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(54) Title: USE OF PYRIMIDINES IN STIMULATION OF PLANT GROWTH AND DEVELOPMENT AND ENHANCEMENT OF STRESS TOLERANCE

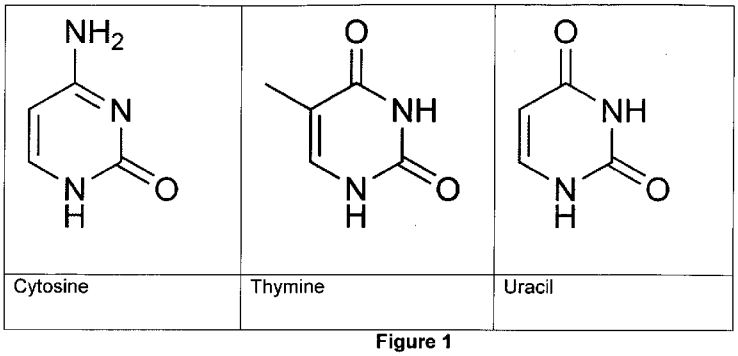

Figure 1

(57) Abstract: The present invention is related with the use of pyrimidines, especially uridine and cytidine chemicals of pyrimidines in stimulation of plant growth and development, as well as use of pyrimidines espacially uridine in enhancement of stress tolerance, reduction of stress, repair of stress-related injury and inhibition of stress, and the methods thereof.

DESCRIPTION

USE OF PYRIMIDINES IN STIMULATION OF PLANT GROWTH AND DEVELOPMENT AND ENHANCEMENT OF STRESS TOLERANCE

The Related Art

The invention relates to use of pyrimidine molecules in the sector of agriculture.

The invention particularly relates to the use of said pyrimidine molecules in promoting growth and development of plants and increasing their tolerances against biotic and abiotic stress factors.

The Prior Art

Various internal and external factors, especially their genetic structures, are effective on growth and development of plants. External factors can be listed as temperature, light, soil etc. environmental or ecological factors. Internal factors comprise hormone, carbohydrate, lipid, enzyme, and secondary metabolites etc. all of the biochemical molecules synthesized within the plant. Almost all of these substances are natural or organic substances that can be produced within the plant, can be transferred from the site of production to other parts of the plant where they are needed, and can be effective even in very low amounts. Among these substances, which are required for various physiological phases and metabolisms of plants, especially the impact of hormones is of significance and specific effects of species or genera are determined and their new effects are being discovered day by day. These substances are formed of growth promoters (auxins, cytokinines, and gibberellins), growth preventers (abscisic acid), ethylene, which is the hormone in the form of a gas that is synthesized in connection with maturation or aging. Besides these ones, studies about the use of brassinosteroids, salicylic acid, jasmonic acid, and polyamines are also present, which are also recently obtained from plants and their hormonal effects being proven. Therefore, these substances are frequently applied externally with the purpose of control and management of growth and development of plants. Synthetic commercial productions of these natural substances that are synthesized from plants are used in external applications. Since obtaining of natural hormones synthesized in the plant via purification is a very difficult, inefficient, and costly process, substances with similar characteristics can be produced synthetically. Except ethylene, the chemical structures of these hormones that are produced synthetically are not same with the natural plant hormones. However, they may have the same or similar effect when they are administered.

Use of these substances in agriculture is subject to pesticide applications and requires registration and authorization by the Ministry of Food, Agriculture and Livestock. Therefore, all kinds of research and trials of these substances that are synthetically produced by the companies are made in an extremely sensitive manner so that they can have place in practical use according

to the results of these research and trials. The impacts of the hormones on the plants can vary according to the concentration of application, time of application, the physiological stage in which the plant is found, the age of the plant, the type and the genus of the plant, the member of the plant, and the ecological condition of the plant at the time of administration. Therefore, it is of great importance to follow the recommended authorized instructions in use of these substances in control and management of growth and development of plants. While these substances that are synthetically produced and used can cause harmful effects on the plants in case of overdose or faulty application situations, they may also pose threat on food safety by means of leaving residue in the food.

Although stress is physically defined as force applied to unit area, in biological terms, it is defined as the impact of an external factor on an organism (Levitt, 1980). Stress factors affecting agricultural production are classified as biotic and abiotic factors. While patogens, microorganisms, weeds, insects etc. are evaluated as biotic stress factors; temperature, drought, radiation, salinity, plant nutrients, light, flood, mechanical impacts (wind, snow and ice mantle), air pollution, toxins etc. environmental factors are defined as abiotic stress.

Abiotic stress conditions, besides negatively affecting growth and development of plants, also cause increase in lose of efficiency more than 50% in fundamental products (Wang et. al., 2004). Various external and internal factors, especially the genetic structures are effective in tolerance of plants against abiotic stresses and defence mechanisms in physiological and molecular levels play role against these problems.

Stress causes some physiological, biochemical, and molecular changes in plant metabolism (Levitt 1980). In plants, most of the changes that occur during adapting to high temperatures are reversible. However, if the magnitude of the stress is high, changes that are irreversible may occur and may cause death of the plant.

Environmental factors of a region significantly affects the growth of plant types or kinds and the most important one among these factors is the temperature. Temperature stress recently increasing together with global warming causes efficiency and dry substance ratio losses especially in moderate climate regions (Levitt 1980, Giaveno and Ferrero 2003, Wahid et al. 2007). Human activities cause increase of carbondioxide, methane, chlorofluorocarbon, and nitrogen oxide etc. greenhouse gas concentrations found in the atmosphere, which contributes to global warming (Wahid et. al. 2007). According to IPCC (Intergovernmental Panel on Climatic Change) 2012 report; global temperature is expected to increase at around 1-3 °C towards the mid-21st century, while it is expected to increase 2-5 °C until the end of the 21st century.

Temperature stress is generally defined as the increase of temperature above the threshold for a certain time that causes irreversible damages in plant growth and development. Temporary

increase in environmental temperature around 10-15 °C is defined as temperature shock or temperature stress. However, temperature stress occurs according to density (degree temperature), time period, and rate of increase of temperature (Eriş 2003).

Cellular damages are caused by the presence of reactive oxygen derivatives (ROS) occurring due to oxidative stress triggered by temperature (Kumar et. al. 2007). Superoxide ($O2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), which are known as ROS, are formed as a result of interaction of metabolism with oxygen. ROS inhibit the enzymes and have harmful effect on important cellular components and their production is significantly increased under high stress conditions (McKersie and Lehsem 1994). Plants and other organisms have developed various mechanisms in order to reduce and repair the damages caused by ROS. Molecular defence mechanism of plants is a part of environmental stress factors and enables gaining stress tolerance (Peet and Willits 1998).

For instance; under stress conditions, various plant types accumulate various osmolites such as sugars and sugar alcohols (poliols), proline, tertiary, and quaternary ammonium compounds, and tertiary sulfonium compounds (Sairam and Tyagi 2004). Hormones such as abscisic acid (ABA) and ethylene (C_2H_4) regulate various physiological events by means of triggering signal molecules during stress (Larkindale and Huang 2005). Moreover, high temperature stress causes accumulation of phenolic compounds, which are the most significant secondary metabolites taking part in tolerance against abiotic stress in plants (Wahid and Ghazanfar 2006, Wahid 2007). Another defence system developed by plants against stress is the antioxidant defence system (Foyer et. al. 1994). The antioxidant defence system of plants is formed of antioxidant molecule and enzymes (Alscher et. al. 1997). By means of complex cooperation of enzymatic and non-enzymatic antioxidants, control of ROS concentrations and repairment of oxidative damages are possible (Smirnoff 2005).

Another one of the defence systems taking part in abiotic stress tolerance is the synthesis of stress proteins. Protection of protein structures and functions under stress conditions is very important for survival of the cell (Wang et. al. 2004). Temperature stress also has negative impacts on the protein structure and activity (Wery et. al. 1993). It is found that normal cellular proteins are reduced, whereas heat shock proteins (HSP) are increased when plants are exposed to high temperature. HSP's are responsible for protein folding, mounting, translocation, and destruction in various normal cellular process and prevent re-folding and denaturation of proteins under stress conditions (Hartl 1996, Boston et. al. 1996, Wang et. al. 2004).

Exposure to extreme temperature changes, salinity, drought etc. environmental stresses leads to lack of water in plant tissue. Under these conditions, plants try to survive by synthesizing and accumulating various osmolites (or osmoprotectants) in order to be able to prevent the loss of the water found in their cells (Williamson et. al. 2002). Metabolic regulation made by means of

accumulation of various organic substances is a fundamental strategy for survival of plants and their protection against extreme environmental conditions. Besides their ability of regulating the osmotic pressure of cellular cytoplasm under stress conditions such as freezing, drought etc., these metabolites also stabilize the cellular membranes and proteins (Bohnert and Jensen 1996; McNeil et. al. 1999). Osmolites are generally found in stable state within the cell, can not be metabolized easily, and do not have any toxic effect against cellular functions even when they are accumulated in high concentrations (Charron et. al. 2002, Peel et. al. 2009). Therefore, they are important for adaptation of plant cells against various negative environmental conditions (Yancey 1994).

Among the quaternary ammonium compounds of osmolites responding to dehydration stresses in plants, the most commonly known is glycine betain (GlyBet) (Venkatesan and Chellappan 1998, Mansour 2000, Mohanty et. al. 2002, Yang et. al. 2003). With the accumulation of these compounds, low water potential occurs in the cell and in this case water enters into cell. There are various studies about the healing effects of GlyBet on the damages formed at cellular membranes and proteins due to stress (Brady et. al., 1984; Paleg et. al., 1984; Arakawa and Timasheff, 1985; Incharoensakdi et. al., 1986; Ashihara et. al., 1997; Mansour 1998).

As disclosed above, following determination of the internal roles of GlyBet under stress conditions for various plant types, studies have been made showing that external administration of this molecule is also effective. It is shown that external GlyBet administrations increase low temperature stress tolerance in plant species such as *Arabidopsis thaliana, Solanum tuberosum, Fragaria x ananassa, Medicago sativa, Triticum aestivum, Zea mays* etc. (Zhao et. al. 1992, Somersalo et al. 1996, Allard et. al. 1998, Sakamoto and Murata 2000, WeiBing and Rajashekar 2001, Xing and Rajashekar 2001, Park et. al. 2003, Park et. al. 2006). For example, it is shown that external administration of GlyBet increases tolerance against frost in cabbage (Sakai and Yoshida 1968) and clover (Zhao et. al. 1992).

In external administration of GlyBet, it can easily be taken from the leaves (Park et. al. 2006). For instance, it is found that big portion of GlyBet administered on the leaves of tomato plant is absorbed by the leaves and transferred to cytosol (Park et. al. 2006). It is reported that radioactive-labelled GlyBet is transferred from the leaves of turnip plant (Brassica rapa L.) to the roots within 2 hours. In the study, it is also reported that, after 24 hours, all members of the plant carry glycine betain (Makela et. al. 1996). In tomato plant, following application of GlyBet through the leaf, it is found to be accumulated in all meristamatic cells comprising sprout and shoot of the flower. In this study, it is found that GlyBey is transported to the actively growing and developing regions via phloem (Park et al. 2003).

As one of the substances used in external applications for tolerance against stress, spermidine, among the polyamine class, is shown to increase high temperature tolerance in tomato at 4 mM concentration (Murkowski 2001).

Besides these, the abscisic acid (ABA) and the jasmonic acid among the hormones for increasing stress tolerance are known to have protective effects in cellular base. However, these substances are not commonly used in agricultural production. Moreover, since the protective effect of the plant nutrient copper against stress is known, external applications of copper-containing preparations have practical significance in commercial applications. These preparations are commonly used in agricultural production since they are inexpensive and easy to apply. However, since these preparations are applied on the plants via spraying, washed by rain water and thus accumulate in soil and water sources, they have the potential to cause environmental pollution. Therefore, there is a need for alternative strategies to improve stress tolerance in plants. In this context, pyrimidines, having proven effects against cellular damages in animal organisms, are believed to have potential protecting, stress tolerance-increasing, and stress supressing etc. effects also in plants.

Pyrimidines are heterocyclic organic aromatic compounds having chemically similar characteristics with benzene and pyridine. The pyrimidine nucleosides uridine, cytidine, and thymidine and their phosphate-bound nucleotide forms are normally found in the body and take part in various physiological functions. Among these, the functions which have been studied especially well are the ones that are related to the roles they take in glycogen metabolism and nucleic acid synthesis.

Although there is no example about plantal application of pyrimidine compounds, a patent about increasing of growth hormones, especially auxin synthesis by means of uridine extracts has been encountered (WO1997000614A1). In this study, it is found that, uridine, which is one of the substances formed as a result of degradation of cellular structures in culture medium, increases cellular regeneration (compared to only using auxin in culture medium) by means of increasing the effect and/or synthesis of auxin.

Polyamine extracts obtained from plants have been reported to prevent stress in plants (EP2486920A1). In this invention, a patent document is mentioned (Japanese Unexamined Patent Application Publication No. 2005-330213) wherein the stress-preventive effect of uridine is examined in relation to nucleic acids.

A patent is published about providing strength against stress together with growth and increase in efficiency by means of synthesis of enzyme in relation to cellulose and transfer of the DNA index controlling this to a plant cell through bacteria (CA2264957A1). Here, in synthesis of enzymes related to cellulose, the precursor is emphasized to be uridine diphosphate glucose (UDP-

glucose). As a result of culture studies, presence of uridine diphosphate glucose is stated to be indirectly important in terms of growth, efficiency, and stress tolerance in plants.

However, in above given patents, no findings have been encountered in relation with the effects of pyrimidine compounds of the present invention under stress conditions.

PATENT REFERENCES

Patent cited	File Admission Date	Publication Date	Owner of Application	Title
WO1997000614A1	24 Jun 1996	09 Jan 1997	Instituut Voor Agrobiologisch	Influencing the activity of plant growth regulators
EP2486920A1	28 Sep 2009	15 Aug 2012	Toyo Boseki Kabushiki Kaisha	Stress-alleviating agent comprising plant-derived polyamine-containing extract as active ingredient
CA2264957A1	09 Sep 1997	19 Mar 1998	B.C. Research Inc.	A process of increasing plant growth and yield and modifying cellulose production in plants

Chemical Structures of Pyrimidine Compounds

Pyrimidine compounds are heterocyclic organic aromatic compounds chemically similar to benzene and pyridine, and carry one each nitrogen atom at the positions of 1 and 3 of the 6-membered chemical ring. Pyrimidines are found in base, nucleoside, and nucleotide forms.

Pyrimidine bases are principally included in the structure of DNA and RNA. While cytosine is found in both DNA and RNA structure, thymine is only found in DNA and uracil is only found in RNA structure. Chemical structure of pyrimidine bases are given in Figure 1.

Figure 1 shows the chemical structures of pyrimidine bases. Pyrimidine nucleosides are formed by addition of a sugar molecule in the form of ribose or 2-deoxyribose to pyrimidine bases. In this reaction, the carbon no 1 of the sugar molecule is combined with the nitrogen no 1 of the pyrimidine base. The pyrimidine nucleoside formed is defined by means of adding "-idine" suffix at the end of the base name (for instance uridine). When ribose is added to bases, the nucleosides formed do not have any suffix, but when 2-deoxyribose is added, the prefix "d-" is added before the name. Chemical structures of pyrimidine nucleosides are given in Figure 2.

Figure 2 shows the chemical structures of pyrimidine nucleosides. Pyrimidine nucleotides are formed by addition of phosphate groups to nucleosides. The number of phosphate groups added determines the name of that nucleotide; and the prefixes mono-, di-, or tri- phosphate are added to the nucleoside name when one, two, or three phosphate groups are added, respectively. The nucleotides formed when three phosphate groups are added to pyrimidine nucleosides are shown in Figure 3 (In Figure 3, the chemical structures of the pyrimidine nucleotides comprising three phosphate group are given).

The Purpose of the Invention

The present invention relates to use of pyrimidine compounds in promoting growth and development of plants and increasing their stress tolerances, which meets above said requirements, eliminates some of the drawbacks, and brings about some additional advantages.

The primary purpose of the invention is to provide the uridine and cytidine chemicals with the purpose of promoting the growth and development of plants.

A purpose of the invention is to prevent harm on growth of plants by use of said uridine or cytidine chemicals in appropriate doses, prevent formation of carcinogenic affect on humans, since it is naturally found in human body, and be nontoxic.

Another purpose of the invention is to provide easy storage, since it is durable against decomposition at room temperature in powder form.

In order to achieve above said purposes, the invention comprises use of uridine or cytidine chemicals in promotion of plant growth and development.

In order to achieve the purposes of the invention, said uridine or cytidine solution is prepared between 10^{-9} – 1 molar.

In order to achieve the purposes of the invention, said solution comprises 0,000243– 243000 mg uridine in 1 litre of water.

In order to achieve the purposes of the invention, said solution comprises 0,000244– 244000 mg uridine in 1 litre of water.

In order to achieve the purposes of the invention, said uridine or cytidine solution is preferably prepared between 10^{-6} to 10^{-4} molar.

In order to achieve the purposes of the invention, said solution comprises 0,243 – 24,3 mg cytidine in 1 litre of water.

In order to achieve the purposes of the invention, said solution comprises 0,244 – 24,4 mg uridine 1 litre of water.

In order to achieve the purposes of the invention, sowing is made in a way that 1 seed would be present in each viol cell.

In order to achieve the purposes of the invention, said germination is made at 25 °C.

In order to achieve the purposes of the invention, plants are kept under light at 24 °C and 22 °C for 16 hours per day and kept under dark conditions at 20 °C for 8 hours.

The primary purpose of the invention is to use uridine for increasing the stress tolerances of plants.

A purpose of the invention is to prevent harm on growth of plants by use of said uridine chemical in appropriate doses, prevent formation of carcinogenic affect on humans, since it is naturally found in human body, and be nontoxic.

Another purpose of the invention is to provide easy storage of said chemical, since it is durable against decomposition at room temperature in powder form.

In order to achieve above said purposes, the invention comprises use of uridine chemical in promotion of plant growth and development.

In order to achieve the purposes of the invention, said uridine solution is prepared between 10^{-9} – 1 molar.

In order to achieve the purposes of the invention, said solution comprises 0,000244– 244000 mg uridine in 1 litre of water.

In order to achieve the purposes of the invention, said uridine solution is preferably prepared between 10^{-5} to 10^{-4} molar.

In order to achieve the purposes of the invention, said solution comprises 0,244 – 24,4 mg uridine 1 litre of water.

In order to achieve the purposes of the invention, sowing is made in a way that 1 seed would be present in each viol cell.

In order to achieve the purposes of the invention, said germination is made at 25 °C.

In order to achieve the purposes of the invention, plants are kept under light at 24 °C and 22 °C for 16 hours per day and kept under dark conditions at 20 °C for 8 hours.

For high temperature stress applications, the temperature of the growth cabinet is increased gradually to 35, 40, and 45 °C and kept for 24 hours at each temperature level.

Following application of 45 °C, total amount of soluble protein is measured in the leaf samples taken from the plants.

Figures for Better Understanding of the Invention

Figure 1 shows the chemical structures of pyrimidine bases.

Figure 2 shows the chemical structures of pyrimidine nucleosides.

Figure 3 shows the chemical structures of pyrimidine nucleotides comprising three phosphate groups.

Figure 4: is the graphical view showing the effect of the 10 µM concentration solution prepared from uridine (A) or cytidine (B) molecule of the invention on the hypocotyl height of the seedlings.

Figure 5: is the graphical view showing the effect of the 10 µM concentration solution prepared from uridine (A) or cytidine (B) molecule of the invention on the epicotyl height of the seedlings.

Figure 6: is the graphical view showing the effect of the 10 µM concentration solution prepared from uridine (A) or cytidine (B) molecule of the invention on the plant height of the seedlings.

Figure 7: is the graphical view showing the effect of the 10 µM concentration solution prepared from uridine (A) or cytidine (B) molecule of the invention on the 1st actual leaf area of the seedlings.

Figure 8: is the graphical view showing the effect of the 10 µM concentration solution prepared from uridine (A) or cytidine (B) molecule of the invention on the 2nd actual leaf area of the seedlings.

Figure 9: is the view of the parts of a plant.

Figure 10 shows the total soluble protein amounts in control cucumber plants (high temperature and no uridine application), plants with application of only uridine at various concentrations, and plants with application of uridine and high temperature stress (45 °C) together.

Figure 11 is view of control plants (high temperature and no uridine application), plants with application of only uridine (A) and plants with and without application of uridine (B) under high temperature stress (45 °C).

Detailed Description of the Invention

In this detailed description, the preferred embodiments of the use of pyrimidine compounds of the invention in promoting growth and development of plants and increasing their stress tolerances are described for better understanding of the invention without forming any limiting effect.

The invention relates to use of uridine and cytidine chemicals and other pyrimidine compounds in agriculture sector; especially for promoting growth and development of plants and increasing their stress tolerances.

Pyrimidine is the general name of nitrous aromatic bases generally found in nucleic acids and also in some coenzymes and vitamins.

The most basic pyrimidine structure is $C_4H_4N_2$ and the pyrimidines are the derivatives of this main structure.

Three pyrimidine bases (cytozine, thymine, and uracil) are found in biologic systems. Uracil is only found in ribonucleic acid (RNA), thymine in deoxyribonucleic acid (DNA), and cytozine in both DNA and RNA. The shapes and sizes of pyrimidines and also the ability of forming hydrogen bonds with the purines provide the three-dimensional structures and the biological functions of nucleic acids.

Besides their uracil, cytozine, and thymine base forms, pyrimidine compounds can have the structure of nucleoside such as uridine, cytidine, and thymidine, respectively, which are formed by addition of a ribose ring to these bases through beta-N-glycosidic bond; deoxy nucleoside structure such as deoxyuridine, deoxycytidine, and deoxythymidine, respectively, which are formed by addition of a deoxyribose ring to these bases through beta-N-glycosidic bond; nucleotide structure such as uridine-5'-monophosphate, uridine-5'-diphosphate, uridine-5'-triphosphate, cytidine-5'-monophosphate, cytidine-5'-diphosphate, cytidine-5'-triphosphate, thymidine-5'-monophosphate, thymidine-5'-diphosphate, thymidine-5'-triphosphate, which are the one-, two-, or three-phosphate added forms of these nucleosides; deoxy forms of these nucleotides; and structures such as cytidine-5'-diphosphate choline, cytidine-5'-diphosphate ethanolamine, uridine-adenosine tetraphosphate, which are the structures wherein choline, ethanolamine, adenosine etc. are added to these nucleotides.

Cytidine is a pyrimidine nucleoside. It is found in plant (Ross, 1965) and animal (Traut, 1994) tissues. In plants, it takes part in synthesis of cytidine-5'-diphosphate (CDP) and cytidine-5'-triphosphate (CTP) (Ross and Cole, 1968). It is included in the structure of RNA in the same ratio with uridine (Ross and Cole, 1968). In addition, following deamination reaction in plants, some part of the cytidine is transformed into uridine (Ross and Cole, 1968). While cytidine is the major pyrimidine in blood circulation of rats (Traut, 1994), in human blood circulation the major pyrimidine is uridine (Wurtman et. al., 2000). Moreover, as in the plants, also in humans, cytidine provided externally to the body is quickly transformed into uridine as a result of deamination

(Wurtman et. al., 2000). Cytidine is transformed into CTP and cytidine-5'-diphosphate choline (CDP-choline) through Kennedy pathway and thus takes part in membrane phospholipid synthesis (Kennedy and Weiss, 1956).

CDP-choline, which is derived from cytidine, is studied extensively in terms of its neuroprotective effects in animal experiments and some clinical studies. CDP-choline reduces damage in hypoxic and ischemic brain injuries and improves the learning and memory functions which are impaired with aging (Secades, 2011). With these features, it is suggested to be useful as neuroprotective in cases of stroke, traumatic brain injury, and Alzheimer disease (Secades, 2011).

Uridine is a pyrimidine nucleoside and a constituent of plant (Ross, 1965) and animal (Pelling, 1959) tissues. Uridine is also the constituent of nucleotides comprising mono-(uridine-5'-monophosphate [UMP]), di-(uridine-5'-diphosphate [UDP]) and tri-phosphate (uridine-5'-triphosphate [UTP]), nucleotide sugars (UDP-glucose and UDP-galactose) (Ross and Cole, 1968) and phospholipid intermediate metabolites (Kennedy and Weiss, 1956) cytidine-5'-triphosphate (CTP) (Genchev and Mandel, 1974) and cytidine-5'-diphosphate choline (CDP-choline) (Cansev et. al., 2005) compounds. Uridine plays role in various physiological functions such as glycogen and phospholipid biosynthesis and protein and lipid glycosylation (Lecca and Ceruti, 2008). RNA synthesis has a vital role in plant growth and development (Oota, 1964) and experimental disintegration of RNA affects growth and development (Brachet, 1954). Membrane phospholipids are also the most important components of cell membranes and cell growth and reproduction are associated with the increase of membrane phospholipid synthesis in both plants (Xue et. al., 2009) and animal cells (Bashir et. al., 1992) and tissues (Wurtman et. al., 2009). It is also shown that UMP, which is a source of uridine, is transformed into uridine after entering into body and reaches the brain (Cansev et. al., 2005) and improves phospholipid production (Wurtman et. al., 2006) or neuron branching and thus neural communication in infant (Cansev et. al., 2009) or adult (Sakamoto et. al., 2007) experimental animals. The uridine added to the neurons in the culture also increases the growth and branching of these cells (Pooler et. al., 2005). With above said characteristics, uridine treatment is found to increase learning and memory functions in experimental animals (Teather and Wurtman, 2006; Holguin et. al., 2008a) and also in environmentally impoverished animals (Holguin et. al., 2008b). In addition, uridine reduces brain damage of laboratory animals in experimental models. For instance, in experimental Parkinson model, uridine administered in the form of UMP ameliorated brain lesion and reduced rotational behaviour, which is the typical indication of damage (Cansev et. al., 2008). Moreover, uridine treatment significantly reduced the level of damage in infant rats, which are exposed to hypoxic ischemic brain damage (Cansev et. al., 2013). Prevention of programmed cell death (apoptosis) mechanism of brain cells by uridine mediated to this effect (Cansev et. al., 2013).

In the prior art, when uridine is used on humans; it is known that it causes diarrhea when it is taken in high oral dosage such as 10 g per day (van Groeningen et. al., 1991) and the dose of 10 g/m^2 administered intravenously is known to cause shaking (Leyva et. al., 1984).

Experiment 1: Promotion of plant growth and development through administration of uridine or cytidine

In the present invention, said uridine or cytidine chemical is used on plants.

Amount of Uridine Chemical Usage in the Invention:

RAW MATERIAL	PREFERRED AMOUNT (gr)	USABLE AMOUNT (gr)
Uridine	10^{-5} – 10^{-4} molar (1 – 100 micromolar) : 2,44 –24,4 mg	10^{-9} – 1 molar (1 nanomolar – 1 molar) : 0,000244– 244000 mg
Water	1 litre	1 litre

Said uridine is administered on cucumber (Cucumis sativus) plants in the preferred embodiment of the invention.

Amount of Cytidine Chemical Usage in the Invention:

RAW MATERIAL	PREFERRED AMOUNT (gr)	USABLE AMOUNT (gr)
Cytidine	10^{-6} – 10^{-4} molar (1 – 100 micromolar) : 0,243 –24,3 mg	10^{-9} – 1 molar (1 nanomolar – 1 molar) : 0,000243– 243000 mg
Water	1 litre	1 litre

Said cytidine is administered on cucumber (Cucumis sativus) plants in the preferred embodiment of the invention.

- Their sowing is preferably made into vials of 72 such that 1 seed would be present per vial.
- Said seeds are germinated at 25 °C in plant growth cabin.

- Following the stage of germination, 10 ml of uridine or cytidine solution prepared at 10 or 100 µM concentration is administered to the plants twice a week.
- Water is used as dissolver in order to dissolve uridine or cytidine (it is preferably dissolved in pure water and at room temperature).

Preparation of Uridine Solution:

For the usable amount of the invention:

- In order to prepare 10^{-9} M (1 nano molar) solution; 0,000244 mg uridine is dissolved in 1 L of water.
- In order to prepare 1 M (1 molar) solution; 244000 mg uridine is dissolved in 1 L of water.

For the preffered amount of the invention:

- In order to prepare 10 µM solution; 2,44 mg uridine is dissolved in 1 L of water.
- In order to prepare 100 µM solution; 24,4 mg uridine is dissolved in 1 L of water.

- Uridine solution should be prepared fresh for each administration.

Preparation of Cytidine Solution:

For the usable amount of the invention:

- In order to prepare 10^{-9} M (1 nano molar) solution; 0,000243 mg cytidine is dissolved in 1 L of water.
- In order to prepare 1 M (1 molar) solution; 243000 mg cytidine is dissolved in 1 L of water.

For the preffered amount of the invention:

- In order to prepare 10 µM solution; 2,43 mg cytidine is dissolved in 1 L of water.
- In order to prepare 100 µM solution; 24,3 mg cytidine is dissolved in 1 L of water.

- Cytidine solution should be prepared fresh for each administration.

Plants are grown under light for 16 hours at 24 °C and 22 °C and under dark conditions for 8 hours at 20 °C daily in growth cabinet for 3 weeks until they have 2 actual leaves.
Afterwards, measurement of plant parts are made as shown in Figure 9 with below given details:

1. Hypocotyl Height (mm)
2. Epycotyl Height (mm)
3. Plant Height (mm)
4. First Actual leaf area (mm2)
5. Second Actual leaf area (mm2)
6. Cotyledon leaves

Experiment 2: Increase of temperature stress tolerance of plants having uridine chemical administration

In the present invention, said uridine chemical is used on plants.

The Amount of Uridine Chemical Usage in the Invention:

RAW MATERIAL	PREFERRED AMOUNT (gr)	USABLE AMOUNT (gr)
Uridine	10^{-5} – 10^{-4} molar (1 – 100 micromolar) : 2,44 –24,4 mg	10^{-9} – 1 molar (1 nanomolar – 1 molar) : 0,000244– 244000 mg
Water	1 litre	1 litre

Said uridine is administered on cucumber (Cucumis sativus) plants in the preferred embodiment of the invention.

- Their sowing is preferably made into vials of 72 such that 1 seed would be present per vial.
- Said seeds are germinated at 25 °C in plant growth cabin.
- Following the stage of germination, 10 ml of uridine solution prepared at 10 or 100 µM concentration is administered to the plants twice a week.
- Water is used as dissolver in order to dissolve uridine (it is preferably dissolved in pure water and at room temperature).

Preparation of Uridine Solution:

For the usable amount of the invention:

- In order to prepare 10^{-9} M (1 nano molar) solution; 0,000244 mg uridine is dissolved in 1 L of water.
- In order to prepare 1 M (1 molar) solution; 244000 mg uridine is dissolved in 1 L of water.

For the Preferred Amount of the Invention:

- In order to prepare 10 µM solution; 2,44 mg uridine is dissolved in 1 L of water.
- In order to prepare 100 µM solution; 24,4 mg uridine is dissolved in 1 L of water.
- Uridine solution should be prepared fresh for each administration.
- Plants are grown under light for 16 hours at 24 °C and 22 °C and under dark conditions for 8 hours at 20 °C daily in growth cabinet for 3 weeks until they have 2 actual leaves.
- For high temperature stress applications, the temperature of the growth cabin is increased gradually to 35, 40, and 45 °C and kept for 24 hours at each temperature level.
- Following application of 45 °C, total amount of soluble protein is measured in the leaf samples taken from the plants.
- Total soluble protein extraction is made by using the method of Arora et. al. (1992, 1997) with some modifications suggested by Gulen and Eris (2003).

  Solution components used in total soluble protein extraction are:

  • 50 mM Borax (Sodium tetraborate)

  • 50 mM ascorbic acid

  • 1 mM PMSF (Phenylmethylsulphonyl)

  • %1 β-mecaptoethanol

- 5 ml of the extraction solution prepared as given above is taken and homogenized together with 1 g of leaf sample in mortar. Homogenized samples are taken into 15 ml centrifuge tubes and centrifuged for 1,5 hours at 26 000 g and 4 °C. Following centrifuge, the above liquid phase is taken and passed through 0,22 µm diameter filters.

- Total soluble protein amount is determined according to Bradford (1976) method as proposed by Arora and Wisniewski (1994). The amount of protein in the supernatant obtained from the protein extraction is determined according to spectrophotometric measurements. Measurements are made by using single use polycarbonate basins at 595 nm wavelength and 0, 10, 20, 30, 40, 50 µg/µl BSA (Bovine serum albumin) standards are used for calculating total soluble protein amount. BSA stock solution is prepared as 5 mg BSA/ml extraction solution.

In a preferred embodiment of the invention; uridine solutions can be used in the form of application to the soil together with liquid fertilizers at certain ratios (liquid fertilizer components).

In a preferred embodiment of the invention; uridine solutions can be used by being added to the plant nutrient components in soilless agriculture applications (plant nutrient components).

In another preferred embodiment of the invention; uridine solutions can be used by being buried into soil after tabletting with suitable filling materials (tablet composition).

In another preferred embodiment of the invention; uridine solutions can be used together with irrigation water in drip irrigation system (drip irrigation).

In another preferred embodiment of the invention; uridine solution can be used in the form of coating by being sprayed onto leaves of plants and to fruits (spraying).

The effects of uridine on plants depends on concentration of application (application dose and frequency) and also application time, the physiological stage of the plant, age of the plant, species or type of the plant, member of the plant and the ecological conditions at the time of application (temperature, light, moisture, wind, soil etc.). As disclosed in the purpose and the method of application of the invention, the applications can be made when the plants are at the seed phase, plantlet, sapling, seedling, trees at the period of yield etc. different physiological and morphological phases. Moreover, it can also be applied on perennial, annual, herbaceous, ligneous, deciduous, evergreen etc. all plant types.

As a pyrimidine nucleoside, cytidine can also cause similar impacts with uridine on stress tolerances of plants, since both some part of it is transformed to uridine in plants and also it uses common pathways (e.g. Kennedy pathway) with uridine during its metabolism. The impacts of cytidine on the plants depends on concentration of application (application dose and frequency) and also application time, the physiological stage of the plant, age of the plant, species or type of the plant, member of the plant and the ecological conditions at the time of application (temperature, light, moisture, wind, soil etc.). As disclosed in the purpose and the method of application of the invention, the applications can be made when the plants are at the seed phase, plantlet, sapling, seedling, trees at the period of yield etc. different physiological and

morphological phases. Moreover, it can also be applied on perennial, annual, herbaceous, ligneous, deciduous, evergreen etc. all plant types.

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CLAIMS

1. The invention is characterized with the use of pyrimidine-containing compounds in increasing stress tolerances of plants.

2. Compounds according to claim 1 containing pyrimidines for increasing stress tolerances of plants, and it is characterized in that; it comprises use of uridine among said pyrimidine compounds.

3. Compounds according to claim 1 containing pyrimidines for increasing stress tolerances of plants, and it is characterized in that; it comprises use of cytidine among said pyrimidine compounds.

4. The invention is characterized with the use of pyrimidine- containing compounds in promoting growth and development of plants.

5. Use according to claim 4 in promoting growth and development of plants, and it is characterized in that; it comprises use of uridine among said pyrimidine compounds.

6. Use according to claim 4 in promoting growth and development of plants, and it is characterized in that; it comprises use of cytidine among said pyrimidine compounds.

7. Compounds according to claims 1 to 6 containing pyrimidines for increasing stress tolerances of plants, and it is characterized in that; it comprises use together with at least one or a few of the group consisting of cytidine, thymidine; uracil, cytozine, and thymine, which are the base forms of these nucleosides; uridine-5'-monophosphate, uridine-5'-diphosphate, uridine-5'-triphosphate, cytidine-5'-monophosphate, cytidine-5'-diphosphate, cytidine-5'-triphosphate, thymidine-5'-monophosphate, thymidine-5'-diphosphate, thymidine-5'-triphosphate, which are the one-, two-, or three-phosphate-added forms of these nucleosides; and cytidine-5'-diphosphate choline, cytidine-5'-diphosphate ethanolamine, uridine-adenosine tetraphosphate, wherein choline, ethanolamine, adenosine etc. structures are added to nucleotides.

8. Use according to any one of the previous claims, and it is characterized in that; said solution is prepared between 10^{-9} – 1 molar.

9. Use according to any one of the previous claims, and it is characterized in that; said solution comprises 0,000244– 244000 mg uridine in 1 litre of water.

10. Use according to any one of the previous claims, and it is characterized in that; said uridine solution is preferably prepared between 10^{-5} to 10^{-4} molar.

11. Use according to any one of the previous claims, and it is characterized in that; said solution comprises 2,44 – 24,4 mg uridine in 1 litre of water.

12. Use according to any one of the previous claims, and it is characterized in that; said solution is applied on the soil together with liquid manner.

13. Use according to any one of the previous claims, and it is characterized in that; said solution is applied in soilless agriculture by means of being added to plant nutrients.

14. Use according to any one of the previous claims, and it is characterized in that; said solution is applied by being buried into soil after tabletting with filler materials.

15. Use according to any one of the previous claims, and it is characterized in that; said solution is applied together with irrigation water in drip irrigation system.

16. Use according to any one of the previous claims, and it is characterized in that; said solution is applied in the form of spraying.

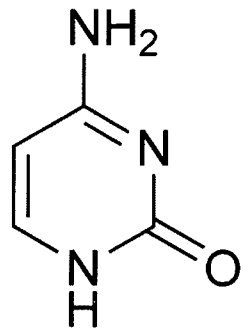	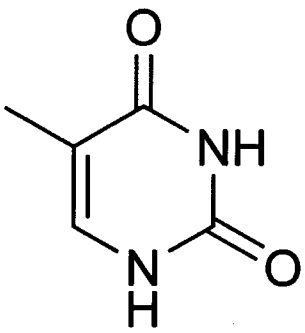	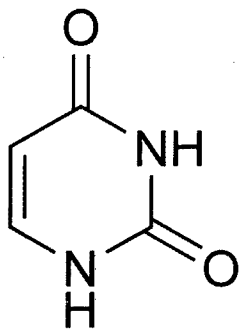
Cytosine	Thymine	Uracil

Figure 1

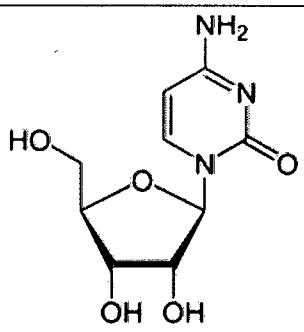	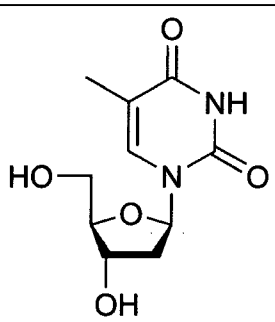	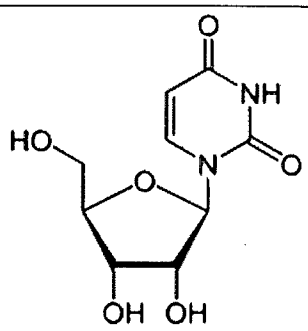
Cytidine	Thymidine	Uridine

Figure 2

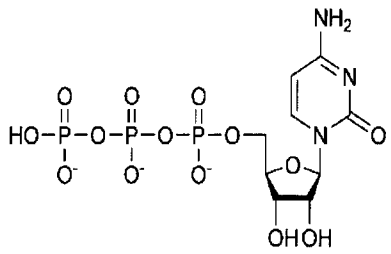	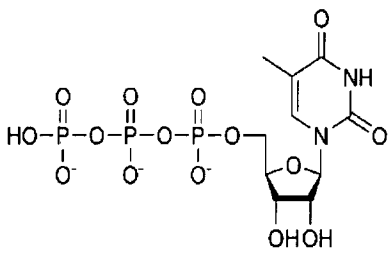	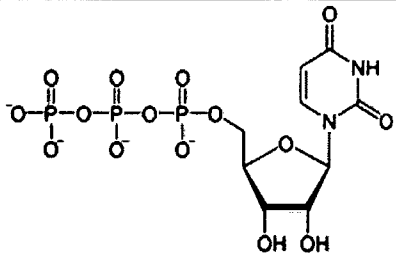
Cytidine-5'-triphosphate (CTP)	Thymidine-5'-triphosphate (TTP)	Uridine-5'-triphosphate (UTP)

Figure 3

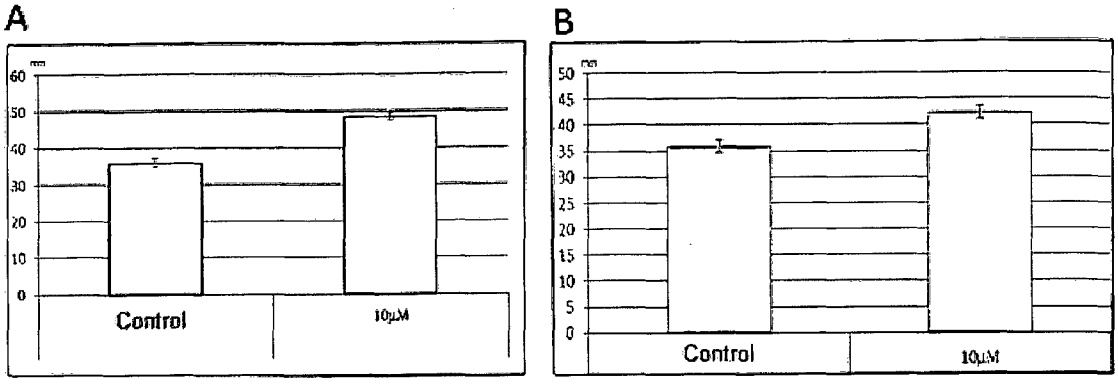

Figure 4

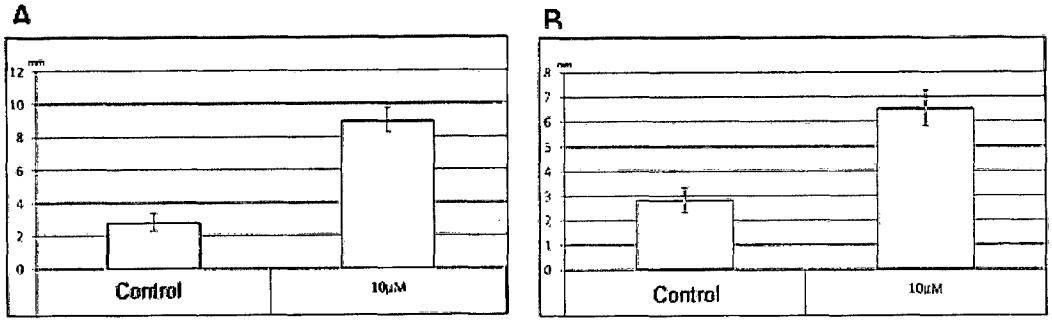

Figure 5

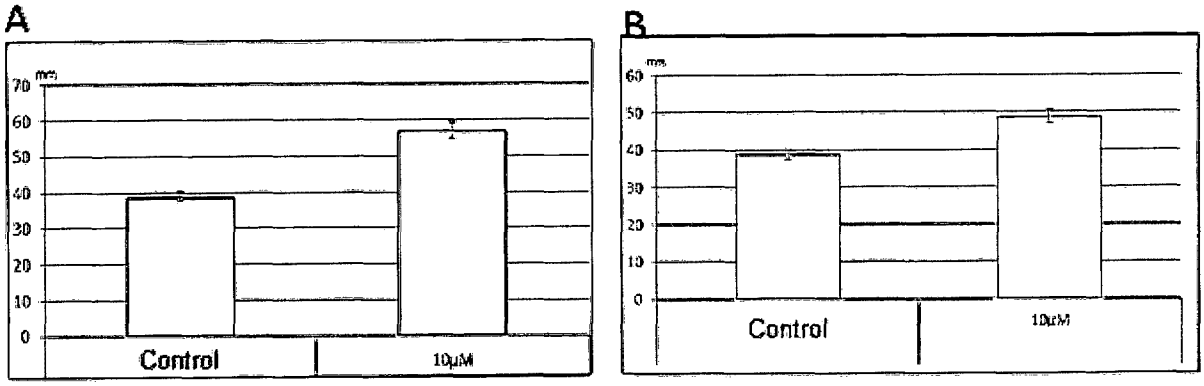

Figure 6

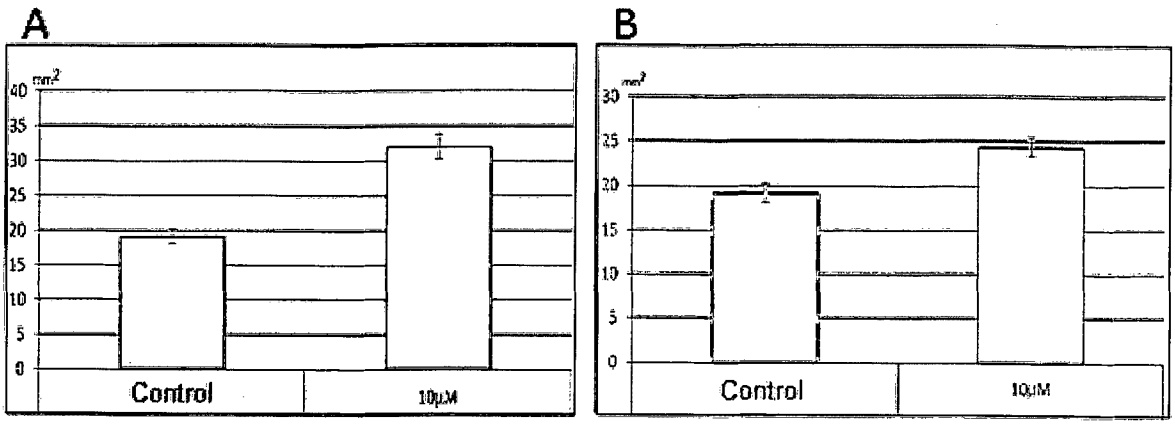

Figure 7

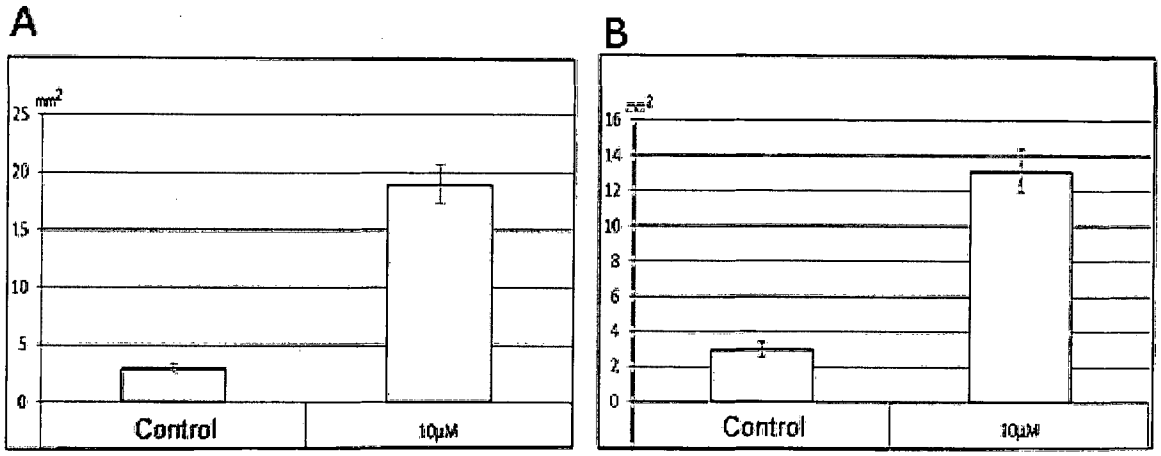

Figure 8

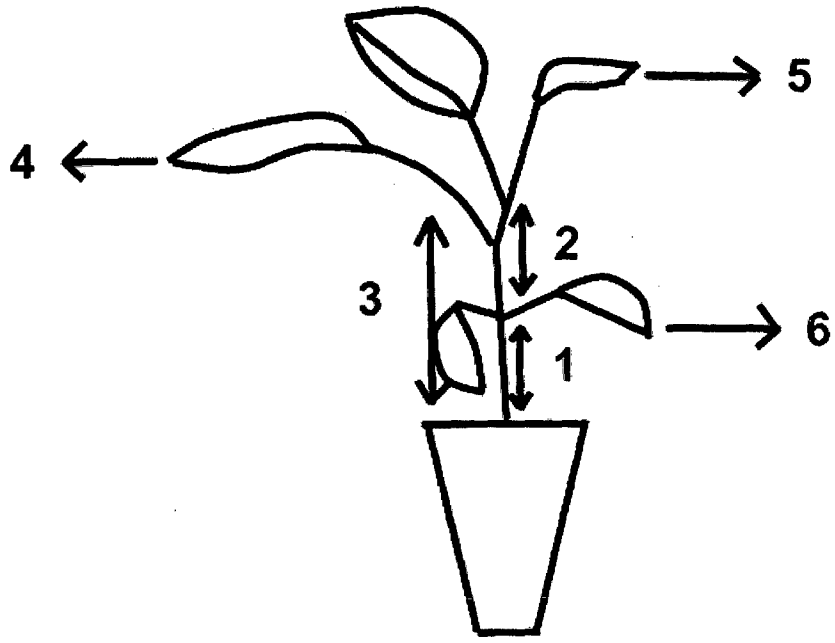

Figure 9

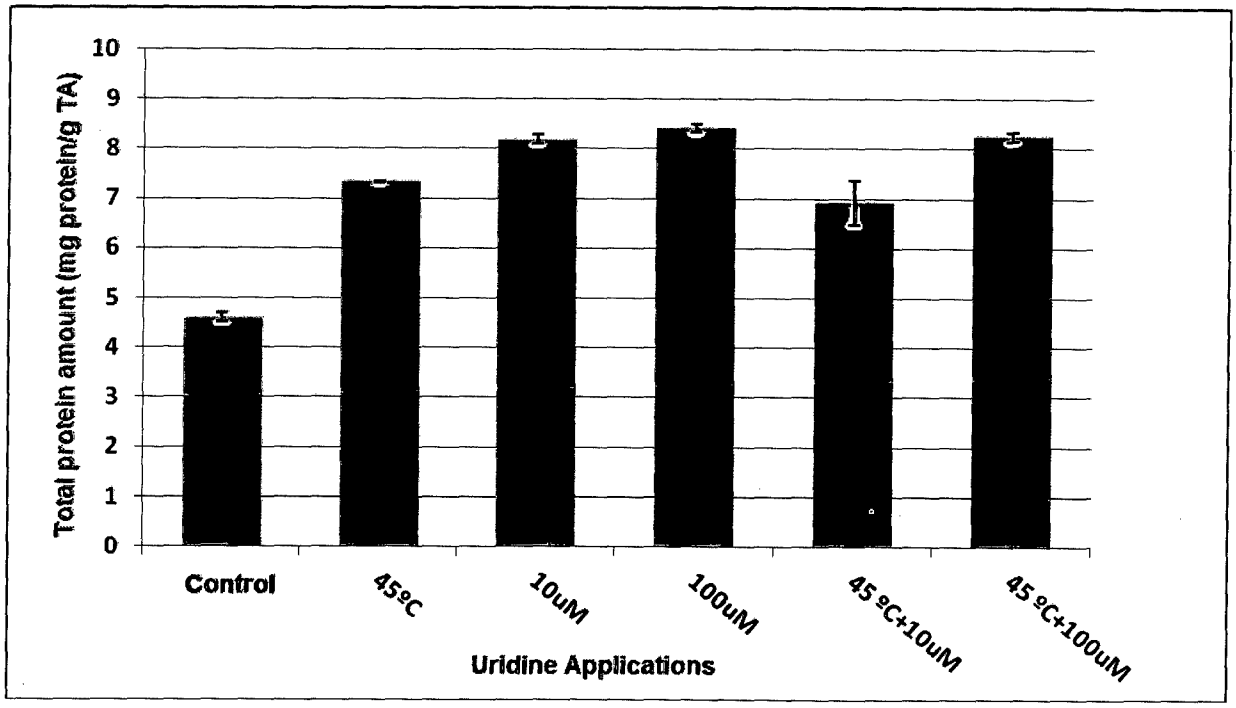

Figure 10

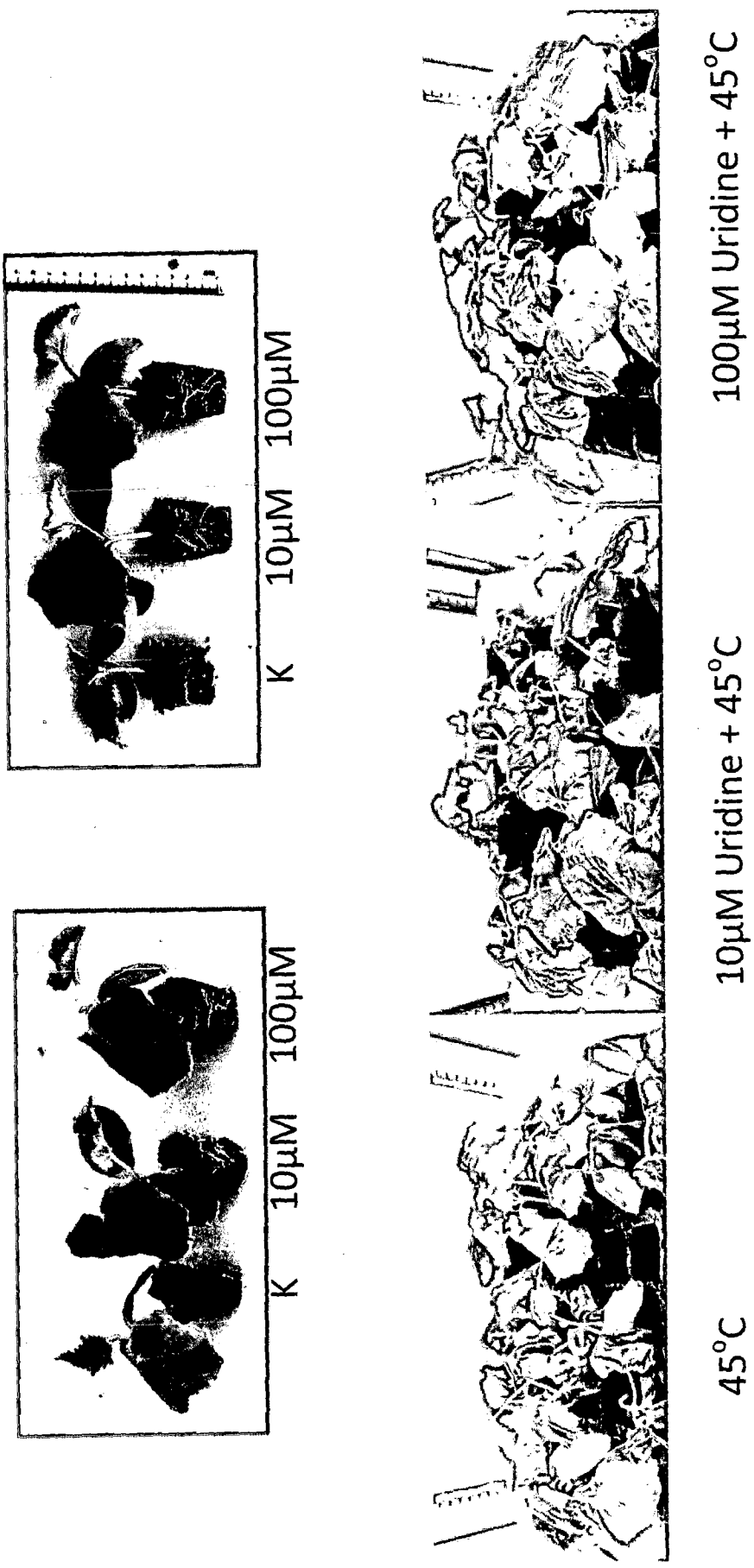
K
10μM
100μM
K
10μM
100μM
45°C
10μM Uridine + 45°C
100μM Uridine + 45°C

Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/TR2014/000051

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N43/54 A01P21/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/130285 A1 (WATANABE KAZUHIKO [JP] ET AL) 2 June 2011 (2011-06-02) claims 1-13; example 5 paragraph [0042] - paragraph [0048] paragraph [0089]; example 5; table 5 -----	1-16
X	US 3 291 592 A (EVANS ARLYN W) 13 December 1966 (1966-12-13) the whole document -----	4

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[X] Further documents are listed in the continuation of Box C.

[X] 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

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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	Date of mailing of the international search report
4 July 2014	21/07/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kamdzhilov, Yavor

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INTERNATIONAL SEARCH REPORT

International application No
PCT/TR2014/000051

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Suge Hiroshi ET AL: "Chemical control of plant growth and development. IV. Promotion of flowering induced by uracil , uridylic acid, and several growth regulators in winter wheat", Nippon Sakumotsu Gakkai Kiji, 1 January 1963 (1963-01-01), XP055126675, DOI: 10.1626/jcs.32.77 Retrieved from the Internet: URL:https://www.jstage.jst.go.jp/article/jcs1927/32/1/32_1_77/_pdf [retrieved on 2014-07-03] the whole document	4,5, 8-11,15, 16
X	JP 2001 199812 A (KOYAMA HIGHTECH KENKYUSHO KK) 24 July 2001 (2001-07-24) abstract	4
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1990, TOMAR R ET AL: "EFFECT OF PLANT GROWTH REGULATOR AND THYMINE TREATMENT ON ROOTING OF CUTTINGS OF PROSOPIS-CINERARIA L MACBRIDE", XP002726650, Database accession no. PREV199191118094 abstract & TOMAR R ET AL: "EFFECT OF PLANT GROWTH REGULATOR AND THYMINE TREATMENT ON ROOTING OF CUTTINGS OF PROSOPIS-CINERARIA L MACBRIDE", AGRICULTURAL AND BIOLOGICAL RESEARCH, vol. 6, no. 2, 1990, pages 94-97, ISSN: 0970-1907	4
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