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2. Full name, address and postcode of the applicant or of each applicant	Acadian Seaplants Limited 30 Brown Avenue Dartmouth B3B1X8 Nova Scotia Canada 13259288001		
Patents ADP number (if you know it)			
3. Title of the invention	Compositions containing an amino-acid betaine for mitigating abiotic stress in plants		
4. Name of your agent (if you have one) "Address for service" to which all correspondence should be sent. This may be in the United Kingdom, the Channel Islands or Gibraltar (see warning note below) (including the postcode)	Barker Brettell LLP 100 Hagley Road Edgbaston Birmingham B16 8QQ United Kingdom 10220036001		
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Description: **21**

Claim(s): **3**

Abstract: **1**

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Signature: **Subject: Andrew Tranter 15171; Issuer: European Patent Office, European Patent Office CA G2** Date: **06 May 2022**

12. Name, e-mail address, telephone, fax and/or mobile number, if any, of a contact point for the applicant	Tranter, Dr Andrew Email: bham@barkerbrettell.co.uk Telephone: +44 121 456 0000
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COMPOSITIONS CONTAINING AN AMINO-ACID BETAINES FOR MITIGATING ABIOTIC STRESS IN PLANTS

TECHNICAL FIELD

[0001] This disclosure generally relates to the field of compounds and compositions for mitigating abiotic stress in plants.

BACKGROUND OF THE ART

[0002] Various strategies are currently used to mitigate the effects of different forms of abiotic stress. Many of these are directed at physically mitigating the effect of specific stressors e.g. the application of water or shielding material to address temperature extremes or light intensity. Other approaches involve genetically engineering plants to be more resilient to stresses such as elevated salinity or temperature extremes.

[0003] These types of measures can be labour intensive and consequentially expensive, may only be useful for mitigating the effects of specific stressors and/or may only be applicable to specific plant species.

[0004] As a consequence, there remains a need for alternate methods, compounds and compositions that are effective at mitigating the impact of abiotic stress on plants, while being sustainable, cost-effective, and simple to use via current agricultural practices.

[0005] Disclosed herein, the present invention overcomes previous shortcomings in the art by providing novel compositions for the treatment and mitigation of abiotic stresses in plants.

SUMMARY OF THE INVENTION

[0006] The present invention provides compositions of amino-acid betaines and uses thereof to mitigate the effects of abiotic stress in plants.

[0007] In one embodiment, there is provided a method of preventing or mitigating the effects of abiotic stress in a plant comprising administering to the plant, a seed of the plant or to a growth medium of the plant an effective amount of an amino-acid betaine.

[0008] In one embodiment, the method comprises administering a combination of the amino-acid betaine and a seaweed extract.

[0009] In some embodiments, the treatment is a seed treatment, and 50–800 g of the amino-acid betaine is administered per tonne of seed and 50–800 g seaweed extract is administered per tonne of seed.

[0010] Preferably, the amino-acid betaine is L-carnitine. Preferably, the seaweed used to produce the extract is from the class *Phaeophyceae*, preferably from the species *Ascophyllum nodosum*. The seaweed extract may be produced using an alkaline hydrolysis extraction.

[0011] Mitigating abiotic stress may include one or more of: increased plant vigour; increased root growth and development; increased shoot growth and development; increased plant growth rate; increased photosynthetic rate and capacity; improved yield; compared to a control plant that is not administered the amino-acid betaine while subjected to an abiotic stress, or a crop grown under similar environmental conditions that is not administered the amino-acid betaine while encountering abiotic stress.

[0012] In a further embodiment, there is provided a plant biostimulant composition comprising an amino-acid betaine and a seaweed extract. Preferably, the amino-acid betaine is L-carnitine and the seaweed extract is from the class *Phaeophyceae*, more preferably from the species *Ascophyllum nodosum*.

[0013] In some embodiments, the weight ratio of the amino-acid betaine to the seaweed extract in the composition is between 10:1 and 1:10, in some embodiments between 2:1 to 1:2. The composition may contain approximately equals amounts by weight of the amino-acid betaine and the seaweed extract.

[0014] In some embodiments, the composition is an aqueous composition containing 100 to 250 g/L of seaweed extract and 100 to 250 g/L of the amino acid betaine.

[0015] Also provided is a seed treatment comprising a composition as defined herein.

[0016] In use, the biostimulant or seed composition may prevent or mitigate one or more of the indicia of abiotic stress identified herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1 provides an example of improved vigour 21 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0018] Fig. 2 provides an example of improved vigour 42 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0019] Fig. 3 provides an example of increased foliar fresh weight 50 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0020] Fig. 4 provides an example of increased root fresh weight 50 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0021] Fig. 5 provides an example of increased plant height 22 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to salinity stress (irrigation with 8 g/l NaCl) four days post-emergence, in comparison to a stressed and unstressed control.

[0022] Fig. 6 provides an example of increased plant height 35 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to salinity stress (irrigation with 8 g/l NaCl) four days post-emergence, in comparison to a stressed and unstressed control.

[0023] Fig. 7 provides an example of increased relative chlorophyll content (SPAD) 29 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to salinity stress (irrigation with 8 g/l NaCl) four days post-emergence, in comparison to a stressed and unstressed control.

[0024] Fig. 8 provides an example of increased plant vigour 43 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to salinity stress (irrigation with 8 g/l NaCl) four days post-emergence, in comparison to a stressed and unstressed control.

[0025] Fig. 9 provides an example of increased fresh foliar biomass 50 days after sowing in winter wheat (cv. Marius) seed-treated with ANE, CAR, or combinations thereof when subjected to salinity stress (irrigation with 8 g/l NaCl) four days post-emergence, in comparison to a stressed and unstressed control.

[0026] Fig. 10 provides an example of increased plant vigour 51 days after sowing in soybean (cv. Mungo) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0027] Fig. 11 provides an example of increased foliar fresh weight 51 days after sowing in soybean (cv. Mungo) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0028] Fig. 12 provides an example of increased foliar dry weight 51 days after sowing in soybean (cv. Mungo) seed-treated with ANE, CAR, or combinations thereof when subjected to drought stress (50 % reduced irrigation) four days post-emergence, in comparison to a stressed and unstressed control.

[0029] Fig. 13 provides an example of increased root length 10 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to drought stress (75 % reduced irrigation) three days post-sowing, in comparison to a stressed and unstressed control.

[0030] Fig. 14 provides an example of increased photosynthetic efficiency (Φ_2) 10 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to drought stress (75 % reduced irrigation) three days post-sowing, in comparison to a stressed and unstressed control.

[0031] Fig. 15 provides an example of reduced non-photochemical quenching (NPQt, an indicator of plant stress level) 10 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to drought stress (75 % reduced irrigation) three days post-sowing, in comparison to a stressed and unstressed control.

[0032] Fig. 16 provides an example of increased linear electron flow (LEF) of the plant photosystems 10 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to drought stress (75 % reduced irrigation) three days post-sowing, in comparison to a stressed and unstressed control.

[0033] Fig. 17 provides an example of increased photosynthetic efficiency (Phi2) 11 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to salinity stress (10 ml of 58 g/l NaCl) three days post-sowing, in comparison to a stressed and unstressed control.

[0034] Fig. 18 provides an example of increased linear electron flow (LEF) of the plant photosystems 11 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to salinity stress (10 ml of 58 g/l NaCl) three days post-sowing, in comparison to a stressed and unstressed control.

[0035] Fig. 19 provides an example of reduced non-photochemical quenching (NPQt, an indicator of plant stress levels) 11 days after sowing in corn (cv. H140354) seed-treated with ANE, CAR, or a combination thereof when subjected to salinity stress (10 ml of 58 g/l NaCl) three days post-sowing, in comparison to a stressed and unstressed control.

DETAILED DESCRIPTION OF THE INVENTION

[0036] As used herein, “abiotic stress” refers to the negative impact of non-living factors on plants; these factors can include, but are not necessarily limited to: extremes of heat and cold, drought or flooding water stress, high or low light intensity, elevated salinity, and soil toxicity. Thus, the consequences of abiotic stress are typically those consequences which negatively impact crop yield and quality.

[0037] As used herein, “plants” may refer to any organism of the kingdom Plantae. In preferred embodiments, the plant is a terrestrial plant. In some embodiments, a crop. Unless

context dictates otherwise, it will be understood that administering a compound or composition as described herein to a plant comprises administering the compound or composition to any part of the plant and at any stage of growth, e.g. to a seed, roots or leaves of a plant.

[0038] As used herein, a “biostimulant” refers to a product composed of substances, micro-organisms, and/or other materials able to stimulate nutrition processes independently of its nutrient content, either in the plant or its surrounding growing environment, that improve the plant’s nutrition use efficiency, tolerance to abiotic stress, and/or the crop quality.

[0039] As used herein, “mitigating the effects of abiotic stress” refers to reducing, reversing or preventing one or more indicia of abiotic stress better than a control plant or part thereof (i.e., a plant or part thereof that has been exposed to the same abiotic stress but has not been contacted with the compositions of the present invention).

[0040] Methods of identifying and measuring indicia of abiotic stress in plants are known to those of skill in the art and can include visual assessments of plant vitality, such as a reduction in the number or size of plants or parts thereof, a reduction in seed germination or emergence, or a reduction in seedling growth rates or vigour; gravimetric assessment of biomass yield, such as fresh or dry weights of shoots or roots; optical scanner-based assessments of plant parts, such as scanning of leaves or root systems and algorithmic determinations of leaf area or root lengths; physiological or biochemical assessments, such as cell membrane stability or relative leaf water content; and photosynthetic assessments of plant stress levels using reflectance or spectroscopy-based methods, such as determination of photosynthetic efficiency, linear electron flow, non-photochemical quenching, and relative chlorophyll levels.

[0041] As used herein, an “effective amount” is an amount sufficient to mitigate the effects of abiotic stress, which may be evaluated using one or more of the indicia discussed above. The effective amount will vary with type and condition of the plant, as well as the nature and duration of the abiotic stressor (or expected abiotic stressor in the case of a preventative treatment).

[0042] As used herein, “growth medium” is used generically to refer to the substrate on which a plant is anchored or grown, and may include soil or man-made mediums, including e.g. mediums suitable for hydroponic applications, such as rockwool or vermiculite.

[0043] As used herein, “amino-acid betaine” is any amino acid-derived zwitterion in which the ammonium nitrogen carries methyl substituents and bears no hydrogen atoms. Amino acid betaines include glycine betaine, aminobutyric acid betaine, and aminovaleric acid betaine.

[0044] As used herein, “L-carnitine” (β -hydroxy- γ -N-trimethylaminobutyric acid) is a derivative of the amino acid lysine. It was first isolated from meat in 1905 and has a critical role in the oxidation of long chain fatty acids for energy production in animal tissues. Commercial uses of L-carnitine include as a dietary supplement and as a foam booster in some cosmetic products.

[0045] Naturally occurring amino acids which include carnitine, phenylalanine, lysine, etc. can be obtained by extraction, fermentation, enzymatic reaction and synthesis. Methods for the synthetic manufacture of L-carnitine are known to those of skill in the art, and L-carnitine is commercially available from a variety of sources.

[0046] Marine algae have been used as agricultural inputs for hundreds of years. It was first used as a soil amendment where the seaweed was incorporated into the growing media and broken down by soil microbes. More recently it has served as a raw material for the production of “seaweed extract” which is used as a biostimulant. Species such as *Ascophyllum nodosum*, *Durvillaea potatorum*, *Ecklonia Maxima*, *Ulva Lactuca*, and *Gelidium robustum* have been used in the manufacture of plant biostimulants and, in some embodiments, may be used in the methods and compositions provided herein.

[0047] As used herein, “seaweed extract” refers to products obtained from marine algae, preferably red, brown or green macroalgae, through a process that extracts and concentrates bio-stimulating compounds. Methods of obtaining extracts from seaweed are known to those of skill in the art, see e.g. EL Boukhari MEM, Barakate M, Bouhia Y, Lyamlouli K. *Trends in Seaweed Extract Based Biostimulants: Manufacturing Process and Beneficial Effect on Soil-Plant Systems. Plants* 2020, 9, 359. doi : 10.3390/plants9030359. Further, the seaweed extract may be prepared from a combination of seaweed species.

[0048] Seaweed processing/extraction can be made using solvents, acids, bases, enzymes or mechanical means eventually in any combination. Preferably, the processing/extraction is made by contacting the seaweed with an aqueous solution comprising an alkaline extraction agent.

[0049] For the purpose of the present invention, the base is preferably an inorganic base selected from: NaOH, KOH, Na₂CO₃, K₂CO₃, or any combination thereof. The alkaline solvent concentration ranges from 1 % to 10 % w/w, more specifically 2 % to 5 % w/w.

[0050] Preferably, the temperature of the extraction step ranges preferably between 20°C and 100°C and the extraction time ranges from 30 minutes to 18 hours at pressures ranging from 1 to 6 Bar.

[0051] The extraction step may be followed by a further step of separating/removing the non-solubilized components when it is desirable using only the extract in the formulation. The removing/separating step is preferably performed by decantation, filtration or centrifugation. Alternatively, a suspension comprising both the extracted components and the non-extracted components can be used.

[0052] According to a preferred embodiment, the seaweed extract used in the invention is prepared from the brown marine algae *Ascophyllum nodosum*. The resulting extract may be in the form of a soluble liquid, a liquid concentrate, or a water-soluble powder.

[0053] Provided herein is a plant biostimulant composition comprising an effective amount of at least one amino-acid betaine and a seaweed extract. Preferably, the amino-acid betaine is L-carnitine and the seaweed extract is from the class *Phaeophyceae*, more preferably from the species *Ascophyllum nodosum*. In some embodiments, the active ingredients of the biostimulant composition are limited to the at least one amino-acid betaine, preferably L-carnitine, and the seaweed extract, preferably *Ascophyllum nodosum*. In some embodiments, the biostimulant composition, consists or consists essentially of the at least one amino-acid betaine, preferably L-carnitine, and the seaweed extract, preferably *Ascophyllum nodosum* and, optionally, water.

[0054] In one embodiment, the biostimulant composition may be in the form of a powder containing the amino-acid betaine, preferably L-carnitine, and a seaweed extract powder. The composition may be dispersed in a liquid medium to facilitate application.

[0055] In some embodiments, the weight ratio of the amino-acid betaine to the seaweed extract in the composition or used in the method provided is between 10:1 and 1:10, in some embodiments between 2:1 to 1:2. In some embodiments, approximately equals amounts by weight of the amino-acid betaine and the seaweed extract are used.

[0056] In some embodiments, the biostimulant composition containing L-carnitine and *Ascophyllum nodosum* extract may be in the form of a liquid, which may be particularly suitable for foliar application or root drenches.

[0057] In some embodiment, the compositions provided herein may be formulated as an aqueous concentrate containing *Ascophyllum nodosum* extract at a range of 100 to 250 g/l in combination with L-carnitine at 100 to 250 g/l.

[0058] In some embodiments, the compositions as described herein may be used as a seed treatment. Seed treatment formulations and technologies are commercially available and methods of applying seed treatments are known to those of skill in the art and can include direct application of a seed treatment to the seed using commercially available seed treating equipment.

[0059] In one embodiment, L-carnitine, can be formulated as a seed treatment. In a preferred embodiment, seaweed extract is further formulated into the seed treatment.

[0060] According to the scope of conditions, an effective amount typically ranges from 50–800 g L-carnitine per tonne of seed and 50–800 g *Ascophyllum nodosum* extract per tonne of seed for seed treatment.

[0061] In a preferred embodiment, the composition described herein for use as a seed treatment may be formulated as an aqueous concentrate containing *Ascophyllum nodosum* extract at a range of 100 to 250 g/l in combination with L-carnitine at 100 to 250 g/l.

[0062] The compositions provided herein may also contain suitable formulation excipients known to persons of skill in the art and are commercially available. In some embodiments, formulation excipients are suitably selected from, but not exclusively, surfactants, wetting and dispersing agents, stickers/binders, antifreeze, antifoams, preservatives, rheological aids and, if appropriate, colourants.

[0063] The treatments described herein may also be preventative, e.g. compounds and compositions provided herein may be provided as a seed treatment prior to an expected stressor event, such as a cold snap or heat wave to mitigate the effects of the stressor.

[0064] While in its broadest embodiments, the methods, compounds and compositions described herein may be used in the cultivation of any plant, in some embodiments, they may

be used in the cultivation of commercially important plants, which can include, without being limited to, cereals (including maize, wheat, alfalfa, barley, rye, oat), vegetables (including pepper, tomato, lettuce, carrots, potatoes), and other important crops, such as cotton, rice, soybean, canola, oilseed rape.

[0065] All documents referenced herein are incorporated by reference, however, it should be appreciated that any patent, publication, or other disclosure material, in whole or in part, that is incorporated by reference herein is incorporated only to the extent that the incorporated material does not conflict with definitions, statements, or other disclosure material set forth in this disclosure. As such, and to the extent necessary, the disclosure as explicitly set forth herein supersedes any conflicting material incorporated herein by reference.

[0066] It will be understood that numerous modifications thereto will appear to those skilled in the art. Accordingly, the above description and accompanying drawings should be taken as illustrative of the invention and not in a limiting sense. It will further be understood that it is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein before set forth, and as follows in the scope of the appended claims.

EXAMPLES

Example 1: Wheat drought stress greenhouse trial.

[0067] Winter wheat seed (cv. Marius) was treated at a total application volume of 10 ml/kg with water (unstressed control and stressed control treatment groups), or an aqueous solution of *Ascophyllum nodosum* extract (ANE) at 1 ml/kg, or an aqueous solution of L-carnitine hydrochloride (CAR) at 0.3 g/kg, or combinations of *Ascophyllum nodosum* extract at 1 ml/kg with either 0.1, 0.2, 0.3, or 0.4 g/kg L-carnitine hydrochloride. Treated wheat seed was sown in soil in pots with 10 biological replicates per treatment group in a randomized complete block design. Four days after full emergence, drought stress was imposed on all but the unstressed control treatment group through a 50 % reduced irrigation regime relative to the full irrigation received by the unstressed control group.

[0068] As evidenced in Figure 1 and Table 1, plant vigour was increased by up to 17.6 % compared to the stressed control in the plants seed-treated with ANE at 1 ml/kg and CAR at 0.4 g/kg at 21 days after sowing. By 42 days after sowing, this difference had increased to 20.8 % compared to the stressed control, as shown in Figure 2 and Table 2.

Table 1

Treatment group	Mean	Difference (%)
Unstressed	8.45	19.0
Stressed	7.10	0.0
ANE 1 ml/kg	7.25	2.1
CAR 0.3 g/kg	7.60	7.0
ANE 1 ml/kg; CAR 0.1 g/kg	7.50	5.6
ANE 1 ml/kg; CAR 0.2 g/kg	8.05	13.4
ANE 1 ml/kg; CAR 0.3 g/kg	8.25	16.2
ANE 1 ml/kg; CAR 0.4 g/kg	8.35	17.6

Table 2

Treatment group	Mean	Difference (%)
Unstressed	8.95	19.3
Stressed	7.50	0.0
ANE 1 ml/kg	8.40	12.0
CAR 0.3 g/kg	8.35	11.3
ANE 1 ml/kg; CAR 0.1 g/kg	8.76	16.9
ANE 1 ml/kg; CAR 0.2 g/kg	8.59	14.5
ANE 1 ml/kg; CAR 0.3 g/kg	8.53	13.7
ANE 1 ml/kg; CAR 0.4 g/kg	9.06	20.8

The improved plant vigour afforded by seed treatment with the combination of ANE and CAR translated to increased biomass yield assessed 50 days after sowing, with an up to 22.1 % increase in foliar fresh weight (Figure 3, Table 3) and a 62 % increase in fresh root weight in the plants seed-treated with ANE at 1 ml/kg and CAR at 0.4 g/kg (Figure 4, Table 4).

Table 3

Treatment group	Mean	Difference (%)
Unstressed	18.77	107.6
Stressed	9.04	0.0
ANE 1 ml/kg	9.83	8.7
CAR 0.3 g/kg	9.94	9.9
ANE 1 ml/kg; CAR 0.1 g/kg	10.64	17.6
ANE 1 ml/kg; CAR 0.2 g/kg	10.59	17.1
ANE 1 ml/kg; CAR 0.3 g/kg	10.78	19.2
ANE 1 ml/kg; CAR 0.4 g/kg	11.04	22.1

Table 4

Treatment group	Mean	Difference (%)
Unstressed	4.39	100.9
Stressed	2.18	0.0
ANE 1 ml/kg	3.02	38.2
CAR 0.3 g/kg	3.66	67.5
ANE 1 ml/kg; CAR 0.1 g/kg	3.12	42.8
ANE 1 ml/kg; CAR 0.2 g/kg	3.42	56.5
ANE 1 ml/kg; CAR 0.3 g/kg	3.11	42.3
ANE 1 ml/kg; CAR 0.4 g/kg	3.54	62.0

Example 2: Wheat salinity stress greenhouse trial.

[0069] Winter wheat seed (cv. Marius) was treated at a total application volume of 10 ml/kg with water (unstressed control and stressed control treatment groups), or an aqueous solution of *Ascophyllum nodosum* extract (ANE) at 1 ml/kg, or an aqueous solution of L-carnitine

hydrochloride (CAR) at 0.3 g/kg, or combinations of *Ascophyllum nodosum* extract at 1 ml/kg with either 0.1, 0.2, 0.3, or 0.4 g/kg L-carnitine hydrochloride. Treated wheat seed was sown in pots containing soil with 10 biological replicates per treatment group in a randomized complete block design. Four days after full emergence, salinity stress was imposed on all but the unstressed control treatment group through irrigation with saline water (8 g/l NaCl) for the remainder of the trial. The unstressed control treatment group was irrigated with non-saline water.

[0070] As evidenced in Figure 5 and Table 5, plant height was increased by up to 10.9 % compared to the stressed control in the plants seed-treated with ANE at 1 ml/kg and CAR at 0.4 g/kg at 22 days after sowing. By 35 days after sowing, the height increase in this treatment group had increased to 23.3 % in comparison to the stressed control (Figure 6, Table 6).

Table 5

Treatment group	Mean	Difference (%)
Unstressed	27.75	7.6
Stressed	25.80	0.0
ANE 1 ml/kg	26.10	1.2
CAR 0.3 g/kg	27.55	6.8
ANE 1 ml/kg; CAR 0.1 g/kg	27.75	7.6
ANE 1 ml/kg; CAR 0.2 g/kg	28.10	8.9
ANE 1 ml/kg; CAR 0.3 g/kg	28.40	10.1
ANE 1 ml/kg; CAR 0.4 g/kg	28.60	10.9

Table 6

Treatment group	Mean	Difference (%)
Unstressed	35.30	10.7
Stressed	31.90	0.0
ANE 1 ml/kg	33.85	6.1
CAR 0.3 g/kg	37.39	17.2
ANE 1 ml/kg; CAR 0.1 g/kg	37.33	17.0
ANE 1 ml/kg; CAR 0.2 g/kg	36.80	15.4

ANE 1 ml/kg; CAR 0.3 g/kg	38.16	19.6
ANE 1 ml/kg; CAR 0.4 g/kg	39.33	23.3

[0071] At 29 days after sowing, the plant relative chlorophyll content (SPAD) showed a 25.5 % increase in the same treatment group in comparison to the stressed control (Figure 7, Table 7). The increased stress tolerance of the plants was likewise reflected in the plant vigour assessment at 43 days after seeding, with an up to 16.6 % increase over the stressed control (Figure 8, Table 8). By 50 days after seeding, an up to 42.7 % increase in fresh foliar biomass was observed in the plants seed-treated with ANE at 1 ml/kg and CAR at 0.3 g/kg, demonstrating strong induction of long-lasting salinity stress tolerance from the combined treatment of ANE and CAR (Figure 9, Table 9).

Table 7

Treatment group	Mean	Difference (%)
Unstressed	37.46	24.3
Stressed	30.14	0.0
ANE 1 ml/kg	35.52	17.8
CAR 0.3 g/kg	35.30	17.1
ANE 1 ml/kg; CAR 0.1 g/kg	35.24	16.9
ANE 1 ml/kg; CAR 0.2 g/kg	36.94	22.6
ANE 1 ml/kg; CAR 0.3 g/kg	37.98	26.0
ANE 1 ml/kg; CAR 0.4 g/kg	37.81	25.5

Table 8

Treatment group	Mean	Difference (%)
Unstressed	8.95	13.3
Stressed	7.90	0.0
ANE 1 ml/kg	8.40	6.3
CAR 0.3 g/kg	8.68	9.9
ANE 1 ml/kg; CAR 0.1 g/kg	9.00	13.9
ANE 1 ml/kg; CAR 0.2 g/kg	8.25	4.4
ANE 1 ml/kg; CAR 0.3 g/kg	8.40	6.3
ANE 1 ml/kg; CAR 0.4 g/kg	9.21	16.6

Table 9

Treatment group	Mean	Difference (%)
Unstressed	17.5	113.4
Stressed	8.2	0.0
ANE 1 ml/kg	9.0	9.8
CAR 0.3 g/kg	10.6	29.3
ANE 1 ml/kg; CAR 0.1 g/kg	9.4	14.6
ANE 1 ml/kg; CAR 0.2 g/kg	9.6	17.1
ANE 1 ml/kg; CAR 0.3 g/kg	11.7	42.7
ANE 1 ml/kg; CAR 0.4 g/kg	10.7	30.5

Example 3:

drought stress greenhouse trial.

Soybean

[0072] Soybean seed (cv. Mungo) was treated at a total application volume of 10 ml/kg with water (unstressed control and stressed control treatment groups), or an aqueous solution of *Ascophyllum nodosum* extract (ANE) at 1 ml/kg, or an aqueous solution of L-carnitine hydrochloride (CAR) at 0.3 g/kg, or combinations of *Ascophyllum nodosum* extract at 1 ml/kg with either 0.1, 0.2, 0.3, or 0.4 g/kg L-carnitine hydrochloride. Treated soybean seed was sown in pots containing soil with 10 biological replicates per treatment group in a randomized complete block design. Four days after full emergence, drought stress was imposed on all but the unstressed control treatment group through a 50 % reduced irrigation regime relative to the full irrigation received by the unstressed control group.

[0073] As evidenced in Figure 10 and Table 10, plant vigour was increased by up to 25 % compared to the stressed control in the plants seed-treated with ANE at 1 ml/kg and CAR at 0.2 g/kg at 51 days after sowing. Correspondingly, the foliar fresh weight of this treatment group showed a 34.4 % increase compared to the stressed control (Figure 11, Table 11), and a 66.7 % increase in foliar dry weight (Figure 12, Table 12). This demonstrates the clear mitigation of the yield-reducing effects of the induced drought stress afforded by the combined seed treatment of ANE and CAR.

Table 10

Treatment group	Mean	Difference (%)
Unstressed	7.2	-5.3
Stressed	7.6	0.0
ANE 1 ml/kg	8.6	13.2
CAR 0.3 g/kg	9.4	23.7
ANE 1 ml/kg; CAR 0.1 g/kg	9.2	21.1
ANE 1 ml/kg; CAR 0.2 g/kg	9.5	25.0
ANE 1 ml/kg; CAR 0.3 g/kg	9.1	19.7
ANE 1 ml/kg; CAR 0.4 g/kg	9.0	18.4

Table 11

Treatment group	Mean	Difference (%)
Unstressed	39.0	-0.8
Stressed	39.3	0.0
ANE 1 ml/kg	43.9	11.7
CAR 0.3 g/kg	49.1	24.9
ANE 1 ml/kg; CAR 0.1 g/kg	42.0	6.9
ANE 1 ml/kg; CAR 0.2 g/kg	52.8	34.4
ANE 1 ml/kg; CAR 0.3 g/kg	47.2	20.1
ANE 1 ml/kg; CAR 0.4 g/kg	47.7	21.4

Table 12

Treatment group	Mean	Difference (%)
Unstressed	8.99	10.9
Stressed	8.11	0.0
ANE 1 ml/kg	10.17	25.4
CAR 0.3 g/kg	12.64	55.9
ANE 1 ml/kg; CAR 0.1 g/kg	11.54	42.4
ANE 1 ml/kg; CAR 0.2 g/kg	13.52	66.7
ANE 1 ml/kg; CAR 0.3 g/kg	12.07	48.8
ANE 1 ml/kg; CAR 0.4 g/kg	12.85	58.4

Example 4: Corn drought stress controlled environment room trial.

[0074] Corn seed (cv. H140354) was treated at a total application volume of 10 ml/kg with water (unstressed control and stressed control treatment groups), or an aqueous solution of *Ascophyllum nodosum* extract (ANE) at 2 ml/kg, or an aqueous solution of L-carnitine hydrochloride (CAR) at 0.3 g/kg, or a combinations of *Ascophyllum nodosum* extract at 2 ml/kg and 0.3 g/kg L-carnitine hydrochloride. Treated corn seed was sown in field soil inside cell packs set in greenhouse trays with 16 biological replicates per treatment group in a randomized complete block design. Three days after seeding, drought stress was imposed on all but the unstressed control treatment group through a 75 % reduced irrigation regime relative to the full irrigation received by the unstressed control group, and assessments were made ten days after seeding.

[0075] As evidenced in Figure 13 and Table 13, seed treatment with ANE at 2 ml/kg and CAR at 0.3 g/kg resulted in a 30.6 % increase in corn root length in comparison to the stressed control at 10 days after sowing. The combined seed treatment of ANE and CAR furthermore resulted in a 43.2 % increased level of photosynthetic efficiency (photosystem II efficiency, Φ_2) (Figure 14, Table 14), a 40.2 % reduction in non-photochemical quenching (NPQt, an indicator of plant stress level) (Figure 15, Table 15), and a 46.2 % increase in linear electron flow (LEF) of the plant photosystems (Figure 16, Table 16). The latter measurement in particular demonstrated a surprising and unexpected synergistic effect, where ANE at 2 ml/kg resulted in an 18 % increase in LEF, CAR at 0.3 g/kg resulted in a 4.3 % decrease in LEF, while their

combined treatment resulted in a 46.2 % increase. These results demonstrate an increased capacity to conduct photosynthesis and maintain root growth in corn plants exposed to drought stress, afforded by the combined seed treatment with ANE and CAR.

Table 13

Treatment group	Mean	Difference (%)
Unstressed	365.3	61.4
Stressed	226.3	0.0
ANE 2 ml/kg	286.8	26.8
CAR 0.3 g/kg	263.3	16.4
ANE 2 ml/kg; CAR 0.3 g/kg	295.4	30.6

Table 14

Treatment group	Mean	Difference (%)
Unstressed	0.68	124.0
Stressed	0.30	0.0
ANE 2 ml/kg	0.40	29.8
CAR 0.3 g/kg	0.32	5.7
ANE 2 ml/kg; CAR 0.3 g/kg	0.44	43.2

Table 15

Treatment group	Mean	Difference (%)
Unstressed	0.77	-70.0
Stressed	2.57	0.0
ANE 2 ml/kg	1.60	-37.8
CAR 0.3 g/kg	2.32	-10.0
ANE 2 ml/kg; CAR 0.3 g/kg	1.54	-40.2

Table 16

Treatment group	Mean	Difference (%)
Unstressed	23.24	119.4
Stressed	10.59	0.0
ANE 2 ml/kg	12.50	18.0
CAR 0.3 g/kg	10.14	-4.3
ANE 2 ml/kg; CAR 0.3 g/kg	15.49	46.2

Example 5: Corn salinity stress controlled environment room trial.

[0076] Corn seed (cv. H140354) was treated at a total application volume of 10 ml/kg with water (unstressed control and stressed control treatment groups), or combinations of *Ascophyllum nodosum* extract (ANE) at 2 ml/kg with either 0.2, 0.3, or 0.4 g/kg L-carnitine hydrochloride (CAR). Treated corn seed was sown in field soil inside cell packs set in greenhouse trays with 16 biological replicates per treatment group in a randomized complete block design. Four days after seeding, salinity stress was imposed on all but the unstressed control treatment group through irrigation with saline water (10 ml of 58 g/l NaCl). The unstressed control treatment group was irrigated with non-saline water. Assessments were made eleven days after seeding.

[0077] As evidenced in Figure 17 and Table 17, the combined seed treatment with ANE 2 ml/kg and CAR 0.3 g/kg resulted in an 87.8 % increased level of photosynthetic efficiency (photosystem II efficiency, Φ_2) in the salt-stressed corn plants. An up to 118.5 % increase in linear electron flow (LEF) of the plant photosystems (Figure 18, Table 18) was furthermore observed, as well as an up to 39.5 % reduction in non-photochemical quenching (NPQt, an indicator of plant stress level) (Figure 19, Table 19).

Table 17

Treatment group	Mean	Difference (%)
Unstressed	0.67	398.4
Stressed	0.13	0.0
ANE 2 ml/kg; CAR 0.2 g/kg	0.21	53.8
ANE 2 ml/kg; CAR 0.3 g/kg	0.25	87.8
ANE 2 ml/kg; CAR 0.4 g/kg	0.24	81.9

Table 18

Treatment group	Mean	Difference (%)
Unstressed	18.89	431.1
Stressed	3.56	0.0
ANE 2 ml/kg; CAR 0.2 g/kg	7.77	118.5
ANE 2 ml/kg; CAR 0.3 g/kg	6.81	91.5
ANE 2 ml/kg; CAR 0.4 g/kg	7.54	112.2

Table 19

Treatment group	Mean	Difference (%)
Unstressed	1.06	-95.0
Stressed	21.05	0.0
ANE 2 ml/kg; CAR 0.2 g/kg	18.77	-10.8
ANE 2 ml/kg; CAR 0.3 g/kg	12.72	-39.5
ANE 2 ml/kg; CAR 0.4 g/kg	14.36	-31.8

[0078] As can be seen therefore, the examples described above and illustrated are intended to be exemplary only. The scope is indicated by the appended claims.

[0079] Many further features and combinations thereof concerning the present improvements will appear to those skilled in the art following a reading of the instant disclosure.

WHAT IS CLAIMED IS:

1. A method of preventing or mitigating the effects of abiotic stress in a plant comprising administering to the plant, a seed of the plant or to a growth medium of the plant an effective amount of an amino-acid betaine.
2. The method of claim 1, comprising administering a combination of the amino-acid betaine and a seaweed extract.
3. The method of claim 2, wherein the method is a seed treatment, and wherein the method comprises administering 50–800 g of the amino-acid betaine per tonne of seed and administering 50–800 g of seaweed extract per tonne of seed.
4. The method of any one of claims 1 to 3, wherein the amino-acid betaine is L-carnitine.
5. The method of any one of claims 1 to 4, wherein the seaweed used to produce the extract is from the class *Phaeophyceae*.
6. The method of claim 5, wherein the seaweed extract is produced from the species *Ascophyllum nodosum*.
7. The method of claim 5 or 6, wherein the seaweed extract is produced using an alkaline hydrolysis extraction.
8. The method of any one of claims 1 to 7, wherein mitigating abiotic stress comprises one or more of:
 - a) increased plant vigour;
 - b) increased root growth and development;
 - c) increased shoot growth and development;
 - d) increased plant growth rate;
 - e) increased photosynthetic rate and capacity;

f) improved yield;

compared to a control plant that is not administered the amino-acid betaine while subjected to an abiotic stress, or a crop grown under similar environmental conditions that is not administered the amino-acid betaine while encountering abiotic stress.

9. A plant biostimulant composition comprising an amino-acid betaine and a seaweed extract.
10. The composition of claim 9, wherein the amino-acid betaine is L-carnitine.
11. The composition of claim 9 or 10, wherein the seaweed extract is from the class *Phaeophyceae*.
12. The composition of claim 11, wherein the seaweed extract is from the species *Ascophyllum nodosum*.
13. The composition of any one of claims 9 to 12, wherein the weight ratio of the amino-acid betaine to the seaweed extract in the composition is between 10:1 and 1:10.
14. The composition of claim 13, wherein the weight ratio of the amino-acid betaine to the seaweed extract in the composition is between 2:1 to 1:2.
15. The composition of any one of claims 9 to 14 containing approximately equals amounts by weight of the amino-acid betaine and the seaweed extract.
16. The composition of any one of claims 9 to 15, wherein the composition is an aqueous composition containing 100 to 250 g/L of seaweed extract and 100 to 250 g/L of the amino acid betaine.
17. The composition of any one of claims 9 to 16, wherein when applied in an effective amount to a plant, a seed of the plant or to a growth medium of the plant, the composition mitigates abiotic stress in the plant, comprising one or more of:
 - a) increased plant vigour;
 - b) increased root growth and development;

- c) increased shoot growth and development;
- d) increased plant growth rate;
- e) increased photosynthetic rate and capacity;
- f) improved yield;

compared to a control plant that is not administered the composition and is subjected to the same or equivalent abiotic stress as the plant.

18. A seed treatment comprising the composition as defined in any one of claims 9 to 17.

ABSTRACT

A method of preventing or mitigating the effects of abiotic stress in a plant involves administering to the plant or to the growth medium of the plant an effective amount of an amino-acid betaine, preferably carnitine.

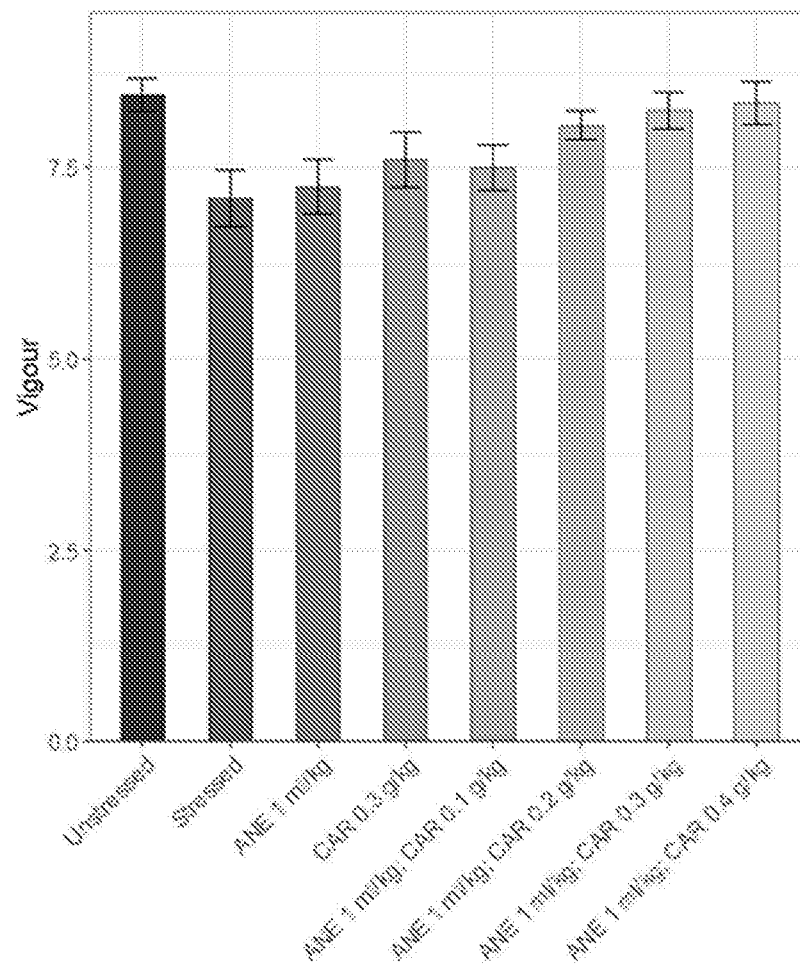


FIGURE 1

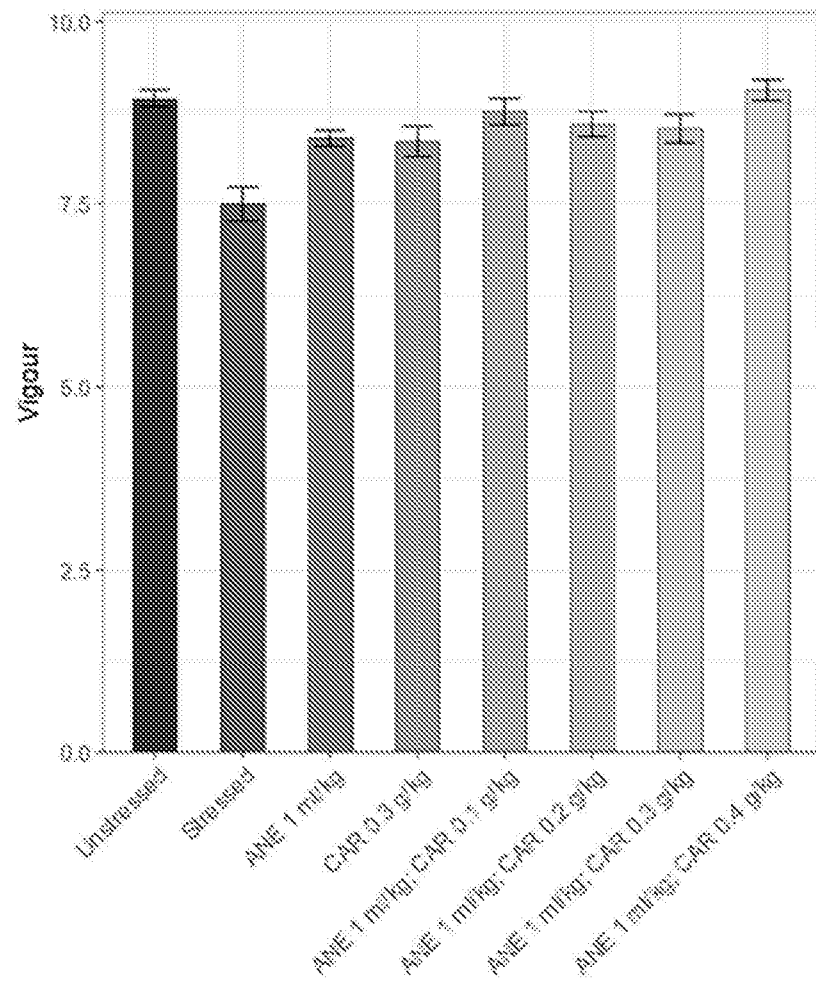


FIGURE 2

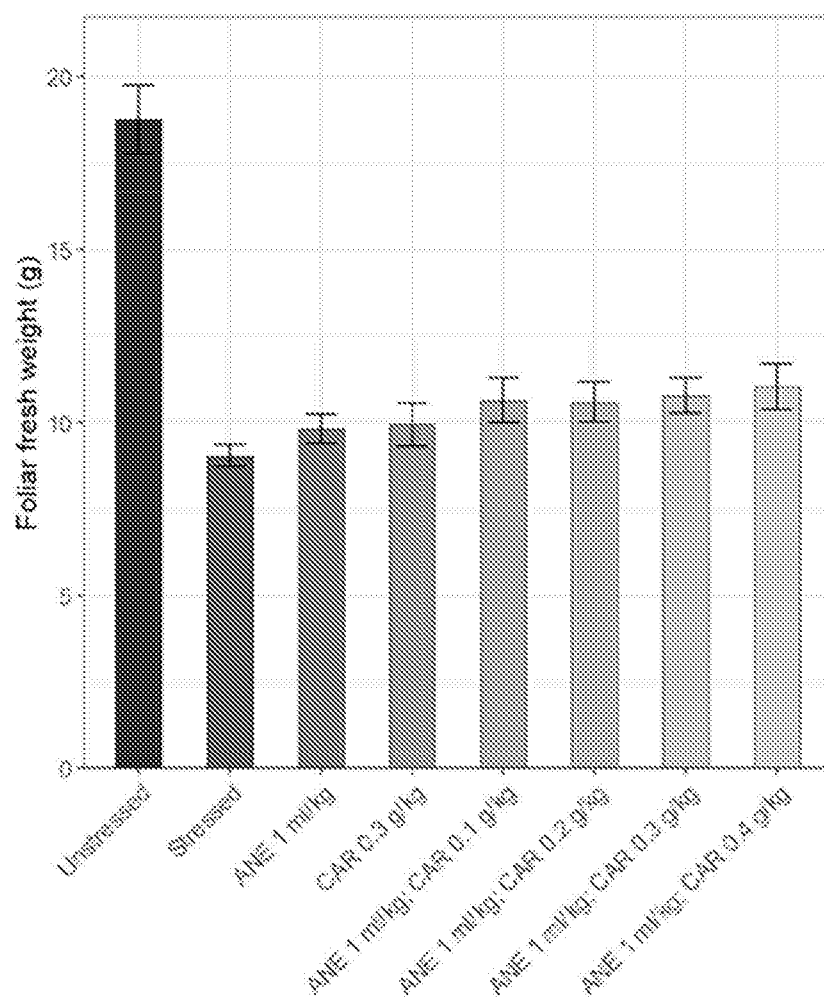


FIGURE 3

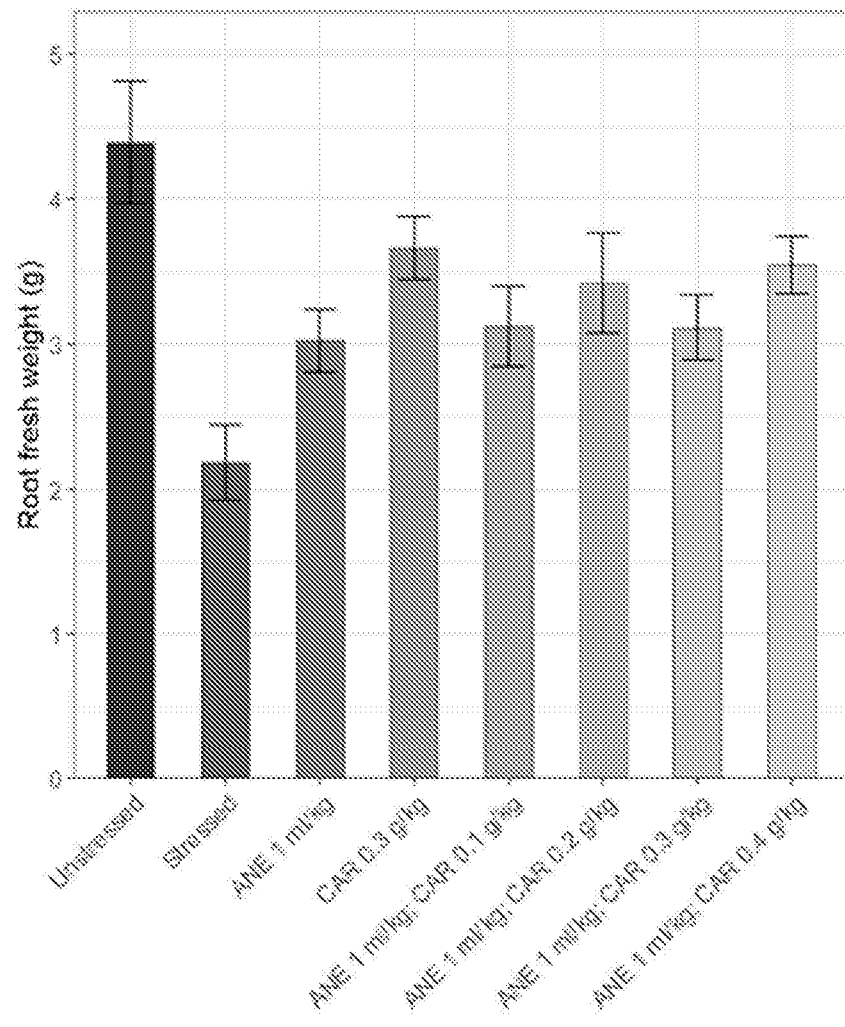


FIGURE 4

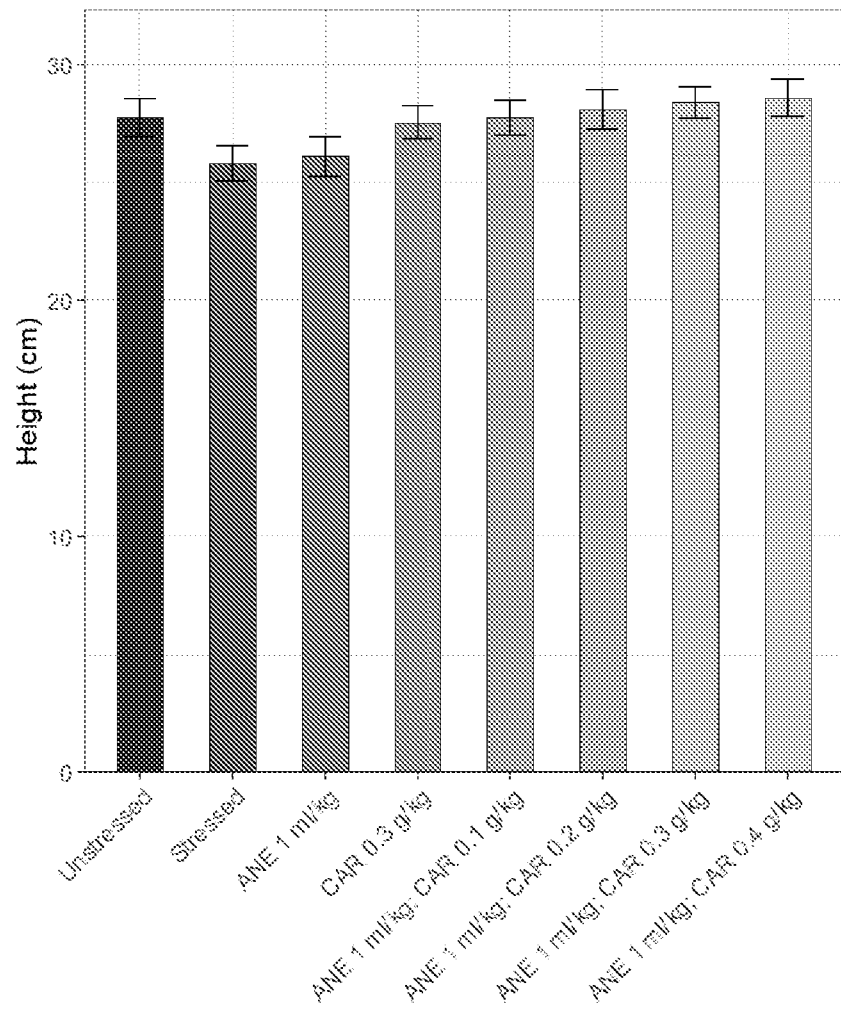


FIGURE 5

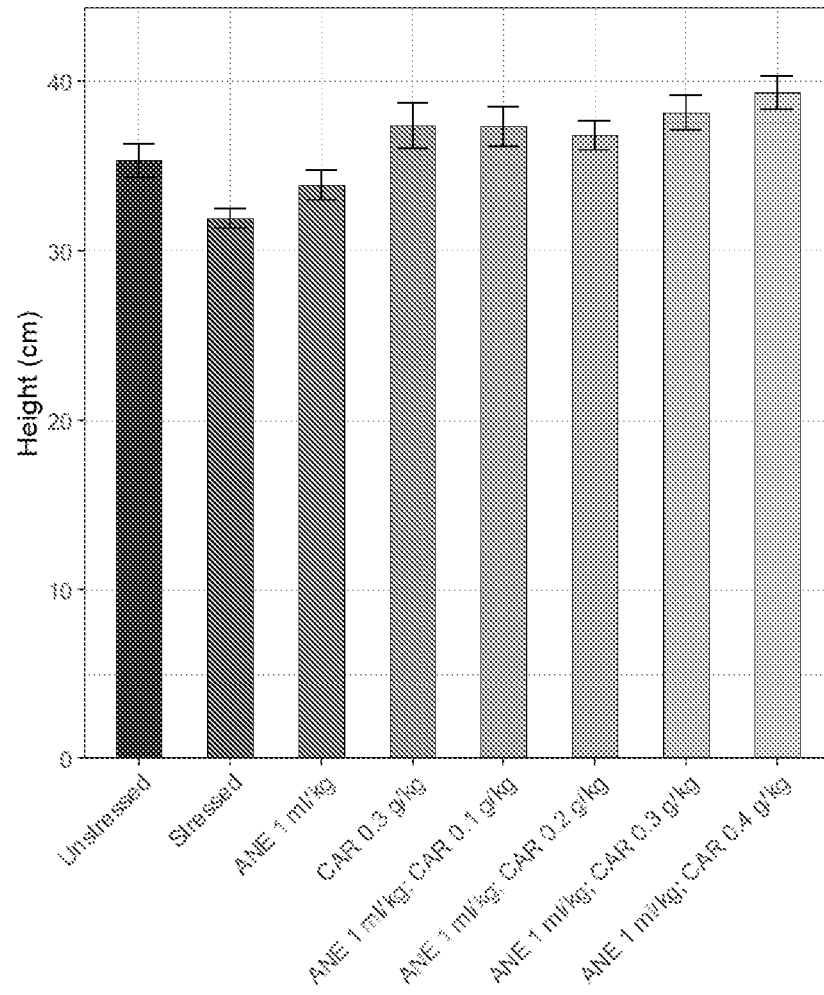


FIGURE 6

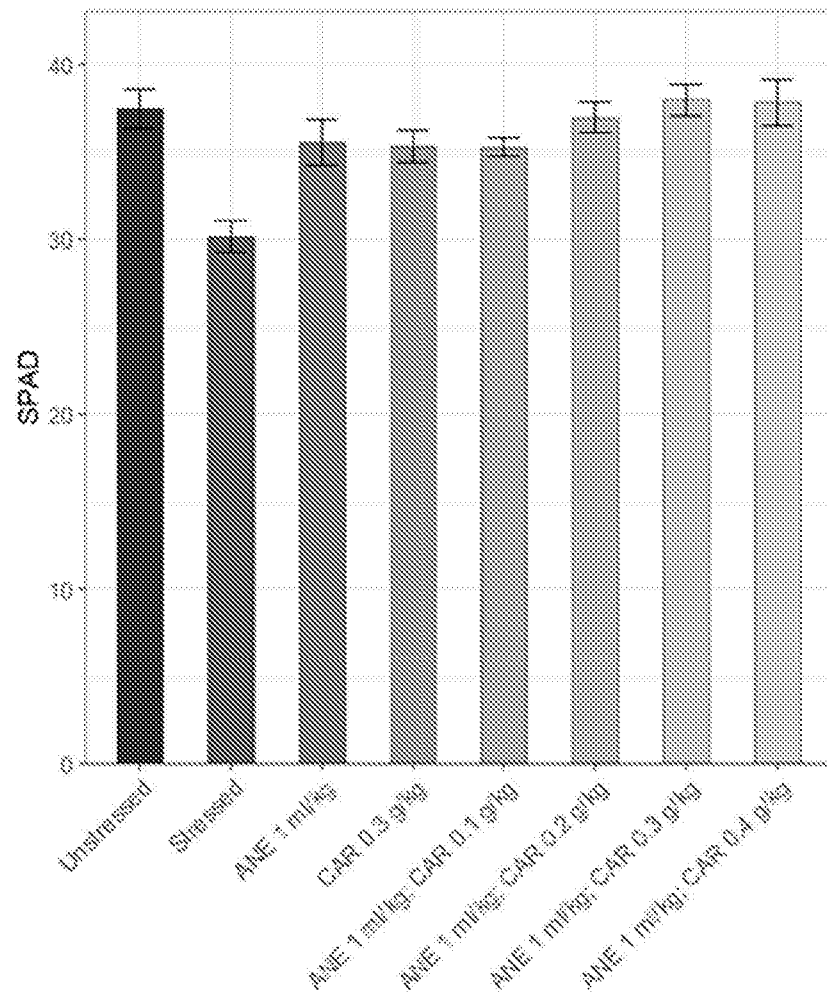


FIGURE 7

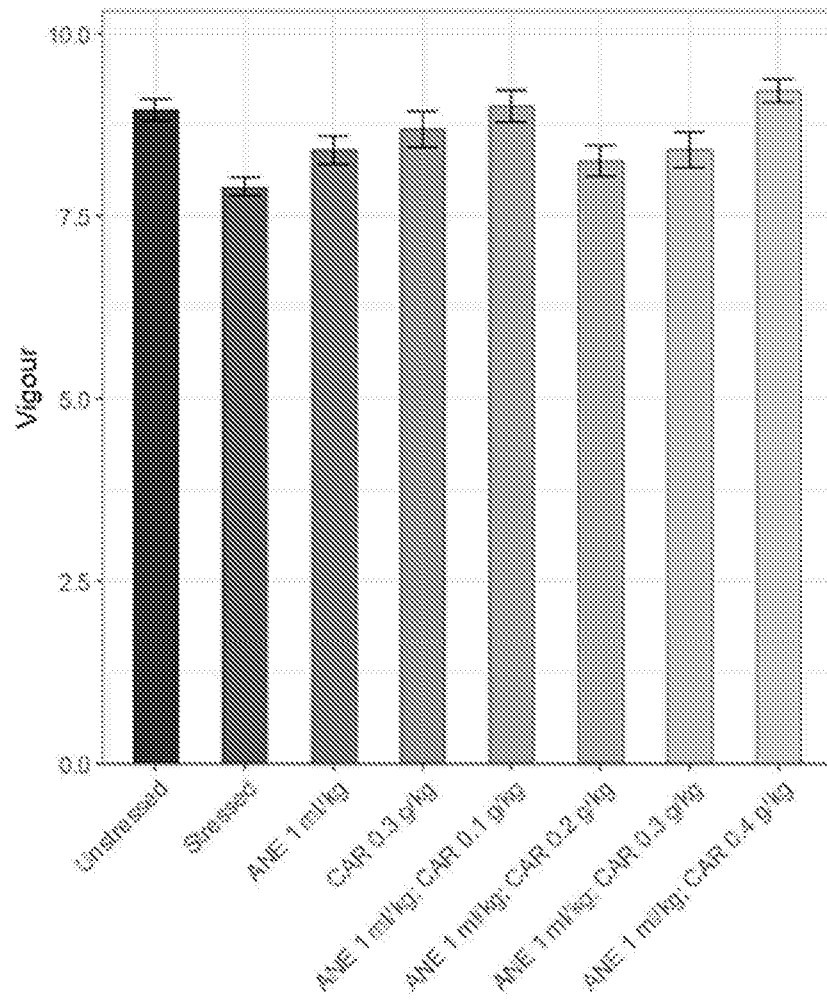


FIGURE 8

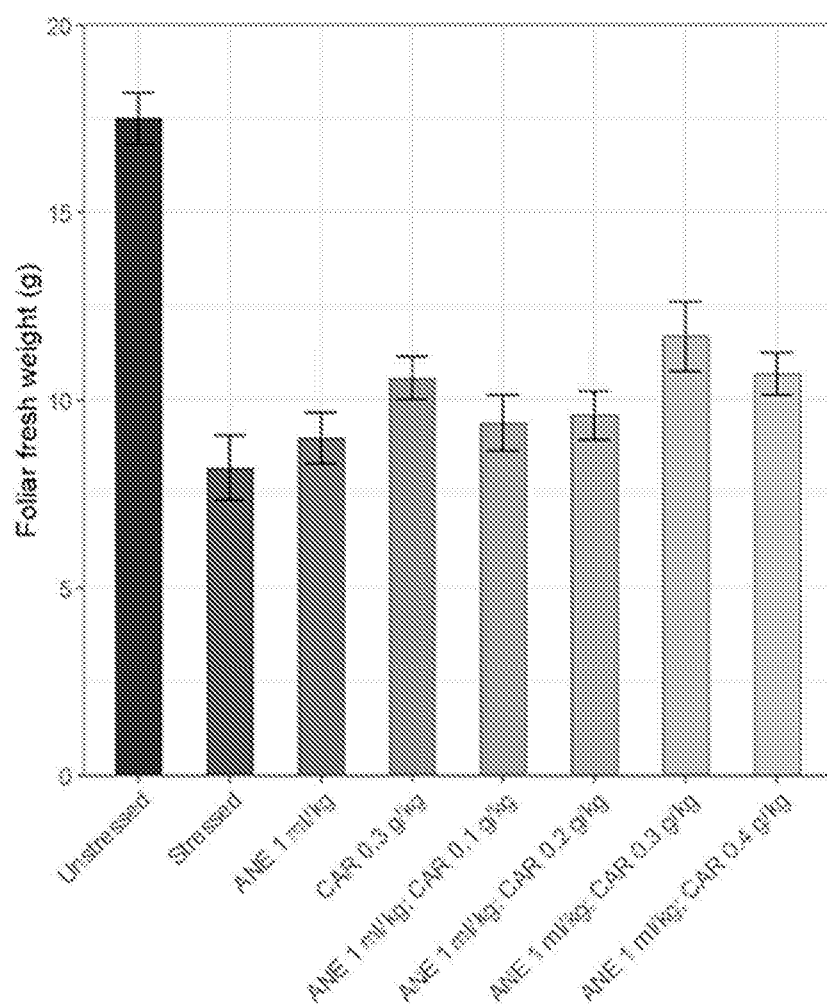


FIGURE 9

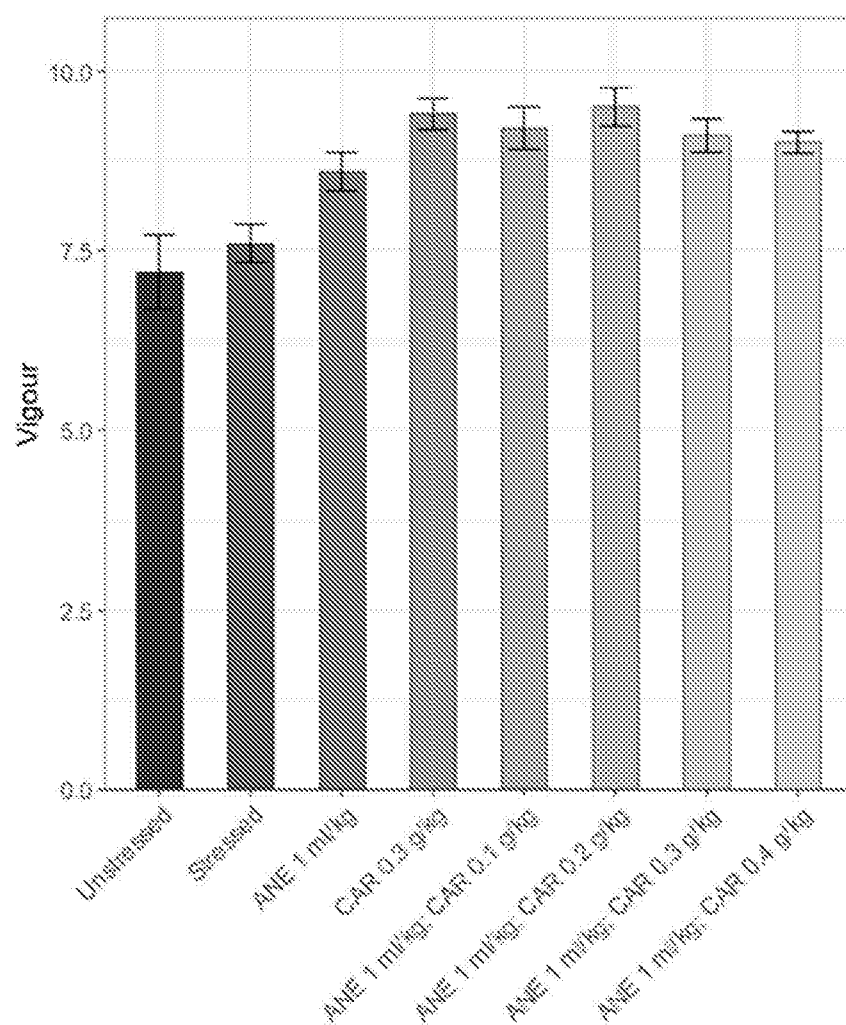


FIGURE 10

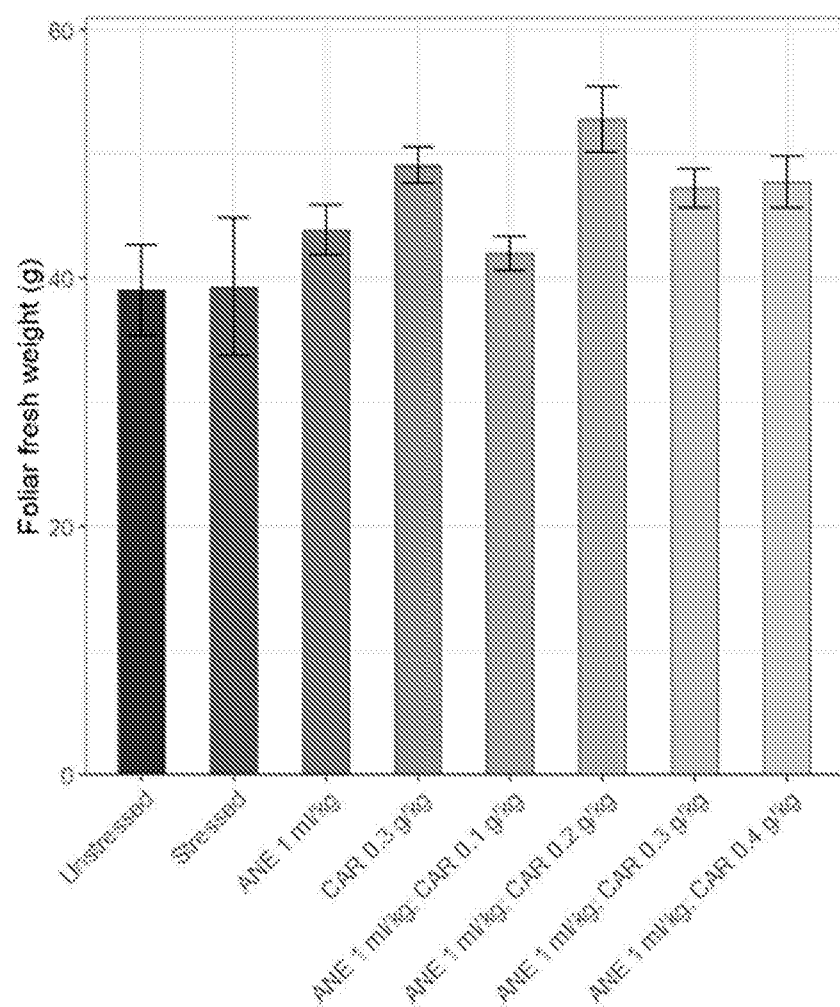


FIGURE 11

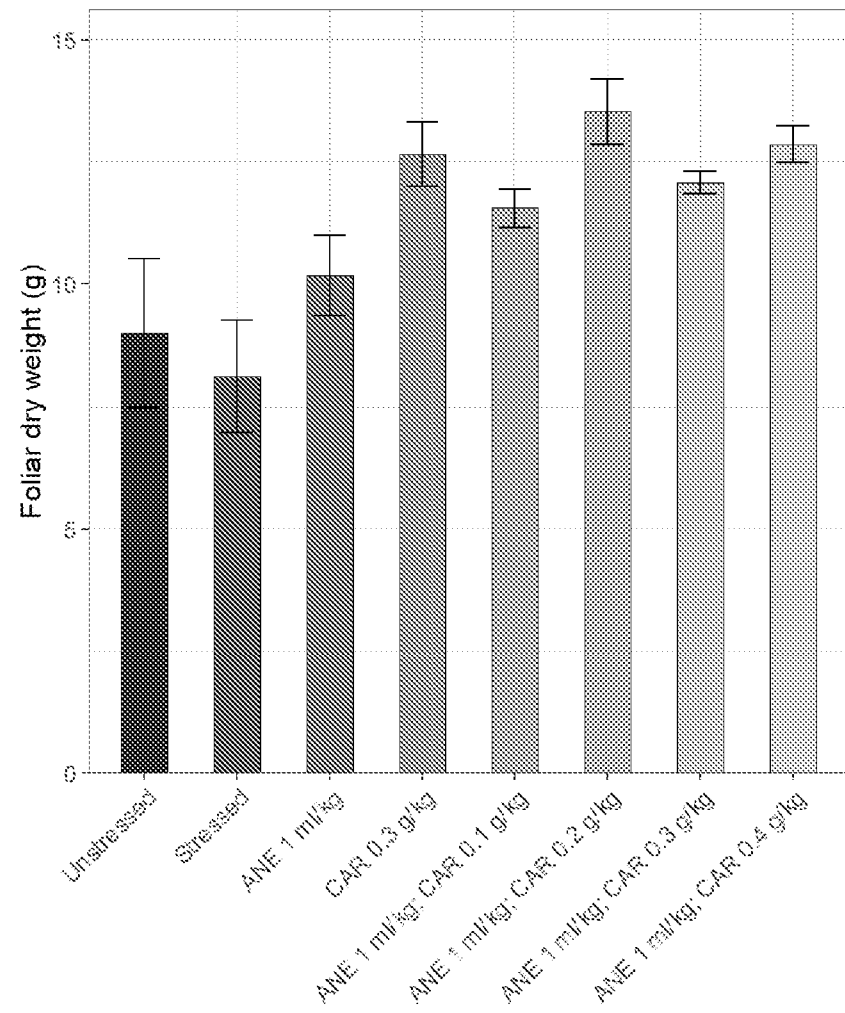


FIGURE 12

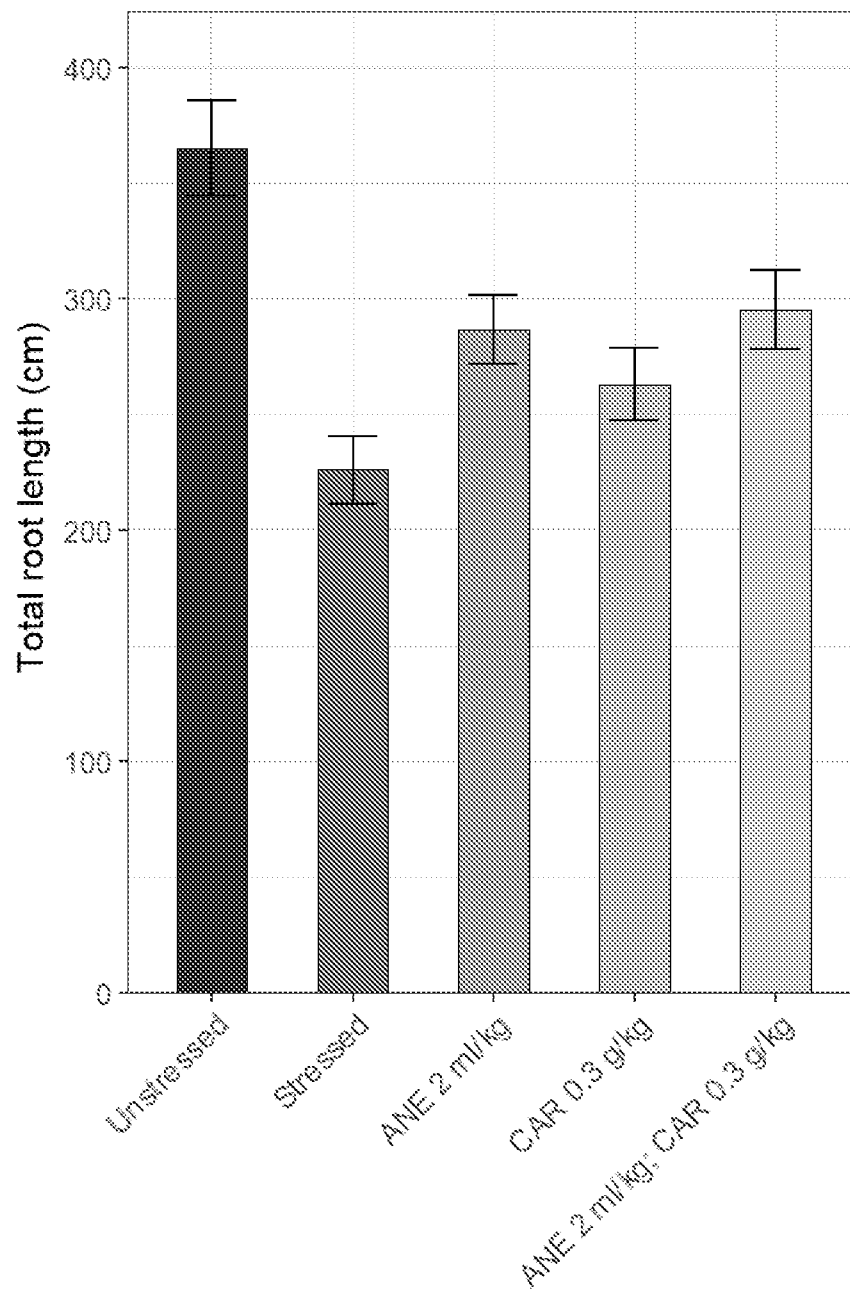


FIGURE 13

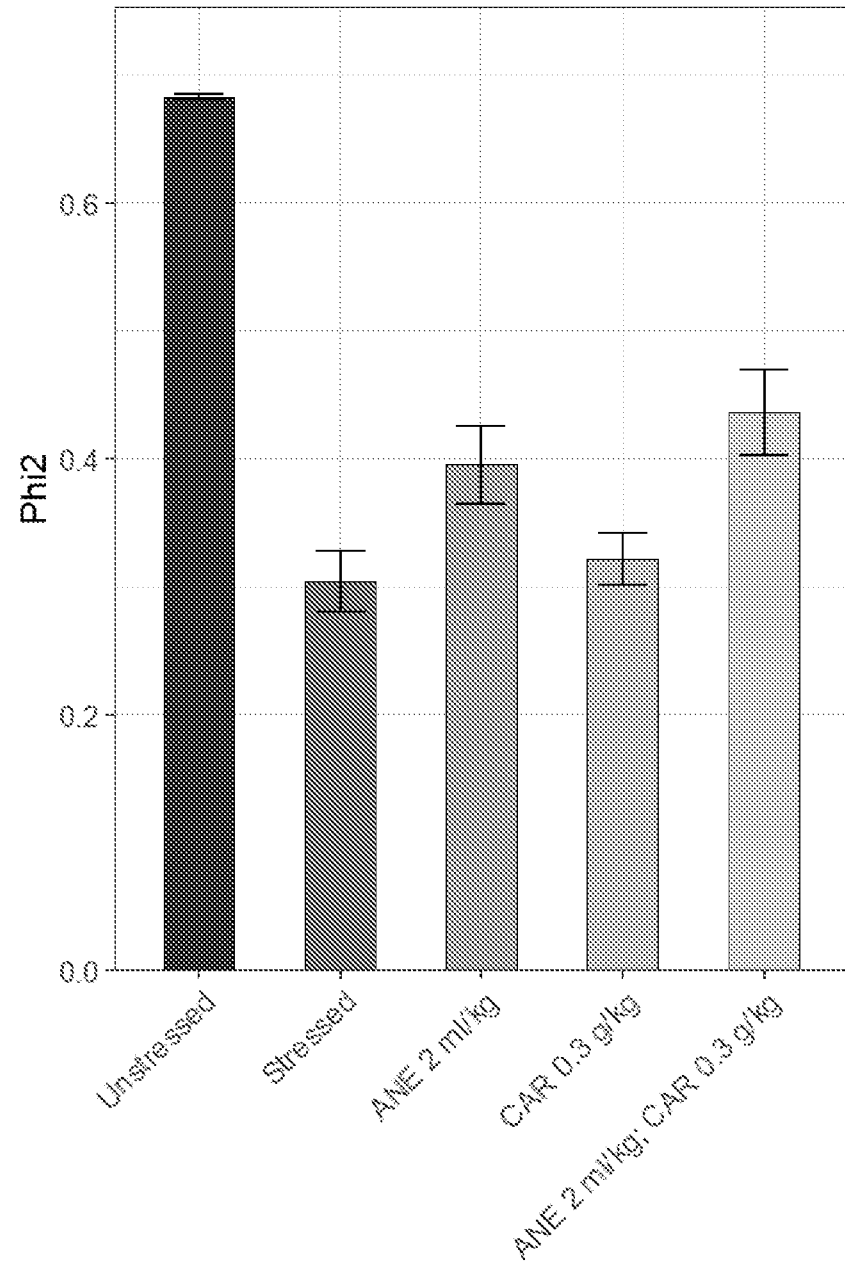


FIGURE 14

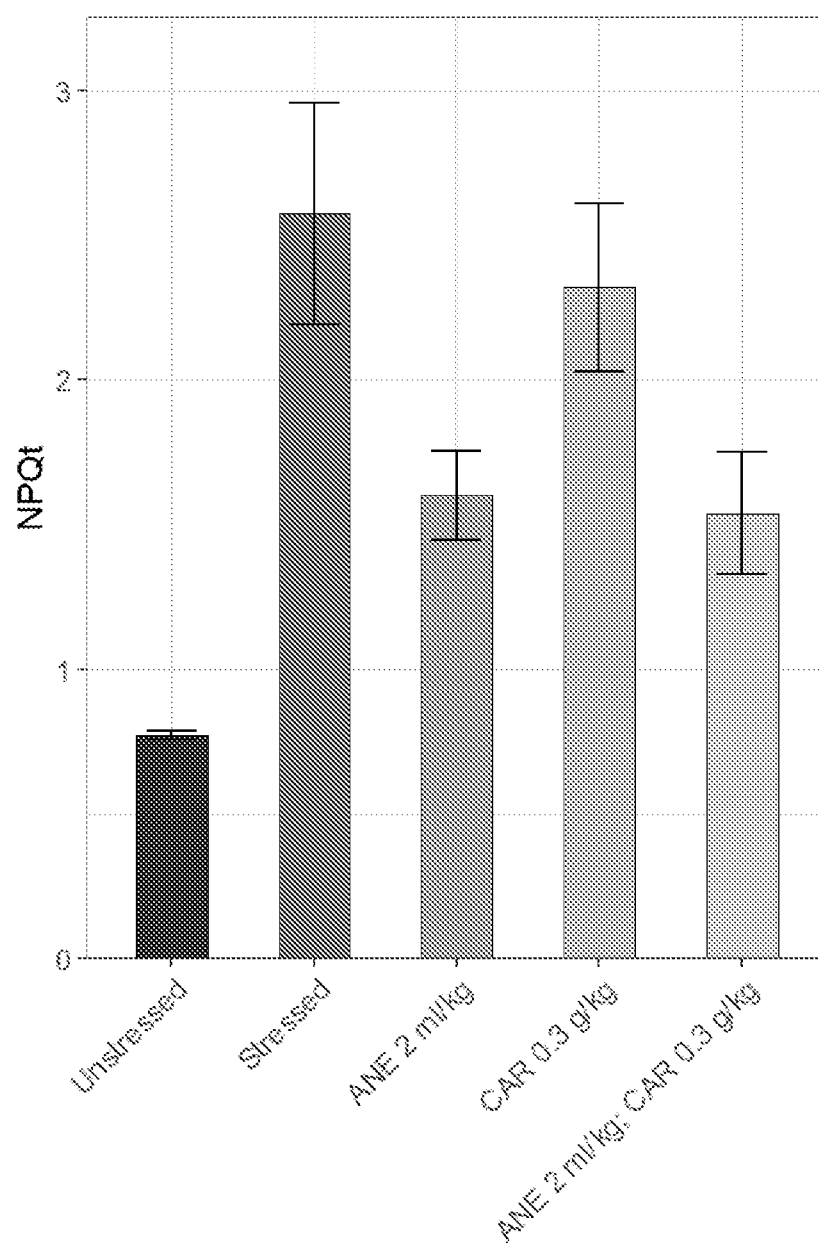


FIGURE 15

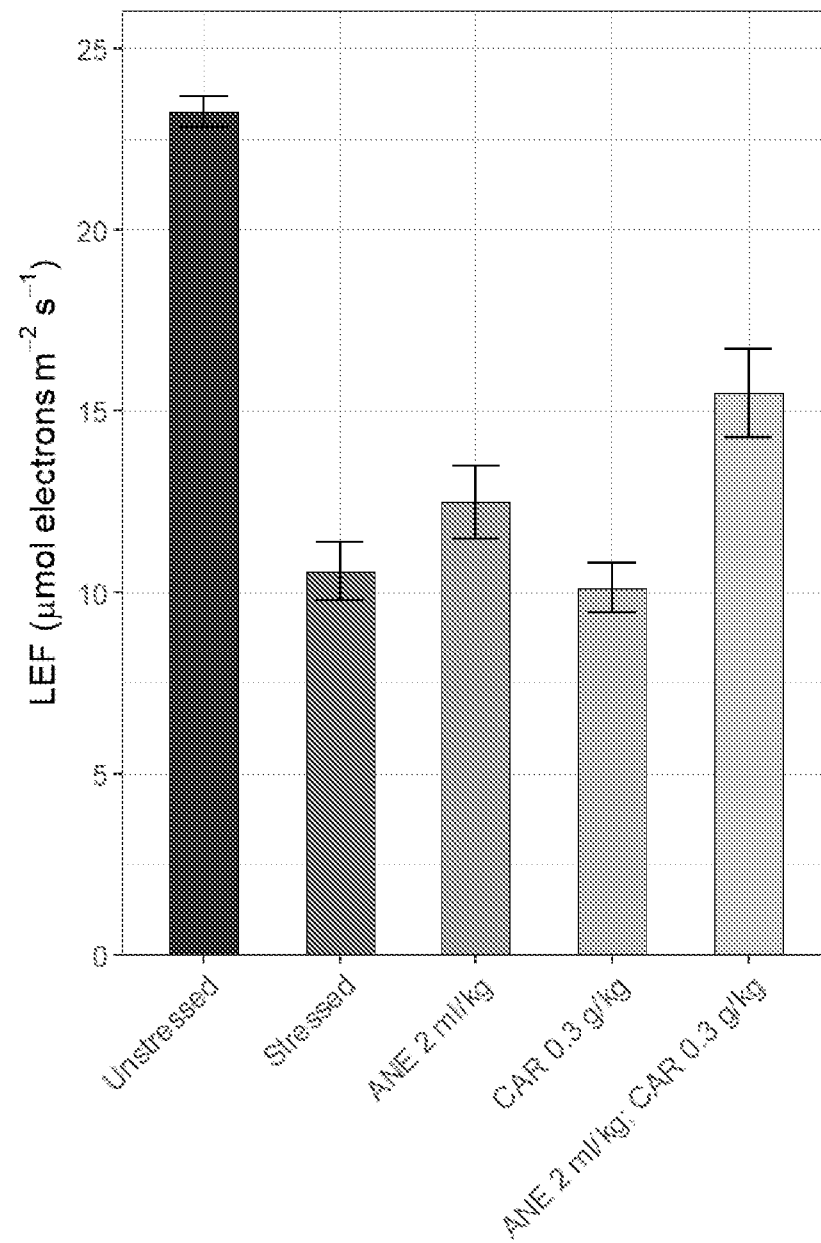


FIGURE 16

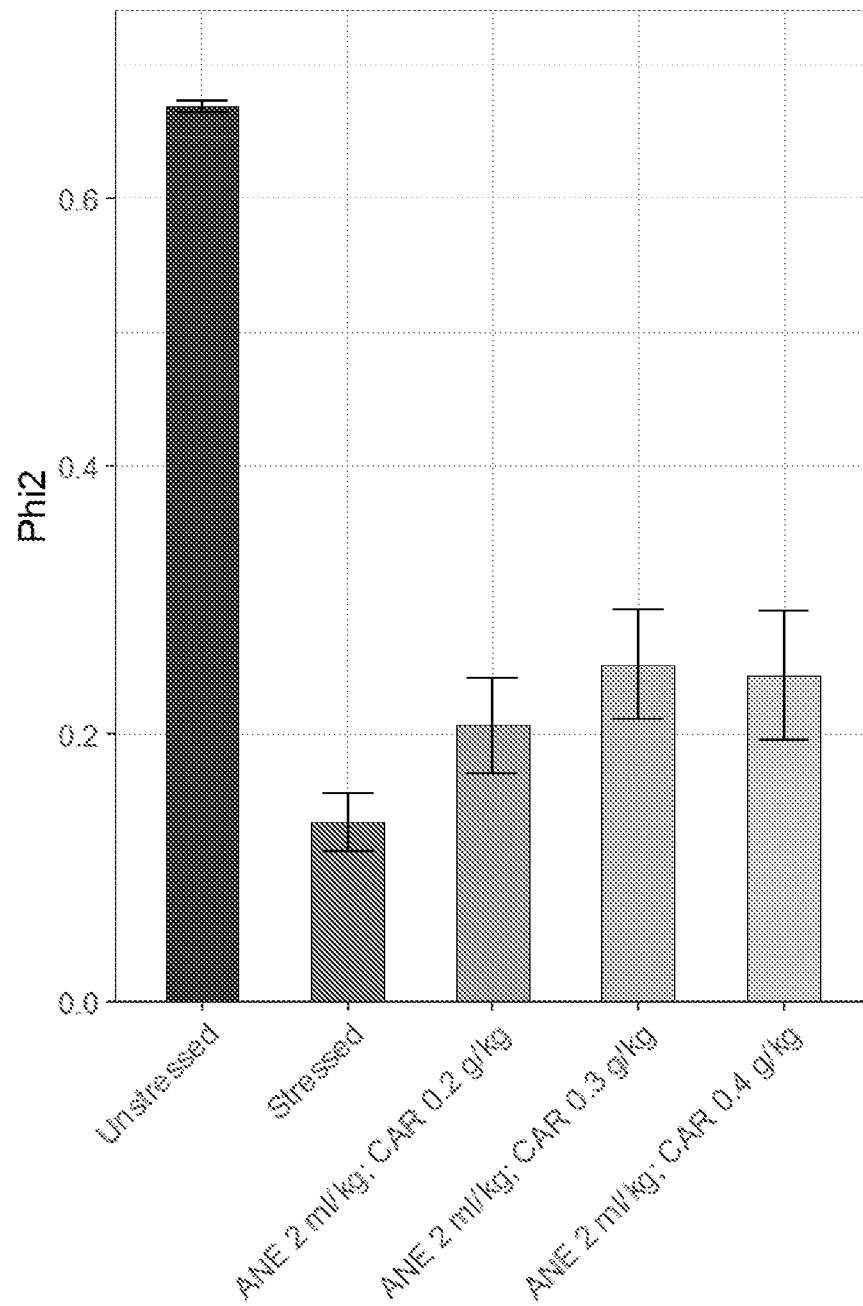


FIGURE 17

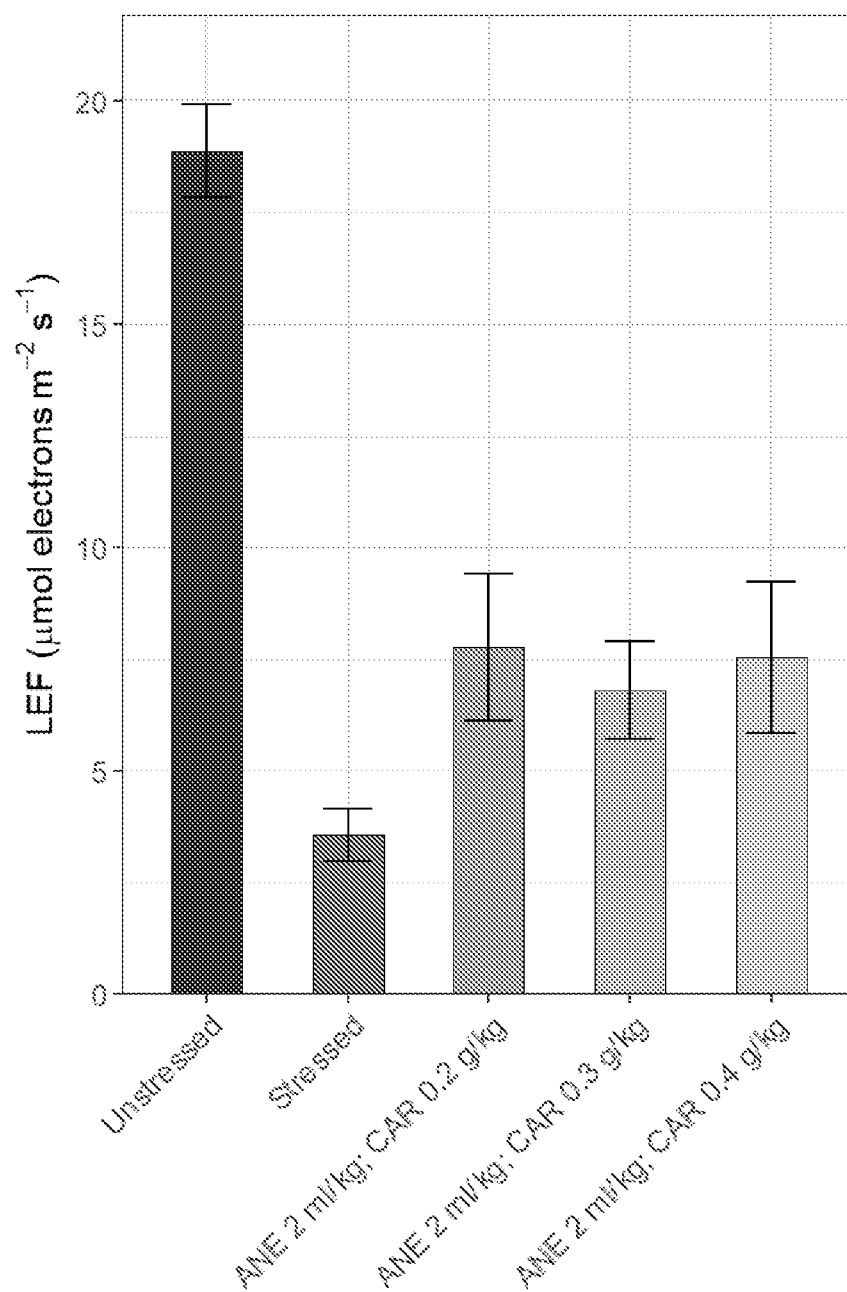


FIGURE 18

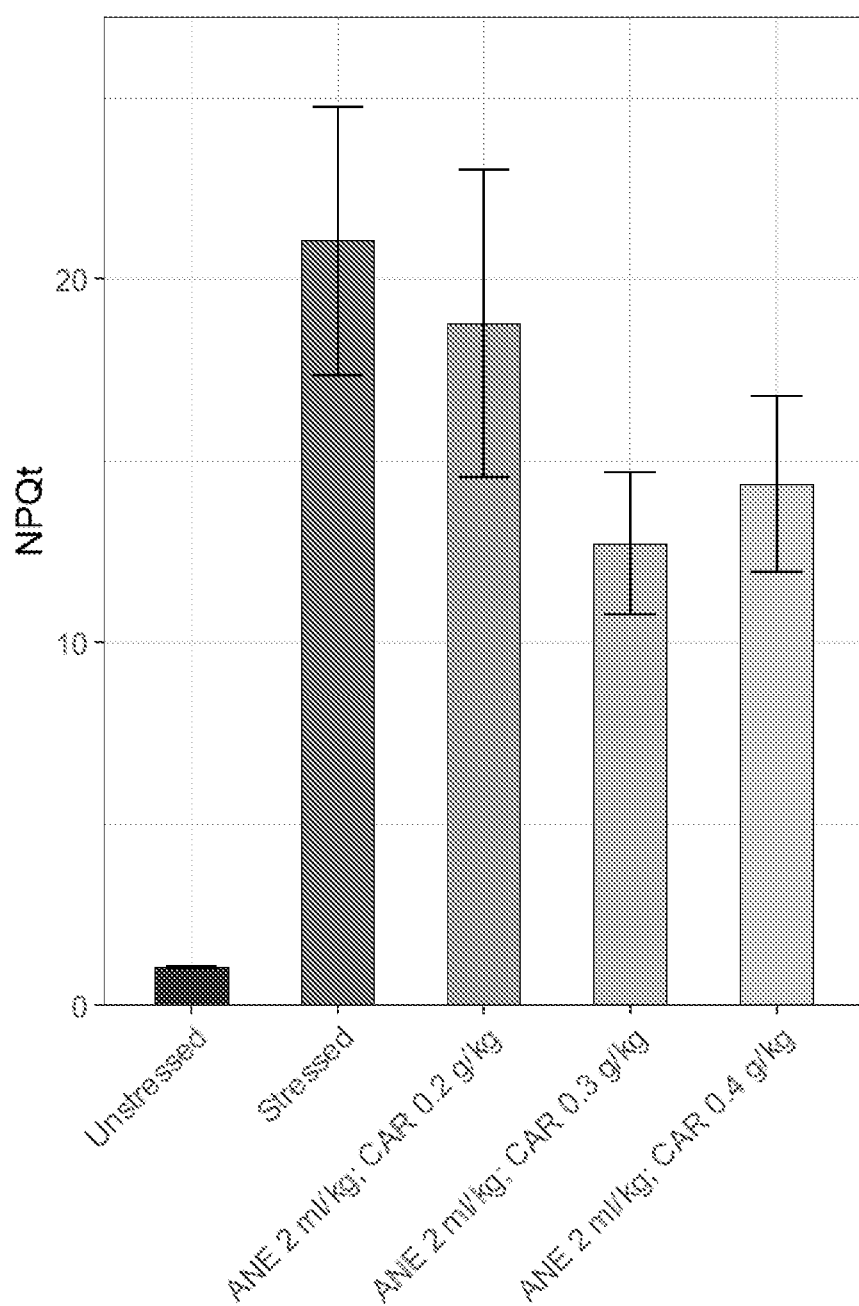


FIGURE 19