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Revised Final Report
of the research grant titled

Genomics and proteomic approaches of identifying dehydration stress responsive genes from selected rice varieties in Sri Lanka

(Grant Number: RG/2012/BT/01)

submitted by

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FR 1709.

Section 1

Information regarding Project/Project Personnel

i). Contract number: RG/2012/BT/01

ii). Title of the project: Genomics and proteomic approaches of identifying dehydration stress responsive genes from selected rice varieties in Sri Lanka

iii). Principal investigator: Dr. H.A.M. Wickramasinghe, Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya

iv). Co-investigators: Dr. H.M.V.G. Herath, Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya

v). Institute(s) where research is being carried out: Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya.

vi). Date of award/date Project was initiated: 20.09.2012 (The project was initiated 01.01.2013 because of unavailability of principle investigator)

vii). Date of completion of the project: 08. 05. 2015

viii). Total allocation of funds (Rs.): 2,761,350.00

ix). Total spent (Rs.): 2,537,414.21

x). Number of research students employed: one

Ms. J.K.P.T.P. Jayaweera

xi). Postgraduate degree completed with dates:

- Ms. J.K.P.T.P. Jayaweera registered for M. Phil. degree in February, 2013 in the Board of Study in Agricultural Biology, Postgraduate Institute of Agriculture, University of Peradeniya. Title of the research is Genomics and proteomic approaches of identifying dehydration stress responsive genes from selected rice varieties in Sri

Lanka. She completed all course work requirement and sat for the comprehensive examination. The research component is completed and the thesis has to be submitted.

xii). Number of Technical Assistants/Labourers employed: No technical assistants were employed. Labourers were hired when required only.

x). Publications/Communications arising from the project during the reporting period:

- Jayaweera, J.K.P.T.P, Herath, H.M.V.G.., Jayatilake, D.V., Udumulla, G.S. and Wickramasinghe, H.A.M. (2015). Physiological, biochemical and proteomic responses of rice (*Oryza sativa* L.) variety Godaheenati and Pokkali for drought stress at the seedling stage. – Paper submitted to 27th Annual Congress, Post Graduate Institute of Agriculture, University of Peradeniya
- Jayaweera, J.K.P.T.P., Herath, H.M.V.G., Wickramasinghe, H.A.M. (2015). Evaluation of the salinity and drought stress responses of selected Sri Lankan rice (*Oryza sativa* L.) varieties based on germination and seedling emergence - Paper submitted to Journal of Agricultural Sciences
- Wickramasinghe, H.A.M. (2014). Guide to Two-Dimensional gel electrophoresis: Theory and Practice. Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka – A laboratory guide book prepared based on protocols developed and tested through the grant and equipment grant RG/2012/EQ/05 (in print)
- Weerasinghe, W.M.M.E., Senevirathne, W.M.A, Wickramasinghe, H.A.M. and Herath, H.M.V.G. (2015). Computational analysis of stress responsive *ABRE* gene in *Oryza* genus. Paper submitted to 27th Annual Congress, Post Graduate Institute of Agriculture, University of Peradeniya (Ms. Weerasinghe conducted the research as her Directed study for M.Sc (Biotechnology) program).
- Rukshana, M.M.F., Herath, H.M.V.G., Wickramasinghe, H.A.M. (2014). *Ab-initio* identification of dehydratin responsive promoter elements in selected rice varieties of Sri Lanka. Proceedings of the undergraduate research, Department of Agricultural Biology. Faculty of Agriculture. University of Peradeniya. 4, 91-94 (Ms. Rukshana carried out this part of the study as her undergraduate research project)

Section 2

Executive summary of the project

Dehydration of crop plants due to several environmental conditions such as drought and high salinity is one of the most crucial factors that may impair plant growth and development and thereby resulting in poor crop productivity. In recent years, the physiological and molecular basis for plant responses to dehydration tolerance has been a subject of intense research. However, the plant tolerance mechanisms are poorly understood. Thus, the identification of novel genes, determination of their differential expressions, and understanding of their functions are important in improving plants' levels of tolerance against such stress.

Dehydration stress is becoming a serious problem in rice cultivation in Sri Lanka due to low quality water supply, high input agriculture and climatic changes. Recently, a breeding program for the development of drought resistant rice has been launched with exotic parents because of lack of knowledge on genetic potential of dehydration stress tolerance of Sri Lankan rice germplasm. Therefore, the present study is proposed with the main objective of identifying genes and proteins responsible for dehydration tolerance in selected rice varieties using both genomics and proteomic approaches with gene regulation and 2-Dimensional gel electrophoresis.

A series of laboratory and plant house experiments were conducted to achieve the above objective over a period of two years under 5 identified specific objectives (page 8). At the end of the project period, all the objectives except 4th specific objective (given in section 3), were completed fully and valuable research findings were produced as follows.

- Morphological screening at germination level revealed that varieties such as *Goda heenati*, Bg 360, Bg 357, Bg 304 and Bg 450 were performed well as similar to salinity check *Pokkali*. *Pokkali* showed higher tolerance even tested highest level of chemical induced drought. Furthermore, 100 mM NaCl concentration and 10% PEG were identified as the two screening levels for both drought and salinity stress at germination stage of Sri Lankan rice through the experiment.
- Biochemical analysis was performed for *Goda heenati* and *Pokkali* by analyzing chlorophyll content, total proteome and relative water content against drought stress. Based on findings *Goda heenati* showed a resistance against drought stress.

- Protein extraction was done from two weeks old both drought affected and well watered rice seedlings of rice variety; *Goda heenati* by saline phosphate buffer and extracted proteins were separated by 2DE gel electrophoresis. About 50 reproducible proteins spots were recorded on 12% acrylamide gels and they were spread over a pH range of 5-8 with molecular weights (15-75 kDa). Among them, three proteins were identified as up or down regulated during drought stress and those detected proteins were similar to the proteins which identified by previous studies.
- Genomic assay was completed through PCR and sequencing of targeted transcription factors was done. Sequencing results of PCR products were analyzed using molecular tools and detected variations of presence of *cis*-elements in targeted transcription factors of all tested varieties. Also we identified a specific deletion/ insertion in *Goda heenati* and *Pokkali* within an analyzed promoter region of ABRE gene.

Based on the findings in the present study, *Goda heenati* could be identified as a dehydration stress tolerating rice and further studies could be conducted to confirm its stress tolerance.

Section 3

Report in detail

i). Introduction/background:

Rice (*Oryza sativa*) is one of the three major cereal crops which is consume by nearly one third of the world population as their staple food ((Ndjiondjop *et al.*, 2010). Since it is the staple food of large number of people all over the world, it spreads throughout the agricultural lands. Rice is expected to be the most vulnerable crop to numerous abiotic and biotic stresses because of its' wide adaptation throughout the world (Mohanty *et al.*, 2013). Abiotic stresses are key limiting factors that affect the plant growth and development which leads to an ultimate yield loss. Among them, drought (Zapico *et al.*, 2008) and salinity (Pattanagul & Thitisaksakul, 2008) are one of the key limiting factors which determine the crop growth as well as the productivity. Yamaguchi-Shinozaki and Shinozaki (1994) reported that both drought and high-salt conditions collectively induce dehydration stress in plants which may trigger physiological and biochemical responses against such stresses and finally affect the growth and development of plants.

Dehydration of crop plants due to several environmental conditions such as drought and salinity is one of the most crucial factors that may impair plant growth and development thereby resulting poor crop productivity (Boyer, 1982). In recent years, the physiological and molecular basis of plant responses to dehydration tolerance has been a subject of intense research mainly at DNA and RNA levels (Seki *et al.* 2001; Fowler & Thomashow 2002; Gulick *et al.* 2005). However, dehydration response in plants is a complex phenomenon, and the exact structural and functional modifications caused by dehydration are poorly understood.

All such changes are basically a result of either transcriptional activation or inactivation of specific genes which is commonly known as dehydration responsive genes (Shinozaki and Yamaguchi-Shinozaki, 1994). Dehydration stress-related genes can be classified either as `effectors' or `regulators'. Effector genes are directly involved with various adaptive physiological processes. Regulator genes function as controllers of other genes. They facilitate integration of adaptive responses by coordinating the expression of the various

effectors (Brown, *et al.*, 2007; Nakashima *et al.*, 2009). Regulator genes bind to effector genes at specific sequence signatures. Recent findings suggest that these regulatory elements play a major role in determining stress responses and they are responsible for the different levels of tolerance of such stress conditions among cultivars (Park *et al.*, 2010; Yun *et al.*, 2010). Therefore, there is a need to explore the frequency and combinations of regulatory *cis*-elements at the promoter regions of key dehydration responsive genes in order to understand the regulatory impact on drought sensitivity spectrum observed among varieties.

The dehydration-responsive genes are presumed to function not only in protecting cells from water deficit but also in regulating genes for signal transduction (Xiong *et al,* 2002; Shinozaki *et al,* 2003). During drought conditions, a variety of genes are induced and the products of these genes are thought to have functions in stress tolerance as well as in regulation of gene expression through signal transduction pathways (Nakashima *et al.*, 2009). During stress tolerance, plants tend to accumulate numerous proteins and they could be either proteins for stress tolerating or products of regulatory genes; transcription factors (TF) which regulating downstream genes during dehydration stress.

Knowledge of the full complement of proteins expressed by the genome of a cell, tissue or organism at a specific time point (i.e. proteome) is necessary to understand the biology of a cell or an organism. The proteome reflects the actual state of the cell or the organism and is an essential bridge between the transcriptome and the metabolome. Compared to the analysis of the transcriptome, the analysis of the plant proteome in response to abiotic and biotic stresses is still limited, but technical progress has been achieved in the separation of proteins and their identification.

Two-dimensional gel electrophoresis (2-DE) developed about 30 years ago is still the most frequently used method to investigate differential protein abundance in large scale proteomics experiments on crude protein mixtures (O'Farrell 1975). Furthermore, different peptide fingerprinting and sequencing technologies together with bioinformatics tools make proteome analysis very successful especially for plants such as rice as the complete genome sequence is published. However, analysis of proteins using 2DE is a novel approach which was not used to analyze protein profile of rice in Sri Lanka.

Identification of drought or salt tolerance attributes as morphological, physiological and biochemical parameters in rice would be an advantage during crop improvement. (Bunnag and Pongthai, 2013, Usman *et al.*, 2013, Rajiv *et al.*, 2010). In Sri Lanka, there is no evidence in analyzing biochemical attributes such as chlorophyll content, relative water content (RWC) and proteome approaches to screen Sri Lankan rice varieties against drought or salinity.

The most successful strategy of increasing rice productivity would be the development of new varieties with improved qualities including dehydration tolerance. However, prior knowledge about the genetic potential of dehydration tolerance of Sri Lankan rice is not sufficient enough for selecting better parents for such breeding programmes. Therefore, understanding the genetic potential of dehydration tolerance of Sri Lankan rice varieties is very much useful for further strengthening such breeding programmes. The present study was planned to identify dehydration stress tolerant rice varieties based on their morphological, biochemical and molecular attributes during dehydration stress conditions by achieving following objectives.

General objectives:

1. To identify genes and proteins involved in tolerance to dehydration stress of rice leaves
2. To identify potential regulatory element/s that can be used to improve the drought tolerance in cultivated rice varieties.
3. To understand the molecular basis of the dehydration tolerance spectrum observed in traditional rice varieties in Sri Lanka.

To satisfy above general objectives, following specific objectives were achieved.

Specific Objectives:

1. To extract total proteome of leaves affected by dehydration stress
2. To separate total proteome of leaves subjected to dehydration stress by 2 DE
3. To compare the proteome of rice leaves affected by dehydration stress with that of normal rice leaves
4. To purify and identify selected proteins differentially expressed with dehydration stress
5. To investigate the unique sequence variations of rice cultivars that potentially would contribute to their dehydration tolerance gradient by sequencing both genic and intergenic regions of their major dehydration responsive genes

ii). Materials and Methods

Plant Materials

Rice varieties; At 354, Bg 300, *Goda heenati, Kurukaruppan, Pokkali*, were collected from Plant Genetic Resources Center, Gannoruwa and multiplied at the plant house Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya. The above rice varieties were selected based on previous literature (Subasinghe *et al.*, 2007; De Costa *et al.*, 2012). Rice variety *Pokkali* is the standard check variety for salinity tolerance (Moons *et al.*, 1995). In addition to above rice varieties, Japonica rice variety, *Niponbare* and indica rice variety, Cultivar 93-11 were used as standard genomic check varieties and they were kindly donated by Prof. Wang Cailin, Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Xiaolingwei, Nanjing, China. Seeds were harvested and stored at 4^0C for further experiments. Before using them for proteomic and genomic analysis, they were screened for morphological, physiological and biochemical characters related to dehydration stress tolerance at the vegetative stage.

Morphological screening:

Ten rice varieties (*Goda heenati, Kurukaruppan, Pokkali* At 354, Bg 300, Bg 304, Bg 357, Bg 360, Bg 450 and At 303) were initially screened against drought and salinity at their germination stage though 5 of them were initially proposed. A total of fifteen seeds of each variety were subjected to two treatments as fifteen replicates and the experiment was arranged in a complete randomized design (CRD). One set of seeds were exposed to polyethylene glycol (PEG™ 8000) (Promega, USA) at 0%, 10%, 15%, and 20% concentration levels and other set was subjected to salinity stress using 0 mM, 50 mM, 100 mM and 150 mM NaCl concentrations with electrical conductivity 0 dS/m, 4.7 dS/m, 9.9 dS/m and 16.1 dS/m respectively. Seeds were allowed to germinate and after 24 hours the number of seeds that germinated was counted. Seeds were considered germinated when the radicle and plumule were emerged and extended to a length of more than 2 mm (Islam & Karim, 2011). Subsequent recordings were done every 24 hours for 5 days. Plumule length and radicle length were measured at the 6^{th} day. The germination percentage and length reduction in radicle and plumule was calculated using the following equations.

$$\text{Germination percentage (\%)} = \frac{\text{No. of seeds germinated in each treatment}}{\text{No. of seed tested in each treatment}} \times 100$$

$$\text{Length reduction in radicle/plumule (\%)} = \frac{\text{Length at control - Length at treatment}}{\text{Length at control}} \times 100$$

Analysis of variance (ANOVA) was performed using Microsoft Excel (Windows 7) and mean separation was done with Least Significant Difference (LSD) to compare the variation of stress tolerating ability among the varieties and treatments.

Biochemical analysis:

Seeds of *Goda heenati* and *Pokkali* were selected for biochemical analysis. Seeds were planted in 8" length soil column with 1" diameter in mixture of soil containing compost, top soil and clay with the ratio of 1:1:1 as a single plant per bag. Plants were fertilized according to the recommendation of Department of Agriculture and were arranged in a complete randomized design (CRD) with three replications. Dehydration condition was applied on the 4-week-old seedlings by withdrawing water and tissues were harvested at every 24 h for 5 days. The unstressed and the stressed plants were kept in parallel in the same condition. The samples from the unstressed (control) plants were collected every day during the course of the dehydration experiment and following experiments were carried out.

Determination of Relative Water Content (RWC)

Rice leaf tissues were collected and immediately weighed (fresh weight (FW)). Then tissues were rehydrated in water for 24 h until fully turgid, then surface-dried and reweighed (turgid weight (TW)). Finally tissues were oven dried at 80 °C for 48 h, and reweighed (dry weight (DW)). The RWC was calculated by the following formula (Bhushan *et al.*, 2007).

$$\text{RWC (\%)} = (\text{FW- DW/TW- DW})^* 100$$

Estimation of Total Protein Content

About 200 mg of leaf tissue was ground in cold mortar with 1 ml of extraction buffer containing 50 mM Tris-HCL buffer (pH 8.0), 1 mM phenylmethanesulfonyl fluoride (PMSF),

10% (v/v) glycerol and then homogenized with hand held homogenizer (PRO200, PRO Scientific, USA). The homogenate was centrifuged at 12000 rpm for 20 minutes at 4^0C and the supernatant was taken. Protein concentration was measured by Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as a standard (Rajiv *et al.*, 2010).

Pigment Estimation

Tissues harvested at each day were ground in 80% chilled acetone. The supernatant was taken for determination of photosynthetic pigments using spectrophotometer. The absorbance values at 663 nm, 645 nm and 470 nm were measured and the pigment concentrations were calculated using following formula (Bhushan *et al.*, 2007).

Chlorophyll a (mg/g) = [(12.7*A 663 - 2.69*A 645) v/w]

Chlorophyll b (mg/g) = [(22.9*A 645 - 4.68*A 663) v/w]

Carotenoid (mg/g) = {[(1000*A470) - (3.27*chlorophyll a + 1.04*chlorophyll b)] /227 v/w}.

Specific objective no. 1: Extraction of total proteome of leaves affected by dehydration stress

Rice variety *Goda heenati* was used for further studies at proteomic level based on the morphological and biochemical analysis. Rice seeds were sorted manually to eliminate broken, small and infected seeds. Healthy seeds were surface sterilized in 5% (v/v) NaOCl solution for 20 minutes. The seeds were rinsed repeatedly with sterilized distilled water and air-dried at room temperature (Jamil *et al.*, 2012). Seeds were planted in plastic pots in mixture of soil containing compost, top soil and clay with the ratio of 1:1:1 as a single plant per bag. Plants were fertilized according to the recommendation of Department of Agriculture. Tissues were harvested from two weeks old plants which subjected for drought stress by withdrawing water for 10 days. The unstressed and the stressed plants were kept in parallel in the same condition. The samples from the unstressed (control) plants were also collected every day during the course of the dehydration experiment. A portion (400 mg) of fresh rice leaf tissues was homogenized with 1 mL of phosphate buffer (pH 7.6) containing 65 mM K_2HPO_4, 2.6 mM KH_2PO_4, 400 mM NaCl and 3 mM NaN_3. The homogenate was centrifuged at 15000 rpm for 10 minutes at 4^0C. Supernatant was transferred to new 1.5 ml

microcentrifuge tube and proteins were purified and precipitated with BioRAD 2-D clean up kit. Resulted pellet was dissolved in 350 µl of 2D rehydration buffer. Furthermore, the total proteins were extracted using TCA acetone method with slight modifications (Zhao *et al.*, 2013).

Specific objective no.2 Separation of the total proteome of leaves subjected to dehydration stress by 2 DE

IPG strip with 18 cm in length (pH 3-10, non-linear, BioRAD) was rehydrated with 350 µL of rehydration buffer with the sample for 24 h. Isoelectric focusing was performed at 250 V for 30 min, followed by 1000 V for 30 min with a gradient, 2500 V for 1h 30 min with a gradient and 2500 V for 5 h. The first-dimensional isoelectric focusing gel electrophoresis (IEF) was conducted at 20^0C. After first-dimensional electrophoresis, IPG strips were subjected to equilibration by two step procedure. First, reduction of IPG strips was done using equilibration buffer containing DTT with the shake for 15 min and second equilibration (Alkylation) was performed using equilibration buffer containing idoacetamide. Second dimensional SDS-PAGE was performed in 12% acrylamide gels using OmniPAGE WAVE (Cleaver Scientific Ltd, UK). Two gels were simultaneously run with the current of 35 mA at 20^0C and protein spots were visualized by coommassie staining.

Specific objective no.3. Comparison of the proteome of rice leaves affected by dehydration stress with that of normal rice leaves

Spots were counted in both control and drought affected gels manually and up or down regulated spots were compared with previous studies.

Specific objective no.4. Purification of identify selected proteins differentially expressed with dehydration stress

This was not achieved due to the unavailability of service providers with available funds. I have informed the NSF regarding this change (copies of the letters attached)

Specific objective no.5. Investigation of the unique sequence variations of rice cultivars that potentially contribute to their dehydration tolerance gradient by sequencing both genic and intergenic regions of their major dehydration responsive genes

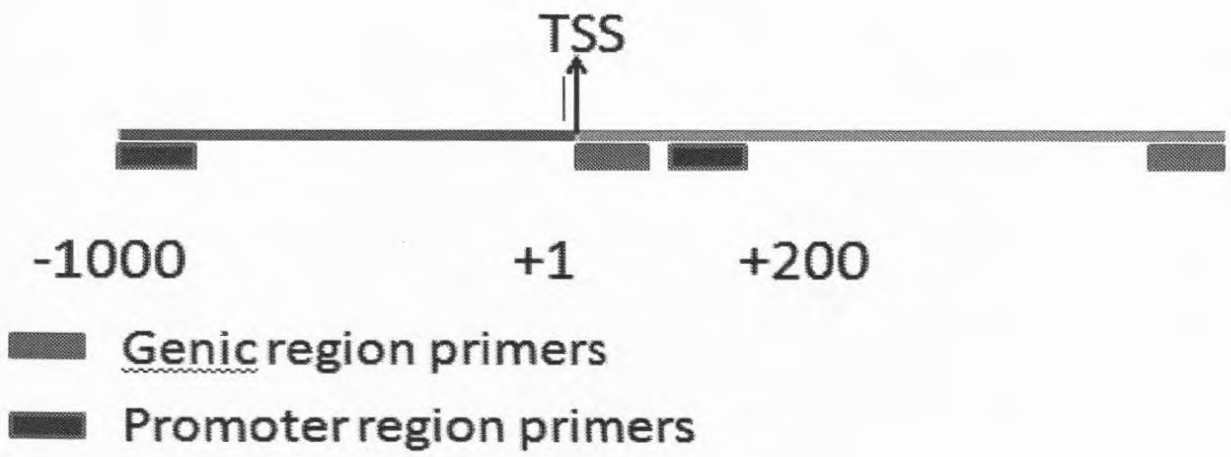
Seeds of five rice varieties (Bg 300, At 354, *Goda heenati, Kurkaruppan* and *Pokkali*) were planted in soil pots and grew under plant house. Immature leaves from 2 weeks old seedlings were harvested for DNA extraction and insect damaged and disease infected leaves were eliminated. Harvested tissues were ground in liquid nitrogen until get fine powder and DNA extraction was done by using CTAB method (Murray and Thompson, 1980). Powdered tissues (3 g) were added to 15 ml of preheated CTAB buffer and incubated for 45 minutes. After incubation, samples were cooled and 15 ml of Chloroform- isoamyl alchohol (24:1) was added and contents were mixed and centrifuged at 4000 rpm for 20 minutes. The top aqueous supernatant was carefully transferred in to clean 1.5ml microcentrifuge tubes (as aliquots). 1/6 volume of ice-cold isopropanol was added and contents were mixed by gentle inversion until the white, thread like DNA appears. Samples were centrifuged at 12000 rpm for 5 minutes and DNA pellets were washed with 500 µl of 70% ethanol. DNA was dried by inverting on the paper towel until ethanol is evaporated. 100 µl of 1 M TE buffer was added and dissolved by a little spin. Then the samples were stored in -20^{0}C for further experiments.

DNA Quantification

Extracted DNA was quantified using both spectrophotometer and 0.8% agarose gels. According to the requirement for PCR protocol original DNA samples were diluted for solutions of 25 ng/µl.

Primer Designing

Primers were designed for both genic and promoter regions of selected transcriptional factors; ABRE, DREB2a, bZIP and AP2/ERF using Primer3PLUS software (Table 3) . Original primers were diluted to working solutions of 10 ng/µl.



PCR amplification:

PCR was performed according to the instruction manual in Promega master mix.

Table 1. Chemical constituent for PCR reactions

Constituent	Concentration in the final reaction	Amount for one reaction
Master Mix (2X)	1X	10 µl
Primer F (10 ng/µl)	10 ng	1µl
Primer R (10 ng/µl)	10 ng	1 µl
DNA (25ng/µl)	100 ng	4 µl
Sterilized Distilled water	-	4 µl
Total		20 µl

Table 2. Thermal conditions for PCR performed

Step	Temperature (^{0}C)	Time duration	
Initial denaturation	95	2 min	
Denaturation	95	30 sec	30 cycles
Annealing	Changed according to the primer	30 sec	
Extension	72	1 min	
Final Extension	72	5 min	
Hold	4	hold	

Table 3. Details of the primers designed for the experiment

Gene	Targeted region	Forward		Reverse		Expected product size (bp)
		Sequence	Tm (°C)	Sequence	Tm (°C)	
ABRE	Genic	5' - AATGGAGTTGGAAGCTGAGG – 3'	58.4	5'- CGAATTGGTTCGATAGAGTTAGC- 3'	61.1	996
	Promoter	5'- ACCTTTTTACCTTCTGAACTTTCG - 3'	60.1	5'- TGATTAGTTGGGGGAGTGATG-3'	59.4	999
DREB2a	Genic	5'- TCAGGGAAAATATCCACATAACC-3'	59.3	5'- TCTTTGTTGCTGTACACTGCTG-3'	60.3	991
	Promoter	5'- AGGCTTCAGTAATTAGCATAACGAG-3'	62.5	5'- CAGCCTACCAGAGGACCAAG-3'	62.5	935

bZIP	Genic	5'-CATGCCAAGCACATTAGCTC-3'	58.4	5'-ATGGACAAGCTCGCCAAC-3'	56.1	915
	Promoter	5'-ATCAAGCCCATCGGTCATAG-3'	58.4	5'-GAGCATATGCACCATCTTAACG-3'	60.3	976
AP2	Genic	5'-ATGGACGACTCCCACGAC-3'	58.4	5'-TTGCTGGATCACTTTTTACCAC-3'	58.4	980
	Promoter	5'-TTTGCAAATTTGGTGTTTGG-3'	52.3	5'-TCGACGTAGACGAAGACGAC-3'	60.5	988
NAC	Genic	5'-GAATTTCTCCGTGCAAACG-3'	55.2	5'-AGCGACCAAGCAAAATAAGC-3'	56.4	981
	Promoter	5'-GTTTGTTCATGTGGATATGTTGTTC -3'	60.9	5'-TTTGGCATGGAACTAAACACAC-3'	58.4	999

PCR products were confirmed in 1% agarose gels and sent to Macrogen, Korea for sequencing.

Multiple alignment and transcription factor analysis of DNA sequences:

DNA sequences were aligned by CLC sequence viewer 6 software and promoter region were analyzed using JASPAR, PLANTCARE and TRANSFAC software to find transcriptional factor analysis.

Data analysis:

Compared the sequential variations through multiple alignments and identified Single Nucleotide Polymorphism (SNPs) present in aligned sequences. According to the results obtained through promoter region analysis, corresponding TF Binding sites were inspected with the relative score of 80% and identified TFs which involved in dehydration stress responsive gene expression in both Abscisic Acid (ABA) dependent and ABA independent pathways.

Except above mentioned objectives, we were carried out morphological screening at germination stage against drought and salinity stresses and biochemical analysis at the seedling stage to validate the findings by genomic assay.

iii. Results

Morphological screening:

Effect of salinity on germination

Figure 2 illustrates the variation in germination percentage of rice varieties at different salt concentrations and germination percentage of rice varieties were reduced with a significant difference along with the increase of salt concentration. Except At 303, all the other varieties germinated with 60% to 100 % of germination percentage even at the 100 mM salt concentration. Even at highest tested salt concentration (150 mM salt concentration), both Bg 450 and Bg 360 obtained 80% germination while Bg 357 gave 73% of germination. At 303 showed no germination at all tested salt concentrations. 150 mM salt concentration was identified as a critical level of salinity and 100 mM could be used as to screen rice varieties for salinity tolerance at their germination and early seedling growth.

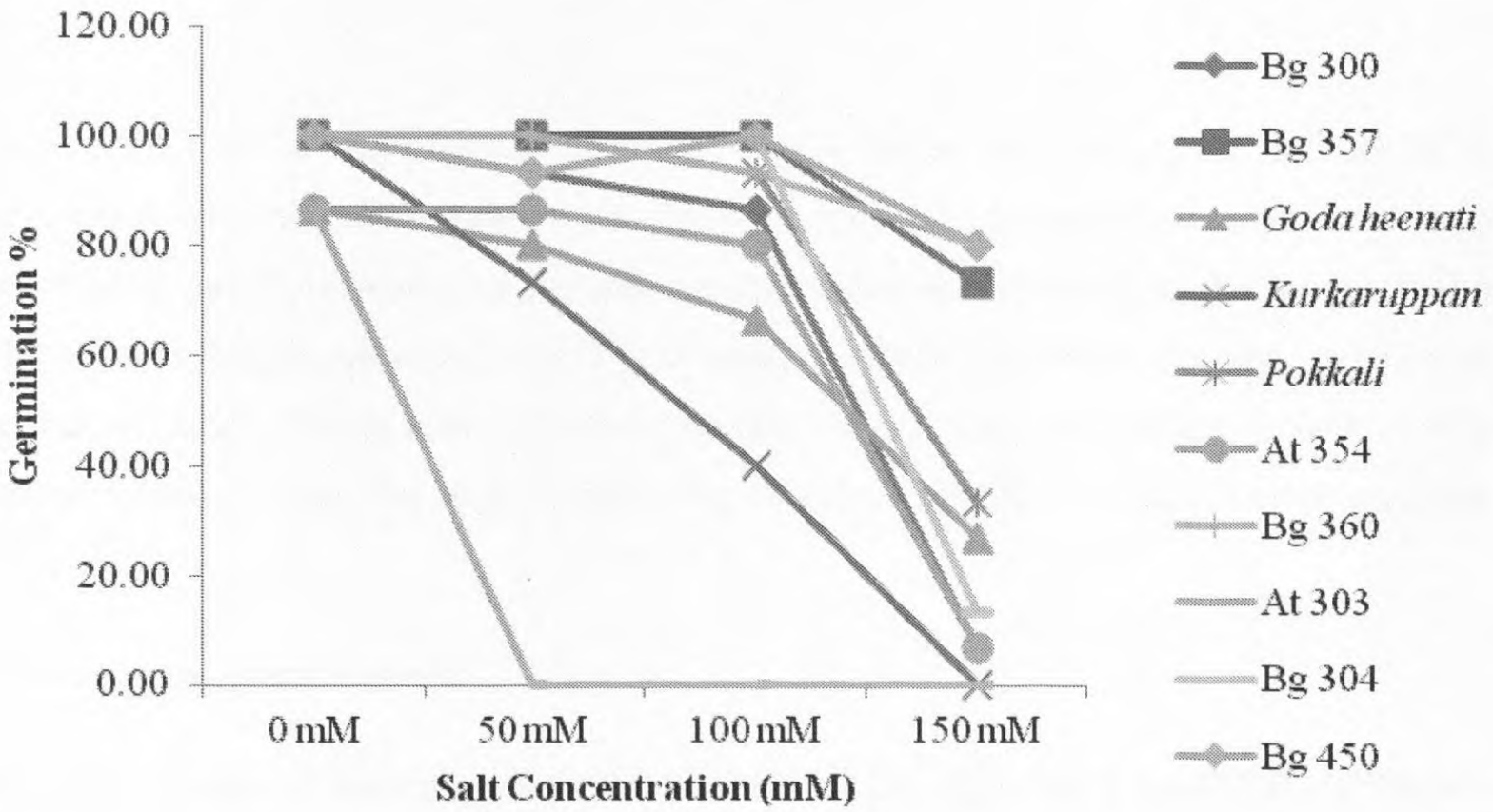


Figure 2. Effect of increased salt concentration on germination percentage of selected rice varieties

Table 4. Effect of salt concentration on radicle and plumule length reduction of rice varieties

Variety	Plumule length reduction			Radicle length reduction		
	50 mM	100 mM	150 mM	50 mM	100 mM	150 mM
Bg 300	0.32 ^{bc}	0.81 ^{bc}	0.98 ^{a}	0.34 ^{ab}	0.54 ^{ab}	0.83 ^{ab}
Bg 357	0.21 ^{bc}	0.58 ^{ef}	0.84 ^{b}	(-)0.06 ^{bc}	0.08 ^{d}	0.74 ^{b}
Godaheenati	0.39 ^{b}	0.72 ^{cd}	0.95 ^{a}	0.27 ^{ab}	0.22 ^{cd}	0.63 ^{c}
Kurkaruppan	0.18 ^{bc}	0.87 ^{b}	1.0 ^{a}	0.55 ^{a}	0.77 ^{a}	0.94 ^{a}
Pokkali.1	0.01 ^{c}	0.76 ^{bc}	0.95 ^{a}	(-)0.8 ^{d}	(-)0.4 ^{e}	0.22 ^{d}
At 354	0.45 ^{b}	0.8 ^{bc}	0.98 ^{a}	(-)0.2 ^{c}	0.47 ^{abc}	0.95 ^{a}
Bg 360	0.27 ^{bc}	0.61 ^{de}	0.83 ^{b}	0.02 ^{bc}	0.13 ^{d}	0.74 ^{b}
At 303	1.0 ^{a}	1.0 ^{a}	1.0 ^{a}	0.53 ^{a}	0.61 ^{ab}	0.97 ^{a}
Bg 304	0.32 ^{bc}	0.54 ^{ef}	0.97 ^{a}	0.29 ^{ab}	0.36 ^{bcd}	0.65 ^{b}
Bg 450	0.14 ^{bc}	0.47 ^{f}	0.67 ^{c}	0.4 ^{ab}	0.62 ^{ab}	0.66 ^{b}

The length of both radicle and plumule of tested rice varieties showed significant variation even at the control with no NaCl (Table 4). Hence, we used length reduction in either radicle or plumule of each variety at every tested salt concentration as a fraction to the length at the control. The radicle length reduction was significantly ($p \leq 0.05$) different among varieties at all tested concentrations. While most of the tested rice varieties restricted their growth at 150 mM salt concentration, *Goda heenati, Pokkali*, Bg 304 and Bg 450 showed a better seedling growth.

Effect of drought on germination

Germination percentage of tested varieties was reduced by significant ($p \leq 0.05$) difference with the increasing concentration of PEG (Figure 3).

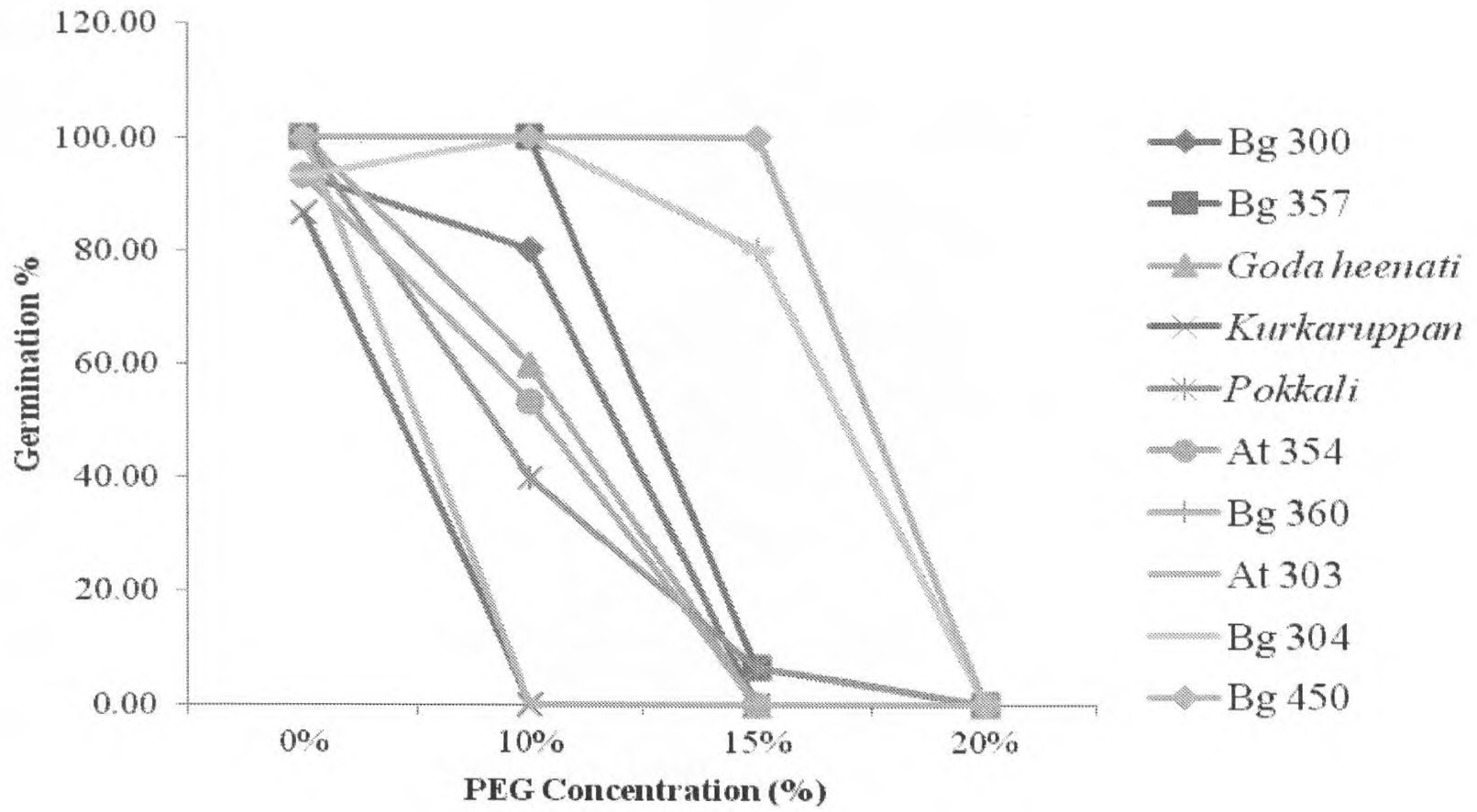


Figure 3. Effect of increased PEG concentration on germination percentage of rice varieties

At 10% PEG concentration, except *Kurukaruppan* and At 303, all other varieties germinated with the range from 40% to 100% and salinity tolerant *Pokkali* reduced its' germination percentage to 40% Therefore, at 10% PEG concentration, rice varieties were grouped in to several groups clearly than the other tested PEG concentrations with significant differences ($p<0.05$). In 15% PEG concentration, Bg 450 maintained 100% germination and Bg 304 and Bg 360 showed 80% germination and other varieties were drastically reduced germination. No any single varieties were able to germinate in 20% PEG concentration. Furthermore, at 10 % PEG concentration, screened varieties were separated into several groups with significant difference ($p \leq 0.05$).

Similar to salinity experiment, early seedling growth of rice varieties were restricted by the increase of PEG concentration (Table 5). *Pokkali,* Bg 304 and Bg 450 showed better growth compared to other tested varieties at 15% PEG concentration.

Table 5. Effect of increased PEG concentration on radicle and plumule length reduction of rice varieties

Variety	Plumule length reduction			Radicle length reduction		
	10 %	15%	20 %	10%	15%	20%
Bg 300	0.81 [b]	1.0 [a]	1.0 [a]	0.48 [ab]	0.76 [ab]	1.0 [a]
Bg 357	0.63 [cd]	0.98 [a]	1.0 [a]	0.19 [abc]	0.76 [ab]	1.0 [a]
Godaheenati	0.79 [bc]	1.0 [a]	1.0 [a]	0.54 [a]	0.78 [ab]	1.0 [a]
Kurukaruppan	1.0 [a]	1.0 [a]	1.0 [a]	0.38 [abc]	0.91 [a]	1.0 [a]
Pokkali.1	0.92 [ab]	0.99 [a]	1.0 [a]	(-) 0.02 [c]	0.45 [c]	0.9 [b]
At 354	0.81 [b]	1.0 [a]	1.0 [a]	0.43 [ab]	0.88 [a]	1.0 [a]
Bg 360	0.6 [d]	0.83 [b]	1.0 [a]	0.21 [abc]	0.77 [ab]	1.0 [a]
At 303	1.0 [a]	1.0 [a]	1.0 [a]	0.12 [abc]	(-) 0.008 [d]	1.0 [a]
Bg 304	0.27 [e]	0.64 [c]	1.0 [a]	0.03 [bc]	0.6 [bc]	0.83 [c]
Bg 450	0.24 [e]	0.3 [d]	1.0 [a]	0.09 [abc]	0.11 [d]	0.86 [bc]

Biochemical analysis:

Relative Water Content

Pokkali showed a gradual reduction of RWC during whole experimental period whereas *Goda heenati* showed an increase of RWC at fourth day of drought after a gradual reduction of RWC only up to the third day. It again reduced on the fifth day and there was no significant difference between RWC in both *Goda heenati* and *Pokkali* during whole experimental period in this study.

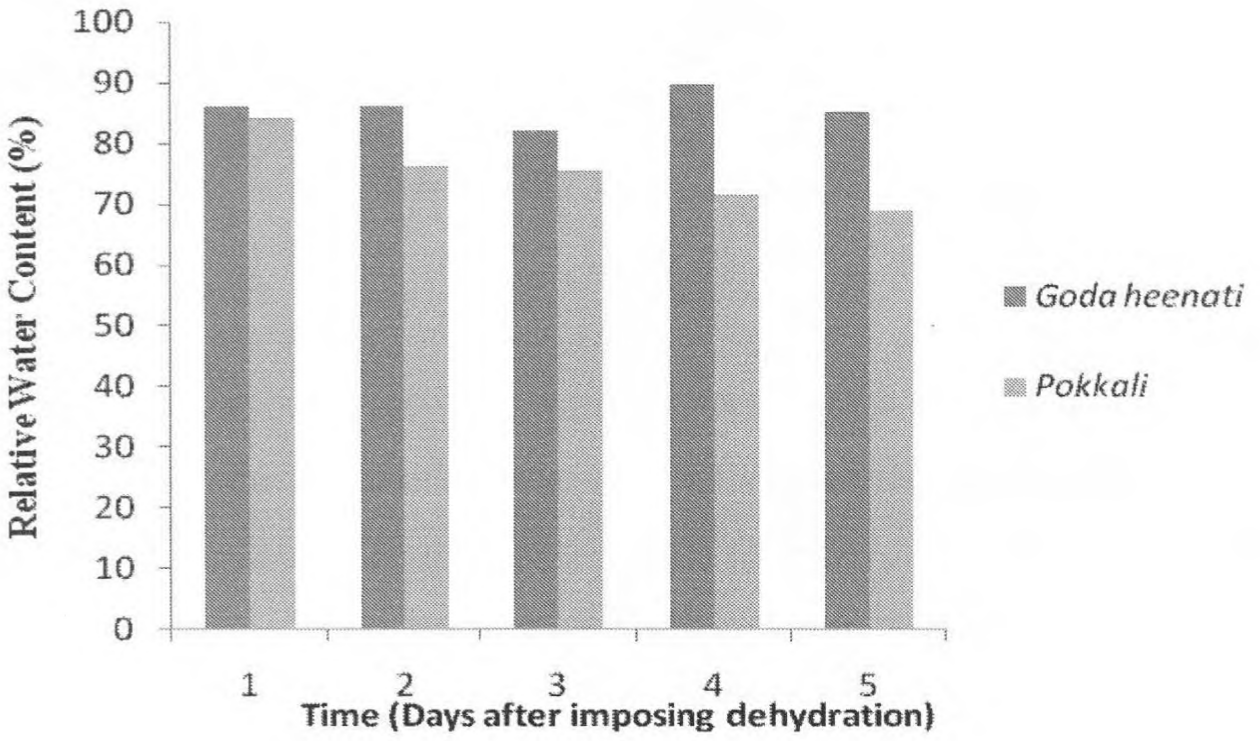


Figure 4. Effect of Drought on Relative Water Content in Rice varieties *Goda heenati* and *Pokkali*

Total protein content

Goda heenati showed negligible changes in the protein content at the first and second day. However a severe reduction of total protein content was shown at the third day followed by a drastic increase at the fourth day onwards. *Pokkali* showed a similar pattern of variation in the total protein content as in *Goda heenati.* However unlike first, second and fifth days, *Pokkali* showed higher protein content than *Goda heenati* at third and fourth days.

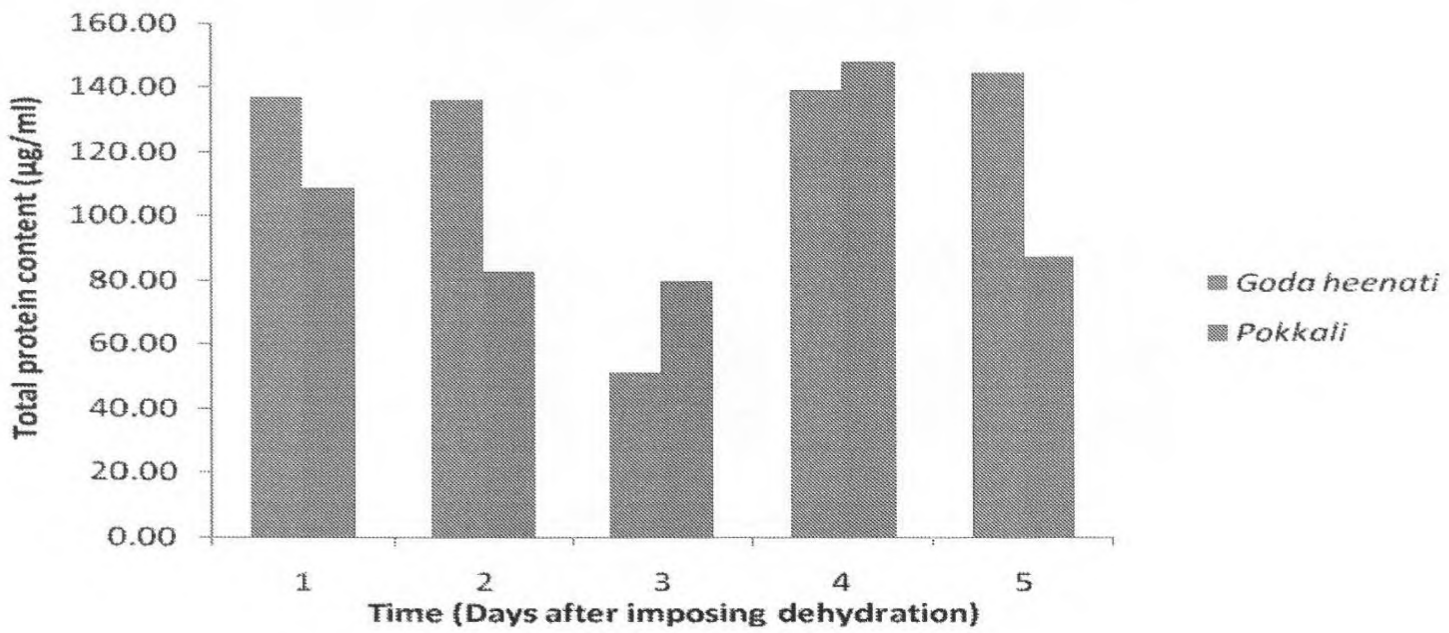


Figure 5. Effect of drought on total protein content in *Goda heenati* and *Pokkali*

Photosynthetic pigments

Chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and carotenoid contents showed similar pattern of variation in both varieties during drought phase (Figure 6 A, B and C). Both *Goda heenati* and *Pokkali* showed a gradual reduction ($p \leq 0.05$) of all three pigments during first three days and a rapid increase at fourth day followed by a reduction at fifth day again with a significant difference ($p \leq 0.05$). Furthermore, there is no significant difference ($p \leq 0.05$) among two tested varieties in all three pigment concentrations during drought period except for fourth day. At the fourth day, *Goda heenati* showed a rapid increase of photosynthetic pigments which was significantly ($p \leq 0.05$) higher than those of *Pokkali*.

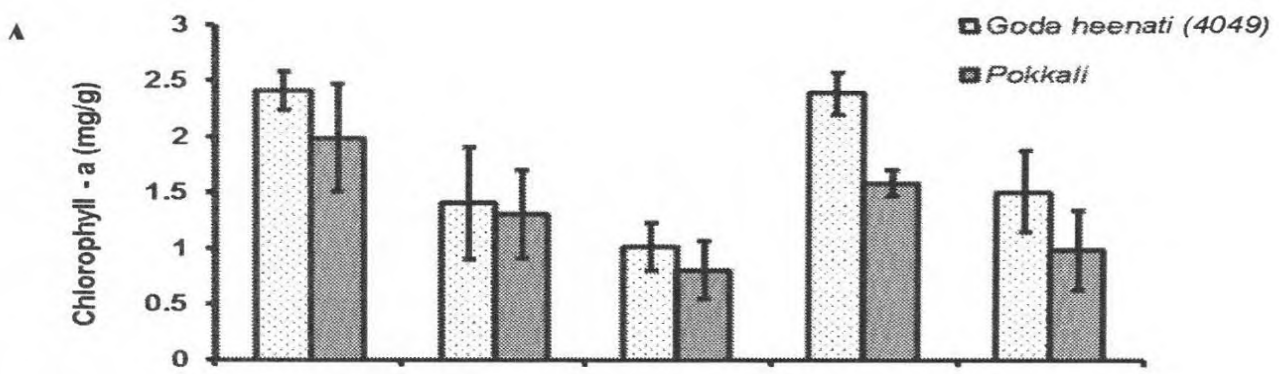


Figure 6. Effect of drought on chlorophyll-a (A), chlorophyll-b (B) and carotenoid (C) in *Goda heenati* and *Pokkali*

Specific objective no. 1: To extract total proteome of leaves affected by dehydration stress

This specific objective was achieved completely. Total protein content was extracted successfully and obtained a protein pellet after purification process. Figure 7 shows the total protein separated on SDS-PAGE.

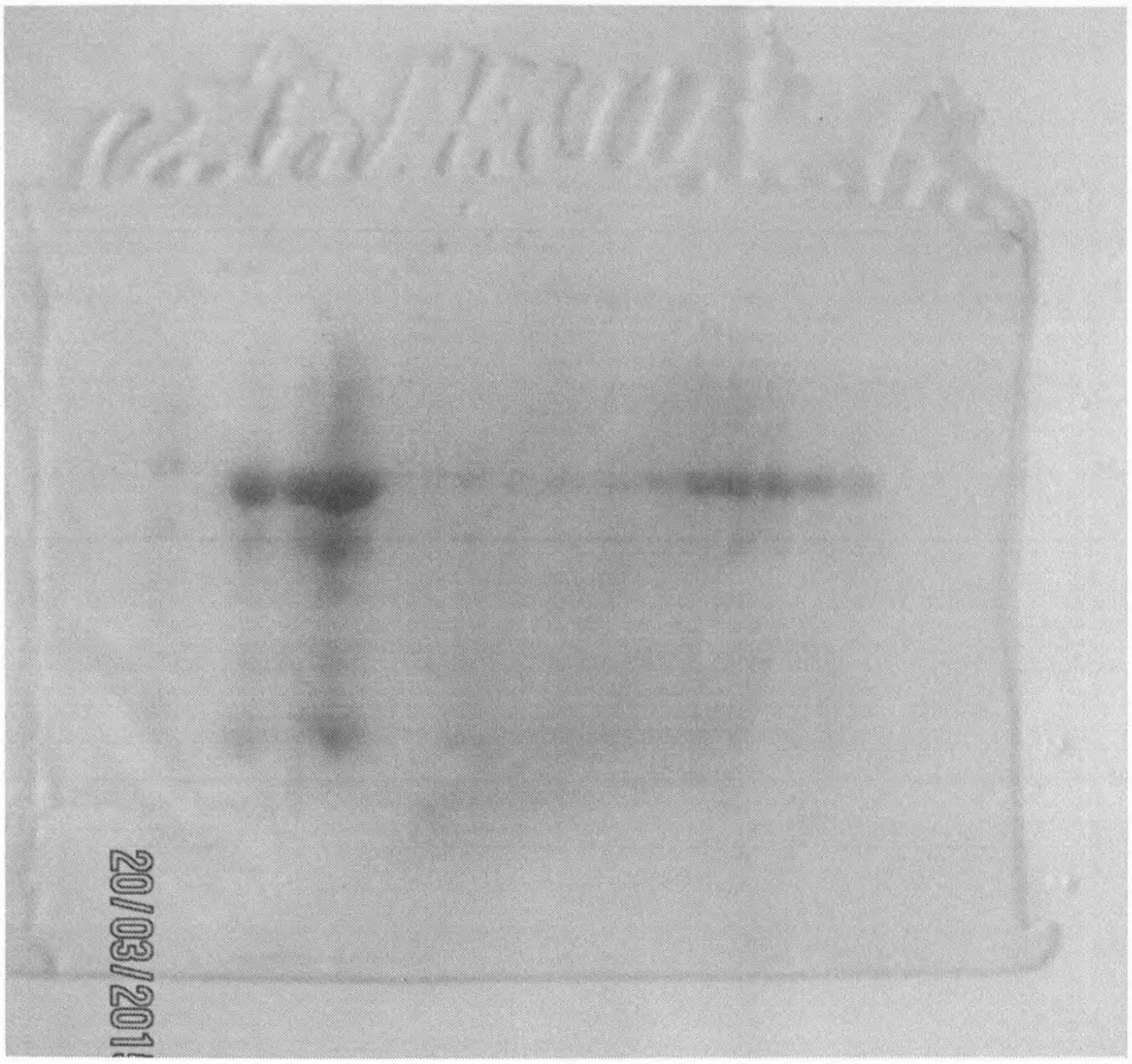


Figure 7. Separation of total protein from leaves of variety *Goda heenati* on SDS-polyacrylamide gel electrophoresis

Specific objective no. 2: Separation of total proteome of leaves subjected to dehydration stress by 2 DE

This specific objective was achieved completely. About 50 reproducible proteins spots were recorded on 12% acrylamide gels stained with coommassie blue (Figure 08). They were spread over a pH range of 5-8 with molecular weights ranging from 15-75 kDa.

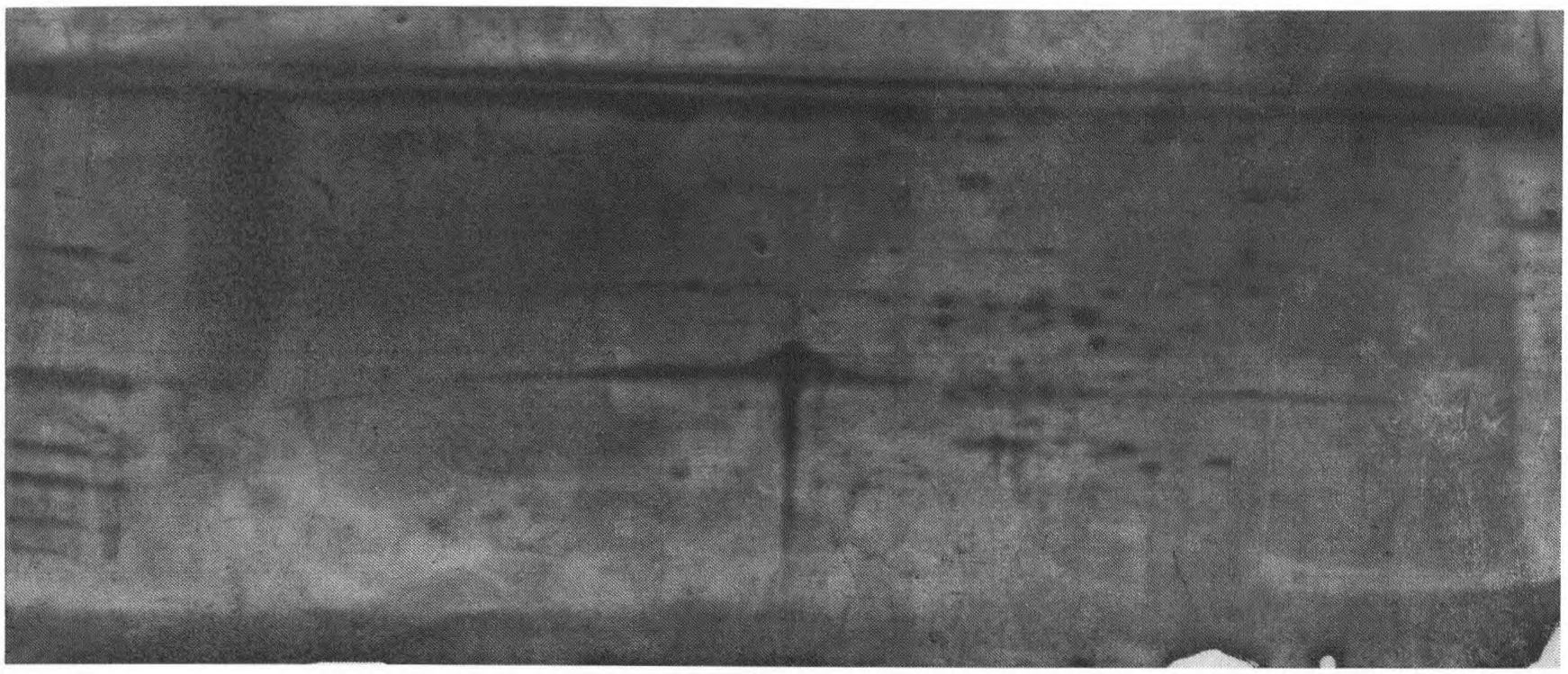


Figure 8 – Separated total proteins of rice leaves of *Goda heenati* after 2DE

Specific objective no. 3: To compare the proteome of rice leaves affected by dehydration stress with that of normal rice leaves

This specific objective was achieved completely.

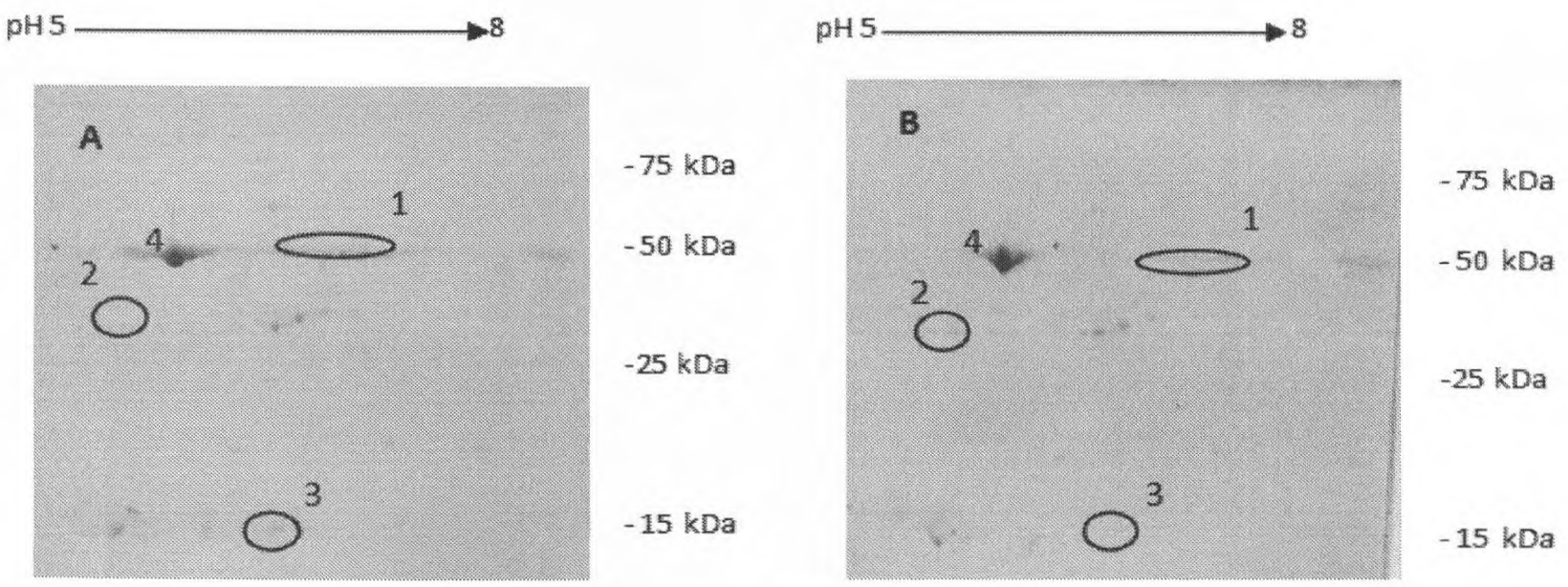


Figure 09. Differentially expressed proteins of two weeks old rice leaves of *Goda heenati* *(A representative fraction of 12% acrylamide gel):* Control (A) and Drought affected (B)

Proteins which numbered as No. 1 and 3 in Figure 9 were up regulated in control plants (Figure 9A) but not in drought affected plants (Figure 9B) and protein which denoted as No.2 was up regulated in drought affected plants (Figure 9B). Protein No. 4 could be RuBisCO

protein, because the size and pH range is quite similar to previous reports available. Generally, the molecular weight and the iso-electric focusing point of one protein is not similar to another protein.

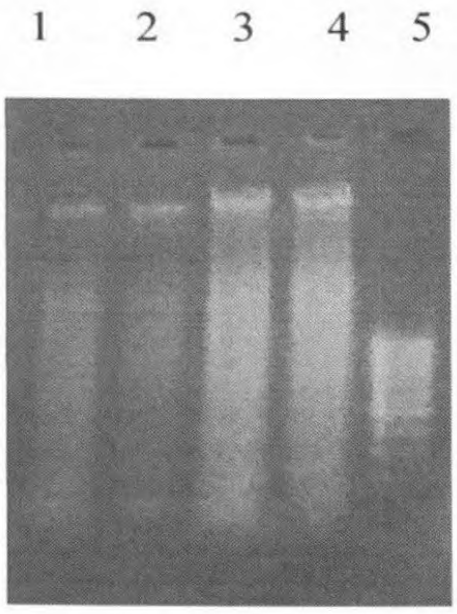
Specific objective no. 5: Investigation of the unique sequence variations of rice cultivars that potentially contribute to their dehydration tolerance gradient by sequencing both genic and intergenic regions of their major dehydration responsive genes

This specific objective was achieved completely.

All tested rice varieties were given good quality genomic DNA (Figure 10) with satisfactory quantity after extraction by CTAB method (Table 6).

Table 6. Concentration of DNA by Spectrophotometer

Rice variety	DNA concentration µg/µl	Ratio between A_{260}/A_{280}
At 354	0.2400	1.7735
Kurukaruppan	0.4500	1.8192
Goda heenati	0.5598	1.7614
Bg 300	0.3900	1.8842
Pokkali	0.4398	2.0333



1 – At 354
2 – *Kurukaruppan*
3 - *Goda heenati*
4 – Bg 300
5 - Ladder

Figure 10. Extracted DNA of four rice varieties in 0.8% agarose gel (for 20 minutes at 50V)

Amplified products obtained through PCR were around 1000 bp as targeted (Figure 11).

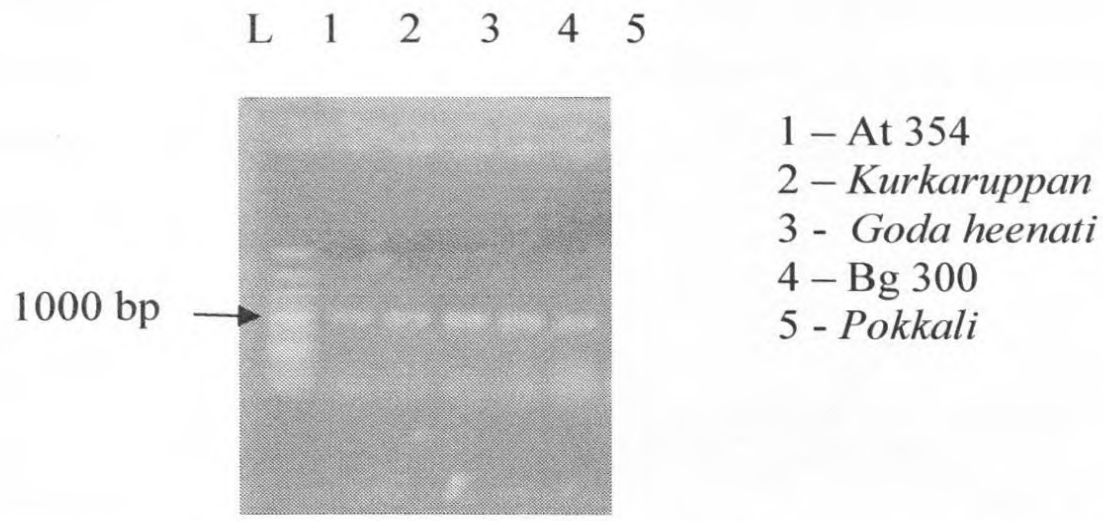


Figure 11. Amplified products of rice varieties obtained through PCR in 1.0% agarose gels

Genomic assay was completed through PCR and sequencing of targeted transcription factors was done. Figure 12 represented a chromatogram for DNA sequencing of promoter region of ABRE in variety *Goda heenati.*

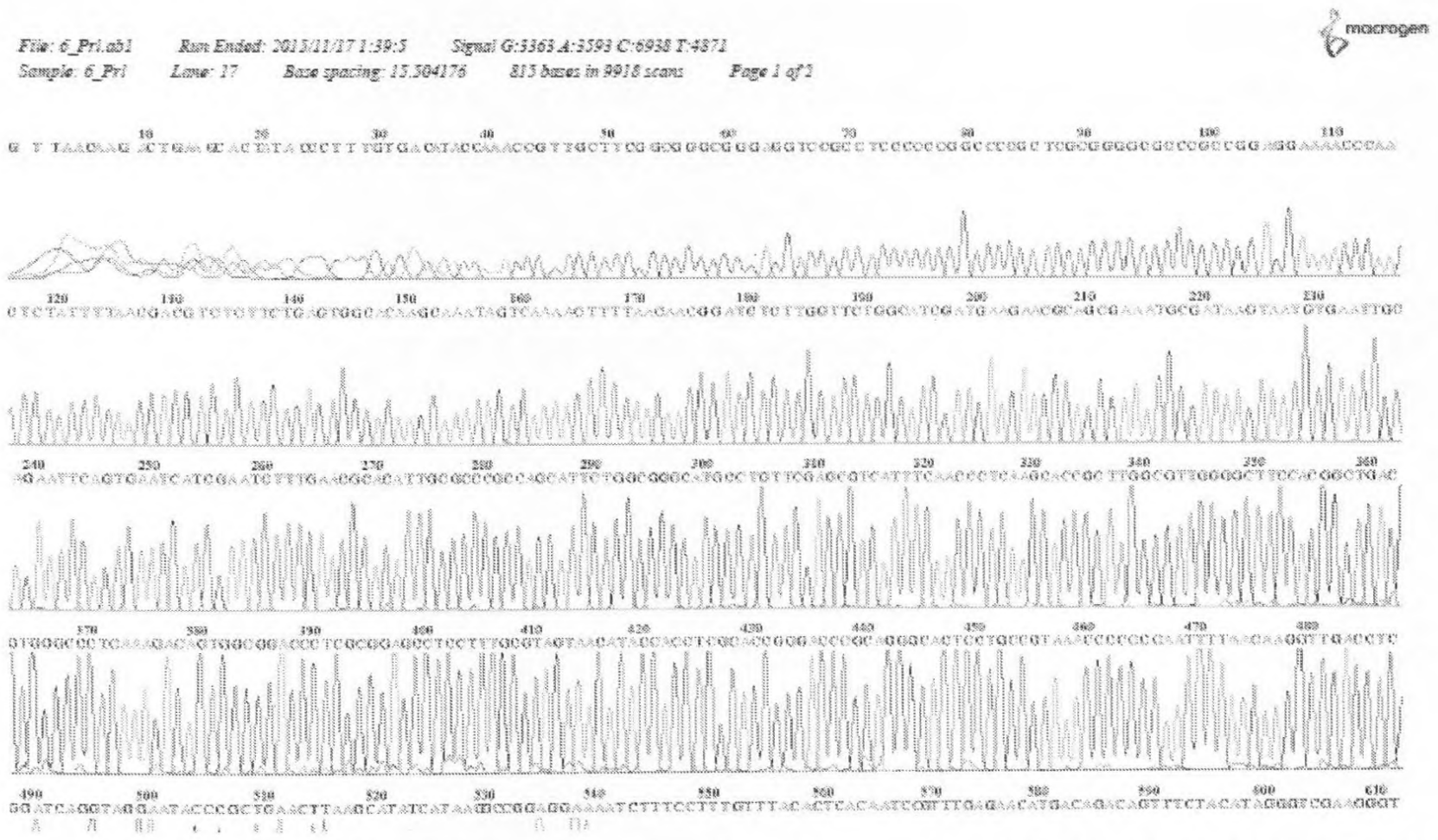


Figure 12. Representative Chromatogram for DNA sequencing of promoter region of ABRE

Sequencing results of PCR products were analyzed using molecular tools and detected variations of presence of *cis*-elements in targeted transcription factors of all tested varieties. Multiple alignments of tested varieties with the sequences of the two standard Japonica and Niponbare varieties showed base differences among varieties in certain regions of the promoters and genic regions (Figure 13). Also identified specific deletion/ insertion in *Goda heenati* and *Pokkali* within an analyzed promoter region, ABRE.

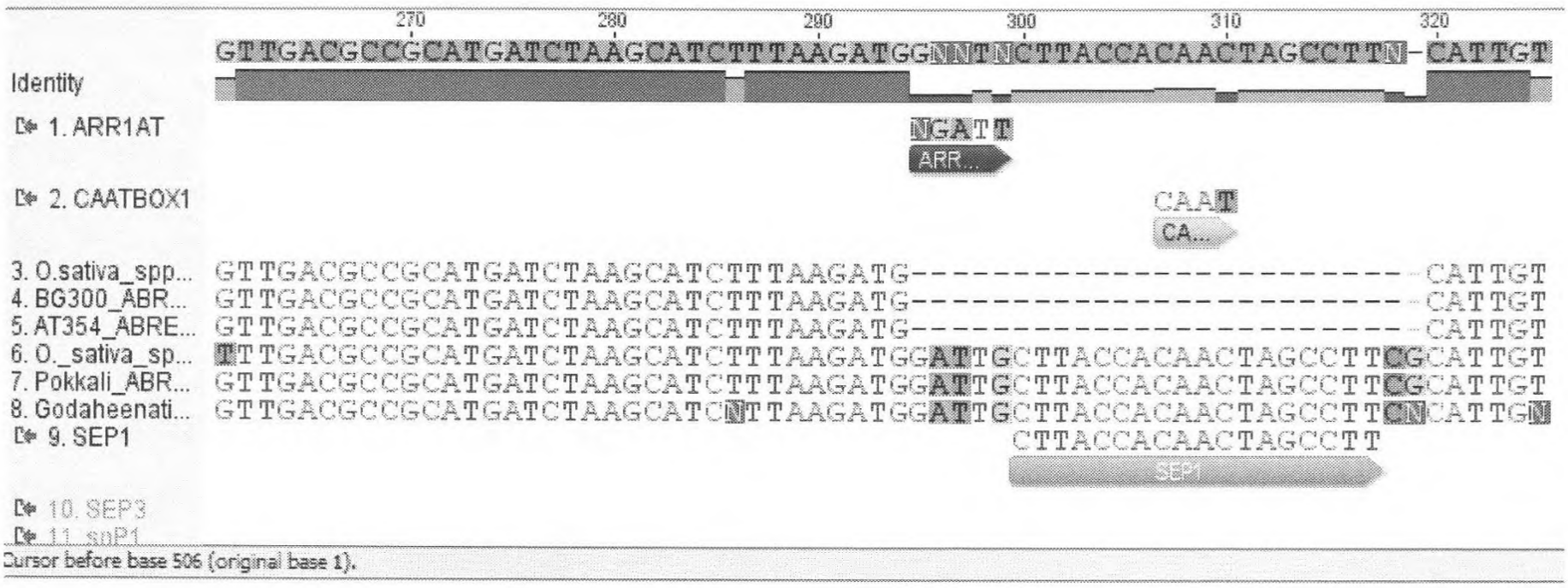


Figure 13. Multiple alignment of ABRE promoter region of tested varieties with standard indica and japonica varieties by Geneious 7.1.3 software

Three 3 TFs biding sites were identified in indel among the tested varieties at ABRE promoter region.

- ARR1AT
- SEP1
- CAATBOX1

iv. Discussion

Effect of salinity on seed germination

During the screening at germination there were no significant ($p \leq 0.05$) difference between tested varieties at the control level and all of them showed better germination ranging from 86% to 100 %. Therefore, it could be considered that the seed lots used for the study were in adequate viability since all of the varieties obtained >80% of germination. At 50 mM salt concentration, except At 303, all the other varieties germinated with >73% germination and therefore, At 303 could be considered as a highly sensitive rice variety to salinity at germination for future studies.

Bg 357, Bg 304 and Bg 450 while Bg 360 and *Pokkali* showed comparatively higher germination at both 100 mM and 150 mM salt concentrations and therefore, they could be considered as salt tolerant varieties at germination stage as well as early vegetative phase. Our results of this study are in agreement with the findings by Costa *et al.*, 2012 and Pradheeban *et al.*, 2014.

Bg 300 and At 354 showed 86% and 80% of germination percentage at 100 mM NaCl respectively and based on mean separation, they were categorized in to one group which are significantly different from the group consisting salinity check variety *Pokkali*. Therefore, they can be considered as rice varieties which possessing moderately tolerance to salinity. Both *Goda heenati* and *Kurukaruppan* were grouped together which showed 66.6% and 40% of germination respectively at 100 mM salt concentration. Compare to other tested improved varieties, performance of both traditional varieties were poor at salt stress. Therefore, it could be concluded that those two varieties were less tolerant to salinity at their germination stage.

Since some of the tested varieties (Bg 304, Bg 300 and At 354) showed drastic reduction of germination when increasing salt concentration from 100 mM to 150 mM and *Kurukaruppan* did not germinate at 150 mM salt concentration, 150 mM salt concentration could be considered as a critical level of salinity. Furthermore, when consider about the grouping behaviour of tested varieties, there were two distinct groups at 150 mM concentration. But at 100 mM concentration rice varieties were separated into four groups with significant difference ($p \leq 0.05$). Therefore, according to the results obtained, 100 mM salt concentration (9.96 dS/m) would be better than 50 mM and 150 mM salt concentrations to screen rice varieties for salinity tolerance at their germination and early seedling growth.

Early seedling growth was highly affected by the salt stress. The radicle and plumule length reduction was significantly ($p \leq 0.05$) different among varieties at each and every salt concentrations. Results obtained in seedling growth, are agreed with germination results which could be identified Bg 357, Bg 304 and Bg 450 while Bg 360 and *Pokkali* as salt tolerant varieties while At 354 and Bg 300 as moderately tolerant. *Goda heenati* and *Kurukaruppan* showed less tolerant and At 303 showed susceptibility to salt stress by reducing their early seedling growth.

Similar to germination, tested varieties were separated into significant groups based on both radicle and plumule length reduction at 100 mM NaCl concentration and clustered rice varieties in to several groups. Therefore, 100 mM NaCl concentration would be considered as an optimum level to screen rice varieties against salinity based on their early seedling growth.

Effect of drought on seed germination

Chemically induced drought by PEG concentrations reduced germination of rice seeds. Except *Kurukaruppan* and At 303, all other varieties germinated in the range from 40% to 100% germination percentage at 10% PEG concentration and salinity tolerant *Pokkali* reduced germination to 40%, illustrating that there is less survival under induced drought conditions. At 303 obtained no germination even at lowest PEG concentration tested and as in salinity experiment and At 303 reflecting that its' susceptibility to both drought and salinity. However, in our study screened rice varieties showed significant differences at 10% PEG and grouped rice varieties in to several groups clearly than the other tested PEG concentrations.

In 15% PEG concentration, Bg 450 maintained 100% germination and Bg 304 and Bg 360 showed 80% germination and no any single variety were able to germinate in 20% PEG concentration illustrating that 20% PEG level is a detrimental concentration for seed germination for selected rice in the experiment. Based on findings in our study, Bg 450 Bg 360 and Bg 304 could be considered as highly tolerant varieties for chemically induced drought and Bg 357, Bg 300, *Goda heenati*, At 354 and *Pokkali* as moderately tolerant for drought. Both *Kurukaruppan* and At 303 were drought sensitive.

Zapico *et al.*, 2008, proved that 15% of PEG concentration was inhibitory to germination of rice seeds effect for a drastic reduction of germination and at 20% PEG some of the tested rice varieties were not germinated. Li *et al.*, 2013 revealed that even *Eremosparton*

songoricum (Litv.) Vass (drought-resistant desert plant) reduced their germination along with the increase of PEG concentration which observed a dramatically reduction at -0.9 MPa (15 %) PEG.

Both radicle length and plumule length were arrested the increase of PEG concentration, showing significant difference ($p \leq 0.05$) among the varieties. Lower reductions in radicle and plumule length were obtained for Bg 304 and Bg 450 even at the highest tested PEG concentration of 15 % while other varieties reduced their seedling growth. Therefore, Bg 450 and Bg 304 could be considered as highly tolerant varieties for chemically induced drought. A previous study identified that there was not significant effect for seedling growth at 10% PEG (Zapico *et al.*, 2008). However, results of our study revealed that rice varieties showed significant variation on their radicle growth at 10% PEG. According to both germination and early seedling growth, 20% PEG is a crucial level of determining both parameters. Since 10% PEG level gave considerable variation among both parameters, 10% PEG would be useful to screen rice varieties against chemically induced drought by PEG at laboratory conditions.

As a summary, *Pokkali* performed well in both germination and early seedling growth exhibiting its salinity tolerance and Bg 360, Bg357, Bg 304 and Bg 450 also showed better germination and lower reduction of both radicle and plumule length compared to other tested varieties at both stresses in this study. At 303 performed as a susceptible variety for both stresses and it was identified as a salinity susceptible variety by previous studies as well. At 354 which is known as salinity tolerant variety and Bg 300 showed moderately tolerance for both stresses. The two traditional varieties tested, performed differently for both stresses. Based on their performances *Goda heenati* could be categorized as a moderately tolerant variety for both stresses, but *Kurukaruppan* was not able to survive under both stresses and therefore, it could be categorized as susceptible variety for both drought and salinity.

Effect of drought – Relative water content

The RWC was identified as a key parameter to detect plants against drought stress (Bunnag and Pongthai, 2013; Chaudhary, *et al.*, 2009,) but it was not used to screen drought affected Sri Lankan rice varieties. In this experiment, *Goda heenati* showed a gradual reduction of RWC only up to the third day of drought induction and the fourth day of drought, it showed an increase of RWC followed by a reduction on the fifth day. Chaudhary *et al.*, 2009 screened four weeks old rice seedlings against drought and all tested rice varieties showed a

steady increase of RWC around 48-72 hours (2-3 days) and thereafter gradual decline during initial stages. Furthermore, they stated that, the increase of RWC during first 2-3 days would be for the OA due to the increase of proline content during 24-48 hours. Based on our results, *Goda heenati* showed an increase of RWC at the fourth day which was around after 72 hours and it would be due to the same reason suggested in previous study. However, *Pokkali* did not show the similar result and Chaudhary *et al.*, 2009 stated that varietal differences in RWC during stress period may be due to different rates of stress development and responses shown by different varieties.

Kumar *et al.*, 2014 stated that in water stress condition, higher value of RWC was recorded in drought stress tolerant rice genotypes as compared to susceptible genotypes. Therefore, according to results obtained in this study it could be concluded that *Goda heenati* has more drought stress tolerating ability than *Pokkali*

Variation in total protein content due to drought

Variation of total protein content in leaves of rice seedlings in both *Goda heenati* and *Pokkali* during drought period would be resulted due to the changes occurred in regular metabolic activities because of the adjustments in gene expression. Qureshi *et al.*, 2007 revealed that abiotic stresses has an ability to alter the concentrations of a number of proteins which produce inside the plant cells as a response to a particular stress to develop defence mechanisms by alteration in the pattern of gene expression. Therefore, due to alteration in gene expression under stress, changes in the quality and quantity in proteins are obvious. Variations in protein content within plants during drought stress was observed by Chaudhary *et al.*, 2009. Based on their findings, rice varieties which are acquiring higher protein contents during drought stress conditions could be considered as a indication of their drought tolerance ability. Further, the variations among protein contents in each variety could be caused by its own genetic composition.

When compare the results obtained in our study with the previous findings (Chaudhary *et al.*, 2009; Qureshi *et al.*, 2007), it could be assumed that the changes in total protein content in both *Goda heenati* and *Pokkali*, during drought stress would be the result of alteration of gene expression under the water deficit condition. In this study, comparatively higher protein content was obtained in *Goda heenati* and it could be due to its' drought tolerance ability and this agreed with the findings by Chaudhary *et al.*, 2009 who stated that rice plants are

acquiring higher protein contents during drought stress conditions which indicate their drought tolerance ability. However, *Pokkali* showed highest protein content at the fourth day and it could be assumed that *Pokkali* might have the ability to withstand drought stress and it initiates after few days of induction of drought.

Effect of drought on photosynthetic pigments

The effect of drought on the photosynthesis was studied by analyzing the status of photosynthetic pigments. Chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and carotenoid contents showed similar pattern of variation in both varieties during drought phase (Figure 07 A, B and C). According to figure 07 (A), both *Goda heenati* and *Pokkali* showed a gradual reduction ($p \leq 0.05$) of all three pigments during first three days and a rapid increase at fourth day followed by a reduction at fifth day again with a significant difference ($p \leq 0.05$). Based on the present study, it would be concluded that dehydration stress causes the fluctuation of photosynthetic pigments in rice leaves along with the time. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Based on the previous findings, the chlorophyll content would be either increase or decrease or even unchanged during drought stress and it depends on the duration and the severity of drought (Anjum *et al.*, 2011).Our results are also in agreement with (Pandey and Shukla, 2015), who revealed that loss of or reduction in synthesis of photosynthetic pigments during drought stress is a common phenomena which closely associated to reduction of plant biomass and yield.

Our results indicated that RWC in *Goda heenati* reduced at first three days and increased at the fourth day which is similar to the pattern of chl-a and chl-b variation in the same variety. Since plant cells reached to their usual status due to the increase of water content, that rapid increase of chlorophyll content would be an evidence for the incidence of photosynthesis inside the plant (Chaudhary *et al.*, 2009). Further they revealed that RWC would increase due to proline accumulation in rice plants which are under drought stress during 48-72 hours to facilitate the maintenance of regular metabolic activities and physiological processes by the osmotic adjustment within plant at lowered potential conditions. Therefore, our findings are supported by the observations made by Chaudhary *et al.*, 2009 reflecting that the increase of photosynthetic pigments at the fourth day in *Goda heenati* would be due to the increase of water status inside the cell which would be lead to create normal condition inside the plant.

Furthermore. Chaudhary *et al*., 2009 stated that higher carotenoid levels might result in improved protection from damage caused by dehydration via preventing the production of reactive oxygen species. Therefore, according to the our results, increased carotenoid content at the fourth day would be facilitated to the recover damaged cells due to drought and because of that cells would able to proceed photosynthesis.

Proteomic analysis (Specific objectives 1-3)

About 50 reproducible proteins spots were recorded on 12% acrylamide gels stained with coommassie blue (Figure 01 A and B). They were spread over a pH range of 5-8 with molecular weights (15-75 kDa). However, previous studies reported higher number of spots than obtained in this study. 698 proteins were identified in rice leaf sheaths of two-week-old rice seedlings which were exposed to drought stress from 2 to 6 days (Ali and Komatsu, 2006) and Abbasi and Komatsu, 2004 reported that 500 rice leaf sheath proteins were detected in two-week-old seedlings which were exposed to salt stress (50 mM NaCl) for 24 h. In this study it was only 50 spots detected and it could be due to either degradation of proteins during extraction and gel running or less sensitivity of staining process. We initiated proteomic analysis for the first time with this research grant and we believe the purchased protease inhibitors may not work properly during the study period. We changed the gel staining protocols and could not achieve much difference in number of protein spots.

According to Ali and Setsuko Komatsu, 2006, protein spots which obtained from drought stress exposed two-week-old rice leaf sheaths were separated within basic side of the gel (pH 5 to 9) and it is agreed with the findings of this study which obtained in the range of pH range of 5-8. Furthermore, their proteins were separated within the size of 15-70 kDa molecular weights and, even though few protein spots were obtained in this study, they were also spread over the range of 15-75 kDa. According to the findings by Zhao *et al*., 2005, most of the rice leaf proteins in field grown rice plants were located within a narrow pH range of 5–7 and they were ranged from 14 to 66 kDa Therefore, our finding are also in agreement with Zhao *et al*., 2005 though we observed a broader range (15-75 kDa and pH range of 5-8).

Proteins which numbered as No. 1 and 3 in Figure 4 were up regulated in control plants (Figure 1A) but not in Drought affected plants (Figure 1B) and protein which denoted as No.2 was up regulated in Drought affected plants (Figure 1B). Ali and Setsuko Komatsu,

2006 identified 12 proteins clearly responded to drought as early as 2-5 days after stress application. Among them, 10 proteins were up regulated during drought stress while 2 proteins were down regulated. One such down-regulated protein was identified a as RuBisCO small chain (100) (N-XQVWPIEGIK) which was 17 kDa size and appeared in pH 6.4. In this study, protein No. 3 was in pH range of 6-7 and the size is around 15 kDa. Even though this protein was not further analyzed due to unavailability of protein identification techniques, we may suggest that the protein No.3 as small subunit of RuBisCO.

Ali and Setsuko Komatsu, 2006 further identified a protein of 40 kDa size in pH 5.6 called Photosystem II oxygen evolving complex protein (N-EGVPPXLTFD) and they stated that it involves in light harvesting which could potentially yield crop plants that are more resistant to environmental stress and it prevents inhibition of photosynthesis. In our experiment protein No. 2 was up regulated during drought stress and it was appeared around pH 5.0 with the size of 37-40 kDa. Therefore, Protein No. 2 would be possible to be the same protein.

During this study, a large protein spot (No. 4) was identified around 50 kDa size on pH 5.5. Ali and Setsuko Komatsu, 2006 identified RuBisCO large chain (100) protein (N-blocked I-MTLGFVDLLR) on pH 6.0 and 50 kDa size. Therefore, protein No. 4 could be RuBisCO protein, because the size and pH range is quite similar. Zhao *et al.*, 2005 identified 52 kDa size protein spot around pH 6.5 as RuBisCO large subunit in field grown rice plants. However, further studies such as protein fingerprinting by Mass Spectrometry are needed to confirm our findings.

Genomic analysis (specific objective 5)

Targeted promoter regions in this study which appeared in upstream region of dehydration responsive genes and they are known as important plant transcription factors that regulate the expression of many stress inducible genes and play a critical role in improving the abiotic stress tolerance of rice (Shinozaki and Yamaguchi-Shinozaki, 2007).

Sequencing was given good quality results according to the chromatogram and multiple alignments of tested varieties with the sequences of the two standard Japonica and Niponbare varieties showed base differences among varieties in certain regions of the promoters and genic regions. Also identified specific indel in *Goda heenati* and *Pokkali* within an analyzed

promoter region, ABRE and these differences leads to changed the sequence of *cis*-elements which responsible for the binding of certain transcription factors during a stress. When consider about the indel presence in ABRE promoter region, tree TFs were identified which bind to that particular indel and they are functioning in specific tasks.

- ARR Transcription factors – Cytokinin mediated development – Root development
- CCAAT box - motifs which present in many eukaryotic promoters. coordinate with the pre-initiation complex
- SEP1 – Floral organ development

vi. Conclusions

Based on the findings of the present study following conclusions can be made:

- Germination and early seedling growth of rice varieties were reduced by salinity and drought and tested varieties performed different manner for two different stresses. Bg 360, Bg 357 Bg 304 and Bg 450 showed their salinity tolerance as well known salt tolerant *Pokkali*. At 354, Bg 300 and *Goda heenati* showed moderately tolerance for salinity stress while At 303 and *Kurukaruppan* were susceptible.

- Bg 450, Bg 304, Bg 357, Bg 300 and Bg 360 showed tolerance for chemically induced drought while *Pokkali*, *Goda heenati* and At 354 performed as moderately tolerant. Similar to salinity test, both At 303 and *Kurukaruppan* were susceptible for drought.

- Both 100 mM salt concentration and 10% PEG concentration would be useful to screen rice varieties under laboratory conditions against salinity and drought respectively.

- Relative water content, photosynthetic pigment concentration and total protein content were fluctuated during vegetative stage in both *Goda heenati* and *Pokkali* due to drought stress. The studied two varieties responded differently in RWC, protein concentrations and pigment levels during analysis and that could be due to their difference in stress development.

- Based on the results obtained in RWC analysis, Goda *heenati* may possess drought tolerating ability than *Pokkali* due to its recovering nature.

- CTAB method is a successful method for DNA extraction in rice seedlings.

- Variation was observed in presence of *cis*-elements in targeted transcription factors of all tested varieties. Identified specific deletion/ insertion in *Goda heenati* and *Pokkali* within an analyzed promoter region, ABRE would be caused differential responses in *Goda heenati* and *Pokkali* against dehydration stress.

- Three proteins were identified as up or down regulated during drought stress by 2DE analysis of *Goda heenati* and those detected proteins were similar to the proteins which identified by previous studies. Further analysis of the protein profiles by 2DE, would be a great advantage to identify and confirm the drought tolerant ability in *Goda heenati*.

Based on the findings in the present study, *Goda heenati* could be acquiring dehydration stress tolerating ability and further studies could be conducted to confirm its stress tolerance.

vii. References

Abbasi. F. M., and Komatsu, S. (2004) A proteomic approach to analyze salt responsive proteins in rice leaf sheath. Proteomics. 4, 2072–2081

Ali, G. M. and Komatsu, S. (2006). Proteomic analysis of rice leaf sheath during drought stress [online]. [Accessed on 16.07.2015]. Available at http://www.researchgate.net/publication/7315891

Anjum, S. A., Xie, X., Wang, L., Saleem, M.F., Chen, M. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research Vol. *6(9)*, 2026-2032.

Attanayake, M. B. N. W. M., Weerasekara, W. M. L., Weerathunga, D. B. and Chithral, G. M. W. (2010). Past achievements and future directions of the seed paddy sector in Sri Lanka. Rice Congress. 2-3 of December, 2010. Plant Genetic Resources Center, Gannoruwa.

Bhushan, D., Pandey, A., Choudhary, M.K., Datta, A., Chakraborty, S. and Chakraborty, N. (2007). Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Molecular & Cellular Proteomics *6(11).* 1868-1884.

Boyer J. S. (1982) Plant productivity and environment. Science **218:** 443

Bradford. M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry *7*, 248-54.

Brown, C. D., Johnson, D. S., & Sidow, A. (2007). Functional architecture and evolution of transcriptional elements that drive gene coexpression. *Science (New York, N.Y.)*, *317*(5844), 1557-60. doi:10.1126/science.1145893

Bunnag, S. and Pongthai, P. (2013). Selection of rice (*Oryza sativa* l .) Cultivars tolerant to drought stress at the vegetative stage under field conditions. American Journal of Plant Science. *4*, 1701–1708.

Choudhary, M. K., Basu, D., Datt, A., Chakraborty, N. and Chakraborty, S. (2009). Dehydration responsive nuclear proteome of rice (*Oryza sativa* L.) illustrates protein network, Novel regulation of cellular adaptation and evolutionary perspective. Molecular and Cellular Proteome. *8*, 1579-1589.

De Costa, W.A.J.M., Wijeratne, M.A.D and De Costa, D.M. (2012). Identification of Sri Lankan rice varieties having osmotic and ionic stress tolerance during the first phase of salinity stress. Journal of National Science Foundation Sri Lanka. 40*(3)*, 251-280

Devos, M. K. and Gale, D.M. (2000). Genome relationships: The grass model in current research. Plant Cell, 12: 637-646

Fowler, S. and Thomashow, M.F. (2002). *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell, 14: 1675–1690.

Gulick, P.J., Drouin, S., Yu, Z.H., Danyluk, J., Poisson, G., Monroy, A.F. and Sarhan, F. (2005). Transcriptome comparison of winter and spring wheat responding to low temperature. Genome, 48: 913–923.

Islam, M. M. and Karim, M. A. (2011). Evaluation of rice (*oryza sativa* L .) Genotypes at germination and early seedling stage for their tolerance to salinity. The Agriculturists. *8*(2), 57–65

Jamil, M., Bashir, S., Anwar, S., Bibi, S., Bangash, A., Ullah, F. and Rha, E.S. (2012). Effect of salinity on physiological and biochemical characteristics of different varieties of rice. Pakistan Journal of Botany. *44*, 7–13.

Kirch, H. H., Nair, A. and Bartels, D. (2001). Novel ABA- and dehydration-inducible aldehyde dehydrogenase genes isolated from the resurrection plant *Craterostigma plantagineum* and *Arabidopsis thaliana*. Plant Journal, 28:555–67.

Kreps, J. A., Wu, Y., Chang, H. S., Zhu, T., Wang, X., and Harper, J. F. (2002) Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiology. 130, 2129–2141

Kumar, S., Dwivedi, S. K., Singh, S. S., Jha, S. K., Lekshmy, S., Elanchezhian, R., Singh, O. N. and Bhatt, B. P. (2014). Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. SABRAO Journal of Breeding and Genetics. *46 (2),* 217-230.

Li, H., Li, X., Zhang, D., Liu, H. and Guan, K. (2013). Effects of drought stress on the seed germination and early seedling growth of the endemic desert plant *Eremosparton songoricum* (fabaceae). Experimental and Clinical Sciences International online journal for advances in science. *12,*89-101.

Mohanty, S., Wassmann, R., Nelson, A., Moya, P. and Jagadish, S. V. K. (2013). Rice and climate change : Significance for food security and vulnerability. IRRI Discussion Paper Series No. 49. Los Banos: International Rice Research Institute. 14

Moons, A., Gielen, J., Vandekerckhove, J., Van der Straeten, D., Gheysen, G., and Van Montagu, M. (1997). An abscisic-acid– and salt-stress–responsive rice cDNA from a novel plant gene family. Planta 202, 443–454

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 8,4321-4325.

Nakashima, K., Ito, Y., & Yamaguchi-Shinozaki, K. (2009). Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant physiology*, *149*(1), 88-95. doi:10.1104/pp.108.129791

Ndjiondjop, M., Cisse, F., Futakuchi, K., Lorieux, M., Manneh, B., Bocco, R. and Fatondji, B. (2010). Effect of drought on rice (*Oryza* spp .) genotypes according to their drought tolerance level. Second Africa Rice Congress.

O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. Journal of Biological Chemistry, 250: 4007–4021.

Pandey, A., Chakraborty, S., Datta, A., and Chakraborty N. (2008) Proteomics approach to identify dehydration responsive nuclear proteins from chickpea (*Cicer arietinum* L.) Molecular Cellular Proteomics. 7, 88–107

Pandey, V. and Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. Rice Science. *22(4).*

Park, M.-R., Yun, K.-Y., Mohanty, B., Herath, V., Xu, F., Wijaya, E., Bajic, V. B., et al. (2010). Supra-optimal expression of the cold-regulated OsMyb4 transcription factor in transgenic rice changes the complexity of transcriptional network with major effects on stress tolerance and panicle development. *Plant, cell & environment.* doi:10.1111/j.1365-3040.2010.02221.x

Pattanagul, W. and Thitisaksakul, M. (2008). Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Indian Journal of Experimental Biology. *46*(10), 736–742.

Pradheeban, L., Nissanka, N.A.A.S.P and Suriyagoda, L.D.B. (2014). clustering of rice (*Oryza sativa* l.) varieties cultivated in jaffna district of sri lanka based on salt tolerance during germination and seedling stages. Tropical Agricultural Research. *25*(3), 358 – 375.

Qureshi, M. I., Qadir, S. and Zolla, L., (2007). Proteomics-based dissection of stress-responsive pathways in plants. Journal of Plant Physiology. *164*, 1239—1260.

Rabbani, M. A., Maruyama, K., Abe, H., Khan, M. A., Katsura, K., Ito, Y., Yoshiwara, K., Seki, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2003) Monitoring

expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA get-blot analyses. Plant Physiology, 133, 1755–1767

Rajiv, S., Thivendran, P. and Deivannai, S. (2010). Genetic divergence of rice on some morpholoical and physiochemical responses to drought stress. Pertanika Journal of Agricultaral Science. *33(2)*. 315-328.

Salekdeh, G. H., Siopongco, J., Wade, L. J., Ghareyazie, B., and Bennett, J. (2002) Proteomic analysis of rice leaves during drought stress and recovery. Proteomics. 2, 1131–1145

Seki, M., Narusaka, M., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y. and Shinozaki, K. (2001). *Arabidopsis* encyclopaedia using full-length cDNA s and its application. Plant Physiology and Biochemistry, 39: 211–220.

Shinozaki, K and Yamaguchi-Shinozaki, K., (2007). Gene networks involved in drought stress response and tolerance, Journal of Experimental Botany. 58(2), 221–227.

Shinozaki, K., Yamaguchi-Shinozaki, K., and Seki, M. (2003) Regulatory network of gene expression in the drought and cold stress responses. Current Opinion Plant Biology. **6**. 410–417

Subasinghe, A. Nissanka, N.A.A.S.P. and Weerakoon, W.M.W. (2007). Identification of salt tolerant rice varieties at the seed germination stage and its relationship to seed husk thickness and ion absorption. Tropical Agricultural Research, 19, 219-228.

Usman, M., Raheem, Z., Ahsan, T., Iqbal, A., Sarfaraz, Z.N. and Haq, Z. (2013). Morphological, physiological and biochemical attributes as indicators for drought tolerance in rice (*Oryza sativa* L.). European Journal of Biological Sciences. *5(1)*. 23-28.

Xiong, L., Schumaker, K. S., and Zhu, J. K. (2002) Cell signaling during cold, drought, and salt stress. Plant Cell, 14, S165–183

Yamaguchi-Shinozaki, K and Shinozaki, K. (1994). A novel cis-acting element in an arabidopsis gene is involved in responsiveness to drought, low temperature, or high-salt stress. The Plant Cell. *6*, 251-264

Yun, K.-Y., Park, M. R., Mohanty, B., Herath, V., Xu, F., Mauleon, R., Wijaya, E., et al. (2010). Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress. *BMC plant biology*, *10*(1), 16. doi:10.1186/1471-2229-10-16

Zapico, F. L., Miranda, J. G. and Pare, M. I. (2008). Physiological characterization for drought tolerance of selected rice varieties in Lake Sebu, Philippines. USM R and D Journal. *16*(1), 13–16.

Zhao, X., Ren, J., Cui, N., Fan, H., Yu, G. and Li, T. (2013) Preparation of Protein Extraction from Flower Buds of *Solanum lycopersicum* for Two-Dimensional Gel Electrophoresis. British Biotechnology Journal, 3(2): 183-190.

Zhao, C., Wang, J., Cao, M., Zhao, K., Shao, J., Lei, T., Yin, J., 2, Hill, G., Xu, N and Liu,S. (2005). Proteomic changes in rice leaves during development of field-grown rice plants. Proteomics.*5*, 961–972.

viii. Problems if any, encountered during the implementation of the project

- Delays in the process of purchasing chemicals and the equipment
  Some of the chemicals required (especially for proteomic analysis) were quoted by local suppliers however, did not supply. At that time, there were no chemical agents to supply proteomic chemicals/kits from companies providing chemicals for proteome analysis. Therefore, we had to purchase them directly from BioRad, USA. Therefore, for some chemicals, we had to call quotations many times.

ix. Major findings and follow up activities

- Identification of variations in *cis*-elements presence in promoter regions of transcription factors and specific deletion/ insertion in *Goda heenati* and *Pokkali* within an analyzed promoter region, ABRE.

- Optimization of protein extraction and 2DE protocols for rice seedlings.

- Prediction of three proteins which up or down regulated during drought stress by 2DE analysis of *Goda heenati* that similar to the proteins which identified by previous studies.

- Identification of rice varieties tolerant and susceptible for salinity and drought at their germination stage.

- Identification of parameters; 100 mM salt concentration and 10% PEG concentration for screen rice varieties under laboratory conditions against salinity and drought respectively.

Following activities are intended to conduct in the near future:

- Further improvement of protein separation through 2DE analysis in order to obtain more number of spots. Based on the available facilities and other restrictions (cost of analysis, sending proteins abroad for analysis), it would be difficult to identify proteins. However, comparison of protein profiles would be possible
- Confirmation of dehydration stress tolerating ability in *Goda heenati* by expression assays at either RNA or protein levels

Section 4

Impact of research results

1. Relevance of results achieved to scientific advancement

This study was expected to result in the discovery of the most essential genes/ regulatory sequences involved in the dehydration genetic network. Diversity analysis of DNA sequences of selected genes gave new findings however, they need to be re-confirmed by expression analysis at either RNA or protein level.

We found new information on drought tolerance of Sri Lankan rice in terms of varietial diversity and based on our findings, we can conclude that *Goda heenati* as a potential variety for tolerance to dehydration. Furthermore, screening Sri Lankan rice for drought tolerance has not been studied previously and therefore, our findings would be useful for scientists and breeder in Sri Lanka. The investigators are willing to share outcome with breeders and scientists at Rice Research and Development Institutes of the country. Then outcome of the proposed study can be directly used in rice breeding programs targeting the development of varieties for dehydration stress.

Training a person for proteome analysis based on 2-DE (through M. Phil degree programme) is another achievement in this project.

ii. Relevance of results achieved to national/socio-economic development

Rice is the staple food and it is clearly identified that rice production has to be increased by 12% to meet consumer demand derived from increasing population. Even though the genetic yield potential of rice is still not achieved; its production is now believed to be stagnated at actual field conditions. It is mainly due to marginal field / environmental conditions. Therefore, breeding for abiotic stress factors (drought, salinity, etc) has been identified as a breeding target for next 10 years (Rice Congress, 2010 at PGRC, Gannoruwa) in order to achieve food security of the country.

Rice production contributed significantly to the economic development in Sri Lanka as it is the staple crop. More than 1.8 million people are depended on paddy production. Expanding the availability of different rice varieties suitable for dehydration stress would be one of the possibilities of increasing paddy production

and economy of the country. Increased rice production will improve food security, stable prices and economical and social well being of the people in the country

Though this research is more towards the basic sciences, understanding the genes/mechanisms involved in dehydration stress tolerance in Sri Lankan rice varieties would be directly used in developing tolerant rice varieties to drought and salinity in Sri Lanka and then by which rice productivity especially in marginal lands or with marginal inputs could be increased.

Department of Agric. Biology had no facility for proteome research earlier and because of this research, the Department got the opportunity to build up proteome research facility. Facility develop for proteome analysis can be directly used for,

- Teaching and research for both undergraduate and postgraduate students
- Improving knowledge and skills of other interested scientists through workshops/training programmes or on request

iii. Dissemination/application of research output

- Publication of the findings in scientific journals
- Suggesting Rice Research Institute to conduct comprehensive research work to introduce *Goda heenati* to their breeding strategies

Section 5

Miscellaneous

i. List of major equipment acquired during the project period and their functionality

A desktop computer (Dell Optiplex) with a colour printer (RicohSPC250) and a UPS – attached to the 2DE gel scanner purchased through equipment grant RG/2012/EQ/05 at the Molecular Genetics Laboratory, Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya. They are in good condition

ii. List of publications/communications arising from the project and/or presentations made at seminars, workshops etc. (please attach copies)

Jayaweera, J.K.P.T.P, Herath, H.M.V.G.., Jayatilake, D.V., Udumulla, G.S. and Wickramasinghe, H.A.M. (2015). Physiological, biochemical and proteomic responses of rice (*Oryza sativa* L.) variety Godaheenati and Pokkali for drought stress at the seedling stage. – Paper submitted to 27th Annual Congress, Post Graduate Institute of Agriculture, University of Peradeniya

Jayaweera, J.K.P.T.P., Herath, H.M.V.G., Wickramasinghe, H.A.M. (2015). Evaluation of the salinity and drought stress responses of selected Sri Lankan rice (*Oryza sativa* L.) varieties based on germination and seedling emergence - Paper submitted to Journal of Agricultural Sciences

Wickramasinghe, H.A.M. (2014). Guide to Two-Dimensional gel electrophoresis: Theory and Practice. Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka – A laboratory guide book prepared based on protocols developed and tested through the grant and equipment grant RG/2012/EQ/05 (in print)

Weerasinghe, W.M.M.E., Senevirathne, W.M.A, Wickramasinghe, H.A.M. and Herath, H.M.V.G. (2015). Computational analysis of stress responsive *ABRE* gene in *Oryza* genus. Paper submitted to 27th Annual Congress, Post Graduate Institute of Agriculture, University of Peradeniya

Rukshana, M.M.F., Herath, H.M.V.G., Wickramasinghe, H.A.M. (2014). *Ab-initio* identification of dehydratin responsive promoter elements in selected rice varieties of Sri Lanka. Proceedings of the undergraduate research, Department of Agricultural Biology. Faculty of Agriculture. University of Peradeniya. 4, 91-94

At present, Rukshana *et. al.* (2014) is available in its printed format. Therefore, a copy of that abstract is attached. I will submit the other publications once published.

Section 6

Summary statement of Expenditure

Total Allocation - Rs. 2,761,350.00

Total amount received - Rs. 2,635,425.00

Total spent - Rs. 2,544,040.41

Item	**Allocation**	**Amount Received**	**Spent**
Personal (Research student)	895,000.00	895,000.00	895,000.00
Personal (Other)	46,850.00	23,425.00	1,900.00
Consumables	1,360,000.00	1,360,000.00	1,361,286.00
Equipment	240,000.00	240,000.00	189,085.66
PG Registration Fee	44,500.00	44,500.00	44,500.00
Travel and Subsistence	25,000.00	12,500.00	3,800.00
Lab services and Sample	90,000.00	45,000.00	48,418.75
Calibration of Instruments	20,000.00	10,000.00	0
Miscellaneous	40,000.00	5,000.00	50.00
Total	**2,761,350.00**	**2,635,425.00**	**2,544,040.41**
Balance sent to NSF on 27.05.2015*			5308.51
Balance**			86076.08

* Based on the financial statement on 08.05.2015 (end of the grant period) Rs. 5308.51 was sent back to NSF to settle the grant

** The balance calculated to the last date of the research period (08.05.2015) was with some committed money. However, after settling all committed money thereafter, Rs 86076.08 was available due to

1. A part of the postgraduate registration payment already done before receiving it on 02.07.2015 (Rs. 25,000.00)
2. Total equipment vote was kept as commitments, but only Rs. 189085.66 was spent and therefore Rs. 50914.34 not used
3. VAT allocation for all payments during 2015 was reduced from 12% to 11%

(Out of this balance, payment for DNA sequencing of 26 samples is still pending. Thereafter, the remaining balance will be sent to NSF)

Section 7

i. Grantee's signature

H.M.V.G Herath

H A M Wickramasinghe

ii. Comments of the Head of the Department/Signature

iii. Head of the Institution's signature

Annexure

1. Chemicals/consumables/equipments purchased

Chemical	**Remarks**	**Price (Rs.)**
Tris Base		8321.60
PCR Master Mix		15612.80
CTAB		32496.80
Tris HCl		19012.00
Plasmid purification kit		39200.00
TA cloning kit		200366.40
RNase A		32946.00
Ultrapure Agarose		41040.00
100bp DNA ladder		14820.00
Platinum PCR super mix		95144.00
Immobilized pH Gradient Strips*	Purchased from BioRAD	45760.00
Ampholytes/ IPG buffer (2 ml)*	Purchased from BioRAD	9620.00
Rehydration buffer*	Purchased from BioRAD	11700.00
Reduction kit*	Purchased from BioRAD	30680.00
Alkylation kit*	Purchased from BioRAD	
Protein purification kit/ 2D cleanup kit*	Purchased from BioRAD	39520.00
Sucrose		1777.44
SDS		5880.00
Urea		2016.00
Thiourea		3640.00
Phenol		2844.80
Ammonium acetate		2393.05
Triton X-100*	Purchased from BioRAD	4290.00
Nonidet P -40		15607.80
EDTA		7280.00
Dithiothreitol*	Purchased from BioRAD	22360.00
N,N'-methylenebisacrylamide		12544.00
Glycine		6160.00
Ethanol		16844.80
CHAPS [(3-cholamidopropyl) dimethylamino]-1-propanesulfonate*	Purchased from BioRAD	21450.00
Acetic acid		3623.20
Bovine Serum Albumin		26431.60
Iodoacetamide*	Purchased from BioRAD	18980.00
Polyvinyl pyrrolidone		3435.04
Trichloroacetic acid		10640.00
Formaldehyde		1668.80
Monosodium phosphate		1898.40
Protease inhibitor cocktail		21495.80
Mineral oil*	Purchased from BioRAD	3640.00
Ammonium bicabonate		1215.20

Agarose		16863.75
Methanol		2576.00
Silver nitrate		7500.00
Nuclease free water		11760.00
Potassium hydroxide		4334.40
Thymol blue		6000.00
Protein marker		21044.80
PCR Master Mix		15859.20
Bradford reagent		24495.00
PMSF (phenylmethylsulfonyl fluoride)		29315.00
Disodium phosphate		1215.20
100 bp DNA ladder		22200.00
10 bp DNA ladder		21040.05
Go Taq Green Master Mix		15717.60
AluI		14008.20
NheI		12526.35
PvuI		22089.00
Glycine		16883.10
Methanol		8602.50
Coomasie Brilliant Blue G 250		14385.60
Coomasie Brilliant Blue R 250		11266.50
Tris base		16045.05
Total		**1170082.83**

Note: *These chemicals were purchased from international company (Bio-RAD Laboratories Private Limited), because they were cheaper than local suppliers and some were not quoted by local suppliers. Only unit price of those chemicals were given and shipping and air freight costs were excluded

Consumable	**Price (Rs.)**
Tissue	240.00
Gloves	2625.00
Al Foil	640.00
Spray cans	220.00
Wash bottles	740.00
Dropper	190.00
Plastic rack	375.00
Plastic buckets	2767.00
Brushes	145.00
Permanent markers	150.00
Brush holder	40.00
Paper cutter	25.00
Rubber bands	40.00
Brown paper bags	175.00
Price tag roll	25.00
Chemifix	98.00
Files	168.00

Paper fastner	140.00
Detergent (Teepol)	290.00
Hand wash	470.00
Plastic buckets	2975.00
Mesh	280.00
Tintes nails	25.00
Note books	200.00
Pens	40.00
glue	26.00
Ruler	30.00
Polythene	250.00
Fertilizer	40.00
Flexible conduit	1500.00
Insulation	100.00
Hose Roller	900.00
Hose clips	50.00
Clear Hose	135.00
Silicone tube	210.00
Fertilizer	120.00
Fungicide	260.00
Polythene	3060.00
Rubber bands	150.00
Polythene	200.00
Plastic trays	240.00

Equipment	**Price (Rs.)**
Computer	137810.00
UPS	8775.66
Printer	42500.00
Total	**189085.66**

Department of Agricultural Biology

Faculty of Agriculture
University of Peradeniya
Peradeniya 20400
Sri Lanka

H.A.M. Wickramasinghe

26th of May, 2015

Director,
National Science Foundation,
47/5, Maitland Place,
Colombo 7.

Dear Sir,

Research Grant - RG/2012/BT/01

I received the letter dated on 15th May, 2015 regarding the release of funds. Due to delays in protocols of purchasing chemicals and consumables, we could not utilize funds fully before the termination of the research grant. However, we managed to complete all activities proposed except one activity; sending protein samples for identification. The Senior Assistant Bursar of the Faculty did not grant permission to send samples as funds were not available for that activity. The balance money (Rs. 5,308.51) will be returned to NSF by the account branch of the Faculty.

The research assistant has completed her research activities and at present, she is preparing her M. Phil thesis. As requested previously, we would be thankful to you, if you could release postgraduate registration fees. We have already sent relevant documents for the purpose.

The last financial statement as at the last date of the research period (08.05.2015) is also attached. The final report of the research grant will be sent before 08.08.2015.

I thank you and the staff of the NSF for providing financial assistance and support in conducting this proposed research.

Yours Sincerely,

H.A.M. Wickramasinghe
Principal Investigator
RG/2012/BT/01

Tel : 08-2388375, Fax : 08-2388041, E-mail : postmast@agri.pdn.ac.lk

Department of Agricultural Biology

Faculty of Agriculture
University of Peradeniya
Peradeniya 20400
Sri Lanka

10th of December, 2014

The Director,
National Science Foundation of Sri Lanka
Maitland place
Colombo 07

Dear Sir,

Progress Report of the research: Grant No. RG/2012/BT/01

Here with I submit the progress report for the period of 16.06.2014 to 15.12.2014 together with the financial statement of our research project on "Genomics and proteomic approaches of identifying dehydration stress responsive genes from selected rice varieties in Sri Lanka". The progress is satisfactory and activities proposed under the research project are carried out on planned except for protein identification.

As stated in the progress report, we wish to send separated protein for identification to National Center of Biomedical Analysis, China as they quoted the least for peptide fingerprinting ($150 per single protein spot, the e-mail copy is attached). The allocated money for sample analysis may enough for maximum of 5 protein spots. If the allocated money is not enough for analysis, please grant permission to utilize money allocated for miscellaneous expenses for the purposes.

Previously, we sent a request (by e-mail and registered post) to purchase a computer and a printer with out a software for protein analysis. Based on the prices quoted by suppliers, we can not purchase the software as it is expensive than the allocated money. The computer is needed to couple to the gel scanner at the laboratory and we wish to perform the analysis using a free software. I would be grateful to you if you make necessary arrangement to make this alteration to the original plan and grant us permission to proceed with purchasing.

Thank You.

Yours Sincerely,

.....
HAM Wickramasinghe
(Grantee
RG/2012/BT/01)

Tel : 081-2395250

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