ha)	Average yield increase compared to UTC (%)
Untreated (UTC)	6512	n/a
30	6741	+3.5
32	6820	+4,7
35	6931	+6.4
39	6757	+3.6

TABLE 5: Multi-year analysis for 2015-2016 (total 17 trials, each with 4 replicates) of the effect of formula (Ia) applied at different growth stages on rice yield

BBCH at application	Yield at 14% MC (kg/ha), with % change compared to UTC in brackets	Thousand grain weight (g/1000 seed)	Empty spikelets (number per 12 rice hills)
Untreated (UTC)	6318	17.01	5636
30	6583 (+4.1 %)	17.24 (+1.3%)	4927 (-12.5%)
32	6727 (+6.5 %)	17.25 (+1.4%)	5115 (-9.2%)
35	6713 (+6.3 %)	17.23 (+1.3%)	5206 (-7.6%)
39	6643 (+5.2 %)	17.25 (+1.4%)	5170 (-8.3%)

## CLAIMS

- 5 1. A method for improving the tolerance of rice plants to heat stress, comprising treating rice plants or the locus at which rice plants are growing, with a compound of formula (I)



10 wherein R denotes one to five substituents independently selected from the group consisting of hydrogen, halogen, hydroxyl, amino, alkyloxy and alkyl; and R2 is selected from the group consisting of amino, halogen, nitro, thio, alkylthio and alkyl; wherein at the time of treatment the rice plants are at reproductive growth phase, and heat stress is present or will be present within 10 days after treatment.

- 15 2. A method for improving the tolerance of rice plants to heat stress, comprising (a) growing rice plants, (b) monitoring the air temperature at or near the locus at which said rice plants are growing, and (c) applying a compound of formula (I) to rice plants that are at a reproductive growth phase or the locus at which said rice plants are growing, when heat stress is present or will be present within 10 days after treatment.

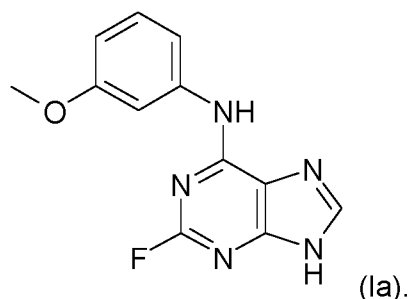
20 3. A method according to claims 1 or 2, wherein the rice plants are treated before the heading growth stage.

25 4. A method according to any one of the preceding claims, wherein the rice plants are at the panicle initiation or panicle formation growth stage.

5. A method according to any one of the preceding claims, wherein the rice plants are at the panicle formation growth stage.



6. A method according to any one of the preceding claims wherein the heat stress is a minimum daily temperature of at least 24°C, and a maximum daily temperature of at least 34°C.
- 5 7. A method according to any one of the preceding claims wherein the heat stress is an average daily minimum temperature of at least 25°C, and an average daily maximum temperature of at least 37°C.
8. A method according to any one of the preceding claims, wherein the compound of  
10 formula (I) has the formula (1a):



9. A method according to any one of the preceding claims, wherein the compound of  
15 formula (I) is applied at a rate from 1 to 100g ai/ha.
10. A method according to any one of the preceding claims, wherein the compound of  
formula (I) is applied at a rate from 5 to 20g ai/ha.
11. A method according to any one of the preceding claims, wherein the compound of  
20 formula (I) is applied simultaneously, or in successive applications with a further  
compound or product that improves tolerance to heat stress.
12. Use of a compound of formula (I) for improving tolerance of rice plants to heat stress,  
by applying the compound to the plants during the reproductive growth phase, when  
25 heat stress is present or will be present within 10 days after treatment.

INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2017/063823

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A01N43/90 A01N43/54  
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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/003428 A2 (UNIVERZITA PALACKEHO V OLOMOUC [CZ]; UNIV BERLIN FREIE [DE]; SPICHAL L) 8 January 2009 (2009-01-08) page 1, line 11 - page 3, line 16 page 7, line 24 - line 27; claims; examples	1-12
Y	----- JOSEPH J. KIEBER ET AL: "Cytokinins", THE ARABIDOPSIS BOOK, vol. 12, 1 January 2014 (2014-01-01), page e0168, XP055397239, DOI: 10.1199/tab.0168 Cytokinin and abiotic stress; page 24 - page 25 -----	1-12

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
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search <b>9 August 2017</b>	Date of mailing of the international search report <b>16/08/2017</b>
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer <b>Butkowskyj-Walkiw, T</b>
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/063823

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