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THE MORPHOLOGICAL, ANATOMICAL AND PHYSIOLOGICAL ASPECTS OF MAIZE (ZEA MAYS L.) PLANT AFTER APPLYING THE DIFFERENT LEVELS OF TEMPERATURE TREATMENTS ON PRE-SOWING MAIZE SEEDS

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Abstract

Maize (*Zea mays* L.) belongs to poaceae family and used as a staple food throughout the world. *Z. mays* L. ranks third after wheat and rice. Four levels of temperature viz. 0^0C (T_1), 6^0C (T_2), 35^0C (T_3) and 60^0C (T_4) respectively for 24hrs were applied to elucidate the effects on pre-sowing *Z. mays L.* seeds. The experiment was performed in pots with triplicates under CRD and sown at 35^0C soil temperature. Chlorophyll content was highest at 6°C and lowest at 0°C except carotene because carotene was lowest at 60°C. The two methods for chlorophyll content extraction were used and 80% acetone proved to be better than distilled water. The results of all anatomical parameters of 0°C and 6°C was worse than 6°C and 35°C like morphological characters because cell was initially damaged. Seeds which treated with both high and chilling temperatures were shown the thin stem, root, low amount of chlorophyll content, lowest value of morphological parameters and narrow leaf after emergence but vernalized seeds were shown the antagonistic characters as compared to heat shock plants. At high and low temperature, the growth of the plants stopped, their color became dull green and the inner cells were damaged. The results of vernalized (6°C) plants were significantly higher than 35°C in all morphological, physiological and anatomical aspects. The seeds did not affect at 35^0C because seeds were vernalized before sowing. This study helps to understand the relationship between temperature and biochemical reactions in plants.

Keywords
Z. Mays L., Heat Shock, Vernalization, Chlorophyll Content, Anatomical Study, Morphological Analysis

1. Introduction

Z. mays L. is a member of poaceae family and versatile plant in the agricultural world. Maize first time grown by native people in Mexico. Z. mays L. is at 3rd position in Pakistan followed by rice (5.54 million tons) and wheat (24.23

million tons). It is an angiospermic plant and seeds linked with cob structure. The flowers of both male and female inflorescence are present on the same plant and plant height is 3m-12m. It's root is primary and endogenously (Hochholdinger, 2012). The optimum temperature for Z. mays L. growth is 10^{0}C-30^{0}C (Malik *et al*., 2006). The period between emergence and seed sowing is shortening due to low temperature treatment and this treatment provides the ability to plants for fighting against diseases (Kim *et al*., 2009). The chilling injury is the result of extremely low temperature due to crystal formation in cells (Sanghera *et al*., 2011). If the temperature is above 40°C then chlorophyll content in leaves begins to decrease (Gosavi *et al*., 2014).

Z. mays L. seeds treated at 0^{0}C for 1-4 days before sowing and then transferred to 35^{0}C, but the germination % of this seeds is higher than those seeds which directly transferred to 35^{0}C (Mei & Song, 2012). The incorporation of solar radiations rises the soil temperature up to 50^{0}C (Farrell & Gilliand, 2011). The trichome frequencies increases and size of the cell is trim down at high temperature (Bnone *et al*., 2004). The endosperm of Z. mays L. seed is destroyed at high temperature owing to disturbance in starch metabolism and cell division (Monjardino *et al*., 2005). The discrepancy in temperature is an immense matter in Pakistan. The first decade of 21st century increases the 0.7^{0}C temperature of the world and optimistically 4oC temperature increases at the end of 21st century. The temperature at the end of 19th century rises up to 0.3oC-0.6oC (Rasul *et al*., 2011).

The annual loss of Z. mays L. in developing countries is approximately 10 million tons and affected the 140 million people. Plants reach early at a maturity stage with a low yield at high temperature due to a disturbance in many biochemical reactions (Hassanuzman *et al*., 2013). The high temperature slows down the chlorophyll biosynthesis and photosynthetic activity (Coskun *et al*., 2011). Naturally, there are many changes in the weather. The main reasons for the decline in agricultural production are the rapid changes in the climate. Lack of agricultural production is a huge threat to the future (Christensen & Christensen, 2007). The objective of this research to indicates the vernalized and optimum temperature range for Z. mays L. growth in southern Punjab of Pakistan**.**

2. Materials and Methods

2.1 Experimental Site

Z. mays L. seeds (Pak 1 Gloi) were collected from seed corporation Bahawalnagar. Experiment was performed in pots with triplicates under CRD in the Botanical Garden of The Govt. Sadiq College Women University Bahawalpur.

2.1.1 Water Analysis and Soil Testing

The water analysis and soil testing of the field was also done.

Table 1: Soil Analysis

EC mS/cm	pH	Organic matter%	Available Potassium ppm	Available Phosphorus ppm	Saturation%	Texture
0.74	7.5	0.85	9.6	145	31	loam

Table 2: Water Analysis

TSS ppm	Ca+ Mg meq/L	Carbo-nate meq/L	Bi-carbonate meq/L	Chloride meq/L	SAR meq/L	RSC meq/L	Sodium meq/L	pH	EC dS/m
683	2.97	0	6.3	1	3.98	2.01	5.5	7.1	0.8

Table 3: Metrological Data of Studied Region

Months	Temperature (°C)		Total rainfall (mm)	Avg. relative humidity %	Evaporation (mm)		Wind speed (Km/h)	
	Max.	Min.			Max.	Min.	Max.	Min.
June	49	24.5	40.7	42.5	14.2	5.1	5.4	9.8
July	45	26	87.6	29.7	10.3	4.6	6.3	11.6
August	42.5	23.4	13.5	53.5	6.2	2.9	2.9	3.7
September	40.3	23	71.6	51.5	5.7	2.1	3	3.8
October	35.5	16	73.4	49.8	5.4	2.6	3.6	3.2
Average	42.6	22.58	57.36	45.4	8.36	3.46	4.24	6.42

2.2 Treatments

The seeds were placed in petri-dish and incubated at 60^0C for 24hrs in an oven and some seeds were placed in a refrigerator at 6°C and 0°C for 24hrs. The remaining seeds were stored at room temperature (35^0C) for further use. The moisture content of seeds was measured before treatment and after treatment. The germination % was measured after emergence and measured the morphological, anatomical and physiological attributes.

Germination percentage= Germinated seeds/Total seeds× 100

2.3 Chlorophyll Content

Leaves were collected early morning at 28^0C for chlorophyll content analysis before cutting of final harvest. The 100mg fresh leaves were crushed with pestle and mortar. Chlorophyll content extracted with 2 extraction methods (i) 80% acetone (10ml) (ii) Distilled H_2O (10ml). The solution was centrifuged at 3000rpm for 10mins and read. Take the supernatant and discard the pellet. The absorbance read at 645nm (Chl a); 663nm (Chl b) and 652nm (carotene) wavelength by atomic absorption spectrophotometer (AAS). The chlorophyll content was found out by using Arnold equation (1949).

2.4 Dissection of Plants

The plants were preserved in FAA solution after the final harvest for anatomical study. For staining purpose, the sections were taken from the FAA solution and cut. After sections cutting, these were mounted on watch glass for staining purpose. For staining, sections were dehydrated by alcohol. Different alcoholic ranks (10%, 30%, 50%, 70%, 90% and 100%) were used in staining. Safranin and Fast green were used as dyes. Canada balsam was used for making permanent slides. Sections were observed with the help of electron microscope at 3 magnifications (4X, 10X and 40X) after staining. All anatomical parameters were measured in millimeter (mm).

2.5 Statistical Analysis

Correlation (Pearson) analysis was done by using Statistics 8.1. The sign of significant and highly significant results was (* and **) at 5% and 1% probability level respectively. Duncan test at 5% probability level was done with SPSS 16.0 version to compare the mean values of chlorophyll content.

3. Results and Discussion

The 33% moisture content of seeds was decreased due to drying in the oven before sowing. The germination percentage of four treatments were 0^0C, 6^0C, 35^0C, 60^0C is 33%, 87%, 64% and 53% respectively. A vernalized plant had shown the highest values in all studied (morphological, physiological and anatomical) parameters except No. of leaves which were highest in heat stressed plants. The heat stressed plants reached early at maturity stage than other plants and produced the minute yield. The yield of four different treatments was 175gm/cob, 320gm/cob, 291gm/cob and 235gm/cob respectively. Plants from vernalized seeds were grown three days earlier than other plants. The vernalized seeds showed the thick stem, root and broad leaves after emergence. Vernalized seeds had shown the highest value in studied anatomical parameters than other three stresses.

The emergence period of oat is shortening after low temperature treatment (Holland et al., 2002). The heat shock disturbed the cell division and many biochemical reactions in maize plants. The high temperature causes thermal injury in maize. The high temperature affected the plant growth until the optimum moisture content is available. 30-46% reduction in crops was predicted at the end of 21^{st} century (Rasul *et al*., 2011).

3.1 Morphological Aspect

The morphological attributes affected at both 0°C and 60°C temperature due to the damaged structure of seeds before sowing. The texture and color of stressed plants were not good like 6°C and 35°C. The results of the 6°C temperature were significantly higher than 35°C. High temperature affected the photosynthesis and respiration process. The life cycle was shortened and production also decreased (Barnabas *et al*., 2008). All selected morphological attributes showed a positive and significant link with each other except some traits. CL showed the non-significant relation with FW, NL and LA. NL had also shown the non-significant relationship with FLL, RL and CL.

Table 5: Correlation among Anatomical Traits Of *Z. Mays* L. At 4x Magnification Power

	SD	RD	LD
SD	1.000		
RD	0.936**	1.000	
LD	0.941*	0.958^{ns}	1.000

Legends= SD; Stem Diameter, RD; Root Diameter LD; Leaf Diameter
* = Correlation is significant at 0.05 level; ** = Correlation is highly significant at 0.01 level; ns= Non significant

Table 6: Correlation among Anatomical Traits of *Z. Mays* L. At 10x Magnification Power

	SVB	SEC	SGT	RVB	CC	LVB	LC	LEC
SVB	1.000							
SEC	0.917*	1.000						
SGT	0.891*	0.988**	1.000					
RVB	0.971**	0.951*	0.942*	1.000				
CC	0.951*	0.987**	0.985**	0.985**	1.000			
LVB	0.977**	0.963*	0.980**	0.979*	0.991**	1.000		
LC	0.977*	0.965*	0.973*	0.992**	0.995**	0.997**	1.000	
LEC	0.976**	0.964*	0.981**	0.979*	0.992**	1.000**	0.997*	1.000

Legends= SVB; Stem Vascular Bundle, SEC; Stem Epidermal Cell, SGT; Stem Ground Tissue, RVB; Root Vascular Bundle, CC; Cortex Cell, LVB; Leaf Vascular Bundle, LC; Leaf Cell, LEC; Leaf Epidermal Cell
* = Correlation is significant at 0.05 levels; ** = Correlation is highly significant at 0.01 levels

Table 7: Correlation amid Anatomical Traits of Stems at 40x Magnification Power

	SX	SP	SVB	SEC	SGT
SX	1.000				
SP	0.998*	1.000			
SVB	0.957*	0.940**	1.000		
SEC	0.976*	0.964*	0.997*	1.000	
SGT	0.937*	0.918*	0.998*	0.990**	1.000

Legends= SX; Stem Xylem, SP; Stem Phloem, SVB; Stem Vascular Bundle, SEC; Stem Epidermal Cell, SGT; Stem Ground Tissue
* = Correlation is significant at 0.05 levels; ** = Correlation is highly significant at 0.01 level

Table 8: Correlation amid Anatomical Traits of Roots at 40x Magnification Power

	RX	RP	RVB	CC
RX	1.000			
RP	0.999**	1.000		
RVB	0.994*	0.992*	1.000	
CC	0.989*	0.988*	0.999**	1.000

Legends= RX; Root Xylem, RP; Root Phloem, RVB; Root Vascular Bundle, CC; Cortex Cell
* = Correlation is significant at 0.05 levels; ** = Correlation is highly significant at 0.01 level

Table 9: Correlation amid Anatomical Traits of Leaves at 40x Magnification Power

	LX	LP	LVB	LC	LEC
LX	1.000				
LP	0.986*	1.000			
LVB	0.969*	0.996*	1.000		
LC	0.989*	0.952*	0.922**	1.000	
LEC	0.943*	0.877*	0.832**	0.982*	1.000

Legends= LX; Leaf Xylem, LP; Leaf Phloem, LVB; Leaf Vascular Bundle, LC; Leaf Cell, LEC; Leaf Epidermal Cell
* = Correlation is significant at 0.05 level; ** = Correlation is highly significant at 0.01 level

3.3 Physiological Aspects

Heat stress and chilling temperature showed an adverse effect on chlorophyll contents. Chlorophyll contents were more destroyed by chilling temperature than heat stress. The performance of vernalized plants was slightly better than plants which treated at 35°C temperature. The two methods were used to extract the chlorophyll content from the leaves, but results proved that 80% acetone was better than distilled water, because it had a higher amount of chlorophyll content. The degradation of chlorophyll content was a good indicator to know the effect of heat stress. Chlorophyll contents were sensitive towards high temperature and increased the accumulation of reactive oxygen species (ROS) which is responsible for the cellular injury (Gosavi *et al*., 2014).

Table 10: Effect of Different Temperature Levels On Chlorophyll Content Of *Z. Mays* L

Treatments	**Distilled water**				**80% acetone**			
	Chl a	**Chl b**	**TCC**	**Carotene**	**Chl a**	**Chl b**	**TCC**	**Carotene**
0°C	16.1 ±1.09	29.2 ±2.28	46.3 ±1.21	0.40 ±0.01	16.3 ±0.90	30.9 ±2.03	47.1 ±1.96	0.42 ±0.03
6°C	16.8 ±1.03	36.7 ±2.02	48.1 ±2.03	0.44 ±0.03	16.9 ±1.05	37.2 ±2.24	48.1 ±2.76	0.45 ±0.01
35°C	16.9 ±1.10	31.4 ±2.07	47.8 ±1.98	0.44 ±0.01	16.8 ±1.07	31.5 ±2.02	47.9 ±2.24	0.45 ±0.01
60°C	16.5 ±0.98	31.1 ±2.12	47.4 ±2.31	0.41 ±0.02	16.9 ±1.13	32.2 ±2.18	47.6 ±1.78	0.41 ±0.02

Legends= Chl; Chlorophyll, TCC; Total Chlorophyll Content

Table 11: ANOVA Table of Chlorophyll Contents of *Z. Mays* L

	Traits	**0°C**		**6°C**		**35°C**		**60°C**	
		Treat-ments	**Error**	**Treat-ments**	**Error**	**Treat-ments**	**Error**	**Treat-ments**	**Error**
A	Chl a	15.14**	17.54	15.25*	17.35	18.64*	16.35	16.23*	15.89
	Chl b	51.32*	14.23	49.87*	16.25	53.49*	14.32	45.89*	16.78
	TCC	134.85*	125.3	145.1*	132.5	105.5*	113.2	182.7*	145.3
	Carotene	5.213**	0.180	4.341*	1.382	6.678*	0.984	3.739*	1.74
B	Chl a	15.62*	16.48	16.57*	17.32	18.85*	16.27	16.46*	15.45
	Chl b	51.49*	14.37	49.96*	16.29	53.67*	14.40	45.95*	16.84
	TCC	134.93*	125.8	145.6*	132.7	105.8*	113.3	182.8*	145.38
	Carotene	5.317*	0.193	4.538*	1.395	6.763*	0.973	3.84**	1.79

Legends= A; Distilled Water, B; 80% Acetone
* = Correlation is significant at 0.05 level; ** = Correlation is highly significant at 0.01 level

4. Conclusion

From the present study, it was concluded that the vernalization or low temperature treatment at (6°C) stimulate the plant growth and emergence process of maize. Due to seed vernalization, coming out of shoot and root from maize seeds are earlier than other seeds which treated at 0°C, 35°C and 60°C for 24 hrs. The heat shock and chilling treatment on seeds showed bad effect on plant growth and emergence processes because it disturbs the many morphological and anatomical aspects of maize seeds. 33% moisture content of seeds was decreases due to heat stress. Vernalized plants show the highest values in all morphological, anatomical and physiological parameters except no. of leaves than other treated plants. The growth period between emergences was decreased due to applying the artificial low temperature.

5. Future Perspectives

The *Z. mays* L. seeds were treated at different temperatures before sowing to know the vernalized temperature of seeds which would help them to fight against the high temperature of the studied area after they grow. I chose this topic for research because Pakistan's farmers could not afford the more expensive treatments and crops are destroyed due to high temperature.

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