CELL MEMBRANE STABILITY AND CHLOROPHYLL STABILITY INDEX AS SELECTION CRITERIA FOR SCREENING OF DROUGHT TOLERANCE IN GROUNDNUT CULTIVARS

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Abstract : To select physiological tests for the screening of drought tolerance in groundnut cultivars, we have studied the changes in cell membrane stability (CMS), chlorophyll stability index (CSI), total chlorophyll content and malondialdehyde (MDA) content in two high yielding cultivars of groundnut (*Arachis hypogaea* L. K-134 and JL-24, drought tolerant and drought sensitive respectively) under different regimes of water stress. The CMS and CSI were significantly decreased in both cultivars under water stress conditions. However, the per cent decrease was relatively less in K-134 than in JL-24. Total chlorophyll content decreased during water stress conditions at all levels of stress in both cultivars, but a more pronounced decrease was observed in JL-24 than in K-134. The level of lipid peroxidation as indicated by MDA formation was greater in cultivar JL-24 than in K-134. The magnitude of physiological responses was dependent on stress severity and varied between the cultivars. These results may contribute to identify useful physiological traits for breeding programs concerning drought tolerance in groundnut.

***Index Terms* - Groundnut, Cell membrane stability, Chlorophyll, MDA, Drought tolerance**

I. INTRODUCTION

Drought stress is one of the major abiotic stresses in agriculture worldwide and recent progressive global climate change and increasing shortage of water resources has made this problem more serious. There is a great need for research aimed at understanding drought tolerance, screening for drought tolerant varieties and breeding crops with an improved water use efficiency. Understanding drought tolerance, however, is quite difficult because of the complex nature of stress and the wide range of plant responses. Groundnut (*Arachis hypogaea* L.) is cultivated predominantly on arid and semi-arid agricultural fields and often experiences drought stress conditions during its growth cycle leading to drastic reduction in productivity [1]. This necessitates development of cultivars which can withstand water stress, and still can be productive. An understanding of the physiological mechanisms and identification of specific physiological traits associated with drought tolerance should be considered in the groundnut breeding programs [2].

The chlorophyll breakdown or destruction commences rapidly at critical temperature and this property of chlorophyll stability has been used to evaluate genotypic tolerance potentials and found to correlate well with drought tolerance [3]. MDA, a product of lipid peroxidation is regarded as a biomarker for evaluation of the damages in organelle membranes caused by oxidative stress and was found to enhance with increase in drought stress, especially in sensitive cultivars [4, 5]. The maintenance of the physical–chemical integrity of membranes under drought stress can be considered as one of the best physiological indicators of protoplasmic tolerance in plants [6]. Consequently, tests based on evaluation of membrane resistance (CMS) could be used for screening parental varieties in breeding. This work concerns in two high yielding cultivars of groundnut (*Arachis hypogaea* L. K-134 and JL-24) differing in drought sensitivity grown widely in Rayalseema region of Andhra Pradesh. Drought constitutes an important factor limiting their production. The aim of this research is to evaluate their chlorophyll stability, membrane tolerance and associated parameters to water stress and the possibility of using these physiological indices as evaluating tools for drought tolerance in groundnut.

II. RESEARCH METHDOLOGY

Seeds of groundnut (*Arachis hypogaea* L.) cultivars namely (K-134 and JL-24) were sown in earthen –pots containing 8kg of red loamy soil and farm yard manure (3:1 proportion). Pots were maintained for one month in the departmental botanical garden under natural photoperiod of 10-12 h and temperature 28 ± 4 ^{0}C. One-month-old plants of each cultivar were divided into four-sets and arranged in randomized complete black design. One set of pots received water daily to field capacity and served as control (100 %). Water stress was induced by adding of water daily to 75, 50 and 25 % soil moisture levels respectively. Leaf samples were collected on day-12 after stress induction for analysis of various parameters.

The total chlorophyll content was estimated in the leaves according to the method of [7]. Chlorophyll stability index was determined by adopting the method of [8]. The chlorophylls was estimated after heating the excised leaf discs of stressed plants incubated in distilled water for 30 minutes at 56 ± 1^0 C. Excised leaf discs incubated in distilled water without heating served as control. CSI was expressed as the difference between these two values.

The levels of malondialdehyde content was determined by the thiobarbituric acid (TBA) reaction as described by [9]. CMS was assessed by determining the extent of membrane leakage. Leaf discs of 1 cm diameter were prepared using cork borer from control and stressed groundnut plants and leaf discs were incubated in 10 ml of water for 2 hours. The solution was filtered and OD was examined at 273 nm (*Initial OD*). Subsequently leaf discs were boiled in the same solution for 30 minutes, cooled, filtered and OD was examined at 273 nm (*Final OD*). Percent leakage was calculated according to the method of [10]. The data were analyzed statistically using Duncan's multiple range (DMR) test to drive significance [11].

III. RESULTS AND DISCUSSION

Chlorophyll content of leaf is an indicator of photosynthetic capability of plant tissues [12]. The decrease in total chlorophyll content in the leaves of water stressed plants was reported in groundnut [13] and other plants [14]. As a confirmation, in the present work, we also observed decline in chlorophyll content in both cultivars under drought stress, but with a greater degree of decline in cultivar JL-24 than in K-134 cultivar (table 1). The maintenance of better chlorophyll levels during stress may be viewed as one of the criterion to decide the tolerance [13]. A reason for decrease in chlorophyll content as affected by water deficit is that drought or heat stress by producing reactive oxygen species (ROS) such as O_2^- and H_2O_2, can lead to lipid peroxidation and consequently, chlorophyll destruction [15] as evidenced in our study which will be discussed later in this paper. Chlorophyll stability index is an important method and forms a basis of the indices for estimating resistance to dehydration [3]. The CSI values were correlated with drought tolerance in mulberry, and the drought tolerant cultivar showed lower CSI value than sensitive cultivar [14]. In this study (figure 1), the loss or breakdown of chlorophyll subjected to the heating processes is comparatively less in K-134 (i.e., loss of chlorophyll in K-134 is about 47% whereas JL-24 it is 62% at the end of experiment). A higher CSI helps to plants with-stand stress through better availability of chlorophyll which leads to increased photosynthetic rate, more dry matter production and higher productivity. The chlorophylls are closely associated with drought tolerance and these parameters are used as biochemical markers for the identification of drought tolerant genotype in groundnut and cluster bean respectively [13, 16], and as such cultivar K-134 seems to be relatively drought tolerant by maintenance of better chlorophylls and CSI.

Table 1. Total Chlorophylls and Malonialdehyde (MDA) content of control and water stressed groundnut cultivars on day-12, after induction of water stress (mean ± SD). The mean values (n=5) in a row followed by different letter for each plant species are significantly different (P≤0.05) according to Duncan's multiple range (DMR) test. Figures in parentheses represent per cent of control.

Parameters	Cultivar	Control	Mild	Moderate	Severe
Total chlorophylls (mg g^{-1} FW)	JL-24	1.887a (100) ±0.062	1.636b (86.73) ±0.084	1.234c (65.42) ±0.049	0.768d (40.72) ±0.072
	K-134	1.692a (100) ±0.064	1.512b (89.37) ±0.058	1.263c (74.67) ±0.077	0.938d (55.47) ±0.069
MDA (µ mol g^{-1} FW)	JL-24	10.04a (100) ±0.84	14.98b (149.17) ±0.86	20.84c (207.53) ±1.02	27.29d (271.86) ±1.32
	K-134	9.01a (100) ±0.91	12.43b (137.53) ±1.10	16.08c (178.46) ±1.21	19.83d (220.15) ±1.34

Lipid membranes are vulnerable targets for stress induced cellular damage and the extent of damage measured in the levels of lipid peroxidation in terms of MDA can be used as an indication to evaluate the tolerance of plants to imposed stress [17]. Water stress resulted a significant increase in the MDA content in both the cultivars throughout the experimentation (table 1). The per cent increase in the MDA content was less in cultivar K-134 when compared to cultivar JL-24, at all stress levels indicating high rate of lipid peroxidation in cultivar JL-24. The increase in MDA levels under drought stress suggests that water stress could cause the formation of membrane lipid peroxidation by means of ROS production [4]. The lower level of MDA in K-134 cultivar suggests that this is better protected from oxidative damage under water stress. These results are in a good agreement with the studies of [4,5], who found that low concentrations of MDA are associated with drought stress tolerance in artichoke plants, and melon respectively. Lipid peroxidation brings about the loss of cell membrane integrity leading to electrolyte leakage and chlorophyll breakdown reducing the rate of photosynthesis [18]. Most often cellular membranes represent the first target of various abiotic stresses and this parameter characterizes their integrity [19]. The maintenance of membrane integrity and there by reducing the amount of solute leakage as a result of desiccation either from water stress or any other stress is considered as a stress tolerance. It is assumed that the amount of electrolyte leakage is a function of membrane integrity. In the present study, the rate of membrane injury was significantly increased with the intensity of water stress in both cultivars. However the per cent membrane injury was found to be lesser in cultivar K-134 (51%) than in cultivar JL-24 (78%) under severe stress (figure 2), characterizing cultivar K-134 as the less susceptible to membrane damage provoked by drought. Thus it is likely that CMS has often been used to assess drought tolerance in groundnut as reported by few authors [13, 20]. The less injury index in K-134 during water stress reflects maintenance of equilibrium in favour of synthetic processes. The less amount of electrolyte leakage has been correlated with the greater membrane integrity under stressful conditions and those are characterized as stress tolerant genotypes [21]. It has been reported that drought stress tolerant genotypes were superior to susceptible ones in maintaining membrane stability and lower lipid peroxidation under drought stress condition [21, 22]. As a good confirmation with these findings, in our study, the drought tolerance of the cultivar K-134, seems to be related to lower lipid peroxidation which would maintain better integrity of cellular components when compared to cultivar JL-24.

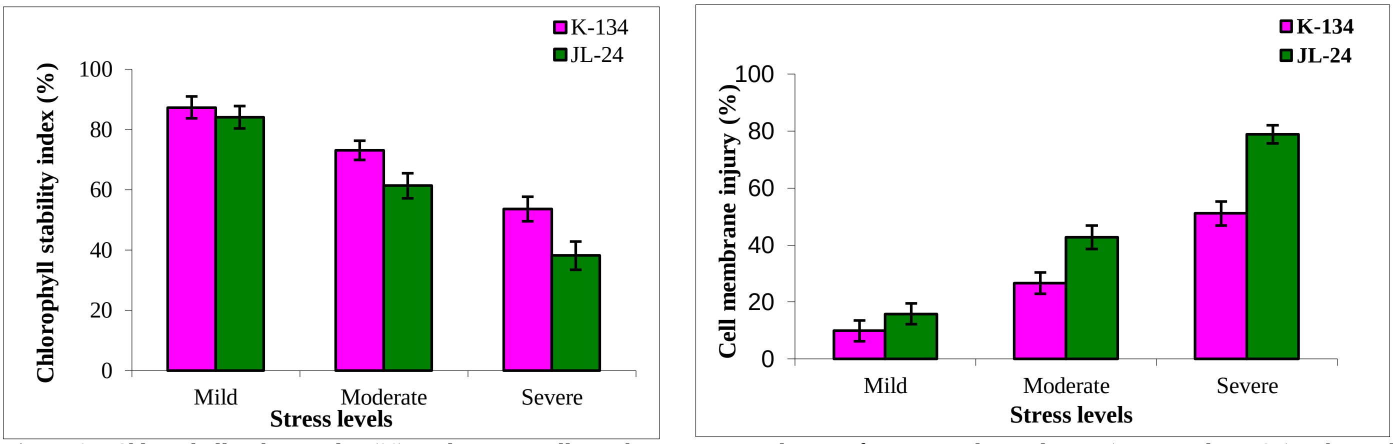

Figure 01. Chlorophyll stabiity index (%) and Percent cell membrane injury in leaves of two groundnut cultivars (JL-24 and K-134) subjected to different stress levels on day-12. Vertical bar indicates mean ± SD

IV. CONCLUSIONS

The drought tolerance of cultivar K-134 could be ascertained from the present study, based on relatively lesser levels of MDA content coupled with better CSI and CMS. Thus, these physiological traits can be used as screening tools for drought tolerance in groundnut. This information is also essential for further studies where these cultivars will be compared in terms of differential gene expression during drought stress.

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