PEG induced drought stress in ash gourd (*Benincasa hispida* (thumb.) Cogn.) seedlings and its mitigation with salicylic acid

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Abstract

Background: Drought is, one of the environmental stresses, plays crucial role in reduction in plant production on majority of agricultural fields in the world. This present study mainly focuses to evaluate the effect of drought stress in ash gourd seedlings and also mitigate its effect with salicylic acid. **Methods:** The 15 days old seedlings were treated with various concentrations of PEG as such 0%,5%,10% and 15%. Anti-stress treatment was given with salicylic acid in seedlings which shown 50% decline in growth (15% PEG) with a concentration of 0.50mM. **Findings**: PEG caused negative influence on plant growth which was mitigated by the salicylic acid pre-soaking treatment. This was accompanying with some physiological processes modification such as enhancing in photosynthetic pigments and metabolites like protein. Moreover, these findings indicate that salicylic acid can be used to improve the plant growth and development under drought conditions. **Novelty and application**: This study discovers that salicylic acid can be applied to improve the growth, photosynthetic pigments and some organic osmolytes of ash gourd plant grown under drought stress conditions. This study will help the farmers to use the SA seed-presoaking doses so as to increase the drought stress tolerance.

1.Introduction

Stress in plants is a condition in which the plant growing in non-optimal or poor state that negatively influences the plant growth, crop productivity, reproductive capacity or death if it exceeds the plant tolerance limits. Abiotic stress factors includes various environmental issues that disturbs plant growth such as light, water-logging, temperature, salinity, drought and heavy metal toxicity, whereas biotic stress factor is a biological harm like pathogen and pest attack, which a plant faces during its life period. Drought is one of the most severe environmental stresses affecting plant productivity. About 80-95% of the fresh biomass of the plant body is comprised of water, which plays a vital role in various physiological processes including many aspects of plant growth, development, and metabolism [1],[4]. The effects of drought in agriculture are aggravated due to the depletion of water resources and the increased food demand from an alarming world population growth [19]. Drought stress in crop plants is characterized by reduced leaf water potential and turgor pressure, stomatal closure, and decreased cell growth and enlargement [7]. Drought stress reduces the plant growth by influencing various physiological as well as biochemical functions such as photosynthesis, chlorophyll synthesis, nutrient metabolism, ion uptake and translocation, respiration, and carbohydrates metabolism ([14] ; [7]; [18]).

This work mainly focuses on preliminary study on effects of drought and drought tolerance of seedlings of ash gourd, (*Benincasa hispida)* which belongs to the family cucurbitaceae. Here, PEG-6000 (polyethylene glycol) was used to induce drought stress. Simulation of drought stress by PEG induces drought stress on the plants. It is reported that PEG induced significant water stress in plants and not having any toxic effects [6].

The application of plant growth regulators known to be involved in the survival from stress related effects. Among the plant growth substances, salicylic acid, cytokinin and abscisic acid have been reported to play a key role in drought tolerance. Under water stress conditions, plant growth regulator treatments can significantly increase the water potential and the chlorophyll content. Salicylic acid (SA) is a phenolic compound involved in the regulation of growth and development of plants, and their responses to biotic and abiotic stress factors [20]. Exogenously sourced SA to stressed plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying was reported to induce major abiotic stress tolerance mechanisms [12] .

The main objectives of present study includes; to study the effect of drought stress on the growth of ash gourd seedlings, to analyze the different morphological, physiological and biochemical activity of the plant under various concentrations of polyethylene glycol, PEG and to mitigate the effects of drought stress by treating with salicylic acid.

2. Materials and methods

2.1 Plant material

Seeds of Ash gourd (*Benincasa hispida*) were bought from Anakkayam seed farm centre at Agricultural research station, Kerala Agricultural University. These seeds were washed with a soap solution (Teepol solution,50% diluted) and later it was surface sterilised with 0.1%(w/v) aqueous solution of mercuric chloride for 5 minutes with constant stirring and washed with distilled water. These seeds were then sowed in 7 polythene bags in such a way that, five seeds per bag. Total four replicates were done for obtaining morphological and biochemical data and were watered regularly twice in a day for 15 days until the seedlings are in a size to induce the stress.

2.2 Drought treatment

15 days old seedlings were treated with different concentrations of 100 ml PEG 6000(Polyethylene glycol) in 5%,15% and 20% concentrations along with control for 4 days. The watering was done at a low rate during this time interval in drought induced seedlings. After the 4 days of drought treatment, the seedlings were uprooted carefully and various parameters such as morphological and biochemical analysis were done.

2.3 Anti-stress treatment

The drought treatment which caused 50% decline in growth (15% PEG) was selected to treat with the growth regulator salicylic acid. Concentration of salicylic acid taken was 0.50 mM which is ideal for crop plants to impose drought tolerance. In this study, seed treatment is opted. Seeds were soaked in 0.50mM salicylic acid for 24 hr and then sowed in the pots. After 15 days, they were treated with PEG to induce drought (15%PEG) along with control. After 4 days, they are uprooted for further studies.

2.4 Morphological/Physiological studies

2.4.1 Determination of shoot length and root length

The length of the shoot and root was measured using a graduated scale and was expressed in centimetres.

2.4.2 Determination of leaf area index

Leaf area index can be measured by using a graph paper. The leaf was traced in a graph paper and calculate the area by adding squares inside it.

2.4.3 Determination of dry weight percentage

Samples were weighed in pre weighed containers using electronic balance (CAY 220). Fresh weight obtained was recorded and the weighed samples were then placed in hot air oven at 100°C for 1 hour, followed by at 60°C overnight. Dry weight of each sample was taken on the next day and drying and weighing were repeated until values became constant and dry weight percentage was calculated.

2.5 Biochemical studies

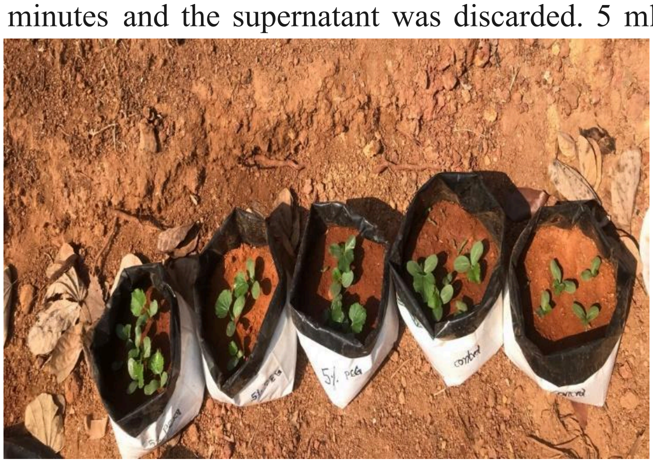
2.5.1 Estimation of pigment composition

Pigment composition of leaves was estimated according to the method of Arnon (1949). Fresh leaves of the control as well as the experimental plants were washed with water and blotted between sheets of filter paper. To estimate the chlorophyll and carotenoids, 80% acetone was used as the extracting medium. One gram of fresh leaf sample was weighed in an electronic balance and weight of each recorded, crushed using chilled mortar and pestle in 5ml of 80% acetone(w/v). Then the homogenate was centrifuged at 5000 rpm for 10 minutes at 4°C and the supernatant was collected. The residue was again washed with 80% acetone and centrifuged. The process was repeated till the pellet become colourless. The final volume of the pooled supernatant was noted. The absorbance was read at 470,646,663 and 750 nm against the solvent blank (80% acetone) in UV-VIS double beam spectrophotometer (ELICO 159). Then the amount of chlorophyll and carotenoid present in the extract was calculated as µg/g fresh weight.

2.5.2 Analysis of metabolites

2.5.2.1 Estimation of Total protein

Protein content of the plant material was estimated using Folin-Ciocalteau reagent according to the method of Lowry et al.,1951. **Extraction**: Fresh tissue weighing 0.5g was macerated in 20% trichloroacetic acid using mortar and pestles. The homogenate was then centrifuged at 600 rpm for 30 minutes and the supernatant was discarded. 5 ml of 0.1 N NaOH was added to the pellet and it was



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saved for the estimation of protein. **Estimation**: To 0.5 ml of the extract, 5 ml of copper reagent C was added (reagent C: mixture of reagents A and B in the 50:1 ratio; Reagent A: 2% Na_2CO_3 in 0.1 N NaOH; Reagent B: equal volume of 1% CuSO4 and 2% sodium potassium tartrate. The tubes were shaken well and allowed to stand in dark for 10 minutes at room temperature. Then 0.5 ml of properly diluted Folin-Ciocalteau was added to the solution and mixed thoroughly. The tubes were kept in dark for 30 minutes for colour development. The absorbance was read in colorimeter. Bovine Serum Albumin Fraction V powder used as standard. The calculations were made in μg/g fresh weight.

Figure.1: Seedlings in various stages of growth: A: 15 days old seedlings B: control (18th day), C: 17 days old seedlings D: 5% PEG treated at 18th day

3. Results

3.1 Standardization of growth and concentration

The seedlings of ash gourd (*Benincasa hispida*) which is treated with various concentrations of PEG along with control were used to elucidate the growth parameters. Pre-treated seedlings with salicylic acid were also analysed for the morphological studies. Various parameters such as shoot length, root length, leaf area index, fresh weight and dry weight were analysed. Analysis and further studies were done on 18th day after germination.

Table 1: Shoot length, root length, leaf area index, fresh weight and dry weight of seedlings grown under different concentrations of PEG and salicylic acid treatment

Concentration of PEG	**Shoot length (cm)**	**Root length (cm)**	**Leaf area Index(cm^2)**	**Fresh weight (g)**	**Dry weight (g)**
Control	7.1098±0.63552	6.3258±0.37558	325.65±0.25487	7.158±0.6125	0.561±0.2365
5%	6.9023±0.58972	4.7636±0.48561	308.48±0.36945	7.252±0.3684	0.494±0.3668
10%	6.3455±0.45583	4.2574±0.56798	312.23±0.25687	6.458±0.5963	0.382±0.5968
15%	5.2287±0.88965	3.9254±0.35654	259.36±0.58967	6.358±0.3621	0.312±0.4896
SA treatment with 15% PEG	7.3225±0.48984	6.7896±0.56896	330.9±0.59874	7.024±0.6354	0.5063±0.5689

The effect of drought on various morphological parameters is shown in table 1. Among the drought induced plants, those treated with 15% PEG, showed approximately 50% reduction in different morphological parameters when compared to control. Upon increasing concentration of PEG from 0-15%, we can see a gradual decrease in the values of characters studied. From the table 1, a highest leaf area could be seen in SA treated plants with a value of 330 cm^2**.** The lowest rate can be observed in 15% PEG with a value of 259 cm^2. This also indicates the mitigation effects of SA. Usually the plant growth is measured in terms of height, weight (both fresh weight and dry weight) etc. and growth is based on two processes, cell division and cell enlargement. Fresh weight and dry weight decreased on increasing concentrations of PEG. An increased fresh weight and dry weight could be seen in control plants as compared to SA treatment. Inducing drought with 15% PEG on 15 days old seedlings emerged from seeds soaked in 0.50mM salicylic acid, shoot length, root length, leaf area index, fresh weight and dry weight got increased than that of plants treated with 15% PEG alone, and is approximately equivalent to control plants. These results indicated that salicylic acid played as drought ameliorating agent in ash gourd seedlings and helped somewhat in stress tolerance.

Figure.2 18 day old seedlings under different concentrations of PEG: A: Control, B: 5% PEG , C: 10% PEG , D: 15% PEG , E: SA treated(15% PEG

C

D

3.2 Pigment composition

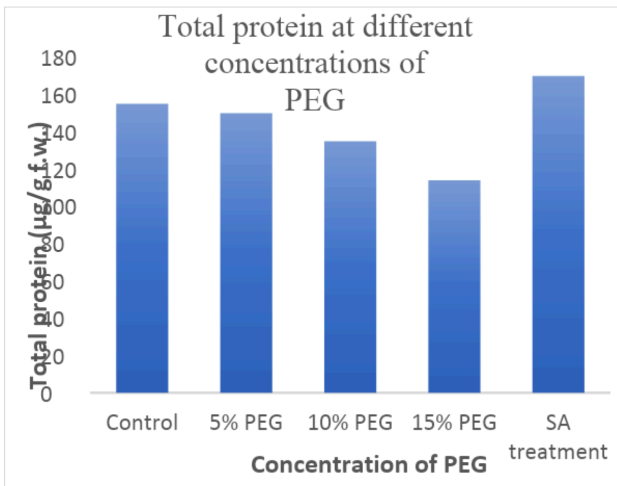
Table 2: The pigment composition (µg/g. fresh weight) of seedlings in different concentration of PEG and SA treatment.

Concentration of PEG	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Control	40.4501±.7585	15.6874±.5896	56.1375±.7857	15.876±.4344
5%	45.3695±.6547	18.3654±.5879	63.7349±.6531	35.476±.6745
10%	43.6872±.5489	14.6398±.4523	58.3273±.5634	17.452±.5748
15%	38.4789±.3256	12.6548±.6547	51.1337±.45647	11.549±.6342
SA treatment (15% PEG)	50.9876±.45678	16.7564±.4536	67.7443±.6578	65.943±.4276

Another plant response to drought stress is the change in photosynthetic pigment content (table 2). The contents of both chlorophyll a and b can change under drought stress. Photosynthetic pigments play important roles in harvesting light. Carotenoids play fundamental roles and help plants to resist drought stress. Amount of chlorophyll a has decreased in increasing the concentration of PEG and an increased amount of chlorophyll a can be observed in seedlings which are treated with salicylic acid. In the case of chlorophyll b, is also decreased along with increasing the concentration of PEG. Carotenoids can be observed as highest amount in salicylic acid treatment and it also decreases along with increasing concentrations of PEG.

3.3 Analysis of protein

The amount of carbohydrate was higher in salicylic acid treated plants when compared to control. The rate of protein can be obtained as decreasing along with increasing the concentrations of PEG. The highest value for protein content is observed in salicylic acid treated and lowest value can be observed in 15% PEG.



4. Discussion

Drought stress has negatively influences on the growth of crop plants and thus decreasing the crop productivity. Thus adopting methods for mitigating the effects of drought stress was very essential for the proper crop growth and production. Drought stress is one of the major factors, which inhibit the growth of a plant. It is well reported that injury in plants takes place due to drought stress at vegetative and reproductive stages of development [5]. Creation of drought stress by application of PEG on the growth of plant showed remarkable deviation from normal levels of moisture. PEG enhances the solute potential in plant, which causes drought stress [25].

In the present study, the application of PEG affects the growth of the ash gourd seedlings in various ways. Similar observation was also observed that PEG decreased the overall growth of plant by inducing drought stress [21]. The plants which are treated with salicylic acid shows an increased value for all morphological and biochemical parameters. Growth is a pattern of change in size, volume, or weight which comprises the stages of cell division, elongation, and differentiation which were affected under drought conditions due to reduced loss of turgor, less energy and reduced enzyme activities ([16];[7];[22]). In this study, the shoot length has a lowest value of 5.22 cm which is treated with 15% PEG. A highest value of 7.32 can be observed in plants treated with salicylic acid along with 15% PEG.

SA treatment also significantly increased shoots and roots fresh weight and dry weight. A stimulation of shoot and root growth by the SA treatment under water stress in cucumber was also reported [3]. SA increased fresh weight and dry weight of shoots and roots in water stress conditions in muskmelon plants [17].

Due to drought stress the comparable results such as reduction in shoot weight, flower fresh weight and dry weights of marigold (*Tagetes erecta* L.) plants were reported [2]. Reduction in leaf area due to loss of turgor and reduced leaf numbers were reported in rice [7]. Drought stress significantly decreases shoot and root dry weights in Asian red sage (*Salvia miltiorrhiza* L.) [18]. Leaf area is a determinant factor in radiation interception, photosynthesis, biomass accumulation, transpiration and energy transfer by crop canopies. It is also important with respect to crop-weed competition and soil erosion [15]. In root length also, a lowest value can be seen in 15% PEG treatment with a value of 3.92 cm and a highest value in plants treated with salicylic acid with a value of 6.78 cm. On the other hand, the external application of SA appeared to ameliorate the influence of drought stress on the growth vigour of shoots and roots of both the controlled and the droughted plants. Leaf, stem and root growth rate are very sensitive to drought stress because they are dependent on cell expansion ([13] [11]).

Photosynthesis is considered to be a vital plant metabolic pathway. The conservation of plant growth under drought stress requires maintenance the right photosynthetic rate [26]. The obtained results showed that drought led to a significant reduction in the photosynthetic pigment contents in the leaves (Table 2). It was observed that the leaves became yellow when they had minimum water potentials in a certain period. These results are in conformity with those obtained with *Plectranthus tenuiflorus* [14]. Drought stress affected photosynthesis by closing of stomata, transfer of CO_2 in chloroplasts and a decrease in cell water potential, which could result in a marked reduction in the plant's productivity [8]. Moreover, the results in Table 2 indicated that the pre-soaking seeds in SA caused significant increase in the photosynthetic pigment contents in stressed as well as nonstressed ash gourd leaves. Furthermore, in other investigations, the application of SA improved the pigment contents in maize, mustard and wheat, under stress conditions.

The reduction in protein concentration was observed, as reduction is directly proportional to the PEG concentration in nutrient medium. The reduction in protein content was may be due to enhancement of hydrolysis of protein [24] or low synthesis of protein [9].

5. Summary and conclusions:

Drought has been considered as one of the most acute abiotic stresses presently affecting agriculture. Drought stress can significantly reduce photosynthesis and stomatal conductance, inhibit photosynthetic pigments synthesis and ultimately lead to reduction in growth of plants [10].

Cucurbits being warm season crops are mainly cultivated during summer season in arid regions, during April to June the environmental temperature increases up to 42 and goes beyond 45 which drastically reduced the yield of cucurbits. In this study, drought caused negative influence on plant growth which was mitigated by the salicylic acid pre-soaking treatment. This was accompanying with modification of some physiological processes such as enhancement in photosynthetic pigments and metabolites like protein. Moreover, these findings indicate that salicylic acid can be used to improve the plant growth and development under drought conditions.

This study discovers that salicylic acid can be applied to improve the growth, photosynthetic pigments and some organic osmolytes of ash gourd plant grown under drought stress conditions and it helps to ameliorate the effects of drought. This study suggests the use of SA treated seeds for cultivation by farmers to reduce the effect of drought stress and thus increases the production and yield.

6. References

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