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Proline: Use as an indicator for assessment of thermotolerance under TIR technique in two Black gram varieties

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ABSTRACT

Two black gram varieties LBG-806 (resistant variety) and LBG-823 (sensitive variety) were selected (as per TIR protocol) for identification of thermotolerance. Among three temperature treatments (control, induced, non-induced) as per TIR protocol, plants acclimatize for severe temperatures under induced treatment and then eventually able to survive even in the followed lethal temperature. In the estimation of proline content, thermo-tolerant variety (LBG-806) at 0^{th} day, showed significant increase in content in induction treatment (38.28%) and significant decrease in non-induction treatment (70.18%), over control was observed. In thermo-sensitive variety (LBG-823) at 0^{th} day, increased content in induction treatment (37.50%) and decreased content in non-induction treatment (69.67%) over control was significant at $P \leq 0.01$. Proline content was appeared high especially in induction treatment of thermo-tolerant variety (LBG-806) explained its cellular and acquired thermo-tolerance.

Key words: TIR, Proline, Thermo-tolerance.

INTRODUCTION

Heat stress imposes challenges for legume crops and has deleterious effects on the morphology, physiology, and reproductive growth of plants (Lobell and Asner, 2003). Black gram [*Vigna mungo* (L.) Hepper] is a tropical leguminous plant, very nutritious as it contains high levels of proteins and vitamins (USDA National Nutrient Database for Standard Reference). Heat stress can be defined as the temperature above the level of ambient which causes irreversible damage to plant growth and development. Extreme temperature causes a

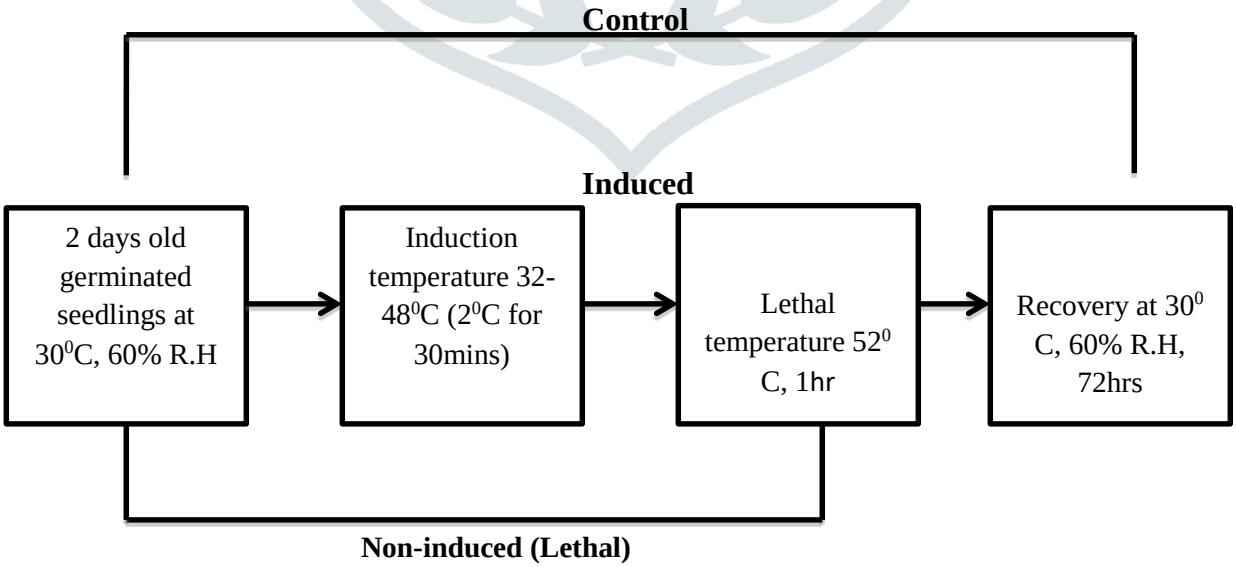
reduction in growth and productivity (Southworth *et al.*, 2000). The phenomenon of adapting to designated severe stress following mild stress is known as acquired thermo-tolerance (Vierling, 1991). Plants adapt tolerance to high temperature stress by inherent basal level as well as acquired (Hikosaka *et al.*, 2005). The T.I.R. (Temperature Induction Response) is potential and versatile one for identifying highly thermo-tolerant genotype from a large population (Srikantbabu *et al.*, 2002).

Proline accumulation in higher plants in response to abiotic and biotic stresses is a good indication of stress tolerance, where it acts as an Osmo protectant and antioxidant (Cechin et al., 2006). In plants proline accumulation to various stresses was an important adaptive physiological response (Hare et al., 1998). Proline acts as an Osmo protectant and was frequently studied for its osmoregulatory protective role (Wahid et al., 2007).

Present investigation aimed to evaluate genotypic variations associated with thermo-tolerant and sensitive varieties of black gram, regarding proline content elevated under non-induction (lethal) and induction temperature treatments using proline estimation protocol coupled with TIR treatment.

II. MATERIALS AND METHODS:

1) **PLANT SAMPLINGS:** Black gram temperature resistant variety LBG-806 and susceptible variety LBG-823 were selected based on the previous study through TIR technique (Sujatha B et al., 2018) TIR technique involves series of experiments i.e., identification of lethal/challenging temperature, sub-lethal temperatures and finally screening of thermo-tolerant genotypes from large populations by subjecting aseptically germinated seedlings from each variety to three temperature treatments i.e. control, induced and non-induced.



This method was developed by Srikanthbabu *et al.*, (2002), followed by Vijayalakshmi *et al.*, 2015 (rice), Babu *et al.*, 2013 (ragi), Chandola *et al.*, 2016 (tomato), Vidya *et al.*, 2017 (banana).

2) Proline Estimation:

Proline content was estimated by the acid ninhydrin method of Troll and Lindsley (1955) as modified by Tully *et al.*, (1979).

A) Reagents:

1. 1.Glacial acetic acid
2. 2.Ninhydrin reagent
3. 3.Toluene
4. 4.Preparation of ninhydrin reagent: The desired amount of the reagent was prepared using the proportions of 125 mg of ninhydrin in 3 ml of glacial acetic acid and 2 ml of 6 M phosphoric acid and heating to 70^0C. The reagent is stable for at least 24 hours.

B) Sample extraction: The extraction of proline was carried by grinding 200 mg of plant material from control, induced and non-induced treatments of both thermotolerant (LBG-806) and thermo-sensitive (LBG-823) black gram cultivars, in 5 ml of water and heating at 100^0C for 30 min in sealed tubes, the cooled content was centrifuged and the supernatant was made up to a known volume.

C)Amino acid Assay: For estimation of proline, 5 ml of sample extracts were taken, 5 ml of glacial acetic acid and 5 ml of ninhydrin reagent were added to each sample extract and heated in a water bath for 1hr in test tubes with plastic screw caps. The solutions were cooled to room temperature and the color was extracted using 5 ml of toluene by shaking them vigorously for 5 min in separating funnels. The phases were allowed to separate and the toluene phases were transferred to colorimeter tubes and the absorbance was determined at 515 nm on BIO-RAD smart spec plus UV visible spectrophotometer. The standard curve was prepared by using proline.

III. Results:

In thermo-tolerant variety (LBG-806) at 0^{th} day, significant increase in content in induction (38.28%) treatment and significant decrease in content in non-induction (70.18%) treatment over control was observed. Significant difference was found in all treatments and in all days at $P \leq 0.01$. In the subsequent recovery period i.e. from 0^{th} to 3^{rd} day rise in content was observed both in induction and non-induction treatments (19.44%; 15.17%) respectively at 3^{rd} day over 0^{th} day of respective treatments. At the end of 3^{rd} induction treatment showed increase in content (33.99%) and non-induction treatment showed decrease in content (72.13%) over the same day of control treatment (Fig. 1).

In thermo-sensitive variety (LBG-823) at 0^{th} day, increased content in induction (37.50%) treatment and decreased content in non-induction (69.67%) treatment over control was significant at $P \leq 0.01$. Difference in all

treatments and in all days was significant at P≤0.01. In recovery period from 0th to 3rd day both induction and non-induction treatments showed increase in content (17.76%; 21.07%) respectively over 0th day of respective treatments. At the end of 3rd day induction treatment showed increased content (41.00%) and non-induction treatment showed decreased content (68.02%) over the same of control treatment (Fig. 2). Control treatment showed increase in content from 0th to 3rd day both in thermo-tolerant and sensitive varieties (23.26%; 14.84%) respectively.

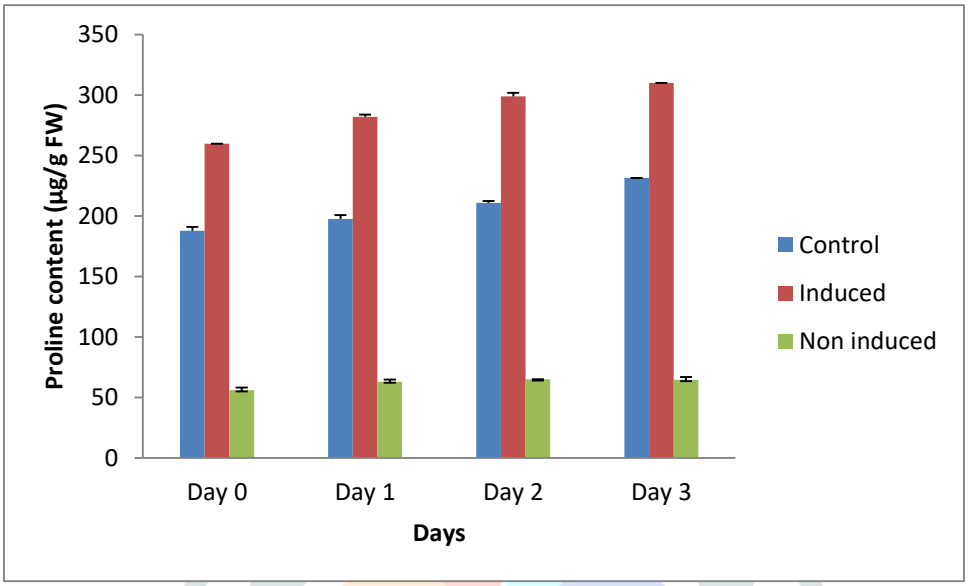


Fig 1: Thermo-tolerant variety (LBG-806)

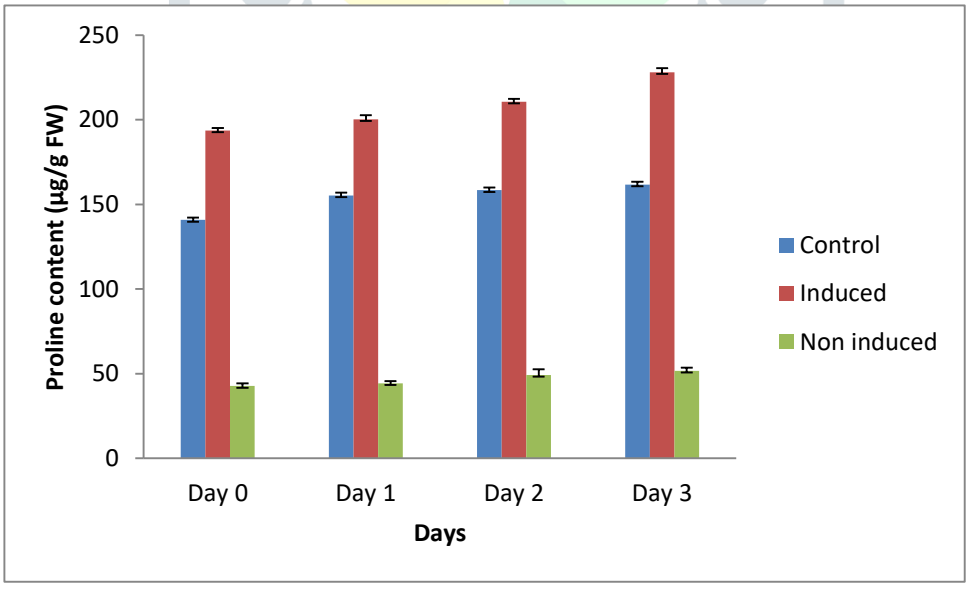


Fig 2: Thermo-sensitive variety (LBG-823)

*Mean followed by different letters on the column were significantly different at p≤0.01

III. Discussion:

On the 0th day (i.e. immediately after treatment), proline content in induction treatment of both thermo-tolerant and sensitive varieties showed higher values (i.e. 38.28% and 37.50%) respectively over their respective control treatment. In contrast, a significant reduction in content was observed in non-induction treatment both in thermo-tolerant (70.18%) and sensitive (69.67%) varieties over their respective control treatment, where the reduction was nearly similar. A gradual rise in proline content during induction temperature protects the plant at a severe temperature from osmotic injury. The rise in proline content in induction treatment might be due to its ability to mediate osmotic adjustment to provide acquired thermo-tolerance, stabilize sub-cellular structures and scavenge free radicals. Accumulation of a high proline pool could be a mechanism that could enforce resistance to plants at transient periods of high temperature stress. A similar increase in induction has been reported in pigeon pea (Sridevi, 1999); sugarcane (Wahid and Close 2007); in tomato (Gomati et al., 2014) and under high temperature in rice (Kumar et al., 2016). High levels of proline synthesis may reduce cell induced cellular acidification, increased NADP+ /NAD(P)H ratio, enhanced activity of oxidative pentose phosphate pathway which provide increased secondary metabolite synthesis as well as nucleotide synthesis accompanying the accelerated rate of cell division upon relief from stress. Data showed that drastic fall in content at the 0th day of thermo-sensitive variety in non-induction treatment might be due to protein denaturation of proline synthesizing enzymes or interconversions of proline and P5C at severe temperatures (Hare and Cress, 1997). The percent increase from 0th to 3rd day was higher in subsequent days than their control and induction treatments due to its recovery growth. High accumulation of proline under high temperature stress was observed in tomato (Kuo et al., 1986); in cowpea (Mayer et al., 1990). Drought stress induced proline accumulation in maize (Mohammadkhani and Heidari, 2008); in tomato (Handa et al., 1986) was observed.

IV. Conclusions:

Accumulation of high proline content, an osmotic adjuster was high in induction treatment than non-induction treatment provided resistance to plants at transient periods of high temperature stress. Protein content was appeared high especially in induction treatment of thermo-tolerant variety (LBG-806) explained its cellular and acquired thermo-tolerance.

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