Assessment of acquired thermotolerance of Ragi (*Eluesina coracana* (L.) Gaertn) genotypes by quantitative and qualitative analysis of protein content.

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***Abstract*:** Ragi (*Eluesina coracana*(L)Gaertn) is the most important small millet in the tropics and is cultivated in more than 25 countries in Africa and South Asia predominantly as a staple food grain. Temperature is one of the most important factors which affect plant growth and its productivity. High temperature stress affects a number of physiological and biochemical processes in plants. According to TIR technique, the seedlings are exposed to optimum induction temperature before being exposed to a severe challenging temperature and subsequently allowed to recovery at room temperature. One resistant genotype (Indaf-8) and one susceptible genotype (CO-7) were selected for the estimation of total protein content and SDS-PAGE analysis. For both selected genotypes, the protein content values of induced seedlings were very high and the values of non-induced seedlings were very low compared to control seedlings. In resistant genotype varied protein banding was observed in the three treatments i.e. control, induced and non-induced. In the induction treatment the total number of bands and the intensity of banding had increased compared to control and non-induction treatments. These new protein bands may be the HSP's or the enzymes of the anti-oxidant systems which play very important role in providing tolerance against oxidative burst. The induced seedlings had acquired thermotolerance during gradual increasing temperatures and had shown high % survival though they experienced lethal temperature in TIR technique. Hence this method is unique for screening of thermotolerance.

Key words: Temperature induction response, acquired thermotolerance, SDS-PAGE, heat shock proteins, anti-oxidants and acclimated.

I. INTRODUCTION

Ragi (*Eluesina coracana*(L)Gaertn) is the most important small millet in the tropics and is cultivated in more than 25 countries in Africa and South Asia predominantly as a staple food grain (Sub sectoral Analysis). For present population Ragi is the major food as it is considered as the power house of health benefits. The production of Ragi is coming down slowly due to the climatic factors like temperature and drought. Temperature is one of the most important factors which affect plant growth and its productivity. High temperature causes irreversible damage to plant functions and developments like inhibition of plant growth, alternation of photosynthesis, phenology, dry matter partitioning, yield reduction of crop quality and also alters the oxidative stress also.

Breeding of selected genotypes with increased heat tolerance is therefore one of the most vital objectives in crop improvement Programme. Based on preliminary studies, an efficient screening technique referred to as the Temperature Induction Response (TIR) technique has been developed to identify thermotolerant lines by Senthil Kumar et al., (2003). To assess acquired thermotolerance of induced seedlings by studying the total protein content quantitatively and qualitatively (SDS-PAGE) is another objective of the present study.

Plants adopt to high temperature stress by inherent basal level tolerance as well as acquired tolerance to severe temperature stress. Acquired thermotolerance is quite rapid and has been shown to be induced during cell

acclimation to moderately high temperature periods (Hikosaka et al.,2006; Larkindale et al., 2005; Massie et al.,2003) Many earlier studies have demonstrated that genetic variability for high temperature tolerance is noticed only upon induction treatment prior to severe stress (Burke,2001; Srikanthbabu et al.,2002) This TIR technique has been used to screen thermotolerant varieties of Ragi (Sujatha et al.,2018, Venkateshbabu 2013), Black gram (Sujatha et al., 2018), Rice (Vijayalakshmi et al 2015; SapnaHarihar et al.,2014; Renukadevi et al.,2013; Sudhakar et al.,2012), Cotton (Kheir et al 2012), Groundnut (Kumar et al.,2006), Sunflower (Senthil Kumar et al.,2003), Pea (Srikanthbabu et al.,2002). Protein synthesis is maintained significantly higher in acclimated seedlings compared to non-acclimated seedlings on being exposed to severe temperature stress (Kumar et al.,1999). Enzyme functions also sensitive to changes in temperature. Heat induced alteration in enzyme activity can lead to imbalance in metabolic pathways or heat cause complete enzyme inactivation due to protein denaturation (Vierling 1991). The best characterized aspect of acquired thermotolerance is production of heat shock proteins (HSP's) (Vierling 1991 and Burke 2001).

II. METHODS AND MATERIALS

Following standardized lethal (non-induction) and sub lethal (induction) temperatures the thermotolerance of 24 Ragi genotypes has been tested using Temperature Induction Response (TIR) technique. (Based on our previous study Sujatha et al.,2018)

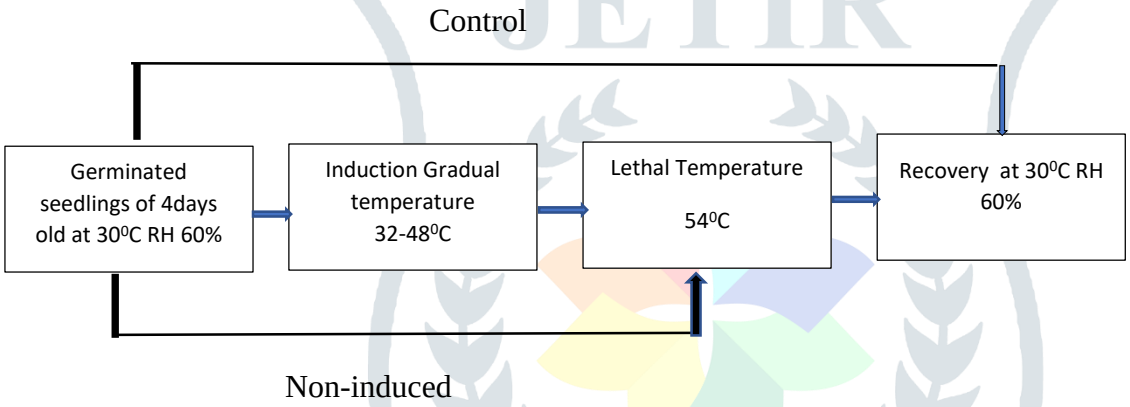

Figure .1 Standardized Temperature Induction Response (TIR) Protocol for Ragi

The 5-day old Ragi seedlings were exposed to gradual increase in temperature range of 32-48^{0}C for 5hrs and later subjected to the lethal temperature of 54^{0}C for 2 hrs. These treated seedlings were allowed to recover at 30^{0}C and 60% relative humidity for 2 days. (Figure.1) After recovery a) percent survival, b) Percent reduction of root growth and c) percent reduction of shoot growth was calculated. After TIR treatment one resistant genotype (Indaf-8) and one susceptible genotype (CO-7) were selected for further experiments namely the total protein content, SDS-PAGE analysis.

Protein from the plant tissue of different treatments (as per TIR protocol) control, induced, non-induced of selected genotypes were extracted by the method Grimplet J., et al., (2009) and estimated by the method of Lowry et al., (1951). SDS-PAGE was developed for protein profiling by adopting the method of Laemmli U.K. (1970) with minor modifications.

III. RESULT AND DISCUSSION

Total protein content

In the seedlings of control treatments for both selected genotypes the protein content had increased gradually from 0 to 3 days of treatments. Whereas the total protein content initially decreased in the seedlings of induced treatment for both selected genotypes, later increased from 0 to 3 days of treatments. The total protein content again gradually increased in the seedlings of non-induced treatment for both selected genotypes days from 0 to 3 days of treatments.

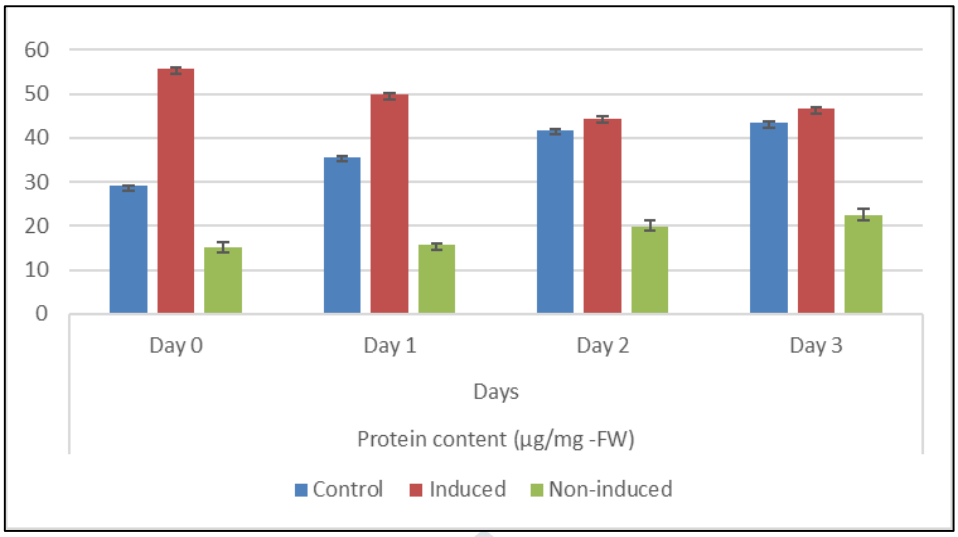

Figure.2 Total protein content of resistant genotype.

For the resistant genotype (Indaf-8) in the 'Day 0' immediately after TIR treatment (Figere.2) the protein content of the induced seedlings showed 91.06% positive growth over control seedlings and the non-induced seedlings showed 48.11% negative growth over control seedlings. In the 'Day 1' immediately after treatment the protein content of the induced seedlings showed 39.49% positive growth over control seedlings and the non-induced seedlings showed 55.46% negative growth over control seedlings. In the 'Day 2' immediately after treatment the protein content of the induced seedlings showed 06.47% positive growth over control seedlings and the non-induced seedlings showed 52.5% negative growth over control seedlings. In the 'Day 3' immediately after temperature induction treatment the protein content of the induced seedlings showed 07.37% positive growth over control seedlings and the non-induced seedlings showed 48.6% negative growth over control seedlings.

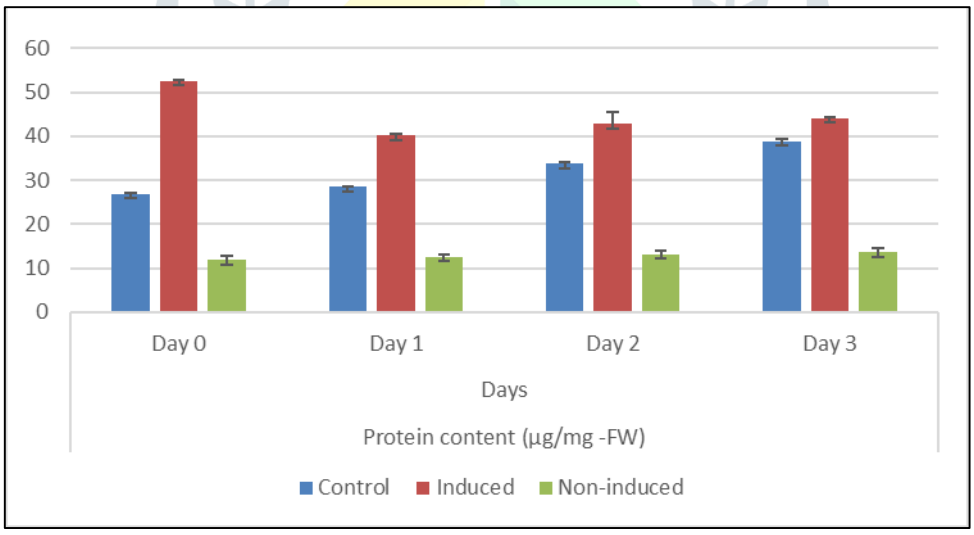

Figure .3 Total protein content of resistant genotype.

For the susceptible genotype (CO-7) in the 'Day 0' immediately after TIR treatment (Figure.3) the protein content of the induced seedlings showed 95.17% positive growth over control seedlings and the non-induced seedlings showed 55.76% negative growth over control seedlings. In the 'Day 1' immediately after temperature induction treatment the protein content of the induced seedlings showed 40.7% positive growth over control seedlings and the non-induced seedlings showed 55.79% negative growth over control seedlings. In the 'Day 2' immediately after treatment the protein content of the induced seedlings showed 27% positive growth over control seedlings and the non-induced seedlings showed 61.12% negative growth over control

seedlings. In the 'Day 3' immediately after treatment the protein content of the induced seedlings showed13.37% positive growth over control seedlings and the non-induced seedlings showed 25.3 % negative growth over control seedlings.

For both selected genotypes, the protein content values of induced seedlings were very high and the values of non-induced seedlings were very low compared to control seedlings (Figure2 and 3). In the TIR treatment, the total protein content of induced seedlings of resistant genotype had shown drastic increase with positive growth compared to control seedlings in the Day 0. This increase was statistically significant at P value 0.05.

This increase may be due to elevation of Heat Shock Proteins (HSP's) or Anti-Oxidant enzymes with response to high temperature stress. Plant adopt several strategies to optimize thermo tolerance. One of the most widely studied aspect is the enhancement of HSP's Srikanthbabu et al., (2002). Gradual increase in induction temperature would also lead to synthesis of higher level of anti-oxidant enzymes Bhoominathan et al., (2016). Upon exposure to acclimation temperature stress many of HSP's and other stress response genes are up regulated Senthil Kumar et al., (2003); Srikanthbabu et al., (2002). Significant increase in soluble protein upon induction treatment could possibly due to the synthesis of heat shock proteins Xu et al., (2006) in turf grass and Wahid and Close (2007) in sugar cane. Plant exposed to heat stress respond to a characteristic set of conserved cellular and metabolic processes. Higher values of soluble protein in acclimated settings than non- acclimated settings indicated the stability of induced seedlings under stress conditions Gomathi et al., 2014.The heat stress causes a decrease in normal protein synthesis accompanied by an accelerated synthesis of new proteins known as heat shock proteins. Mohmed et al., (2013). The induced seedlings had acquired thermotolerance during gradual increasing temperatures and had shown high % survival though they experienced lethal temperature in TIR technique.

SDS-PAGE

The selected two genotypes i.e. resistant (Indaf-8) and susceptible (CO-7) were further studied by SDS PAGE(Figure.4). In Lane-1 control of resistant genotype (CR), in Lane -2 induced of resistant genotype (IR) and in Lane-3 non-induced of resistant genotype (NIR) were taken. In Lane-4 control of susceptible genotype (CS), in Lane-5 induced of susceptible genotype (IS) and in Lane-6 non-induced of susceptible genotype (NIS) were taken.

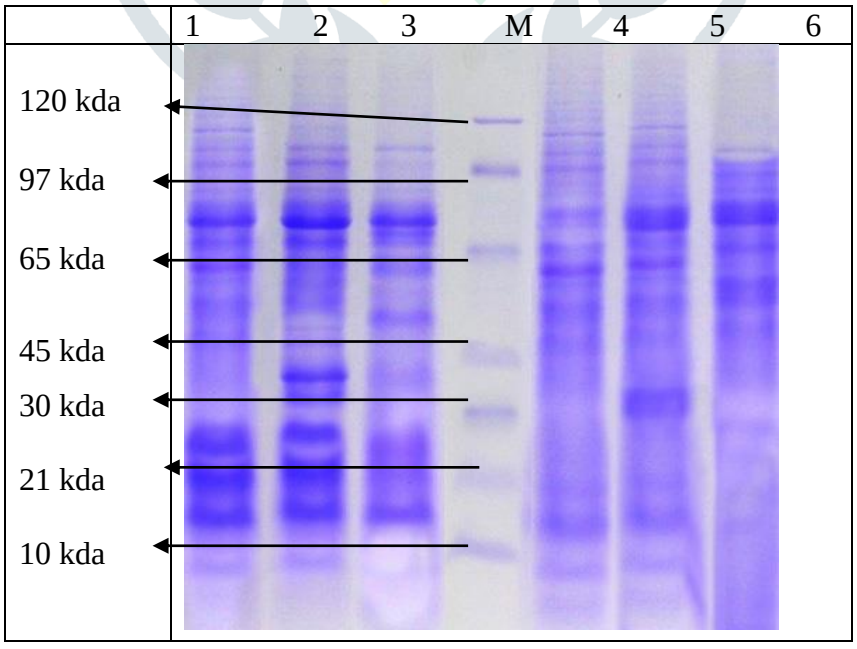

Figure.4 Protein proliferation of selected genotypes on SDS-PAGE.

Lane 1: Resistant genotype (VR - 900) Control, Lane 2: Resistant genotype (VR - 900) Induced, Lane 3: Resistant genotype (VR-900) non-induced, Lane 4: Susceptible genotype (VR -1138) Control, Lane 5: Susceptible genotype (VR-1138) Induced, Lane 6: Susceptible genotype (VR-1138) non-induced. Lane-M: Protein marker.

At molecular weight of 10Kda CR had a prominent protein band, at the same place IR had very light protein band and there was no band in NIR. Between molecular weight of 21-30Kda CR had a normal protein band, at the same place IR had more prominent protein band and NIR had very light protein band. At molecular weight of 30Kda a protein band is observed in IR and there was no band in CR and NIR. Between molecular weight of 30-45Kda a prominent protein band was observed in IR, at the same place NIR had very light protein band and there was no band in CR. At molecular weight of 45Kda CR had a protein band, at the same place there was no band in IR and NIR. Between molecular weight of 45-65Kda CR, IR and NIR had protein band but was prominent only in NIR and was light in CR and IR. At molecular weight of 65Kda also CR, IR and NIR had protein band but was prominent only in CR and IR and was light in NIR. Between molecular weight of 65-97Kda IR had a prominent protein band, at the same place CR and NIR had light protein band. At molecular weight of 97Kda CR and IR had a protein band, at the same place NIR had no band. At molecular weight of 120Kda CR had protein band, at the same place IR and NIR had no band.

At molecular weight of 10Kda, between molecular weight of 10-21Kda and 65Kda CS and IS had a protein band, at the same place NIS had no band. At molecular weight of 30Kda IS had a protein band, at the same place CS and NIS had no band. Between molecular weight of 45 -65 andat120Kda NIR had a protein band, at the same place CS and IS had no band. Between molecular weight of 65-97Kda IS and NIS had a prominent protein band, at the same place CS had no band.

Three sets of modifications were observed in the protein patterns of SDS-PAGE (figure.4)

1)The resistant genotype had shown thick and significant protein bands compared to the susceptible genotype.

2)In resistant genotype varied protein banding was observed in the three treatments i.e. control, induced and non-induced. In the induction treatment the total number of bands and the intensity of banding had increased compared to control and non-induction treatments. Some protein bands selectively increased in the induction treatment both in resistant and susceptible genotypes.

3)where as in non-induced treatment some bands had disappeared and the remaining bands were with less intensity compared to control and induction treatments

These results were in accordance with Kumar et al., (2011) who found that there was gradual increase in the number of new protein bands in Wheat cultivars with the increase in heat shock temperature. Similar expression of Hsp's upon heat stress was reported Sanjam et al., (2010). One of the most widely studied aspect of thermotolerance is the enhanced expression of heat shock proteins (Hsp's). Synthesis and Localization of a few Hsp's have been shown to trigger several physiological and biochemical processes Cushman and Bohnert (2000).These new protein bands may be the HSP's or the enzymes of the anti-oxidant systems which play very important role in providing tolerance against oxidative burst which is conformity with the observations made by Yamaguchi et al., (1995) Kumar et al., (2011)who observed that the expression of many new protein bands in case of resistant compared to susceptible one in Wheat cultivars under heat stress treatment which was also observed by Amako et al.,(1994)

Plants have developed many strategies to tolerate stress that include expression of some novel proteins Wang et al., (2003). There is convincing evidence to show that the stress-responsive proteins and genes are predominantly expressed during the sub-lethal induction stress that would bring the required changes in the plant metabolism necessary for withstanding the subsequent severe stress Lindquist and Craig (1988).

IV. CONCLUSION

Plants alter their cellular and physiologies in an attempt to counter the imbalance created by high temperature stress is referred as thermotolerance, which can be acquired by gradual increase in temperature. This acquired thermotolerance can be assessed by analysing the total protein content quantitatively and qualitatively (SDS-PAGE). Using this TIR technique it is easy to identify thermotolerant lines from a large range of population at the seedling level itself. Hence this method is unique for screening of thermotolerance.

V. ACKNOWLEDGMENT

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