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Responses of *In-Vitro* Regenerated *Colocasia esculenta* (L.) Schott to PEG induced drought stress

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***Abstract*: The changes in the water potentials in the environment can cause drastic negative effect in the cellular activities of plants. Drought stress being one of the major abiotic stresses results in reduced growth and productivity. Various morphological, biochemical and physiological mitigations occur within the plant system to cope up with effects of such stress. Results of the study intiated to evaluate the responses of the PEG- 6000 induced drought stress in *in-vitro* regenerated plants of Sree Reshmi cultivar of *Colocasia esculenta* are presented here under. The induced stress had the negative effect on fresh and dry weight, plant height, relative water content and total chlorophyll content. Levels of certain oxidative stress markers viz., hydrogen peroxide, proline, malondialdehyde, ascorbic acid and glutathione were elevated relative to control. Antioxidative enzymes, ascorbate peroxidase, gauaicol peroxidase and glutathione reductase were significantly enhanced, whereas, activity of catalase was reduced in time dependent manner. β- Amylase, a metabolic enzyme was moderately enhanced in the stressed plants. The plants were able to rehydrate and grow upon re-watering. The paper discusses contribution of above mentioned factors to abiotic stress response in in-vitro regenerated *Colocasia esculenta*.**

***Keywords*: *Colocasia esculenta*, Sree Reshmi cv. induced water stress, PEG-6000, antioxidant enzymes, drought tolerance.**

I. INTRODUCTION

Drought is defined as a condition with decrease in the availability of soil water. This decreased water availability can be quantified as change in water potential [1]. Decreased water potential makes it more difficult for the plant to take up water which in turn, elicits a range of responses [2]. With an increasing degree of drought stress, osmotic pressure of the soil solution increases, this often results in water shortage, cell dehydration, plant wilting and withering, and occasionally, death [3]. The drought resistance of plants is quite complex process involving multiple players. Different categories of mechanisms confer drought resistance either by avoidance or by tolerance mechanisms [4].

The capability of plants to maintain normal physiological processes under moderate drought stress conditions by adjusting certain morphological structures is referred as Drought Avoidance mechanism [5]. The immediate response to avoid negative effects of water deficit is through leaf rolling. This response of leaves to drought stress is crucial to reduce water loss and promote water use efficiency [6]. The dehydration caused by drought condition is avoided by reducing transpiration through increasing wax accumulation and stomatal closure [7]. The CDPK gene, CPK10 in *Arabidopsis* is reported to mediate stomatal movement via the ABA and Ca^{2+} Signaling pathways in response to drought stress [8]. The water uptake ability is enhanced through a well-developed root system. Beside these changes, cuticular wax and relative water content are also used as avoidance mechanism in plants under drought stress [9].

Plants tolerate severe drought stress through a series of metabolic pathways and through regulation of genes to cope with stress damage [10]. Plants accumulate various organic and inorganic substances (such as sugars, polyols, amino acids and

inorganic ions) to regulate the osmotic potential, thereby protect the enzymes and plasma membrane [11]. Late embryogenesis abundant (LEA) proteins, prevent excessive dehydration of plant tissues, and control the expression of other genes by binding to nucleic acids, aquaporins and molecular chaperones have also been involved in osmotic adjustment in plant cells [12].

Prolonged drought stress leads to accumulation of ROS resulting in Oxidative stress. ROS attack the biological macromolecules to induce lipid peroxidation, protein carbonylation, and DNA damage, resulting in a catastrophic cascade of events [13]. To protect cells against the deleterious effects of excessive ROS, plants have evolved enzymatic and non-enzymatic antioxidant defense machinery to maintain the homeostasis of the intracellular redox state. The antioxidant enzymes include superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, glutathione reductase etc., The non-enzymatic antioxidant system comprises of ascorbic acid, glutathione, carotenoids, α-tocopherol. Certain drought responsive genes are characterized for their functions in drought responses viz., OsMYB2, is found to be associated with increased tolerance to salt, cold, and dehydration stresses in transgenic transporters in rice [14].

Drought stress can be induced either by withholding water or by adding osmoticum to the soil or to the hydroponic solution. One of the apt drought inducer is polyethylene glycol (PEG), a high molecular weight compound, non-penetrating, inert osmoticum. It does not enter the apoplast or the pores of plant cells, thus causes cytrrhysis rather than plasmolysis [15]. It causes blockage of the water movement pathways, reducing water absorption and causing desiccation of the plant [16] by lowering the water potential without being taken up or being phototoxic. It maintains uniform osmotic potential [17-19]. In general, addition of PEG leads to withdrawal of water from the soil which mimics the actual dry soil. Hence, PEG is used frequently as an artificial drought stress inducer in many studies.

Colocasia esculenta (L.) Schott, commonly known as Taro, is one of the crops which utilize less water [20], [21]. Both the quantity and distribution of available water have a significant role on taro growth, which may ultimately interfere with the yield. Globally, taro is the 14th most productive staple crop, with a yield of approximately 12 million tons from 2 million ha of land [22]. The nutritional composition of taro tuber is found to be high in carbohydrates and protein but low in fat. It is a good source of potassium and provides moderate level of phosphorus. The plant contains high amount of vitamin B complex [23], and is rich in minerals and starch. Taro starch, known for its small granule size [24], increases its efficiency in digestion and absorption, because of which it is being used in food industrial applications [25].

Production of taro is often threatened by water scarcity, leading to 90% of yield loss to total crop failure [26]. The severity in water scarcity is expected to have a growing negative impact on this important subsistence crop [27], [3]. There are a few reports on physicochemical and thermal properties [28]; cytotoxic and antimicrobial properties [29]; Antioxidative enzyme studies [30] in different taro genotypes.

Thus, the response of taro to varying water regimes must be critically evaluated to understand the physiological changes, biochemical regulations and ROS defense machineries under induced stress conditions in order to develop a stress-tolerant line. Sree Reshmi cultivar, a low oxalate content cultivar of *Colocasia esculenta*, is widely cultivated throughout the arid and semiarid regions of India, since all the parts are edible. It is scientifically under exploited with a solitary report on *in vitro* regeneration [31]. The results of the present study shed light on the effect of induced water stress by polyethylene glycol-6000.

II. MATERIALS AND METHODS

Plant Source and Drought stress

Tubers of Sree Reshmi cultivar of *Colocasia esculenta* were procured from Centre for Tuber Crop Research Institute, Thiruvananthapuram, Kerala. These tubers were selected as a main plant source for *invitro* studies. Cultures were raised on MS media supplemented with NAA and BAP [31]. Thus obtained *invitro* plants were acclimatized and hardened for 30 days. Drought stress was induced by PEG-6000 for the time interval of 24 h, 48 h and 72 h to the hardened plant. Control plants were watered daily without using PEG.

A factorial experimental design was performed by watering 5% to 25% w/v Polyethylene glycol-6000, possessing osmotic potential of -0.50 to -7.35 bars which was calculated according to Michael and Kauffman [32]. Leaf samples from both treated and control plants were collected after 24 h, 48 h and 72 h of applying stress for screening antioxidants and antioxidant enzymes under different drought regimes. All the experiments were carried out in triplicates.

$$O.P= (1.18\times10^{-2}) C - (1.18\times10\text{-}4) C+ (2.67\times10\text{-}4) C T + (8.39\times10\text{-}7) C 2T$$

O.P= Osmotic potential

C= Concentration of PEG

T = Temperature

Determination of morphological and physiological parameters

Samples from control and stress induced plants were randomly selected to measure the plant height; fresh and dry weight of whole plant was noted to determine the relative water content (RWC) according to the method of Turner and Kramer [33]. Fresh leaf disks of 10 mm diameter were weighed to determine the fresh weight (FW), then soaked in distilled water at 25˚C for 4 h and weighed to get the turgid weight (TW); finally, oven-dried at 80˚C for 24 h to determine the dry weight (DW).

$$RWC = \frac{FW-DW}{TW-DW} X100.$$

FW= Fresh weight

DW=Dry weight

TW=Turgid weight

Estimation of antioxidants and stress markers

Hydrogen peroxide (H_2O_2): Hydrogen peroxide levels in stressed and control samples were determined according to the method of Velikova et al [34]. 500 mg of treated and control samples were homogenized individually in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10,000 rpm for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance was measured at 390 nm.

Ascorbic acid (ASC): Ascorbic acid was estimated according to the procedure of Sadasivam and Manickam [35]. The leaf segments were homogenized in 4% oxalic acid and centrifuged at 10,000 g for 10 min. The assay mixture consisted of 0.1 ml brominated sample extract which was made upto 3.0 ml with distilled water, 1.0 ml of 2% DNPH reagent and 1-2 drops of thiourea. The orange red osazone crystals were formed after incubation at 37^{o}C for 3 hr. This was dissolved by addition of 7.0 ml of 80 % sulphuric acid and absorbance was read at 540 nm.

Glutathione (GSH): Determination of Glutathione (GSH) was done according to Beutler method [36]. The samples were homogenized with 3% metaphosphoric acid. DTNB (5, 5'-dithiobisnitrobenzoic acid) was added to supernatants. 5-thio-2-nitrobenzoic acid, which is formed, is proportional to the total glutathione concentration, which was monitored at 412 nm against control.

Chlorophyll content: Chlorophyll content was determined according to Mackinney [37] using acetone (80%) extracts. The concentrations of total chlorophyll, chlorophyll-a, and -b were calculated by the formula of Arnon [38].

$$\text{mg total chlorophyll/ g tissue} = 20.2(A645) + 8.02\ (A663)\ x\ \frac{V}{1000\ X\ W}$$

A= absorbance at specific wavelengths

V= Final volume of chlorophyll extract in 80% acetone

W= Fresh weight of tissue extracted.

Proline: Proline content was determined according to the method of Bates et al [39]. Free proline was extracted from 0.5 g of fresh samples in 10 ml sulphosalicylic acid (3%) and the extract was filtered through Whatman no. 1 filter paper. A known quantity of the filtrate was mixed with 2 ml of acid ninhydrin reagent. The contents were boiled for 1 h and cooled rapidly on ice. 4 ml toluene was added by vigorous shaking and the organic phase was recorded at 520 nm against toluene as blank. Standard curve was prepared for different concentrations of proline.

Lipid peroxidation: The product of lipid peroxidation was determined according to the method of Heath and Packer [40], with suitable modification. 0.5 g of sample was homogenized with 5.0 ml of 0.1% TCA, 0.5% butylated hydroxytoluene and 1.0% PVP. The homogenate was centrifuged at 12,000 g for 30 min. 4.0 ml of supernatant was mixed with 4.0 ml of

substrate (0.5% thiobarbituric acid and 20% trichloroacetate). The mixture was boiled for 30 min, chilled on ice, and again centrifuged at 12,000 g for 10 min. the absorbance of supernatant was measured at 532 nm and absorbance at 600 nm was subtracted. The malondialdehyde content was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1}.

Assay of Antioxidant Enzymes

Extraction of Enzyme:

Leaf samples were homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 5mM β-mercaptoethanol and 1mM EDTA (Ethylenediaminetetraacetic acid). The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as source of enzymes. Soluble protein was determined with BSA as a standard according to the method of Lowry et al [41].

Catalase (CAT, E.C.No.1.11.1.6)

Catalase activity was assayed by following the decline in optical density of H_2O_2 at 240 nm (ε= 39.4 $M^{-1}cm^{-1}$) according to the method of Aebi [42]. The reaction mixture consisted of 50 µl of enzyme extract and 50mM sodium phosphate buffer (pH 7.0). The reaction was started by addition of H_2O_2, to a final concentration of 10 mM, and its consumption was measured for 2 min. One unit of activity was defined as the amount of enzyme that catalyzes the oxidation of 1µmol H_2O_2 min^{-1} under the assay conditions.

Ascorbate peroxidase (APX, E.C. 1.11.1.11)

The activity of APX was determined spectrophotometrically as described by Webb and Allen [43]. The assay mixture contained 50 mM HEPES buffer (pH 7.0), 1 mM EDTA, 1 mM H_2O_2, 0.5 mM sodium ascorbate, and 50µl of enzyme extract in a total volume of 2.0 ml. The oxidation of ascorbate was followed by a decrease in the A_{290} nm (ε = 2.8 mM^{-1} cm^{-1}). One unit of ascorbate peroxidase is defined as the amount of enzyme necessary to oxidize 1µmol of ascorbate per min at 25ºC.

Guaiacol peroxidase (GPX, E.C.No.1.11.1.7)

The activity of guaiacol peroxidase was measured according to the method of Chance and Maehly [44]. 3.0 ml of reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 100 µl enzyme extract. The formation of tetraguaiacol was determined by an increase in absorbance at 470 nm [ε=26.6 mM^{-1} cm^{-1}]. One unit of peroxidase is defined as the amount of enzyme required to convert 1µmol of H_2O_2 min^{-1} at 25° C.

Glutathione reductase (GR, E.C. No. 1.6.4.2)

Glutathione reductase was assayed according to the method of Carlberg and Mannervik [45] by monitoring oxidation of NADPH at 340 nm (ε= 6220 $M^{-1}cm^{-1}$). The reaction mixture contained 50 mM Tris buffer (pH 7.5), 3 mM $MgCl_2$, 500 nM GSSG, 200 nM NADPH and 250 µl of enzyme extract in a total volume of 1.5 ml. One unit of enzyme is defined as the amount of enzyme that catalyzes the oxidation of 1µmol of NADPH min^{-1} under the assay conditions.

β- Amylase (AMY, E.C. No. 3.2.1.1)

Amylase activity was assayed according to the method of Bernfeld [46]. The reaction mixture consisted 0.5 ml of 2% starch solution prepared in 50 mM phosphate buffer and 0.5 ml of enzyme extract.

Statistical Analysis

All data are expressed in the study are means of triplicate experiments. The mean differences were compared by lowest standard deviations test using GraphPad prism 5.0 software. Differences in mean values were considered significant at $p \leq 0.05$.

III. RESULTS AND DISCUSSIONS

Morphological changes

Growth retardation under stressful condition is a usual phenomenon. Under decreased water potential, plant cell turgor and growth decline [47]. Decrease in plant height may be due to decrease in cell elongation resulting from water shortage [48]. In *Colocasia esculenta*, drought stress caused significant growth retardation in concentration and time-dependent manner. At 25% of PEG stress for 72h, fresh and dry weight (1.9±0.01g and 0.14±0.03g) showed drastic reduction

relative to control (3.2±0.08g and 022±0.04g) (TABLE I), resulting in curling of leaf (Fig. 1). Similarly, plant height also showed gradual decrease with increasing PEG of upto 25% (8.08±0.03cm - 6.34±0.05cm). Savitha et al [49] have reported similar results in *Macrotyloma uniflorum* under 5% to 25% PEG concentration.

Physiological changes

The water status of a plant is highly sensitive to any stress and thus a major factor in determining plant responses to water stress [50]. The RWC of plants is considered as a measure of plant water status, which reflects its metabolic activity in tissues. Hence, it is used as an important index for dehydration tolerance [51]. While control plants exhibited increase in RWC from 87% to 94% over 72h plants stressed with 10% PEG showed reduction to the extent of 76% (Fig. 2). Further increase beyond 15% PEG showed steeper decline in the RWC, similar to observations of Sahoo et al [30], who observed reduction in RWC level under PEG induced drought stress in different taro genotypes.

Total Chorophyll content is the primary visible change during stress, reduction of which causes chlorosis and reduced growth and yield [52], [53]. Drought stress is known to produce reactive oxygen species (ROS), resulting in the lipid peroxidation and finally, destruction of the photosynthetic apparatuses and chlorophyll degradation [54], [55]. A Gradual decrease in chlorophyll under lower PEG (5%) and relatively moderate drop in chlorophyll over 72 h of observation at higher PEG (15-25%) (TABLE II) suggested a stronger protective mechanism against PEG induced drought stress. On the same lines, Mafakheri et al [56] and Hanci and Cebeci [57] have observed decreased Chlorophyll content under water deficit conditions in Chickpea and onion, which also exhibited better tolerance to PEG- induced drought.

Stress markers

Under abiotic stress, plants generate increased level of ROS, of which H_2O_2 is earliest ROS to be produced. Reduction of oxygen in PSII during electron transport chain produces H_2O_2 [58]. A Significant increase in the amount of H_2O_2 with increasing time of exposure and concentration of PEG was observed in *C.esculenta* (TABLE II). *Macrotyloma uniflorum* [49] also exhibited similar response during PEG imposed drought stress. At relatively low concentrations, H_2O_2 can diffuse over long distances causing redox changes in surrounding tissues. Hence, H_2O_2 accumulation may trigger an antioxidative response in the tissue [54].

Increased levels of H_2O_2 through Haber-Weiss reaction produces greater OH^-, which in turn, causes lipid peroxidation leading to the formation of Malondialdehyde (MDA). MDA increased by 2-fold at 15% PEG level during 72 h stress treatment (TABLE II). But on further increase in stress level, not much change was recorded. This may be due to the onset of scavenging activity of the antioxidant system, which peaks at the same time, as observed in *Zea mays*, *Brassica napus*, Pepper cultivar, Verset [59], [60], [50]. Lipid peroxidation continues to spread through the membranes when the capacity to scavenge radicals is saturated [61].

Glutathione (GSH) and Ascorbate (ASC) are the major antioxidants which together can effectively scavenge ROS. GSH protects biological macromolecules against oxidation by acting as a proton donor in presence of ROS, yielding GSSG [62]. ASC can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce hydrogen peroxide to water *via* ascorbate peroxidase reduction. GSH compliments reduction of ASC by donating its electron in GSH-ASC cycle [63]. Imposition of drought stress elevated the levels of both reduced GSH and ASC (TABLE II). The increase in ASC pool under stress suggests its role as a powerful reducing agent, in free radical scavenging. The changes in ASC and GSH level may be due to their interrelationship in Halliwell-Asada cycle, which suggests the tolerance mechanism under stress [64]. Shamsun et al [65] recorded similar ASC-GSH levels in rice.

For the survival under drought stress, plant cells are found to accumulate organic osmolyte, Proline. It balances osmotic condition by buffering cellular redox potential [66]. It acts as a protein stabilizer [67]. Proline level increased proportionately in concentration dependent manner (TABLE II), which indicated that Sree Reshmi cultivar of *C. esculenta* could adapt to drought stress through an osmotic adjustment mechanism. This result is in consonance with Zhang et al [68] for soybeans and Mohammadkhani and Heidari [69] for maize.

Antioxidants enzymes

Scavenging of excess ROS is regulated by an efficient cooperative system of antioxidative enzymes [70], [71]. These enzymes are upregulated under oxidative stress. The detoxification of H_2O_2, a potent ROS, results from the action of peroxidases and catalases. Increased activity of Ascorbate peroxidase (APX) (Fig. 3) and guaiacol peroxidase (GPOX)

(Fig. 4) showed detoxification of H_2O_2 in *C.esculenta*. APX is a key enzyme in ascorbate-glutathione cycle. It catalyzes the reduction of H_2O_2 using ascorbate as an electron donor. GPOX detoxifies H_2O_2 using GSH as a reducing agent. It is associated with defense system in detoxifying ROS, biosynthesis of IAA and wound healing [72]. These results are in good agreement with those observed in drought tolerant faba bean [73]. Unlike peroxidases, catalase activity decreased with time of exposure and concentration of PEG (Fig. 5), suggesting the primacy of scavenging capacity of peroxidases in *C.esculenta*. Similar pattern of CAT activity was observed by Sheoran et al [74] in wheat. Contrary observations made by Boldaji et al [17], who recorded increased activity of CAT enzyme along with GPOX and APX activity in drought stressed alfalfa, is in general agreement with the countervailing activities of POX and CAT under abiotic stress.

Glutathione reductase (GR) is involved in GSH/ASC cycle which provides reducing equivalents to plants. GR catalyzes the reduction of GSSG to GSH, which becomes a reducing equivalent donor for the reduction of dehydroascorbate. Increased activity of GR under drought stress in concentration and time dependent manner in *C.esculenta* (Fig. 6), correlates with the increased levels of reduced GSH, which is associated with better tolerance to stress through GSH/ASC cycle. Kholova et al [71] witnessed similar pattern of GR activity in drought stressed Pearl millet.

Apart from antioxidant enzymes, metabolic enzyme levels are also enhanced during abiotic stress. Cell turgidity and structural integrity of macromolecules are protected by sugars. Stimulation of starch degradation by β- amylase raises the levels of maltose, an osmoprotectant, which protects the cellular integrity under stress [75], [76]. β- amylase activity increased by Two-fold at 25% PEG for 24 h stress exposure. But a moderate activity was observed at later stages of stress exposure in *C esculenta* (Fig. 7), which suggested a better mechanism of protecting structural integrity and well maintained turgidity of the cell under imposed drought stress. Similar findings were reported by Kaplan and Guy [75] in abiotic stressed *Arabidopsis.*

IV. CONCLUSION

In summary, abiotic stress in *C.esculenta* appears to be resisted by efficient operation of ASC-GSH cycle with enhanced levels of antioxidants, ASC, GSH and the antioxidant enzyme, GR. Increased levels of proline suggested a tolerance partly due to osmotic adjustment. The plant is also endowed with antioxidant enzymes, POX and CAT, major ROS detoxifiers. Thus, apparent drought tolerance in the cultivar, Sree Reshmi is due to combined effects of various physiological and biochemical factors, may serve as markers of abiotic stress tolerance *in C. esculenta*, and the cultivar may be good choice for future breeding programs.

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APPENDICES – A

List of Figures:

Fig. 1: Morphological changes under PEG induced drought stress of *C. esculenta* .
A: Control plant; B: Curling of leaf after 48h stress treatment; C: Yellowing of leaf after 72 h of exposure .

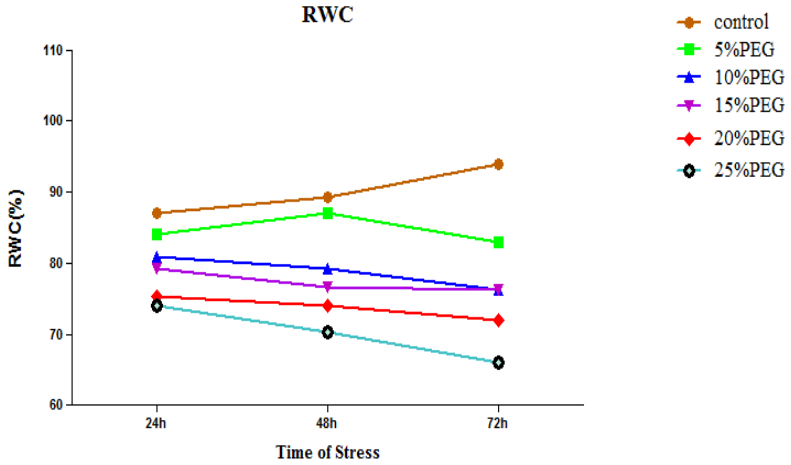

Fig . 2: Relative water content of leaves of *C. esculenta* under 24-72 h PEG induced drought stress

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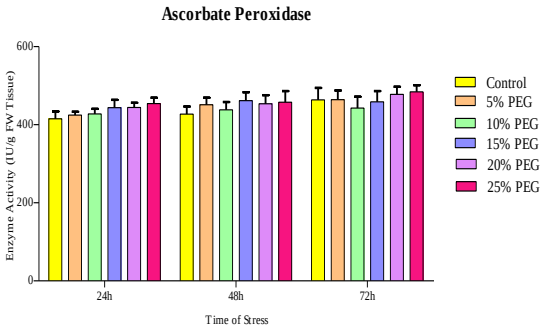

Fig. 3: Effect of drought stress on Ascorbate Peroxidase activity in leaves of Taro. Results are Mean± SE (P ≤ 0.05) obtained from three replicates.

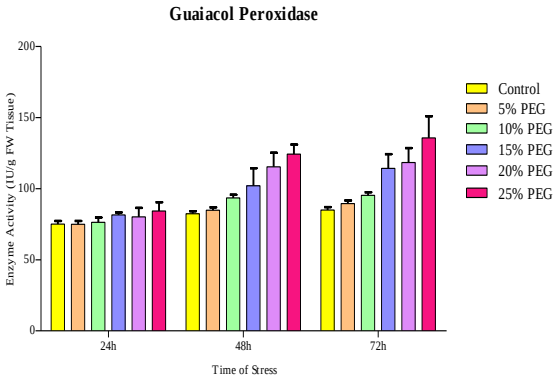

Fig. 4: Effect of drought stress on Guaiacol Peroxidase activity in leaves of Taro. Results are Mean± SE (P ≤ 0.05) obtained from three replicates.

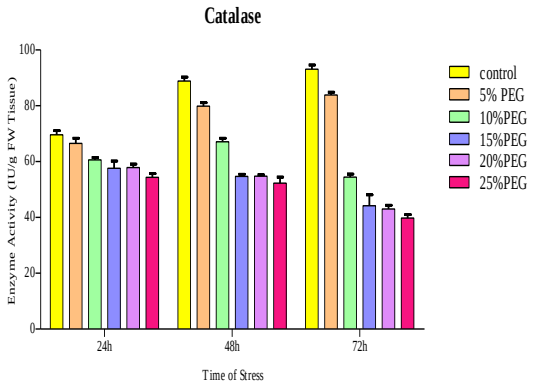

Fig. 5: Effect of drought stress on Catalase activity in leaves of Taro. Results are Mean± SE (P≤0.05), obtained from three replicates.

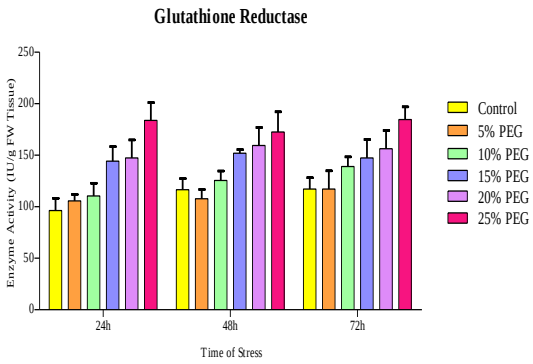

Fig. 6: Effect of drought stress on Glutathione Reductase activity in leaves of Taro. Results are Mean± SE (P≤0.05), obtained from three replicates.

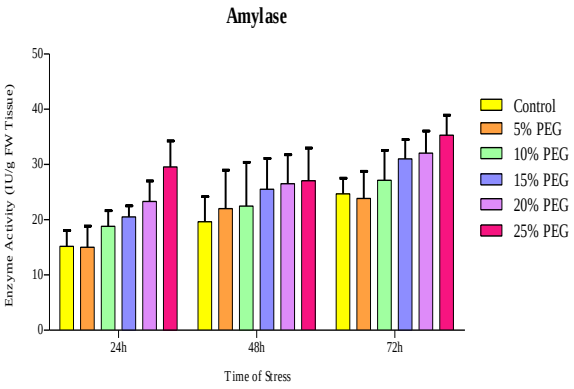

Fig. 7: Effect of drought stress on amylase activity in leaves of Taro. Results are Mean± SE (P≤0.05), obtained from three replicate

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List of Table:

TABLE I: Effect of PEG induced drought stress on morphological paramerters of *C.esculenta*

PEG level	Time Interval	Plant Height (cm)	FW (g)	DW (g)
Control	24h	8.38±0.19	1.8±0.04	0.19±0.05
	48h	8.04±0.11	2.4±0.04	0.20±0.02
	72h	8.16±0.06	3.2±0.08	0.22±0.04
5% PEG	24h	8.08±0.03	1.8±0.02	0.19±0.03
	48h	8.6±0.21	2.3±0.09	0.18±0.08
	72h	8.5±0.18	2.8±0.04	0.18±0.00
10% PEG	24h	7.94±0.04	1.7±0.10	0.17±0.00
	48h	7.86±0.02	2.1±0.05	0.17±0.05
	72h	7.92±0.05	2.6±0.08	0.17±0.02
15% PEG	24h	7.6±0.08	1.4±0.03	0.16±0.06
	48h	7.74±0.06	1.9±0.10	0.16±0.03
	72h	7.68±0.03	2.3±0.00	0.16±0.01
20% PEG	24h	6.88±0.06	1.2±0.04	0.15±0.10
	48h	6.94±0.05	1.7±0.05	0.15±0.10
	72h	6.8±0.04	2.1±0.05	0.15±0.04
25% PEG	24h	6.48±0.05	1.1±0.08	0.14±0.05
	48h	6.3±0.05	1.5±0.03	0.14±0.01
	72h	6.34±0.05	1.9±0.01	0.14±0.03

Leaf samples of *C.esculenta* were stressed for 24-72 h. Results are mean ±SE.

TABLE II: Differential levels of Stress markers in drought induced *C.esculenta*.

Time	Stress Marker	Control	Stress (% of PEG)				
			5	10	15	20	25
24h	H_2O_2 [a]	26.86±1.4	26.49±1.2	28.04±1.8	29.62±1.9	30.56±2.35	29.8±1.14
	Total Chlorophyll [b]	16.23 ±0.45	15.6±0.4	15.06±0.73	14.53±0.31	13.83±0.3	12.43±0.31
	Proline [b]	234.27±6.16	237.63±8.35	245.00±9.43	277.6±7.65	277.60±9.98	348.20±8.68
	MDA [c]	4.24±0.44	4.29±0.59	4.71±0.81	4.99±0.81	5.69±0.78	5.46±1.23
	GSH [a]	71.83±2.74	72.63±4.35	78.07±5.10	81.00±7.82	86.43±6.64	86.27±10.52
	ASC [b]	24.7±1.28	23.3±1.11	23.53±1.21	24.12±0.92	25.0±1.04	25.76±0.6
48h	H_2O_2 [a]	26.52±2.0	27.27±1.4	28.83±1.31	29.74±1.7	31.37±2.49	33.25±1.85
	Total Chlorophyll [b]	17.9±0.45	15.5±0.3	15.1± 0.73	14.13±0.31	13.4±0.45	11.8±0.3
	Proline [b]	227.63±9.56	242.27±6.63	283.63±8.86	334.97±630	334.97±7.68	357.03±7.01
	MDA [c]	3.91±0.20	4.19±0.49	4.30±0.73	7.12±1.01	6.01±0.71	5.73±1.02
	GSH [a]	79.33±2.55	84.80±6.99	94.43±5.21	98.73±9.89	122.20±5.71	135.07±9.48
	ASC [b]	25.7±0.72	24.2±0.69	24.16±0.98	24.17±1.15	25.43±1.19	27.48±0.74
72h	H_2O_2 [a]	28.09±1.5	29.21±1.9	30.27±2.4	33.74±1.68	28.16±1.65	31.47±1.5
	Total Chlorophyll [b]	18.8±0.45	15.1±0.25	14.8±0.3	13.53±0.40	12.43±0.35	11.37±0.42
	Proline [b]	350.60±5.99	372.50±8.39	385.87±9.51	428.20±8.25	428.20±9.10	451.13±9.92
	MDA [c]	3.19±0.60	4.39±0.19	7.13±0.58	7.02±0.97	6.45±0.94	6.12±1.26
	GSH [a]	95.87±1.36	115.37±7.42	125.46±8.64	132.67±7.43	145.87±6.47	164.93±10.56
	ASC [b]	24.26±0.65	24.28±1.37	24.06±1.84	25.27±1.43	25.93±1.64	27.87±1.69

* [a] - µg/g tissue; [b] - mg/g tissue; [c] - m moles/g tissue.