Plant growth regulator

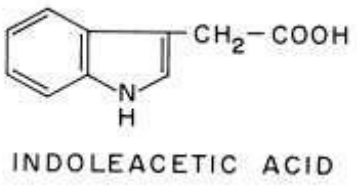
Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Plant hormones1 have also been referred to as 'phytohormones' though this term is infrequently used.

Phytohormones are chemical messengers produced in one part of plant and translocated to the other parts,

Plant hormones identified five major classes: abscisic acid, auxin, cytokinin,ethylene and gibberellins.

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinin, they control the growth of stems, roots, and fruits, and convert stems into flowers.

Indole-3-acetic acid (IAA, 3-IAA) is the most common, naturally occurring, planthormone of the auxin class. It is the best known of the auxins, and has been thesubject of extensive studies by plant physiologists.

Chemical formula of IAA	 INDOLEACETIC ACID $C_{10}H_9NO_2$

Types of stress

1- **Biotic stress** is **stress** that occurs as a result of damage done to an organism by

other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants

2- **Abiotic stress** is defined as the negative impact of non-living factors on the living organisms in a specific environment.

Abiotic stress such as cold, drought, salt, and heavy metals largely influences plant development and crop productivity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses.

physiological
changes1-
carbohydrates
2-proteins

3-amino acid
4-proline

Methods Expressing

Concentration1- Molarity, M = moles solute/liter of solution

2-Normality, N = equivalents of solute/liter of solution

3-ppt part per thousand g/l

4- Parts per million (ppm) mg/l 5-parts per billion (ppb) µg/l 1PPt =1000 **ppm** = 1000000 **ppb Sterilization**

Sterilization (or sterilisation) refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms, prions, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. Sterilization can be achieved through various means, including: heat, chemicals, irradiation,

high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents which are present.

Methods of seed sterilization Methods of seed sterilization 1-

Ethanol 70%

70ml absolute ethanol+30ml dist. H_2O

To surface-sterilize seeds immersed them in 70% ethanol for 2 min., rinsed with distilled water

2-$HgCl_2$ mercuric chloride 0 .1%

Mercuric chloride is highly antimicrobial, with action against both fungi and bacteria, but frequently also kills the seeds/plant materials. At low concentrations (upto 0.1 %) it is perhaps the most effective disinfective agent for seeds with soil-borne and the epiphytic fungi.Treat seeds with 0.1% Mercuric chloride for 2min Then carefully washed 3-4 times with distilled water in order to ensure safe removal of any sterilizing agent

3-H_2O_2 30%

Soak seeds in hydrogen peroxide for five minutes, then rinse them off with water.

4-sodium hypochlorite (NaOCl) . Surface sterilize seeds with 0.5% sodium hypochlorite (NaOCl) for 4-5minutes. Then carefully washed 3-4 times withdistilled water in order to ensure safe removal of any sterilizing agent.

Sterilizing glass Petri dishes

Sterilizing glass Petri dishes depends on the temperature of the oven. It is recommended to place the oven at 160 degrees C for one to two hours, or 180 degrees C for 20 minutes

The role of phytohormones in alleviating salt stress in plants

1- Prepare the following concentration of NaCl by Dilution Law:

0 50 mM 100 mM 150 mM

Prepare 1 M of NaCl as stock solution

$$g = \frac{M*V*M.\ Wt}{1000}$$

M=1 V=1000 ml M. Wt of NaCl = 58.45

g= 58.5 gram of NaCl in 1 liter of dist. Water give 1M OF NaClDilution Law

M*V (before) = M*V (after)

To prepare 0.1 M of NaCl 50 ml from stock solution 1M

1000*V = 50* 1000

V=50 ml take 5ml of stock solution and complete it to 1000 ml with dist. Water (950ml)

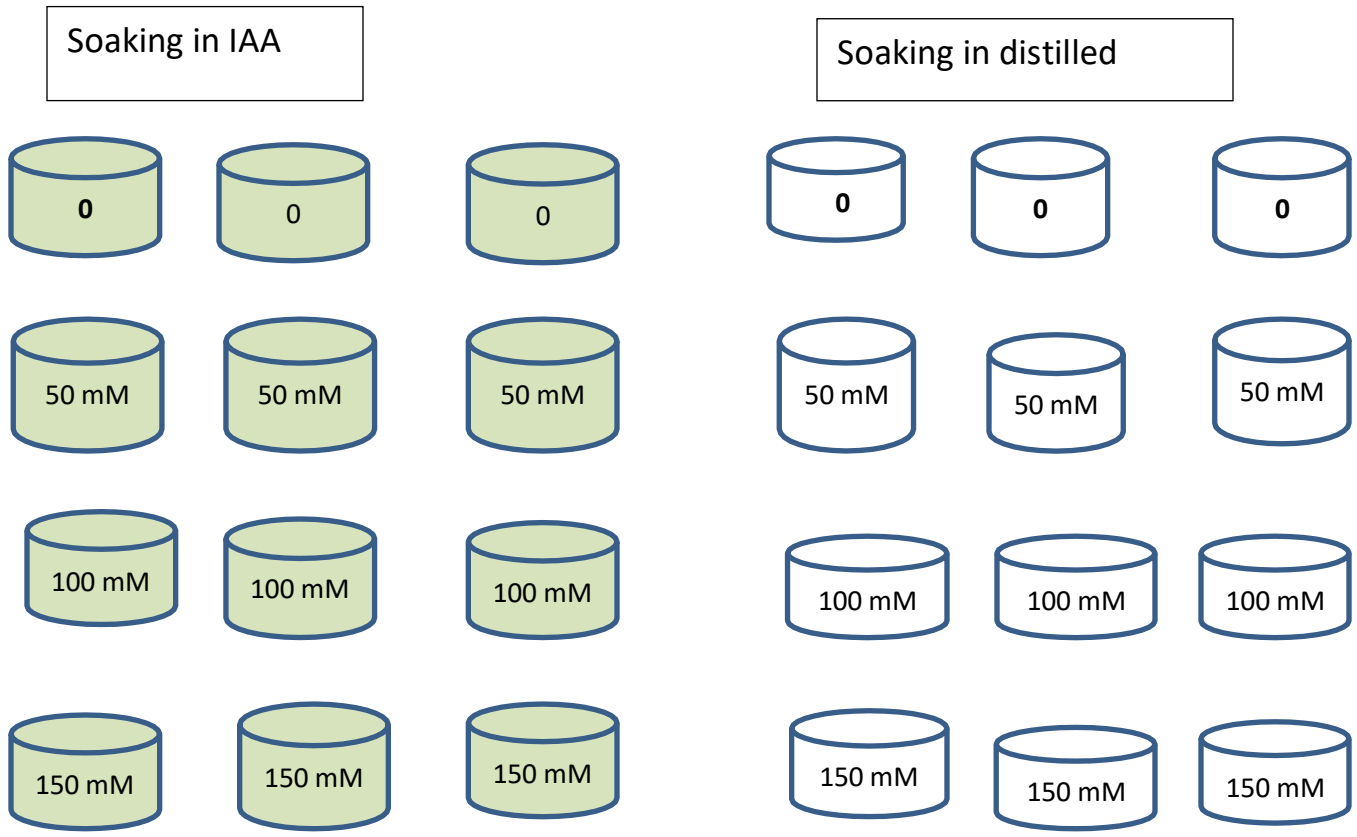
concentration	NaCl volume	dist. Water volume	Final volume
0	0 ml	1000ml	50 ml
50 mM	50 ml	950ml	50 ml
100 mM	100 ml	900 ml	50 ml
150 mM	150 ml	850 ml	50 ml

2- Prepare 100 PPM OF IAA take 100mg of IAA in 1Liter of distilled water

3- Divide the seeds into two groups. The first group is soaked in IAA for 12 hours and the other group is soaked in distilled water for 12 hours.

4- Soaked seeds are taken and placed in petri dishes where a fixed number of seeds are placed in all dishes and irrigated at 30 ml of the appropriate concentration of sodium chloride.

5- Three replicates are made from each concentration



6- Petri dishes are covered and germination rate is calculated daily

7- After germination of most seeds, the dishes open to exposed to light.

8-Leave dishes open until seedlings grow and reach a suitable length

9- Seedlings are taken from each dish and the length of the shoot and root are calculated

10- Then the average is calculated for each dish.

11- Root and shoot lengths of each seedling were measured. Fresh weights of seedling organs (roots or shoots) were determined. The dry weights of roots and shoots of seedlings were determined after drying the freshly harvested organs in an aerated oven at 70°C to constant weight for 48 hr. Then water content was calculated as: water content (%) = (fresh weight – dry weight)/ fresh weight ×100).

Germination rate

Soaking in water							
	1st	2nd	3rd	4th	5th	6th	7th
0							
50 mM							
100 mM							
150 mM							
Soaking in IAA							
	1st	2nd	3rd	4th	5th	6th	7th
0							
50 mM							
100 mM							
150 mM							

Effect of different of NaCl on growth parameter

treatments		Shoot				Root			
Soaking in	**NaCl (mM)**	**Length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**Water content (%)**	**Length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**Water content (%)**
Water	0								
	50 mM								
	100 mM								
	150 mM								
IAA	0								
	50 mM								
	100 mM								
	150 mM								

1-Estimation of photosynthetic pigments

1- Estimation of photosynthetic pigments

The photosynthetic pigments were extracted from a known fresh weight of leaves in 85% aqueous acetone. The extract was taken and diluted by 85% aqueous acetone to a certain volume for spectrophotometric measurements, using spectrophotometer.

-Determination

The photosynthetic pigments which include (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method recommended by **(Metzner, et al., 1965).**

The pigments extracts were measured against a blank of pure 85% an aqueous acetone at three wavelengths of 663,644 and 452.5 nm. Taking in your mind the dilution factor, it was possible to determine the concentration of pigment fractions (Chl.a, Chl.b and carotenoids) as µg/ml using the following equations:

Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$= µg/ml

Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$ = µg/ml

$$\text{Carotenoids} = 4.2\ E_{452.5} - \left\{ \begin{array}{c} 0.0264\ \text{Chl.a} \\ + \\ 0.4260\ \text{Chl.b} \end{array} \right\} = \quad \mu g/ml$$

Finally these pigment fractions were calculated as mg/g dry matter.

2-Water- soluble Carbohydrates

Extraction

To estimate water soluble carbohydrates, a known weight of the dried tissue material was put in 10 ml of distilled water which was boiled in water bath at 100°C for 2 hours, after cooling the hydrolyses was filtered and then completed to definite volume.

Determination

The soluble carbohydrates were determined by the method of anthrone sulphoric acid which was stated by **(Fales, 1951and Schlegel, 1956)** and adopted by **(Badour, 1959)**.

3-Reagents

Anthrone sulphoric acid reagent:

The anthrone sulphoric acid reagent consists of 0.2 gm anthrone,8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml of concentrated H_2SO_4 (D = 1.84).These substances were successively mixed in a beaker under continuous cooling. This reagent must be always freshly prepared.

Procedures:

1- One ml of the plant tissue extract was put in a clean Pyrex test tube of about 16x 160 mm and mixed with anthrone reagent.

2- This sample was heated at 100°C in water bath for 7 minutes, and directly cooled under tap water.

3- The extinction of developed blue green colour was measured at wavelength of 620 nm against a blank, which contained only distilled water and anthrone reagent, using spectrophotometer.

4- A calibration curve using pure glucose was constructed from which it was indicated that one extinction is equivalent to 210 mg glucose. Then 4.5 ml of anthrone reagent was added to 1 ml of the prepared unknown solution in a clean dried test tube.

The carbohydrates content were calculated as mg/g dry weight of the plant organ.

Estimation of proteins

- **Water soluble proteins**

1-Extraction:

Powdered tissue samples (50 mg) were boiled in 10 ml of distilled water for two hours. After cooling, the water extract was centrifuged and the supernatant was decanted and completed to a definite volume using distilled water.

2-Determination:

The soluble proteins were determined according to the method adopted by (**Lowery, et al., 1951)**

Reagents:

Reagent A (2 % Na_2CO_3 in 0.1 N NaOH)

It was prepared by dissolving 2 g of sodium carbonate in 100 ml of 0.1 N NaOH.

Reagent B (0.5 % $CuSO_4$ in 1 % sodium potassium tartarate)

It was prepared by dissolving 0.5 g CuSO4, $5H_2O$ in 1 % sodium potassium tartarate.

The alkaline reagent solution:

It consists of 50 ml of reagent A and 1 ml of reagent B.

This reagent should be always freshly prepared.

Procedures:

1- 5 ml of the alkaline reagent solution were added to 0.1 ml of the test solution (water extract) in a clean test tube.

2- Both were mixed thoroughly and allowed to stand at room temperature for at least 10 minutes.

3- 0.5 ml of the diluted foline-ciocatteau reagent (1:1 v/v) was added to the above mixture and mixed immediately.

4- After 30 minutes the extinction against appropriate blank was measured at 700 nm.A calibration curve was constructed using egg albumin and the data were expressed as mg protein/g dry matter.

◊ Determination of total free amino acids:

Free amino acids were extracted from plant tissues and determined according to the method of **(Moore and Stein, 1948).** However, in this method traces of proline and hydroxyl proline are encountered. A calibration curve was constructed using glycine. The free amino acids concentration was calculate as mg/g dry matter.

◊ Determination of proline

1- Extraction

A definite weight of macerated dry matter tissue was homogenized in 5 ml of 3% sulfosalicylic acid, and then filtered through whatman 2 filter paper.

2-Determination

Free proline was determined according to **(Bates, et al., 1973)**.

Two ml of the filtrate were mixed with 2 ml glacial acetic acid and 2 ml of acid ninhydrin in a test tube for one hour at 100 °C. The reaction mixture was extracted with 4 ml toluene, mixed vigorously in test tube for one 15-20 sec. The chromophore containing toluene was aspirated from aqueous phase and warmed to room temperature. The absorbance was measured at 520 nm using a standard curve and calculated on a dry weight basis as mg proline/g dry matter.