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(54) Title: METHOD FOR REDUCING ABIOTIC STRESS IN PLANTS

(57) Abstract: The present invention provides: a method for reducing abiotic stress in plants, comprising treating a plant that has been or is to be exposed to abiotic stress with an effective amount of a compound represented by the following formula (1); and so on.



DESCRIPTION

METHOD FOR REDUCING ABIOTIC STRESS IN PLANTS

Technical Field

5 The present invention relates to a method for reducing abiotic stress in plants.

Background Art

10 When plants encounter abiotically stressful environments, various disorders may emerge due to either a slow or rapid decrease in the physiological function of the cells. Some chemical substances are known to be effective in reducing the effect of abiotic stress by controlling the physiological conditions of plants (see, for example, *Physiologia Plantarum* 38: pp. 95-97 (1976); and *Plant and Cell Physiology*, 22(3) pp. 15 453-460 (1981)). However, in fact, their effectiveness is not satisfactory.

Disclosure of Invention

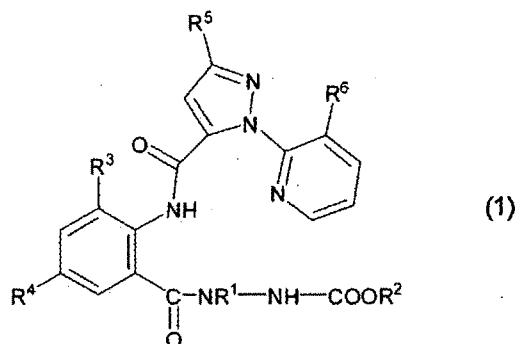
20 An object of the present invention is to provide a method for reducing abiotic stress in plants, among others.

 The present invention is based on the finding that when exposed to abiotic stress, a plant treated with a specific compound shows an abiotic-stress reducing effect.

25 More specifically, the present invention provides:

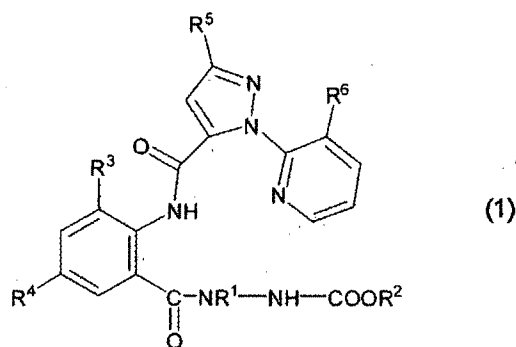
 [1] A method for reducing abiotic stress in plants, comprising treating a plant that has been or is to be exposed to abiotic stress with an effective amount of a compound

represented by the following formula (1):



wherein R^1 represents a C1-C6 alkyl group which may be substituted with a halogen atom; R^2 represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkenyl group which may be substituted with one or more halogen atoms, or a C3-C6 alkynyl group which may be substituted with one or more halogen atoms; R^3 represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^4 represents a hydrogen atom, a halogen atom, a cyano group, or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^5 represents a hydrogen atom, a halogen atom, a cyano group, a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C1-C6 alkoxy group which may be substituted with one or more halogen atoms, a C1-C6 alkylthio group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfinyl group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms; and R^6 represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms;

[2] The method of [1], wherein the compound represented by the formula (1) is a compound selected from the following compound group A:



5 wherein, the compound group A consists of:

(1) a compound of the formula (1) in which R^1 is an ethyl group, R^2 is a methyl group, R^3 is a bromo atom, R^4 is a bromo atom, R^5 is a bromo atom, and R^6 is a chloro atom; and

10 (2) a compound of the formula (1) in which R^1 is a methyl group, R^2 is a methyl group, R^3 is a methyl group, R^4 is a cyano group, R^5 is a bromo atom, and R^6 is a chloroatom;

[3] The method of [1] or [2], wherein the treatment is spraying treatment, soil treatment, seed treatment or hydroponic treatment;

15 [4] The method of any one of [1] to [3], wherein the treatment is seed treatment which is to treat seeds with 5 g or more and 500 g or less of the compound per 100 kg of seeds;

[5] The method of any one of [1] to [3], wherein the treatment is seed treatment which is to treat seeds with 250 g
20 or more and 500 g or less of the compound per 100 kg of seeds;

[6] The method of any one of [1] to [5], wherein the plant is rice, corn or wheat;

[7] The method of any one of [1] to [6], wherein the

plant is a transgenic plant;

[8] The method of any one of [1] to [7], wherein the abiotic stress is low-temperature stress;

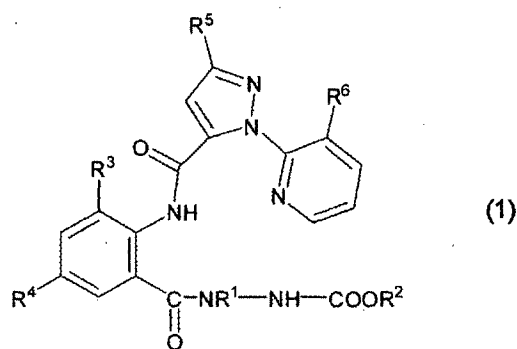
[9] The method of any one of [1] to [7], wherein the
5 abiotic stress is drought stress;

[10] The method of any one of [1] to [9], wherein the reduction of the abiotic stress in plants is indicated by a change in at least one of the following plant phenotypes:

<Plant phenotypes>

- 10 (1) Germination rate,
- (2) Seedling establishment rate,
- (3) Leaf mortality rate,
- (4) Plant length,
- (5) Plant weight,
- 15 (6) Leaf area,
- (7) Leaf color,
- (8) Number or weight of seeds or fruits,
- (9) Quality of harvested products,
- (10) Rates of flower setting, fruit setting, seed setting and
20 seed filling,
- (11) Chlorophyll fluorescence yield,
- (12) Water content,
- (13) Leaf surface temperature, and
- (14) Transpiration capacity;

25 [11] Use of an effective amount of a compound represented by the following formula (1):



wherein R^1 represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^2 represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkenyl group which may be substituted with one or more halogen atoms, or a C3-C6 alkynyl group which may be substituted with one or more halogen atoms; R^3 represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^4 represents a hydrogen atom, a halogen atom, a cyano group or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^5 represents a hydrogen atom, a halogen atom, a cyano group, a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C1-C6 alkoxy group which may be substituted with one or more halogen atoms, a C1-C6 alkylthio group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfinyl group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms; and R^6 represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms;

for reducing abiotic stress in plants; and

[12] The use of [11], wherein the reduction of the abiotic stress in plants is indicated by a change in at least one of the following plant phenotypes:

- 5 <Plant phenotypes>
- (1) Germination rate,
 - (2) Seedling establishment rate,
 - (3) Leaf mortality rate,
 - (4) Plant length,
 - 10 (5) Plant weight,
 - (6) Leaf area,
 - (7) Leaf color,
 - (8) Number or weight of seeds or fruits,
 - (9) Quality of harvested products,
 - 15 (10) Rates of flower setting, fruit setting, seed setting and seed filling,
 - (11) Chlorophyll fluorescence yield,
 - (12) Water content,
 - (13) Leaf surface temperature, and
 - 20 (14) Transpiration capacity.

The method of the present invention may be used to reduce abiotic stress.

25 Mode for Carrying Out the Invention

In the present invention, "abiotic stress" refers to stress such as temperature stress, i.e., high-temperature or low-temperature stress, salt stress, and water stress, i.e.,

drought stress or excessive moisture stress. When plants encounter abiotic stress, physiological functions of their cells are reduced, the physiological conditions of the plants get worse, and their development is inhibited. The high-temperature stress refers to a stress that plants experience when they are exposed to a temperature exceeding the suitable temperature for their growth or germination. Specifically, the high-temperature stress may be caused under conditions in which the average growth temperature is 25°C or higher, more harshly 30°C or higher, and even more harshly 35°C or higher in the environment in which the plants are cultivated. The low-temperature stress refers to a stress that plants experience when they are exposed to a temperature lower than the suitable temperature for their growth or germination. Specifically, the low-temperature stress may be caused under conditions in which the average growth temperature is 15°C or lower, more harshly 10°C or lower, and even more harshly 5°C or lower in the environment in which the plants are cultivated. The drought stress refers to a stress that plants experience when they are exposed to an environment that retards their growth by preventing water absorption due to a reduction in the water content of the soil caused by a shortage of rainfall or irrigation. Specifically, the drought stress may be caused under conditions in which the water content in the soil in which the plants are grown is 15% by weight or less, more harshly 10% by weight or less, and even more harshly 7.5% by weight or less, although these values may vary depending on the type of the soil, or in which the pF value of the soil in

which the plants are grown is 2.3 or more, more harshly 2.7 or more, and even more harshly 3.0 or more, although these values may vary depending on the type of the soil. The excessive moisture stress refers to a stress that plants experience when they are exposed to a moisture environment in which the water content in the soil is excessively high, so that the growth of the plants is inhibited. Specifically, the excessive moisture stress may be caused under conditions in which the water content in the soil in which the plants are grown is 30% by weight or more, more harshly 40% by weight or more, and even more harshly 50% by weight or more, although these values may vary depending on the type of the soil, or in which the pF value of the soil in which the plants are grown is 1.7 or less, more harshly 1.0 or less, and even more harshly 0.3 or less. The pF value of soil may be determined according to the principle described in "Encyclopedia of Soil, Plant Nutrition and Environment" (in Japanese, TAIYOSHA Co., Ltd., 1994, Matsuzaka et al.), in the section entitled "Method for pF Value Measurement," on pages 61 and 62. The salt stress refers to a stress that plants experience when they are exposed to an environment that retards their growth by preventing water absorption due to an increase in the osmotic pressure caused by accumulation of salts contained in the soil or hydroponic solution in which the plants are cultivated. Specifically, the salt stress may be caused under conditions in which the osmotic potential due to the salts contained in the soil or hydroponic solution is 0.2 MPa (NaCl concentration of 2,400 ppm) or higher, harshly 0.25 MPa or higher, and more

harshly 0.30 MPa or higher. The osmotic pressure in the soil can be calculated according to Raoult's equation, shown below, by diluting the soil with water and analyzing the supernatant for salt concentration:

5

Raoult's Equation: π (atm) = cRT

R = 0.082 (L•atm/mol•K)

T = Absolute temperature (K)

c = Ion molar concentration (mol/L)

10 1 atm = 0.1 MPa

Effects of abiotic stress on plants can be understood by comparing changes in the plant phenotypes described below between the plants that have been exposed and those that have not been exposed to abiotic stress. That is, the following plant phenotypes serve as indicators of abiotic stress:

<Plant phenotypes>

- (1) Germination rate,
- (2) Seedling establishment rate,
- 20 (3) Number (Rate) of healthy leaves,
- (4) Plant length,
- (5) Plant weight,
- (6) Leaf area,
- (7) Leaf color,
- 25 (8) Number or weight of seeds or fruits,
- (9) Quality of harvested products,
- (10) Rates of flower setting, fruit setting, seed setting and seed filling,

- (11) Chlorophyll fluorescence yield,
- (12) Water content,
- (13) Leaf surface temperature, and
- (14) Transpiration capacity.

5

These indicators can be measured in the following manner.

(1) Germination rate

Seeds of a plant are sown, for example, in the soil, or on a filter paper, agar culture medium or sand for germination, and then the ratio of the number of germinated seeds to the number of seeds sown is examined.

(2) Seedling establishment rate

Seeds of a plant are sown, for example, in the soil, or on a filter paper, agar culture medium or sand, and allowed to grow for a given period of time. During the entire or a partial cultivation period, temperature stress is applied and then the ratio of surviving seedlings is examined.

(3) Number (or rate) of healthy leaves

The number of healthy leaves is counted for each plant, and the total number of the healthy leaves is examined. Alternatively, the ratio of the number of healthy leaves to the number of all leaves of the plants is examined.

(4) Plant length

For each plant, the length from the base of the stem in the aerial part to the tip of the branches and leaves is measured.

(5) Plant weight

The above-ground part of each of plants is cut and

weighed to determine the fresh weight. Alternatively, the cut sample is dried and weighed to determine the dry weight.

(6) Leaf area

Plants are photographed with a digital camera and the green area in the photograph is quantified using image analysis software, such as Win ROOF (manufactured by MITANI CORPORATION), to obtain the leaf area of the plants.

(7) Leaf color

Leaves of plants are sampled, and the chlorophyll content is measured using a chlorophyll gauge (for example, SPAD-502, Konica Minolta) to determine the leaf color. The plants are photographed with a digital camera and the green area in the photograph is quantified by color extraction, using image analysis software, such as Win ROOF (MITANI CORPORATION), to obtain the green area of the plants.

(8) Number or weight of seeds or fruits

Plants are grown until they reach fructification or ripening of seeds or fruits, and then the number of seeds or fruits per plant is counted or the total weight of seeds or fruits per plant is measured. Alternatively, a yield component, such as panicle number, percentage of ripened grains or thousand kernel weight, is examined after plants are grown until they bear ripe seeds.

(9) Quality of harvested products

Plants are grown until they reach ripening of seeds or fruits, and the sugar content of the fully matured fruits is measured using a saccharimeter, for example. Alternatively, the quality of harvested products is evaluated by determining

the protein and lipid content by performing component analysis.

(10) Percentages of flower setting, fruit setting, seed setting and seed filling

Plants are grown until they reach fructification, and then the numbers of set flowers and set seeds are counted to calculate the percentage of seed setting (%) ($(\text{Number of set seeds} / \text{Number of set flowers}) \times 100$). After seeds are ripe, the numbers of set seeds and filled seeds are counted to calculate the percentages of seed setting (%) ($(\text{Number of set seeds} / \text{Number of set flowers}) \times 100$) and seed filling (%) ($(\text{Number of filled seeds} / \text{Number of set seeds}) \times 100$).

(11) Chlorophyll fluorescence yield

The chlorophyll fluorescence yield is determined by measuring the chlorophyll fluorescence (F_v/F_m) of plants by using a pulse modulation chlorophyll fluorometer, such as IMAGING-PAM (WALZ).

(12) Water content

At each developmental stage of plants, the fresh and dry weights of the plants are determined according to the method described above in "(5) plant weight", and the value obtained by subtracting the dry weight from the fresh weight is regarded as the water content of the plants. Also, the water content of plants is determined in a nondestructive manner, by near infrared irradiation and measuring the absorption (transmission) at this specific wavelength. The water content is measured using, for example, Scanalyzer (LemnaTec).

(13) Leaf surface temperature

At each developmental stage of plants, the leaf surface

temperature is monitored using thermography (for example, Scanalyzer (LemnaTec)).

(14) Transpiration capacity

At each developmental stage of plants, transpiration of water from the leaf surface is measured using a porometer (for example, AP4, manufactured by Delta-T).

As described herein, the abiotic stress can be quantified with the "intensity of stress" represented by the following equation.

Equation: "Intensity of stress" = $100 \times \frac{\text{"any one of the plant phenotypes of plants not exposed to abiotic stress"}}{\text{"the one of the plant phenotype of plants exposed to abiotic stress"}}$

The method of the present invention may be applied to plants that have been exposed to or are to be exposed to a abiotic stress whose "intensity of stress" represented by the above equation is from 105 to 450, preferably from 110 to 200, and more preferably from 115 to 160.

When plants are exposed to abiotic stress, at least one of the phenotypes listed above would be affected. More specifically, events such as described below are observed and they may be used as indicators to measure the abiotic stress in plants:

(1) Decrease in germination rate,

- (2) Decrease in seedling establishment rate,
- (3) Decrease in number (or rate) of healthy leaves,
- (4) Decrease in plant length,
- (5) Decrease in plant weight,
- 5 (6) Decrease in leaf area increasing rate,
- (7) Fading of leaf color,
- (8) Decrease in number or weight of seeds or fruits,
- (9) Deterioration in quality of harvested products,
- (10) Decrease in rate of flower, fruit or seed setting or seed
- 10 filling,
- (11) Decrease in chlorophyll fluorescence yield,
- (12) Decrease in water content,
- (13) Increase in leaf surface temperature, and
- (14) Decrease in transpiration capacity.

15

The present invention is directed to a method for reducing the effect of abiotic stress in plants that have been exposed to or are to be exposed to abiotic stress by treating plant with a compound represented by the formula (1). The effect of the reduction of abiotic stress can be evaluated by comparing the indicators described above between plants treated with the compound represented by the formula (1) and those untreated with the compound after the plants are exposed to the abiotic stress.

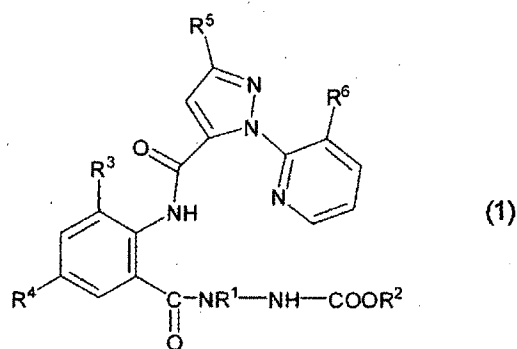
25 In the method of the present invention, when a plant is treated with the compound, the plant may be an entire plant or part thereof (e.g., stem and leaf, shoot, flower, fruit, panicle, seed, bulb, tuber and root). Also, the plant may be

at any of the various stages of growth of the plant (e.g., the germination period, including preseeding time, seeding time, and the period before and after the seedling emergence after sowing; the vegetative growth period, including the nursery
5 period, the time of seedling transplantation, the time of planting or nursing cuttings and the growth period after field planting; the reproductive growth period, including the periods before, during and after flowering, immediately before heading or the heading period; and the harvest period,
10 including a period before the expected harvest date, a period before the expected ripening date and the time of initiation of fruit coloration. As used herein, the term bulb refers to a scaly bulb, corm, rhizome, root tuber and rhizophore. The seedlings may include cuttings and sugar cane stem cuttings.

15

Stages at which a plant of interest in the present invention can be exposed to abiotic stress include all the stages of plant growth, including the germination stage, nursery stage, vegetative growth stage, reproductive growth
20 stage and harvesting stage.

The compound of the present invention (hereinafter may be referred to as "the present compound") is a compound represented by the following formula (1):



wherein R¹ represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R² represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkenyl group which may be substituted with one or more halogen atoms, or a C3-C6 alkynyl group which may be substituted with one or more halogen atoms; R³ represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R⁴ represents a hydrogen atom or a halogen atom, a cyano group or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R⁵ represents a hydrogen atom, a halogen atom, a cyano group, a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C1-C6 alkoxy group which may be substituted with one or more halogen atoms, a C1-C6 alkylthio group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfinyl group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms; and R⁶ represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms.

Then, the details of the compound of the present invention, represented by the formula (1) are described below.

Examples of the groups represented by R^1 to R^6 indicated in the formula (1) include the following groups:

The halogen atom may be, for example, a fluorine, chlorine, bromine, or iodine atom.

The C1-C6 alkyl group which may be substituted with one or more halogen atoms may be, for example, a methyl, trifluoromethyl, trichloromethyl, chloromethyl, dichloromethyl, fluoromethyl, difluoromethyl, ethyl, pentafluoroethyl, 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, propyl, isopropyl, heptafluoroisopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, or hexyl group.

The C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms may be, for example, a 2-methoxyethyl, 2-ethoxyethyl, or 2-isopropoxyethyl group.

The C3-C6 alkenyl group which may be substituted with one or more halogen atoms may be, for example, a 2-propenyl, 3-chloro-2-propenyl, 2-chloro-2-propenyl, 3,3-dichloro-2-propenyl, 2-butenyl, 3-butenyl, 2-methyl-2-propenyl, 3-methyl-2-butenyl, 2-pentenyl, or 2-hexenyl group.

The C3-C6 alkynyl group which may be substituted with

one or more halogen atoms may be, for example, a 2-propynyl, 3-chloro-2-propynyl, 3-bromo-2-propynyl, 2-butynyl, or 3-butynyl group.

5 The C1-C6 alkoxy group which may be substituted with one or more halogen atoms may be, for example, a methoxy, ethoxy, 2,2,2-trifluoroethoxy, propoxy, isopropoxy, butoxy, isobutyloxy, sec-butoxy, or tert-butoxy group.

10 The C1-C6 alkylthio group which may be substituted with one or more halogen atoms may be, for example, a methylthio, trifluoromethylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, sec-butylthio, tert-butylthio, pentylthio, or hexylthio group.

15 The C1-C6 alkylsulfinyl group which may be substituted with one or more halogen atoms may be, for example, a methylsulfinyl, trifluoromethylsulfinyl, ethylsulfinyl, propylsulfinyl, isopropylsulfinyl, butylsulfinyl, 20 isobutylsulfinyl, sec-butylsulfinyl, tert-butylsulfinyl, pentylsulfinyl, or hexylsulfinyl group.

25 The C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms may be, for example, a methylsulfonyl, trifluoromethylsulfonyl, ethylsulfonyl, propylsulfonyl, isopropylsulfonyl, butylsulfonyl, isobutylsulfonyl, sec-butylsulfonyl, tert-butylsulfonyl, pentylsulfonyl, or hexylsulfonyl group.

By way of example, embodiments of the present compound include the following compounds:

5 A compound of the formula (1), wherein R^1 is a methyl, ethyl, or isopropyl group, R^2 is a methyl or ethyl group, R^3 is a halogen atom or a methyl group, R^4 is a halogen atom or a cyano group, R^5 is a halogen atom or a trifluoromethyl group, and R^6 is a halogen atom.

10 A compound of the formula (1), wherein R^1 is a methyl group, R^2 is a methyl group, R^3 is a chlorine or bromine atom or a methyl group, R^4 is a chlorine or bromine atom or a cyano group, R^5 is a chlorine or bromine atom or a trifluoromethyl
15 group, and R^6 is a chlorine atom.

20 A compound of the formula (1), wherein R^1 is an ethyl group, R^2 is a methyl group, R^3 is a chlorine or bromine atom or a methyl group, R^4 is a chlorine or bromine atom or a cyano group, R^5 is a chlorine or bromine atom or a trifluoromethyl
25 group, and R^6 is a chlorine atom.

Specific examples of the present compound include Compounds 1 to 26, in which R^1 to R^6 indicated in the formula
25 (1) are the groups shown in Table 1.

Table 1

Compound	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶
1	CH ₃ CH ₂	CH ₃	Br	Br	Br	Cl
2	CH ₃	CH ₃	CH ₃	Cl	Br	Cl
3	CH ₃	CH ₃	Br	Br	Br	Cl
4	(CH ₃) ₂ CH	CH ₃	Br	Br	Br	Cl
5	CH ₃	CH ₃	CH ₃	Cl	CF ₃	Cl
6	CH ₃	CH ₃	CH ₃	CN	Br	Cl
7	CH ₃	CH ₃	Cl	Cl	Br	Cl
8	CH ₃	CH ₃ CH ₂	Cl	Cl	Br	Cl
9	CH ₃ CH ₂	CH ₃	Cl	Cl	Br	Cl
10	CH ₃ (CH ₂) ₂	CH ₃	Br	Br	Br	Cl
11	CH ₃ CH ₂	CH ₃	CH ₃	Cl	Br	Cl
12	CH ₃ CH ₂	CH ₃	CH ₃	CN	Br	Cl
13	CH ₃ CH ₂	CH ₃	Br	Br	Cl	Cl
14	CH ₃ CH ₂	CH ₃	Cl	Cl	Cl	Cl
15	CH ₃ CH ₂	CH ₃	CH ₃	Cl	Cl	Cl
16	CH ₃ CH ₂	CH ₃	CH ₃	CN	Cl	Cl
17	CH ₃ CH ₂	CH ₃	Br	Br	CF ₃	Cl
18	CH ₃ CH ₂	CH ₃	Cl	Cl	CF ₃	Cl
19	CH ₃ CH ₂	CH ₃	CH ₃	Cl	CF ₃	Cl
20	CH ₃ CH ₂	CH ₃	CH ₃	CN	CF ₃	Cl
21	CH ₃	CH ₃	Br	Br	CF ₃	Cl
22	CH ₃	CH ₃	Br	Br	Cl	Cl
23	CH ₃	CH ₃	Cl	Cl	Cl	Cl
24	CH ₃	CH ₃	CH ₃	Cl	Cl	Cl
25	CH ₃	CH ₃	CH ₃	CN	Cl	Cl
26	CH ₃	CH ₃	Cl	Cl	CF ₃	Cl

When the present compound has at least one acidic group, the compound may be a salt with a base. Examples of such a salt include: metal salts such as alkali metal salts and alkaline earth metal salts (e.g., salts of sodium, potassium or magnesium); salts with ammonia; and salts with organic amines such as morpholine, piperidine, pyrrolidine, mono-lower

alkylamines, di-lower alkylamines, tri-lower alkylamines, monohydroxy lower alkylamines, dihydroxy lower alkylamines and trihydroxy lower alkylamines.

The present compound may have stereoisomers such as optical isomers based on the asymmetric carbon atoms, and isomers such as tautomers. In the present invention, any isomers may be contained and used, either alone or in any isomer ratio.

The present compounds are the compounds described in Published Japanese Patent Application No. 2007-182422. They can be produced according to the method described in WO 2008/12693.

When used in the method of the present invention, the present compound may be used alone or formulated with various inert components, as described below.

Examples of the solid carrier used in formulation include fine powders or granules such as minerals such as kaolin clay, attapulgite clay, bentonite, montmorillonite, acid white clay, pyrophyllite, talc, diatomaceous earth and calcite; natural organic materials such as corn rachis powder and walnut husk powder; synthetic organic materials such as urea; salts such as calcium carbonate and ammonium sulfate; and synthetic inorganic materials such as synthetic hydrated silicon oxide; and as a liquid carrier, aromatic hydrocarbons such as xylene, alkylbenzene and methylnaphthalene; alcohols

such as 2-propanol, ethylene glycol, propylene glycol, and ethylene glycol monoethyl ether; ketones such as acetone, cyclohexanone and isophorone; vegetable oil such as soybean oil and cotton seed oil; petroleum aliphatic hydrocarbons, esters, dimethylsulfoxide, acetonitrile and water.

Examples of the surfactant include anionic surfactants such as alkyl sulfate ester salts, alkylaryl sulfonate salts, dialkyl sulfosuccinate salts, polyoxyethylene alkylaryl ether phosphate ester salts, lignosulfonate salts and naphthalene sulfonate formaldehyde polycondensates; nonionic surfactants such as polyoxyethylene alkyl aryl ethers, polyoxyethylene alkylpolyoxypropylene block copolymers and sorbitan fatty acid esters; and cationic surfactants such as alkyltrimethylammonium salts.

Examples of the other formulation auxiliary agents include water-soluble polymers such as polyvinyl alcohol and polyvinylpyrrolidone, polysaccharides such as Arabic gum, alginic acid and the salt thereof, CMC (carboxymethyl-cellulose), Xanthan gum, inorganic materials such as aluminum magnesium silicate and alumina sol, preservatives, coloring agents and stabilization agents such as PAP (acid phosphate isopropyl) and BHT.

In general, the method of the present invention is carried out by applying an effective amount of the present compound to plants or their growing area. Examples of the

plants to be treated include foliage, shoots, flowers, fruits, panicles, seeds, bulbs, stem tubers, roots and seedlings. As used herein, bulbs mean discoid stem, corm, rhizoma, root tuber and rhizophore. In the present specification, the
5 seedlings include cutting and sugar cane stem cutting.

Examples of the growing sites of plants include soil before or after sowing plants.

When the present compound is applied to plants or growing sites of plants, the present compound is applied to
10 the target plants once or more.

Specifically, examples of the treatment in the method of the present invention include, for example, treatment of foliage, floral organs or panicles of plants, such as foliage
15 spraying; treatment of cultivation areas of plants, such as soil treatment; treatment of seeds, such as seed sterilization, soaking or coating; treatment of seedlings; and treatment of bulbs.

Specifically, examples of the treatments of foliage, floral organs or panicles of plants in the method of the present invention include treatment of the surface of plants, such as foliage spraying and trunk spraying. Also, examples of the treatments include spray treatment of floral organs or
20 entire plants in the flowering stage including before, during and after flowering. For crop plants and the like, the treatments include spray treatment of panicles or entire
25 plants in the heading stage.

Examples of the soil treatment method in the method of the present invention include spraying onto the soil, soil incorporation, and perfusion of a chemical liquid into the soil (irrigation of chemical liquid, soil injection, and dripping of chemical liquid). Examples of the place to be treated include planting hole, furrow, around a planting hole, around a furrow, entire surface of cultivation lands, the parts between the soil and the plant, area between roots, area beneath the trunk, main furrow, growing soil, seedling raising box, seedling raising tray and seedbed. Examples of the treating period include before seeding, at the time of seeding, immediately after seeding, raising period, before settled planting, at the time of settled planting, and growing period after settled planting. In the above soil treatment, two or more kinds of present compounds may be simultaneously applied to the plant, or a solid fertilizer such as a paste fertilizer containing the present compound may be applied to the soil. Also, the present compound may be mixed in an irrigation liquid, and, examples thereof include injecting to irrigation facilities (irrigation tube, irrigation pipe, sprinkler, etc.), mixing into the flooding liquid between furrows, mixing into a hydroponic medium and the like. Alternatively, an irrigation liquid may be mixed with the present compound in advance and, for example, used for treatment by an appropriate irrigating method including the irrigation method mentioned above and the other methods such as sprinkling and flooding. Alternatively, the present compound can be applied by winding a crop with a

resin formulation processed into a sheet or a string, putting a string of the resin formulation around a crop so that the crop is surrounded by the string, and/or laying a sheet of the resin formulation on the soil surface near the root of a crop.

5

Examples of the seed treatment in the method of the present invention includes treatment of seeds, bulbs and the like of plants to be protected from abiotic stress.

Specifically, the treatment includes a spraying treatment by which a suspension of the present compound is atomized to be
10 sprayed onto the surface of seeds or bulbs; a spreading treatment by which the present compound in the form of wettable powder, emulsion, a flowable agent or the like is applied, directly or after being added with a small amount of
15 water, onto seeds or bulbs; a soaking treatment in which seeds are soaked into a solution of the present compound for a certain period of time; a film coating treatment; and a pellet coating treatment.

20 Examples of the treatment of seedlings in the method of the present invention include spraying treatment of spraying to the entire seedlings a dilution having a proper concentration of active ingredients prepared by diluting the present compound with water, immersing treatment of immersing
25 seedlings in the dilution, and coating treatment of adhering the present compound formulated into a dust formulation to the entire seedlings. Examples of the method of treating the soil before or after sowing seedlings include a method of spraying

a dilution having a proper concentration of active ingredients prepared by diluting the present compound with water to seedlings or the soil around seedlings after sowing seedlings, and a method of spraying the present compound formulated into a solid formulation such as a granule to soil around seedlings after sowing seedlings.

When plants or growing areas of plants are treated with the present compound, the treatment amount generally can vary according to the kind of plants to be treated, the formulation form, the timing of the treatment and the weather conditions; the effective amount per 1,000 m² generally ranges from 0.1 g to 1,000 g, and preferably from 0.5 g to 50 g.

In such treatment, an emulsion, wettable powder, flowable agent, microcapsule or the like is generally diluted with water before application. In such applications, the concentration of present compound generally ranges from 1 ppm to 10,000 ppm, and preferably from 10 ppm to 300 ppm. Agents such as powders and granules are generally used directly without dilution.

In the treatment of seeds, the amount of the present compound is generally 1 g to 1,000 g, preferably 5 g to 500 g, and more preferably 250 g to 500 g per 100 kg of seeds.

In the treatment of seedlings, the amount of the present compound per seedling is generally 0.1 mg to 50 mg, preferably

0.5 mg to 50 mg. In the treatment of the soil before or after planting seedlings, the amount of the present compound per 1,000 m² is generally 0.1 g to 100 g, and preferably 0.5 g to 50 g.

5

In the treatment of culture solution, the concentration of the present compound in the culture solution is 0.1 ppm to 1,000 ppm, and preferably 5 ppm to 500 ppm.

10 The method of the present invention may be carried out either in agricultural land, such as upland field, paddy field, lawn, and orchards, or in non-agricultural land.

The plants in which temperature stress can be reduced by the present invention are exemplified as follows:

15

Crops: corn, rice, wheat, barley, rye, oat, sorghum, cotton, soybean, peanut, buckwheat, beet, oilseed rape, sunflower, sugar cane, tobacco, hop, etc.;

20 Vegetables: Solanaceous vegetables (eggplant, tomato, potato, pepper, sweet pepper, etc.), Cucurbitaceous vegetables (cucumber, pumpkin, zucchini, water melon, melon, oriental melon, etc.), Cruciferous vegetables (Japanese radish, turnip, horseradish, kohlrabi, Chinese cabbage, cabbage, brown
25 mustard, broccoli, cauliflower, etc.), Compositae vegetables (burdock, garland chrysanthemum, artichoke, lettuce, etc.), Liliaceous vegetables (Welsh onion, onion, garlic, asparagus, etc.), Umbelliferase vegetables (carrot, parsley, celery,

parsnip, etc.), Chenopodiaceous vegetables (spinach, Swiss chard, etc.), Labiatae vegetables (Japanese mint, mint, basil, etc.), Leguminous vegetables (pea, common bean, azuki bean, broad bean, Garbanzo bean, etc.), strawberry, sweet potato,
5 yam, aroid, Amorphophallus konjac, ginger, okra, etc.;

Fruits: pomaceous fruits (apple, pear, Japanese pear, Chinese quince, quince, etc.), stone fleshy fruits (peach, plum, nectarine, Prunus mume, cherry fruit, apricot, prune,
10 etc.), citrus fruits (Citrus unshiu, orange, lemon, lime, grapefruit, etc.), nuts (chestnuts, walnuts, hazelnuts, almond, pistachio, cashew nuts, macadamia nuts, etc.), berries (blueberry, cranberry, blackberry, raspberry, etc.), grape, kaki fruit, olive, Japanese plum, banana, coffee, date palm,
15 coconuts, oil palm, etc.;

Trees other than fruit trees: tea, mulberry, flowering trees (Rhododendron indicum, camellia, hydrangea, sasanqua, skimmia, cherry, tulip tree, crape myrtle, orange osmanthus,
20 etc.), street trees (ash tree, birch, dogwood, eucalyptus, ginkgo, lilac, maple tree, oak, poplar, cercis, Chinese sweet gum, plane tree, zelkova, Japanese arborvitae, fir tree, Japanese hemlock, needle juniper, pine, spruce, yew, elm, horse-chestnut, etc.), sweet viburnum, Podocarpus
25 macrophyllus, Japanese cedar, Japanese cypress, croton, spindle tree, Chinese hawthorn, etc.;

Grasses: Zoysia grasses (Japanese lawn grass, mascarene

grass, etc.), Bermuda grasses (*Cynodon dactylon*, etc.), bent
grasses (creeping bent grass, *Agrostis stolonifera*, *Agrostis*
tenuis, etc.), bluegrasses (Kentucky bluegrass, rough
bluegrass, etc.), fescues (tall fescue, Chewing fescue,
5 creeping fescue, etc.), ryegrasses (darnel, perennial grass,
etc.), orchard grass, timothy grass, etc.;

Other plants: ornamental flowers (rose, carnation,
chrysanthemum, *Eustoma grandiflorum* Shinners, gypsophila,
gerbera, pot marigold, salvia, petunia, verbena, tulip, aster,
10 gentian, lily, pansy, cyclamen, orchid, lily of the valley,
lavender, stock, ornamental cabbage, primula, poinsettia,
gladiolus, cattleya, daisy, cymbidium, begonia, etc.), biofuel
plants (*Jatropha*, safflower, camelina alyssum, switchgrass,
miscanthus, reed canary grass, *Arundo donax*, kenaf, cassava,
15 willow, etc.), foliage plants, etc.

Examples of the plants in which abiotic stress can be
reduced by the present invention preferably include rice, corn
and wheat.

20 The "plants" described above include plants to which
resistance to the following agents is conferred using a
classical breeding method or a genetic engineering technique:
4-hydroxyphenylpyruvate-dioxygenase inhibitors, such as
25 isoxaflutole; acetolactate synthetase (hereinafter referred to
as ALS) inhibitors, such as imazethapyr and thifensulfuron-
methyl; 5-enolpyruvylshikimate-3-phosphate synthase
(hereinafter referred to as EPSPS) inhibitors such as

glyphosate; glutamine synthetase inhibitors, such as
glufosinate; acetyl-CoA carboxylase inhibitors, such as
sethoxydim; protoporphyrinogen oxidase inhibitors, such as
flumioxazin; dicamba; auxin herbicides, such as 2,4-D; and
5 herbicides such as bromoxynil.

Examples of a "plant" on which resistance has been
conferred by a classical breeding method include oilseed rape,
wheat, sunflower and rice resistant to imidazolinone ALS
10 inhibitory herbicides such as imazethapyr, which are already
commercially available under a product name of Clearfield
(registered trademark). Similarly, there is soybean on which
resistance to sulfonylurea ALS inhibitory herbicides such as
thifensulfuron-methyl has been conferred by a classical
15 breeding method, which is already commercially available under
a product name of STS soybean. Similarly, examples on which
resistance to acetyl-CoA carboxylase inhibitors such as trione
oxime or aryloxy phenoxypropionic acid herbicides has been
conferred by a classical breeding method include SR corn.

20 The plant on which resistance to acetyl-CoA carboxylase
inhibitors has been conferred is described in Proceedings of
the National Academy of Sciences of the United States of
America (Proc. Natl. Acad. Sci. USA), vol. 87, pp. 7175-7179
(1990).

25 Examples of a "plant" on which resistance has been
conferred by genetic engineering technology include
glyphosate-resistant varieties of corn, soybean, cotton,

oilseed rape, and sugar beet that have an EPSPS inhibitor resistance EPSPS gene. These varieties are already commercially available under the product names of RoundupReady (registered trademark), Agrisure (registered trademark) GT, Gly-Tol, etc. Similarly, there are corn, soybean, cotton and oilseed rape varieties that are conferred with resistance to glufosinate by genetic engineering technology, and they are already commercially available under the product name of LibertyLink (registered trademark). Similarly, bromoxynil-resistant cotton generated by genetic engineering technology is already commercially available under the product name of BXN. Similarly, there are varieties of corn and soybean that are resistant to both glyphosate and ALS inhibitors, and they has been announced to enter the market under the name of Optimum (registered trademark) GAT (registered trademark). Also, an imazapyr-resistance soybean variety produced by genetic engineering has been announced to enter the market under the name of Cultivance (registered trademark).

20 A variation of acetyl-CoA carboxylase resistant to an acetyl-CoA carboxylase inhibitor is reported in Weed Science, vol. 53, pp. 728-746 (2005) and a plant resistant to acetyl-CoA carboxylase inhibitors can be generated by introducing a gene of such an acetyl-CoA carboxylase variation into a plant
25 by genetically engineering technology, or by introducing a variation conferring resistance into a plant acetyl-CoA carboxylase. Furthermore, plants resistant to acetyl-CoA carboxylase inhibitors or ALS inhibitors or the like can be

generated by introducing a site-directed amino acid substitution variation into an acetyl-CoA carboxylase gene or the ALS gene of the plant by introduction a nucleic acid into which has been introduced a base substitution variation

5 represented Chimeraplasty Technique (Gura T. 1999. Repairing the Genome's Spelling Mistakes. Science 285: 316-318) into a plant cell.

A crop plant, such as soybean, with resistance to
10 dicamba can be produced by introducing a gene encoding a dicamba-degrading enzyme, including dicamba monooxygenase isolated from *Pseudomonas maltophilia* (Behrens et al., "Dicamba Resistance: Enlarging and Preserving Biotechnology-Based Weed Management Strategies," Science 316: 1185-1188
15 (2007)).

A crop plant with resistance to both the phenoxy and aryloxyphenoxypropionic acid herbicide systems mentioned below by introducing a gene encoding aryloxyalkanoate dioxygenase: phenoxy herbicides, such as 2,4-D, MCPA, dichlorprop and
20 mecoprop, pyridine oxyacetic acids, such as fluroxypyr and triclopyr, and aryloxyphenoxypropionic acid herbicides, such as quizalofop-P-ethyl, haloxyfop-P-methyl, fluazifop-P-butyl, diclofop, fenoxaprop-P-ethyl, metamifop, cyhalofop-butyl and clodinafop-propargyl (WO 05/107437, WO 07/053482, WO
25 08/141154). The resultant crop plant is called a DHT crop plant.

A plant with resistance to HPPD inhibitors can be

produced by introducing a gene encoding HPPD which shows resistance to HPPD inhibitors (US2004/0058427). By introducing genes that allows for synthesis of homogentisic acid, which is produced from HPPD through another metabolic pathway even when HPPD is inhibited by an HPPD inhibitor, a plant resistant to HPPD inhibitors can be produced (WO 02/036787). A plant with resistance to HPPD inhibitors can be produced by introducing a gene for overexpressing HPPD so that HPPD is produced at a level sufficient for plant growth even in the presence of HPPD inhibitors (WO 96/38567). By introducing a gene encoding prephenate dehydrogenase to increase the production of p-hydroxyphenylpyruvate, a substrate of HPPD, in addition to the introduction of a gene for overexpressing HPPD, a plant with resistance to HPPD inhibitors can be produced (Rippert P et al., "Engineering plant shikimate pathway for production of tocotrienol and improving herbicide resistance," Plant Physiol. 134: 92-100 (2004)).

The "plants" described above include plants to which resistance to nematodes and aphids is conferred using a classical breeding method. Examples of such plants includes the soybean plant into which the RAG1 (Resistance Aphid Gene 1) gene, which confers aphid resistance, is introduced.

The aforementioned "plants" include genetically engineered plants produced using such genetic engineering techniques, which, for example, are able to synthesize selective toxins as known in genus *Bacillus*.

Examples of toxins expressed in such genetically engineered plants include: insecticidal proteins derived from *Bacillus cereus* or *Bacillus popilliae*; δ -endotoxins such as Cry1Ab, Cry1Ac, Cry1F, Cry1Fa2, Cry2Ab, Cry3A, Cry3Bb1 or Cry9C, derived from *Bacillus thuringiensis*; insecticidal proteins such as VIP1, VIP2, VIP3, or VIP3A; insecticidal proteins derived from nematodes; toxins generated by animals, such as scorpion toxin, spider toxin, bee toxin, or insect-specific neurotoxins; mold fungi toxins; plant lectin; agglutinin; protease inhibitors such as a trypsin inhibitor, a serine protease inhibitor, patatin, cystatin, or a papain inhibitor; ribosome-inactivating proteins (RIP) such as lycine, corn-RIP, abrin, luffin, saporin, or briodin; steroid-metabolizing enzymes such as 3-hydroxysteroid oxidase, ecdysteroid-UDP-glucosyl transferase, or cholesterol oxidase; an ecdysone inhibitor; HMG-COA reductase; ion channel inhibitors such as a sodium channel inhibitor or calcium channel inhibitor; juvenile hormone esterase; a diuretic hormone receptor; stilbene synthase; bibenzyl synthase; chitinase; and glucanase.

Examples of toxins expressed in such genetically engineered plants also include: hybrid toxins of δ -endotoxin proteins such as Cry1Ab, Cry1Ac, Cry1F, Cry1Fa2, Cry2Ab, Cry3A, Cry3Bb1, Cry9C, Cry34Ab or Cry35Ab and insecticidal proteins such as VIP1, VIP2, VIP3 or VIP3A; partially deleted toxins; and modified toxins. Such hybrid toxins are produced from a

new combination of the different domains of such proteins, using a genetic engineering technique. As a partially deleted toxin, Cry1Ab comprising a deletion of a portion of an amino acid sequence has been known. A modified toxin is produced by substitution of one or multiple amino acids of natural toxins.

Examples of such toxins and genetically engineered plants capable of synthesizing such toxins are described in EP-A-0 374 753, WO 93/07278, WO 95/34656, EP-A-0 427 529, EP-A-451 878, WO 03/052073, etc.

Toxins contained in such genetically engineered plants are able to confer resistance particularly to insect pests belonging to Coleoptera, Hemiptera, Diptera, Lepidoptera and Nematodes, to the plants.

Genetically engineered plants, which comprise one or multiple insecticidal pest-resistant genes and which express one or multiple toxins, have already been known, and some of such genetically engineered plants have already been on the market. Examples of such genetically engineered plants include YieldGard (registered trademark) (a corn variety for expressing Cry1Ab toxin), YieldGard Rootworm (registered trademark) (a corn variety for expressing Cry3Bb1 toxin), YieldGard Plus (registered trademark) (a corn variety for expressing Cry1Ab and Cry3Bb1 toxins), Herculex I (registered trademark) (a corn variety for expressing phosphinotricine N-acetyl transferase (PAT) so as to confer resistance to Cry1Fa2

toxin and glufosinate), NuCOTN33B (registered trademark) (a cotton variety for expressing Cry1Ac toxin), Bollgard I (registered trademark) (a cotton variety for expressing Cry1Ac toxin), Bollgard II (registered trademark) (a cotton variety for expressing Cry1Ac and Cry2Ab toxins), VIPCOT (registered trademark) (a cotton variety for expressing VIP toxin), NewLeaf (registered trademark) (a potato variety for expressing Cry3A toxin), NatureGard (registered trademark) Agrisure (registered trademark) GT Advantage (GA21 glyphosate-resistant trait), Agrisure (registered trademark) CB Advantage (Bt11 corn borer (CB) trait), and Protecta (registered trademark).

The aforementioned "plants" also include plants produced using a genetic engineering technique, which have ability to generate antipathogenic substances having selective action.

A PR protein and the like have been known as such antipathogenic substances (PRPs, EP-A-0 392 225). Such antipathogenic substances and genetically engineered plants that generate them are described in EP-A-0 392 225, WO 95/33818, EP-A-0 353 191, etc.

Examples of such antipathogenic substances expressed in genetically engineered plants include: ion channel inhibitors such as a sodium channel inhibitor or a calcium channel inhibitor (KP1, KP4 and KP6 toxins, etc., which are produced by viruses, have been known); stilbene synthase; bibenzyl synthase; chitinase; glucanase; a PR protein; and

antipathogenic substances generated by microorganisms, such as a peptide antibiotic, an antibiotic having a hetero ring, a protein factor associated with resistance to plant diseases (which is called a plant disease-resistant gene and is described in WO 03/000906). These antipathogenic substances and genetically engineered plants producing such substances are described in EP-A-0392225, WO95/33818, EP-A-0353191, etc. A recombinant papaya variety produced by introducing the coat protein gene of papaya ringspot virus (PRSV) is already commercially available under the product name of Rainbow Papaya (registered trademark).

The "plant" mentioned above includes plants on which advantageous characters such as characters improved in oil stuff ingredients or characters having reinforced amino acid content have been conferred by genetically engineering technology. Examples thereof include VISTIVE (registered trademark) (low linolenic soybean having reduced linolenic content) or high-lysine (high-oil) corn (corn with increased lysine or oil content).

Stack varieties are also included in which a plurality of advantageous characters such as the classic herbicide characters mentioned above or herbicide tolerance genes, harmful insect resistance genes, antipathogenic substance producing genes, characters improved in oil stuff ingredients or characters having reinforced amino acid content are combined.

Examples

While the present invention will be more specifically described by way of formulation examples, seed treatment
5 examples, and test examples in the following, the present invention is not limited to the following examples. In the following examples, the part represents part by weight unless otherwise specified.

10 Formulation Example 1

An emulsion is obtained by fully mixing 3.75 parts of any one of compounds 1 to 26, as shown in Table 1, 14 parts of polyoxyethylene styrylphenyl ether, 6 parts of calcium dodecyl benzene sulfonate and 76.25 parts of xylene.

15

Formulation Example 2

A wet-pulverized slurry is obtained by mixing 75 parts of any one of compounds 1 to 26, as shown in Table 1, 15 parts of propylene glycol (Nacalai Tesque), 15 parts of Soprophor
20 FLK (Rhodia Nicca), 0.6 parts of Antifoam C Emulsion (Dow Corning) and 120 parts of ion exchange water, followed by wet-pulverization of the slurry. A thickener solution is obtained by mixing 0.3 parts of Kelzan S (Kelco), 0.6 parts of Veegum granules (R.T. Vanderbilt) and 0.6 parts of Proxel GXL
25 (Archchemicals) with 72.9 parts of ion exchange water. A flowable formulation is obtained by mixing 75.2 parts of the wet-pulverized slurry and 24.8 parts of the thickener solution.

Formulation Example 3

Fifteen (15) parts of any one of compounds 1 to 26, as shown in Table 1, 1.5 parts of sorbitan trioleate and 28.5 parts of an aqueous solution containing 2 parts of polyvinyl alcohol are mixed and pulverized by wet-pulverization. To the pulverized mixture, 45 parts of an aqueous solution containing 0.05 parts of Xanthan gum and 0.1 parts of aluminum magnesium silicate is added, and 10 parts of propylene glycol is further added, and then mixed while stirring, thereby obtaining a flowable formulation.

Formulation Example 4

A bulk slurry is prepared by mixing 45 parts of any one of compounds 1 to 26, as shown in Table 1, 5 parts of propylene glycol (Nacalai Tesque), 5 parts of Soprophor FLK (Rhodia Nicca), 0.2 parts of Antifoam C Emulsion (Dow Corning), 0.3 parts of proxel GXL (Arch Chemicals) and 49.5 parts of ion exchange water. Into 100 parts of the slurry, 150 parts of glass beads (diameter = 1 mm) are placed to grind the slurry for 2 hours while cooling with cooling water. After the grinding, the glass beads are removed by filtration, thereby obtaining a flowable formulation.

Formulation Example 5

An AI premix is obtained by mixing 50.5 parts of any one of compounds 1 to 26, as shown in Table 1, 38.5 parts of NN kaolin clay (Takehara Chemical Industrial), 10 parts of Morwet

D425 and 1.5 parts of Morwer EFW (Akzo Nobel). This premix is ground with a jet mill to obtain powder formulations.

Formulation Example 6

5 A granule formulation is obtained by fully milling and mixing 5 parts of any one of compounds 1 to 26, as shown in Table 1, 1 part of synthetic hydrated silicon oxide, 2 parts of calcium lignin sulfonate, 30 parts of bentonite and 62 parts of kaolin clay, and then fully kneading the mixture with
10 adding water, followed by granulation and drying of the mixture.

Formulation Example 7

15 A powder formulation is obtained by fully milling and mixing 3 parts of any one of compounds 1 to 26, as shown in Table 1, 87 parts of kaolin clay and 10 parts of talc.

Formulation Example 8

20 A wettable powder is obtained by 22 parts of any one of compounds 1 to 26, as shown in Table 1, 3 parts of calcium lignin sulfonate, 2 parts of sodium lauryl sulfate and 73 parts of synthetic hydrated silicon oxide.

Seed Treatment Example 1

25 Treated seeds are obtained by dressing 10-kg dried seeds of oilseed rape with 120 ml of a flowable formulation prepared according to Formulation Example 2 or 3 using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

Seed Treatment Example 2

Treated seeds are obtained by dressing 10 kg of dried corn seeds with 200 ml of a flowable formulation prepared according to Formulation Example 3 using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

Seed Treatment Example 3

A mixture is prepared by mixing 5 parts of a flowable formulation prepared according to Formulation Example 4, 5 parts of pigment BPD6135 (Sun Chemical) and 35 parts of water. Treated seeds are obtained by dressing 10 kg of dried cottonseeds with 600 ml of the mixture using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

Seed Treatment Example 4

Treated seeds are obtained by powder coating 10 kg of dried corn seeds with 50 g of a powder formulation prepared according to Formulation Example 5.

Seed Treatment Example 5

Treated seeds are obtained by powder coating 100 kg of dried rice seeds with 9 kg of a powder formulation prepared according to Formulation Example 7.

Seed Treatment Example 6

Treated seeds are obtained by dressing 10 kg of dried soybean seeds with 180 ml of a flowable formulation prepared

according to Formulation Example 2 using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

Seed Treatment Example 7

5 Treated seeds are obtained by dressing 10 kg of dried wheat seeds with 180 ml of a flowable formulation prepared according to Formulation Example 3 using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

10 Seed Treatment Example 8

 Treated seeds are obtained by dressing 10 kg of dried sunflower seeds with 600 ml of a mixture of 5 parts of a flowable formulation prepared according to Formulation Example 4, 5 parts of pigment BPD6135 (Sun Chemical) and 35 parts of
15 water using a rotary seed treatment machine (seed dresser, Hans-Ulrich Hege GmbH).

Seed Treatment Example 9

 Treated seeds are obtained by powder coating 10 kg of
20 dried sugar beet seeds with 50 g of a powder formulation prepared according to Formulation Example 5.

Application Example 1

 Treated seeds are obtained by dressing 10 kg of potato
25 tuber pieces with 1,000 ml of a mixture of 5 parts of a flowable formulation prepared according to Formulation Example 4, 5 parts of pigment BPD6135 (Sun Chemical) and 35 parts of water using a rotary seed treatment machine (seed dresser,

Hans-Ulrich Hege GmbH).

Test Example 1: Evaluation Test for reduction of Low-Temperature Stress by Corn Seed Treatment (Plant weight)
5 (Test plants)

Corn (cultivar: Kuromochi)

(Seed treatment)

A blank slurry solution containing 5% (V/V) color coat red (Becker Underwood, Inc.), 5% (V/V) CF-Clear (Becker
10 Underwood, Inc.) and 0.4% Maxim XL (Syngenta) is prepared. Slurry solutions are prepared by dissolving Compound 1 or 6, as shown in Table 1, in the blank slurry solution so that 100 kg of corn seeds (cultivar: Kuromochi) are treated with 250-1,000 g of Compound 1 or 6. In a 50-ml centrifuge tube (BD
15 Japan), 0.48 ml of the slurry solution is placed for each 20 kg of corn seeds (cultivar: Kuromochi) and stirred until the solution is dried, thereby coating the seeds. As a control, seeds coated with the blank slurry solution are used as the untreated seeds.

20 (Low-temperature treatment)

Two treated corn seeds were sown in the culture soil (AISAI) in each plastic pot (55 mm in diameter × 58 mm in length), grown for 10 days under the following conditions and subjected to the test: temperature, 27°C; illuminance, about
25 5,000 lux; day length, 16 hours.

The pots at day 10 after the sowing were placed in a phytotron under the following conditions in order to expose the plants to low-temperature stress for 7 days: temperature,

3 ± 2°C; day length, 16 hours; illuminance, about 5,000 lux; humidity, 35 to 80%.

(Evaluation process)

After the low-temperature treatment, the plants were grown for another 4 days under the following conditions: temperature, 27°C; humidity of 50 to 75%, illuminance, about 5,000 lux; day length, 16 hours. Then the fresh weight of the above-ground part of the plants was measured. The experiment was performed in four replications and the average weight per individual was calculated.

As a result, the reduction in the fresh weight of the above-ground part caused by the low-temperature stress was reduced in the plots treated with Compound (1) in an amount of 250 g and 500 g per 100 kg of the seeds, as compared with the untreated plot.

Table 2

	Amount of Compound (g/10 kg seeds)	Average fresh weight of above- ground part (g)	Relative value (%)
Compound 1	0 (Untreated plot)	1.70	100
	250	2.09	123
	500	2.46	145

Test Example 2: Evaluation Test for Reduction of Low-Temperature Stress Immediately after the Germination by Corn Seed Treatment (Plant weight)
(Test plants)
Corn (cultivar: Kuromochi)
(Seed treatment)

A blank slurry solution containing 5% (V/V) color coat red (Becker Underwood, Inc.), 5% (V/V) CF-Clear (Becker Underwood, Inc.) and 0.4% Maxim XL (Syngenta) is prepared.

Slurry solutions are prepared by dissolving Compound 1
5 or 6, as shown in Table 1, in the blank slurry solution so that 100 kg of corn seeds (cultivar: Kuromochi) are treated with 250 g to 1,000 g of Compound 1 or 6. In a 50-ml centrifuge tube (BD Japan), 0.48 ml of the slurry solution is placed for each 20 kg of corn seeds (cultivar: Kuromochi) and
10 stirred until the solution is dried, thereby coating the seeds. As a control, seeds coated with the blank slurry solution are used as the untreated seeds.

(Low-temperature treatment)

Two treated corn seeds were sown in the culture soil
15 (AISAI) in each plastic pot (55 mm in diameter × 58 mm in length), grown for 4 days under the following conditions and subjected to the test: temperature, 27°C; illuminance, about 5,000 lux; day length, 16 hours.

20 The pots at day 4 after the sowing were placed in a phytotron under the following conditions in order to expose the plants to low-temperature stress for 7 days: temperature, $3 \pm 2^\circ\text{C}$; day length, 16 hours; illuminance, about 5,000 lux; humidity, 35 to 80%.

25 (Evaluation process)

After the low-temperature treatment, the plants were grown for another 7 days under the following conditions: temperature, 27°C; humidity of 50 to 75%, illuminance, about

5,000 lux; day length, 16 hours. Then the fresh weight of the above-ground part of the plants was measured. The experiment was performed in eight replications and the average weight per individual was calculated.

5 As a result, the reduction in the fresh weight of the above-ground part caused by the low-temperature stress was alleviated in the plots treated with Compounds 1 and 6, as compared with the untreated plot.

10 Table 3

	Amount of Compound (g/10 kg seeds)	Average fresh weight of above- ground part (g)	Relative value (%)
Compound 1	0 (Untreated plot)	1.09	100
	500	1.22	111
Compound 6	250	1.17	107

Test Example 3: Evaluation Test for Reduction of Drought
Stress by Rice Seed Treatment (Plant Weight)
(Seed treatment)

15 A blank slurry solution containing 5% (V/V) color coat
red (Becker Underwood, Inc.), 5% (V/V) CF-Clear (Becker
Underwood, Inc.) and 0.4% Maxim XL (Syngenta) was prepared.
Slurry solutions were prepared by dissolving Compound 1 or 6,
as shown in Table 1, in the blank slurry solution so that 100
20 kg are treated with 250 g to 1,000 g of Compound 1 or 6. In a
50-ml centrifuge tube (BD Japan), 0.48 ml of the slurry
solution was placed for each 20 kg of rice seeds (cultivar:
Nipponbare) and stirred until the solution was dried, thereby
coating the seeds. As a control, seeds coated with the blank

slurry solution are used as the untreated seeds.

Also, as a control, seeds coated with the blank slurry solution in place of the slurry solution described above were used as untreated seeds.

5 (Test plants)

On the wells of a 406-well plug plate, filter paper was placed and rice seeds subjected to the above seed treatment were sown on the filter paper. A two-fold diluted Kimura B water culture solution (Plant Science 119: 39-47 (1996)), was
10 used to grow the rice for 14 days under the following conditions: temperature, 28°C/23°C (day/night); illuminance of 8,500 lux; day length, 12 hours. The plants were then subjected to the test.

(Drought Stress Treatment and Recovery Treatment)

15 Each 5 seedlings of the test plants were placed in an empty 35 ml flat-bottomed test tube (ASSIST/ Sarstedt) and allowed to stand uncovered for 2 days, in order to apply drought stress. As a test plot without the drought stress, each 5 seedlings of the test plants were placed in a
20 centrifuge tube filled with 10 ml of a two-fold diluted Kimura B water culture solution, and allowed to stand uncovered for 2 days. After the incubation for 2 days, each 5 seedlings of the plants were transplanted into a plastic pot (N-71-130G, TOKAN KOGYO) filled with sterilized field and allowed to grow
25 for 14 with bottom-watering under the following: temperature, 28°C/23°C (day/night), illuminance, 8,500 Lux; day length, 12 hours.

(Evaluation)

After the drought stress treatment, the fresh weight of the above-ground part of the five individuals of the test plants in each test plot was collectively measured and the weight of each plot was determined. The results are shown in Table 4. The results show that the drought stress was reduced in the test plots for the present invention since the fresh weight of the above-ground part of the test plots is apparently larger than that of the untreated plot.

10 Table 4

Present Compound	Amount of Compound (g/10 kg seeds)	Fresh weight of above-ground part (g/5 plants)	Percentage to untreated plot (%)
None (Untreated plot)	0	0.79	100
Compound 1	250	0.89	113
Compound 6	250	0.86	110

Test Example 4: Evaluation Test for Reduction of Drought Stress by Rice Seed Treatment (Plant Weight)
(Test plants) Wheat (cultivar: Apogee)
15 (Seed treatment)

A blank slurry solution containing 5% (V/V) color coat red (Becker Underwood, Inc.), 5% (V/V) CF-Clear (Becker Underwood, Inc.) and 0.4% Maxim XL (Syngenta) is prepared. Slurry solutions are prepared by dissolving any one of
20 Compound 1 to 26, as shown in Table 1, in the blank slurry solution so that 1 g of seeds (cultivar: Apogee) are treated with 0.05 to 0.25 mg of the compound. The seeds are coated by mixing the seeds with 1.3 ml of the slurry solution per 50 g of the wheat seeds using a rotary seed treatment machine (seed

dresser, Hans-Ulrich Hege GmbH), and then the seeds are spread and dried. As a control, seeds coated with the blank slurry solution are used as the untreated seeds. Five of the coated wheat seeds are sown in the culture soil (AISAI) in each plastic pot (55 mm in diameter × 58 mm in length), and they are grown for 3 weeks at 18°C. The plants are thinned to select 3 seedlings showing good growth.

(High-temperature stress treatment)

At week 3 after the sowing, the plants are grown for 7 days under the following conditions to treat them with high-temperature stress: temperature, 36°C (day)/32°C (night), humidity, 60 to 70%; illuminance, about 6,000 lux; day length 12 hours. Subsequently, the plants are grown for a week under the following conditions: temperature, 18°C; illuminance, about 6,000 lux; day length 16 hours.

(Evaluation process)

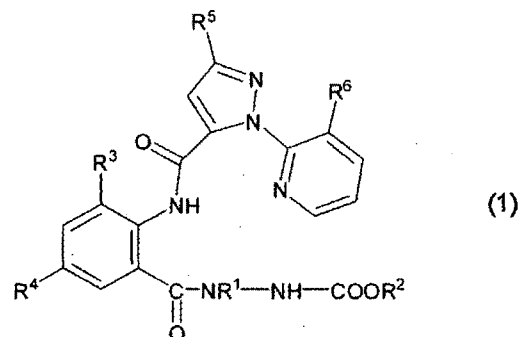
The fresh weight of the above-ground part of the test plants is then examined in 8 replications. Alleviation of reduction in the fresh weight of the above-ground part is observed in the present-compound-treated plots as compared with the untreated plot.

Industrial Applicability

Use of the method of the present invention allows for reduction of abiotic stress.

CLAIMS

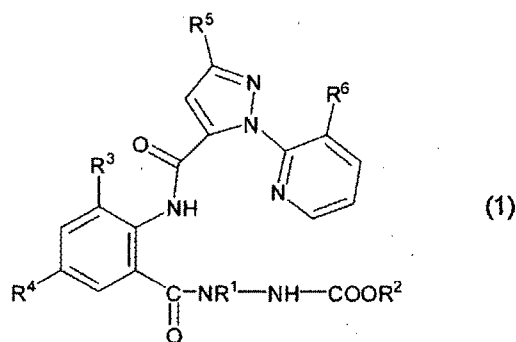
1. A method for reducing abiotic stress in plants,
comprising treating a plant that has been or is to be exposed
5 to abiotic stress with an effective amount of a compound
represented by the following formula (1):



wherein R¹ represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R² represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkenyl group which may be substituted with one or more halogen atoms, or a C3-C6 alkynyl group which may be substituted with one or more halogen atoms; R³ represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R⁴ represents a hydrogen atom, a halogen atom, a cyano group, or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R⁵ represents a hydrogen atom, a halogen atom, a cyano group, a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C1-C6 alkoxy group which may be substituted with one or more halogen atoms, a C1-C6 alkylthio group which may be substituted with one or

more halogen atoms, a C1-C6 alkylsulfinyl group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms; and R⁶ represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms.

2. The method of claim 1, wherein the compound represented by the formula (1) is a compound selected from the following compound group A:



wherein, the compound group A consists of:

(1) a compound of the formula (1) in which R¹ is an ethyl group, R² is a methyl group, R³ is a bromo atom, R⁴ is a bromo atom, R⁵ is a bromo atom, and R⁶ is a chloro atom; and

(2) a compound of the formula (1) in which R¹ is a methyl group, R² is a methyl group, R³ is a methyl group, R⁴ is a cyano group, R⁵ is a bromo atom, and R⁶ is a chloroatom.

3. The method of claim 1 or 2, wherein the treatment is spraying treatment, soil treatment, seed treatment or hydroponic treatment.

4. The method of any one of claims 1 to 3, wherein the treatment is seed treatment which is to treat seeds with 5 g or more and 500 g or less of the compound per 100 kg of seeds.

5 5. The method of any one of claims 1 to 3, wherein the treatment is seed treatment which is to treat seeds with 250 g or more and 500 g or less of the compound per 100 kg of seeds.

6. The method of any one of claims 1 to 5, wherein the
10 plant is rice, corn or wheat.

7. The method of any one of claims 1 to 6, wherein the plant is a transgenic plant.

15 8. The method of any one of claims 1 to 7, wherein the abiotic stress is low-temperature stress.

9. The method of any one of claims 1 to 7, wherein the abiotic stress is drought stress.

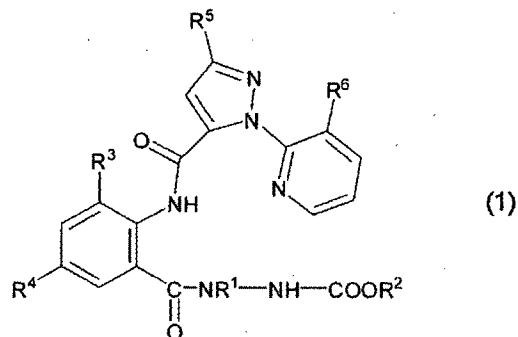
20 10. The method of any one of claims 1 to 9, wherein the reduction of the abiotic stress in plants is indicated by a change in at least one of the following plant phenotypes:

<Plant phenotypes>

- 25 (1) Germination rate,
(2) Seedling establishment rate,
(3) Leaf mortality rate,
(4) Plant length,

- (5) Plant weight,
 (6) Leaf area,
 (7) Leaf color,
 (8) Number or weight of seeds or fruits,
 5 (9) Quality of harvested products,
 (10) Rates of flower setting, fruit setting, seed setting and seed filling,
 (11) Chlorophyll fluorescence yield,
 (12) Water content,
 10 (13) Leaf surface temperature, and
 (14) Transpiration capacity.

11. Use of an effective amount of a compound represented by the following formula (1):



15 wherein R^1 represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R^2 represents a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkoxyalkyl group which may be substituted with one or more halogen atoms, a C3-C6 alkenyl group which may be substituted with one or more halogen atoms, or a C3-C6 alkynyl group which may be substituted with one or more halogen atoms; R^3 represents a halogen atom or a C1-C6

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alkyl group which may be substituted with one or more halogen atoms; R⁴ represents a hydrogen atom, a halogen atom, a cyano group or a C1-C6 alkyl group which may be substituted with one or more halogen atoms; R⁵ represents a hydrogen atom, a
5 halogen atom, a cyano group, a C1-C6 alkyl group which may be substituted with one or more halogen atoms, a C1-C6 alkoxy group which may be substituted with one or more halogen atoms, a C1-C6 alkylthio group which may be substituted with one or more halogen atoms, a C1-C6 alkylsulfinyl group which may be
10 substituted with one or more halogen atoms, a C1-C6 alkylsulfonyl group which may be substituted with one or more halogen atoms; and R⁶ represents a halogen atom or a C1-C6 alkyl group which may be substituted with one or more halogen atoms;
15 for reducing abiotic stress in plants.

12. The use of claim 11, wherein the reduction of the abiotic stress in plants is indicated by a change in at least one of the following plant phenotypes:

20 <Plant phenotypes>

- (1) Germination rate,
- (2) Seedling establishment rate,
- (3) Leaf mortality rate,
- (4) Plant length,
- 25 (5) Plant weight,
- (6) Leaf area,
- (7) Leaf color,
- (8) Number or weight of seeds or fruits,

- (9) Quality of harvested products,
- (10) Rates of flower setting, fruit setting, seed setting and seed filling,
- (11) Chlorophyll fluorescence yield,
- 5 (12) Water content,
- (13) Leaf surface temperature, and
- (14) Transpiration capacity.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/059948

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. A01N47/24 (2006.01) i, A01N25/00 (2006.01) i, A01P21/00 (2006.01) i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. A01N47/24, A01N25/00, A01P21/00

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

Published examined utility model applications of Japan 1922-1996
 Published unexamined utility model applications of Japan 1971-2012
 Registered utility model specifications of Japan 1996-2012
 Published registered utility model applications of Japan 1994-2012

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 2010-265200 A (Sumitomo Chemical Co., Ltd.) 2010.11.25, Claims, Table1(compound 1, compound 6) & WO 2010/131770 A1	1-12
Y	JP 2010-275202 A (Sumitomo Chemical Co., Ltd.) 2010.12.09, Claims, Table1(compound 2, compound 8) & WO 2010/137677 A1	1-12
Y	JP 2010-222342 A (Sumitomo Chemical Co., Ltd.) 2010.10.07, Claims, Table1(compound 2, compound 8) & KR 10-2011-0131193 A & WO 2010/098489 A2	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

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

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

27.06.2012

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/059948

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 2010-222343 A (Sumitomo Chemical Co., Ltd.) 2010.10.07, Claims, Table1(compound 2, compound 8) & KR 10-2011-0132341 A & WO 2010/098494 A2	1-12
Y	WO 2008/126858 A1 (Sumitomo Chemical Co., Ltd.) 2008.10.23, Claims, [0223], Table1(compound 3-2, compound 3-8) & CN 101715446 A & EP 2143720 A1 & JP 2008-280336 A & KR 10-2009-0127918 A & US 2010/0113794 A1	1-12
Y	JP 2008-280335 A (Sumitomo Chemical Co., Ltd.) 2008.11.20, Claims, Table1(compound 3-2, compound 3-8) & CN 101679352 A & KR 10-2009-0129462 A & US 2010/0120866 A1 & WO 2008/126933 A2	1-12
Y	JP 2010-510193 A (BASF SE) 2010.04.02, Claims, [0118] & CN 101541178 A & US 2010/0095396 A1 & WO 2008/059054 A2	1-12