International Journal of Environmental Science and Toxicology Research (ISSN: 2408-7262) Vol. 3(8) pp.134-143, November, 2015
Available online http://www.internationalinventjournals.org/journals/IJESTR

Full Length Research Paper

Cytomorphological and Biochemical Impact of Temperature stress in Buckwheat (*Fagopyrum esculentum* Moench)

G. Kumar and A. Srivastava*

Department of Botany, University of Allahabad, Allahabad 211002, India

Abstract

Inspite of the great nutritive values of buckwheat, data about the temperature stress under abiotic stress tolerance of this plant sp. is a very major aspect for concern. The aim of this work is to find out the cytological, morphological and biochemical response of buckwheat plant against temperature stress such as heat and cold stresses. Changes in chromosome level, morphological parameters including germination and survival percentages, plant height (cm), internodal length (cm), no. of seed etc. as well as changes in content of carbohydrate, proline and chlorophyll with respect to heat and cold stress, have been investigated. Reasons for selecting this plant for temperature stress are due to its temperature stress sensitivity as well as to search possibilities for enhancing stress tolerance on different parameters. Over all it has been concluded that temperature stress especially heat stress severely affects the plant at cytological, morphological and biochemical levels as compared to cold stress.

***Keywords*:** Carbohydrate content; Chlorophyll content; *Fagopyrum esculentum* Moench; Proline content; Temperature stress; Total abnormality percentage.

INTRODUCTION

For the limitation of plant growth and productivity, the environmental stress is becoming the vital aspect for plant scientists worldwide due to climatic changes. The temperature stress under abiotic stresses is a major environmental factor that limits the agricultural productivity and adversely affects the morphological, physiological, biochemical and molecular changes in plants. Though the plants have capability to cope and survive under temperature stress but extreme temperature caused various changes in plant growth and metabolism with regard to cytological, morphological as well as biochemical parameters. Therefore, we have discussed the effect of heat and cold stresses on different parameters in response to plant growth and metabolism.

The impact of high temperature stress is more devastating than cold stress. It is defined as the rise in temperature beyond a critical threshold for a period of time adequate to cause permanent damage to plant growth and development. Innumerable biochemical reactions come under the growth and developmental stages of plant which are very sensitive at some degree of temperature. This stress plays a very significant role in various aspects such as altering the cell division, cell elongation rate due to which leaf size and weight are affected. Almost all the growth stages of plant from emergence to ripening and harvesting are affected through exposure of heat stress (Shah et al., 2011).

Cold or low temperature stress involving chilling (< 20^0C) and freezing temperature (< 0^0C) reduce the plant growth and development in many ways which causes significant crop losses (Gulzar et al., 2012). The plants affected by cold stress are sensitive to chilling stress and lack the mechanism of cold acclimation related to tropical and sub tropical origins. Besides, the plants of temperate climate regions are less sensitive to chilling and have mechanism of acclimation (Levitt, 1980). It affects the germination, growth and morphology,

*Corresponding Author Email: srivas.akanksha20@gmail.com

reproductive development, cell membrane damage, photosynthesis etc.

Buckwheat (*Fagopyrum esculentum* Moench) is a pseudocereal which belongs to family polygonaceae having 16 chromosomes in diploid set (2n=16). The plant can be easily grown everywhere in favourable season. The temperature factor plays a very important role in development and growth of buckwheat plant because temperature requirement is almost fix during growth and developmental stages of plants, especially at the time of flowering and seed setting. The temperature stress evokes the complex cellular responses which have been explained by many progresses, exploring and understanding the plant under abiotic response at different level. Plant growth and metabolism shows negative effects under this stress as a result of cell inhibition and cell alternation.

The objective of the present work is to investigate the response of heat and cold stresses on buckwheat plant, with special attribute to cytological, morphological and biochemical changes.

MATERIAL AND METHODS

Plant Material

The germ plasm of *Fagopyrum esculentum* was obtained from National Bureau of Plant Genetic Resources, Shimla, Phagli. During the present study, VL-7 variety of this plant was used for cytological, morphological and biochemical analysis.

Germination of seed

For the germination, the seeds were presoaked in a petridish by using filter paper, then covered and kept into an incubator for 2-3 days at 22-25^{0}C.

Heat and Cold stress Treatment

After the germination, seeds were kept in labelled petriplates in oven at 40^{0}C and in refrigerator at 2^{0}C with regard to heat and cold stress treatment for different durations (3 hrs, 5 hrs. and 7 hrs.) and one set kept for control at room temperature. Then, the root tips were washed thoroughly in distilled water and seeds were sown in three pots per treatment for cytological, morphological and biochemical study.

Cytological analysis

The few buds were plucked with appropriate size and fixed in carnoy's fixative (1:3) for 24 hrs and preserved in 90% alcohol for cytological study. The anther excised's from the buds then teased with the help of sharpen object and stained with 2% acetocarmine for 15 minutes. Finally, slides were prepared by using squash technique for cytology and observation was done at 40X resolution under light microscope and photographs were taken by using PCTV Vision Photography Software.

Following formulae used for calculation of abnormality percentage –

Total Abnormality percentage (TAP) %

$$= \frac{\text{No. of Abnormal Pollen mother cell (PMC)}}{\text{Total no. of Pollen mother cell observed}} \times 100$$

Morphological analysis

The different morphological parameters were taken such as germination (7 days) and survival percentage (14 days), plant height (cm), internodal length (cm), leaf area (cm^2), no. of flowers, no. of seeds for studying the impact of heat and cold stress.

Biochemical analysis

Proline content analysis

The proline content was quantified according to Bates et al. (1973). 250 mg sample of leaves of plant material was homogenized in 10 ml of aqueous solution of 3% sulfosalicylic acid to prepare crude extract and centrifuged at 3000 rpm for 10 minutes. This supernatant was reacted with 2ml acid ninhydrin and 2 ml of 3% glacial acetic acid in a test tube. The mixture was kept in water bath at 100^{0}C for 1 hour and for the termination of reaction, the test tube was placed in an ice bath. The reaction mixture was transferred in a separating funnel and extracted with 4.0 ml of toluene then vortexed in a mixer for 10-15 second. The toluene layer containing organic and inorganic phase was separated, warmed at room temperature and the absorbance was read at 520 nm of the organic toluene phase containing the chromophore in a spectrophotometer (UV Visible Spectrophotometer 118, Systronic) using toluene for a blank. The concentration of proline in a plant tissue was determined from a standard curve and calculated on the basis of fresh weight (FW).

Carbohydrate content analysis

The carbohydrate content was estimated by anthrone method (Hedge and Hofeiter, 1962). 100 mg of fresh leaf sample was taken into a boiling tube. The content was hydrolyzed in a boiling water bath for 3 hours by adding 5 ml of 2.5 N HCl and cooled at room temperature. Hydrolyzed sample centrifuged at 8000 rpm for 3 minutes and supernatant collected. Standard

was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard. '0' served as a blank. Added distilled water to bring the volume to 1 ml in all test tubes including the sample tubes. Then 4 ml of anthrone reagent (200 mg anthrone+100 ml H_2SO_4) was added. The samples were heated in a boiling water bath for 8 minutes, cooled and then the absorbance was read at 630 nm. Concentration of carbohydrate in plant tissues is expressed on a fresh weight basis and determined from standard curve. From this standard curve, the amount of carbohydrate was calculated.

Pigment content analysis

Chlorophyll and Carotenoid contents were determined according to procedure described by Lichtenthaler and Welburn (1983). Leaf tissues (0.5 mg) were homogenized in 5ml of acetone 80%. The homogenate was centrifuged and the O.D. was taken at the wavelengths of 663, 646 and 470 nm in a spectrophotometer and calculated chl a, chl b and carotenoid content.

Statistical analysis

For using Statistical analysis in the table, three replicates for each treatment were used. Statistical analysis was performed using the SPSS 16.0 software. A one way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT, $P < 0.05$) was conducted for mean separation and the graph was plotted by using sigma plot 10.0 software. Actual mean and standard error were calculated and the data was subjected to analysis of variance.

RESULT

Temperature stress leads to reduction of yield in response of plants and shows various changes in developmental stages of plant growth and metabolism. The effect of this abiotic stress in buckwheat plant clearly monitored at cytological, morphological as well as biochemical level.

Cytological Results

The cytological result revealed the diploid chromosome no. of *Fagopyrum esculentum* (2n= 16). The present study showed the normal pattern of chromosomes and abnormal behaviour of chromosomes due to lower and higher exposure of temperature stress from optimal threshold level. The meiosis with respect to control was perfectly normal with 8 bivalents arranged at metaphase I (Figure 1A) and 8:8 separation at anaphase I. In cytological observation, Figure 1B and C showed normal second division of meiosis such as metaphase II and anaphase II, respectively. Exposure of heat and cold stress changes the behaviour of chromosomes and abnormal dividing PMC's were observed.

The rate of chromosomal abnormality and pollen fertility along with increased duration of both stresses has been represented in Table 1. Abnormality percentage increased with increasing duration of temperature stress (Table 1). The value of abnormality percentage increased from 3.63±0.10 to 8.35±10.09 in cold stress and from 6.49±0.17 to 14.60±0.07 in heat stress on increasing the duration from 3-7 hours. Pollen fertility rate was also studied in control set and it was recorded to be 99.87±0.02 whereas it decreased from 98.74±0.03 to 90.40±0.47 and from 95.10±0.06 to 78.04±0.15 in both stresses, respectively.

Morphological Results

The control set exhibited 100% germination and seeds with exposure of cold and heat stress for a duration of 3, 5 and 7 hours exhibited germination 90%, 80%, 70% and 50%, 40%, 30% respectively (Table 2). In both temperature stresses, the germination percentage reduced along with increased duration as compared to control. The survivability also decreased depending on the duration of temperature stress. The seeds treated with cold stress have higher percentages of germination and survivability as compare to heat stress.

Other morphological parameters such as plant height, internodal length, leaf area, no. of flowers and no. of seeds were measured along with stress treatment set with respect to control. The value of average plant height in control was observed as 43.16±1.217 and it decreased from 35.13±0.617 to 24.76±0.560 in cold stress treatment and from 28.63±0.712 to 23.66±0.688 in heat stress treatment depending on the duration of temperature exposure (Figure 2A). Cold and heat stresses also reduced the internodal length (i.e. 11.83±0.166 in control) from 10.56±0.808 to 8.13±0.185 in cold stress and from 7.83±0.375 to 7.10±0.360 in heat stress, respectively (Figure 2B). Leaf area also decreased from 21.36±0.317 to 16.43±0.290 and from 16.86±0.202 to 13.33±0.233 in cold and heat stresses, respectively with dependent duration of exposure as compared to control value i.e. 27.36±0.260 (Figure 2-C). Declinations of no. of flowers and seeds with leading duration of temperature stress were observed as shown in Figure 2- D and E.

Biochemical Results

Proline

The proline content (μg/g) was observed as 5.07±0.148

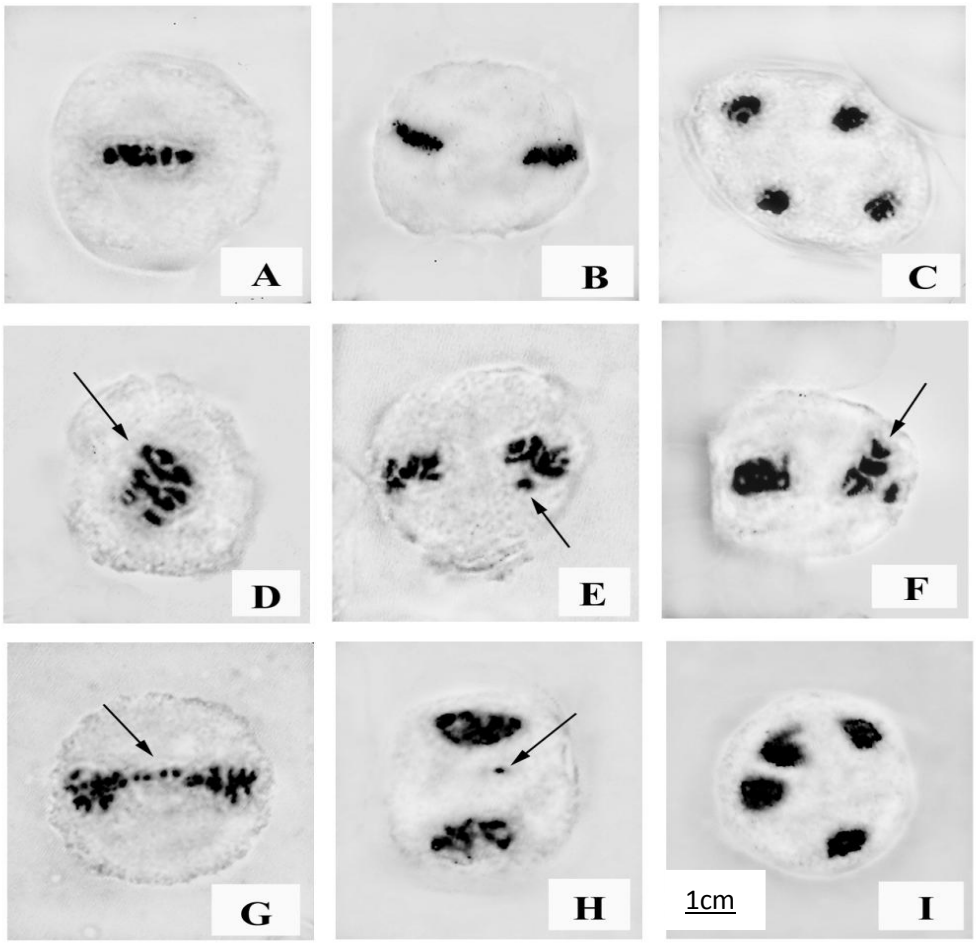

Figure 1. Chromosomal abnormalities induced by Temperature stress in VL-7 variety of *Fagopyrum esculentum* Moench.
A. Normal Metaphase I; **B.** Normal Metaphase II; **C.** Normal Anaphase II; **D.** Unorientation with multivalent formation at Metaphase I; **E.** Precocious movement at Metaphase II; **F.** Unorientation at Metaphase II; **G.** Fragmentation at Metaphase II; **H.** Laggard formation at Anaphase I; **I.** Unorientation with stickiness at Anaphase II. [**Scale Bar** = 8.08 µm]

Table 1. Abnormality induced by Cold and Heat stress in meiosis and pollen fertility of *Fagopyrum esculentum* Moench

Treatment	Doses	Metaphasic I/II Abnormality (%)					Anaphasic I/II Abnormality (%)				Total Abnormality (%)	Pollen Fertility (%)
		Multi	Frag	Pre	St	Un	Lg	St	Un	Oth		
Cold Stress (2^0C)	Control	-	-	-	-	-	-	-	-	-	-	99.87±0.02[a]
	3 hrs.	0.45±0.11[b*]	0.57±0.11[a]	0.56±0.11[a]	0.34±0.01[a]	0.68±0.01[a]	0.34±0.01[a]	0.51±0.17[a]	0.45±0.11[a]	0.51±0.17[a]	3.63±0.10[c]	98.74±0.03[b]
	5 hrs.	0.65±0.13[b]	0.91±0.26[a]	1.09±0.30[a]	0.78±0.01[a]	0.65±0.13[a]	0.78±0.22[a]	0.52±0.13[a]	1.17±0.22[a]	0.58±0.19[a]	6.39±0.12[b]	96.27±0.18[c]
	7 hrs.	1.42±0.15[a]	0.85±0.01[a]	0.99±0.14[a]	0.84±0.24[a]	1.13±0.37[a]	0.71±0.14[a]	0.85±0.24[a]	0.98±0.36[a]	0.56±0.13[a]	8.35±0.09[a]	90.40±0.47[d]
Heat Stress (40^0C)	Control	-	-	-	-	-	-	-	-	-	-	99.87±0.02[a]
	3 hrs.	1.37±0.19[b]	1.02±0.19[ab]	0.57±0.11[b]	0.68±0.34[a]	0.51±0.17[b]	0.79±0.11[a]	0.57±0.11[b]	1.03±0.35[b]	0.68±0.19[a]	6.49±0.17[c]	95.10±0.06[b]
	5 hrs.	2.04±0.21[ab]	0.81±0.24[b]	1.09±0.37[ab]	0.81±0.22[a]	0.54±0.14[b]	0.67±0.13[a]	1.02±0.23[ab]	1.35±0.49[b]	0.60±0.21[a]	8.41±0.10[b]	87.60±0.05[c]
	7 hrs.	2.20±0.26[a]	1.61±0.15[a]	1.61±0.28[a]	1.17±0.14[a]	1.32±0.25[a]	1.02±0.14[a]	1.46±0.14[a]	3.09±0.26[a]	1.10±0.21[a]	14.60±0.07[a]	78.04±0.15[d]

Where, **Multi-** Multivalent formation,
Frag- Fragmentation,
Pre- Precocious movement, **St-**Stickiness,
Un- Unorientation, **Lg-** Laggard, **Oth-**Others
*Means followed by lower case letter are statistically significant at $p<0.05$ in Duncan's Multiple Range Test.

Table 2. Germination and Survivability percentages induced by Temperature stress in *Fagopyrum esculentum* Moench

Treatment	Doses	Germination Percentage (in 7 days)	Survivability Percentage (in 21 days)
Cold Stress (2^0C)	Control	100%	100%
	3 hrs.	90%	90%
	5 hrs.	80%	80%
	7 hrs.	70%	60%
Heat Stress (40^0C)	Control	100%	100%
	3 hrs.	50%	50%
	5 hrs.	40%	30%
	7 hrs.	30%	20%

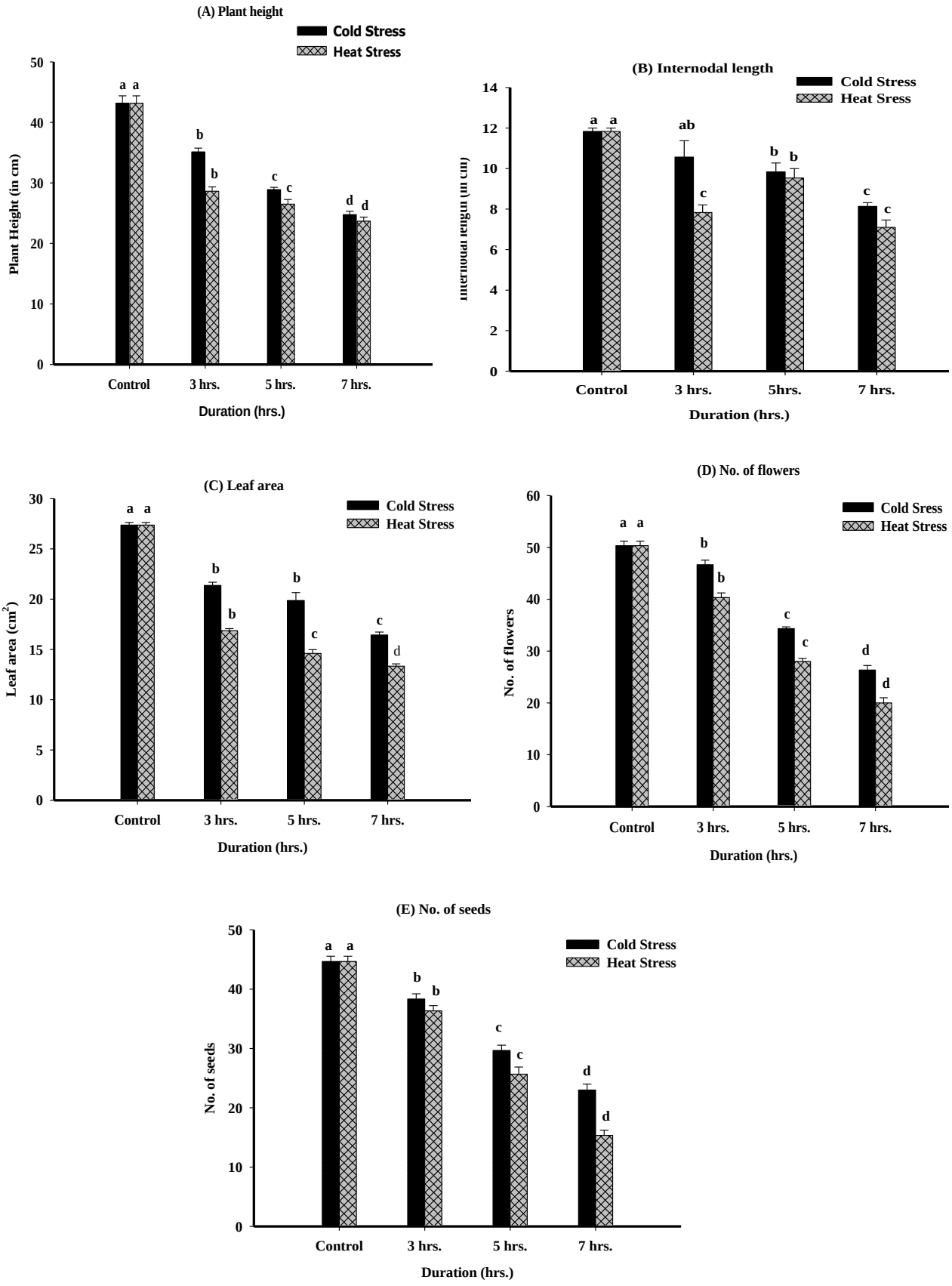

Figure 2. Comparative account of Morphological Parameters induced by Temperature stress in VL-7 variety of *Fagopyrum esculentum* Moench.

in control set but it was increased along with increasing duration of cold and heat stresses (Figure 3A). Plant treated with heat stress showed positive gradation from 7.14±0.099 to 12.14±0.749 as compared to cold stress treated plant (From 5.68±0.218 to 8.57±0.139) with the increase in respective durations of stress (Table 3).

Carbohydrate

The control exhibited 126.67±0.881 of carbohydrate content (µg/ml). The carbohydrate content in plant treated with cold and heat stresses was observed from 109.67±0.881 (3 hrs.) to 89.00±0.577 (7 hrs.) and from 86.66±0.881 (3 hrs) to 68.00±1.154 (7 hrs), respectively. The plant with the treatment of cold stress has higher content of carbohydrate in contrast to heat stressed plant (Figure 3B).

Pigment

Chlorophyll and carotenoid content in the fresh green leaves with the stress of cold and heat treated plant were measured and the results have been presented in Figure 3 including C-I, C-II and C-III. The values were 2.63±0.268, 0.91±0.078 and 0.72±0.036 with respect to chl a, chl b and carotenoid content at control set. Cold and heat stresses showed the reduction of chlorophyll and carotenoid content along with increased duration but the lowest content of pigment in heat stress treatment was observed (Table 3).

DISCUSSION

Temperature stresses influenced the developmental stages of plant and metabolism at cytological, morphological as well as biochemical level. Buckwheat is sensitive to high temperature stress. Due to this reason it does not tolerate high duration of heat stress. The plants were influenced by cold stress also but chilling treatment (1^0C -7^0C) is tolerable wherever the lowest temperature below 0^0C involves frost condition which rapidly ceases plant growth and metabolism. Based on this observation, Buckwheat can be considered as heat sensitive plant species.

Cytological discussion

The above result indicated that the heat stress was more effective to cause chromosomal anomalies in higher percentage as compared to cold stress. Heat stress alters the cell division and changes the behaviour of chromosomes in contrast to normal pattern. A long term exposure of heat stress causes severe cellular injury and even cell death may occur within minutes which could be attributed to a catastrophic collapse of cellular organization (Schoffl et al., 1999).

Under temperature stress, the most common anomalies were observed including univalent and multivalent formation, precocious movement, stickiness, laggard and fragmentation at metaphasic and anaphasic I / II division. These abnormalities lead to increased dependent duration of temperature. Multivalent formation (Figure 1D) is the results of primary pairing at zygotene and chiasma formation among two or more homologous chromosomes (Kumar and Chaudhary, 2013). Another abnormality such as precocious movement (Figure 1E) was observed formed by complete lacking of homologous chromosome pairing or improper spindle functioning. Laggard formation (Figure 1H) is due to delayed terminalisation, chromosomal stickiness or failure of chromosomal movement (Reddy and Munirajappa, 2012). Another reason of laggards formed at anaphasic I/II, is temperature stress which may directly affect the spindle fibres due to which chromatin bridge breaks down resulting into irregular separation of chromosomes. Correa et al. (2005) affirmed that the rate of pollen fertility shows the close relationship to success of meiotic process and resulting in the changes of meiotic behaviour consider as pollen viability in theoretical aspect. Chromosomal anomalies observed during the course of meiosis reflected on pollen fertility which clearly showed declination of fertility percentage along with increased duration of temperature stress.

Morphological

Declination of different parameters occurs in case of both stresses with respect to control. But heat stress suppressed the all morphological parameters as compared to cold stress. Optimal temperature should be required for germination of seeds and if the temperature stress maximized or minimized with respect to optimal threshold level then all developmental stages and metabolism of plant were affected. From above going result, heat stress has shown negative response in the plant. Generally, the higher exposures are usually inhibitors of the seed germination in angiosperm and gymnosperm (Dhakshsnamoorthy et al., 2011; Akhaury and Singh, 1993; Majeed and Muhammad, 2010).

When the temperature exceeds 40^0C (up to optimal temperature) in *Fagopyrum esculentum*, its seed germination, seedling and vegetative growth, no. of flowers, seed set and seed ripening are adversely affected. It has also been seen in tomatoes i.e. when the ambient temperature exceeds 35^0C, its developmental stages and growth of the plant are affected and under such conditions plants tend to divert resources to cope with the heat stress and thus limited photosynthetic would be available for reproductive development (Wahid et al., 2007).

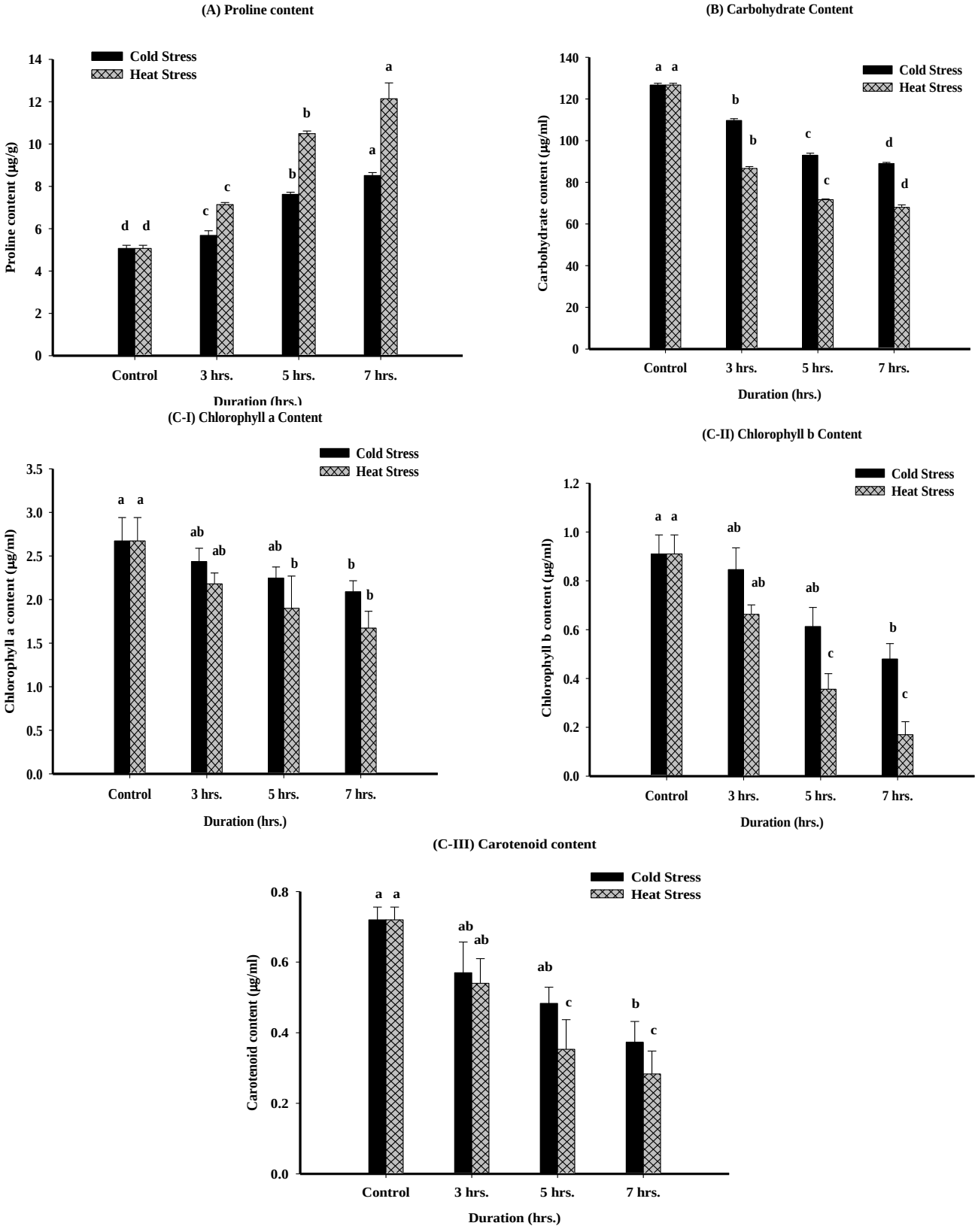

Figure 3. Comparative account of Biochemical Characteristics induced by Temperature stress in VL-7 variety of *Fagopyrum esculentum Moench*

Table 3. Temperature stress induced changes in proline, carbohydrate and pigment content in VL-7 variety of *Fagopyrum esculentum* Moench

Treatment	Doses	Proline [µg/g]	Carbohydrate (µg/ml)	Pigment Content (µg/ml)		
				Chlorophyll a	Chlorophyll b	Carotenoid
Cold stress (2^0C)	Control	5.07 ±0.148^{d}	126.67±0.881^{a}	2.67±0.268^{a}	0.91±0.078^{a}	0.72±0.036^{a}
	3 hrs.	5.68 ±0.218^{c}	109.67±0.881^{b}	2.43±0.153^{ab}	0.85±0.089^{ab}	0.57±0.087^{a}
	5 hrs.	7.62 ±0.099^{b}	93.00±1.000^{c}	2.24±0.128^{ab}	0.61±0.078^{bc}	0.48±0.046^{ab}
	7 hrs.	8.51 ± 0.139^{a}	89.00±0.577^{d}	2.09±0.126^{b}	0.48±0.063^{c}	0.37±0.059^{b}
Heat stress (40^0C)	Control	5.07 ±0.148^{d}	126.67±0.881^{a}	2.67±0.268^{a}	0.91±0.078^{a}	0.72±0.036^{a}
	3 hrs.	7.14 ±0.099^{c}	86.66±0.881^{b}	2.18±0.124^{ab}	0.66±0.038^{b}	0.54±0.070^{ab}
	5 hrs.	10.49±0.124^{b}	71.66±0.333^{c}	1.90±0.370^{b}	0.35±0.063^{c}	0.35±0.084^{c}
	7 hrs.	12.14±0.749^{a}	68.00±1.154^{d}	1.67±0.193^{b}	0.17±0.052^{c}	0.28±0.065^{c}

Severe damaging effect of heat stress monitored in vegetative stage such as ceasing of stem growth, damaging of leaf gaseous exchange property and during reproductive stage, short period of heat stress can cause significant increases in floral buds and opened flowers abort. However there are great variations in sensitivity within and among plant species (Guilioni et al., 1997; Young et al., 2004). Cold stress also influences the growth and metabolism of plant but buckwheat is a cool season crop for which 0^0C is often the best predicted base temperature (Miller et al., 2001). Based on this literature study, 2^0C was not more effective with respect to plant growth as compared to heat stress.

Biochemical

Proline

Proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kavi Kishore et al., 2005). Under temperature stress, the plant plays a role as a key adaptive mechanism. Due to this property, certain organic compounds of low molecular mass accumulated in the plant which generally referred to as compatible osmolytes (Hare et al., 1998; Sakamoto and Murata, 2002). The accumulation of such solutes can help them to enhance stress tolerance (Wahid et al., 2007) which is very significant in response to sensitive plant. In heat stress treatment, this accumulation leads with increasing duration stress as compared to cold stress because heat stress disrupts the sugar metabolism and proline transport due to which proline content accumulated with dependent duration of stress.

Carbohydrate

From the above observation, water soluble carbohydrate decreased in both stresses along with increased duration in respect to control. But plants usually had the highest concentration when grown under drought and salinity stress (Mafakheri et al., 2011). However, the cold stress causes little disturbance in enzymatic process due to this reason normal functioning of sugar metabolism occurs in cold stress as compare to heat stress.

Pigment

Alternation processes in photosynthetic attributes are the good source of thermo tolerance of the plants because they show direct relationship with growth in the case of heat stress. Any constituent changes in the process of photosynthesis can limit the growth of plant at high temperature stress (up to 35^0C). The heat stress causes injury on the primer site of photochemical reaction and carbon metabolism (Wise et al., 2004).

In heat stress, degradation of chlorophyll and carotenoid content was more pronounced with increased duration as compare to cold stress because it is directly subjected to the production of active oxygen species (Camejo et al., 2006; Guo et al., 2006). From above researchers view, the normal process of photosynthesis is affected which leads to the reduction of chlorophyll and carotenoid contents and also influenced the soluble protein etc. The plant treated with cold stress involves increased chlorophyll accumulation, reduced photosynthesis activity because chloroplast and photosynthesis are major sites of injury. Tolerance of these aspects is expressed in native vegetation adapted to growing under cool condition (Sanghera et al., 2011).This study supports views in favour of *Fagopyrum esculentum*. The low temperature in respect to cold stress reduced the sensitivity of photosynthesis has also been reported in maize which is partly related to specific enzymatic process (Singh, 2000).

CONCLUSION

Plant exhibits a negative response in respect to heat stress which affects the plant growth and metabolism

and depicted various changes at cytological, morphological as well as biochemical level as compared to cold stress treatment. Buckwheat is cool season adaptive plant and therefore, it showed positive results in respect to cold stress such as chilling treatment which is not much influenced by the changes at various levels and increased the seed production compared to heat stress. In general, the buckwheat is very economically plant because of medicinal drugs, oils, also used as a food and its market value is very high because of lower production if agriculture farmers can prefer chilling treatment to this plant to increase its productivity in positive way and lowers the cost also. Which can be easily available to all people but heat stress plays an injurious role in all developmental stages and metabolism of the plant because the plant is heat sensitive and does not tolerate high temperature up to optimal threshold level. For improving plant yield and productivity whether the heat tolerance ability improved in the plant or plant can be grown under required threshold temperature which varies plant to plant of crop plants because greater addition in temperature stress against threshold level resulted and species to species. Such insights are playing an important role in response of heat tolerance capacity to lead the highest proportion of sterility. Without developing of this capacity, plant cannot be making beneficial for environmental and economical purpose in case of heat stress than cold stress.

ACKNOWLEDGEMENT

This effort is supported by National Bureau of Plant Genetic and Resources (NBPGR), Shimla, Phagli for providing the inbred seeds of *Fagopyrum esculentum* and all members of the Plant Genetics Laboratory who have given reinforcement for this work. One of the authors (Akanksha Srivastava) is extremely thankful to University Grant Commission (UGC) for financial support. The author is greatly thankful to the Head of Department, University of Allahabad for procuring experimental facilities.

REFERENCES

Akhaury KDN, Singh AK (1993). “Effect of gamma-rays on the seed output of *Vicia* species”, Neo Botanica,Vol. 1, No. 1-2, pp. 63- 67.

Bates L, Waldren RP, Teare ID (1973). “Rapid determination of free proline for water-stress studies”, Plant and Soil, Vol.39,No.1, pp. 205-207.

Camejo D, Jim´enez A, Alarc´on JJ, Torres W, G´omez JM, Sevilla F (2006). “Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants”, Funct. Plant Biol., 33, pp. 177–187.

Corrêa MGS, Viegas J, Silva JB, Avila PFV, Busato GC, Lemes JS (2005). “Meiose eviabilidade polínica na família Araceae”, Acta Botânica Brasileira, 19, pp. 295-303.

Dhakshanamoorthy D, Selvaraj R, Chidambaram ALA (2011). “Induced mutagenesis in *Jatropha curcas* L. using gamma rays and detection of DNA polymorphism through RAPD marker”, C.R. Biol.,Vol.33,pp.24-30.

Guilioni L, Wery J, Tardieu F (1997). “Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs?”, Ann. Bot., Vol.80, pp. 159–168.

Guo YP, Zhou HF, Zhang LC (2006). “Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species”, Sci. Hort., Vol.108, pp. 260–267.

Hare PD, Cress WA, Staden JV (1998). “Dissecting the roles of osmolyte accumulation during stress”, Plant Cell Environ., Vol.21, pp. 535–553.

Hedge JE, Hofreiter BT (1962). “In: Methods in Carbohydrate Chemistry. Whistler, R.L., Be Miller, J. N., Eds”, Academic Press, New York, Vol. 17, pp. 420.

Kavi Kishore PB, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005). “Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance”, Curr. Sci., Vol. 88, pp. 424–438.

Kumar G, Chaudhary N (2013). “Secondary chromosomal association in kidney bean (*Phaseolus vulgaris* L.)”, Jordan Journal of Biological Sciences, Vol.7, pp. 71-74.

Levitt J (1980). “Response of plants to environmental stress. In Chilling, Freezing and High Temperature stress”, New York Academic Press, 1.

Lichtenthaler HK, Wellburn AR (1983). “Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents”. Biochemical Society Transactions, Vol.11, pp. 591 - 592.

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2011). “Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (Cicer arietinum) cultivars”, Australian Journal of Crop Science, Vol. 5, pp. 1255-1260.

Majeed A, Muhammad Z (2010). “Gamma irradiation effects on some growth parameters of *Lepidium sativum* L.”, World Journal of Fungal and Plant Biology, Vol.1, pp. 8-11.

Miller P, Lanier W, Brandt S (2001). “Using Growing Degree Days to Predict Plant Stages”, Ag/Extension Communications Coordinator, Communications Services, Montana State University-Bozeman, Bozeman, MO.

Reddy PM, Munirajappa M (2012). “Gamma ray induced meiotic abnormalities IN S13 Mulberry”, International Journal of Science & Nature, Vol.3, pp. 170-172.

Sakamoto A, Murata N (2002). “The role of glycine betaine in the protection of plants from stress: clues from transgenic plants”, Plant Cell Environ, Vol. 25, pp. 163–171.

Sanghera GS, Wani SH, Hussain W, Singh NB (2011). “Engineering cold stress tolerance in crop plant”, Current Genomics, Vol.12, pp. 30-43.

Schoffl F, Prandl R, Reindl A (1999). “Molecular responses to heat stress. In: Shinozaki, K., Yamaguchi-Shinozaki, K. (Eds.), Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants”, R.G. Landes Co.,Austin, Texas, pp. 81–98.

Shah F, Huang J, Cui K, Nie L, Shah T, Chen C, Wang K (2011). “Impact of high temperature stress on rice plant and its traits related to tolerance”, Journal of Agricultural Science, Vol. 10, pp. 1-12.

Singh BD (2000) “Plant Breeding”, pp. 443-460.

Wahid A, Gelani S, Asraf M, Foolad MR (2007). “Heat tolerance in plants: An overview”, Environmental and Experimental Botany, Vol. 61, pp. 199-223.

Young LW, Wilen RW, Bonham-Smith PC (2004). “High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production”, J. Exp. Bot., Vol.55, pp. 485–495.

How to cite this article: Kumar G, Srivastava A (2015). Cytomorphological and Biochemical Impact of Temperature stress in Buckwheat (*Fagopyrum esculentum Moench*). Int. J. Environ. Sci. Toxic. Res. Vol. 3(8):134-143