Effect of Sodium Chloride and Polyethylene Glycol on Some Physiological and Biochemical Activity on Growth of *Cajanus Cajan* L., Cv Upas-120.

Mubashar N Mintoo, R.K. Agnihotri, Rajendra Sharma, Mubashar Nazir Mintoo

***Abstract*— This study examined the expression of salinity and osmotic adjustment in the photosynthetic pigments and accumulation of compatible solute and other metabolites in stress imposed by water and salt in *Cajanus cajan* L. cv. UPAS-120. Pigeonpea (*Cajanus cajan* L. cv. UPAS-120) plant was grown in Hoagland nutrient medium with or without different concentration of NaCl (0.1, 0.2, 0.3 and 0.5 M) and polyethylene glycol 6000 (-0.45, -0.60, -0.75 MPa) and four harvests (15, 20, 30 and 40 d after treatment) were analysed. After three weeks of growth, there was decrease in root and shoot length, total chlorophyll content and protein content in leaves. The major effect was imposed by PEG at (-0.75 MPa) and NaCl (0.5M). However, lower concentration of NaCl and PEG has less effect on these parameters. At 0.5M concentration of NaCl and –0.75MPa of PEG, there was about 92.27% and 90.41% reduction in total chlorophyll content. Proline content and total soluble sugars were the only parameters which were increased significantly when the concentration of NaCl and PEG was increased. The accumulation of compatible solutes is more a consequence of damage produced by salt stress than of a protective strategy. This study may be useful to farmer of this area for screening of the salinity level upto which extent the *Cajanus cajan* L. cv. UPAS-120 line could be grown to enhance the crop production, as this region is particularly having high salinity level and osmotic stress.**

***Index Terms*— Cajanus cajan, drought stress, pigment composition, proline, salt stress, Soluble sugars.**

Abbreviations:Chl Chlorophyll, NaCl :Sodium Chloride,PEG : polyethylene glycol 6000 Ψ_w Water potential, MPa = Mega Pascals

I. INTRODUCTION

Plants when imposed to various abiotic stress factors like drought, salinity, heat, temperature and heavy metals show adverse affect on growth, metabolism and productivity of diverse crops. These stress show dehydration which result in less availability of water to plant cells and may cause several agricultural problems in arid and semi arid regions like India (Abdul et al., 2006; Rodriguez et al., 2005; Zhang *et al*., 2006). These stress ranges from synthesis of limited quantities of specialized metabolites and large shift in primary metabolite composition which causes many physiological changes. This causes tilt in equilibrium beyond normal fluctuation boundaries which constitute a stress parameter. Salinity and Drought are one of the prevalent environmental conditions that cause adverse effect on growth of plant and limit the crop productivity (Ncube et al. 2013).

Plants absorb very less amount of water from saline environment and thus they cannot easily survive. Salinity stress affects nutrient uptake and metabolic activities in plants (Singh and Hoque, 2007). About 20 % of the world agricultural lands are affected by salinity which mainly causes reduction in the growth, low germination rate, accelerated leaf senescence and accumulation of ROS, such as superoxide radical ($O^{\cdot}_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) which seriously disrupt normal metabolism by oxidative damage causing lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Chaves et al. 2009; Scandalios, 1993).

The photosynthetic process provides mainly carbon and nitrogen precursors which drive these diverse complex metabolic processes. The reduction of water potential that occurs in both salinity and osmotic stress alters the ratio between the rate of light absorption and photosynthetic capacity which lead to photoinhibition (Angelopoulos et al. 1996).

Plants have developed a number of strategies to cope with stress, such as production of osmoregulators as proline. Proline accumulation usually occurs in primary response to stresses that dehydrate plant tissue such as Salinity, drought and freezing (Verslues et al, 2006). Under certain circumstances of salinity and drought stress, proline accumulation serve as osmoregulator by acting as compatible solute to increase cellular osmolarity thereby avoiding deleteriously high ionic strength and preventing cell against oxidative and osmotic stresses. Besides this, it also has important role in the redox buffering, energy transfer, protecting cellular enzymes, storage of carbon and nitrogen for rapid growth, proteins and cellular degradation, detoxification of various free radicals formed by stress by forming long lived adducts with them and decontamination procedures for exclusion and compartmentalization of sodium ions in the vacuoles (Smiroff and Cumbes, 1989; Hare and Cress, 1997; Yeo 1998).

Pigeonpea (*Cajanus cajan* L.) is one of the drought tolerant legume of the tropics and subtropics needing low to medium rainfall, though it is well adapted to several environmental conditions (Troedson et al., 1990). The crop represents about 5% of the world legume production (Hillocks et al. 2000) with more than 70% being produced in India. It is cultivated in the

Mubashar N Mintoo, Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra, India
R.K. Agnihotri, Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra, India
Rajendra Sharma, Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra, India
Mubashar Nazir Mintoo, Corresponding author

gross cropped area of 3.58 million ha under pulses, providing 20% of the national pulse production (2.51 m tones) which accounts for 90% of the world's Pigeonpea production (Kumar *et al.*, 2011).

NaCl and Polyethylene glycol 6000 (PEG-6000) have been used to simulate salinity and drought stress in *in vitro* and pots for plants to maintain uniform water potential throughout the experimental period. Polyethylene glycol 6000 is commonly used to impose water stress because it is inert, non-ionic and having high molecular weight and thus cannot pass through root cell walls making it cell impermeable. They are small enough to influence osmotic pressure, but large enough unabsorbed by plants (Berg and Zeng, 2006). Therefore, they are frequency used to stimulate osmotic stress (Bhargava and Paranjpe 2004; Radhouane 2007 Kaydan and Yagmur; 2008). The aim of the present study was to compare the effects of drought and salt stress and identify how pigeonpea variety *(Cajanus cajan* L. cv. UPAS-120) grown in Agra region respond to different level of salinity and drought stress induced by NaCl and polyethylene glycol at seedling level. The studies were conducted to evaluate the effect of such a stress on seedling survival, photosynthetic pigment content, carbohydrate content and accumulation of proline in *Cajanus cajan*. To ensure sustainable crop productivity, it is imperative to design and evolve improved versions of crop plants that can endure the detrimental effects of changing environmental factors.

II. MATERIAL AND METHODS

The experiments were conducted at the Botanical garden of the Department of Botany, School of Life Science, Dr.B.R. Ambedkar University, Agra, India, during the month of August – July.

Plant material and growth conditions

Seeds of Pigeonpea (*Cajanus cajan L.)* cultivar UPAS-120 cultivar were obtained from National Seed Corporation, Sikandra, Agra, India. The seeds were soaked in 10 % bavistin for 8-10 min and then washed under running tap water for 10 min to remove the remaining Bavistin. This was followed by quick rise with 70% ethanol for 10 s. After evaporating ethanol, seeds were surface sterilized by 0.1% mercuric chloride (w/v) solution for 10 min. The seeds was subsequently washed for 6-7 times with sterile distilled water to remove all the traces of mercuric chloride and later imbibed in distilled water for one day. Seeds were germinated with distilled water in Petri dishes in plant growth chamber (Model; 3500 G; Saveer Biotech Ltd., New Delhi) in Agra (26" 44' north and 77" 26' E) at 25 ± 2ºC and humidity ranging between 72±5% RH in the dark, following recommendation of International Seed Testing Association (Anonymous, 1996). After one week ten uniform seedlings per pot of *Cajanus cajan* were transplanted in earthen pots containing 3:1 soil and FYM supplemented with Hoagland's nutrient medium (Hoagland and Arnon, 1950). After two weeks of growth, the plants were exposed to stress for 3 weeks. To create salinity stress and water stress, sodium chloride (NaCl) and polyethylene glycol (PEG-6000) solution (0.1M, 0.2M, 0.3M, and 0.5M NaCl; Ψ_w of -0.40, -0.50, -0.60 and -0.75MPa) representing different salinity and osmotic levels. Plant grown without NaCl and PEG addition were taken as control. Sodium chloride and polyethylene glycol were given twice a week. Fourteen days after initiation of stress treatment, samples were taken from each treatment separately. These samples were processed for seedling survival and various biochemical parameters such as total chlorophyll, proline content and total soluble sugar content.

Seedling survivals were observed during the seedling growth period, the seedlings were daily observed and the number of dead seedlings were counted and discarded from the pots (Agnihotri, et al., 2009).

Total Chlorophyll and Carotenoid Content

Total chlorophyll and carotenoid content in fresh leaves was estimated by using Brougham (1960) method. The fresh leaf tissues (200 mg) from were ground in a mortar and pestle containing 80% acetone (up to 20 ml). After centrifugation for 10 min at 10,000 rpm, absorbances (OD) were read at 663 and 646 nm (chlorophyll a and b) and 470 nm (carotenoids) using a spectrophotometer (Spectronic 20D, Milton Roy, USA). Concentration of chlorophyll 'a' and 'b' and total carotenoids (mg g^{-1} FW) were calculated according to Arnon's (1949).

Total protein content

For the estimation of protein in leaves and shoot parts, Folin-Lowry method (1975) was used. For each replicate, 100 mg of plant material was homogenized in 0.8 ml of 0.2M ice cold potassium phosphate buffer. After centrifugation at 7,000 x g, 30 min at 4ºC, a aliquot of 0.1 ml of supernatant was mixed with 0.5 ml of freshly prepared alkaline solution. After 10 min, 0.5 ml of Folin ciocalteau reagent was added and change in absorbance at 750 nm was measured spectrophotometrically. Total protein content was calculated using Bovine Serum Albumin (BSA, Sigma : 20 – 200 µg) as a standard.

Free proline content

Free proline content was estimated by following the method of Bates et al, (1973). 1gm of fresh leaves samples were homogenized in 10 ml of 3% (w/v) sulphosalicylic acid by using mortar and pestle. 5 ml of extract was in test tube and to it 5 ml of glacial acetic acid and 5 ml of ninhydrin reagent was added. The reaction mixture was boiled in water bath at 100°C for 30 min. After cooling the reaction mixture; 10 ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and absorbance of red color developed was read at 520 nm. Standard curve was prepared by using pure proline (BDH).

Estimation of Total soluble sugars

For estimation of total soluble sugars Dubois *et al*., (1956) method was used. Dried powder (50 mg) was extracted with 80% ethanol at room temperature for one hour and centrifuged at 1000 x g at 25ºC for 60 minutes. About 0.5 ml phenol reagent was added. The colour was developed by rapidly adding 5 ml conc. H_2SO_4. The absorbance was recorded at 485 nm. Standard curve was prepared by using D-Glucose.

Statistical Analysis

Experiments were conducted in triplicate and data was subjected to one way ANOVA and the correlation between different physiological traits was evaluated with the help of a SYSTAT programme of SPSS Software Inc., version 10.0 Chicago, USA. The mean was further separated using Least Significant Differences.

III. RESULTS

Saline conditions bring the stunting of plant growth, restrict the lateral shoot development and reduce the size of leaves and fruit. Increasing NaCl and PEG concentration gradually decreases the growth of Pigeon pea plant, its total chlorophyll content, protein content but the only biochemical parameters i.e. total soluble sugars and proline content was increased.

Effect of salinity stress on seedling survival

As the concentration of NaCl and PEG increases, the seedling survival decrease significantly. Control plant exhibit 96.94% survival, while as the plant grown under 0.1M, 0.2M, 0.3M and 0.5M of NaCl concentration, showed 80.77%, 55.56%, 46.43% and 45.83% survival percentage, respectively. While as plant grown in -0.40MPa, -0.50MPa, -0.60MPa and -0.75MPa of PEG solution, the survival percentage was 71.43%, 70.23%, 54.33%and 44.45% respectively.

But the treatment with PEG poses less harmful for seedling survival than NaCl (Fig. 1). The seedling survival decreased by 16.68% under 0.1M, 42.68% under 0.2M, 52.10% under 0.3M and 45.83% under 0.5M of NaCl. PEG reduced seedling survival by 26.31% under -0.40MPa, 27.55% under -0.50MPa, 43.96% under -0.60MPa and 54.14% under -0.75 MPa. (Table 1)

Total chlorophyll and carotenoids contents

The pigment displayed a characteristic decrease with the increase in salinity and osmotic stress chlorophyll and carotenoids contents of pigeon pea plant were affected significantly by osmotic stress induced by NaCl and PEG. The maximum total chlorophyll (1.65 mg g^{-1} fresh weight) was recorded in 0.1M NaCl and minimum (0.16 mg g^{-1} fresh weight) in 0.5M NaCl; whereas in PEG treated plants, the maximum chlorophyll content (1.098 mg g^{-1} fresh weight) was recorded in -0.40 MPa and minimum (0.1648 mg g^{-1} fresh weight) in 0.50 MPa and maximum carotenoid content was (0.210 mg g^{-1} fresh weight) recorded in 0.1M NaCl, whereas minimum (0.08 mg g^{-1} fresh weight) in 0.3 M NaCl.

In PEG treated plants, the maximum carotenoids (0.76 mg g^{-1} fresh weight) was recorded in -0.40 MPa and minimum (0.038 mg g^{-1} fresh weight) was recorded in -0.50 MPa.The chlorophyll content was decreased by 20.21% under 0.1M NaCl, 29.45% under 0.2M NaCl, 84.54% under 0.3M and 92.27% under 0.5 M NaCl concentration (Table 1; Fig. 2). However in case of PEG, total chlorophyll was decreased by 46.96% under -0.40 MPa, 92.04% under -0.50 MPa, 89.88% under 0.60 MPa and by 90.41% under -0.75 MPa.

Similar to the total chlorophyll content, carotenoid content was also decreased by the induction of osmotic stress of NaCl and PEG. Carotenoids concentration decreased by 78.79% under 0.1 M, and 87.88% under 0.2M, 91.92% under 0.3M and 78.79% under 0.5M NaCl whereas in PEG treated plants, the carotenoids contents decreased 23.23% under -0.40 MPa, 45.45% under -0.50 MPa, 59.60% under -0.60 MPa and 33.33% under -0.75 MPa (Table 1; Fig. 2).

Total Protein content.

Proteins are the main components of nucleic acid, cell membrane and other cell organelles. Salts are known to reduce the protein contents in plants. Data presented in Table 2, demonstrate the deleterious effects of NaCl and PEG on protein content. This detrimental effect was more due to NaCl compared to PEG. As evident from the Table 2 control plants exhibit 99.2mg^{-1}g FW while as plant grown under 100 mM, 200 mM, 300 mM and 500 mM stress shows 93, 45.88, 46.19 and 46.19 mg^{-1}g FW of protein content respectively and 80.6, 54.56, 58.90 and 86.80 mg^{-1}g FW was found under -0.40MPa, -0.50 MPa, -0.60MPa and -0.75MPa of PEG, respectively (Table 2). The protein content of pigeon pea plant decreased by 6.25% under 0.1M NaCl, 53.75% under 0.2M NaCl under 54.44% under 0.3M NaCl, 54.44% under 0.5M NaCl.

PEG decrease the protein content of pigeon pea by 18.75% under -0.40MPa, 45% under -0.50, 40.63% under -0.60MPa, and 12.5 under -0.75MPa (Fig 3, Table 2).

Total carbohydrate content.

The carbohydrate content was decreased with the increase in concentration of NaCl and PEG. Control plant exhibit maximum (1.87 mg^{-1}g FW) carbohydrate content and the minimum (0.63 mg^{-1}g FW) reported in 0.3M NaCl concentration. Carbohydrate content was reduced by 44.39% under 0.1M NaCl, 55.61% under 0.2M NaCl, 66.31% under 0.3M NaCl and 52.94% under 0.5M NaCl. PEG proved less harmful for carbohydrate content and reduced carbohydrate contents by 17.64% under -0.40MPa, 23.53% under -0.50MPa, 52.94% under -0.60MPa and 40.64% under -0.75MPa (Fig. 3: Table 2) and correlation between carbohydrate content and the carotenoids have also shown in Fig 3.

Effect of NaCl and PEG on proline synthesis

The proline content increased significantly when concentration of NaCl and PEG increases. The plant grown with NaCl and PEG showed rapidly accumulation of proline. In case of NaCl treated plants maximum proline content (0.259 mg^{-1}g FW) was recorded in 0.5M NaCl and minimum (0.064 mg^{-1}g FW) in control.However in case of PEG treated plants, maximum proline content (0.231 mg^{-1}g FW) were recorded in -0.75MPa and minimum (0.064 mg^{-1}g FW in control) (Table 2). Correlation between proline content and the seedling survival have also depicted in Fig 4.

For the significance of different biochemical parameters, the regression equations of different growth parameters were estimated separately. The Correlation between NaCl and PEG induced stress on the carbohydrate and carotenoid contents of pigeonpea is shown in graph (Fig: 6) and that of proline and plant survival (Fig: 7) show significant role in different salinity levels.

IV. DISCUSSION

In India, plants growing under rainfed conditions (without irrigation) are often exposed to period of water deficit that may have a negative effect on photosynthesis, growth and yield. Drought and salt stress invariably affect the photosynthesis leading to imbalances between absorbed light energy and energy utilized through metabolism. The main target for the photoinhibition to photosynthetic capacity under these stresses is photosystem II (PSII) (Martinez et al, 2012). The long progressive stress along with some other environmental factor may affect photosynthetic ability of the plant system. In the present study, the total chlorophyll content of pigeonpea leaves was decreased with the increasing concentration of NaCl and PEG. Maximum reduction in the total chlorophyll content was observed in 0.5M NaCl and -0.50 MPa of PEG. However, in case of PEG treated plants, reduction was increased in -0.50 MPa and then decreased in the proceeding concentration. The reduction in chlorophyll content under stress at various developmental

stages is commonly reported phenomenon in various studies. This may be because of membrane deterioration (Ashraf and Bhatti, 2000); inhibition of chlorophyll synthesis or an acceleration of its degradation (Reddy and Vora, 1986); destruction of chlorophyll pigments and the instability of the pigment protein complex (Levit, 1980, Ncube, 2013); interference of salt ions with the *de novo* synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (Jaleel et al., 2007); inhibition of photosynthesis through stomatal closure, which decreases biomass (Yousfi et al, 2010). There is also a reason for decrease in chlorophyll content that the drought or salinity stress induced by NaCl or PEG produce reactive oxygen species (ROS) such as O_2 and H_2O_2, can lead to lipid peroxidation and consequently, chlorophyll destruction also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Martine et al, 2012). Similar trend was observed in case of protein content of Pigeon pea where maximum reduction was is 0.2M and -0.50 MPa. The values obtained in this study fall within the range reported by earlier workers (Juliana et al, 1966; Devi, 1980, Martinez et al., 2012).

Plants when exposed to stresses by NaCl or PEG, accumulated starch and soluble carbohydrates (Greenway and Munns, 1980). This accumulation has been attributed to impaired carbohydrate utilization (Dhanapackiam and Ilyas, 2010). It is apparent from the results that the soluble carbohydrate content in the leaves was higher in salt stress plants compared with control. An increase in the accumulation of sugars was also reported in many legumes, when exposed to salinity (Gilbert et al., 1997). As the photosynthesis is the main source of carbohydrates accumulation, Munns (1993) has reported that the concentration of sugars and reserve polysaccharides always rise after plants are exposed to salinity in both growing and fully expanded tissues. This is consistent with a blockage in utilization of sugars in the growing tissues and a subsequent build-up in the rest of the plant. A reduction in photosynthesis could be due to feed back inhibition by the high sugar concentrations in the mesophyll cells.

During salinity and drought stress, the accumulation of proline results in balancing the osmotic potential of cytoplasm with that of environment. The accumulation of proline is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and is only achieved when the rate of synthesis prevails over that degradation, probably because too much proline is toxic to plant cell (Yokota et al. 2006). The transcriptional upgradation of proline biosynthesis and down regulation of its degradation is thought to control the proline accumulation during stress (Roshandel and Flowers, 2009; Stines et al, 1999). In present work, increase in the concentration of both NaCl and PEG enhanced the concentration of proline synthesis in pigeon pea seedlings. The sharp increased in proline content at lower concentration might theoretically attribute to the genes for synthesis and degradation of proline which are up-regulated strongly under salinity and drought stress. It might be an adaptation to the purpose of which is to overcome the stress condition and it could supply energy for growth and survival and thereby help the plant to tolerate stress (Sankar et al., 2007). Furthermore, Proline may play a role as an enzyme-stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanein, 2004). As proline has hydrophilic property, it might replace water molecules around nucleic acid, protein and membranes during water shortages. It might also prevent interaction between destabilize ions and cellular components by replacing the water molecules around these components, thereby protecting against destabilization during drought (Yokota et al., 2006). Similar results were observed by Priyanka et al, 2010 and Turan et al., 2007. The maximum increase in the proline content was observed in 0.5M NaCl and -0.75 MPa of PEG whereas minimum proline was reported in control plants. Furthermore, proline perform an important role in free radical scavenging and subcellular structure stabilizing (Ashraf and Fooland, 2007). Therefore, increase accumulation of these organic solutes might improve salt tolerance of *Cajanus cajan*. The proline accumulation was higher in NaCl compared to PEG.

Table : 1 Effect of NaCl and PEG induced stress on the seedling survival of pigeon pea.

Osmotic stress	Concentration	Seedling survival percentage
NaCl (M)	Control	96.94±0.1
	0.1	80.77 ± 0.15
	0.2	55.56 ± 0.04
	0.3	46.43 ± 0.13
	0.5	45.83 ± 0.1
PEG (MPa)	-0.45	71.43 ± 0.12
	-0.50	70.23 ± 0.05
	-0.60	54.33 ± 0.05
	-0.75	44.45 ± 0.03

Table : 1 Effect of NaCl and PEG on the Total Chlorophyll and Carotenoids contents of pigeon pea

Osmotic stress	Concentration	Chlorophyll a (mg g^{-1} FW)	Chloroplyll b (mg g^{-1} FW)	Total chloroplyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
NaCl (M)	Control	1.08 ± 0.01	0.984 ± 0.02	2.069 ± 0.04	0.991 ± 0.09
	0.1	0.877^{ns} ± 0.17	0.771^{ns} ± 0.05	1.651* ± 0.038	0.206^{ns} ± 0.06
	0.2	0.222* ± 0.01	1.234* ± 0.10	1.450^{ns} ± 0.07	0.12* ± 0.01
	0.3	0.164* ± 0.01	0.159^{ns} ± 0.00	0.323* ± 0.04	0.08* ± 0.02
	0.5	0.073** ± 0.01	0.089** ±0.00	0.160** ± 0.00	0.07** ± 0.01
PEG (MPa)	-0.40	0.005** ± 0.00	1.094* ± 0.03	1.098* ± 0.03	0.76 ** ± 0.06
	-0.50	0.157^{ns} ± 0.01	0.008** ± 0.00	0.165* ± 0.02	0.54 ** ± 0.07
	-0.60	0.124* ± 0.01	0.086** ±0.05	0.209^{ns} ± 0.02	0.42** ± 0.11
	-0.75	0.162** ± 0.027	0.162^{ns} ± 0.03	0.199** ± 0.03	0.66** ± 0.12

Data represent average percentage value of three replicates.
Values represent Mean ± standard errorns = Non significant,
* = significant at 5% level of probability, ** = significant at 1% level of probability

Table : 2 Effect of NaCl and PEG induced stress on the carbohydrate content mg g^{-1} of pigeon pea

Osmotic stress	Concentration	mg of Protein gram^{-1} FW	mg of carbohydrate gram^{-1} FW	mg g^{-1} proline
NaCl (M)	Control	99.20 ± 0.19	1.87 ± 0.40	0.064 ± 0.43
	0.1	93.00 ** ± 0.12	1.06 ^{ns} ± 0.14	0.113 ^{ns} ± 0.32
	0.2	45.88 ** ± 0.15	0.83 ** ± 0.16	0.188 ^{ns} ± 0.05
	0.3	46.23** ± 0.13	0.63 ** ± 0.22	0.225* ± 0.03
	0.5	45.19 ** ± 0.22	0.88 ** ± 0.31	0.259 ^{ns} ± 0.05
PEG (MPa)	-0.45	80.60 ** ± 0.15	1.54 ** ± 0.53	0.071 ^{ns} ± 0.20
	-0.50	54.56 ** ± 0.13	1.43 ** ± 0.22	0.101* ± 0.21
	-0.60	58.90 ** ± 0.22	0.88** ± 0.18	0.161 ^{ns} ± 0.27
	-0.75	86.80 ** ± 0.04	1.10 ^{ns} ± 0.03	0.231**± 0.41

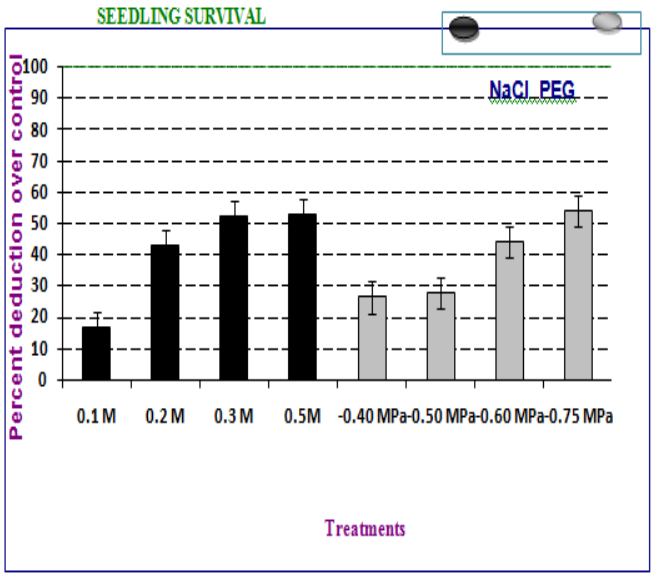

Fig. 1 : Effect of NaCl and PEG induced stress on seedling survival percentage of Pigeon pea. Vertical bars indicate reduction over control.

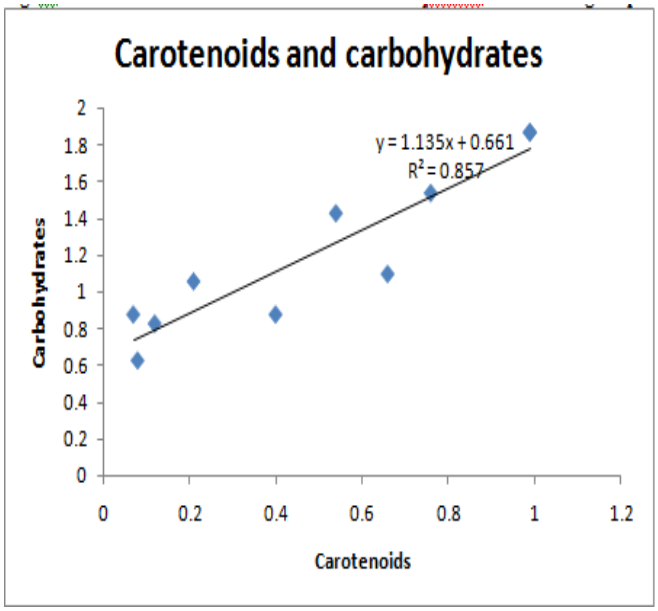

Fig. 2 : Effect of NaCl and PEG induced stress on the proline content of Pigeon pea.

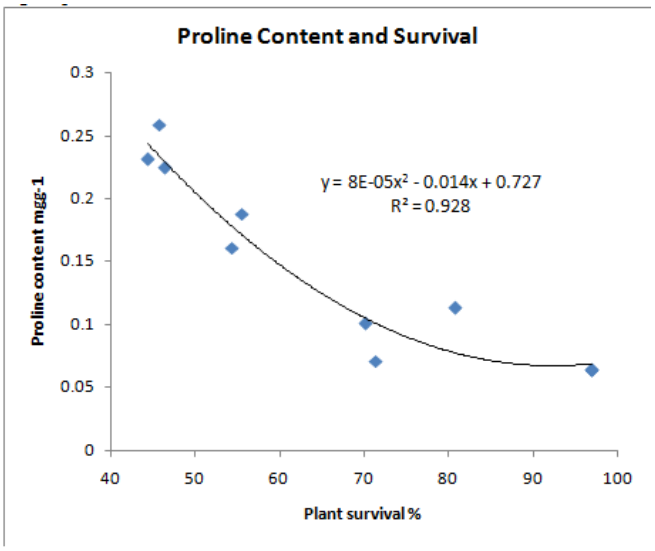

Fig. 3 :Correlation between NaCl and PEG induced stress on the carbohydrate and carotenoid contents of Pigeon pea.

REFERENCES

[1] Abdul, W Ishfaq, AM. Ghulam Q, Ghulam M., Tariq M, and Muhammad A, (2006): Effect of salinity on germination, growth, yield, ionic balance and solute composition of Pigeonpea (*Cajanus cajan* L Millsp.), Pak. J. Bot., 38 (4): 1103-1117.

[2] Agnihotri RK, Palni LMS, Chandra S, Joshi SC (2009): Gas exchange variability and water use efficiency of thirty landraces of rice still under cultivation in Kumaun region of the Indian Central Himalayas. Physiol. Mol. Biol. Plant 15(4) 303-310.

[3] Angelopoulos K, Dichio B, Xiloyannis C (1996): Inhibition of photosynthesis in olive trees (*Olea eropaea* L) during water stress and rewatering. J Exp. Bot. 47: 1093-1100

[4] Anonymous (1996): International rules for seed testing. Seed Sci. & Tech. 24 : 253 – 270.

[5] Arnon, DT (1949): Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15.

[6] Ashraf, MY and Bhatti, AS (2000): Effect of salinity on growth and chlorophyll content of rice. Pak. J. Sci. Ind. Res., 43: 130-131

[7] Ashraf M and Foolad MR (2007): Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environm Exp Bot 59:206–216. doi:10.1016/j.envexpbot.2005.12.006

[8] Bates, LS, Waldren, RP. Teare, ID. (1973). Rapid determination of free proline for water stress studies. Plant and Soil. 39 : 205 – 207.

[9] Berg V L. and Zeng YJ (2006): Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *South Afri. J. Bot.*, 72: 284-286.

[10] Brougham, RW (1960): The relationship between the critical leaf area, total chlorophyll content and maximum growth rate of some pastures and crop plants. Ann. Bot. 24: 463 – 473.

[11] Chaves MM, Flexas J, Pinheiro C (2009): Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560.

[12] Devi, P. (1980): Studies of the properties and cooking characteristics of new varieties of rice. M.Sc. thesis submitted to GBPUA & T, Pantnagar, 95 p.

[13] Dhanapackiam, S and Ilyas, Muhammad MH (2010): Effect of salinity on chlorophyll and carbohydrate contents of *Sesbania grandiflora* seedlings, Indian Journal of Science and Technology 3:64-66

[14] Dubois, M. Gilles KA, Hamilton, JK, Rebers PA, and Smith, F (1956): Colorimetric method for determination of sugars and related substances, Analytical Chemistry, 28:350

[15] Gilbert, GA. Wilson, C and Madore MA (1997): Root zone salinity alters raffinose family oligosaccharide metabolism and transport in Coleus, Plant Physiology, 115:1237

[16] Greenway, H and Munns, R (1980): Mechanisms of salts tolerance in non halophytes. Annual Review of Plant Physiol. Plant Mol. Biol. 31: 149–190.

[17] Hare PD and Cress WA (1997): Metabolic implications of stress induced proline accumulation in plants, Plant Growth Regul. 21: 79-102

[18] Hassanein A (2004). Effect of relatively high concentrations of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (*Alhagi graecrum*) and stress sensitive (*Lycopersicon esculentum* L) plant species. Bulg. J. Plant Physiol. 30: 19-36.

[19] Hillocks, RJ., Minja, E., Nahdy, MS., Subrahmanyam, P. (2000).Diseases and pests of pigeonpea in eastern Africa.International Journal of Pest Management, 46: 7–18.

[20] Hoagland DR, Arnon DI (1950): The water culture method for growing plants without soil. Calif Agric Exp Stn Circ 347:1–32

[21] Jaleel, CA., Gopi, R., Sankar, B., Manivannan, P., Kishore kumar, A., Sridharan, R., and Panneerselvam, R (2007): Studies on Germination, Seedling Vigour, Lipid Peroxidation and Proline Metabolism in *Catharanthus roseus* Seedlings under Salt Stress, *South Afr. J. Bot*, vol. 73, pp. 190–195.

[22] Jaleel, CA., P. Manivannan and GMA Lakshmanan, (2007): NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus*. C. R. Biologies, 330: 806-813.

[23] Juliano, BO, Onate, LU and Delmunds, AM (1966): Relation of starch composition, protein content and gelatinization

[24] Kaydan, D and M. Yagmur, (2008): Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl, Afr. J. Biotech., 7 (16): 2862-2868.

[25] Kumar, RR., Karjol, K. and Naik, GR. (2011): Variation of sensitivity to drought stress in pigeonpea (*Cajanus cajan* L. Millsp) cultivars during seed germination and early seedling growth. World Journal of Sci. Technol. 1(1): 11-18

[26] Levit, J., (1980): Responses of Plants to Environmental Stresses, Academic Press, Vol. II, New York.

[27] Martinez AP, Grana E, Reigosa MJ and Sanchez Moreiras AM (2012): Early Photosynthetic Response of *Arabidopsis thaliana* to Temperature and Salt Stress Conditions *Russ J Plant Physio (59), 5, 640–647*.

[28] Munns R (1993): Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell Environ. 16, 15-24.

[29] Ncube, B, Finnie JF, Staden, JF (2013): Dissecting the stress metabolic alterations in in vitro *Cyrtanthus* regenerants. Plant Physiol and Biochem (65) 102-110

[30] Priyanka B, Sekhar, K, Reddy VD and Rao KV.(2010): Expression of pigeonpea hybrid-proline-rich protein encoding gene (CcHyPRP) in yeast and *Arabidopsis* affords multiple abiotic stress tolerance. Plant Biotechnol Journal (2010) 8, pp. 76–87

[31] Reddy, MP and Vora AB (1986): Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (*Pennisetum typhoides* S and H) leaves under NaCl salinity. Photosynthetica, 20: 50- 55.

[32] Rodriguez, ME. Canales and Hidalgo OB (2005): Molecular aspects of abiotic stress in plants, BiotechlogiaAplicada, 22: 1-10.

[33] Roshandel P and Flowers T (2009): the ionic effects of NaCl on physiology and gene expression in rice genotypes differing in salt tolerance, Plant Soil 315: 135-147

[34] Sankar B, Jaleel C, Manivannan P, Kishorekuma A, Somasundaram R, Panneerselvan R (2007): Drought-induced biochemical modification and proline metabolism in *Abelmoschus esculentus* L. Moench.Acta. Bot. Croat. 66:43-56

[35] Singh R, Hoque MS (2007): Changes in growth and metabolic activity in seedling of lentil (*Lens culinarys* Medik.) genotypes during salt stress.Indian J. Pl. Physiol. 6: 406-410.

[36] Smirnoff N and Cumbes, QJ (1989): Hydroxyl radical scavenging activity of compatible solute s, Phytochemistry 28 1057-1060.

[37] Stines AP, Naylor DJ, Hoj PB, Heeswijck R (1999): Prolien accumulation in developing grape wine fruit occurs independently of changes in the level of Δ^1-pyrroline-5-carboxylase synthetase mRNA or protein, Plant Physiol 120: 923-931

[38] Troedson, RJ, Wallis, ES, Singh, L. (1990): Pigeonpea: Adaptation. In: Nene, Y., Hall, S.D., Sheila, V.K. (Eds.), The Pigeonpea. CABI, Wallingford, pp. 159–177

[39] Turan AM, Turknen N, Taban N (2007): Effect of Novel on stomatal resistance and praline, chlorophyll, Na, Cl and K concentration of lentil plants. J. Agro. 6(2): 378-381.

[40] Verslues PE, Agrawal M, Katiyar- Agrawal S, Zhu JH, Zhu, JK (2006): Methods and concepts in quantifying resistant to drought salt and freezing, abiotic stress that affects plant water status, Plant J. 45: 523-539

[41] Yeo, A,(1998): Review Article, Molecular Biology of Salt Tolerance in the Context of Whole Plant Physiology, *J. Exp. Bot.*, vol. 49, pp. 915–929.

[42] Yokota A, Takahara K, Akashi K (2006).Physiology and Molecular Biology of stress tolerance in plants. In: Madhavarao K, Raghavendra, Janardhanreddy K (eds) , Springer. pp. 15-40.

[43] Yousfi, S., Maria Dolores S., Jordi V and Araus LJ., (2010): Effect of salinity and water stress during the reproductive stage on growth, ion concentrations, $D^{13}C$ and $d^{15}N$ of durum wheat and related amphiploids. J. Exp. Botany, 61: 3529-3542.

[44] Zhang, X., S. Zhou, Y. Fu, Z. Su, X. Wang, C. Sun, (2006): Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.), Plant Mol. Biol., 62: 247-259.