JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

The Sodium azide induced expression of Heat Shock Proteins in Vigna mungo (L.) Hepper in conjunction with high temperature

P. G. Bansod[1] and S. N. Malode[2]
1- Vidya Bharati mahavidyalaya, Amravati
2- Government Vidarbha Institute of Science and Humanities

Abstract
The analysis was carried out to investigate the induction of heat shock proteins during the seedlings stage of *Vigna mungo* L. Hepper. In this study three day old seedlings of *Vigna mungo* L. Hepper are exposed to high temperature stress along with the integrated high temperature and mutagen stress independently under the control laboratory conditions. Two types of treatments such as dry treatment and pre-soaked water treatments were used for seeds, in dry set seeds were directly treated to various concentrations of NaN_3 (0.2%, 0.4%, 0.6 % an0.02%, 0.03%, 0.04% and 0.05%) for 18 hrs, whereas, pre-soaked water treatments (PSW) seeds were soaked in distilled water for 12 hrs and then subjected the NaN_3 (0.02%, 0.06%, 0.1% and 0.14%) treatment for 6hrs.The enormous fluctuation in expression of total proteins as well as in SDS-profile has been observed. Many small HSPs of low molecular weight are reported in this analysis such as 7.5 KDa, 5.7 KDa and 3.6 KDa along with a protein with molecular weight 43 KDa which was constitutively expressed in all seedlings irrespective of the high temperature and high salinity treatments.

Key words – High temperature, Sodium azide, Vigna mungo, SDS-PAGE, HSPs.

Introduction

A major part of human diet all over the world consists of cereal and legumes (Mandal and Mandalm, 2000). Legumes are considered as major source of proteins and dietary amino acids for man and farm animals (Boudoin and **Marechal**, 1999). According to estimation of FAO, 70% of human food comprises cereals and legumes and the remaining 30% comes from animals (FAO/IAEA, 1970). *Vigna mungo* (L) Hepper commonly known as black gram is an important pulse crop occupying a unique position is Indian agriculture. Among the pulse, it stands fourth in production and acreage (Deepalakshmi and Anandkumar, 2004). Black gram belongs to the family Leguminosae and sub-family Papilionaceae. The chromosome number of this crop is 2n=22 (Bhatnagar *et al*, 1974). In the area like Vidarbha the black gram is among the important crop plant which is taken in Kharip and Ragbi i.e. twice in a year. It grows enormously in the saline soil and drought region of Vidarbha, which indicates its salinity and drought resistance status, Therefore to elaborate this resistance status of *Vigna mungo* at molecular level the present analysis is carried out.

Sodium azide induced mutation also affects the level of total phenolics, anthocyanins and pro-anthocyanidines, and various kinds of antioxidant activities. NaN_3 induced mutations also affects the monomeric anthocyanidines with two or three anthocyanins were detectable in the seed coat. These higher level of total phenolics, anthocyanins etc. results into increased antioxidant activity.

Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. It has been reported that sodium azide affects plant physiology and decrease cyanide resistance respiration in tobacco callus (Wen and Liang, 1995). The mutagenicity of sodium azide is mediated through the production of organic metabolites of azide compounds (Owais and Kleinhofs, 1988). These metabolites enter into the nucleus, interact to DNA and create point mutations in the genome. Maize embryonic cell derived from immature embryos of inbred line 18-599 was treated with gamma rays and sodium azide (NaN_3) and selected on high osmotic medium containing 1.0% NaCl for salinity and drought tolerant mutant (He *et al*. , 2009). A combination of 20 Gy of gamma rays and 1mM of NaN_3 was identified to be most effective for the mutation. The drought tolerance of mutated line 18-599 M was significantly higher than its parental 18-599.

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

The mutagens like sodium azide and ethyl methyl sulphonate are used to develop variation in crop plants during plant breeding programs since long time. An attempt has been made investigate whether these mutagens can certainly affect the expression of HSPs which in turn enhance abiotic stress tolerance of crop plants. Some of the researcher as mentioned earlier, successfully developed the mutants of crop plants having higher antioxidant activity, greater drought resistance capacity and increased herbicide resistance property. These studies suggest that sodium azide and EMS are something to do with physiology of crop plants under stress.

Materials and Methods

Procurement of germplasm:-

Parental material of *Vigna mungo* (L) Hepper six cultivars were obtained from various Institutes. Four cultivars namely AKU-15, AKU-9904, BDU-1 and TAU-1 were obtained from PKV, Akola and two remaining cultivars i.e. Azad-1 and Shekhar were obtained from Department of Pulses, Pusa, New Delhi.

Determination of (LD_{-50}) doses:-

Lethal dose 50 (LD_{50}) is used as a criteria to define the optimum chemical dose. For determination of (LD_{50}) treatments of various concentrations were given to the dry as well as pre-soaked seeds. Treated seeds then tested for germination. After 48 hours, germinated seeds were counted and recorded the germination percentage. Doses were selected on the basis of preliminary LD_{50} experiments on seed germination. The LD-50 was recorded using germination percentage and final doses or concentrations were selected for further experimentation.

Method of treatment:-

Pure and homogenous seeds of ***Vigna mungo*** (L) Hepper were treated with different concentrations of chemicals sodium azide (SA). For treatments dry as well as presoaked in water (PSW) seeds were utilized. All the treatments were carried out in triplicates. In case of dry treatment seeds were directly treated in various concentrations of chemicals solution for 18hrs, whereas, pre-soaked water treatments (PSW) seeds were soaked in distilled water for 12 hrs and then subjected the chemical treatment for 6hrs. All the germination trays were kept in germination chambers and maintained at 25^0C temperature with 70% relative humidity.

Dry and presoaked water treatments:-

For dry seeds treatment uniform seeds were directly soaked in the mutagenic solution, for about 18 hrs. In each treatment, 30 seeds were added in 50 ml of chemical solution. All treatments were carried out in triplicates. For SA the concentrations used were 0.02%, 0.03%, 0.04% and 0.05%.

In pre-soaked water (PSW) treatments dry seeds were soaked in distilled water for 12hrs and then exposed to chemical solution for 6 hrs. In each treatment 30 seeds were treated in 50 ml of chemical solution. All treatments were carried out in triplicates at 24 ± 0.5^0c in Remi orbital shaking incubator. For pre-soaked water treatments with SA concentrations used were 0.02%, 0.06%, 0.1% and 0.14%. After completion of treatments the seeds were thoroughly washed in running water 2-3 times to remove the excess chemical stick to the seed coat. After washing seeds were used for analysis of different parameters.

Germination percentage:-

Germination is a fascinating process. The first sign of germination is the absorption of water. This activates an enzyme, respiration increases and plant cells are duplicated. Soon the embryo becomes too large, the seed coat bursts open and the growing plant emerges. The tip of root is the first thing to emerge. For the study of germination percentage, 30 seeds were kept in germination tray. Germination counts were taken from 30 seeds after 3 days, from the time of treatment. Actively emerging radicals were taken as the criteria for germination. Seeds did not germinate were consider as dead.

Quantitative analysis of proteins (Bradford):

Three day old seedlings of *Vigna mungo* (L) Hepper grown in the germination tray after providing relevant treatments and maintained at 25^0C were used for quantification of proteins.

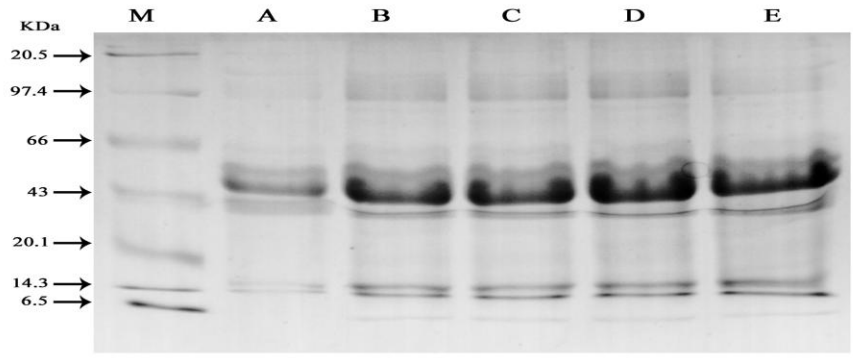
ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

Qualitative analysis of proteins by SDS-PAGE (Lammaeli, 1970)

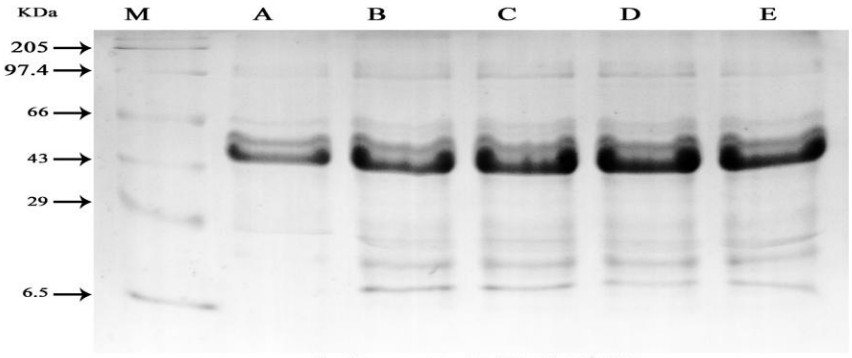
The separation of proteins was carried out on Genei Mini vertical gel electrophoresis assembly. Three day old seedlings of *Vigna mungo* (L) Hepper after treated with selected doses of sodium azide which was maintained at 25^0C in growth chamber are used for SDS-PAGE analysis of polypeptides.

Result and Discussion

PLATE-XIX



Sodium azide/ TAU-1/ 40°C/ Dry.

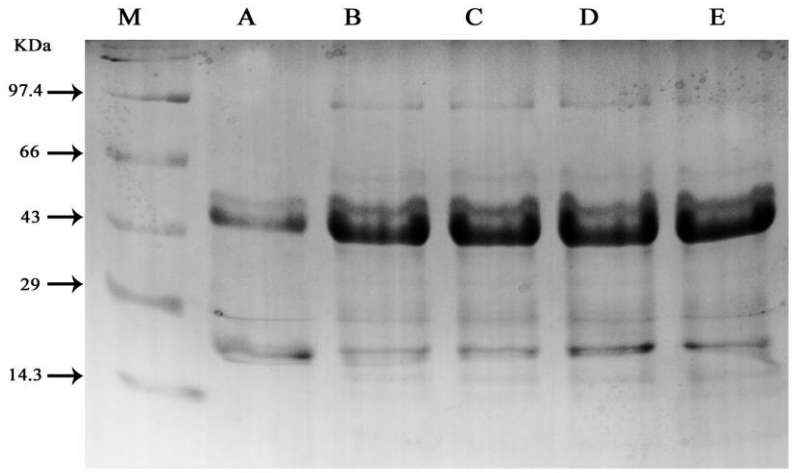


Sodium azide/ TAU-1/ 45°C/ Dry.

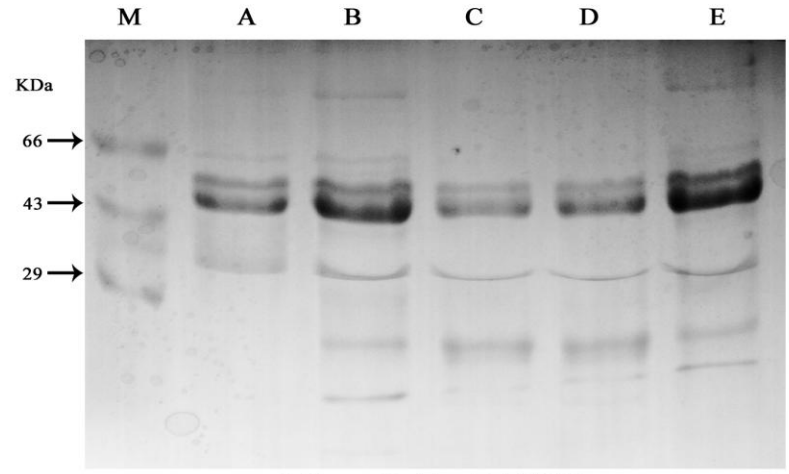
M-Marker, A-Control, B-0.02%, C-0.03%, D-0.04%, E-0.05%.

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

PLATE-XVI



Sodium azide / AKU-15/ 45^{0}C/ Dry.

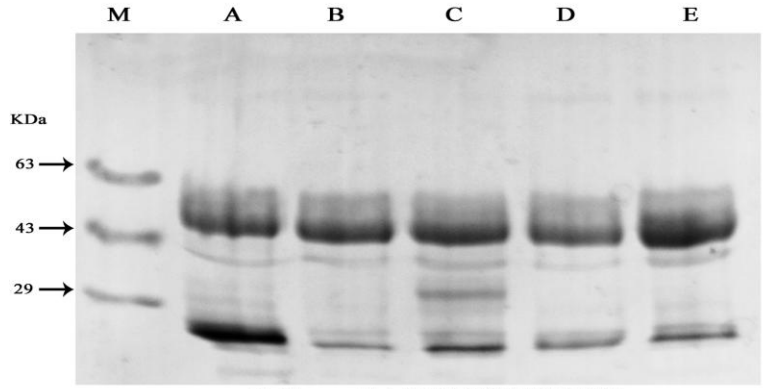


Sodium azide/ Azad-1/ 45^{0}C/ Dry.

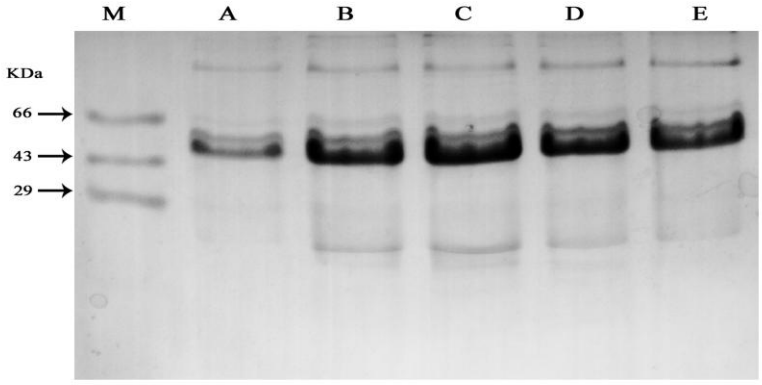
M-Marker, A-Control, B-0.02%, C-0.03%, D-0.04%, E-0.05%.

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

PLATE-XXI



Sodium azide/ AKU-9904/ 45°C/ PSW.



Sodium azide/ Azad-1/ 40°C/ PSW.

M-Marker, A-Control, B-0.02%, C-0.06%, D-0.1%, E-0.14%.

When three day old seedlings of *Vigna mungo* treated with different concentration of sodium azide in dry set, it has been observed that total protein content of seedlings between all the concentrations showed significant variations. Initially when concentration of sodium azide was increased, the amount of protein content was also increases but after that there was decrease in protein amount with increase in sodium azide concentration. Moreover it has been seen that in most of the cases the seedlings treated with 0.04% sodium azide showed highest quantity of protein. Whereas 0.03% sodium azide was another dose responsible for highest production of protein after 0.04% sodium azide. In PSW treatment out of the four concentration of sodium azide, 0.1% was found to be most favorable for protein synthesis in the seedlings. 0.06% sodium azide was the second dose which was responsible for higher induction of protein in some cases

The mutagenic treatment with Sodium azide, in three day old seedlings of *Vigna mungo* (L.) Hepper among the six cultivars revealed that, irrespective of dry and PSW treatment AKU-15 cv. have highest (178.64mg/g) protein amount found in 0.04% Sodium azide treatment which shows 53.69% increase. Whereas Shekhar cv have lowest (0.66mg/g) protein amount found in 0.04% EMS treatment.

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

Arulbalchandran and Mullainathan (2009) tried to improve the quantity and quality of proteins of *Vigna mungo* L. (Hepper) through the mutation using EMS. The result showed that 0.1 % EMS was effective in improvement of protein content among mutated generation.

Muthusami and Narayan Swami Jayabalan (2013) investigated the inference of ethyl methyl sulphonate and sodium azide treatment on yield and protein content of cotton plants by Annamalai EMS and SA at lower concentration effectively improved the yield and protein content.

The induction of biological damage and effects on seed germination, pollen fertility and seedling growth was investigated by Sheikh *et al*., (2012). They treated the seeds of two wheat (*Triticum aestivum* L.) varieties with 0.01 %, 0.02%, 0.03% and 0.04 % sodium azide. In M2 generation, the mean seed protein content of the mutants showed no considerable variation from controls.

Kozgar *et al.*, (2010) to elucidated effect of different doses (0.1% to 0.4%) of EMS on the two species of genus *Vigna* i.e. *V. radiata* and *V. mungo* for isolation of putative mutant. The comparative study of estimation of total seed protein content showed that there was a linear correlation to nitrate reductase and the total plant yield.

The developmental stage of the plant is important in order to withstand or sustain the adverse fluctuation in the environment for examples, sudden or gradual rise in temperature, several chemical and physical regents that being used by the human to increase the plant production. In fact it is the early development stages of living organism which ultimately decides their fate in future. Present study was carried out to investigate the induction of Heat shock proteins during the seedlings stage of *Vigna mungo* L. Hepper.

Temperature stress is one of the important environmental factors that may affect morphology, anatomy and plant biochemistry at all levels of organization. Direct injuries due to high temperatures in plants include protein denaturation.

In response to temperature stress various approaches are being used by the plants, which can mitigate the effect of stress and lead to the adjustment of the cellular milieu and plant tolerance. In nature stress does not generally come in isolation and many stresses act hand in hand with each other. In response to these stress signals that cross talk with each other, plants naturally have developed diverse mechanisms for combating and tolerating them.

In this investigation we have emphasized high temperature stress independently as well as high temperature in conjunction with salinity and mutagens. Various physiological and biochemical mechanisms involved in stress tolerance may include up-regulation of stress responsive genes, modifications in signal transduction pathways, indirect or slower high temperature injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis etc.

The literature available on this aspect with reference to this plant is insufficient to fully understand the role of effects of temperature stress but in most of the cases initially during the rise of temperature i.e. up to 40^0C protein amount also increased. Above 40^0C temperature this protein amount in seedling gradually or suddenly decreased. Our observations in this regards are same, as at 40^0C most of the seedlings shows highest amount of proteins. It may imply the denaturation of protein synthesizing enzymes at higher temperature.

Low molecular weight heat shock proteins in both plants and animals are together aggregated into a protein of higher molecular weight (Neumann *et al.*, 1989). The increase in total protein content after the combined stress can be attributed to these aggregations of higher molecular weight protein. This implies that combined stress can be resulted into the increased gene expression.

In the present investigation control refers to the seedlings which have been treated with temperature alone, as the main aim of the study was to find out the effect of combined stress. The results revealed that temperature alone is more efficient in inducing the higher protein amount as compared to temperature in conjunction with salinity and mutagens.

Among the salinity and mutagens induced stress in conjunction with rise in temperature it has been revealed that at the lower concentrations these stresses improve the amount of proteins in the seedlings. In the seedling stage of the plants active localization of metabolites such as DNA and proteins are required which moves from one cellular compartment to other. This movement in turn is regulated by signal transduction mechanism which is the output of signal received from outside the cell, either in the form of chemical or environmental stress. The increase in seedling protein amount may be attributed to increase in signal transduction rate. The efficiency of the mutagens decreases with increase in temperature. The total protein amount decrease at 45^0C could be related to above property of mutagens.

ISSN- 2394-5125 VOL 7, ISSUE 03 , 2020

References-

1. **Arulbalchandran D. and Mullainathan L. (2009).** Changes in protein and methionine content of Black Gram Induced by Gamma rays and EMS. American - *Eurasion Journal of Scientific Research*., 4 (2) : pp. 68-72.
2. **Baudoin J.P. and Marechal R.(1988).** Taxonomy and Evolution of the genus *Vigna.* In: Shanmugsundaram S., Mclean BT eds. Mungbean : proceedings of the second International Symposium. Taiwan: Asian Vegetables Research and Development center. pp. 2-12.
3. **Bhatnagar C.P., Chandola R.P., Saxena D.K. and Sethi S. (1974).** Cyto taxonomic studies of Genus Phaseolus. *Indian J. Genet*. 34: pp. 800-804.
4. **Boudoin J. and Moquet A. (1999).** Improvement of protein and amino acid contents in seeds of food legumes. A case study of *Phaseolus. Biotechnology Agronomy society of Environment.* 3(4): pp. 220-224.
5. **Deeplakshmi A.J. and Anandkumar C.R. (2004).** Creation of genetic variability for different polygenic traits in black gram (*Vigna mungo* (L) Hepper through induced mutagenesis. *Legume Research.* 27(3): pp. 188-192.
6. **FAO/ IAEA (1970).** Manual on mutation breeding. *Tech. Rep. Ser*. 119, IAFA, Vienna.
7. **He J., Hu Y., Li W.C. and Fu F.L. (2009).** Drought tolerant mutant induced by gamma rays and sodium azide from Maize calli. Maize Genetica Cooperation Newsletter 83.
8. **Kozgar M.I., Sonu Goyal and Samiullah Khan (2010).** EMS induced mutational variability in *Vigna radiata* and *Vigna Mungo. Research Journal of Botany*. pp. 1816-4919.
9. **Mandal S. and Mandalm R.K. (2000).** Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. *Current Science.* 79(5): pp. 576-589.
10. **Muthusami A. and Jayabalan N. (2013).** Variations in seed protein content of cotton (*Gossypium hirsutum* L) mutant lines by in vivo and in vitro mutagenesis. *Journal of Environmental Biology.* Vol. 34: pp. 11-16.
11. **Owais W.M. and Kleinhofs A. (1988).** Metabolic activation of the mutagen azide in biological systems. *Mutation Research*. 1997: pp. 313-323
12. **Sheikh S.A., Wani M.R., Lone M.A., Tak M.A. and Malla N.A. (2012).** Sodium azide induced biological damage and Variability for quantitative traits and protein contents in wheat (*Triticum aestium* L). *Journal of Plant Genomics*. Vol. 2(1) pp. 34-38.
13. **Wen J.G. and Liang H.G. (1995).** Effect of KCN and NaN3 pretreatment on the cyanide resistant respiration in tobacco callus. *Acta Botanica Sinica*. 37: pp. 711- 717.