YS-2. ATDREB1A TRANSGENIC GROUNDNUT SHOWS TOLERANCE TO DROUGHT AND SALINITY STRESSES

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important leguminous cash crop for farmers particularly in the semi-arid tropics and its productivity is adversely affected by various abiotic stresses. Abiotic stress tolerance is a polygenic character making use of regulated expression of large number of genes in response to a particular stress. So, genetic transformation using transcription factor controlling signal transduction pathway and expression of various stress inducible genes offers a viable option to address the problems in groundnut cultivation. *DREB1A/CBF3* transcription factor recognizes dehydration responsive elements (DRE/CRT) of promoter region of stress-inducible downstream genes under abiotic stress; subsequently regulates their expression in *Arabidopsis thaliana* and conferred stress tolerance (Pellegrineschi *et al*. 2004, Bhatnagar-Mathur *et al.* 2007).This research work is aimed to develop transgenic groundnut tolerant to abiotic stress by incorporating the transgene *AtDREB1A* under the control of stress-inducible rd29A promoter in groundnut cv. GG20.

MATERIALS AND METHODS

AtDREB1A transgenic groundnut events were developed using protocol described by Mehta *et al.* (2013). Transgene integration and expression were confirmed by dot blot and reverse transcriptase PCR. Eighteen day old seedlings of three transgenic events (T) and wild type (WT) were used to analyze for their tolerance level at 0, 10, 15, 20 % PEG and 0, 100, 150, 200 mM NaCl induced drought and salinity stress for 7 and 12 days respectively in hydroponic solution. The response and tolerance level of T and WT under both type of stresses were analysed visually by counting the number of wilted leaves on daily basis. Physiological and biochemical characterization were carried out by quantitative estimation of abiotic stress related parameters like proline content, osmotic potential (OP), relative water content (RWC), electrolytic leakage (EC), total chlorophyll content prior to, during and just before withdrawal of both stresses.

RESULTS AND DISCUSSIONS

PCR, dot blot and RT-PCR confirmed that three transgenic events had *AtDREB1A* transgene in their genome and also expressed under soil moisture deficit stress condition. Under normal condition there were no phenotypic difference between T and WT. Appearance of wilted leaves occurred early in WT over T under both drought and salinity stresses. At 15% PEG, WT showed wilting at 3^{rd} day whereas transgenic plants showed the wilting symptoms at 6^{th} day. At 200 mM NaCl, upto 5^{th} day transgenic plants did not reveal wilting symptoms on leaves, but, WT showed wilting of leaves at 3^{rd} day. Tnansgenic and WT showed increased level of proline, OP, EC; while RWC and total chlorophyll content decreased in response to gradual increase in PEG and NaCl concentration. However, transgenic events showed improved physiological, biochemical traits over WT. In our study, *AtDREB1A* transgenic plants showed elevated level of proline resulting in better osmotic adjustment as indicated by increased osmotic potential; and these two phenomenons might be responsible for higher water retention capacity and protection of photosynthetic apparatus; lower level of ion leakage over WT (Table 1).

Table 1. Physiological and biochemical stress related parameters at various levels of PEG and NaCl induced drought and salinity stresses on 7th day

	Genotypes	Without NaCl	Without PEG	100 mM NaCl	10% PEG	150 mM NaCl	15 % PEG	200 mM NaCl
RWC (%)	T	71.53±1.34	70.75±2.62	66.00±2.33	59.76±3.32	62.99±3.24	53.29±2.34	56.01±4.42
	WT	68.37±2.21	65.39±3.41	61.17±3.26	55.52±2.34	42.85±2.41	45.42±2.89	38.01±3.56
Proline (µg/g FW)	T	290.49±12.31	213.52±22.56	3013.40±234.56	4614.88±303.53	4314.47±309.11	5252.06±405.98	5710.99±668.89
	WT	272.14±7.56	177.28±27.11	2015.83±201.72	3519.91±256.12	3304.94±2.98.27	4428.42±378.12	4394.61±409.13
OP (mmol/kg)	T	547.22±57.69	378.67±35.67	761.56±145.29	720.89±89.67	865.89±67.34	858.11±98.11	1200.44±111.19
	WT	486.67±45.34	351.33±31.12	542.33±176.34	693.67±45.22	561.67±23.61	710.33±75.18	827.00±89.11
EC (%)	T	20.81±1.23	19.87±1.14	29.91±2.25	22.86±2.89	35.54±4.41	25.25±1.76	49.20±2.94
	WT	21.74±1.45	21.57±1.56	34.38±2.47	34.31±1.97	47.59±3.91	37.11±2.45	61.89±3.45
Chlorophyll (mg/g FW)	T	1.48±0.11	1.47±0.10	1.49±0.06	1.50±0.05	1.49±0.04	1.42±0.04	1.45±0.03
	WT	1.42±0.05	1.43±0.4	1.41±0.07	1.17±0.04	1.40±0.06	1.13±0.03	1.36±0.06

CONCLUSIONS

Three transgenic events with improved biochemical, physiological traits developed in this study shows enhanced tolerance to drought and salinity stress and these–events could be used for further agronomic performances. The role of transgene in regulation of expression of stress inducible genes under various stressed condition can be analysed further.

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