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Research Article

DROUGHT INDUCED ALTERATIONS IN CHLOROPHYLL, CARBOHYDRATES AND REDUCING SUGARS IN IN VITRO PLANTS OF *MACROTYLOMA UNIFLORUM* (LAM.) VERDC.

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ABSTRACT

In this work, we studied the effect of water stress on chlorophyll, carbohydrate and reducing sugar content in *in vivo* and *in vitro* plants of *Macrotyloma uniflorum*. The water stress was generated using by PEG-6000 in several concentrations ranging from 5% to 25%. 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 and physiological responses. *In vitro* plantlets of *M.uniflorum* were obtained on L_2 + IBA (2.45 µM) + BAP (8.88 µM) under water stress conditions induced by supplementing the medium with PEG at various concentrations ranging from 5% to 25%. The percent of response of the *in vitro* shoots adapted to low concentrations of PEG were more when compared to the plants adapted to high concentrations of PEG. The same kind of treatment was given to *in vivo* plants. The primary metabolites were decreased in the stressed plants both in *in vivo* and *in vitro* plants. Tissue cultured plants as well as *in vivo* plants as well as the *in vivo* plants were compared for their primary metabolite content. Chlorophyll, carbohydrates and reducing sugars increased in the stressed micropropagated plants. *In vitro* plants were better compared to *in vivo* plants.

INTRODUCTION

Legumes are very important component of the vegetation of most countries, more particularly so in the tropics and the subtropics. *Macrotyloma uniflorum* has been identified as a potential food resource of the tropics by the National Academy of Sciences of the USA (1979). Horse gram is a good source of protein, and fairly have high amount of calcium. It is also high in iron but its availability is reduced by the phytates, tannins and oxalic acid (Gopalan *et al.* [1]. *Macrotyloma uniflorum* has the greatest potential for further utilization as food for malnourished and drought prone areas of the world.

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. Stress is measured in relation to plant survival, crop yield, growth or the primary assimilation processes which are related to the overall growth of the plant. Despite the great deal of investigations carried out to understand the stress response in a number of plants, the available information still lacks vital keys that can lead to the understanding of the plant stress interaction and its exploitation to develop more such tolerant varieties for future use. Thus, the investigation would fill the lacunae in the understanding of the phenomenon of stress-response elicited by plants in general and crops in particular.

Drought is one of the most common abiotic stresses reducing the yield of many crops including legumes. The legumes are generally grown under water limiting conditions and as a result, these crops often encounter drought situation that reduces productivity to a large extent by Vadez *et al.* [2]. Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological, physiological and biochemical characters by Mitra, [3].

Tissue culture techniques have opened up many new possibilities of crop improvement since responses are well defined under controlled conditions. The present study involves the comparison of primary metabolites in *in vivo* and *in vitro* plants of *M.uniflorum*.

MATERIALS AND METHODS

Seeds of *Macrotyloma uniflorum* (Lam.) Verdc, var. PHG-9 were procured from GKVK, University of Agricultural

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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 formulated by Phillips and Collins [4] 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 stress tolerant plants were subjected to various biochemical characters to study the tolerant mechanism involved in the plant. Biochemical characters such as chlorophyll, carbohydrates and reducing sugars were discussed here.

Estimation of chlorophyll content

Chlorophyll a, b and total chlorophyll content in fresh leaves of control and stress induced *in vivo* an vitro plants were estimated by Arnon[5] and percent transmission was recorded in a spectrophotometer (Elico SL 171) at 645nm and 663nm against the solvent blank, 80 % acetone.

Chlorophyll stability index (CSI %) was calculated for treated *in vivo* and *in vitro* plants using the formula by Kumari *et al.* [6].

$$CSI = \frac{Chlorophyll\ before\ stress - Chlorophyll\ under\ stress}{Chlorohyll\ under\ stress} \times 100$$

Estimation of primary metabolites

The total Carbohydrates and reducing sugars contents in leaves of stressed *in vivo* and *in vitro* plants were estimated by anthrone and DNS reagent methods respectively by Yemm and Willis [7]; Miller, [8].

RESULTS

Chlorophyll content

The observations revealed, there was a very significant difference in the total chlorophyll content in treated *in vitro* and *in vivo* plants and *in vitro* plants showed better content compared to *in vivo* plants. Chlorophyll a, b and total chlorophyll content were significantly more in *in vitro* stressed plants compared to *in vivo* stressed plants (Fig 1-3).

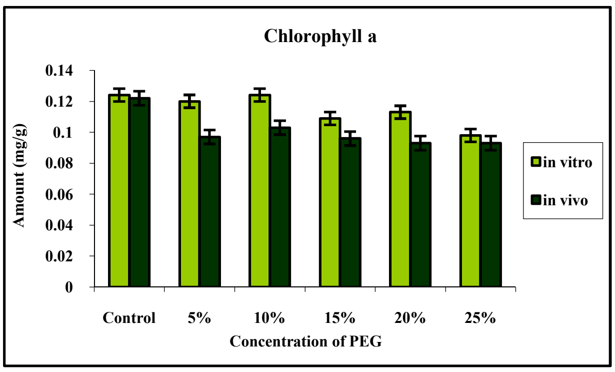

Fig 1 Effect of PEG on Chlorophyll a content of *in vitro, in vivo* leaves of *Macrotyloma uniflorum*

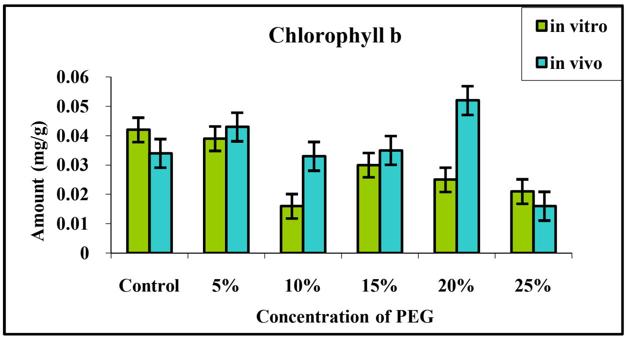

Fig 2 Effect of PEG on Chlorophyll b content of *in vitro, in vivo* leaves of *Macrotyloma uniflorum*

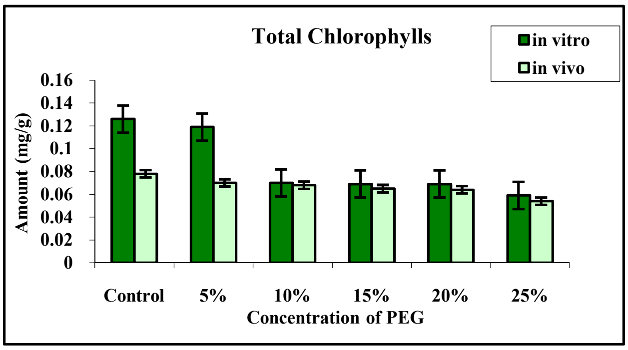

Fig 3 Effect of PEG on total Chlorophyll content of *in vitro, in vivo* leaves of *Macrotyloma uniflorum*

Chlorophyll Stability Index (CSI)

CSI was 14.40% in 5% and 39.49% in 25% PEG treated *in vitro* plants. Similarly, CSI was 11.42% in 5% and 43.11% in 25% PEG treated *in vivo* plants (Fig. 4). Significant differences were observed in both *in vitro* and *in vivo* PEG treated plants. CSI was gradually increased from 5% to 25% PEG treated plants under both *in vitro* and *in vivo* conditions respectively. As the concentration of PEG increased, the CSI increased both in *in vitro* and *in vivo* treated plants. But CSI was more for *in vitro* treated plants compared to treated *in vivo* plants.

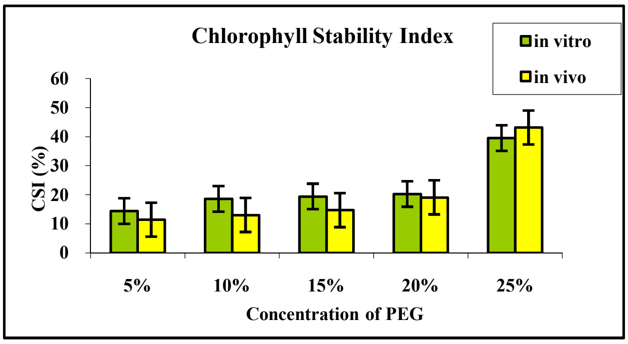

Fig 4 Effect of PEG on Chlorophyll Stability Index of *in vitro, in vivo* leaves of *Macrotyloma uniflorum*

Total carbohydrates

Total carbohydrate content was estimated in treated and untreated *in vitro* and *in vivo* plants Fig. 5). After 45 days, the carbohydrate contents in untreated *in vitro* and *in vivo* leaves was 78.96mg /g DW and 67.15mg /g DW respectively. The content was more in *in vitro* leaf than in *in vivo* leaf. The total carbohydrate contents were 64.35mg /g DW, 48.80mg /g DW, 48.49mg /g DW, 44.66mg /g DW and 44.76mg /g DW at 5%, 10%, 15%, 20% and 25% PEG treatments respectively. Likewise in *in vitro* leaves, at 5%, 10%, 15%, 20% and 25% PEG treatments, the contents were 73.36mg /g DW, 68.08mg / DW, 28.59mg /g DW, 22.38mg /g DW and 17.71mg /g DW respectively.

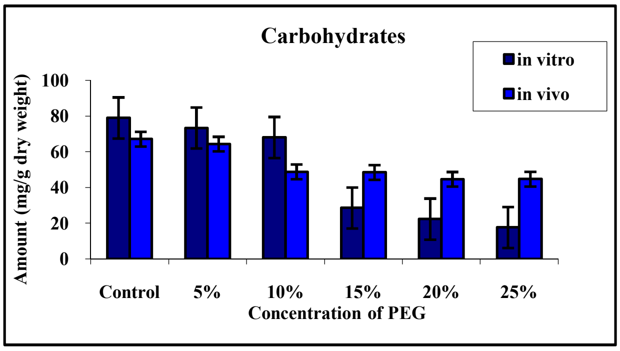

Fig 5 Effect of PEG on Carbohydrates of *in vitro, in vivo* leaves of *Macrotyloma uniflorum*

In both *in vivo* and *in vitro* treated leaves, as the concentration of PEG increased, the total carbohydrate content decreased gradually. The content was more in *in vitro* leaves than *in vivo* leaves. But the carbohydrate content was almost same with a minor difference in 10% and 15% PEG treated plants and 20% and 25% PEG treated plants also showed same amount of content with a minor difference in *in vivo* treated plants.

Reducing sugars

The influence of PEG on reducing sugar content is shown in Fig 6. As compared with control, inducing water stress with PEG treatment resulted in a significant decrease in reducing sugars in *in vivo* plants. The reducing sugar contents were 12.58mg/g DW and 8.92mg/g DW in untreated *in vitro* and *in vivo* leaves respectively. There was a slight reduction in the content of the reducing sugar from 5% to 25% PEG treatments in both *in vitro* and *in vivo* leaves. 12.20mg/g DW and 8.26mg/g DW at 5%, 10.16mg/g DW and 7.59mg/g DW at 10%, 9.25mg/g DW and 6.50mg/g DW at 15%, 7.58mg/gDW and 6.36mg/g DW at 20% and 5.17mg/g DW and 4.45mg/g DW at 25% PEG in both *in vitro* and *in vivo* plants respectively. The content was more in *in vitro* condition compared to *in vivo* condition.

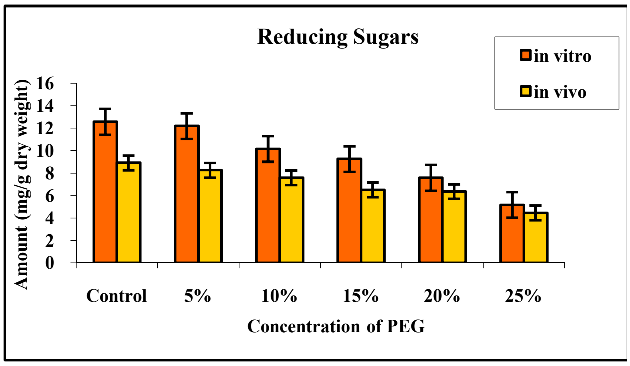

Fig 6 Effect of PEG on Reducing Sugars of in vitro, in vivo leave of Macrotyloma uniflorum

DISCUSSION

The effect of water stress on a plant's physiology varies depending on the species and degree of tolerance, as well as on the magnitude of the water deficit and how fast the plants experience this water deficit. Plant cell cultures have been used as models for studying physiological and biochemical processes Lerner [9].

The physiological mechanisms involved in cellular and whole plant responses to water stress to generate considerable interest and are frequently reviewed (Mohammadkhani and Heidari [10]).

Carbohydrates serve as signaling molecules for sugar responsive genes which leading to different physiological responses like defense responses and turgor driven cell expansion explained by Koch [11] and Sturm and Tang, [12]. Carbohydrates were reduced as the concentration of PEG increased both in *in vivo* and *in vitro* plants. Low chlorophyll content causes a relevant reduction of light absorption by leaves and consequently reduces the biosynthesis of carbohydrates Azooz *et al.* [13].

Water stress detrimentally affects many aspects of carbohydrate metabolism besides photosynthesis. The major carbohydrates *viz*, starch, reducing sugars and non reducing sugars undergo marked changes on the advent of drought in many plant species and changes in carbohydrates during drought have been assigned to altered enzymatic activities.

Chlorophyll-a, chlorophyll-b and total chlorophyll were decreased as the concentration of PEG increased. The contents of chlorophyll-a, chlorophyll-b and total chlorophylls in leaf discs were significantly decreased by osmotic stress simulated by PEG in gerbera (Qi-Xian *et al.*[14]). Reduction in photosynthesis under water stress has been well documented in some legumes indicating reduction in chlorophyll content (Sheoran *et al.*[15]). Water stress considerably lowered the chlorophyll content in the leaves of different cultivars of French bean (Upreti *et al.*[16]). In water stressed plants, loss in chlorophyll is associated with a reduction in the flux of nitrogen into the tissue as well as alterations in the activity of enzyme systems such as nitrate reductase (Begaum and Paul, [17]).

CSI is a measure of the extent the chlorophyll pigments undergo decomposition at higher temperatures. Lower the value of CSI, greater is the stability of chlorophyll at higher temperatures. CSI was an important index for screening of plant cultivar for abiotic stresses. In several instances the CSI has been correlated with drought resistance of certain crop plants. CSI is an indication of the stress tolerance capacity of plants (Madhan Mohan *et al.*[18]). The higher CSI indicates the tolerance of plants under water stress condition. In the present study, CSI was increased as the stress increased. The CSI was more under stressed conditions in field grown cow pea plants (Ramesh and Devsenapathy,[19]). A high CSI value indicates that the stress did not have much effect on chlorophyll content of plants and helps to withstand stress through better availability of chlorophyll, leads to increased photosynthetic rate and more dry matter production (Immanuel and Ganapathy,[20]).

CONCLUSION

In conclusion, *Macrotyloma uniflorum* showed better adaptation towards water stress and this was justified by the biochemical responses. Drought stress effects on the biochemical aspects to provide an insight to the underlying mechanisms responsible for these responses. Since, horse gram is naturally a stress tolerant plant, study of biochemical parameters under control stress conditions will throw light on the mechanisms that are involved in fighting abiotic stress.

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