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Evaluation of biochemical markers for managing drought stress tolerance in a valuable endangered medicinal plant *Withania somnifera*

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Abstract
In four genotypes of *W.somnifera* namely J-20, Nimitly, Chetak and Pratap MDA content, electrolyte leakage and proline content of callus were studied under drought stress (on agar solidified media containing 10, 20 &30g/l polyethylene glycol). Data for each parameter was taken at three time interval i.e.15, 30 & 45 days after inoculation. MDA content, % EL as well as proline content was found to be increased with increasing conc. of PEG i.e. 1%, 2% and 3% PEG in MS media. Maximum MDA content was calculated by Jawahar -20 i.e. 0.141 µmol/mg while Nimitly showed minimum (0.044 µmol/mg) MDA content. Maximum proline content i.e. 3.76 µmol/g was recorded by genotype Nimitly while minimum by Pratap i.e. 2.98 µmol/g. Thus selection and use of the genotypes with higher proline and low MDA content under drought stress may be a practical approach to improve drought tolerance.

Keywords: biochemical markers, *Withania somnifera*, electrolyte leakage

1. Introduction
Water deficit stress is among the global issues to ensure sustainable food production. In plants it may result in drop of water potential and lead to decline in growth and productivity (Chaves *et al*., 2003) [3]. *Withania somnifera* (L.) Dunal commonly known as 'Ashwagandha' has a high repute in traditional Indian medicine, and is one of the most extensively used plant in Ayurveda and Unani medicines. It is known for it's enormous therapeutic value against a large number of ailments such as mental diseases, asthma, inflammation, arthritis, rheumatism, tuberculosis and a variety of other diseases including cancer (Pati *et al.,* 2008) [9]. Ashwagandha is grown as rain-fed crop in many parts of India under arid and semi-arid regions. It grows well in soils having residual moisture and single or two supplementary irrigations during the entire period of its growing season. Despite its drought tolerance capacity, prolonged soil moisture deficit inhibits the growth and development of the crop and adversely affects the crop yield potential and plant productivity (Shah *et al*., 2010) [11].
On one hand drought stress has become a threat for plants; while on the other this valuable medicinal plant (*W. somnifera*) has also been listed in endangered species because of the overexploitation by the pharmaceutical companies. Thus mass multiplications as well as biochemical responses under drought stress are the prerequisite challenges for the researchers to make this valuable plant available for the future use. A study regarding the damage caused by the stress & and the various mechanism that plant exhibit in order to cope with it, becomes highly important. Peroxidation of lipids and electrolyte leakage are among the important indicators of oxidative damage while accumulation of proline is one of the defense mechanisms shown by the plants.
Maintenance of low levels of MDA (product of the lipid peroxidation) is associated with better resistance to drought (Lima *et al*., 2002) [7] as peroxidation of lipids disrupts the membrane integrity of a plant cell. Proline is thought to play a cardinal role as an osmoregulatory solute in plants subjected to drought stress. It not only act as an osmolyte for osmotic adjustment but it also contributes to stabilize sub-cellular structures (e.g., membranes and proteins) under stress conditions. Thus relates the tolerance power of plants against stress (Hayat *et al*., 2012) [5]. In view of varied therapeutic potential of Ashwagandha as well as the increasing drought prone areas, this plant is subject a of considerable modern scientific attention as it requires less water for its growth. Keeping these facts in mind mass multiplication of *W. somnifera* have been done by using in vitro techniques. Simultaneously MDA content and electrolyte leakage were measured as the indicator of membrane damage and proline estimation was done from *in vitro* generated calluses to check the tolerance capacity with increasing levels of stress as well as time interval.

Materials and methods

Leaves of four genotypes of *Withania somnifera* namely Jawahar -20, Nimitli, Chetak and Pratap were used as explants. To induce the callus sterilized excised explants were placed in to culture vessels containing Murashige and Skoog (MS) media having various conc. of plant growth regulators ranging from 0.5 – 2 mg/l (2,4-D and NAA) and 0.2- 0.5 mg/l (Kinetin). Cultures were incubated at 25± 2ºC under white fluorescent light (3000 lux) for 16h photoperiod and 55 – 60% relative humidity. Induced calluses were transferred in to proliferation media having same conc. of plant growth regulators as used for callus induction. Proliferated callus were then transferred on poly ethylene glycol (PEG) containing media for the imposition of drought stress. The different conc. of PEG used for intensifying the stress level were 10, 20 & 30g/l. Medium without PEG was considered as control. Thereafter MDA content, % EL and proline content were recorded after 15, 30 & 45 days of inoculation.MDA content and electrolyte leakage were estimated in stressed and non-stressed callus tissues using the method was estimated according to the procedure of Bates *et al*., 1973.

Table 1: Effect of drought stress on MDA content (µmol/mg fresh wt) in callus of different genotypes of *W.somnifera* at three time intervals.

Treatments	**MDA content (µmol/mg fresh wt. of callus)**					
	J -20			**NIMITLY**		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	0.044 ±0.001	0.023 ±0.006	0.016 ±0.005	0.015 ±0.001	0.021 ±0.006	0.018 ±0.001
10gm/lPEG	0.100 ±0.002	0.080 ±0.009	0.035 ±0.001	0.021 ±0.005	0.022 ±0.003	0.020 ±0.007
20gm/lPEG	0.124 ±0.002	0.090 ±0.009	0.041 ±0.003	0.022 ±0.004	0.029 ±0.006	0.027 ±0.003
30gm/lPEG	0.141 ±0.003	0.125 ±0.007	0.050 ±0.004	0.044 ±0.005	0.034 ±0.008	0.039 ±0.002
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.002	0.002	0.005	0.004	0.004	0.008
C D at 5%	0.007	0.008	0.014	0.012	0.014	0.024
	CHETAK			PRATAP		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	0.002 ±0.001	0.012 ±0.001	0.013 ±0.001	0.018 ±0.008	0.016 ±0.006	0.007 ±0.002
10gm/lPEG	0.009 ±0.001	0.025 ±0.012	0.022 ±0.007	0.023 ±0.007	0.026 ±0.007	0.009 ±0.001
20gm/lPEG	0.011 ±0.001	0.028 ±0.005	0.022 ±0.008	0.078 ±0.012	0.036 ±0.008	0.013 ±0.001
30gm/lPEG	0.045 ±0.009	0.037 ±0.002	0.051 ±0.008	0.082 ±0.008	0.040 ±0.007	0.037 ±0.008
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.006	0.007	0.013	0.008	0.010	0.017
C D at 5%	0.019	0.022	0.039	0.026	0.030	0.052

*Data is average of three replicates (± indicates SEm).

Table 2: Effect of drought stress on Electrolyte leakage (%) in callus of different genotype of *W.somnifera* at three time intervals.

Treatments	**Electrolyte leakage (%)**					
	J -20			**NIMITLY**		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	33.41 ±0.802	35.58 ±0.124	33.74 ±0.264	46.64 ±0.678	49.48 ±0.870	55.25 ±0.157
10gm/lPEG	35.31 ±0.724	45.55 ±0.485	58.86 ±0.504	55.97 ±0.613	50.10 ±0.185	64.59 ±0.038
20gm/lPEG	61.80 ±0.730	61.32 ±0.055	61.76 ±0.215	70.54 ±1.209	75.07 ±0.232	71.80 ±0.396
30gm/lPEG	63.35 ±0.111	65.53 ±2.332	70.94 ±0.186	73.13 ±0.360	77.77 ±0.837	79.79 ±0.240
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.403 0.403	0.465	0.806	0.296	0.342	0.592
C D at 5%	1.17	1.35	2.35	.864	.998	1.72
	CHETAK			PRATAP		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	49.54 ±0.365	44.34 ±0.989	41.29 ±0.221	41.77 ±0.477	44.62 ±0.657	47.29 ±0.374
10gm/lPEG	52.20 ±0.419	56.94 ±0.244	53.89 ±0.138	49.95 ±0.449	65.94 ±0.267	56.46 ±0.349
20gm/lPEG	52.23 ±1.369	69.85 ±0.426	60.71 ±0.227	51.59 ±0.670	66.81 ±1.707	65.65 ±0.164
30gm/lPEG	69.11 ±0.240	78.71 ±0.297	61.32 ±0.247	52.51 ±0.331	71.86 ±0.223	73.37 ±0.237
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.278	0.321	0.557	0.316	0.365	0.632
C D at 5%	0.813	0.939	1.62	0.923	1.066	1.846

*Data is average of three replicates (± indicates SEm).

Table 3: Effect of drought stress on proline content (µmol/g fresh wt) in callus of different genotype of *W. somnifera* at three time intervals.

Treatments	Proline content (µmol/g fresh wt. of callus)					
	J -20			NIMITLY		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	2.678 ±0.154	2.553 ±0.003	2.615 ±0.003	1.970 ±0.090	1.733 ±0.002	2.031 ±0.011
10gm/lPEG	2.777 ±0.011	2.615 ±0.011	2.769 ±0.011	3.519 ±0.003	2.264 ±0.004	3.189 ±0.003
20gm/lPEG	2.888 ±0.024	2.658 ±0.002	2.852 ±0.005	3.620 0±.004	2.759 ±0.008	3.290 ±0.007
30gm/lPEG	3.077 ±0.082	2.756 ±0.008	2.985 ±0.008	3.761 ±0.007	2.790 ±0.003	3.681 ±0.004
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.025	0.029	0.051	0.013	0.015	0.026
C D at 5%	0.074	0.085	0.148	0.039	0.045	0.078
	CHETAK			PRATAP		
	15DAI	30DAI	45 DAI	15DAI	30DAI	45 DAI
CONTROL	1.953 ±0.003	1.704 ±0.007	2.023 ±0.003	2.573 ±0.007	2.328 ±0.005	2.557 ±0.002
10gm/lPEG	2.076 ±0.002	1.730 ±0.002	2.115 ±0.004	2.793 ±0.002	2.363 ±0.003	2.655 ±0.007
20gm/lPEG	2.229 ±0.003	1.895 ±0.007	2.262 ±0.006	2.897 ±0.007	2.451 ±0.002	2.937 ±0.006
30gm/lPEG	3.042 ±0.004	1.975 ±0.006	2.391 ±0.51	2.989 ±0.004	2.559 ±0.003	2.984 ±0.002
	Days (A)	Treatments (B)	A X B	Days (A)	Treatments (B)	A X B
SEm±	0.008	0.009	0.016	0.002	0.002	0.004
C D at 5%	0.023	0.027	0.046	0.006	0.007	0.013

*Data is average of three replicates (± indicates SEm).

Results and discussion

Callus induction: Callus initiation in all the genotypes of *W. somnifera* was observed within 10 -12 days after inoculation of leaf explant on MS media supplemented with 1.5 ppm 2,4-D and 0.2 ppm kinetin for genotypes J-20, Nimitly and Chetak and 0.5ppm 2,4-D + 0.5ppm kinetin for genotype Pratap. The range of callus induction was 90 -95%.

Visual appearance of calluses under PEG treatment: Growth of calluses in all the genotypes were stunted with increasing drought in the media and they turned to brown color. This might be due to the cellular dehydration and water potential imbalance between cell and surroundings caused by PEG which has been established as a potent osmotic substance lowering the water potential (Tsago *et al.*, 2013) [13].

MDA content and Electrolyte leakage: In the present experiment MDA content was found to be increased with increasing conc. of PEG. Maximum MDA content was observed in the callus of Jawahar -20 i.e. 0.141 µmol/mg followed by Pratap (0.082) and Chetak (0.045) while Nimitly showed minimum (0.044 µmol/mg) MDA content. Cells showing low MDA content can be considered as less damaged once (Table 1). An increased MDA accumulation under prolonged drought stress in colonial bentgrass and creeping bentgrass was reported by Dacosta and Houng, 2007 [4] they mentioned the impairment of antioxidant enzyme activities as the prime cause of membrane damage. In our study genotypic variation was clearly observed with respect to MDA content. Genotype J-20 showed decreased MDA content with increasing stress duration in all the treatments including control, showing less damage or more tolerance towards long duration stress while in genotype Nimitly it was first increasing up to 30 days then decreasing in all the treatments except with 3% PEG. Genotype Chetak showed similar results as Nimitly but more MDA content signifies more damage. Notably in genotype Pratap there was an increament found up to 30 days with 1% PEG thereafter it was decreasing whereas in other stress levels viz. 2% and 3% it was following the same pattern as genotype J-20. A supportive statement to our investigation was drawn by Sabir *et al.*, 2012 [10]. They found an enhancement in MDA conc. with increasing conc. of salts in shoots as well as calli of *W. somnifera*. A related study by Tatari *et al.*, 2012 [12] also supports our results in which they reported a significant higher MDA content during stress period in *Agropyron desestorum*. Electrolyte leakage % was also found to be increased with increasing conc. of PEG (Table 2). According to Bajji *et al.*, 2001 [2] an enhancement in EL with increasing level of PEG in Durum wheat was observed whereas Masoumi *et al.*, 2010 [8] reported 50% increased EL at stress treatment compared with control in *Kochia scoparia.*

Proline content: Accumulation of proline in the tissues/plants and it's role in osmotic adjustment is well established. It is non toxic at high cellular concentration and lowers the cellular osmotic potential to sustain water absorption (Ashraf and Foolad, 2007) [1]. During investigation it was worked out that proline content was increased with increasing level of stress i.e. 1 - 3% PEG in MS media. Maximum proline content i.e. 3.76 µmol/g was recorded by the callus of genotype Nimitly followed by Jawahar -20, Chetak and Pratap i.e. 3.07, 3.04, 2.98 µmol/g after 15 days of inoculation under PEG induced stress condition respectively with 3% PEG, while after 30 days of inoculation genotype Nimitly and J-20 showed more or less similar proline content i.e. 2..75 and 2.79 µmol/g with 3% PEG followed by 2.55 and 1.97 µmol/g by Pratap and Chetak respectively. After 45 days of inoculation max. proline content i.e. 3.68 µmol/g was recorded by genotype Nimitly. Whereas minimum i.e. 1.97 µmol/g was recorded in callus of Pratap (Table 3). A unique pattern of proline content was observed with increasing time interval of stress which is first decreasing up to 30 Days after inoculation as compared to 15 days and thereafter a little increment was observed in between 30 days to 45 days after inoculation. The above result shows the cell was not much affected which might have been due to the fact that proline helps the cells to cope up the water stress as it balances the osmotic potential of cells. Karamanas *et al.*, 1983 [6] reported that the free proline increases under water and osmotic stress. Our study also seek support from the study of Sabir *et al.*, 2012 [10], who reported a sudden enhancement in the proline content when the calluses were treated with higher conc. of CaCl2 (200mM).

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References

1. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007; 59:206-216.
2. Bajji M, Kinet JM, Lutts S. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regulation. 2001; 00:1-10.
3. Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought–from genes to the whole plant. Func. Plant. Biol. 2003; 30:239-264.
4. DaCosta M, Huang B. Changes in Antioxidant Enzyme Activities and Lipid Peroxidation for Bentgrass Species in Response to Drought Stress. J. AMER. SOC. HORT. SCI. 2007; 132(3):319-326.
5. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments. Plant Signaling & Behaviour. 2012; 7(11):1456-1466.
6. Karamanos AJ, Drossopoulos JB, Niavis CA. Free proline accumulation during development of two wheat cultivars with water stress. J. Agric. Sci., Camb. 1983; 100:429-439.
7. Lima ALS, DaMatta FM, Pinheiro HA, Totola MR, Loureiro ME. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. Environ. Exp. Bot. 2002; 47:239-247.
8. Masoumi A, Kafi M, Khazaei H, Davari K. Effect of drought stress on water status, elecrolyte leakage and enzymatic antioxidants Of Kochia (*Kochia scoparia*) under saline condition. Pak. J. Bot. 2010; 42(5):3517-3524.
9. Pati PK, Sharma M, Salar RK, Sharma A, Gupta AP, Singh B. Studies on leaf spot disease of *W. somnifera* and it's impact on secondary metabolites. Indian J.Microbiol. 2008; 48:432-437.
10. Sabir F, Sangwan RS, Kumar R, Sangwan NS. Salt stress-induced responses in growth and metabolism in callus cultures and differentiating *in vitro* shoots of Indian ginseng (*W. somnifera* Dunal). J.Plant Growth Regul. 2012; 31:537-548.
11. Shah S, Saravanan R, gajhiye NA. Phytochemical and physiological changes in ashwagandha (*W. somnifera* Dunal) under soil moisture stress. Braz. J. Plant. Physiol. 2010; 22(4):255-261.
12. Tatari1 M, Ghazvini RF, Etemadi N, Ahadi AM, Mousavi A. Analysis of antioxidant enzymes activity, lipid peroxidation and proline content of Agropyron desertorum under drought stress. South Western Journal Of. Horticulture, Biology And Environment. 2012; 3:9-24.
13. Tsago Y, Andargie M, Takele A. *In vitro* screening for drought tolerance in different sorghum (*Sorghum bicolor* (L.) *Moench*) varieties. J. of stress physiology & biochemistry. 2013; 9:73-83.