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Promising drought tolerance associated genes of *Hevea brasiliensis*

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INTRODUCTION

To meet the increasing demand of Natural Rubber (NR), the cultivation is being extended to non-traditional regions like North Konkan, parts of Karnataka, Orissa, Madhya Pradesh where it faces warm temperature, high light and atmospheric and soil drought during summer. Though these areas are not favourable for NR cultivation (Vijayakumar *et al.*, 1998; Jacob *et al.*, 1999), we are left with no option other than identification of suitable clones that can perform better under such extreme agroclimatic conditions. In order to identify/evolve suitable clones, it is essential to identify the genes that are associated with stress tolerance. In this context, a quantitative expression analysis study was carried out with 17 stress related genes of *Hevea* with an objective to identify candidate genes/factors associated with drought tolerance. The promising genes were identified and their relevance in the context of drought tolerance in *Hevea* is discussed in this paper.

MATERIALS AND METHODS

Two year old *Hevea* plants belonging to drought susceptible group (RRII 105) and drought tolerant group (RRIM 600, RRII 208 and RRII 430) grown in Regional Research Station, Dapchari, Maharashtra, India were selected for the present study. The control plants were irrigated on every third day, while the drought imposed plants were given life saving irrigation once in 15 days. Gas exchange parameters (A, g and transpiration rate) were measured using a portable photosynthesis system (Li-6400, Li-COR, USA) on intact, fully mature top leaves on 14th day and leaf samples were collected in liquid nitrogen after confirming the impact of stress by gas exchange parameters.

mRNA was isolated using magnetic beads (Dynabeads, Invitrogen, USA) and cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen, USA). Quantitative gene expression analysis was carried out at least thrice under standard conditions using Applied Biosystems 7500 Real Time PCR System. Reaction efficiency of both the target genes and the endogenous control was calculated based on the formula, Efficiency = $10^{(-1/\text{slope})} - 1$. Primers with slope values between -3.2 and -3.5 only were employed for these reactions. GAPDH gene was used as endogenous control. Three biological replications were included in the qPCR analysis for each treatment. Statistical analysis was performed with the relative quantification data using ANOVA. The ratio with a P value ≤ 0.05 was adopted as significant for up or down regulation.

RESULTS AND CONCLUSIONS

All the three tolerant clones maintained significantly higher rate of photosynthesis than RRII 105 under drought stress when compared to the susceptible clone RRII 105 (data now shown). The relative quantification data of 17 genes in three drought tolerant clones as compared to RRII 105 control plants is furnished in Table 1. Among the genes studied, only two genes were found significantly up-regulated in all the three drought tolerant clones. HbDRT 5b and HbTPD 24 which have homology to NAC transcription factor (NAC tf) got expressed significantly under drought conditions. Another gene which

Table 1. Relative quantification of seventeen genes under drought conditions with reference to irrigated plants of RRII 105 (RQ values)

Genes	RRII 105 C	RRII 105 D	RRIM 600 C	RRIM 600 D	RRII 208 C	RRII 208 D	RRII 430 C	RRII 430 D	CD
HbDRT 5b	1	1.04	1.44	14.26**	1.11	7.16**	0.61	4.30**	2.10
HbTPD 24	1	0.89	1.34	14.41**	0.69	3.82**	0.60	3.47**	1.90
HbTPD27	1	1.83	2.99	1.99	4.58**	4.97**	3.04	9.83**	3.50
HbDRT50	1	1.65	4.58**	3.75*	3.90*	3.15	2.20	4.72**	2.41
HbNRG18	1	0.47	4.34	5.23**	74.39**	7.22**	3.25	29.65**	3.63
HbNRG 21	1	1.28	4.93	3.23	6.24**	4.27	5.39**	16.45**	4.13
HbDRT 82b	1	0.99	1.40	1.37	2.50	1.10	0.95	1.66 -	
Peroxidase	1	2.195	42.31**	95.74**	1.441	12.84	5.140	26.65**	18.63
LEA 5	1	0.856	1.150	5.001**	2.583**	3.219**	0.902	2.973**	1.029
CRT/DRE bf	1	1.922**	0.827	0.845	0.639	1.404	0.698	0.784	0.581
WRKY tf	1	0.897	2.063	3.778	4.524	3.729	2.258	7.508*	4.345
Tf MBF	1	1.075	2.448**	1.582**	0.957	1.435	1.3421	1.624**	0.524
GPX	1	1.204	2.237**	2.756**	3.198**	4.418**	2.480**	2.852**	1.012
ABCT	1	1.672	2.813**	4.865**	3.034**	3.175**	1.068	2.059**	0.9718
Hb 33 HP	1	0.631	0.835	1.149	0.694	1.897**	1.358	1.386	0.545
Hb 22HP	1	1.54	2.28	4.54*	3.39*	3.16*	2.06	2.56	1.878
Hb 20 HP	1	1.27	1.65	3.86**	3.34**	3.060**	1.308	2.853**	1.478

*P value ≤ 0.05 ** P value ≤ 0.01

got up-regulated significantly in all the three tolerant clones is LEA 5 protein when compared to RRII 105 control plants. This was also found significantly up-regulated in RRIM 600 and RRII 430 when compared with their respective control.

Peroxidase gene was also found up-regulated in all three tolerant clones when compared to RRII 105 control and their respective control plants though it was significant only in RRIM 600 and RRII 430. Looking at the overall relative quantification results, maximum up-regulation was found with only peroxidase (about 96 fold in RRIM 600). Two genes such as ABCT protein and Hb20 HP were found significantly up-regulated in all three clones when compared to RRII 105 control plants though they are significantly up-regulated only in two clones (RRIM 600 and RRII 430) when compared with their respective control. The other genes investigated neither exhibited any trend with the drought treatment when compared with the RRII 105 control nor with their own respective control.

Among the 17 genes analyzed, only two transcripts exhibited significant up-regulation in all the tolerant clones (HbDRT 5b and Hb TPD 24). The other two genes which are very promising are LEA 5 protein and peroxidase. LEA 5 protein is significantly up-regulated in all three clones when compared to RRII 105 control. The level of peroxidase is remarkable by its magnanimity (about 97 fold) when comparing the expression of other genes. The rest of the genes which did not change much under drought could be treated as not drought responsive. Quantitative gene expression analysis of genes is only recently being attempted for the identification of candidate genes/factors that are associated with the drought tolerance in *Hevea*. With the advent of qPCR technique, it is easier to quantify each gene and establish their relevance under the given stress situations. This study reveals the existence of strong association of three genes such as NAC tf, LEA 5 protein and peroxidase with drought stress tolerance in *Hevea* for the first time. This opens up the possibility of using these genes as marker for drought tolerance in *Hevea*.

REFERENCES

Vijayakumar, K.R., Dey, S.K., Chandrasekhar, T.R., Devakumar, A.S., Mohankrihna, T., Rao, P.S. and Sethuraj, M.R. (1998). Irrigation requirement of rubber trees (*Hevea brasiliensis*) in the sub humid tropics. *Agricultural Water Management*, **35**: 245-259.

Jacob, J., Annamalainathan, K., Alam, B., Sathik, M.B.M., Thapliyal, A.P. and Devakumar, A.S. (1999). Physiological constraints for cultivation of *Hevea brasiliensis* in certain unfavorable agroclimatic regions of India. *Indian Journal of Natural Rubber Research*, **12(1&2)**: 1-16.