Screening and identification of black gram(*Vigna mungo* (L.)) genotypes under cold stress

A

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In Partial fulfilment of the Requirements

for the degree of Master of Science in Agriculture

(Agricultural Biotechnology)

By

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ANTI-PLAGIARISM CERTIFICATE

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This is to certify that the thesis entitled **"Screening and Identification of blackgram genotypes under cold stress"** submitted by **Miss Dipsikha Sahoo**, Master of Science in Agriculture (Agricultural Biotechnology) bearing Adm. No. 18122B05 is plagiarism checked and has not crossed the limit as per the Anti-Plagiarism Policy of OUAT.

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CERTIFICATE –I

This is to certify that the thesis entitled **" Screening and identification of black gram (*Vigna mungo* (L.)) genotypes under cold stress "** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (AGRICULTURAL BIOTECHNOLOGY**) to the Orissa University of Agriculture and Technology is an authentic record of *bona fide* research work carried out by DIPSIKHA SAHOO(Adm. No. 18122B05) under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma .

It is further certified that the evidence and help obtained 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 " **Screening and identification of Black gram*(Vigna mungo* (L.)) genotypes under cold stress" submitted by **DIPSIKHA SAHOO(Adm. No. 18122B05)** to Orissa University of Agriculture and Technology , Bhubaneswar in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Agricultural Biotechnology)** has been approved by the students' Advisory Committee and the external examiner.

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ABSTRACT

Cold stress is one of the major abiotic stress factors that reduces the yield and productivity of pulses . Black gram is an important pulse crop that is susceptible to cold stress which in turn affects it's productivity in India. Hence , there is a need for identification of black gram genotypes that are tolerant to cold stress and can give it's full inherent yield potential by minimising the effect of cold stress. Present study was carried out using 34 genotypes of black gram (*Vigna mungo* (L.)) to study their physiological, morphological and biochemical characteristics in relation to cold tolerance . Cold screening of 34 black gram genotypes at 10 degree classified the genotypes into 3 categories resistant, moderately resistant and susceptible . Among these, the best resistant and highest susceptible ones are used for polybag experiment. Various morphological characters were studied during their growth period in the natural environment. After 15 days , the 1st cold stress was given for 3 days and then the biochemical analysis was planned to be carried out both for cold stressed and control plants. Unfortunately due to shut down of university , I was unable to conduct biochemical analysis . But on the basis of screening parameters(germination percentage, germination index , root and shoot length characteristics) and morphological observations, the genotypes KVK Nayagarh 3 , C3PU31 are resistant and C2Ujala , C4OBG 31 are susceptible to 10 degree centigrade temperature.

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LIST OF ABBREVIATIONS

µg = Microgram

µl = Microlitre

Mm = Millimolar

Pro = Proline

TSC = Total soluble carbohydrate

CAT = catalase

Chl = Chlorophyll

SDS = Sodium dodecyl sulphate

APS = Ammonium persulfate

ABREs = ABA-responsive elements

CDPKs =Calcium dependent protein kinases

CBF = C-repeat - binding factors

COR = Cold responsive

DREB = Dehydration responsive element

DAS = Days after sowing

INTRODUCTION

Pulses constitute an important part of human 1diet and are hence grown primarily for human consumption , besides human consumption also used as livestock forage and silage and also as green manure that enhances soil health . According to Food and Agriculture Organisation, legume crops that are harvested solely for dry seed are given the term ***pulse.*** Since most of the pulses are legumes , they undergo biological nitrogen fixation due to presence of root nodules and hence play an important role in crop rotation.

Black gram (*Vigna mungo*) also known as urd bean is an important pulse crop of India. It is a leguminous plant and generally sown in almost all districts of Odisha. It is a highly nutritious crop as it is rich in proteins(around 25g/100g),vitamins and minerals. It is often choosen as a fallow crop after rice cultivation. Among the pulse crops grown in Odisha , black gram contributes around 27% to the total pulse area and it's production share is around 25%.

Cold stress is one of the primary abiotic stresses that puts adverse effect on productivity of crop,quality and post harvest life depending upon the severity degree , stage of growth and duration of exposure. Every individual plant passes through various growth stages and each stage of plant is completed under unique set of temperatures for proper development. Lowering of temperature thermodynamically reduces the kinetics of metabolic reactions. Different symptoms shown by plants in response to chilling stress such as dessication, osmotic inbalance ,discoloration, tissue breakdown, accelerated senescence ,ethylene production ,shortening of life span and undergoing decay at a faster rate due to leakage of plant metabolites.(sharma et al. 2005) due to loss of membrane permeability. Chilling has also been known to cause DNA strands disruption , lowered enzymatic activity and specificity, membrane rigidification ,destabilisation of protein complexes, stabilisation , destruction of photosynthetic pigments of RNA secondary structures, production and accumulation of reactive oxygen intermediates(ROIs) ,destruction of photosynthetic pigments and leakage across membranes(Nayyar et al .2005). Formation of reactive oxygen species (ROS) during temperature stress also causes disruption in the flow of electrons in eletron transfer chains resulting in disturbance of the redox potential of various metabolic pathways. Thus ROS formation causes injuries of cells and apoptopsis resulting in death of plant due to impairment of photosystem II reaction center and membrane lipids(Suzuki and Mittler 2006). Antifreeze proteins(AFPs) , dehydrins(DHN),late embryogenesis abundant proteins(LEA) ,heat shock proteins(HSPs), chaperonins, pathogen related proteins and those related

to transduction, transcription regulation and signalling pathways are the types of proteins expressed in response to cold stress.

The production , productivity and yield components of black gram are greatly hampered by various biotic and abiotic stress conditions. Among the abiotic stress factors , low temperature or chilling or cold stress is highly detrimental for growth and development of the crop which reduces it's yield significantly. So there is a need to identify cold tolerant genotypes of black gram which can survive under cold stress conditions without affecting the yield . Cold stress is a type of abiotic stress in which the temperature is too low for the normal growth and development of plant .It generally occurs in tropical and subtropical crop species. Cold tolerant genotypes are those which can grow normally under cold stress conditions without affecting the yield or other parameters.Research is carried out with the following objectives :

1) Screening of 34 germplasms .

2) Physiological & Biochemical analysis of tolerant and non-tolerant genotypes

3) Yield attribute analysis of tolerant and non-tolerant genotypes .

REVIEW OF LITERATURE

Boyer (1982) and Wang et al(2003) reported that stress exposure is the major limiting factor to agricultural productivity, where more than 50 % yield loss in crops is attributed to abiotic stress. Sanghera et al (2011) stated that agricultural expansion and crop productivity in hilly terrains is limited by the major environmental factor that is cold stress. Thomashow (1999) reported that both chilling and freezing can have harmful effect on plant functions. Lukatkin et al (2012) found that prolonged exposure of plant species which are sensitive to chilling stress results in tissue necrosis or plant death. Osmond et al (1987)and Kranner et al (2010) reported that when a plant's environment prevents it from achieving it's full genotypic potential ,then the plant is said to be under stress. Qin et al (2011) reported that to overcome stress ,in accordance with environmental change plants must also alter their biological processes. Wani et al (2016) reported that plant growth physiology is detoriated by non-freezing low temperatures by the induction of chilling injuries like photosynthesis-associated damages, chlorosis, unregulated apoptosis, loss of membrane fluidity and ultimately wilting. Wani et al (2013) reported that depending on the extent of sensitivity among plants, cold 3stress is sub -divided into two types. Chilling stress is characterized by 0-15 degree centigrade whereas freezing stress is caused by temperature below 0 degree centigrade. Yamaguchi-Shinozaki and Shinozaki (2006) stated that By virtue of cold acclimation and associated alterations at the molecular and biochemical levels, plants of temperate climate exhibit greater ranges of cold tolerance compared to their tropical and sub- tropical counterparts. Rikin and Richmond (1976),Ciardi et al (1997) and Morgan and Drew (1997) stated that cold tolerance mechanism involves a series of biochemical and physiological changes that leads to increase in ABA ,alteration in lipid composition in cell membrane by Graham and Patterson (1982) ,Murata (1983) and Tasaka et al (1990) .According to McKhann et al (2008) ,Plant's capacity for cold acclimation determines their survival in freezing temperatures. Dahal et al (2012)reported that there exists a significant correlation between accumulation of sugars and development of cold tolerance in plants. Levitt in 1980 stated that cold acclimation is characterized by extensive physiological and biological changes which starts with reduction in the growth rate and water content of various plant tissues. Minami et al (2009) ,Takahashi et al (2013)stated that many proteins such as P-type ATPases, aquaporins and tubulins tend to accumulate under low temperature treatment . Gery et al (2011) reported that *Arabidopsis* plant has been used in majority of studies

involving improvement of cold tolerance of plants. Li et al (2012) stated that for the investigation of molecular mechanism of low -temperature tolerance in core Pooideae species, *Brachypodium distachyon* can be an important model since it has cold responsive IRIP. Seki et al (2002) reported that low temperature sensitive genes are classified into two main groups: first group involves LEA proteins(Cushman & Bohnert 2000) that respond to cold stress and second group involves protein having role in regulation of gene expression under cold stress conditions. Mizoi et al (2012) stated that the expression of COR genes is induced by CBF gene that play an important role in plant cold tolerance improvement. Zarka et al .(2003) reported that there exists a significant co-relation between the expression of CBF/DREB genes and change in temperature that is with the decrease in temperature, the expression of these genes increases. Tyystjarvi (2013) stated that ROS acts as an important secondary messengers that responds to various abiotic stresses. Rao et al (2006) reported that an oxidative burst is caused by abiotic stresses and an increase in calcium ion influx into the cytoplasm is induced by a low level of ROS. Ton et al.(2009) reported that several physiological ,molecular and developmental progressions occur in plants that results in adaptation of plants to the stress environment. Lee and Luan (2012) reported that ABA synthesis is induced by abiotic stresses that results in activation of expression of stress-related genes and closure of stomata. Guy(1990) reported that on the basis of cooling conditions, Ice formation can be either intracellular or extracellular. Guy et al (1998) reported that due to low temperature, protein denaturation occurs that results in cellular damage. According to Takahashi et al (2013) , one of the most important adaptation mechanisms for plant cold tolerance is the alteration of plasma membrane compositions and functions during cold acclimation. Xin and Browse (2000) reported that cold acclimation is a process in which plants are acclimatized to the low growth temperatures due to a series of events making them more freezing tolerant. According to Hughes and Dunn (1996),cold stress protein expression is involved in cold acclimation. Strand et al in 2003 found that sucrose is accumulated during cold acclimation. Xin and Browse (1998) ,Wanner and Junttila (1999) ,Bravo et al (2001) reported that cryoprotectans like proline are also accumulated under cold acclimation. Castonguay and Nadeau (1998) reported that carbohydrate metabolism is affected due to low temperatures during autumn. Dalmanndottir et al (2001) reported that lipid metabolism is also affected due to low temperatures during autumn. Bravo et al (2001) and Larcher (2003) reported that Thermal analysis is a good method for finding ice formation dynamics in plant tissues. According to Lejeune-Henaut et al in 1999,after the commencement of floral initiation in plants,they become more susceptible

to frost. In contrast, Bourion et al (2003) ,indicated the most freezing sensitive stage in plants is the stage before floral initiation. Stoddard et al (2006) , found that abiotic stress studies in plants such as frost testing and screening on the basis of plant physiology changes are quite difficult to carry out due to unpredictability of severity of abiotic stresses in field experiments. Margesin et al (2007) reported that during cold acclimation ,photosynthesis rate decreases that results in reduction in plant growth and water contents in tissues and solute accumulation . Siddique et al (1999) reported that flowering,early pod formation and seed filling stages are the most susceptible stages in legumes. Margesin et al (2007) reported that plants suffering damage when exposed to low and non-freezing temperature between 0-12 degree centigrade are termed as chilling sensitive plants whereas plants from high tropical mountains are frost resistant. Choudhary (2013) and Pooniya et al (2015) reported that the gaps in yield in pulses at research farms and farmer's field varied to the extent of 368-492 kg/ha in urdbean,220-417 kg/ha in kidney bean,477-563kg/ha in pigeon pea,372-494 kg/ha in cowpea,225-601kg/ha in chickpea and 253-510 kg/ha in lentil. Knight et al (1996) reported that in *Arabidopsis thaliana,*various genes and factors have been identified which are dependent on Ca2+ signal transduction via kinases. Seki et al(2002) reported that plant's tolerance to abiotic stresses is not a function of one gene infact it is a coordinated action of a number of genes corresponding to enzymes with multiple metabolic functions. Prasad et al (1994) reported that cold stress leads to oxidative stress in plants due to elevated levels of reactive oxygen species that results in chilling damage. Dat et al (2000) and Gechev et al (2006) reported that during cold and other unfavorable environmental conditions ,ROS accumulation is observed. Morsey et al (2007) reported that an effective ROS scavenging system is possessed by cold stress tolerant rice genotypes than the susceptible genotypes of rice plants. Croser et al (2003) and Clark et al(2005) reported that at the vegetative stage, low temperature had an appreciable result on plant growth and dry matter production whereas there is abortion of flowers and pods, infertile pods and small shriveled seeds at the reproductive stage resulting in reduced seed yield. According to Kaur et al 2009, more number of pods per plant, green pods per plant ,less damaged pods and very few black spots on grains are observed in cold tolerant genotypes during cold stress whereas all pale and damaged pods and blackened seeds were found in susceptible genotypes under cold stress. According to Lee et al 2007,for improved tolerance to abiotic stresses and increased antioxidative defense ,over-expression of SOD and APX genes which are utilized in scavenging ROS reported to be one of the mechanisms involved in this. According to Pearce (2001) ,growth and development of agronomic species throughout the world is affected by

low temperature. Pearce & Fuller (2001) reported that the geographical distribution of plant species gets limited and the yield of several crops around the world significantly decreases due to low temperature . Stushnoff et al (1984) reported that according to low temperature tolerance, plants can be classified into three different classes. Knight and Knight (2000) reported that from extracellular and intracellular calcium stores, the free cytoplasm Ca2+ comes that is observed under low temperature. Marc et al (2010) reported that stoichiometry of lipids initiates physical changes in membrane fluidity that triggers calcium influx into the cell. Miura and Furumoto (2013) found that after cold treatment, Nuclear Ca2+ increases and in comparison to the peak of cytosolic calcium, the maximum increase is delayed at 5-10 s. Mauger (2012) found that nuclear envelope leads to an increase in nuclear Ca2+ and is considered as the major store of Ca2+ . Zhou et al (2008) ,Zhang et al in 2009 and Lv et (2010) reported that in different plant species, many cold regulated miRNAs have been found to be utilizing bioinformatics, cloning and sequencing tools. According to Miura and Furumoto (2013) , under low temperature, very little is known about the mi target genes that are not regulated. Schwender et al (2004) and Fernie et al (2005) reported that plant regulatory mechanisms become active and function to restore normal metabolic levels most importantly metabolic fluxes under temperature stress. Steponkus (1984) and Steponkus et al (1993) reported that plasma membrane damage is the adverse effect of cold stress in plants which is due to cold stress -induced dehydration .Steponkus et al (1993) reported that relative proportion of saturated and unsaturated fatty acids in the lipids of plasma membrane determines the fluidity of membrane . Meijer and Munnik (2003) found that phosphatidic acid is transiently and rapidly generated in response to various stresses in response to various stresses and functions as a second messenger. Laloi et al (2004) and Mahajan and Tuteja (2005) reported that phospholipase D is linked with Reactive Oxygen Species that are involved in abscisic acid and in cold stress responses. Katagiri et al (2001) reported that abscisic acid and cold in Arabidopsis plants induces the AtPLDd expression. According to Xiong et al (2001), a significant reduction in the expression of osmotic stress induced genes and cold is shown by los5 mutant plants. Fowler and Thomashow (2002) reported that C-repeat binding factors induces cold responsive genes approximately 12 % in *Arabidopsis* . Arbaoui et al (2008 a) reported that as the physiological and molecular mechanisms have considerable importance ,so there is a need for biochemical selection criteria for cold or frost resistance. Arbaoui and Link (2008) reported that frost tolerance in faba bean is associated with the fatty acid composition. Herzog (1987) ,(1989) ,Badaruddin and Meyer (2001) ,for evaluation of cold tolerance in faba bean, several non-destructive methods such as visual

scoring of freezing injuries in leaves ,through measurement of leaf conductivity by Herzog in 1987 or by evaluation of chlorophyll fluorescence by Herzog and Olszewski (1998) . Toker et al (2007) reported that cold related stress can be defined either in terms of chilling(0-12 degree centigrade) or freezing (below 0 degree centigrade) without snow cover. According to Toker 2005 and Toker et al (2007) ,for both cool season food legumes and their wild relatives ,improvement of cold tolerance is of utmost importance. According to Bond et al (1994) and Arbaoui and Link (2008) , the main component for winter growing is cold tolerance . Mc William and Naylor (1967) reported that between 10-15 degree centigrade temperature ran7ge , newly formed chlorophyll gets destroyed due to rapid photoxidation before complexing in the chloroplast lamellae . Taylor and Craig (1971) found that high light intensities amplifies photo-oxidation. Van Hasselt and van Berlo (1980),Baker et al (1983),Wise and Naylor (1987) ,Smillie et al (1987) reported that the effects are more pronounced when the temperature is lower than 10 degree centigrade because, despite of protective action of carotenoids ,the membrane bound chlorophyll gets destroyed by the free radicals of oxygen. Miedema et al (1987) reported that metabolic and respiratory processes are greatly affected in addition to effect on chlorophyll. Popelka et al (2004) stated that preceded by cereals and oilseeds, legumes ranks third in it's production. Legumes contribute approximately 27 % of total crop production according to Graham and Vance (2003) .

Cell membrane

Jewell et al (2010) reported that membrane become more static under low temperatures thereby reduction in fluidity occurs resulting in rigidity of membrane and it may become non-functional that is loses it's function. Chang et al (2001) reported that an irreversible chilling injury is observed on five-day old seedlings when subjected to low temperature that is concluded from increased electrolyte leakage.

Photosynthesis and respiration

Hikosaka et al (2006) stated that photosynthesis rate varies in different species of plants and is dependent upon temperature. Aro et al (1990) reported that the activity of ribulose activase enzyme is affected under low temperature, large and small subunits of rubisco's availability changes and PS-II oxygen evolving complex also disrupts and structure and functioning of D1 and D2 polypeptides of PS-II damages under low temperature. Smillie and Hetherington (1984) reported that low temperature affects photosynthesis and it directly influences performance of photosystem and activities of photosynthetic apparatus. According

to Krause (1994) ,the efficiency of photosynthetic electron transport in plant is decreased under cold stress resulting in an excessive energy generation and trigger of photoinhibition. Kee et al (1986) reported that there is involvement of the whole electron transport chain and photochemistry catalyzed by PS II in cold induced inhibition. Chen et al (2013) reported that when plants are subjected to conditions of photo inhibition, reactive oxygen species (ROS) are formed that results in serious injury of components of PS II and the extent of damage depends on the balance between damage and repair of components of PSII.

ABA (Abscisic acid)

Zhou and Guo (2005) reported that the ABA content decreases under the action of ABA biosynthesis inhibitor sodium tungstate ,sodium tungstate also inhibits the antioxidant enzyme activity induced by ABA resulting in serious chilling injury. According to Anderson et al (1994) , Zhou et al (2002) and Lu et al (2005) ,chilling resistance of crops like *Zea mays*, *Litchi chinensis* and *Eremochloa ophiruoides* is enhanced by exogenous application of ABA. According to Jiang and Zhang (2001) ,antioxidant enzymatic activity is enhanced by the treatment of ABA in various species such as *Zea mays* and according to Lin et al in 2001,application of ABA also enhances antioxidant enzymatic activities in various species such as *Oryza sativa*. According to Zhou et al .in 2005,Luet al .in 2003 ,antioxidant enzymatic activity is enhanced by ABA in S.guianensis and turfgrasses. According to Jiang and Zhang (2002 a,b) , Hu et al (2005a) and Zhou et al (2005b) , in the antioxidant enzymatic activities induced by ABA ,H2O2 and NO acts as signal molecules.

Ca2+

Krot et al (2006) reported that under stressful environmental conditions ,Ca2+ concentration in the cytosol increases .Increased Ca2+ concentration in cytosol regulates expression of gene and relative physiological and biochemical reactions according to Bush in 1995,Monroet al y and Dhindsa (1995) and Gong et al (1998) . Cousson (2007) reported that in the closing of stomata induced by ABA ,Ca2+ is involved. Pei et al (2000) and Murata et al (2001) ,cytosolic Ca2+ concentration increases by ABA by induction of Ca2+ influx from extracellular space and release of Ca2+ from intracellular stores.

Abiotic stress

Choudhary and Vijaykumar (2012) reported that breeding for abiotic stress is considered more difficult because of following reasons:-

a)complex conditions that cause abiotic stress.b)Existence of complex nature of abiotic resistance in a variety.c)Often occurrence of one stress in conjuction with other.d)Heritability of abiotic resistance is quite low.e) Intensity of abiotic stress is variable under field condition. Arora et al (2002) and Srivalli et al (2003) reported that plant metabolism is affected, cellular homeostasis is disrupted and major physiological and biochemical processes are uncoupled by abiotic stresses.

Effect of low temperature

Yong et al (2002) and Sandhu et al (2007) reported that variations in genotype for cold tolerance in pigeon pea particularly for survival traits are well documented. Choudhary (2007) reported that primary effect of low temperature is retardation of growth ,development and opening of flower buds. Andrews (1987) reported that many species of tropical origin when exposed to temperature below 20 degree centigrade suffer cold injury. According to Roy and Basu (2009) ,each plant species requires an optimum range of temperature for it's normal growth and development which varies among the genotypes within a species, the specific temperature is dependent upon state of growth and development of particular genotypes. Hedhly et al (2008) and Thakur et al (2010) reported that in a plant life cycle, among all stages, reproductive phase is the most temperature vulnerable to many external constraints.

Germination

According to Chen et al (1983) , the first 930 minutes of imbibition is the period of greatest sensitivity to cold. According to Ellis et al (1986) ,the germination rate and percentage is positively correlated with temperature under optimal temperature condition after that it becomes negatively correlated.

Vegetative phase

According to Wery (1990) ,the minimum temperature for survival of chickpea is -8 degree centigrade.

Reproductive and maturation phase

According to Varma and Kumari (1978) , sudden drop in temperature during reproductive phase results in flower and pod drop. According to saxena (1980) ,when the night

temperature falls below 10 degree centigrade,then it is the main reason behind abortion of floral parts of chickpea that is field grown.

According to Fiehn (2002) ,the end products of cellular regulatory processes are called as metabolites and their levels estimated as the ultimate response of biological systems to genetic or environmental changes. According to Stitt and Hurry (2002) ,during plant's response to abiotic stress ,small soluble metabolites such as sucrose, proline and fructan are involved. According to Levitt (1980) ,when plants are exposed to cold stress, that is when temperature is not enough to kill tissues, sugars are frequently accumulated in plants. According to Zhu et al (2007) ,three main types of metabolic signals that is ROS, soluble sugars and tetrapyrrole intermediate Mg-protoporphyrin might be pivotal for cold signalling.

Black gram

According to Lakhanpaul et al (2000) ,three times more protein is present in dry mature seeds in comparison to cereals and in a vegetarian diet, it constitutes an important source of protein. According to Sivaprakash et al (2004) ,a number of traditional land races of black gram possessing unique traits such as disease tolerance, abiotic stress tolerance and pest tolerance are cultivated in many parts of India as an intercrop in rice, sugarcane, cotton, groundnut and sorghum cultivating season.

According to Thomashow (2001) ,cold inducible genes plays a fundamental role in protecting plant cells against cellular dehydration with the CRT/DRE (C-repeat/dehydration responsive genetic element) that imparts responsiveness to both dehydration and chilling. According to Streb et al (2003) ,there was accumulation of high quantities of glucose-6-phosphate,mannose-6-phosphate,fructose-6-phosphate and sucrose in pea leaves under chilling stress. According to Busheva and Apostolova (`1997,exogenous glycine betaine application helps in preventing changes in antenna size and relative proportion of PS-II in pea during cycles of freeze-thaw. According to Welbaum et al (1997) , exogenous application of ABA improved tolerance to freezing but failed to replace cold hardiness in peas. Goodwin et al (1996) reported that there was induction of BNPRP gene in *Brassica napus* when the plants were subjected to cold stress . Castonguay et al (1994) reported that in cold tolerant alfa-alfa ,in comparison with cold sensitive plants, there was higher levels of cold regulatory gene MSACIC .

Proline

Hare and Cress (1997) , Saradhi et al (1995) and Siripornadulsil et al (2002) reported that accumulation of proline in plants occurs after low temperature, high temperature ,heavy metal ,atmospheric pollution, nutrient deficiency ,pathogen infection, anaerobiosis and UV irradiation. Hare and Cress (1997) reported that proline acts as a compatible osmolyte and is a way for storage of carbon and nitrogen. Smirnoff and Cumbes (1989),invitro studies done earlier, proved that proline can also be a ROS scavenger. According to Maggio et al (2002) , accumulation of proline is a type of adaptive response influenced by stress signal. In *Arabidopsis thaliana* , Fabro et al (2004) and Szekely et al (2008) found that two P5CS isoenzymes exist that play specific role in proline biosynthesis control . According to Fabro et al (2004) and Szekely et al (2008) ,not P5CS2 gene rather P5CS1 gene is required during stress for accumulation of proline. Elthon and stewart (1981) ,Rayapati et al (1989) and Szoke et al (1992) reported that synthesis of proline occurs in cytosol and plastids whereas degradation of proline occurs in mitochondria.

Glutathione reductase

Gilles and Vidaver (1990) stated that mechanism that is induced due to low temperature injury to plants is known as photo-oxidation. Esterbauer and Grill (1978) ,Anderson et al (1992) and Doulis et al (1993) reported that activities of glutathione reductase increase during cold hardening of conifers and activities increase throughout winter in tissues that are dormant. Guy and Carter (1984) found that there was a difference in kinetic characteristics of glutathione reductase isolated from hardened spinach leaves and the tissues that was not hardened.

Roychoudhury and Nayek (2014) and Banerjee and Roy Choudhury (2015a)and (2015b) reported that to overcome stressful conditions, plants exhibit vivid molecular responses at molecular and biochemical level. Yamaguchi- Shinozaki and Shinozaki (2006) stated that to design a stress-tolerant genetic model is extremely challenging and difficult . According to Bartels and Sunkar (2005) , plants which are stress tolerant like *Craterostigma plantagineum* ,*Thellungiella halophila* and *Mesembryanthemum crystallinum* can be used as valuable models in which study of system biology and it's subsequent integration can be done in crop plants to enhance stress tolerance.

Osmolytes

Hayat et al (2012) and Roychoudhury and Chakraborty (2013) reported that the common osmolytes that are accumulated during stresses include sugar alcohols like sorbitol , mannitol adonitol, complex sugars like trehalose, fructans and raffinose, free amino acids like glycine betaine and proline, organic acids like malate, citrate, lactate ,succinate, salicylate, gamma - amino butyric acid (GABA), quarternary ammonium compounds like beta -alanine-betaine, probetaine and hydroxy -proline betaine and tertiary sulfonium salts like dimethylsulfoniopropionate and choline-o-sulfate are major carbohydrates like fructose, glucose and sucrose, sugar alcohols like pinitol, cyclitol and ononitol, polyols like mannitol, sorbitol, myoinositol.

Molassiotis and Fotopoulos (2011) reported that the earliest signals in abiotic stresses are ROS and reactive nitrogen species(RNS).

Croser et al (2003) ,Singla and Garg (2005) reported that filling of grains in legumes is drastically affected by low or high temperature, drought and salinity. Chinnuswamy et al (2005) reported that advancement in understanding of tolerance mechanisms involved in plant against abiotic stress and advent of molecular genetic technology helps in addressing such issues more efficiently than in the past.

Glycine betaine

Rhodes and Hanson (1993) and Chen and Murata (2011) reported that glycine betaine is a" compatible osmolyte " and it is one of the members of a group of small molecules such as proline, prolinebetaine, β-alaninebetaine, choline-o-sulfate and 3-dimethylsulfoniopropionate. Dawson et al (1969) and Wyn Jones et al (1977) reported that , improving plant's tolerance to abiotic stresses is one of the main function of glycinebetaine and other compatible osmolytes. Kurepin et al (2013a) reported that glycine betaine is also associated with maintenance of performance of photosynthesis and stability of PS-II in abiotically stressed plants i.e it increases plant growth and reproductive yield i.e the traits that are regulated by plant hormones. Rhodes and Hanson (1993) and Chen and Murata (2011) reported that , accumulation of glycine betaine occurs in plants due to abiotic stresses and it is directly correlated with increasing tolerance of plants to abiotic stresses . According to Kiba et al (2011) ,Dodd and Davies (2010) ,Kurepin and Pharis (2014)and Huner et al (2014), various types of abiotic stresses such as fluctuations in temperature and mineral levels,

changes in water availability and light are dealt by plants via multiple pathways that are regulated by changes in biosynthesis of plant hormones and their perception.

Pulse protein

Kato and Nakai (1980) reported that there exists a relation between surface hydrophobicity, interfacial tension and emulsifying properties of proteins. Meng and Ma (2001),Tang et al (2009) , Tang and Sun (2010) and Shevkani et al (2015a,b) reported that the major storage protein in kidney bean, mung bean, cowpea, urad bean and red bean is Vicilin which accounts upto 88% of total globulins. Chavan et al (1988) reported that the major globulins in chickpeas are the legumins. Boulter and Croy (1997) and Boy et al (2010) reported that molecular weight of pulse albumins are generally low that is about 5-80 kilodaltons and the cysteine and methionine content in pulse albumin is higher than pulse globulin .

Seed priming

Basra et al (2005) reported that seeds that are primed usually exhibit an increased rate of germination, greater uniformity of germination and total germination percentage also increases at times. Lin and Sung (2001) stated that improvement of germination is observed under sub-optimal conditions. Bray et al (1989) reported that metabolic repair during imbibition is the reason behind increased rate of germination and uniformity. Basra et al (2005) reported that the reason for increased rate of germination and uniformity is build up of metabolites that enhances germination.

Kumar et al (1991) found that there is a variation at genetic level for the ability to germinate and length of root under low temperature(14 degree centigrade) . Kumar et al (1991) and (1995) reported that at seedling stage ,greater root:shoot ratio can be used as a selection criterion for cold tolerance for pigeon pea. Wery et al found that in plants, formation of intracellular ice causes dehydration of cell and destruction of cell membrane due to freeze thaw cycle resulting in death of plants under cold conditions.

Oquist et al (1987) and Huner (1991) reported that an important environment stress is low temperature that makes photosynthesis more sensitive to photoinhibition due to which photoinhibition is caused by very low photon flux densities.

There is a reduction in photosystem II repairing ability at low temperatures. According to Chow et al (1989) ,Gong and Nilsen (1989) and Aro et al (1990) , there is a slowing down

of D1 protein synthesis in the reaction centre and it's degradation under the effect of low temperature. Kyle (1987) reported that at low temperatures, there is a slowing down of migration rate, assembly of proteins and co-factor ligation.

Demmig and Bjorkman (1987) and Demmig-Adams et al (1990) reported that under low temperature, zeaxanthin formation ability which is thought to quench the excitation energy of photosystem II antenna may be inhibited.

Sims et al (2012) and Genisel et al (2013) reported that under optimum environmental conditions ,plants species evolve and function and any deviation in these conditions may result in reduction of growth of plants,photosynthetic inhibition ,ion uptake inbalance and oxidative stress. Lambers et al (2008) reported that low temperature directly or indirectly puts impact on the array of photosynthetic processes that occur in chloroplasts such as electron transport in thylakoid membrane, carbon reduction cycle and stomatal conductance control. Alam and Jacob (2002) further stated that this leads to reactive oxygen species generation which represses the net rate of photosynthesis because of photo-energy accumulation.

ROS accumulation puts detrimental effect on growth of plants because they cause destruction to lipids, nucleic acids and proteins. Apel and Hirt (2004) reported that for protection against ROS ,higher species of plants generally utilise a defence system that involves antioxidative compounds or enzymes. Kuk et al (2002) reported that plants that are cold tolerant exhibit lower reductions in chlorophyll content and higher leaf moisture content. Huang and Guo (2005) reported that in comparison to cold sensitive plants ,cold tolerant plants exhibit higher anti-oxidative enzymatic activity. Tan et al (2012) and Yamamoto et al (2012) reported that various metabolites and plant growth regulators such as melatonin, spermidine and proline play significant role in improving resistance of plants to cold stress.

CBF Genes

Gilmour et al (1998) reported that when plants are transferred to low temperature, transcripts for gene CBF1,CBF2 and CBF3 are accumulated that can be detected within 15 mins . According to Chinnusamy et al (2003) , first transcription factor ICE and the proteins that have role in expression of CBF have been identified.

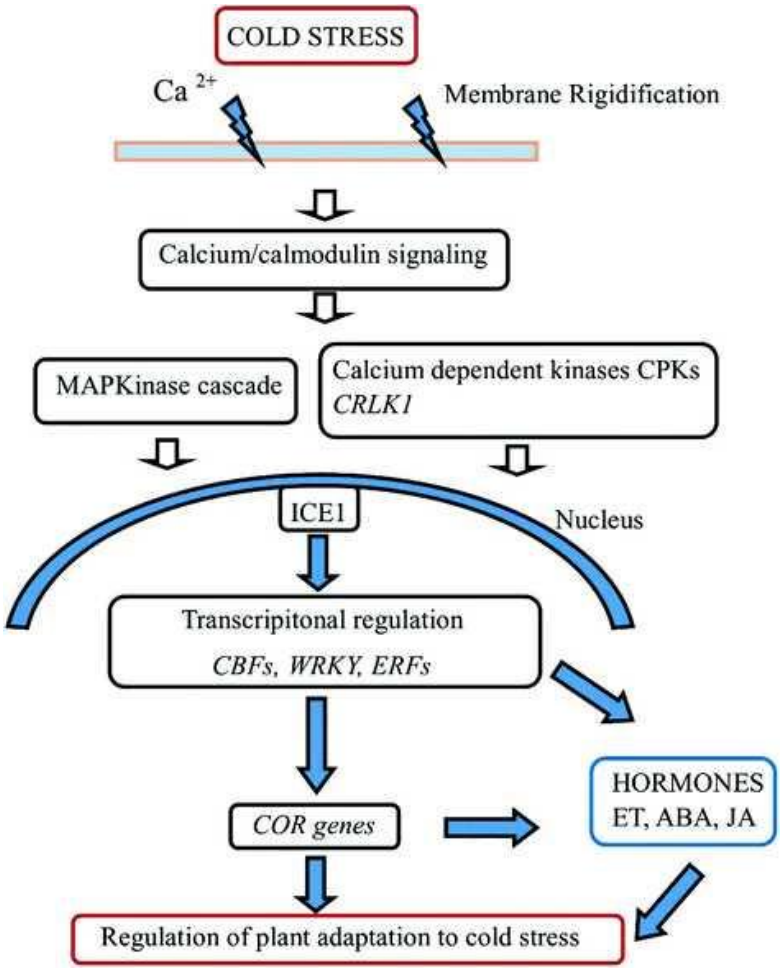

Fig 1 : Cold response mechanism in plants

Proteins having positive role in CBF expression.

According to Chinnusamy et al (2003) , in regulation of CBF3 , MYC- like bHLH protein is encoded by a nuclear gene ICEI:ICEI that has a fundamental role. Chinnusamy et al (2003) again reported that there are 5 bHLH binding sites in CBF3 promoter gene and all can bind with ICE1 protein. Chinnusamy et al (2003) again reported that the ice-1 mutation least affects the CBF1 and CBF2 transcripts that are accumulated under low temperature. Riechmann et al (2000) , reported that 139 bHLH proteins are encoded by *Arabidopsis.* According to Gong et al (2002) , a RNA helicase like protein is encoded by a nuclear gene LOS4 that has a positive effect on the expression of CBF gene. Again Gong et al (2002) reported that there is a reduction in accumulation of transcripts of CBF under the effect of low temperature and also reduction in accumulation of CBF induced genes transcripts such as RD29A and COR15A due to los4-1 recessive mutation. Gong et al (2002) reported that the

chilling sensitive phenotyopes are suppressed due to over-expression of CBF3 which indicates that the CBF regulon has a role in chilling tolerance.

Proteins having negative role in the expression of CBF

Ishitani et al (1998) reported that the nuclear encoded gene HOS1 has a role in the expression of CBF genes that are induced under cold stress. Lee et al (2002) reported that protein that is degraded by the 26s proteosome may be targeted by HOS1 protein. Guo et al (2002) reported that the expression of CBF genes are affected by a nuclear gene LOS1.

Role of enzymatic and non-enzymatic antioxidants in plants during abiotic stress

Ahmad et al (2008a) , Bhatnagar-Mathur, Vadez &Sharma ,(2008) ,jaleel et al (2009) and Tuteja et al (2009) ,there is an increase in level of several metabolites and proteins whenever a plant is subjected to abiotic stress ,that confers a certain degree of protection to these stresses. Mittler (2002) reported that , electrons having high energy state are transferred to molecular oxygen to form reactive oxygen species (ROS) during stress. Jaleel et al (2007d-f) ,(2008f) and Tuteja et al (2008) reported that, ROS has the capability of inducing damage in almost every cellular macromolecules including DNA. Yakes and Van Houten (1997) reported that due to the absence of organisation of chromatin and lower mitochondrial DNA repair activities ,it is more sensitive to oxidative damage than nuclear DNA.

Superoxide dismutase

McKersie et al (1999) reported that , since tolerance to abiotic stress is a trait that is multigenic ,alteration in the SOD expression may in turn lead to the cascade of expression of other enzymes that are associated with the stress resistance. Slooten et al (1995) reported that overexpression of SOD leads to enhancement of oxidative stress that is MV-dependent provided other important anti-oxidant enzymes such as APX, DHAR ,ascorbate and glutathione are also present at higher levels.

Catalases

Srivalli et al (2003) and Ben Amor et al (2005) reported that catalases that are present in peroxisomes are tetrameric heme that contains enzymes for converting $2H_2O_2$ to O_2 $+2H_2O$. Frugoli et al (1996) reported that many isozyme forms of catalase are present in many plants such as two in castor bean ,six in *Arabidopsis*. Van Breusegem et al (2001)

observed that the important scavenging enzymes that are helpful in directly dismutating H2O2 and are indispensable for detoxification of ROS during stress are catalases.

Glutathione reductase

Bray ,Bailey-Serres &Weretilnyk (2000) reported that the Halliwell-Asada enzyme pathway describes well the role of glutathione and Glutathione reductase in scavenging of H2O2 . According to Lee and Lee (2000) ,GR activity increases significantly in the leaves of cucumber due to chilling stress. Roxas et al (1997) reported that unlike wild type, the levels of oxidized glutathione becomes much higher in transgenic lines of tobacco that over-expresses glutathione –S-transferase(GST) and glutathione peroxidase(GPX).

According to Ding et al (2007) , under chilling temperature stress , the activity of GR increases in mango fruit when salicyclic acid or oxalic acid is exogenously treated.

Non-enzymatic antioxidants

Glutathione

According to Noctor and Foyer (1998) , for keeping ROS under control, GSH is one of the key players.

ROS and redox signalling

Shao et al (2005) reported that ROS signals can be sensed by plants and can be translated into appropriate cellular responses and this process requires the involvement of redox-sensitive proteins which undergoes reversible oxidation/reduction and depending upon cellular redox state ,it either switches on or off. Li and Jin (2007) reported that cellular metabolism may directly be modulated by it's corresponding redox sensitive metabolic enzymes whereas kinases, phosphatases and trasnscription factors like downstream signalling components provide a pathway for the redox-sensitive signalling proteins to execute their function. Coupe et al (2006) reported that towards downstream of the ROS signal, higher plant hormones are located but in many hormone signalling pathways, ROS themselves are also secondary messengers.

Glutathione peroxidases in the plant response to stress.

Levine et al (1994) ,Willekens et al (1997) and Leisinger et al (2001) reported that ROS induces strongly some GPX genes. Herbette et al (2002) reported that novel isoforms of

TRX peroxidase is represented by atleast two plant PHGPXs that are in comparison to lipid peroxidases are more active against H2O2.According to Ursini et al (1995) ,in transgenic plants, over-expression of PHGPX , enhances stress tolerance.

MAPK Cascades

Sheen (2001) and Yang et al(2001) reported that touch,cold and water stress in *Arabidopsis thaliana* induces AtMEKK1 in the downstream of AtHK1 . Matsuoka et al (2002) reported that cold, wounding ,drought and high salt stress also activates AtMEKK1 and also protein kinase activity also elevates for phosphorylation of AtMAPK4. Sangwan et al (2002) reported that SAMK is induced by membrane rigidification during cold treatment and HAMK is activated by membrane fluidisation. Mizoguchia et al (1998) reported that MAPK cascade is induced by H2O2 which in turn induces gene expression that are stress responsive but the auxin action is blocked due to it. Patrick et al (2002) reported that the knockout mutation of ANP leads to stress responses that are upregulated and no apparent change occurs in gene expression that are regulated by auxin.

Role of Cytokinin in abiotic stress responses.

Itai and Vaadia (1965) ,Itai et al (1973) ,Walker and Dumbruff (1981) ,Caers et al (1985) ,Hubick et al (1986) ,Hansen and Dorffling (2003) ,Kudoyarova et al (2007) ,Albacete et al (2008) and Ghanem et al (2008) reported that on the basis of numerous studies conducted, they have found that extended stress decreases the concentration of cytokinin in plants. Whereas Walker and Dumbroff (1981) ,Hansen and Dorffling (2003) and Pospisilova et al (2005) and Alvarez et al (2008) in contrast reported that severe stress leads to increase in cytokinin levels. Jeon et al (2010) and Jeon and Kim (2013) reported cold exposure leads to transient induction in the expression of the RRAs ARR5,6,7 and 15 that depends upon receptors AHK2 ,AHK3 ‘s function along with RRB ARR1 and AHP2,3 or 5. Shi et al (2012 b) reported that the positive regulator of ethylene signalling that is EIN3 helps in repression in the expression of ARR5,7 and 15 upon extended cold treatment. Kang et al (2013) reported that there is a repression in the expression of several RRAs including ARR5,6 and 7 due to induction in the overexpression of the type C RR ARR22 that leads to increased resistance to cold stress .

MATERIALS AND METHODS

The proposed work on " Screening and identification of black gram genotypes (*Vigna mungo)* tolerant under cold stress " was carried out in the department of Agril. Biotechnology ,college of Agriculture , Odisha University of Agriculture and Technology, Bhubaneswar .

Taxonomy of black gram :

- Kingdom-Plantae-Plants
- Subkingdom-Tracheobionta-Vascular plants
- Division-Magnoliophyta-flowering plants
- Class-Magnoliopsida- Dicotyledons
- Subclass-Rosidae
- Order-Fabales
- Family-leguminosae
- Genus-*Vigna*
- Species-*mungo*

EXPERIMENTAL MATERIALS:

Total 34 genotypes of black gram differing in their degree of tolerance to cold were studied for various biochemical and physiological approaches.

Table No.1 : **List of black gram genotypes**

SL.NO.	REF.NO.	ACCESSION NO.
1	P1	C4 CBG 31
2	P5	C3 PU 31
3	P7	KVK Nayagarh 3
4	P8	C2 Ujala
5	P11	CPRBAMGP-231
6	P12	C1 Prasad
7	P13	CPRBAMGP-224
8	P18	C1 Prasad
9	P19	KVK Puri-2
10	P20	C2-Ujala
11	P21	C3 PU 31
12	P25	KVK Nayagarh -1
13	P26	C4-OBG-31
14	P28	KVK jagatsinghpur
15	P29	CPRBAMGP-217
16	P32	CPRBAMGP-236
17	P35	KVK Nayagarh-2
18	P37	CPRBAMGP-240
19	P38	CPRBAMGP-239
20	P42	C4-OBG-31
21	P44	C2 Ujala
22	P46	KVK Puri-3
23	P47	KVK Jajpur
24	P51	C3PU31
25	P53	C1 Prasad
26	P57	C3 PU 31
27	P58	C2 Ujala
28	P61	C4 OBG 31
29	P63	CPRBAMGP -233
30	P68	C1 Prasad
31	P76	KVK Bhanjanagar
32	P77	CPRBAMGP-231
33	P78	CPRBAMGP-229
34	P84	C1Prasad

Table No. 2 : Morphological descriptors of black gram based on DUS guidelines.

Sl.no.	characteristics	states	note	Stage of observation	Type of assesssment
1	Hypocotyls: Anthocyanin colouration	Absent present	1 9	Cotyledons unfolded	VS
2	Time of flowering	Early Medium Late	3 5 7	50% plants with atleast one open flower	VG
3	Plant:growth habit	Erect Semi erect spreading	3 5 7	50% flowering	VG
4	Plant: habit	Determinate indeterminate	1 3	50% flowering	VG
5	Stem : colour	Green Green with purple splashes Purple with green splashes purple	1 2 3 4	50% flowering	VS
6	Stem: pubescence	Absent present	1 9	50%flowering	VS
7	Leaf shape (terminal)	Deltoid Ovate-2 Lanceolate cuneate	1 2 3 4	50% flowering	VG
8	Foliage colour	Green Dark green	1 2	50% flowering	VG
9	Leaf :vein colour	Green purple	1 2	50% flowering	VG
10	Leaf pubescence	Absent present	1 9	50% flowering	VS
11	Petiole colour	Green	1	Fully developed	VG

		Green with purple splashes purple	2 3	green pods	
12	Pod: intensity of green colour of pre-mature pods	Yellowish green Green Dark green	3 5 7	Fully developed green pods	VG
13	Pod pubescence	Absent present	1 9	Fully developed green pods	VG
14	Peduncle length	Small Medium long	3 5 7	Harvest maturity	MS
15	Pod length	Small Medium long	3 5 7	Harvest maturity	MS
16	Pod colour	Buff Brown black	1 2 3	Harvest maturity	VG
17	Plant height	Short Medium long	3 5 7	Harvest maturity	MS
18	Seed shape	Globose Oval Drum shaped	1 2 3	Mature seeds	VG
19	Seed lustre:	Shiny dull	1 2	Mature seeds	VG
20	Seed colour	Green Greenish brown Brown Black Mottled	1 2 3 4 5	Mature seeds	VG
21	Seed size	Small Medium large	3 5 7	Mature seeds	MG

MG : measurement by a single observation of a group of plants or parts of plants.

MS: measurement of a number of individual plants or parts of plants

VG; visual assessment by a single observation of a group of plants or parts of plants

VS: visual assessment by observation of individual plants or parts of plant

Screening experiment :

- The screening experiment was conducted by taking 34 genotypes of black gram and maintaining a artificial cold stress condition of 10 degree centigrade inside plant growth chamber for 5 days . From each genotype , 8 seeds were put in a petriplate containing filter paper soaked with distilled water . Simultaneously control for each genotype was also taken for observing physiological differences between the plants at normal and those at cold stressed conditions. Then physiological parameters like germination percentage and root and shoot length of genotypes were recorded for 5 days .
-

PHYSIOLOGICAL PARAMETERS:

1. **Germination percentage (GP**) = total number of seeds germinated / total number of seeds sown × 100
2. **Germination index (GI)** =(5× N1) +(4× N2) + (3×N3) + (2 ×N4) + (1× N5)

Where N1, N2…N5 are the number of germinated seeds on the 1^{st} , 2^{nd} and subsequent days until 5^{th} day and 5,4,..1 are the multipliers given to the days of germination.

3. **Root and shoot length**

Polybag experiment :

For observing natural inherent cold tolerance of plants ,the polybag experiment was conducted . The resistant and susceptible genotypes of black gram identified through screening technique were taken for pot experiment. The resistant genotypes C3PU-31(P 57) and KVK Nayagarh 3(P7) were taken and the susceptible genotypes that were taken are C2 Ujala(P8) and C4 OBG 31 (P42). 3 replications each of susceptible and resistant variety were taken and sown in polybags on 26^{th} February ,2020. Simultaneously control genotypes were also kept. Total 24 polybags were taken and 3 seeds in each polybag were sown.

Then after 15 days of growth, resistance and susceptible genotypes were transferrred to chamber maintained at 10 degree centigrade for 3 days (1^{st} cold stress).Then after 1^{st} cold stress, the polybags were again transferred to natural environment for 1 week. Then after 1 week, the resistance and susceptible genotypes were again transferred to chamber

maintained at 10 degree centigrade for 3 days.(2nd cold stress) . Till reproductive stage, the process was continued and biochemical analysis of genotypes were performed both before and after cold stress. Then seed yield and it's attribute analysis was also done.

Biochemical analysis

Estimation of chlorophyll :

Chlorophyll estimation was done according to the method of Arnon (1949) .fresh leaves of about 100 mg was homogenized using chilled 80% acetone in a pre-chilled clear glass mortar and pestle. The homogenate was centrifuged for 10 minutes and the supernatant was collected in a test-tube. The residue was again extracted with 80% acetone and centrifuged. The supernatant was pooled together and the combined supernatant volume was noted. The absorbance of solution was measured at 480 nm, 645nm and 663nm against the solvent (80% acetone) used as blank by using a spectrophotometer. The amount of chlorophyll present in the leaf extract was calculated in mg chlorophyll per gram tissue according to the following equation :

Total chlorophyll = 20.2(A 645nm) + 8.02 (A663) × V/ 1000 ×W

Estimation of proline :

Proline content was estimated by using the method of Bates *et al* .(1973)

Reagents

1) 3% aqueous sulphosalicylic acid (w/v)
2) Acid ninhydrin (prepared by dissolving 1.25g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M o-phosphoric acid until dissolved.)
3) Toluene

Extraction

Three hundred mg of fresh leaves were separately homogenized in 5ml of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was collected.

Procedure

2ml of supernatant was taken in a test tube and 2.0 ml reagent acid ninhydrin was added. This mixture was kept in boiling water bath for 1h at 100 degree centigrade and thereafter reaction was terminated by keeping tubes in ice bath. Then toluene 4.0 ml was added .After vigorous shaking, the upper coloured organic phase was taken after attainment of room temperature and absorbance was recorded at 520 nm by using toluene as blank. Standard curve was prepared by using graded concentration of proline (20-100 µg/ml). the proline content was expressed as mg/g DW .

Estimation of total soluble carbohydrate

It was estimated with the method of Yem and Willis (1954) using anthrone reagent .

Extraction

Fresh samples of about two hundred mg were homogenized in 80% ethanol using acid washed sand as an abrasive . three times the homogenate was refluxed with 80 % ethanol . from different extraction, the supernatant was pooled and the volume was made to 5ml with 80 % ethanol. The extract so obtained was used for estimation of TSC .

Reagents

Anthrone reagent :

It was prepared by dissolving 0.4g anthrone in 100 ml concentrated H_2SO_4 .

Procedure:

From the above extract, an aliquot of 0.2 ml was evaporated to dryness in a test tube in a boiling water bath. After cooling the residue left in the tube ,it was dissolved in one ml of distilled water and was mixed with 4ml of anthrone reagent.then the mixture was heated in a water bath for 10 minutes. After cooling, absorbance was recorded at 620nm using spectrophotometer. Standard curve was prepared using graded concentration (20-100 µg/ml) of D-glucose and data were expressed as mg/g DW of the tissue.

Estimation of catalase

Activity of catalase was estimated by the UV method of Aebi ,(1984)

Reagents :

i) 0.1 M H_2O_2

ii) 0.05M potassium phosphate buffer (pH 7)

Procedure : enzyme extract of about 500 µl was taken and to this 0.2ml of 100mM H_2O_2 and 1.5ml of 50 mM potassium phosphate buffer was added . At the time of absorbance recording , immediately the enzyme sample was added and incubated for 3 minutes. The change in absorbance was recorded at 240 nm at an interval of 15 seconds for 1.5 minute. The enzyme activity was expressed as unit/mg protein/min.

Estimation of protein

Total protein content was determined in the leaves of black gram genotypes in the normal and cold tolerant plants at pre-flowering stage by the method of Lowry *et al* .(1951)

Reagents :

a) Ethanol(80 percent)
b) Alkaline copper solution.

Procedure for protein estimation

Leaf sample of 200 mg was homogenized with 10ml of 80% ethanol using mortar and pestle and was centrifuged at 4000 rpm for 20 minutes. The supernatant was kept aside and the residue was hydrolysed with 5ml of 1N NaOH for overnight and next day it was centrifuged again at 4000 rpm for 20 minutes. Then supernatant was collected and residue was again extracted with 5ml of of 1N NaOH after 1 hour of adding it and then centrifuged. Both the supernatant were mixed and volume was made upto 10 ml. Then 0.5ml supernatant and 5ml reagent b(alkaline copper solution) were added and mixture was left for 10 min and after that 0.5mlreagent c(folin reagent) was added and incubated at room temperature for 1 hour. A blue colour was developed and thereafter absorbance of blue colour was recorded at 730nm by using spectrophotometer.

Protein profiling of leaf and seed samples.

Protein profiling of leaf and seed samples were performed by SDS-PAGE.

Procedure for SDS PAGE profiling

1) first of all the electrophoresis unit was asssembled in such a way that the glass plates clamped to the unit along with the spacers placed in between the two vertical edges.

2) Then 1% agarose (0.05g in 5ml of distilled water) and was boiled to dissolve the agarose and poured on a thin horizontal layer at the lower edge of the plates to seal the assembly and was allowed to cool down for 5-10 minutes.

3) Then 12% separating gel was prepared. To prepare separating gel,the following components were added :

a) 30% Acrylamide-bisacrylamide solution -6ml

b) Distilled water -3 ml

c) 2.5 X Tris-SDS Buffer-6ml

d) 10% APS Solution -125µL

e) TEMED- 7.5 µL

Above gel mixture was poured in between the plates and was allowed to solidify for an hour. After pouring the gel, immediately distilled water was added to level the gel .

4) Water was poured off after an hour by inverting the casting assembly.
5) Preparation of 5 % stacking gel - for preparation of stacking gel,following components were added :

a) 30% Acrylamide- bisacrylamide solution -1.3ml
b) Distilled water- 5.1 ml
c) 5X Tris-SDS Buffer -1.6ml
d) 10% APS solution - 75µL
e) TEMED - 15µL

After adding TEMED, all the components were mixed by swirling the beaker and then stacking gel was poured on the top of separating gel and immediately the comb was placed to avoid air bubbles and was allowed to solidify for 30 minutes.

Note: Acrylamide is a potential neurotoxin and should be treated with great care. Always wear a face mask and use gloves.

6) IX Tris-Glycine-SDS Gel Running Buffer was poured in the unit in such a way that the buffer connected the two electrodes and hence completes the flow of current. Carefully the combs were removed carefully from the stacking gel .
7) Sample preparation : 20 µL of each sample were taken in the eppendorf tubes and 5µL of 5X sample loading buffer was added to it ,then the tubes containing protein

samples were boiled at 100 degree centigrade in boiling water bath for 2min. Tube that contained the Prestained Protein ladder was not boiled.

8) Prestained protein ladder of 5µL and samples of 20 µL after the heat treatment were immediately loaded in the wells created by the comb in the stacking gel.
9) To the electrophoretic power supply ,the electric power was connected according to conventions : Red –Anode and Black –Cathode. Electrophoresis was done at 100 volts and 10mA until dye front reaches 0.5 cm above the sealing gel.

10)Afterwards, the gel was carefully removed from in-between the plates using spatula into the plastic tray containing distilled water. Gel was washed in distilled water for 1minute then water was discarded and proceeded for staining and de-staining procedure.

11) Destained protein gel was analyzed subsequently and documented by Multi Imager Gel documentation system (Bio-Rad).

Yield and yield attributes

Pods/plant

The total no. of 5 randomly selected plants was recorded and expressed as number of pod/plant

Seeds/pod

The number of seeds /pod was averaged from randomly taken 10 pods on 5 selected plants.

Test weight

100 seed were counted randomly from each genotypes and the test weight was recorded.

\Biological yield /plant

The completely matured plants were uprooted carefully along with roots and were dried completely. The weight of dried plant along with pods was recorded as biological yield.(g/plant).

Seed yield /plant(g)

The seed weight in grams from each plant was recorded**.**

Harvest index (%)

It is represented in terms of percentage. The harvest index was calculated by dividing the economic yield by the biological yield and multiplied by 100

HI= seed yield / biological yield * 100

RESULTS

Black gram (*Vigna mungo* (L.)) is one of the important pulse crops grown in all the 3 seasons such as Kharif , Rabi and Summer throughout the India. However among the abiotic stresses , cold stress is the primary cause of crop loss reducing average yield by more than 20 % . So there is a need for identification of cold tolerant genotypes of black gram that can survive under cold stress conditions without compromising yield characteristics .

The present investigation " Screening and identification of black gram (*Vigna mungo* (L.)) genotypes under cold stress ." was carried out and the results of the objectives are presented in this section

PHYSIOLOGICAL PARAMETERS :

1) Germination percentage :

Variation in germination was observed during the cold screening of 34 genotypes at 10 degree centigrade kept for 5 days .At the end of 5 DAE , the genotypes having **100 % g*ermination*** are KVK jagatsinghpur, CPRBAMGP-217 , CPRBAMGP-231 , C4 OBG -31 ,CPRBAMGP-240 , KVK Nayagarh _1 and C3 PU 31 . Genotypes CPRBAMGP-241, CPRBAMGP -231, KVK-Puri-2 , C1Prasad , C2 Ujala, KVK Jajpur are having **87.5 % germination ,** KVK Puri 3 , CPRBAMGP-236, CPRBAMGP -233 are having **75 % germination** . Genotypes CPRBAMGP-231 and KVK Nayagarh 2 are having **62.5% germination** and the lowest rate of germination was recorded in C2 Ujala and CPRBAMGP-224 **ie 37.5% .**

Fig 2 : genotypes at control condition

Fig 3 : genotypes in chamber under cold stress

Table No .3 : Data pertaining rate of germination of *Vigna mungo* after 1st day of sowing(control condition)

SL.no.	VARIETY NAME	REF.NO.	NO.OF SEEDS GERMINATED (OUT OF 5)	GERMINATION PERCENTAGE
1	C4-OBG-31	P1	Nil	0%
2	KVK Nayagarh3	P7	4	80%
3	C2 Ujala	P8	1	20%
4	C1 Prasad	P12	4	80%
5	KVK Puri 2	P19	2	40%
6	C2 Ujala	P20	2	40%
7	C3PU31	P21	4	80%
8	KVK Nayagarh 1	P25	4	80%
9	C4OBG 31	P26	3	60%
10	CPRBAMGP217	P29	2	40%
11	KVK Nayagarh 2	P35	2	40%
12	CPRBAMGP240	P37	2	40%
13	CPRBAMGP239	P38	3	60%
14	C2 Ujala	P44	4	80%
15	KVK Puri 3	P46	1	20%
16	C1 Prasad	P53	1	20%
17	C3PU31	**P57**	**5**	**100%**
18	C2 Ujala	P58	2	40%
19	C4OBG 31	P61	1	20%
20	CPRBAMGP229	P78	4	80%

After 1st day of sowing in control condition , 100 % germination rate was observed in the variety C3PU31 ie all 5 seeds were germinated . 4 out of 5 seeds were germinated in the variety KVK Nayagarh 3 , KVK Nayagarh 1, C2Ujala and CPRBAMGP229 . 3 seeds were germinated in the variety C4OBG 31 and CPRBAMGP-239 , 2 seeds were germinated in the variety KVK Puri 2 , C2 Ujala , CPRBAMGP-217 and KVK Nayagarh 2 . Least germination was observed in KVK Puri 3 , C1 Prasad and C4OBG31 where only 1 seed germinated out of 5 seeds.

Table no. 4 : Data pertaining rate of germination of *Vigna mungo* after 1st day of sowing (stress condition)

SL.NO.	VARIETY NAME	REF.NO.	NO OF SEEDS GERMINATED	GERMINATIONPERCENTAGE
1	C4CBG31	P1	3	37.5%
2	C3PU31	P5	2	25%
3	KVK Nayagarh3	P7	2	25%
4	C2Ujala	P8	2	25%
5	CPRBAMGP230	P11	0	0%
6	C1Prasad	P12	4	50%
7	CPRBAMGP224	P13	0	0%
8	C1Prasad	P18	0	0%
9	KVKPuri2	P19	0	0%
10	C2 Ujala	P20	1	12.5%
11	C3PU31	P21	1	12.5%
12	KVK Nayagarh1	P25	0	0%
13	C4OBG31	P26	3	37.5%
14	KVK Jagatsinghpur	P28	0	0%
15	CPRBAMGP 217	P29	3	37.5%
16	CPRBAMGP236	P32	0	0%
17	KVK Nayagarh2	P35	0	0%
18	CPRBAMGP240	P37	3	37.5%

19	CPRBAMGP239	P38	0	0%
20	C4OBG31	P42	0	0%
21	C2Ujala	P44	0	0%
22	KVK Puri3	P46	0	0%
23	KVK Jajpur	P47	0	0%
24	C3PU31	P51	2	25%
25	C1Prasad	P53	0	0%
26	C3PU31	P57	0	0%
27	C2Ujala	P58	0	0%
28	C4OBG31	P61	5	62.5%
29	CPRBAMGP233	P63	4	50%
30	C1Prasad	P68	1	12.5%
31	KVK Bhanjanagar	P76	0	0%
32	CPRBAMGPGP-231	P77	0	0%
33	CPRBAMGP-229	P78	0	0%
34	C1Prasad	P84	0	0%

After 1st day of cold exposure , highest germination percentage ie 62.5% germination was observed in C4OBG31 where out of 8 , 5 seeds germinated. 4 out of 8 seeds were germinated in the variety C1Prasad and CPRBAMGP 233 . 3 out of 8 seeds were germinated in the variety CPRBAMGP217 and CPRBAMGP 240 . 2 seeds were germinated in the variety KVK Nayagarh 3 , C2 Ujala and C3PU31 . Least germination was observed in C2Ujala and C1Prasad were only 1 seed germinated out of 8 seeds.

Table no.5 : Data pertaining rate of germination of *Vigna mungo* at 2DAS(control condition)

SL.no.	VARIETY NAME	REF.NO.	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4CBG31	P1	4	80%
2	KVK Nayagarh 3	P7	All 5	100%
3	C2 Ujala	P8	1	20%
4	C1 Prasad	P12	4	80%
5	KVK Puri 2	P19	All 5	100%
6	C2 Ujala	P20	4	80%
7	C3PU31	P21	All 5	100%
8	KVK Nayagarh 1	P25	All 5	100%
9	C4OBG31	P26	4	80%
10	CPRBAMGP217	P29	4	80%
11	KVK Nayagarh 2	P35	3	60%
12	CPRBAMGP240	P37	5	100%
13	CPRBAMGP 239	P38	4	80%
14	C2 Ujala	P44	All 5	100%
15	KVK Puri3	P46	4	80%
16	C1Prasad	P53	2	40%
17	C3PU31	**P57**	**5**	**100%**
18	C2Ujala	P58	4	80%
19	C4OBG31	P61	4	80%
20	CPRBAMGP229	P78	4	80%

2 days after sowing , under control condition , all the seeds were germinated in the variety KVK Nayagarh 3, KVK Puri 2 , C3PU31 , KVK Nayagarh 1 and C2 Ujala . 4 out of 5 seeds were germinated in the variety C4CBG31, C1 Prasad, CPRBAMGP-217 , C4OBG31, CPRBAMGP 239 , KVK Puri3 and CPRBAMGP229 . least germination was observed in C2 Ujala where only 1 seed germinated .

Table no.6 : Data pertaining the rate of germination of *Vigna mungo* at 2 DAS(cold stress condition)

SL.NO.	VARIETY NAME	REF.NO.	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4 CBG 31	P1	7	87.5%
2	C3 PU 31	P5	6	75%
3	KVK Nayagarh 3	P7	6	75%
4	C2 Ujala	P8	3	37.5%
5	CPRBAMGP-231	P11	7	87.5%
6	C1 Prasad	P12	6	75%
7	CPRBAMGP-224	P13	Nil	0%
8	C1 Prasad	P18	4	50%
9	KVK Puri-2	P19	5	62.5%
10	C2-Ujala	P20	6	75%
11	C3 PU 31	P21	5	62.5%
12	KVK Nayagarh -1	P25	7	87.5%
13	C4-OBG-31	P26	7	87.5%
14	KVK jagatsinghpur	P28	5	62.5%
15	CPRBAMGP-217	P29	6	75%
16	CPRBAMGP-236	P32	3	37.5%
17	KVK Nayagarh-2	P35	4	50%
18	CPRBAMGP-240	P37	4	50%
19	CPRBAMGP-239	P38	4	50%
20	C4-OBG-31	P42	1	12.5%
21	C2 Ujala	P44	1	12.5%
22	KVK Puri-3	P46	3	37.5%
23	KVK Jajpur	P47	6	75%
24	C3PU31	P51	5	62.5%
25	C1 Prasad	P53	3	37.5%
26	C3 PU 31	P57	8	100%
27	C2 Ujala	P58	3	37.5%
28	C4 OBG 31	P61	6	75%
29	CPRBAMGP -233	P63	6	75%
30	C1 Prasad	P68	6	75%
31	KVK Bhanjanagar	P76	5	62.5%
32	CPRBAMGP-231	P77	5	62.5%
33	CPRBAMGP-229	P78	7	87.5%
34	C1Prasad	P84	4	50%

After 2 days of sowing in cold stress condition, 100 % germination percentage was recorded in the genotype C3PU31 . 75% germination percentage was recorded in the genotypes KVK Nayagarh3 , C2Ujala, CPRBAMGP217 , KVK Jajpur, CPRBAMGP 233 . 50% germination percentage was recorded in C1 Prasad , CPRBAMGP-240 and CPRBAMGP 239. Whereas in the genotype CPRBAMGP224 there was no germination observed .

Table no.7 : Data pertaining rate of germination of *Vigna mungo* at 3DAS (control condition)

SL.no.	VARIETY NAME	REF.NO.	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4CBG31	P1	4	80%
2	KVK Nayagarh3	P7	5	100%
3	C2 Ujala	P8	2	40%
4	C1 Prasad	P12	4	80%
5	KVK Puri 2	P19	5	100%
6	C2 Ujala	P20	4	80%
7	C3PU31	P21	5	100%
8	KVK Nayagarh1	P25	5	100%
9	C4OBG 31	P26	5	100%
10	CPRBAMGP217	P29	4	80%
11	KVK Nayagarh2	P35	3	60%
12	CPRBAMGP240	P37	5	100%
13	CPRBAMGP239	P38	4	80%
14	C2Ujala	P44	4	80%
15	KVK Puri 3	P46	4	80%
16	C1Prasad	P53	2	40 %
17	C3PU31	**P57**	**4**	**80%**
18	C2Ujala	P58	5	100%
19	C4OBG31	P61	4	80%
20	CPRBAMGP229	P78	4	80%

After 3 days of sowing in control condition, 100% germination percentage was recorded in the genotypes KVK Nayagarh 3 , KVK Puri2, KVK Nayagarh 1, C4OBG31 , CPRBAMGP240 and C2 Ujala and least germination was observed in the genotype C1prasad where only one seed germinated out of five .

Table no.8: Data pertaining rate of germination of *Vigna mungo* 3DAS(cold stress condition)

Sl.no	VARIETY NAME	REF.NO	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4CBG31	P1	7	87.5%
2	C3PU31	P5	7	87.5%
3	KVK Nayagarh 3	P7	8	100%
4	C2Ujala	P8	3	37.5%
5	CPRBAMGP231	P11	8	100%
6	C1Prasad	P12	6	75%
7	CPRBAMGP224	P13	3	37.5 %
8	C1Prasad	P18	5	62.5%
9	KVK Puri-2	P19	7	87.5%
10	C2-Ujala	P20	7	87.5%
11	C3PU31	P21	7	87.5%
12	KVK Nayagarh 1	P25	8	100%
13	C4OBG31	P26	8	100%
14	KVK jagatsinghpur	P28	8	100%
15	CPRBAMGP-217	P29	7	87.5%
16	CPRBAMGP-236	P32	5	62.5%
17	KVK Nayagarh-2	P35	4	50%
18	CPRBAMGP-240	P37	8	100%
19	CPRBAMGP-239	P38	4	50%
20	C4-OBG-31	P42	2	25%
21	C2Ujala	P44	5	62.5%
22	KVK Puri3	P46	3	37.5%

23	KVK Jajpur	P47	7	87.5%
24	C3PU31	P51	6	75%
25	C1Prasad	P53	5	62.5%
26	C3PU31	P57	8	100%
27	C2Ujala	P58	7	87.5%
28	C4OBG31	P61	7	87.5%
29	CPRBAMGP233	P63	6	75%
30	C1Prasad	P68	8	100%
31	KVK Bhanjanagar	P76	6	75%
32	CPRBAMGP231	P77	6	75%
33	CPRBAMGP229	P78	7	87.5%
34	C1Prasad	P84	4	50%

In the table no. 8 , 100 % germination percentage was recorded in the genotypes KVK Nayagarh3 , CPRBAMGP 231, KVK Nayagarh 1, KVK Jagatsinghpur, CPRBAMGP 240, C3PU31 , C2 Ujala and C1Prasad at 3 DAS in cold stress condition. In the genotype C2 Ujala lowest germination rate was observed i.e 37.5% .

Table no.9 : Data pertaining rate of germination of *Vigna mungo* at 5DAS(control condition)

SL.no.	VARIETY NAME	REF.NO.	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4CBG31	P1	4	80%
2	KVK Nayagarh3	P7	5	100%
3	C2Ujala	P8	4	80%
4	C1Prasad	P12	4	80%
5	KVK Puri2	P19	5	100%
6	C2Ujala	P20	5	100%
7	C3PU31	P21	4	80%
8	KVK Nayagarh1	P25	4	80%
9	C4OBG31	P26	5	100%
10	CPRBAMGP217	P29	4	80%
11	KVK Nayagarh2	P35	3	60%
12	CPRBAMGP240	P37	5	100%
13	CPRBAMGP239	P38	4	80%
14	C2Ujala	P44	2	40%
15	KVKPuri3	P46	5	100%
16	C1Prasad	P53	2	40%
17	C3PU31	**P57**	**5**	**100%**
18	C2Ujala	P58	5	100%
19	C4OBG31	P61	5	100%
20	CPRBAMGP229	P78	5	100%

In table no. 9, 100 % germination rate was observed in the genotypes KVK Puri2, C4OBG31, KVK Puri 3 , C3PU31 , C2Ujala , C4OBG 31 and CPRBAMGP 229 at 5DAS in control condition. Lowest germination was observed in C1Prasad .

Table no. 10: Data pertaining rate of germination of *Vigna mungo* at 5 DAS(cold stress condition)

Sl.no	VARIETY NAME	REF.NO	NO. OF SEEDS GERMINATED	GERMINATION PERCENTAGE
1	C4CBG31	P1	7	87.5%
2	C3PU31	P5	7	87.5%
3	KVK Nayagarh 3	P7	8	100%
4	C2 Ujala	P8	3	37.5%
5	CPRBAMGP-231	P11	8	100%
6	C1Prasad	P12	6	75%
7	CPRBAMGP 224	P13	3	37,.5%
8	C1Prasad	P18	6	75%
9	KVK Puri2	P19	7	87.5%
10	C2 Ujala	P20	7	87.5%
11	C3PU31	P21	7	87.5%
12	KVK Nayagarh1	P25	8	100%
13	C4-OBG-31	P26	8	100%
14	KVK Jagatsinghpur	P28	8	100%
15	CRBAMGP 217	P29	8	100%
16	CPRBAMGP-236	P32	6	75%
17	KVK Nayagarh 2	P35	5	62.5%
18	CPRBAMGP 240	P37	8	100%
19	CPRBAMGP-239	P38	6	75%
20	C4-OBG-31	P42	4	50%
21	C2 Ujala	P44	7	87.5%

22	KVK Puri-3	P46	6	75%
23	KVK Jajpur	P47	7	87.5%
24	C3PU31	P51	6	75%
25	C1Prasad	P53	6	75%
26	C3PU31	P57	8	100%
27	C2 Ujala	P58	7	87.5%
28	C4OBG31	P61	7	87.5%
29	CPRBAMGP-233	P63	6	75%
30	C1 Prasad	P68	8	100%
31	KVK Bhanjanagar	P76	7	87.5%
32	CPRBAMGP-231	P77	6	75%
33	CPRBAMGP-229	P78	7	87.5%
34	C1 Prasad	P84	4	50%

In table no. 10 , 100 % germination was observed in the genotypes KVK Nayagarh 1, C4OBG31, KVK Jagatsinghpur , CPRBAMGP 217 , CPRBAMGP 240 , C3PU31 and C1Prasad at 5DAS in cold stress condition. Least germination was observed in genotypes CPRBAMGP 224 and C2 Ujala where the germination percentage was only 37.5 % .

2) Table No. 11. Germination index value of control and cold stressed genotypes

SL.no.	VARIETY NAME	REF.NO.	GERMINATION INDEX
1	C4CBG31	P1	40
2	KVK Nayagarh3	P7	70
3	C2Ujala	P8	25
4	C1Prasad	P12	60
5	KVK Puri2	P19	60
6	C2Ujala	P20	53
7	C3PU31	P21	70
8	KVK Nayagarh1	P25	70
9	C4OBG31	P26	61
10	CPRBAMGP217	P29	50
11	KVK Nayagarh2	P35	40
12	CPRBAMGP240	P37	60
13	CPRBAMGP239	P38	51
14	C2Ujala	P44	70
15	KVKPuri3	P46	46
16	C1Prasad	P53	25
17	C3PU31	**P57**	**75**
18	C2Ujala	P58	56
19	C4OBG31	P61	48
20	CPRBAMGP229	P78	61

SL.NO.	Variety name	REF.NO.	GERMINATION INDEX
1	C4 CBG 31	P1	34
2	C3 PU 31	P5	56

3	KVK Nayagarh 3	P7	68
4	C2 Ujala	P8	19
5	CPRBAMGP-231	P11	26
6	C1 Prasad	P12	48
7	CPRBAMGP-224	P13	46
8	C1 Prasad	P18	37
9	KVK Puri-2	P19	57
10	C2-Ujala	P20	48
11	C3 PU 31	P21	63
12	KVK Nayagarh -1	P25	66
13	C4-OBG-31	P26	59
14	KVK jagatsinghpur	P28	49
15	CPRBAMGP-217	P29	44
16	CPRBAMGP-236	P32	51
17	KVK Nayagarh-2	P35	33
18	CPRBAMGP-240	P37	53
19	CPRBAMGP-239	P38	43
20	C4-OBG-31	P42	31
21	C2 Ujala	P44	66
22	KVK Puri-3	P46	40
23	KVK Jajpur	P47	38
24	C3PU31	P51	34
25	C1 Prasad	P53	19
26	C3 PU 31	P57	69
27	C2 Ujala	P58	51

28	C4 OBG 31	P61	42
29	CPRBAMGP -233	P63	47
30	C1 Prasad	P68	32
31	KVK Bhanjanagar	P76	39
32	CPRBAMGP-231	P77	45
33	CPRBAMGP-229	P78	54
34	C1Prasad	P84	50

In the table above depicts the calculated germination index value for both control and cold stressed genotypes. And it is observed that the germination index (GI) value significantly decreased under cold stressed condition as compared to control condition. Under cold stressed condition , highest GI value is observed for the genotype C3PU31 and KVK nayagarh 3. A higher GI value indicates a higher percentage and rate of germination . Bench Arnold *et al* (1991)

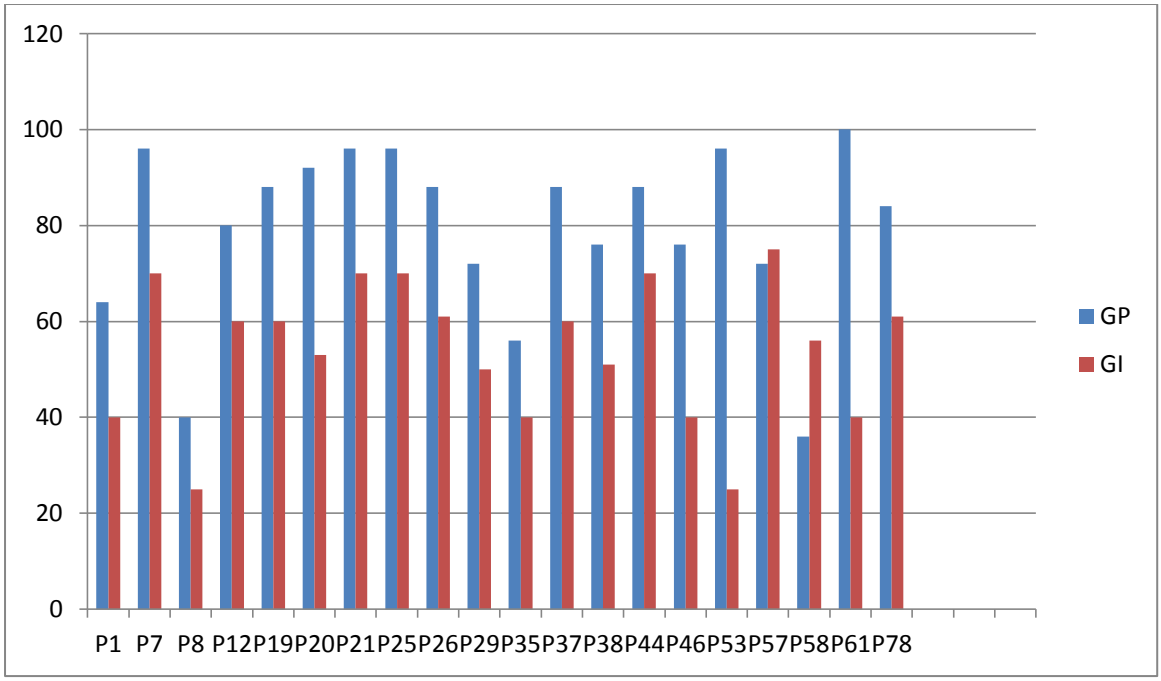

Fig 4 : GP and GI of black gram genotypes at control condition

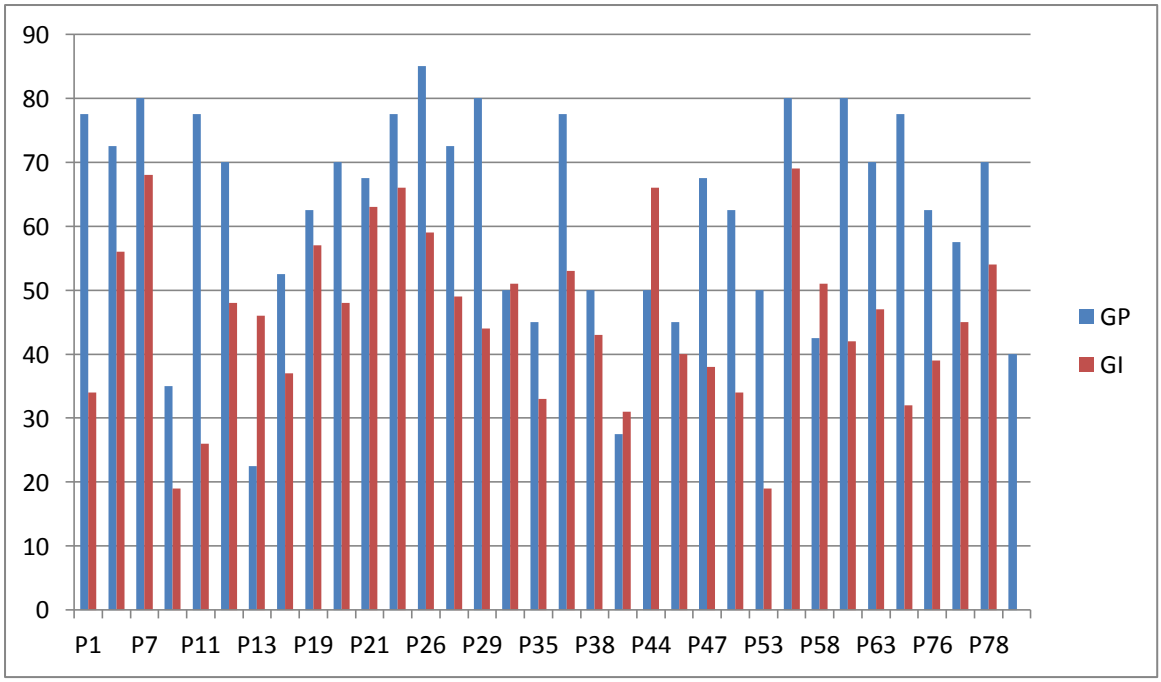

Fig 5 : GP and GI of black gram genotypes under cold stress

3) Root and Shoot length

The length of shoot and root was measured with the help of standard scale. It was observed that radicle emergence was delayed in comparison to plumule emergence. The radicle length and plumule length of the seedlings of 5 days old were recorded for both control and cold stress conditions and presented in the table below.

Table no. 12 : Data pertaining root and shoot length of genotypes 5 DAS(control condition)

SL.no.	VARIETY NAME	REF.NO.	Root length(cm)	Shoot length(cm)
1	C4 CBG31	P1	1.6	4
2	KVKNayagarh3	P7	3	8.7
3	C2Ujala	P8	3.6	6
4	C1Prasad	P12	2	8.2
5	KVKPuri2	P19	3.5	8
6	C2Ujala	P20	3.0	4.2
7	C3PU31	P21	3.8	4.6
8	KVK Nayagarh1	P25	2.8	5.4
9	C4OBG31	P26	4.2	7.5
10	CPRBAMGP217	P29	1.5	5.8
11	KVK Nayagarh 2	P35	4.5	6
12	CPRBAMGP240	P37		
13	CPRBAMGP239	P38	2	5.5
14	C2Ujala	P44		
15	KVK Puri3	P46	2	7
16	C1Prasad	P53	2.5	7.2
17	C3PU31	**P57**	**4**	**4**
18	C2Ujala	P58	3	6
19	C4OBG31	P61	3	7.6
20	CPRBAMGP229	P78	4.5	8.2

Table no. 12 describes the root and shoot length of genotypes at 5DAS in control condition. Highest shoot length was observed in the genotype KVK Nayagarh 3 ie about 8.7 cm and highest root length was observed in the genotype KVK Nayagarh 2 and CPRBAMGP 229 ie about 4.5 cm whereas in the genotype C3PU31, root and shoot length was almost same of about 4 cm.

Table no.13 : Data pertaining root and shoot length of genotypes 5DAS (stress condition)

Sl.no.	VARIETY NAME	Ref.no.	Root length	Shoot length
1	C4CBG31	P1	0	4
2	C3PU31	P5	0	5
3	KVK Nayagarh3	P7	0	5
4	C2Ujala	P8	0	3
5	CPRBAMGP231	P11	0	4
6	C1Prasad	P12	0	3.5
7	CPRBAMGP224	P13	0	<0.5
8	C1Prasad	P18	0	4
9	KVKPuri2	P19	0	3.9
10	C2Ujala	P20	0	4
11	C3PU31	P21	0	4
12	KVK Nayagarh1	P25	0	3
13	C4OBG31	P26	0	4
14	KVK Jagatsinghpur	P28	0	2.5
15	CPRBAMGP217	P29	0	3.6
16	CPRBAMGP236	P32	0	3
17	KVK Nayagarh2	P35	0	2.5
18	CPRBAMGP240	P37	0	3.5
19	CPRBAMGP239	P38	0	3
20	C4OBG31	P42	0	2
21	C2Ujala	P44	0	4.8
22	KVKPuri3	P46	0	0.5
23	KVK jajpur	P47	0	3
24	C3PU31	P51	0	3.8
25	C1Prasad	P53		
26	C3PU31	P57	0	5
27	C2Ujala	P58	0	2.5
28	C4OBG31	P61	0	2.5
29	CPRBAMGP233	P63	0	4
30	C1Prasad	P68	0	4.5
31	KVK Bhanjanagar	P76	0	4
32	CPRBAMGP231	P77	0	5
33	CPRBAMGP229	P78	0	5
34	C1Prasad	P84	0	3

Table no. 13 describes the root and shoot length of genotypes at 5DAS in cold stress condition. It was observed that there was no emergence of radicle under cold stress conditions. So only shoot length was measured and recorded. Highest shoot length of about 5cm was observed in the genotypes C3PU31 and KVK Nayagarh 3 and lowest shoot length was observed in the genotype KVK Puri 3 where only a 0.5 cm of plumule emerged .

Polybag experiment :

The genotypes C3PU31, KVK Nayagarh 3 taken as resistant genotypes and C2Ujala , C4OBG 31 taken as susceptible genotypes for polybag experiment . 3 replications was given for each genotype simultaneously control was also kept.

Fig.6 : Layout of polybags for sowing

Fig 7 : Genotypes in natural environment at 15 DAS

8 Morphological characters of the genotypes sown in natural environment were studied and presented in the table no. 3 . The characters observed were as follows :

1) Hypocotyl : Anthocyanin colouration – The colour of hypocotyl of the genotypes was observed and coded according to DUS guidelines. If the anthocyanin pigmentation was

present , it is coded **9** and if absent then it is coded as **1** .And here in the present investigation all the genotypes possessed the anthocyanin colouration .

2 **) Plant growth habit :** Growth habit of the plant is divided into erect , semi-erect and spreading and coded as 3,5 and 7 respectively according to DUS guidelines.

3 **) Stem colour** : Stem colour of the plant is divided into green, green with purple splashes and purple and coded as 1,2 and 3 respectively according to DUS guidelines.

4 **) Leaf shape** : Leaf shape is divided into Deltoid, Ovate, Lanceolate and Cuneate and coded as 1,2.3 and 4 according to DUS guidelines .

5 **)Leaf colour** : leaf colour is divided into green and dark green and coded as 1and 2 according to DUS guidelines.

6 **) Leaf vein colour** : leaf vein colour is divided into green, greenish purple and purple and coded as 1,2 and 3 .

7 **Petiole colour** : Petiole colour is divided into green, green with purple splashes and purple and coded as 1,2 and 3 respectively.

8 **Plant height** : plant height is divided into short, medium and long and coded as 3,5 and 7 respectively .

Table No. 14: **MORPHOLOGICAL DATA TAKEN ON 15TH DAY**

sl. no	Ref. no.	Hypocotyl : Anthocyanin colouration	Time of flowering	Plant growth habit	Stem colour	Leaf shape	Leaf vein colour	Foliage colour	Petiole colour	Pod colour of mature pods	Pod length	Plant height
1	P1	9	3	5	4	2	1	2	3	2	3	3
2	P5	9	3	7	2	1	2	2	3	2	3	3
3	P7	9	3	7	2	1	1	2	3	3	3	3
4	P8	9	3	7	4	2	1	1	2	3	3	3
5	P11	9	3	3	3	3	1	1	2	3	3	3
6	P12	9	3	7	2	3	1	1	3	3	3	3
7	P13	9	7	3	2	2	2	1	2	3	3	3
8	P18	9	3	5	4	1	1	2	2	3	3	3
9	P19	9	3	5	2	1	1	2	3	3	3	3
10	P20	9	3	7	2	3	2	2	2	3	3	3
11	P21	9	5	7	4	2	1	1	3	2	2	3
12	P25	9	3	7	3	1	1	1	3	2	5	3
13	P26	9	3	5	3	3	2	2	2	3	7	3
14	P28	9	5	5	4	2	1	2	3	3	3	3
15	P29	9	7	7	2	1	2	2	2	2	3	3
16	P32	9	3	7	2	1	1	1	3	2	3	3
17	P35	9	3	5	3	3	2	2	3	3	3	3
18	P37	9	7	7	1	3	1	2	3	3	3	3
19	P38	9	7	7	4	2	1	1	2	3	3	3
20	P42	9	7	7	4	2	2	1	3	3	3	3

21	P44	9	3	5	4	2	1	2	2	2	3	3
22	P46	9	5	7	2	1	2	1	3	2	3	3
23	P47	9	5	7	2	1	2	2	2	3	3	3
24	P51	9	7	5	3	9	2	1	2	3	3	3
25	P53	9	7	7	1	1	1	1	3	3	3	3
26	P57	9	3	7	4	1	3	2	2	3	3	3
27	P58	9	5	7	4	1	2	2	3	3	3	3
28	P61	9	5	7	2	1	1	2	2	1	3	3
29	P63	9	5	5	2	1	3	2	2	1	3	3
30	P68	9	5	7	3	1	2	1	3	3	3	3
31	P76	9	7	7	1	1	2	1	3	2	5	3
32	P77	1	7	5	3	1	2	2	3	3	5	3
33	P78	9	7	7	4	1	2	2	3	3	3	3
34	P84	9	7	7	3	9	3	2	2	1	3	3

Table no. 15 : classification of genotypes into resistant ,moderately resistant and susceptible ones

RESISTANT GENOTYPES	**MODERATELY RESISTANT GENOTYPES**	**SUSCEPTIBLE GENOTYPES**
C2 Ujala	C3PU-31	CPRBAMGP-231
KVK jagatsinghpur	C4CBG-31	KVK Nayagarh-2
CPRBAMGP-217	C1 Prasad	C2 Ujala
C4OBG-31	CPRBAMGP-239	CPRBAMGP-224
KVK Nayagarh 1	KVK Puri-3	
CPRBAMGP-231	C1 Prasad	
KVK Nayagarh 3	CPRBAMGP-236	
C1Prasad	CPRBAMGP-233	
CPRBAMGP-240	C1Prasad	
C3-PU-31		
CPRBAMGP-241		
CPRBAMGP-231		
KVKPuri-2		
C1-Prasad		
C2 Ujala		
C4 OBG -31		
C3 PU 31		
KVK Jajpur		

Chlorophyll analysis :

Chlorophyll content was estimated by Arnon method .

Fig 8 : chlorophyll analysis

Genotype	A470nm	A645.0 nm	A663nm
C2 Ujala R1	0.121	0.049	0.051
C2 Ujala R2	0.001	0.002	-0.000
C2 Ujala R3	0.000	0.000	0.000
C3PU31-R1	0.669	0.301	0.734
C3PU31-R2	0.439	0.243	0.575
C3PU31-R3	0.746	0.314	0.794

The table above depicts the chlorophyll absorption obtained at 470nm, 645 nm, and at 663 nm for control genotypes . Unfortunately for cold stresssed plants, the chlorophyll estimation could not be done due to closure of the department .

With respect to objectives , all the results especially biochemical analysis results could not be obtained due to sudden close down of college on 23rd March, 2020 due to the onset of COVID-19 pandemic.

DISCUSSION

Black gram is the third most important pulse crop in India and occupies a unique position in Indian Agriculture. It's cultivation in India is about 3.25 million hectares with an annual productivity 1.45 million tonnes(Arulbalachandran *et al* .,2010).Although India is the main producer of black gram but it's production is limited due to various biotic and abiotic stresses.(Varalaxmi *et al* .,2007) .Abiotic stresses can be atmospheric like cold, heat and UV irradiation ,or can also be edaphic like salinity, drought and heavy metal toxicity (Wani and Gosal,2011; Surekha et al.,2015). Of all these, cold stress is regarded as a major environmental factor which limits expansion of agriculture and productivity of crop in hilly terrains (Sanghera et al .,2011). Depending on the extent of sensitivity among plants, cold stress has been subdivided into two types. Chilling stress is characterized by 0-15 degree centigrade whereas freezing stress is caused by temperature below 0 degree centigrade. (Wani et al .,2013).

The results that were obtained during the screening experiment at 10 degree centigrade involving 34 genotypes both under normal /control condition and cold stress condition are discussed in this section. After 1st day of sowing in control condition, in the genotype C3PU31 100 % germination was observed where all the 5 seeds germinated. Least germination was observed in KVK Puri 3 , C1 Prasad and C4OBG31 where only 1 seed germinated out of 5 seeds. After 1st day of sowing in cold stress condition, highest germination was observed in the genotype C4OBG31 ie about 62.5% whereas least germination was observed in C2Ujala and C1Prasad were only 1 seed germinated out of 8 seeds. At 2DAS in control condition , all the seeds were germinated in the variety KVK Nayagarh 3, KVK Puri 2 , C3PU31 , KVK Nayagarh 1 and C2 Ujala whereas least germination was observed in C2 Ujala where only 1 seed germinated . At 2DAS in cold stress condition, 100 % germination was observed in the genotype C3PU31 whereas in the genotype CPRBAMGP 224, not a single seed germinated . At 3DAS in control condition , 100% germination percentage was recorded in the genotypes KVK Nayagarh 3 , KVK Puri2, KVK Nayagarh 1, C4OBG31 , CPRBAMGP240 and C2 Ujala and least germination was observed in the genotype C1prasad where only 1 seed germinated out of 5 . At 3DAS in cold stress condition , 100 % germination was recorded in the genotypes KVK Nayagarh3 , CPRBAMGP 231, KVK Nayagarh 1, KVK Jagatsinghpur, CPRBAMGP 240, C3PU31 , C2 Ujala and C1Prasad whereas in the genotype C2 Ujala lowest germination rate was

observed i.e 37.5% . At 5 DAS in control condition , 100 % germination rate was observed in the genotypes KVK Puri2, C4OBG31, KVK Puri 3 , C3PU31 , C2Ujala , C4OBG 31 and CPRBAMGP 229 at 5DAS in control condition. Lowest germination was observed in C1Prasad . At 5 DAS in cold stress condition, 100 % germination was observed in the genotypes KVK Nayagarh 1, C4OBG31, KVK Jagatsinghpur , CPRBAMGP 217 , CPRBAMGP 240 , C3PU31 and C1Prasad whereas least germination was observed in genotypes CPRBAMGP 224 and C2 Ujala where the germination percentage was only 37.5 % . At 5 DAS in control condition ,highest shoot length was observed in the genotype KVK Nayagarh 3 ie about 8.7 cm and highest root length was observed in the genotype KVK Nayagarh 2 and CPRBAMGP 229 ie about 4.5 cm whereas in the genotype C3PU31, root and shoot length was almost same of about 4 cm. At 5DAS in cold stress condition, it was observed that there was no emergence of radicle . So only shoot length was measured and recorded. Highest shoot length of about 5cm was observed in the genotypes C3PU31 and KVK Nayagarh 3 and lowest shoot length was observed in the genotype KVK Puri 3 where only a 0.5 cm of plumule emerged .

Chlorophyll content

Leaf chlorophyll content, a good indicator of photosynthesis activity ,mutations, stress and nutritional state, was reported to be a special significance to precision agriculture (Wu *et al* , 2008) . Some authors have generally attributed the pigment loss to the destruction of chlorophyll structure by excessive ROS and /or by the stimulation of chlorophyllase activity .(Triantaphylides and Havaux , 2009) . Low temperature (10 degree centigrade) is expected to cause a decrease in chlorophyll a,b of all the 4 genotypes at stress(10 degree centigrade) as compared to control conditions(> 10 degree centigrade).

Proline

Proline accumulation has been reported to occur after salt, drought, high temperature, **low temperature**, heavy metal ,pathogen infection, anaerobiosis ,nutrient deficiency, atmospheric pollution and UV irradiation(Hare and Cress 1997; Saradhi et al,1995; Siripornadulsil et al,2002). Early in vitro studies showed that Pro acts as ROS scavenger.(Smirnoff and Cumbes 1989). Proline has been proposed to act as a compatible osmolyte and to be a way to store carbon and nitrogen (Hare and Cress 1997). Proline is an amino acid that under abiotic stresses , performs the role of osmotic regulation.. Under oxidative stress,

it acts as an anti-oxidant . As per review literature , proline accumulation would be greater in leaves of stress plants (10 degree centigrade) compared to control plants.

Total soluble carbohydrates

Sugars frequently accumulate in plants exposure to cold stress, especially if the temperature is insufficient to kill tissues(Levitt,1980). As per review literature , total soluble carbohydrates content(TSC) also expected to increase in the stressed genotypes compared to contol plants.

Protein content :

Protein content of different pulses varies with genotypes, germination, environmental conditions and application of fertilizers during growth and development (Singh 2017). Pulse proteins chiefly comprise of globulins which are soluble in salt solutions and albumins (soluble in water) while prolamins(soluble in alcohol) and glutelins(soluble in dilute acid / base) are minor proteins and constitute a small portion , generally less than 5 % . As per the methodology , I had to estimate the protein content both qualitatively and quantitatively but unfortunately due to COVID-19 pandemic ,I was unable to estimate protein content.

Catalase

Catalases present in peroxisomes are tetrameric heme that contains enzymes for converting peroxide to oxygen and water . (Srivalli et al ; 2003 , Ben Amor et al ;2005). Catalases are the important scavenging enzymes helpful in directly dismutating H2O2 and are indispensable for detoxification of ROS (Van Breusegem et al ;2011). CAT is indispensable for ROS detoxification during stressed conditions .(Willekens et al 1997). Catalase has been reported to induce tolerance to chilling in maize seedlings (Prasad 1997), however there was decrease in catalase activity during chilling stress in cucumber (Lee and Lee 2000).

Yield and it's attributes :

Yield is a quantitative character and is the result of various physiological and biochemical processes . It is expected that there would be a significant variation in the seed yield and it's components among the 4 black gram genotypes sown. They would vary with respect to number of pods/plant, seeds/pod, hundred seed weight, biological yield/ plant and harvest index as per expectation. However , due to COVID -19 pandemic , yield and it's attribute analysis was unable to be performed .

SUMMARY AND CONCLUSION

First of all screening experiment was carried out using 34 genotypes of Black gram (*Vigna mungo* L .) to study their physiological parameters like germination percentage, germination index , root and shoot length characteristics in relation to low temperature and to identify the resistant and susceptible varieties. The cold temperature that was provided inside plant growth chamber is 10 degree centigrade for 5 days. It was observed that germination index value significantly decreased under cold stressed condition in comparison to control condition . The germination index value was highest for the genotype CPU31 followed by KVK Nayagarh 3. So on the basis of germination data obtained , varieties were grouped into 3 categories ie resistant, moderately resistant and susceptible . C2Ujala, KVK jagatsinghpur, CPRBAMGP-217, C4OBG-31, KVK Nayagarh1, CPRBAMGP-231, KVK Nayagarh 3, C1 Prasad, CPRBAMGP-240 , C3PU31,CPRBAMGP-231, KVK Puri -2, C1 Prasad , C4 OBG -31 and KVK Jajpur were classified under **resistant category ,** C4 CBG 31,C1 Prasad, CPRBAMGP 239 , KVK Puri -3 , CPRBAMGP-236 , CPRBAMGP 233 were classified under **moderately resistant** , KVK- Nayagarh-2,CPRBAMGP-224,CPRBAMGP-231 and C2 Ujala were classified under **susceptible category**. Then the most resistant and highly susceptible varieties identified from screening were taken for polybag experiment**. C3PU31** and **KVK Nayagarh 3** were taken as resistant varieties and **C2Ujala** and **C4OBG 31** were taken as susceptible varieties. 3 replications each of susceptible and resistant variety were taken and sown in pots on 26th February ,2020. Simultaneously control genotypes were also kept. Total 24 polybags were taken and 3 seeds in each polybag were sown. Then upto 15 days , morphological features were recorded using DUS guidelines. Then 1st cold stress was given and 1st chlorophyll analysis of control was done . But the chlorophyll analysis was unable to be completed due to close down of department . So the full data was not obtained. But as per review literature , exposure to low temperature (10 degree centigrade) would significantly decrease chlorophyll a, chlorophyll b contents in all the black gram genotypes . Under stress (10 degree) condition , the decline in all photosynthetic pigments is expected to be maximum in C2 Ujala and minimum in C3PU 31.

Since the biochemical analysis and yield attribute analysis was unable to be performed due to the onset of COVID -19 pandemic, so some expected conclusions are drawn regarding to biochemical analysis by observing the review literature and previous studies.

The black gram genotypes C3PU31 and KVK Nayagarh 3 is expected to show higher accumulation of proline and TSC contents than other black gram genotypes because literature says proline and TSC accumulation is greater in resistant genotypes as compared to susceptible ones. These osmolytes is expected to contribute better osmoregulation in C3PU31 and KVK Nayagarh 3 in comparison to all others.

The specific activities of reactive oxygen species (ROS) scavenging enzyme ie catalase is expected to increase in the black gram genotypes at cold stressed condition as compared to their respective control (normal temperature) .

So , the conclusion that the genotypes C3PU31 and KVK Nayagarh 3 were found to be tolerant to cold stress than other genotypes were made on the basis of various physiological features (germination percentage , germination index , root and shoot length) studied. So. these black gram genotypes can be utilised for further crop improvement programme of black gram for cold tolerance.

REFERENCES

Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G., & Sharma, S. 2010.Roles of enzymatic and non enzymatic antioxidants in plants during abiotic stress. *Critical reviews in biotechnology*, ***30***(3), 161-175.

Baisakh, B. Jena, B., Das, T. R., & Panigrahi, K. K. 2013. Genetic architecture of yield and cold tolerance in land races of greengram from Odisha , *Journal of Food Legumes*, ***26***(1and2), 20-25.

Bhandari, K., Sharma, K. D., Rao, B. H., Siddique, K. H., Gaur, P., Agrawal, S. K., ... & Nayyar, H. 2017. Temperature sensitivity of food legumes: a physiological insight, *Acta physiologiae plantarum*, *39*(3), 68.

Choudhary, A. K., Sultana, R., Chaturvedi, S. K., Sharma, R., Bhatt, B. P., & Singh, S. P., 2014,. Breeding strategies to mitigate abiotic stresses in pulses. *Emerging Challenges and Opportunities for Biotic and Abiotic Stress Management (ECOBASM)*, 16-21.

Esra, K. O. Ç., İŞLEK, C., & Üstün, A. S. 2010. Effect of cold on protein, proline, phenolic compounds and chlorophyll content of two pepper (Capsicum annuum L.) varieties, *Gazi University Journal of Science*, ***23*(**1), 1-6.

Gill, S. S., & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants , *Plant physiology and biochemistry*, ***48***(12), 909-930.

Gill, S. S., Anjum, N. A., Hasanuzzaman, M., Gill, R., Trivedi, D. K., Ahmad, I., ... & Tuteja, N. ,2013, Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry*, ***70,*** 204-212.

Kilian, J., Whitehead, D., Horak, J., Wanke, D., Weinl, S., Batistic, O., ... & Harter, K. 2007. The AtGen Express global stress expression data set: protocols, evaluation and model data analysis of UV- B light, drought and cold stress responses, *The Plant Journal*, ***50***(2), 347-363.

Kumar, M., *Morpho-physiological and biochemical traits for cold tolerance of chickpea (Cicer arietinum L.) genotypes* (Doctoral dissertation, CCSHAU).

Leung, J., & Giraudat, J. 1998. Abscisic acid signal transduction, *Annual review of plant biology*, ***49***(1), 199-222.

Mazzucotelli, E., Mastrangelo, A. M., Crosatti, C., Guerra, D., Stanca, A. M., & Cattivelli, L. 2008. Abiotic stress response in plants: when post-transcriptional and post-translational regulations control transcription. *Plant Science*, ***174***(4), 420-431.

Nayak ,S. , *Molecular characterization of black gram and validation of markers for cold tolerance* (Doctoral dissertation, Orissa University of Agriculture and Technology, Bhubaneswar).

Ottander, C., Hundal, T., Andersson, B., Huner, N. P., & Öquist, G. 1993. Photosystem II reaction centres stay intact during low temperature photoinhibition , *Photosynthesis research*, ***35***(2), 191-2000

Rihan, H. Z., Al-Issawi, M., & Fuller, M. P. 2017. Advances in physiological and molecular aspects of plant cold tolerance , *Journal of Plant Interactions*, ***12(***1), 143-157.

Roychoudhury, A., Banerjee, A., & Lahiri, V. , 2015., Metabolic and molecular-genetic regulation of proline signaling and itscross-talk with major effectors mediates abiotic stress tolerance in plants, *Turkish Journal of Botany*, ***39(***6), 887-910.

Shevkani, K., Singh, N., Chen, Y., Kaur, A., & Yu, L.2019. Pulse proteins: Secondary structure, functionality and applications, *Journal of food science and technology*, 1-12.

Singh, R., Singh, M. K., Singh, A. K., & Singh, C. 2018. Pulses production in India: Issues and elucidations, *Pharma Innov* ***, 7***(1), 10-13.

Sivasubramaniam K., Geetha, R., Sujatha, K., Raja, K., Sripunitha, A., & Selvarani, R.2011.Seed priming: triumphs and tribulations, *The Madras Agricultural Journal*, ***98***(7-9), 197-209.

Solanki, M. V., Trivedi, S. K., Kandoliya, U. K., & Golakiya, B. A. (2018). Effect of exogenous application of salicylic acid on biochemical constituent in black gram (Vigna mungo L.) Hepper irrigated with saline water. *European Journal of Biotechnology and Bioscience****, 6***(5), 28-34.

Tripathy SK, Mishra , Rout GR and Das AB.2011.Biochemical and Molecular Basis of Cold Tolerance in Plants, In :Biotechnology: A New Approach,(P.C Trivedi Eds.) Agrobios(India) : pp 161-187

Verbruggen, N., & Hermans, C. 2008.Proline accumulation in plants: a review, *Amino acids*, ***35***(4), 753-759.

Xiaochuang, C., Chu, Z., Lianfeng, Z., Junhua, Z., Hussain, S., Lianghuan, W., & Qianyu, J. 2017. Glycine increases cold tolerance in rice via the regulation of N uptake, physiological characteristics, and photosynthesis, *Plant Physiology and Biochemistry*, ***112***, 251-260.

Yadav, D. K. *MORPHO-PHYSIOLOGICAL, ANATOMICAL AND BIOCHEMICAL CHANGES AS INFLUENCED BY PACLOBUTRAZOL IN MUNGBEAN [Vigna radiata (L.) Wilczek] UNDER FLOODING STRESS* (Doctoral dissertation, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi).

Yu, S. W., & Tang, K. X. 2004. MAP kinase cascades responding to environmental stress in plants, *ACTA BOTANICA SINICA-ENGLISH EDITION*-, ***46***(2), 127-136.

Zwack, P. J., & Rashotte, A. M. 2015. Interactions between cytokinin signalling and abiotic stress responses. *Journal of experimental botany*, ***66***(16), 4863-4871.