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Effect of Osmotic Stress on Growth, RGR, DM and associated Morphological changes in *in vivo* and *in vitro* plants of *Macrotyloma uniflorum* (Lam.) Verdc.

Savitha M Murthy[1*], Anitha, P.[2] and Tejavathi, D.H.[3]
[1] Department of Botany, Mount Carmel College, Bengaluru 560 052
[2] BMS College for Women, Basavanagudi, Bangalore 560 004
[3] Department of Botany, Bangalore University, Bangalore 560 056
***Corresponding Author**'s e-mail: jeejamurthy@gmail.com

ABSTRACT
Macrotyloma uniflorum (Lam.)Verdc., is a drought tolerant plant which will grow under different drought regimes with some differences in their growth and associated morphological characters. In this work, in vivo and in vitro plants of horse gram were induced with different concentrations of osmotic stress inducer PEG – 6000. The results showed that in comparison to in vivo plants in vitro plants showed better adaptation towards stress. RGR, DM and biomass of the treated in vivo plants were decreased as compared to in in vitro treated plants as the stress increased in the medium. Water stress affects many morphological, physiological and biochemical responses. In this study, the effect of induced water deficit by PEG was evaluated observing the morphological changes in vivo and in vitro plants of Macrotyloma uniflorum. The plants have showed different morphological changes to withstand drought stress.
***Key Words**: Macrotyloma uniflorum, Biomass, PEG, RGR, DM*

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INTRODUCTION

Legumes are second only to the cereals in providing food crops for agriculture. Legumes are very important component of the vegetation of most countries, more particularly so in the tropics and the subtropics. The growth and development of plants depend on the environmental conditions. Plants are exposed to a variety of biotic and abiotic stress such as drought, salt loading and freezing that influence their development, growth and productivity. Plants vary greatly in their capability to tolerate stress conditions, hence some of them are unable to endure stress to wilt and die (sensitive plants) while others can tolerate stress by undergoing certain physiological changes in their tissues which thus maintain their cell water potential, turgidity at normal level, in spite of drought tolerant plants [1].

Drought is one of the most common abiotic stresses reducing the yield of many crops including legumes. Improving crop productivities under conditions of abiotic constraints in field is one of the major concerns in many areas of the world where legumes are grown. Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological, physiological and biochemical characters [2,3]. Selection for drought tolerance therefore, must involve molecular, biological, biochemical and physiological approaches using provocative induction treatments [4].

Plant tissue culture research is multidimensional. Perhaps the most heavily researched area of tissue culture today is the concept of selecting disease, insect or stress resistant plants through tissue culture.

Polyethylene glycol (PEG) has been used to simulate osmotic drought stress in plants. The addition of PEG to the nutrient medium of cultured plant cells similarly simulates water stress by acting as a non-penetrating osmotic agent which lowers the water potential of the medium in which the cells are growing [5].

Drought stress effects on the morphological aspects under *in vitro* conditions in this crop have not been studied previously. The main objective of this study was to evaluate the influence of PEG *in vitro* and its comparison with *in vivo* conditions on morphological aspects with the objective of selecting surviving cell

lines under different levels of PEG stress under *in vitro* conditions and study their morphological characters.

MATERIALS AND METHODS

Seeds of *Macrotyloma uniflorum* (Lam.) Verdc, var. PHG-9 were procured from GKVK, University of Agricultural Sciences, Bangalore. Healthy seedlings were developed and maintained in the poly house, Department of Botany, Bangalore University and which served as *in vivo* plants for further studies and the seeds were also raised in test tubes on moist filter paper bridges which served as *in vitro* seedlings for further studies.

L_2 medium [6] was selected for establishment of cultures under both normal and treated conditions. Osmotic/water stress inducing chemicals such as polyethylene glycol (PEG Mol. Wt = 6000), were added at a concentration ranging from 5% to 25% (PEG) to aseptic medium.

The morphological and yield characters were studied in control and stress induced in vivo and in vitro plants after 45 days of planting. Morphological characters like plant height, number of nodes, number of leaves, biomass, leaf area index, stomatal index, relative growth rate (RGR) and dry matter (DM) were observed and recorded both in *invitro* and in vivo plants using standard methods. The heights of the plant were recorded from the ground level to the top of the canopy, number of leaves and nodes per plant were calculated. Biomass was calculated by taking the fresh and dry weight of the plants. LAI was calculated by Stickler *et al.,* [7] and SI was by Stace, [8]. RGR (%) with respect to biomass of the plant both in treated and normal *in vivo* and *in vitro* plants were calculated using the formula [9].

$$RGR = \frac{FW_2 - FW_1}{No.\ of\ days} \times 100$$

Where, FW_1 = Initial fresh weight

FW_2 = final fresh weight

Dry matter percentage was calculated using the formula followed by Sakthivelu *et al*., 2008.

$$DM = \frac{DW_2}{FW_2} \times 100$$

Where, DW_2 = final dry weight

FW_2 = final fresh weight

RESULTS

When explants were inoculated on L_2 medium supplemented with IBA (2.45uM) and BAP (8.88uM), proliferation of multiple shoots were observed. These served as control in vitro plants. On the same medium, when PEG was supplemented at a concentration ranging from 5% to 25%, multiple shoots were developed. These multiple shoots were later transferred to rooting medium and well established rooted plants were acclimatized. These regenerated plants selected for morphological characters.

Similarly, the seeds were grown in pots and were fed with PEG solutions of concentration ranging from 5% to 25%. These served as *in vivo* treated plants. Morphological observations were recorded and comparative study was made between *in vivo* and *in vitro* plants.

Morphological characters

All readings were taken after 45 days of planting for in vivo plants and 3rd subculture for in vitro plants.

Plant height: the height of the plant was recorded in cm in both normal and treated *in vivo* and *in vitro* plants. Though stress was induced in *invivo* plants, there was not much significant difference among the treatments. Where as in vitro plants showed significant difference.

Number of nodes per plant:

It was found that the number of nodes was more in *invivo* untreated plant (6.6), in comparison to *in vitro* untreated plant (2.8). But a significant difference was observed between untreated and treated *in vitro* and *in vivo* plants. There was decrease in nodal number from 1.8 to 1.4 at a concentration of PEG varying from 5% to 25% without much difference in *invitro* plants.

Number of leaves per plant:

Decrease in the number of leaves was observed within the treated plants. In 5% PEG treated *in vitro* plants, number of leaves present was 13 where as in 25% PEG treated *in vitro* plants, only 6 leaves were present. Where as in *in vivo* 5% PEG treated *in vivo* plants 20 leaves were present and in 25% PEG treated *in vivo* plants 12 leaves were present and significant differences were observed between the treatments.

Biomass

The fresh and dry weight of untreated *in vivo* plant was 1500 mg and 60 mg and 836 mg and 50 mg in untreated *in vitro* plant. The fresh weight of the treated *in vivo* plant ranged from 1180 mg to 780 mg at a range of 5% to 25% PEG treatments where as it was 678 mg to 443 mg from 5% to 25% PEG treatments in *in vitro* plants. There was a gradual reduction in the fresh weight of the plants as the concentration of the PEG increased. In general, the treated *in vivo* plants showed better growth compared to the treated *in vitro* plants.

Leaf Area Index (LAI)

The surface area of the leaf was decreased in treated *in vivo* and *in vitro* plants. The LA was much reduced in *in vitro* plants compared to *in vivo* plants. There was a dramatical decrease in the LAI of the treated plants both in *in vivo* and *in vitro* plants ranging from 24.61cm^2 to 20.45cm^2 from 5% to 25% PEG treated *in vivo* plants and 22.22cm^2 to 15.68cm^2 at 5% to 25% PEG treated *in vitro* plants. The LAI remains similar both in untreated *in vivo* and *in vitro* plants.

Stomatal Index (SI)

SI of the leaf was more in untreated *in vivo* and *in vitro* plants. The treated plants showed a tendency of decrease in SI depending on the concentration of PEG. As the concentration of PEG increased, there was a decrease in SI of the leaf both in *in vivo* and *in vitro* plants. Not much significant difference was observed with in *in vitro* and *in vivo* treated plants.

Relative Growth Rate (RGR%)

Relative Growth Rate was more in untreated plants compared to treated plants. There was a tremendous decrease in the RGR in treated plants. RGR was rapid in normal plants and gradually became less in treated plants. RGR was 0.26% in terms of percentage in 5% PEG plants compared to 25% PEG plants where the RGR was 0.06% in *invitro* plants and RGR was 0.275 in 5% PEG plants and 0.12% in 25% PEG plants under *in vivo* conditions. In general, RGR was more in untreated *in vivo* and *in vitro* plants compared to treated *in vivo* and *in vitro* plants.

Dry Matter Percentage (DM%)

Dry matter percentage was calculated for treated and untreated plants. The initial fresh weight was recorded after 15 days of culture and the final fresh weight was recorded after 3rd and last subculture for both treated and untreated plants under *in vitro* conditions. The initial fresh weight was recorded after 7 days of germination and the final fresh weight was recorded after 45 days of germination in *in vivo* plants. The final fresh weights of the untreated *in vivo* plants were 1500 mg and 836 mg in *in vitro* plants, with 60 mg and 50 mg dry weight respectively. The final fresh weight of the *in vitro* treated plants decreased from 678 mg to 443 mg in 5% to 25% PEG treatments and 1180 mg to 780 mg at 5% to 25% PEG treatments in *in vivo* plants. The dry matter percentage was 13.19% in untreated *in vitro* plants and gradually decreased from 8.62% to 5.66% at a range of 5% to 25% PEG treatments with a slight increase in 15% PEG treated plants. Likewise, the dry matter percentage was 14.2% in untreated *in vivo* plants and gradually decreased from 8.20% to 5.26% at a range of 5% to 25% PEG treated *in vivo* plants.

In general, almost in all morphological observations, in vitro treated plants showed better tolerance towards stress compared to in vivo plants.

Table: 1 Effect of induced water stress on plant height, number of nodes and number of leaves per plant in *In vitro* and *In vivo* plants of *Macrotyloma uniflorum*

Parameter	Plant Height (cms)						No. of nodes / plant						No. of leaves / plant					
Treatment	*In vitro* plants			*In vivo* plants			*In vitro* plants			*In vivo* plants			*In vitro* plants			*In vivo* plants		
	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE
Control	2.6	±	0.11^a	25	±	0.20^a	2.8	±	0.20^a	6.6	±	0.60^b	15	±	0.67^a	25	±	0.99^a
5% PEG	2.2	±	0.40^b	25	±	0.48^a	1.8	±	0.37$_b$	4.8	±	0.58^a	13	±	0.48^b	20	±	0.67^b
10% PEG	1.9	±	0.04^c	22	±	0.17^b	1.6	±	0.24^b	2.6	±	0.40^c	11	±	0.74^c	19	±	0 .60^b
15% PEG	1.8	±	0.03^c	22	±	0.12^b	1.4	±	0.24^b	2.4	±	0.40^c	9	±	0.44^d	16	±	0.60^c
20% PEG	1.6	±	0.05^d	22	±	0.17^b	1.2	±	0.20^b	2.2	±	0.20^c	6	±	0.73^e	15	±	0.66^c
25% PEG	1.4	±	0.05^e	21	±	0.08^b	1.4	±	0.00^c	1.6	±	0.24^d	6	±	0.37^e	12	±	0.99^d

The numbers followed by same superscribed alphabets did not differ significantly as determined by Duncan Multiple Range Test ($p<0.05$)

Fig.1 Effect of PEG on Leaf area Index of *in vitro, in vivo* leaves and callus of *Macrotyloma uniflorum*

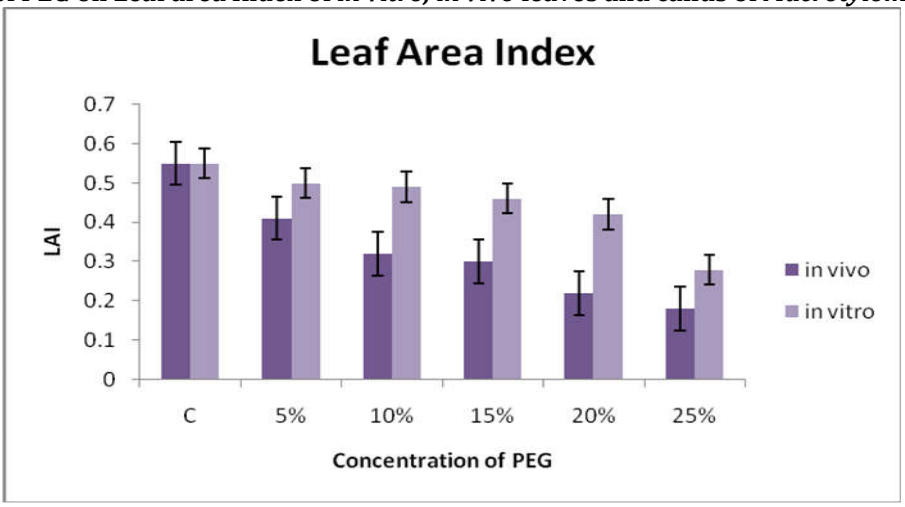


Fig. 2 Effect of PEG on Stomatal Index of *in vitro* and *in vivo* leaves of *Macrotyloma uniflorum*

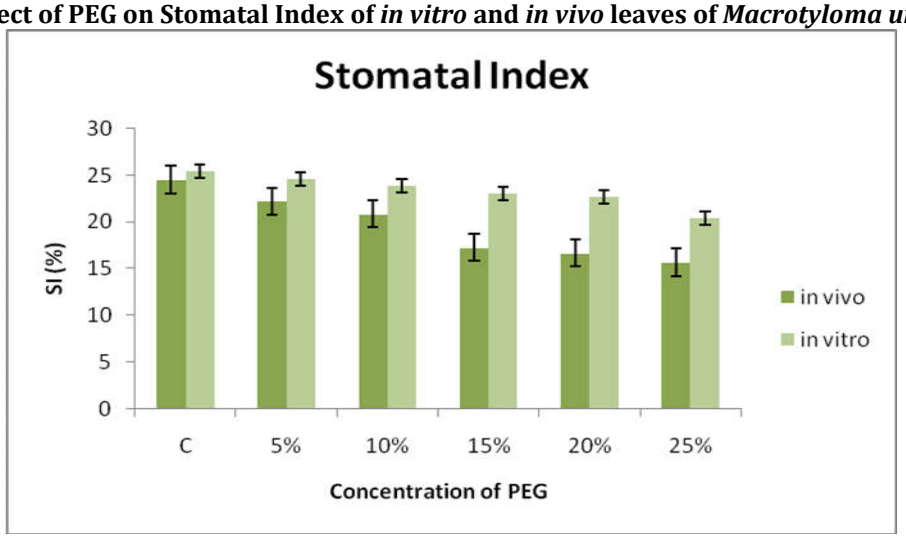


Fig.3 Effect of PEG on Relative growth rate of *in vitro, in vivo* leaves and callus of *Macrotyloma uniflorum*

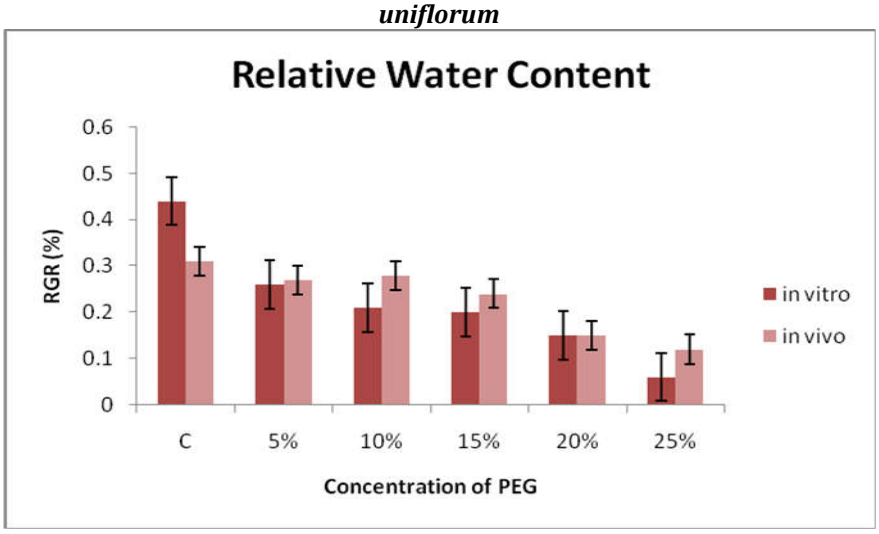
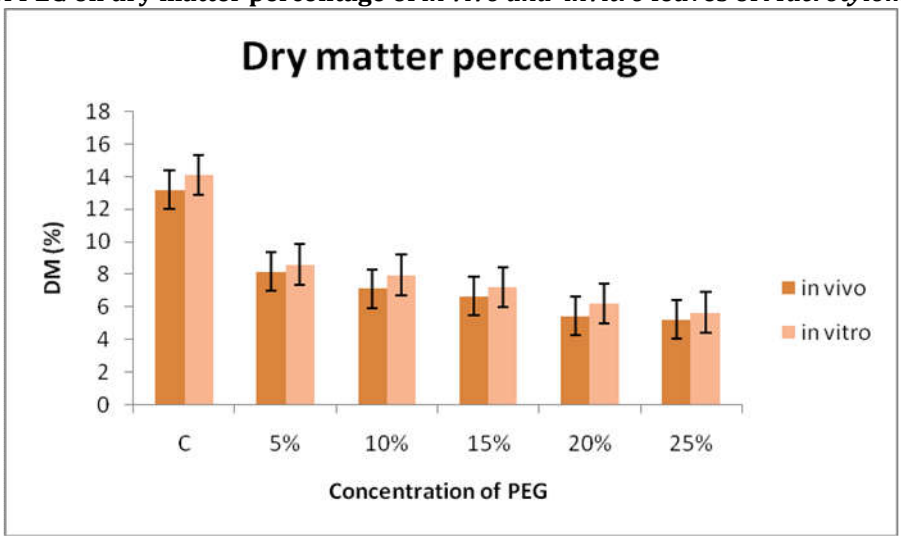


Fig.4 Effect of PEG on dry matter percentage of *in vivo and invitro* leaves of *Macrotyloma uniflorum*



DISCUSSION

Drought is one of the primary abiotic stresses causing not only differences between the mean yield and the potential yield but also causing yield instability. The abiotic stresses are location specific exhibiting variation in frequency, intensity and duration. Stresses can occur at any stage of plant growth and development, thus illustrating the dynamic nature of crop plants and their productivity [10].The effect of water stress varies with the plant species, degree and duration of water stress and growth stage of the plant [11].

Plant cell and tissue culture has been an useful tool to study stress tolerance mechanisms under *in vitro* conditions [12]. Simulation of drought stress under *in vitro* conditions during the regeneration process constitutes a convenient way to study the effects of drought on the morphogenic responses.

Growth is an important parameter used to assess novel phenotypes derived from plant tissue cultures. Hence analysis of growth plays a role in evaluation and characterization of phenotypes. Stress, in general, has a greater effect on the morphology of the plant. In the present work, there was a decrease in the length of the shoots under both treated *in vitro* and *in vivo* conditions compared to non stressed ones. Significant differences were present between non stressed and stressed plants. Similar type of result was reported by Timpa *et al.,* [13] in cotton cultivars. Decrease in shoot length in response to drought may be either due to decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth [14] and /or due to blocking up of xylem and phloem vessels thus hindering any translocation [15]. Sadeghipur [16], reported that there was a reduction in the plant height under water stress conditions in *Vigna radiata* cultivars compared to watered plants. Water stress reduces growth and manifests several morphological, anatomical and biochemical alterations in plants, including modification in gene expression leading to a massive loss in yield [17].

Number of leaves was also reduced from 5% to 25% PEG level in the present study. Reduction in number of leaves due to water stress can be attributed to its direct effect on cell division which arose from reduction in nucleic acid synthesis and /or enhancement of its break down [18]. Likewise, reduction in number of leaves was observed by Timpa *et al.,* [13] in cotton plants. Number of nodes and internodal length per plant drastically decreased from lower level of osmoticum to higher level of osmoticum.

Biomass of the stressed plants on fresh weight basis was decreased compared to non stressed plants in *in vivo* and *in vitro* conditions. The total biomass of the plants decreased with the increase in the salinity of irrigation water in *Salvadora persica* [19]. Osmotic adjustment has been shown to reduce growth sensitivity to water stress or to allow growth to proceed at a slower rate under water stress by monitoring turgor. Water deficit affects plant biomass production partly through its negative effects on leaf area. The reduction in leaf area decreases biomass production and there is a positive correlation between total leaf area and biomass production [20].

Measurements of leaf area are often a necessary for agronomic and physiological study involving plant growth. Drought resistant plants can use several mechanisms to tolerate dehydration. These include reduction of water loss by increased stomatal resistance, reduction in leaf area and decrease in osmotic potential.

LAI was decreased as the osmotica of the medium increased, in the present investigation. Similar reduction in LAI was reported by Timpa *et al.*[13] in cotton cultivars under water stress conditions. Drought may initially inhibit leaf growth and development, significantly reducing leaf area [21]. Total leaf area is a key ecophysiological trait, involved in photosynthesis and biomass production. Reduction in leaf area under drought can be considered as avoidance mechanisms which minimize water loss when stomata are closed [22, 23]. Decreased leaf area in response to water stress was due to reduction in total number of leaves as well as poor expansion of leaves in stressed environment, indicating an adaptation to avoid water loss through transpiration [24].

RGR and DM were decreased as the water potential of the media decreased in the present study. But there was no significant difference in percentage of DM between control and stressed conditions in Soybean cultivars [9]. On contrary, dry weight increase may be attributed to the increased synthetic activity association with cell division and new material synthesis . Stressed plants had greater fresh weights than the control and corresponding large dry matter accumulation was reported in cotton strains [13]. The reduction in dry matter was attributed to accelerated senescence and shedding of leaves under water stress. The dry matter pointed to the improvement of plant photosynthetic systems avoiding the adverse environmental conditions. Water content per dry matter (lyophilized) was found to be a sensitive criterion of metabolic changes during the water stress [25]. PEG induced water deficit produced substances dehydration that led to elevated dry matter content and reduced RGR in plants. The decrease in osmotic potential is considered a potential cellular mechanism of drought resistance as it enables turgor maintenance and growth continuation [12].

CONCLUSION

The plant has well developed to cope with the water stress condition. All the morphological adaptations showed that the plant has better adapted under drought and is helpful in genetic transformation studies where the novel drought tolerant genes from this plant can be transferred to susceptible varieties.

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