Studies on physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress

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By

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This is to certify that the thesis entitled **"Studies on physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress."** submitted inpartial fulfillment of the requirments for the award of degree **of MASTER OF SCIENCE IN AGRICULTURE (PLANT PHYSIOLOGY)** to the Odisha University of Agriculture and Technology is a faithul record of bona fide and original research work carried out by **Mrutyunjay Barik, Adm No.18122M06** under my guidance and supervision. No part of this thesis has been submitted for the award of any other degree ordiploma.

It is further certified that the assistance and help recived by him from various sources during the course of investigation has been duly acknowledged.

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CERTIFICATE-II

This is to certify that the thesis entitled " **Studies on physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress.**" submitted by **Mrutyunjay Barik, Adm No. 18122M06** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirments for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PHYSIOLOGY)** has been approved/or disapproved by the student Advisory Committee and the External Examiner".

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Place : Bhubaneswar **MRUTYUNJAY BARIK**
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ABSTRACT

The present experiment entitled " Studies on Physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress" was conducted in the labarotory of Department of Plant Physiology, OUAT, Bhubaneswar. 11 popular dolichos genotypes, 5 photo-insensitive genotypes (Arka Soumya, Arka Amogh, Arka Vistar, Arka Swagat, Arka Sambhram) and 6 potential accessions (IIHR-B-DB 11, 25, 48, 65, 115 and *Dolichos lalab* var. lignosus) were subjected to the high temperature stress.The response of dolichos genotypes to high temperature was studied by standardizing Temperature Induction Response (TIR) technique and lethal temperature (LT50) standardized at 48^0C in order to identify the genotypes tolerant to high temperature stress. The 3 days old seedlings were subjected to lethal and sub-lethal temperature in incubator and kept at room temperature for 72 hrs for recovery of seedlings. The variation among the high temperature stress treatments of dolichos genotypes in the morphological, physiological and biochemical parmeters over their control were recorded 3 days after the high temperatute stress. The effect of high temperature on their survival percentage, seedling weight, root and shoot length reduction and changes in their metabolites were analyzed statistically and the findings are discussed and shortlisted with a concluding remark. Dolichos bean is a seasonal vegetable crop, mainly grown in winter season and sensitive to high temperature. Through TIR screening; Arka Soumya, Arka Vistar and Arka Amogh among photo-insensitive genotypes and IIHR-B-DB25 among photosensitive genotypes were identified as thermotolerant genotypes and demonostrated higher survival percentage, lower reduction of shoot & root growth and seedling weight. The variety Arka Soumya was observed with negligible reduction in seedling weight as a sign of tolerance. Further, the tolerant genotypes accumulated higher amount of TSS in leaves over control. Osmolyte proline was also incresaed up to 33.33% in 3 days as compared to control. Antioxidant enzymes like catalase, peroxidase increased in its accumulation in temperature stressed treated genotypes than control. A remarkable increase in non enzymatic compounds like GSH (upto 20.59%) and ascorbate (upto 24.0%) in Arka soumya, Arka vistar, Arka Amogh and IIHR-B-DB25. This clearly demonstrated that TIR technique can be effectively used in screening high temperature tolerance genotypes in dolichos. The findings of present study suggest that out of 11 studied genotypes as experimental materials Arka Soumya, Arka Vistar, Arka Amogh (photo-insensitive) can be categorized as tolerant to high temperature stress and IIHR-B-DB25 (photo-sensitive) may be useful for donor parents in improvement programmes related to high temperature stress. The resulted photo-insensitive thermotolerant cultivers can be recommend for summer season cultivation after stress response validation in reproductive stage of plant and genotype IHR-B-DB 25 can be recommended for donor parent in improvement programmes of hyacinth bean.

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LIST OF FLOW-CHART

ABBREVIATIONS USED

%	:	Percentage
^{0}C	:	Degree Celcius
TIR	:	Temperature Induction Response
CD	:	Critical difference
CAT	:	Catalase
POX	:	Peroxidase
GSH	:	Glutathione
AsA	:	Ascorbate
TSS	:	Total soluble sugar
DOT	:	Date of treatment
et al.	:	Co-workers
CRD	:	Complete randomized design
Max	:	Maximum
Min	:	Minimum
Fig	:	Figure
FW	:	Fresh weight
Mg	:	Miligram
S.E.M	:	Standard error of mean
µg	:	Microgram
gm	:	gram
CV	:	Coefficient of Variation

INTRODUCTION

Dolichos lablab L. a member of leguminoceae, usually known as Dolichos bean, Hyacinth bean, Field bean or Sem, is one of the most ancient crop among the culivated legume. It is a bushy, semi-erect and perennial herb. It usually grown as pure or mixed crop with finger millet, castor, maize, pearl millet or sorghum in Asia or Africa. It is a multipurpose crop grown for pulse, vagetable and forage purpose. The crop is grown for its green pods, while dry seeds are used in various vagetable preparations. It is also grown in home gardens as annual crop or on fences as perennial crop. It is one of the major source of protein in the diets in southern states of India. It is also grown as ornamental plant, mostly in USA for its beautiful dark-green, purple vein and foliage with large spikes clusterd with deep-violet and white pea like blossom

Lablab is remarkably adaptable to wide areas under diverse climatic conditions such as arid, semi-arid, sub-tropical and humid regions where temperatures vary between 22°C–35°C, low lands and uplands and many types of soils and the p^H varying from 4.4 to 7.8. Being a legume, it can fix atmospheric nitrogen to the extent of 170 kg/ha besides leaving enough crop residues to enrich the soils with organic matter. It is a drought tolerant crop and grows well in dry lands with limited rainfall. The crop prefers relatively cool seasons (temperature ranging from 14°C- 28°C) with the sowing done in July-August. It starts flowering in short days (11-11.5 hour's day length) and continues indeterminately in spring. Hyacinth bean flowers throughout the growing season. Within India, lablab is a field crop mostly confined to the peninsular region and cultivated to a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra. Karnataka contributes a major share, accounting for nearly 90 per cent in terms of both area and production in the country. In odisha it is also highly cultivated in coastal and western districts in fields as well as backyard of houses during winter season. Outside India, the crop is cultivated in East Africa, with similar uses and in Australia as a fodder crop.

Apart from this raw, immature hyacinth pods carry just 46 calories per 100 g; whereas, dry mature seeds contain 344 calories. Dry hyacinth beans contain 23.90g or 44% of recommended daily allowance of protein. Dry lablab beans indeed are very

good sources of dietary fiber among pole beans. 100 g dry beans carry 25.6 g or 64% of fiber. Dietary fiber works as a bulk laxative that helps to protect the colon mucosa by decreasing its exposure time to toxic substances as well as by binding to cancer-causing chemicals in the colon. Dietary fiber has shown to reduce blood cholesterol levels by decreasing reabsorption of cholesterol binding bile acids in colon. Hyacinth beans are gluten-free food products. They often recommended as alternative gluten-free protein food in gluten-allergy and celiac disease patients. Dry hyacinth beans are one of the finest sources of several B-complex vitamins like thiamine, riboflavin, pyrodoxin, pantothenic acid, folates, and niacin. Most of these vitamins work as co-factors for the enzymes in carbohydrate, protein and fat metabolism. Fresh pods are also excellent sources of vitamin-C. 100 g fresh pods contain 12.6 mg (21% of DV). Vitamin C is a powerful water-soluble antioxidant and helps in wound healing and tissue repair. Dry lablab beans are an excellent sources of minerals. 100 g of dry beans hold copper-148%, calcium-13%, iron-64%, magnesium-71%, manganese-68%, phosphorus-53% and zinc-84%.

With the pace of climate change due to natural or anthropogenic activities like deforestation, use of fossil fuel the concentration of Green House Gases (GHGs) is increasing alarmingly which leads to increasing the global average temperature. However the Inter-Governmental Panel on Climte Change (IPCC) of United Nations in its recent report 2018 has projected that the global average temperature over last 5 years has increased between 1.04°C compared to pre industrial baseline. This would leads to more frequent hot extremes, floods, droughts, cyclones which in turn would result in greater instability in food production. It has been suggested that fluctuations in temperature extremes and water deficit issue will be the key determinant of future climatic condition.

For Indian regions, the IPCC has projected 0.5-1.2^{0}C rise in temperature by 2024, 0.88^{0}C-3.16^{0}C by 2050 and 1.56-5.44^{0}C by 2080. It is estimated that crop production losses in india by 2100 AD could be 10-40% despite benificial effect of higher carbon dioxide on crop growth. Warming will be more pronounced over land areas in Indian subcontinent. Due to this increase in temperature it has been concluded that there will be 10% increase in kharif rainfall and uncertain rainfall and increase in temperature in rabi season. With rise in seasonal temperature the yeild of dolichos bean shown a decreasing trend .

Dolichos has a strong indeterminate growth habitat and generally it produces many flowers but only 50-80% of these devlop into mature pod due to failure of pod set and pod abortion. A few days exposure to high temperature cause heavy yeild losses through accelerating floral drop and pod abortion. In general heat stress has negative effects on growth, flowering and podding. Significance change in water potential in the environment can impose osmotic stress on plants, disrupting the normal cellular activities. Plant subjected to heat stress exhibit a variety of mitigation strategies that include biochemical, physiological, morphological and devlopmental processes. High temperature stress often leads to the production of reactive oxxygen species (ROS) like singlet oxygen, superoxide radicle, hydrogen peroxide and highly destructive effects of hydroxyl radicle. Plant protect cellular and subcellular system from cytotoxic effects of the reactive oxygen species (ROS) using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, ascorbic acid, glutathione and carotenoids (Sairam *et al.* 2000). However, under abiotic stresses the increase in ROS production is always more than the antioxidactive capacity resulting in heat stress induced oxidative damage, in addition to the direct effects of heating (Larkndale *et al.* ;2005). At physiological level, this damage translates into reduced efficiency of photosynthesis, impaired translocation of assimilates and loss of carbon gain. These factors in turn combine to cause altered phenology, reproductive failure and accelerated senescence (Hall 1997). Accumulation of proline has also been reported to occur in plants subjected to high temperature (Oshanina ,1989).

Plant overcome high temperature stress by adopting several physiological and biochemical mechanism (Senthil Kumar *et al.* ;2006). The thermotolerance can also be induced by gradual incraese in temperature from sub lethal to lethal temperature as would be experienced in natural environment (Larkindale *et al.* ;2005). Plants growing in natural habitat at regular ambient temperature may absence of acclimation and hence, ability to acuire thermotolerance is of significant importance to plants. Based on this concept, the temperature induction response (TIR) technique has been devloped (Kumar *et al.* ;2012) and its relevance was recently validated in rice (Sapnaharihar *et al.* 2014). In this technique, the seedlings are initially exposed to a non-lethal induction stress to facilitate expression of stress responsive genes subsiuently followed by lethal temperature. The seedlings that survive after lethal temperature with high recovery growth can be devloped into thermotolerant lines (Senthil Kumar, 2001).

As dolichos bean is a winter season crop and almost all the cultivated varieties are photosensitive in nature. But now a days many photo-insensitive varieties have been devloped through improved programme. These photo-insensitive can be cultivated in both winter and summer season. However summer season is not suitable for cultivation of dolichos bean due to effect of heat stress. Therefore primary screening at seedling stage of these photo-insensitive varieties is important which will show potential heat tolerant varieties suitable for cultivtion during summer. The present investigation is therefore planned to screening the thermotolerant genotypes of dolichos bean with the following objectives:

A) To standardize the lethal temperature in dolichos bean through temperature induction response (TIR) technique.

B) To elucidate physiological and biochemical changes associated with heat stress tolerance in Dolichos bean genotypes.

REVIEW OF LITERATURE

Global warming is predicted to have a great negative impact on plant growth and development in the up coming decades. The increasing threat of this extreme climatological changes including very high temperature might lead to catastrophic loss of crop productivity (Christensen, 2007). High temperature stress is one of the important abotic stress that impacts plants to molecular levels from morphological levels and is visible at all phases of plant development and has a great negative impact on agricultural productivity globally (Zhao *et al.* ;2017). Growth and devlopment of dolichos bean like other legume crops is highly influenced to greater extent by such abiotic stress conditions like high temperature stress and drought. A good deal of research work has been carried out on studies on physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress. An attempt has been made in this chapter to review the findings of various workers pertaining to the effect of high temperature of bean plants and the physiological, biochemical changes associated with them. A brief review of literature cited is discussed in this chapter.

Abiotic stress

A large global land area is affected with the abiotic stresses like drought, high temperature and salinity (Sharma *et al.* ;2001). Though biotic factors equally reduce crop yeilds, the yield losses due to abiotic stresses have been a major concern in many crops in India.In the last two decades phenomenal progress has been made in assessing the stress responses of the plants (Sharma *et al.* ;2001).

Temperature Stress

Effect of high temperature on plants

Heat sterss occurs where temperature are hot enough for sufficient time that they cause irreversible damage to plant function and devlopment of many crop species. Plant can be damaged in different ways by high day or high night temperaturs and either high soil or high air temperature.

Wahid *et al.* ;2007 discussed the detrimental effect of heat stress on reproductive devlopment that has been reported for dolichos bean. Controlled environment studies in which dolichos plants were subjected to separately controlled

high root and shoot and day and night temperature demonostrated that pod was hampered by moderately high temperature (*Warrag et al. ;*2009). Seeds produced under high temperture can be asymmetrical twisted cotyledons.

Morphological characters

Shahinnia (2013) performed studies on physiological basis of yeild variation in lablab bean (*Dolichos lablab* L.). Sixteen genotypes were evaluated for various physiological characters like plant height, branching, dry matter accumulation, percentage of dry matter distribution in various plant and growth parameters. Results showed that dry matter continiously increased up to harvest. Also different growth parameters showed variation among genotypes. At the time of harvest, the daily rate of dry matter production as highest in genotype EC10184.

Hurber *et al.* (1998) reported that seed yeild of all cultivers decreased as sowinng as delayed and the early maturing was the lowest yeilding cultiver. Yeilds of Hodgson and Wells were similar with April and may sowing but Hodgson yeilded more then Wells in later sowing dates.

Ehsanulla *et al.* (2011) reported that a numbaer of days to flowering, pod formation and maturity were observed to be significantly reduced for the month of may sowing in field bean.

Nasreenet *et al.* (2000) studied the morphological and physiological variation in lablab bean. A total of 107 lablab genotypes were evaluated from 20 different countries for major morphological characters. Variations was observed but no definite regional gene pool was identified.

Shinde (2009) studied the effect of sowing dates on the performance of bean sown on the rabi season. He concluded that bean 46 MW gave better performance over 2nd and 4th MW in respect of growth and yeild attributing characters, namely, height of plant, dry matter accumulation per plant.

Joshiet *et al.* (2012) studied the effect of dates of sowing, row spacing and varieties of date of sowing, row spacing and varieties on growth and yeild attributes of rabi Indian bean (*Dolichos lablab* L.). They found that plant height was recorded significantly higher in variety Gujrat Val 1 when sown at 30 cm row spacing.

Mustapha *et al.* (2014) evaluated the effect of moisture stress at vagetative, flowering and post flowering stage on the growth parameters of different soyabean genotypes. The results indicated that at stress conditions in all the genotypes of soyabean showed increasing trend for plant height from stress at vagetative stage to stress at post flowering stage. Among the genotypes TGX1830-2DE genotypes of soyabean showed maximum plant height.

Effect of high temperature on germination and seedling

Poor seedling establishment limits crop production throughout the world. High soil temperature has been shown to reduce bean seedlings germination, emergence and survival of seedlings (Gupta *et al. ;*2005). Bure (2009) reported that seedlings frequently experiences high temperature during emergence and establishment in many regions of the world, which leads to reduction in yeild.

Ruan *et al.* (2010) confinded that, heat stress during legume reproduction causes significant los of seed yeild, primarily by compromising seed setting or subsiquent seed filling.

Patriyawaty *et al.* (2018) reported that failure of seed setting by heat stress imposed at the early reproductive stages can not be rescued, usually leading to fatal and irreversible yeild loss, while compromised seed filling by heat stress imposed at the late reproductive processes including microsporogenesis, megasporogenesis, pollen production and viability, stigma receptivity.

Petkova *et al.* (2008) reported that heat stress just prior to during flowering usually the most detrimental effect on seed settingin legumes like cowpea, dolichos bean.

Paupiere *et al.* (2014) stated that heat stress impairs anther devlopment and reduces pollen production and fertility in chickpea, while heat stress during anthesis diminishes stigma receptivity pollen germination and pollen tube growth, limiting double fertilization.

Nakano *et al.* (1998) find that in extreme cases, high temperature stress causes the abscission of floral buds and flowers in common bean.

Iba (2002) reported that in plants during exposure to excess heat is characteristics set of cellular and metabolic response are triggered. The heat shock is characterized by transient expression of heat shock protein (HSPs). The expressions of HSPs positively correlate ith the acuisition of thermo tolerance. High temperature stress invariably causes denaturation of proteins, resulting in the formation of insoluble aggregates and hampering cell recovery after heat shock.

During heat stress period plants have evolved various mechanism for thriving under higher prevailing temperatures. These include short term avoidance mechanismor long term evolutionry adaptions. In case of sudden heat stress during the germination phase plant shows short term response that is leaf orientation, transpirational cooling and changes in membrane lipid compositionare more important for survival (Wahid *et al. ;*2007).

Relative Water Content (RWC)

Due to high temperature induced higher transpiration,situation similar to water stress is created and RWC becomes important under heat stress.Ahmed *et al*. (2004) reported that heat stress injury involves water defcit and cell turgor loss. Various studies have reported that maintenance of favourable water status is essential for plant tolerance to heat stress. (Sicher *et al.* ;2012 and Jiang and Huang ;2000). Under drought, sensitive bean genotypes are more affected by decline in RWC than tolerant one (Upreti *et al.* ;2000).

Gardener and Ehling (2008) studied the maintenance of higher warer content in leaves ensures better hydration and more favourable internal water relations of tissues with a possibly higher pressure potential.

Kuhad *et al.* (2003) studied the physiological changes during heat stress conditions of cluster bean reported that decreased in relative water content in all the cultiver when stress was created by with holding irrigation at flower initiation stage.

High temperature stress on Physiological and biochemical behaviour

Heat stress affects plants growth as well as physiological processes such as germination, seedling growth and vigour, vagetative growth, flowering and fruit set (Sairam and Tyagi ;2004) by causing cytotoxicity (Munns 2002).

Flowers *et al.* (2002) revealed that the injurious effects of heat stress on plant physiology include oxidative stress, ion-specificity, nutritional and hormonal imbalance. Out of theseeffects cellular disorganization, membrane-instability, reduction of nutrient absorption, generation of toxic metabolites and ROS, that ultimately cause plant death.

Rainey and Griffith (2005) studied the effect of high temperature that cause delyed germination of seeds and loss of seed vigour, reduced plant emergence and patchy crop stand in legumes.

Potters *et al.* (2007) stated at whole plant level, heat stress causes a reduction in cell size and closure of stomata, nagetaively affecting lef water status. It evolved the generation of ROS alters the structural organization of thylakoids and inhibits photosynthesis.

Apel *et al.* (2004) studied the plant show a mechanism of protection cell organels and cellular processes against the damaging effects of ROS with the synthesis of various enzymatic and non enzymatic ROS scavenging and detoxification systems.

Vincour and Altman (2005) shown the stress responsive mechanism established by an initial stress signal are in the form of ionic and osmotic effects or changes in the membrane fluidity. This helps to reestablished homeostasis and to protect, repair the damaged proteins and membranes.

Charng *et al.* (2006) revealed that during heat acclimation, the plant devlop the heat tolerance is genetically controlled process that is triggered by exposing plant to mild or sublethal temperature or by application of compounds or biomolecules to the growth medium. The process involved in temperature acclimation are initiated by the perception of temperature signals and transduction of these signals into biochemical process.

D Souza *et al.* (2010) reported that a number of physiological processes are influenced by the over expresion of OD in plants including, removal of hydrogen peroxidase, oxidation of toxic reductant, biosynthesis and degradation of lignin and auxin.

Heat stress often leads to excess accumulation of ROS such as superoxide radical and hydrogen peroxidase causing oxidative damage to DNA, proteins and lipids, thus the reproductive failure (Laloi *et al.* ;2004).

Andrew *et al.* (1991) studied that in leaves proline accumulation accounts for 30% of total soluble nitrogen in several crop plants and free proline accumulation was used as a relative index for water stress and heat stress resistance.

Nahar *et al.* (2011) found that antioxidnt such as glutathione (GSH), ascorbic acid, proline plays an important role in protecting the plats from oxidative damage by scavenging ROS and thus enhance the heat tolerance of dolichos bean.

Nahar *et al.* (2011) found GSH enhanced bean seedling tolerance of short term high temperature stress (42^0C) by modulating the antioxidant and glyoxalalase systems.

Application of exogenious proline improve chickpea heat tolerance, which is partially attributed to the enhanced biosynthesis of GSH and ascorbic acid in shoots and roots (Kausal *et al. ;*2011).

Kaushal *et al*. (2011) stated heat tolerant chickpea genotypes shown higher levels of ascorbic acid and reduced GSH in leaves than sensitive genotypes have shown higher levels of ascorbic acid and reduced GSH in leaves then sensitive genotypes under heat stress.

Ashraf and Foolad (2007) found under abioic stress such as heat stress plants often over produc different types of compatible organic solutes, among which proline and glycin betaine are important in stress tolerance of plants by acting as osmoprotectants and ROS scavengers.

Proline may enhance heat tolerance of dolichos through allevating the inhibition of heat stress on key enzymes in carbon and oxidative metabolism and oxidative metabolism (Kushal *et al*. ;2011).

Ozga *et al.* (2017) found that hormones play vital roles in plant reproduction under both normal and heat stress conditions. In general auxin, gibberllin and cytoinin positively regulate plant reproductive tolerance tolerance to heat stress. Ex- in

common bean, the heat tolerant cultivers which have a smaller loss in pod and seed number under heat stress, have a smaller reduction of IAA conent in in flowers and young pods.

Dianaguiraman *et al.* (2011) revealed different form of auxin, ethylene may play a negative role in legumess reproduction under heat stress. Heat treatment of soyaben plants increases ET production rate along with induction of oxidative damage, which triggers flower abscission and decreased pod set percentage.

Devi and Sujatha (2014) studied the effect of heat stress on IPCL 85063 and IPCL 87119 cultivers of pigeonpea. They cocluded that increase in free content during stress condition suggested that proline is one of the common compatible osmolytes under heat stress condition.

Kumar *et al*. (2011) studied the effect of water stress on pigeonpea with two progressive stress treatments. They confinded that increase in free proline content during water stress condition suggestes that proline osmolytes accumulated under stress condition. The genotype exhibited lower accumulation of catalase and increased activity of superoxide dismutase and peroxidase under water stress condition.

Mafakheri *et al.* (2010) stated that there were variation in proline accumulation over difference of varieties. The proline content of leaf, however increases at both growth stages in all varierties of dolichos bean in response to stress.

Verbbruggen and Hermans (2018) studied that drought stree during vagetative stages ,increased the proline content about 10 fold, which result in drought stress avoidance in plants. Proline accumulation belived to play an adaptive role in plant stress tolerance .

Sanchez *et al.* (2004) reported that proline is often considered as an osmoregulatory solutes. During the heat stress proline content increases. Cultivers with high proline accumulation having lowerwater contet upon turgor loss.

Gadallah (1999) reported that the external proline application reduced membrane injury under abiotic stress.

Babu and Devraj (2008) repotrted five fold increase in proline in french bean leaves under high temperature (46-48^0 C).

Hoekstra *et al.* (2001) reported that the accumulation of soluble sugars (sucrose, glucose and fructose) is strongly correlated to the acquisition of heat tolerance genotypes of dolichos. Sugar protect the cells of plants during heat.

Guy *et al.* (2002) reported sucrose plays an important role in osmoregulation and cryoprotection inside the plant during the heat stress.

Ciaffi *et al.* (1995) studied that heat stress reduces the pod weight in Indian bean. Starch synthesis is highly sensitive to higher temperature stress due to susceptibility of the soluble starch synthase in devloped pods.

Singh *et al.* (2007) obseved a significant decreased in protein content in chickpea genotypes under very late shown heat stress environment compared to that of late sown.

Gulen and Eris (2004) stated that under the abiotic stress,most of the plants produce reactive oxygen species (ROS) which leads to oxidative stress. The major scavenging enzymes necessary for reducing the ROS are catalase, peroxidase and superoxide dismutase. Decreases in antioxidant activity in stressed tissues results in higher level of active oxygen species (AOS) that may contribute to plant cell injury.

Babu *et al.* (2007) found that significant increase in the activity of guaiacol-specific peroxidase under high temperature stress (46-48^0 C).

Kepova *et al.* (2005) reported that heat stress can cause several cellular abnormalities by over production of ROS causes denaturation of protein, enzymes and damage membranes called oxidative stress. The common adverse effect of heat stress in cells because of production of superoxides, lipid peroxides and hydrogen peroxide.

Gong *et al.* (2005) and Balla *et al.* (2007) reported that to counter the oxidative damage plant cells have well devloped several enzymatic and non enzymatic antioxidant defense systems as a line of defense to remove and detoxify the deleterious effects of ROS which include catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, glutathione S transferase, glutathione, hydrogen peroxide and ascorbic acid.

Babu and Devraj (2008) studied that when plant undergoes heat stress they show tolerance mechanism with increase in antioxidants inside cell.

Almeselmani *et al.* (2009) studied that under high stress plants are found accumulation of enhanced amount of nonenzymatic antioxidant and upregulate the activities of antioxidant enzymes. He observed that the activities of SOD, APX, CAT, GR and POX were increased significantly at all the growth stages in heat stress tolerant cultivers in response to heat stress in dolichos.

Lee *et al.* (2000) found at high temperature plant able to produce small heat shock protein (sHSPs) and these are among the most abudant proteins synthesized in response to high temperature.

Yancey *et al.* (1999) reported that accumulation of compatible solutes (amino acids, sugars) is known to protect cell against high temperature stress in dolichos.

Kuznetsov *et al.* (1999) studied the high temperature induced accumulation of aminoacids and amides especially arginine, prolineand aspargine and enhanced their contribution to the leaf cell sap osmotic pressure. These compounds are not only involved in osmoregulation, but also in nitrogen and total metabolism.

Egli *et al.* (2005) studied the adverse effect of heat stress on devlopmental processes required for pollination, fruit set, seed size, seed number and seed quality of dolichos bean.

Druege *et al.* (2006) reported that plants secrets several amount of plant hormones and chemicals like ethylene, ABA during the high temperature stress.

Khan and Chopra (2006) reported that systemic increase in ascorbate peroxidase in bean when subjected to both drought and high temperature.

Macer and Mkmeei (2008) studied that glutathione reductase activity in ICL-3279 genotypes when exposed to high temperature stress.

Rabiye *et al.* (2009) reported that increase in glutathione reductase activity in common bean cultiver in response to heat stress.

In groundnut variety increase in GSH activity in response to heat stress.K-1375 genotype higher GSH content then heat sensitive genotype R-925 (Sharada and Naik, 2011).

Miller *et al*. (2009) reported that under high temperature stress the thermotolerant bean genotypes accumulated higher proline content than the susceptible genotype.

Randeva *et al.* (2008) reported increase in free proline content in pea plant under high temperature stress. Plant showed increase in proline content as a indicator of highly thermo tolerance.

Khetraphal *et al.* (2009) studied the effect of high temperatute stress in chickpea tolerant variety Pusa 1108 maintained higher proline accumulation compared to that of susceptible genotype Pusa 1053.

Babu and Devraj (2008) reported significant increase of proline content in french bean leaves under high temperature stress (46-48^{0}C).

Kaushal *et al.* (2011) reported that the antioxidant accumulation and the enzyme activity of cataase and peroxidase increased in french bean under high temperature stress. Along increased with these enzyme activity non-enzymatic compounds loke GSH, ascorbate and carotenoids also increased to several fold.

Gur *et al.* (2010) reported that the thermotolerant genotypes of chickpea increased the ascorbate, glutathione and carotenoids content under high temperature stress.

When plant exposed to high temperature they organize various enzymatic defense mechanism to over come, minimize the deleterious effects of ROS which include the enzyme such as SOD, CAT, POX, APX, GR (Keles and Oncel ;2002).

Wang *et al.* (2008) reported the increased level of catalase and peroxidase activity in groundnut in induced genotypes as compared to the control genotypes.

Mafekheri *et al.* (2011) studies the heat stress in french bean an reported increase in glutathione and ascorbte content in plants is an indicator of better tolerance to high temperature stress.

Babu *et al.* (2007) found a significant increase in the activity of guaiacol-specific peroxidase under high temperature stress (46-48^{0}C) french beans.

Mohammadi *et al.* (2011) reported higher antioxidant enzyme activity inder drought sress condition in chickpea cultiver. *Kumar et al.* (2012) observed an increase in the activity of the enzyme peroxidase under drought stress in pigeon pea leaves.

Due to high temperature stress high amount of reactive oxygen species (ROS) produced inside the plant cell. These species includes singlet oxygen, hydrogen peroxide hydroxyl ion and superoxide radical. These ROS species cause lipid peroxidation, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands in plants (Imaly and Linn ;2008).

Glutathione (GSH), Ascorbate and GR in field bean genotypes examined under high temperature stress and reported GSH, GR activity was increased in all the treated genotypes as compared to the fresh control genotypes (Kumar *et al.* ;2012).

MATERIALS AND METHODS

The present study entitled **"Studies on physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress"** was conducted in the laboratory of Department of Plant Physiology, OUAT, Bhubaneswar. The materials used and methodology adopted for the study is described in this chapter.

LOCATION

Bhubaneswar lies in the East & South Eastern coastal Plain Zone of agro-climatic zone of Odisha. Experimental lab was in Department of Plant Physiology, OUAT between latitude 20.24degree North and longitude 85.78degree East.

Experimental details

Design and layout

The experiment was laid out in control randomized design (CRD) with 11 treatments and 3 replications. Particulars about the layout has been given below.

Season of the crop	:	Rabi 2019-20
Layout design	:	Control Randomized Design(CRD)
No of treatments	:	11
No of replications	:	3

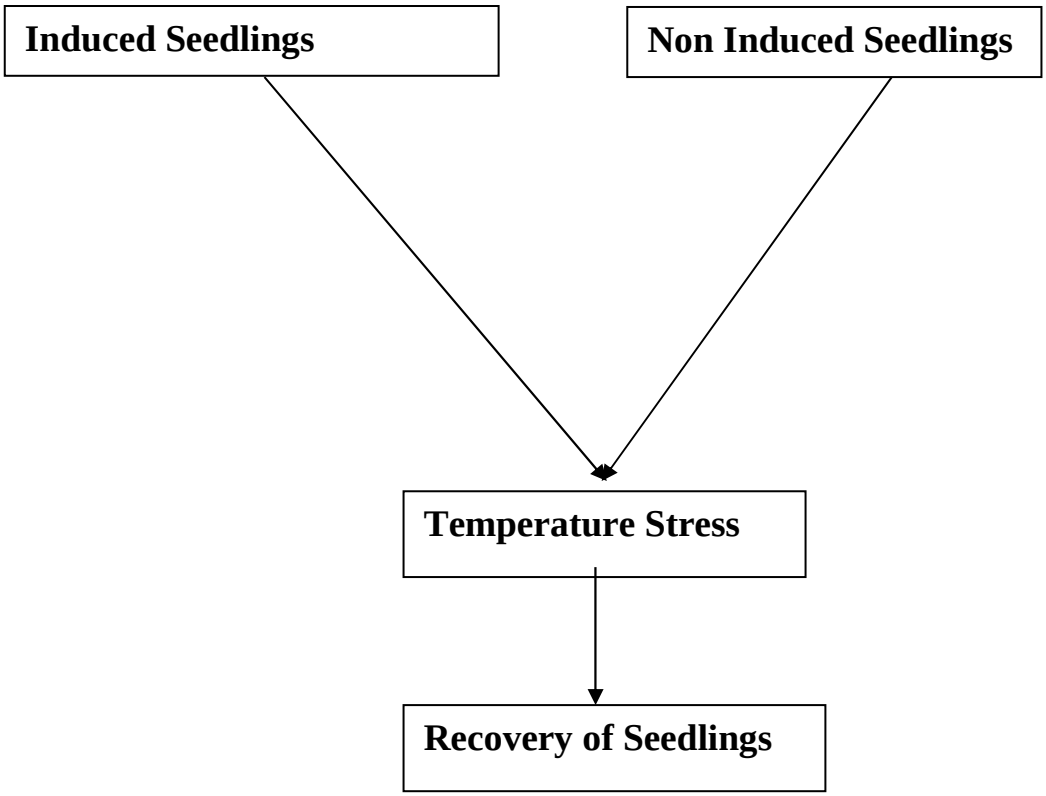
11 dolichos bean genotypes (5 photo insensitive and 6 photo sensitive) are used in this study and screened for thermotolerance using TIR technique. This involves initial identification of optimum challenging and induction temperature for screening genotypes for intrinsic thermotolerance (Senthil Kumar *et al. ;*2005).

Temperature Induction Response(TIR) technique: A method to assess thermotolerance

The principle in this technique is that, initially the seedlings are exposed to mild temperature (induction temperature) following which the seedlings are exposed to severe temperature. Recovery growth is determined as a measure of tolerance.

Chart 1. **General Protocol for TIR technique**

Flow-chart- 1



Preparation of seedlings :

The genotype IIHR-B-DB25 was selected to standardize ptotocol for TIR technique. This technique required for standardization of lethal temperature and induction temperature for dolichos. The dolichos seeds were surface sterilized by treating with 2% bavistin sloution for 15 minutes and washed with distilled water, placed in protrays (filled with cocopit) and then allowed to germinate. Three sets of three days old uniform seedlings were selected for this experiment. Initial seedlings length was measured.

Identificatin of Lethal temperature :

The minimum temperature which causes more then 75% mortality when the dolichos seedlings are exposed without induction cycle is known as lethal temperature. One set of grown dolichos seedlings were directly exposed to standardized the lethal temperature in a humidity controlled (60% humidity) incubator for 3hours. At the end of the treatment, removed the protrays and kept at room

temperature for 72 hours for the recovery of seedlings. Keeping another one set of measured seedling at room temperature throughout the experiment and consider as absolute control.

Identification of optimum Induction temperature :

Induction cycle is the sequence of temperature treatments during which the seedlings are exposed to an optimum sub lethal temperature, followed by recover preiod of 72 hours. Transfer the rest one set of seedlings exposed into humidity controlled growth chamber to standardize the induction temperature treatment.

Optimization of induction and lethal temperature:

The dolichos genotype IIHR-B-DB25 is used to optimise the induction and lethal temperature. The temperature at which 75 percent mortality of seedlings and 50 percentage reductions in seedlings growth is taken as lethal or challenging temperature to assess the difference in cellular thermotolerance. To standardize the optimum induction temperature, 3 days old dolichos seedlings (IIHR-B-DB25) were subjected to different induction temperature cycles, following which they transfered to standardize lethal temperature. The seedlings were exposed to following temperatures gradually for 5 hrs at the rate 2^0 C per hour. After subjecting the seedlings to lethal temperature, seedling are again allowed to recover at 30^0 C for 72hrs.

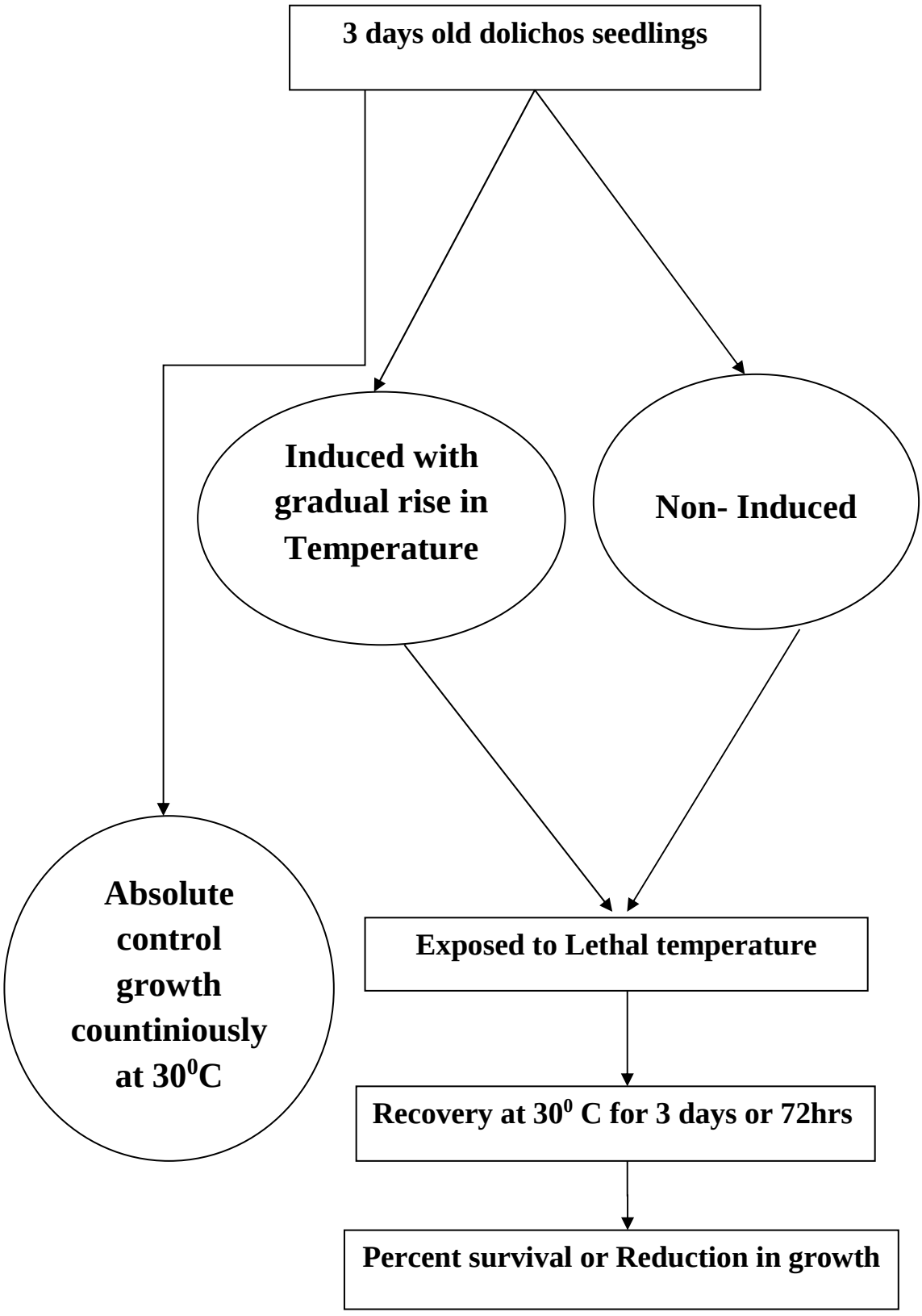
36-44^0 C
38-46^0 C
40-48^0 C
42-50^0 C

At the end of the recovery period, the seedlings survival percentage and recovery growth was measured. Based on percentage seedlings survived and percentage reduction in growth, the thermotolerance genotypes are selected. The percentage reduction over absolute control and the survival percent is calculated by using the formula suggested by Senthil Kumar (2001).

Percentage reduction over absolute control- $[(C-R)/C]\times 100$
Where C= Recovery growth of absolute control
R= Recovery growth of induced seedlings
survival of the seedlings (%)= [No of seedlings survived/Total no. of seedlings] × 100

Protocol for Identification of Optimum Induction Temperature

Flow-chart -2



Biochemical analysis of control and treated by leaf sample

Estimation of Protein Content by Lowry's Method: 1951

Total protein content in leaves were determined by using the method stated by Lowry (1951). Fresh leaf sample of 1 g was macerated in 10 ml TCA (10%) solution using mortar and pestle. After transferring into a centrifuge tube it was taken for centrifugation at 5000 rpm for 10 minutes. The supernatant was thrown out and residue was collected to which 10 ml of 1N NaOH was added followed by mixing thoroughly by using a glass rod. Again centrifuged at 10,000 rpm for 10 minutes at 4^0C. Then 0.2 ml of sample extract was pipetted out into a test tube and the volume was made up to 2 ml with water. In each test tube including blank 10 ml of reagent C was added and allowed to stand for 10 minutes. 1 ml of reagent D was added to it, mixed well and incubated at room temperature and kept in dark for 30 minutes. At last blue colour was noticed. Finally, absorbance was taken at 660 nm. A standard protein curve was prepared and from that sample protein content was calculated as mg/g FW of sample.

Estimation of total soluble sugar : Hedge*et al*. (1962)

Total soluble sugar of the leaves were calculated by using the Hedge *et al.* (1962) method. Weigh 100mg of fresh sample and add 5ml of 2.5N Hcl. Transfer it into hot boiling water bath for 3 hours. Then cool it to room temperature. Neutralizing it with solid sodium carbonate until the effervescence ceases. Further make up the volume to 100 ml and centrifuse. Collect the suprernatant and take 0.5 ml aliuots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard . " 0" as blank.Then make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Add 4ml of anthrone reagent and heat for 8mins in a boiling water bath. Cool rapidly and read the green to dark green colour at 630nm. A standard sugar curve was prepared and from that sample total sugar (TSS) content was calculated as mg/g FW of sample.

Calculation

$$\text{Amount of carbohydrate present in sample (\% mg)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (0.5 or 1ml)}} \times \frac{\text{Total vol. of extract (ml)}}{\text{Wt. of sample (mg)}} \times 100$$

Estimation of Peroxidase Activity: Rotrck *et al.* (1973)

The Peroxidase enzyme activity of leaves were calculated by using Rotruck *et al.* (1973) method. 1 g leaf sample was macerated with 0.4 M sodium phosphate buffer ($p^{H=}$ 7) using a pre-chilled mortar and pestle. Homogenate was centrifuged at 10000 rpm for 15 minutes at 4^0C. Supernatant was collected and used for enzyme estimation. 0.1 ml enzyme extract was pipetted out in a test tube and other reagents such as 0.4 ml buffer, 0.1 ml sodium azide, 0.2 ml glutathione, 0.1 ml hydrogen peroxide was added. Then the volume was made up to 1 ml with 0.1 ml of distil water. The tubes were incubated at 37^0C for 5-10 minutes and 10% TCA was added to stop the reaction. To determine the residual glutathione content, the assay was again centrifuged. Again 3 ml of reaction mixture was prepared by adding 1.9 ml buffer, 1.0 ml DTNB and 0.1 ml supernatant. Thus the enzyme mixture was allowed to take absorbance at 412 nm against blank containing 2 ml of buffer and 1 ml DTNB reagent. The activity of enzyme was expressed as µg of glutathione consumed per minute per mg protein.

Calculation:

$$\textbf{Peroxidase (U/min/g FW)} = \frac{\text{(Change in abs./min) X Total volume(ml) X Dilution factor}}{\text{Extinction coefficient X Volume of sample taken (ml)}}$$

$$\textbf{Specific activity (EAU/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein content (mg/g FW)}}$$

Estimation of Catalase Activity: Dhindsa *et al.* (1981)

The catalase enzyme activity of the leaves were calculated by using the Dhindsa's method. 0.05g of fresh leaf sample was macerated in 1ml of potassium phosphate buffer using mortar and pestle and homogenate was centrifuged at 12000 rpm for 20 minutes in cooling centrifuge at 4^0C. Then the supernatant was collected and diluted to 10ml with distil water. 3 ml of assay was prepared in a cuvette by adding 0.01ml of enzyme, 2.99ml of $H_2O_2.PO_4$ (0.036% w/w) and a blank containing only 2.99 ml $H_2O_2.PO_4$ simultaneously. Absorbance reading was taken at 240 nm agains//t blank.

Calculation:

Catalase(U/min/g FW) =

$$\frac{\text{(Change in abs./min) X Total Volume (ml) X Dilution factor}}{\text{Extinction coefficient X Volume of sample taken (ml)}}$$

$$\text{Specific activity (EAU/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein content (mg/g FW)}}$$

Estimation of Proline content : R .P. Bate *et al.* (1992)

The proline content of leaves were calculated by using this methord. 50mg of fresh weight leaf is macerated with 5ml sulfo salisylic acid and centrifuged it at 4000 r.p.m for 15 mins. Decanted the liquid supernatant to a 50ml test tube. Further add 5ml of Glacial Acetic Acid and 5ml Acid Ninhydrin. Then close the mouth of the test tube with polythene paper and rubber band. Transfer the test tube to hot water bath at 100^0 C for 1hour. After boiling of standard and sample, the reaction of mixture is transferred to 60ml separating funnels. 20 ml toluene is added and shake vigorously. It is then allowed to settle down. The chromophere containing toluene is separated out through the bottom hole of the separating funnels. Measure the OD value at 520nm. Then by the help of standard curve data, amount of proline is present in sample is calculated and ecxpressed as µg/g proline FW.

$$\textbf{µ moles of proline /gm} = \frac{\textbf{µg proline/ ml X ml toluene}}{115.5} \textbf{ X } \frac{5}{\text{g sample}}$$

Estimation of Ascorbate content : Sadasivam *et al.* (1987)

Estimation of ascorbate content in leaves were calculated by using this method.

Reagents

i. 4% Oxalic acid

ii. Dye solution : weigh 42mg of sodium bicarbonate into a small volume of distilled water. Dissolve 52mg, 2, 6-dichlorophenol indophenol in it and make up to 200ml with distilled water.

iii. Stock standard solution: Dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask (1mg/ml)

1v. Working standard: Dilute 10ml of the stock solution to 100ml with 4% oxalic acid. The concentration of working standard is 100µg/ml.

Procedure

Pipette out 5ml of the working standard solution into a 100ml conical flask. Add 10ml of 4% oxalic acid and titrate against the dye (V1 ml). End point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbate . Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V2 ml).

Calculation

Amount of ascorbate

$$\text{(mg/100g sample)} = \frac{0.5\text{mg}}{\text{V1 ml}} \times \frac{\text{V2}}{\text{15 ml}} \times \frac{100\text{ml}}{\text{Wt. of the sample}} \times 100$$

Estimation of Glutathione content : Smith (1985)

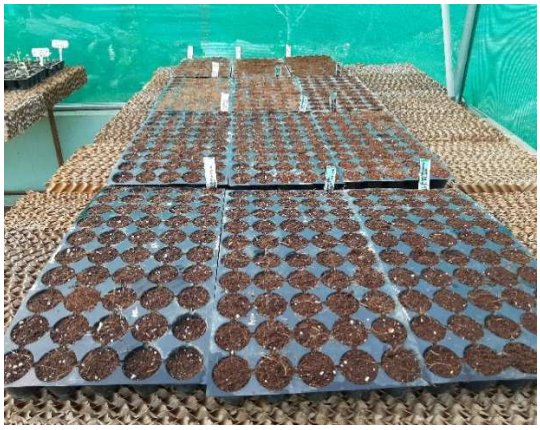
Content of total glutathione in leaves was determined by the methord of Smith (1985)

Reagents

i. 0.1M sodium phosphate buffer ($p^{H=}$ 7.5)
ii. 2mM NADPH
iii. 5mM of DNTB
iv. Glutathione reductase enzyme
v. 4- vinylpyridine

Procedure

Total glutathione was determined by adding 1.75 ml of 0.1M sodium phosphate buffer ($p^{H=}$ 7.5), 0.1ml of 2mM NADPH, 0.1ml of 5 mM DNTB and 0.2 ml of TCA extract. Added one unit (3.4µl) of standard glutathione reductase, mixed well, incubated for 10 ml of TCA extract added 0.95ml of potassium phosphate buffer (0.5M, $p^{H=}$ 7.5) and 50 µl of 4-vinylpyridine. The contents were mixed well and allowed to stand for 1hr to remove GSH . To 0.1ml of vinyl pyridine treated extract added 1.75ml of 0.1M sodium phosphate buffer (pH 7.5), 0.1ml of 2mM NADPH and 0.1ml of DTNB. The reaction was started by adding one unit of standard GR enzyme, incubated for 10 mins and absorbance was recorded at 412nm against reagent blank. Total glutathione was calculated from standard curve. The results were expressed as µ mol/gm DW.



1. **Sowing of seeds in protrays**

2.Germination of dolichos

3. 3days old dolichos seedlings

4. Heat treatment in the incubator

5. Seedlings before heat treatment

6. Seedlings after heat traetment

7. Susceptible dolichos genotype

8.Thermotolerant dolichos genotype

9. Arka Vistar (Control vs Treated)

10. IIHR-B-DB25 (Control vs Treated)

11. IIHR-B-DB48 (Control vs Treated)

12. IIHR-B-DB11 (Treated vs Control)

13. IIHR-B-DB115 (Treated vs Control)

14. IIHR-B-DB65 (Treated vs Control)

15. Lignosus (Treated vs Control)

16.Arka Sambhram (Treated vs Control)

17. Arka Soumya (Control vs Treated)

18. Arka Amogh (Control vs Treated)

19. Optimum induction temperature (44-52^{0}C) (42-50^{0}C) (40- 48^{0}C)

20. Died Thermo-susceptible genotype after heat treatment at lethal temperature (50^{0}C)

21. Recovered Thermotolerant genotype after heat treatment at lethal temperature (50^{0}C)

22. Thermotolerant genotype and susceptible genotypes 72hrs after heat treatment

RESULTS

The present experiment was conducted during rabi 2019 in the laboratory of Department of Plant Physiology, OUAT, Bhubaneswar to assess the effect of physiological responses of Hyacinth bean (*Dolichos lablab* L.) genotypes against high temperature stress. The effect heat stress on different morphological, physiological and biochemical characters recorded during conducting experiment, were tabulated and presented in the following heads and sub heads.

Standardization of optimum Lethal temperature for TIR

The dolichos genotype IIHR-B-DB25 is exposed to the temperature at 50^0C lethal temperature.The experimental data were recorded and the results depicted in (Table-1) showed reduction in shoot and root growth of the treated seedlings over the control with rise in lethal temperature. At the same time with increase in temperature to lethal temperature the survival percentage of the seedlings decreased. At the challenging temperature of 50^0C for 5 hours the lowest survival percentage (38%) and highest growth reduction (51%) of seedlings was observed. The trend observed was similar in all the experiments that conducted before. Both total growth and percentage survival show a significant variation among different challenging temperatures. Based on this above parameters, in this experiment lethal temperature is standardized as 50^0C. The lethal temperature was recorded as 48^0C for pea by (Srikanthbabu *et al. ;*2001), 52^0C for rice by Sapna *et al.* (2011). Highest lehal temperature as recorded in groundnut (55^0C) by Gangappa *et al.* (2006).

Standardization of optimum Induction temperature

The temperature cycle at which least reduction of root and shoot length and higher survival of genotypes as compared to control is considered as optimum induction temperature. The dolichos genotype IIHR-B-DB25 was exposed to induction cycles followed by standardization of lethal temperature. The experimental data were recorded and the results are depicted in (Table-2) showed that the optimum induction cycles were optimized as 38^0C-46^0C as in this temperature range least root and shoot length reduced and higher survival percentage of seedlings found. Higher survival percentage (53%) and least growth reduction (22.56%) of seedlings were

observed when seedlings were induced with a gradual temperature induction range of 36^0C-46^0C for 5 hrs (Table-2). From this table both total growth and percentage of survival varied significantly among different induction temperature cycles. The induction cycle varies from crop to crop species which depend upon intrinsic ability of plant system to acquire thermotolerance. For soyabean it is experimented 34^0C-42^0C (C Viaylaxmi *et al.* ;2015), 38^0C-54^0C for groundnut (K Rekha Rani *et al.* ;2012) and 36^0C-44^0C for rice as optimum induction temperature.

Table : 1 Standardization of lethal temperature in dolichos

Treatments	Shoot and Root length (cm)	Percent Survival	% reduction of shoot and root length over control
Control	**17.47**	**100**	**-**
46^0C	**16.77**	**100**	**4**
48^0C	**14.43**	**76.67**	**17.4**
50^0C	**9.28**	**38.0**	**44.0**
52^0C	**0**	**0**	**100**
54^0C	**0**	**0**	**100**
Mean	**9.74**	**62.93**	
S.E.m (±)	**0.06**	**0.14**	
CD(0.05)	**0.17**	**0.40**	
CV= 2.04			

Table : 2 Standardization of optimum induction temperature in dolichos

Treatments	Shoot and root length(cm)	Percent survival	Percent reduction of shoot and root length over control
Control	**17.57**	**100**	**NA**
34 -42^0C	**12.93**	**31.77**	**26.40**
36 -44^0C	**12.10**	**37.95**	**31.13**
38 -46^0C	**13.57**	**52.46**	**22.56**
40-48^0C	**11.20**	**40.75**	**36.25**
Mean	**13.47**	**52.58**	
S.E.m(±)	**0.14**	**0.32**	
CD(0.05)	**0.40**	**0.93**	
CV=5.35			

Screening of Dolichos genotypes for thermotolerance

Based on the standardized lethal temperature and induction temperature, 11 genotypes of dolichos were screened for tolerance to high temperature. Out of the 11 genotypes 5 genotypes are photo-insensitive and rest 6 genotypes are photosensitive.

The results depicted in (Table-3) that variety Arka Soumya (13.02%) was found to have the least reduction in growth follwed by Arka Amogh (13.39%) and Ara Vistar (24.35%) among the photo-insensitive genotypes while IIHR-B-DB25 variety showed least reduction in growth (20.57%) among the photosensitive varieties.

5 photo-insensitive and 6 photosensitive dolichos (total 11) genotyes were treated at their lethal temperature. However 3 genotypes of photo-insensitive and 1 from photosensitive genotypes recoverd 3 days after the heat stress treatment. Highest survival percentage was recorded in Arka Soumya (90.07%), followed by Akra Vistar (85.17%) and Arka Amogh (41.57%) among the photo-insensitive varierties, while IIHR-B-DB25 (42.96%) was the only survived among the photosensitive genotypes. Rest 7 dolichos genotypes failed to recover 3 days after the heat treatment. In this experiment, all the biochemical and enzymatic tests has done to study the changes came across the died genotypes over their control along with the recoverd thermotolerant genotypes.

Table : 3 Cellular level tolerance of dolichos genotypes based on TIR technique (Survival percent)

Genotypes	Control	Induced	Mean
Lignosus	**100**	**0.00**	**50.0**
IIHR-B-DB11	**100**	**0.00**	**50.00**
IIHR-B-DB65	**100**	**0.00**	**50.00**
IIHR-B-DB115	**100**	**0.00**	**50.00**
IIHR-B-DB25	**100**	**42.96**	**71.48**
IIHR-B-DB48	**100**	**0.00**	**50.00**
Arka Amogh	**100**	**41.57**	**70.87**
Arka Soumya	**100**	**90.07**	**95.03**
Arka Vistar	**100**	**85.17**	**92.58**
Arka Swagat	**100**	**0.00**	**50.00**
Arka Sambhram	**100**	**0.00**	**50.00**
Mean	**100**	**23.61**	
	T	**V**	**T*V**
S.E.m (±)	**1.83**	**4.29**	**6.03**
CD(0.05)	**5.21**	**12.24**	**17.31**
CV= 14.37			

Table : 4 Effect of high temperature on shoot and root length (cm) growth of exposed dolichos seedlings

Genotypes	Control	Induced	Percent growth reduction
Lignosus	**19.93**	**9.50**	**52.33**
IIHR-B-DB11	**17.07**	**10.90**	**36.14**
IIHR-B-DB65	**18.80**	**10.53**	**43.98**
IIHR-B-DB115	**20.77**	**8.33**	**59.89**
IIHR-B-DB25	**17.93**	**14.87**	**20.57**
IIHR-B-DB48	**18.40**	**11.07**	**39.83**
Arka Amogh	**21.13**	**18.30**	**13.39**
Arka Soumya	**18.43**	**16.03**	**13.02**
Arka Vistar	**19.13**	**14.47**	**24.35**
Arka Swagat	**12.83**	**6.97**	**45.67**
Arka Sambhram	**14.70**	**10.23**	**30.40**
Mean	**18.48**	**12.33**	
	T	**V**	**T×V**
S.E.m (±)	**0.24**	**0.57**	**0.81**
CD (0.05)	**0.70**	**1.64**	**2.33**
CV= 9.43			

Seedling weight

The expoure to high temperature significantly decreased the fresh weight of dolichos seedlings. Considering 5 seedlings of each genotypes as an unit . There was a 40.09% decline in fresh weight of the seedlings of Arka Amogh, 16.81% decrease in IIHR-B-DB25,10.96% decline in Arka Vistar. However there was a negligible reduction in seedling weight of Arka Soumya 3 days after the treatment to heat stress. The results are significant for both treatments, genotypes and their interaction.

Table : 5 Effect of high temperature on seedling weight (gm) of survived dolichos seedlings

Genotypes	Control	Induced	Percent decrease over control
IIHR-B-DB25	**14.27**	**11.87**	**16.81**
Arka Amogh	**10.30**	**6.17**	**40.09**
Arka Soumya	**8.50**	**8.50**	**NA**
Arka Vistar	**10.03**	**8.93**	**10.96**
Mean	**10.77**	**8.86**	
	T	**V**	**T×V**
S.Em (±)	**0.018**	**0.042**	**0.060**
CD(0.05)	**0.051**	**0.121**	**0.171**
CV = 2.90			

Effect of High temperature to Biochemical changes

Proline

Plants accumulate compatible solute such as proline under the high temperature stress conditions to ptrotect the cells from water loss. Proline accumulation in treated and untreated plants were recorded in (Table- 6) 3 days after the treatments. The heat stress resulted significant increased in accumulation of proline in all the treated dolichos genotypes over control. In genotype Arka Soumya highest increase in proline (31.81%) accumulation was found followed by Arka Vistar (26.98%), Arka Amogh (20.31%) and IIHR-B-DB25 (15.62%). However genotype Lignosus was found with least increase in proline accumulation (3.63%) three days after the high temperature treatment.

Proline acts as a low molecular weight chaperon and osmoprotectant. Proline helps in the maintenance of membrane intigrity, stabilization and protection of protein and enzyme structure and scavenging of ROS. Thus a higher proline content is a part of an overall defence mechanism against abiotic stress.

Total Protein

Protein content of leaves of the experimental plants analyzed and depicted in (Table-7) which indicated that protein content appreciably decreased in all the treated genotypes, subjected to heat stress over control genotypes. Variety Arka Soumya(6.65%) was found with least decrease in protein content followed by Arka Vistar (9.52%) and Arka Amogh (10.21%) among the photo-insensitive genotypes. While variety IIHR-B-DB25 (10.49%) was found least decreased in protein content among the photosensitive genotypes 3 days after heat treatment. In genotype IIHR-B-DB65 highest decrease in protein content (62.16%) was found. The data was found statistically significant in treatments, varieties and their interaction.

Total soluble sugar(TSS)

Plants accumulate compatible solutes like proline and soluble sugar as a protection strategy for survival against the abiotic stress and has been involved in the establishment and maintenance of tolerance to heat stress. The total sugars were appreciably increased in all the genotypes subjected to heat stress as compared to control. The result showed in (Table-8) that TSS significantly increased in Arka

Soumya (66.26%) followed by Arka Vistar (56.87%),Arka Amogh (46.01%) and IIHR-B-DB25 (45.89%) genotype. In Arka Sambhram lowest increase in TSS (5.03%) content was found 3 days after the heat treatment. The data was found statistically significant in treatments, varieties and their interaction.

Table :6 Effect of high temperature on proline content (mg/gm) of exposed dolichos seedlngs

Genotypes	Control	Induced	Percent increase over control
Lignosus	**0.55**	**0.57**	**3.63**
IIHR-B-DB11	**0.57**	**0.60**	**5.26**
IIHR-B-DB65	**0.50**	**0.56**	**12.0**
IIHR-B-DB115	**0.49**	**0.54**	**10.20**
IIHR-B-DB25	**0.64**	**0.74**	**15.62**
IIHR-B-DB48	**0.56**	**0.59**	**5.35**
Arka Amogh	**0.64**	**0.77**	**20.31**
Arka Soumya	**0.66**	**0.87**	**31.81**
Arka Vistar	**0.63**	**0.80**	**26.98**
Arka Swagat	**0.49**	**0.58**	**18.36**
Arka Sambhram	**0.53**	**0.57**	**7.54**
Mean	**0.56**	**0.64**	
	T	**V**	**T×V**
S.E.m (±)	**0.003**	**0.006**	**0.009**
CD(0.05)	**0.007**	**0.017**	**0.024**
CV= 2.44			

Table :7 Effect of high temperature on protein content (mg/gm) of exposeddolichos seedlings

Genotypes	Control	Induced	Percentage decrease over control
Lignosus	**3.19**	**1.28**	**59.87**
IIHR-B-DB11	**2.38**	**1.75**	**26.47**
IIHR-B-DB65	**3.78**	**1.43**	**62.16**
IIHR-B-DB115	**4.12**	**2.13**	**48.30**
IIHR-B-DB25	**6.48**	**5.80**	**10.49**
IIHR-B-DB48	**2.83**	**1.40**	**50.53**
Arka Amogh	**7.05**	**6.33**	**10.21**
Arka Soumya	**7.66**	**7.15**	**6.65**
Arka Vistar	**6.72**	**6.08**	**9.52**
Arka Swagat	**3.51**	**1.98**	**43.58**
Arka Sambhram	**3.87**	**1.83**	**52.71**
Mean	**4.69**	**3.37**	
	T	**V**	**T×V**
S.E.m (±)	**0.013**	**0.029**	**0.042**
CD(0.05)	**0.036**	**0.084**	**0.113**
CV= 1.78			

Table : 8 Effect of high temperature on TSS (%/mg) of exposed dolichos seddlings

Genotypes	Control	Induuced	Percent increase over Control
Lignosus	**7.11**	**8.78**	**23.48**
IIHR-B-DB11	**8.25**	**9.48**	**14.90**
IIHR-B-DB65	**4.58**	**6.40**	**39.73**
IIHR-B-DB115	**5.21**	**7.39**	**41.84**
IIHR-B-DB25	**6.34**	**9.25**	**45.89**
IIHR-B-DB48	**4.59**	**6.23**	**35.72**
Arka Amogh	**7.15**	**10.44**	**46.01**
Arka Soumya	**6.67**	**11.09**	**66.26**
Arka Vistar	**6.33**	**9.93**	**56.87**
Arka Swagat	**8.74**	**9.19**	**5.03**
Arka Sambhram	**5.54**	**7.51**	**35.55**
Mean	**6.15**	**9.46**	
	T	**V**	**T×V**
S.E.m(±)	**0.04**	**0.10**	**0.15**
CD (0.05)	**0.13**	**0.31**	**0.44**
CV= 3.54			

Effect of high temperature on Antioxidant Enzyme Activities

Catalase Acctivity

Catalase scavenge hydrogen peroxide by breaking it down directly to form water and oxygen. The activity of catalase enzyme was recorded during the experiment period was presented in the (Table-9) which indicated that the catalase activity per minute was high in all the treated genotypes over control genotypes. The catalase activity was found significant among the variety,treatment and their interaction effect. Arka Soumya was found with highest catalase activity of 10.59 mol μA/mg protein/min followed by Arka Amogh (8.56 mol μA/gm protein/minute), Arka Vistar (8.49 mol μA/ mg protein/minute) and IIHR-B-DB25 (6.88 mol μA/mg protein/minute). Lowest catalase activity was recored in IIHR-B-DB48 in control as well as treated. However highest percent increase of catalase activity in induced seedling was recorded in Arka Soumya (62.42%) and lowest in Arka Swagat (14.31%).

Peroxidase Activity

The antioxidant enzyme peroxidase (POX) also scavenge hydrogen peroxidase. The maintenance of higher peroxidase activity provides more oxidative protection through detoxification of hydrogen peroxidase. The exposure of dolichos seedlings to high temperature, the activity of peroxidase enzyme was recorded during the experiment period was presented in the (Table-10) which indicated that the peroxidase activity per minute was high over the control dolichos genotypes. The peroxidase activity was found significant among the variety,treatment and their interaction effect. Variety Arka Soumya accumulated high peroxidase concentration of 10.26 mol μA/mg protein/min followed by Arka Vistar (9.29 mol μA/mg protein/minute), Arka Amogh (9.23mol μA/mg protein/minute) and IIHR-B-DB25 (8.46 mol μA/mg protein/minute) genotypes. Susceptible genotype Arka Sambhram recorded lowest peroxidase activity (3.84mol μA/mg protein/minute) 3 days after the high temperature stress. However, highest percent increase in peroxidase activity in treated seedlings was found in Arka Soumya (64.68%), followed by Arka Vistar and lowest in IIHR-B-DB48 (15.71%) followed by IIHR-B-DB11 (17.66%).

Table : 9 Effect of high temperature on catalase enzyme activity (μ mol/mg protein/ minute) of TIR exposed different dolichos seedlings

Genotypes	Control	Induced	Percentage increase over control
Lignosus	**3.46**	**4.11**	**18.78**
IIHR-B-DB11	**2.61**	**3.42**	**31.03**
IIHR-B-DB65	**4.47**	**5.62**	**25.72**
IIHR-B-DB115	**5.14**	**5.99**	**16.63**
IIHR-B-DB25	**5.35**	**6.88**	**28.59**
IIHR-B-DB48	**2.31**	**3.06**	**32.46**
Arka Amogh	**6.29**	**8.56**	**36.08**
Arka Soumya	**6.52**	**10.59**	**62.42**
Arka Vistar	**6.05**	**8.49**	**40.33**
Arka Swagat	**5.73**	**6.55**	**14.31**
Arka Sambhram	**3.37**	**4.18**	**24.03**
Mean	**4.66**	**6.13**	
	T	**V**	**T ×V**
S.E.m (±)	**0.037**	**0.087**	**0.123**
CD(0.05)	**0.105**	**0.247**	**0.349**
CV= 3.93			

Table :10 Effect of high temperature on peroxidase enzyme activity (µ mol/mg protein /minute) of TIR exposed dolichos seedlings

Genotypes	Control	Induced	Percentage increase over control
Lignosus	**3.43**	**4.30**	**25.36**
IIHR-B-DB11	**5.32**	**6.26**	**17.66**
IIHR-B-DB65	**2.91**	**4.32**	**48.43**
IIHR-B-DB115	**5.20**	**6.17**	**18.65**
IIHR-B-DB25	**5.62**	**8.46**	**50.53**
IIHR-B-DB48	**3.50**	**4.05**	**15.71**
Arka Amogh	**6.39**	**9.23**	**44.44**
Arka Soumya	**6.23**	**10.26**	**64.68**
Arka Vistar	**6.15**	**9.29**	**51.05**
Arka Swagat	**3.28**	**4.13**	**25.91**
Arka Sambhram	**2.81**	**3.84**	**36.65**
Mean	**4.62**	**6.39**	
	T	**V**	**T×V**
S.E.m (±)	**0.023**	**0.053**	**0.075**
CD(0.05)	**0.064**	**0.151**	**0.213**
CV =2.35			

Ascorbate(AsA)

Results presented in the (Table-11) showed the effect of high temperature on ascorbate content (µ mol/gm) of the leaves. The ascorbate contents were increased in all the treated genotypes of dolichos over contol. The AsA content was found significant among the variety, tretment and their interaction. Variety Arka Soumya accumulated high amount of Ascorbate (66.31µ mol/gm) followed by Arka Vistar (58.79µ mol/gm) , Arka Amogh (57.79 µ mol/gm) and IIHR-D-DB25 (55.95 µ mol/gm) in induced seedlings. Lowest ascorbate content was recorded in Arka Sambharam in both control (39.02 µ mol/gm) and induced (43.22µ mol/gm). However highest percent increase of ascorbate content in induced seedling was recorded in Arka Soumya (41.53%) followed by IIHR-B-DB25 (36.03%), Arka Amogh (31.64%) and Arka Vistar (30.87%) while lowest in Arka Swagat (10.69%).

Glutathione(GSH)

Results depicted in the (Table-12) showed the effect of high temperature on Glutathione (µ mol/gm) of the leaves. A small increase in GSH content in all the treated genotypes over their control. Arka Soumya found highest GSH accumulation (381.0 µ mol/gm) followed by Arka Amogh (372µ mol/gm), Arka Visatar (349.2µ mol/gm) and IIHR-B-DB25 (349.2µ mol/gm). Lowest GSH was recorded in IIHR-B-

DB65. However highest percent increase of GSH content in induced seedlings was recorded in Arka Soumya (20.59%) followed by Arka Amogh(19.27%), and lowest in IIHR-B-DB11**5** (2.41%).

Table : 11 Effect of high temperature on ascorbate content (µ mol/gm) of TIR exposed dolichos seedlings

Genotypes	Control	Induced	Percentage increase over control
Ligosus	**38.63**	**47.59**	**23.19**
IIHR-B-DB11	**40.53**	**50.26**	**24.00**
IIHR-B-DB65	**44.11**	**53.70**	**21.74**
IIHR-B-DB115	**42.82**	**51.86**	**21.11**
IIHR-B-DB25	**41.13**	**55.95**	**36.03**
IIHR-B-DB48	**43.34**	**49.64**	**14.35**
Arka Amogh	**43.90**	**57.79**	**31.64**
Arka Soumya	**46.85**	**66.31**	**41.53**
Arka Vistar	**44.92**	**58.79**	**30.87**
Arka Swagat	**42.16**	**46.67**	**10.69**
Arka Sambhram	**39.02**	**43.22**	**10.76**
Mean	**42.49**	**52.88**	
	T	**V**	**T×V**
S.Em (±)	**0.108**	**0.253**	**0.358**
CD(0.05)	**0.116**	**0.725**	**1.027**
CV= 1.30			

Table :12 Effect of high temperature on glutathione content (µ mol/gm) of TIR exposed dolichos seedlings

Genotypes	Control	Induced	percent increase over control
Lignosus	**311.28**	**332.21**	**6.72**
IIHR-B-DB11	**304.52**	**319.00**	**4.75**
IIHR-B-DB65	**318.69**	**327.93**	**2.89**
IIHR-B-DB115	**328.44**	**336.37**	**2.41**
IIHR-B-DB25	**318.84**	**349.26**	**9.54**
IIHR-B-DB48	**307.39**	**330.80**	**7.61**
Arka Amogh	**312.64**	**372.91**	**19.27**
Arka Soumya	**316.66**	**381.78**	**20.56**
Arka Vistar	**320.21**	**349.29**	**9.08**
Arka Swagat	**324.48**	**339.90**	**4.75**
Arka Sambhram	**319.80**	**348.38**	**8.93**
Mean	**316.63**	**348.43**	
	T	**V**	**T×V**
S.E.m (±)	**1.99**	**1.06**	**2.81**
CD (0.05)	**4.08**	**2.18**	**NS**
CV = 7.21			

DISCUSSION

Dolichos lablab L. is a cool season crop sensitive to high (>30◦C) temperature which was found to have negative effects on plant growth, devlopment and grain yeild (Summerfield *et al.* ;2004). Present experiment on dolichos summerized the adverse effects of high temperature on early vagetative phase and screened out the thermo tolerant genotypes among the experimented genotypes based on the TIR response (Kumar *et al.* 2012 ;Sapnaharihar *et al.* ;2014 ;Senthil Kumar *et al.* 2001). High temperature induce oxidative stress by producing reactive oxygen species (ROS) and further aggravate the damage to membranes, protein and nucleic acids (Suzuki and Mittler, 2006). Plants defend themselves from heat stress by producing heat shock proteins, antioxidants, secondary metabolites and osmolytes. The interactive functioning among these molecules in response to heat stress is least understood. An attempt has been made to study the high temperature mediated physiological and biochemical responses of 11 dolichos bean genotypes and to identified the thermotolerance genotypes. Silent points of the results obtained in the present invesigation are discussed below in the light of available literature.

Intrinsic thermotolerance genotypes among dolichos genotypes

Among all the genotypes (5 photo-insensitive and 6 photosensitive) when subjected to their lethal temperature only 4 genotypes are able to recoved. Arka Soumya showed the highest recoverd percentage and least reduction in root and shoot growth followed by Arka Vistar, Arka Amogh in survived percent and Arka Amogh and Arka Vistar in reduction of root and shoot.IIHR-B-DB25 is the only photosensitive genotypes was recovered (42.96%) and (20.57%) reduction in root and shoot length. Thus Arka Soumya, Arka Vistar, Arka Amogh and IIHR-B-DB25 were identified as thermotolerant genotypes.

TIR response technique has been devloped to identify and select the thermotolrerant genotypes. It involves exposing seedlings or plants to induce stress and subsequently challenging severe temperature and selecting the surviving seedlings at the end of the recovery period. By adopting the TIR technique,the existence of significant genetic variability has been demonstrated across the genotypes of pea (Kumar *et al. ;*2012). The thermotolerant lines from the sunflower

were identifiedin open pollinated population (Charng *et al.* (2006), Gangappa *et al.* (2006) in groundnut.

Biochemical and physiological responses to high temperature stress

Proline acts as a low molecular chaperon and osmoprotectant (Gupta *et al.*, 2013). Proline helps in maintaining the membrane intigrity,stabilization and protection of protein and enzyme structure and scavenging ROS. All the exposed genotypes to high temperature accumulate higher proline over control (Kavi Kishore *et al.* 2005; Trovovato *et al ;*2008). Proline content in dolichos leaves was found significantly higher in treated than the control. However Arka Soumya accumulated higher percentage of proline over control followed by Arka Vistar, Arka Amogh and IIHR-B-DB25. Babu and Devraj (2008) also reported significant increase of proline content in french bean leaves under high temperature stress (46-48^{0}C). Khetarpal *et al.* (2009) also reported that under high temperature stress the tolerant chickpea Pusa1108 maintained higher proline accumulation compared to that of susceptible genotype Pusa 1053. Miller *et al.* (2009) also reported that osmolyte proline accumulated higher in the tolerance genotypes of bean than the susceptible genotypes. Randeva and Ilieva (1998) reported in increase in free proline content in pea leaves in response to high temperature stress.

Accumulation of high amount of TSS under heat stress has been implicated in the establishment and maintenance of thermotolerant in plants (Wahid and Close *;*2007). Temperature tolerant genotypes Arka Soumya, Arka Vistar, Arka Amogh and IIHR-B-DB25 exhibited higher TSS. Babu and Devraj (2008) also reported significant increase in total soluble sugar content in french bean that under the high temperature stress (46^{0}C-48^{0}C). Sugar serves as signalling molecules during heat stress in stress tolerant phenotypes (*Rosa et al.* ;2009*).* Greer and Weston (2010) reported that when dolishos seedlings (Lablab purpureus) subjected to heat stress in the thermotolerant genotypes higher accumulation of TSS then the susceptible one.

Gupta *et al.* (2005) have observed a decrease in protein content in the leaves of chickpea due to heat stress. Similarly decrease in soluble protein in french beans leaves in response to high temperature stress (Gorbanli *et al.* ;2007). The results obtained in the present study are contradictory to these reports. The cultiver Arka Soumya was found with least decrease in protein content over its control followed by

Arka Vistar, Arka Amogh and IIHR-B-DB25. While the susceptible genotypes recorded with higher percentage of protein decrease. IIHR-B-DB65 recorded with highest decrease in protein content.

Effect of High temperture on Enzymatic activity

High temperature stress induced production of reactive oxygen species(ROS) and concominant oxidative damesges (Kumar *et al.* 2012) include damage to proteins and nucleic acids. It has been postulated that in leaves,the specific activity of CAT and POX increase on with increase in high temperature which increase in reactive oxygen species generation.

In the present study its was observed that the activity of CAT and POX increased in the leaves of all the treated genotypes finding Arka Soumya highest CAT activity followed by Arka Amogh, Arka Vistar and IIHR-B-DB25 (Babu and Devraj, 2008 ;Kumar *et al.* 2012; Sairam *et al.*1998) indicating higher production of reactive oxygen radicals under high teperature stress. Similar trend of increase in catalase activity was demonostrated in bean (Cao *et al.* 2009).

The POX activity has also increased in similar trend that expose to high temperature (Zhang and Kirkham, 1994). Babu *et al.* (2007) found a significant increase in the activity of peroxidase under high temperature stress (46^0C-48^0C) in french beans. Mohammadi *et al.* (2011) reported higher antioxidant enzyme activity under heat stress condition in chickpea. In this present experiment treated dolichos genotypes observed an increase in peroxidase activity over their control (Kumar *et al.* 2011). It is found Arka Soumya accumulated highest peroxidase (28.08%) activity followed by Arka Vistar, Arka Amogh and IIHR-B-DB25. There are numerous plant studies which indicate the tolerance to temprature stress in plants is positively correlated with increase in enzyme activity (Almeselmani *et al.* ;2009; Gill and Tuteja, 2010; Kaushal *et al.* 2011; Hasanuzzaman *et al.* 2012; Kumar *et al.* 2012).

Effect of High temperature on Non-enzymatic compounds activity

When the plant expose to higher temperature stress they organize various enzymatic defense mechanism to overcome, minimize the deleterious effects of ROS which include the enzymes such as SOD, CAT, POX, APX, GR (Keles and Oncel, 2002). Along with the formation of enzymatic antioxidants various non-enzymatic

compounds like ascorbate, glutathione (GSH) and carotenoids also produced during high temperature stress (*Kumar et al.* 2012).

In this present experiment all the dolichos genotypes that were exposed to high temperature showing small increase in ascorbate and GSH activity over control (Wang *et al.* 2008). The GSH is also important because higher GSH content is an indication for better tolerance to stress (Mafakheri *et al.* ;2011).

Under high temperature stress,it is observed that GHS content is markedly increased over control genotypes. The Arka Soumya genotypes accumulated higher amount of GSH followed by Ark Amogh, Arka Vistar and IIHR-B-DB25 over their control. The similar trend of increase in GSH activity was demonostrated in chickpea (Gur *et al.* 2010). Kaushal *et al.* (2011) reported that significant increase in glutathione content under high temperature stress in french bean.

Kaushal*et al.* (2011) reported that under high temperature stress ascorbate accumulation increased in french beans upto 50%. In this present study ascorbate content increased up to 41.53% in Arka Soumya seedlings when subjected to high temperature stress followed by IIHR-B-DB25, Arka Amogh and Arka Vistar. Arka Swagat found with lowest % increase in ascorbate content 3 days after heat treatment. Similarly under high temperature stress ascorbate content increased in thermotolerant genotype of chickpea (Gur *et al.*, 2011).

SUMMARY AND CONCLUSION

Hyacinth bean (*Dolichos lablab* L.) is a minor legume belong to leguminoceae family cultivated in winter mainly. It is used for multiple purpose like pods, dried seeds, forage and fodder crop. It has a special economic importance for its high proteineous and medicinal value. Due to climate change, high temperature stress is a serious problem now a days. To over come the temperature stress during growing period, thermotolerant genotypes are to be evaluated for thermotolerance.

The major emphasis of the present study was to screen dolichos genotypes for thermotolerance by using TIR technique and to understand the physiological and biochemical variation in the tolerance. The induction response in the seedling was assessed by recovery growth and seedling survival after subjecting to different induction temperatures followed by lethal temperature. Lethal temperature was standardized as 50^0C and optimum induction temperature was found 36^0C -50^0C. Adopting the TIR technique, 11 dolichos genotypes were screened for thermotolerance by assessing the recovery growth. Out of 11 genotypes, 4 genotypes are screened as tolerant and 7 genotypes as susceptible. Variation in the recovery growth among these genotypes was assessed by biochemical and enzymatic activity studies.

Three days old dolichos seedlings that undergo high temperature stress were all died except 4 genotypes. Although the died genotypes showed increse in accumulation of osmolytes proline, total soluble sugar, high catalase and peroxidase activity but simultaniously showed a drastic morphological changes in higher root and shoot growth reduction, reduced seedling weight, zero recovery and decrease in protein content. Cultivars Arka Soumya, Arka Vistar, Arka Amogh and IIHR-B-DB25 showed higher recovery percentage, lowest root and shoot reduction and seedling weight .

Stress indicator proline was also found increase in these 4 genotypes over their control. Arka soumya accumulated highest proline content under high temperature stress. Enzymatic activity of catalase (upto 63%) and peroxidase also increased (upto 65%) in these themo tolerant genotypes. Accumulation of non-enzymaitc compounds like ascorbate and glutathione increased then the contol genotypes after the treatment of high temperature stress.

CONCLUSION

On the basis of results of the present investigation, the following conclusions may be drawn.

High temperature stress has a very detrimental effect on growth and devlopment of dolichos bean during the vagetative growth period. There was a great reduction in seedling height, fresh weight, root and shoot length and survival percentage. However increase in CAT and POX activity, higher proline,soluble sugar content to several fold was found. TIR technique is a robust and constructive technique to identify genetic variability in thermotolerance for dolichos within a short period of time. Irrespective of all adversities the dolichos geotypes i.e Arka Soumya, Arka vistar, Arka Amogh and IIHR-B-DB25 have the ability to with stand the temperature stress condition during the early vagetative period than others and considered as thermotolerant genotypes due to higher survival %, low reduction in shoot and root length, seedling weight and increase in accumulation of proline, TSS, CAT, POX activity and glutathione content in induced condition. The photo-insensitive cultivars Arka Soumya and Arka Vistar shown higher thermotolerance and may be recommend for summer season cultivation after stress response validation in reproductive stage of plant while genotype IIHR-B-DB25 and Arka Amogh shown medium thermotolerance. The genotype IIHR-B-DB25, a photo sensitive but potential in terms of economic yield may be recommended for donor parent in improvement programmes of hyacinth bean after stability analysis.

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