



## Carbohydrate and Nitrogen metabolism in wheat (*Triticum aestivum L.*) in response to salinity in laboratory and field condition.

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### Abstract

Wheat (*Triticum aestivum L.*) is second important crop being next only to rice and contributes about 33 percent of the total food grain production of this country, and salinity is one of the environmental factor that have a critical influence on the germination of seeds and subsequent establishment of seedling in the soil. In order to investigate salinity stress on wheat germination indices, an experiment was carried out at A.N.D.U.A.T, (student in structural farm) and net house in department of MBB, (Kumarganj, Ayodhya) to create salinity stress at the level of T<sub>0</sub> (as control), 25, 75, 125 mM concentration of NaCl, and ten wheat (*Triticum aestivum L.*) cultivars FLW-11, DBW-303, DBW-71, DBW-129, FLW-3, DBW-187, FLW-8, KH-65, HD-2858, KRL-3-4 were tested. For each treatment rate of germination percent, fresh weight of seedling, dry weight of seedling, seedling length, number of tillers, panicle length, plant height, and number of grain per spike, test weight and other biochemical were compared. In conclusion it was observed the increase in salinity level, it hampers the plant growth and development. However, wheat productivity is adversely affected by salt stress, which is associated with a reduction in germination, growth, altered reproductive behavior and enzymatic activity, disrupted photosynthesis, hormonal imbalance, oxidative stress, and yield reductions. Thus, a better understanding of wheat (plant) behavior to salinity stress has essential implications to devise counter and alleviation measures to cope with salt stress. Different approaches including the selection of suitable cultivars, conventional breeding, and molecular techniques can be used for facing salt stress tolerance. As rate of salinity increase there was significant reduction in plant growth. By investigation it was found that the most salinity tolerant variety is KH65, KRL3-4, DBW187, and least tolerant variety were HD2851, followed by FLW11 and other remaining variety are moderate salt to tolerant.

**Keywords:** oxidative stress; conventional breeding; salinity; enzymatic activity.

## 1. Introduction

In terms of production and consumption, wheat (*Triticum aestivum* L.) is the most significant cereal crop globally. The majority of the world's population depends on wheat to meet their nutritional needs, and wheat-based foods like chapati, bread, biscuits, pasta, and fermented items are eaten by people everywhere. A healthy diet with adequate calories, well-balanced proteins, and micronutrients with minimal antinutrients is necessary for a person's normal growth and development.

Wheat is the most important staple food for humans and is farmed on more acreage than any other crop used for commercial purposes. With India contributing 96 million metric tonnes, or the second-highest amount after China, the world's wheat production in 2017 was 754.1 million tonnes (USDA, 2017). According to Curtis et al. (2002), wheat is traded more globally than all other crops combined.

With a higher protein concentration than other main cereals like maize or rice, wheat is the best vegetable protein source for human meals worldwide (Arzani and Ashraf, 2017). According to Singh (2010), this crop provides over 50% of the calories needed by the people who eat it, which makes a significant contribution to the nation's food security. Much like other crops, a variety of biotic and abiotic factors limit the amount of wheat that may be produced. Drought, extreme heat or cold, and salinity are examples of abiotic stresses that impact crop quality and productivity globally. This is particularly true for emerging nations, where the highest population growth will place a significant demand on reliable food sources (Bates et al., 2008). The issue of soil salinization in agriculture has become a global concern. Seawater and irrigation water, which have very little sodium chloride (NaCl) in them, are the primary sources of salt accumulation in farmed soils (Flowers and Yeo, 1995; Tester and Davenport, 2003). Soil salinity limits crop production in about 20% of irrigated land (Flowers and Yeo, 1995). Wheat production is also affected severely due to salt stress. In India, 6.7 Mha land under wheat cultivation is affected by salt including 3 Mha by salinity and 3.7 Mha by sodicity/alkalinity, distributed across 15 of the 28 states. Out of these 15 states, eight contribute ~97% of national wheat production and have ~5.6 Mha affected by salt (Khokharet al., 2017; Lekshmy et al., 2016). About 10% of wheat cultivated area in the world is already salt affected and is predicted to increase in the future (Rajendran et al., 2009).

Salt stress not only reduces yield but also impairs a number of physiochemical processes in plants, including membrane stability, ion toxicity, cell turgor, and the buildup of toxic metabolites (Kumar et al., 2017; Arzani and Ashraf, 2016). Breeders have made progress in creating salt-tolerant lines for numerous crops thanks to recent advances in our understanding of how plants respond to salt (Kumar and Singh, 2016; Kumar et al., 2017). In addition to identifying the genes responsible for salt tolerance and producing new breeding materials, understanding the biochemical, physiological, and molecular components of salt tolerance will be useful in screening germplasm for breeding in saline circumstances (Sairam et al., 2002).

Therefore, the greatest challenge for the coming decades will be increasing the wheat production from the salt affected lands. Understanding abiotic stress and signaling can be very helpful in improving wheat's genetic resistance to abiotic stress.

## 2. Materials and Methods

Ten genotypes of saline wheat viz., FLW -11, DBW-303, DBWW-71, DBW-129, FLW-3, DBW-187, FLW-8, KHARCHIA-65, HD-2851, KRL-3-4. Kharchia-65 is the check variety used as the study's experimental materials, At the Acharya Narendra Deva

University of Agriculture and Technology, located in Kumarganj, Ayodhya, these genotypes were reproduced using a collection of genetic stock kept in the Wheat division of the Department of Plant Molecular Biology and Genetics Engineering. This experiment is totally based on saline conditions of wheat genotype.

### 3. Results and discussions

Wheat is a staple food and a source of carbohydrate and calories for the majority of people across the globe. However, wheat productivity is adversely affected by salt stress which is associated with reduction in germination, growth, altered reproductive behavior and enzymatic activity, disrupted photosynthesis, hormonal imbalance, oxidative stress and yield reduction.

Thus a better understanding of wheat (plant) behavior to salinity stress has essential implications to devise counter all alleviation measures to cope with the salt stress.

The production of salt-tolerant plant genotypes in salt-affected areas requires a thorough understanding of how plants respond to salinity stress at different levels as well as an integrated strategy that combines molecular tools with physiological and biochemical procedures. At the molecular, cellular, metabolic, and physiological levels, recent research has revealed a variety of adaptive responses to salinity stress.

#### 3.1) Response of wheat genotypes during germination under different regimes of salinity treatment

Ten contrasting genotypes of wheat viz., FLW-11, DBW-303, DBW-71, DBW-129, FLW-3, DBW-187, FLW-8, KH-65, HD-2858, KRL-3-4 were subjected to germination under four regimes of salinity control ( $T_0$ ), 25, 75, 125 mM concentration of NaCl  $T_1, T_2, T_3$  respectively by putting their seeds on top of the filter paper in petriplates.

The following observations were recorded during germination of different parameters i.e.:-

##### 3.1) Germination Percent :-

It is expressed in percent and it was found that there was no difference in germination among all genotypes at control treatment. A slight decrease in germination percent in all genotypes except KH-65, and KRL-3-4, where germination was not significantly decreased even at 125 mM NaCl concentration of salt. The maximum reduction was recorded in FLW11, FLW8 and HD2851.

Germination%						
	Genotype	Treatment				Mean
		$T_0$ (Control)	$T_1$ (25mM)	$T_2$ (75mM)	$T_3$ (125mM)	
	FLW11	98.00	95.00	90.00	89.25	3.06
	DBW303	99.00	98.00	98.00	90.00	6.25
	DBW71	98.00	98.00	95.00	95.00	6.50
	DBW129	97.00	98.00	97.00	92.00	6.00
	FLW3	100.00	100.00	95.00	90.00	6.25
	DBW187	100.00	100.00	90.00	90.00	5.00
	FLW8	97.00	96.00	92.00	90.00	3.75
8	KH 65	100.00	100.00	100.00	100.00	00.00
9	HD2851	100.00	100.00	90.00	90.00	5.00
10	KRL3-4	100.00	100.00	99.90	99.00	9.73
	Mean	98.90	98.50	94.69	92.53	

	Factors	SE(d)	SE(m)	C.D.		
	Treatment(T)	1.01	0.714	2.014		
	Variety(V)	1.597	1.129	3.185		
	T xV	3.193	2.258	N/A		

### 3.2) Length of seedling:-

Seedling length were calculated by root and shoot length of individual seedling and summing up by selecting three random seedling from each replication. the mean value is taken from three seedling from each treatment. seedling length was significantly reduced in all genotype with all salinity treatment. table no. (4.2) The maximum reduction was seen in DBW71 followed by HD2851 and least in KH65 and KRL3-4.

Length of seedling (cm)						
S. No.	Genotype	Treatment				Mean
		T <sub>0</sub> (Control)	T <sub>1</sub> (25mM)	T <sub>2</sub> (75mM)	T <sub>3</sub> (125mM)	
1	FLW11	7	6	6	5	6.00
2	DBW303	6	5.5	5	4	5.13
3	DBW71	6	4.5	4.5	4.15	5.79
4	DBW129	6	4	6	5	5.25
5	FLW3	6	5	4.75	4.25	5.00
6	DBW187	6.75	6	6	5	5.94
7	FLW8	6	6	5	4	5.25
8	KH 65	8	7	6.5	5.75	6.81
9	HD2851	5.75	5	5	4.25	5.00
10	KRL3-4	7	7	6.5	5	6.38
	Mean	6.25	5.60	5.53	4.64	
	Factors	SE(d)	SE(m)	C.D.		
	Treatment(T)	0.062	0.044	0.124		
	Variety(V)	0.098	0.069	0.196		
	T xV	0.196	0.139	0.392		

Fresh wt.ofseedling (gm)						
Genotype	Treatment				Mean	
	(Control)	(25mM)	(75mM)	(125mM)		
FLW11	2.55	2.71	0.98	0.92	1.79	
DBW303	1.99	1.68	1.38	1.32	.5925	
DBW71	2.46	1.26	1.47	1.09	1.57	
V129		51			8775	
73					75	
V187					75	
78	21				052	
65						
851					5	
3-4					25	
n	621	051	2	7		

### 3.3) Fresh weight of seedling

It was calculated by adding fresh weight of root and shoot. There was significant reduction in fresh weight of all genotypes with increase in salinity. The maximum reduction was observed in HD2851 followed by DBW71 and least in KH65.

	ors	)	n)			
	mentT	0	4	1		
	ety(V)	2	3	4		
	r	4	5	8		

3.4)Dry weight of seedling:-

Seedling dry weight was calculated by adding root and shoot dry weight of individual seedling by selecting random seedling from treatment. Seedling dry weight is decreased significantly with increasing salinity in all genotypes. Again maximum reduction was observed in HD2851, followed by DBW71. And least in KH65 and DBW129, given below in table

wt.ofseedling(g)						
	tment					
otype	ontrol)	5mM)	5mM)	25mM)	n	
711	1	5	5	4	65	
V303	7	1		1	475	
V71	4	2	2	5	575	
V129	2	5	9	7	825	
73		4	4	9	675	
V187	1	1	3	9	1	
78	2	1	7	8	7	
65	5	7	8	2	05	
851	5	5	2	1	825	
3-4	5	2	2	5	85	
n	72	63	83	91		
ors	)	n)				
tment(T)	2	1	3			
ety(V)	3	2	5			
r	5	4	1			

3.5)Number of Tiller:-

The following record were taken during vegetative phase of plant. Three plants were selected randomly from each treatment from each variety. There were not so much difference observed, but the maximum number of tillers bearing plants are observed from KH65 and least in HD2851

ftillers(Reproductive stage)						
	tment					

	otype	ontrol)	5mM)	5mM)	25mM)	n
	V11					
	V303					
	V71					
	V129					
	V3					
	V187					
	V8					
	V65					
	V851					
	V3-4					
	n					
	ors	)	n)			
	tment(T)	5	9	9		
	ety(V)	6	1	2		
	V	3	2	4		

3.6) Spikelet/panicle length:-

The spikelet length were measured, and the following observation were made. In which the spikelet length decreased mostly in HD2851 followed by FLW3. And minimum reduction were observed in KH65, DBW187, and other remaining varieties have average and nearly same spikelet length.

Spikelet length(cm)(Flowering/Reproductive)						
	otype	ontrol)	5mM)	5mM)	25mM)	n
	V11					
	V303					
	V71					
	V129					5
	V3					5
	V187					5
	V8					5
	V65	5				75
	V851					
	V3-4					
	n			5		
	ors	)	n)			
	tment(T)	0	4	9		
	ety(V)	2	0	3		
	V	4	1	7		

3.7)Plant height:-

Height of plant is significantly changes due to change in salt concentration of different genotypes. The maximum plant height was noted in KRL 3 -4, followed by KH65. And the least plant height was observed in HD2851 and DBW 187. But the height of plant donot effect overall yield. Some varieties with dwarf shoot characters, yield more then the genotypes having more plant height.

Height (Physical maturity)(cm)					
Genotype	Treatment				n
	(control)	5mM)	5mM)	25mM)	
W 11					5
W 303					5
W 71					
W 129					
W 3					
W 187					5
W 8					5
W 5					5
W 851					5
W 3-4					
n					
Means	(n)	(n)			
Treatment(T)	7	1	9		
Variety(V)	6	2	5		
T	1	4	9		

Estimation of enzyme alpha amylase and isoenzyme of peroxidase:-

3.9)Alpha amylase activity:-

The estimation of enzymatic activity were done in wheat seedling (7DAS) in petri dish (in vitro) of different salt concentration of each genotypes. The yellow coloured complex was formed. The minimum alpha amylase activity was observed in DBW303, HD2851, DBW12 and FLW11. The minimum reduction was observed in KRL3-4, FLW8 followed by KH65.

Alpha amylase				
No.	Genotype	Glucose released µg/g Fresh (control)	Treatment	n
	W 11	.2	.25	.725
	W 303	.12	.17	.145
	W 71	.25	.76	.005
	W 129	.36	.78	.07
	W 3	.34	.15	.745
	W 187	.21	.92	.565



	W 8	.02	.66	.84
	65	.27	.21	.74
	2851	.49	.04	.265
	L 3-4	.75		.875
	an	.701	.494	
	tors	d)	m)	p.
	atment(T)	7	2	5
	iety(V)	2	3	88
	7	35	2	04

**3.10) Isoenzyme of peroxidase**

The peroxidase activity was measured in wheat seedling from root and shoot (7DAS). The peroxidase activity was found to be maximum in salt treated condition in KH65 followed by KRL3-4, DBW187, DBW129. Least peroxidase activity was found in HD2851 and FLW11.

**3.11) Estimation of total soluble sugar**

The total soluble carbohydrate was estimated in leaf and grain by phenol sulphuric acid method. The amount of total soluble was greater in grain as compared to leaves. It was recorded to be maximum in KH65, (195mg/ml) and KRL (187.87mg/ml) 3-4, and found to be minimum in FLW3, DBW129, DBW303, DBW71, followed by FLW11.

	otype	ucose sed(mg/ml)Freshwt rol	ucose sed(mg/ml)Freshwt, ment	n
	711			
	V303			
	V71			5
	V129			5
	73			5
	V187			
	78			5
	55			
	851			5
	3-4			5
	n		5	
	ors	d)	n)	
	atment(T)	3	3	
	ety(V)	2	5	3
	7	2	2	5

**3.12) Estimation of protein by folin lowry's method**

True protein content in wheat leaves presented in table no. (4.13) It was observed that the highest protein content was found in KH65, followed by KRL3-4 and minimum in DBW303, DBW129 followed by HD2851.

Genotype	Protein content (g Fresh wt. control)	Protein content (g Fresh wt. treatment)	Mean
FLW11	18	11	19.5
DBW303	12	12	12
FLW71	11	11	10.5
DBW129	11	11	11
FLW3	13	13	11.5
FLW187	14	14	12
FLW8	13	13	11.5
DBW55	12	12	11.5
DBW851	11	11	11
KRL3-4	13	13	11.5
Mean	17.8	17.9	
Factors	(D)	(n)	
Treatment (T)	5	2	1
Genotype (V)	9	2	9
Replication	4	9	7

**To estimate nitrate reductase (NR) and nitrite reductase (NiR) enzyme activity in leaves in response to NaCl salinity**

**3.13) Nitrate reductase (NR)**

The nitrate reductase activity was assayed from wheat leaves of ten different genotypes, and it was observed that the nitrate was found to be maximum in KH65 in treatment of different salt concentration followed by KRL3-4 and least in DBW129, FLW11, HD2851 and DBW303.

Genotype	Nitrate con. (µg NO <sub>2</sub> /g fresh wt./hr) control	Nitrate con. (µg NO <sub>2</sub> /g fresh wt./hr) treatment	Mean
FLW11	32	32	16
DBW303	22	28	15
FLW71	21	32	15.5

	V129	24	312	276
	73	51	71	11
	V187	72	29	505
	78	27	73	5
	65	71	63	67
	851	26	23	745
	3-4			5
	n	076	5502	
	ors	)	n)	
	ment(T)			4
	ety(V)			89
	r			41

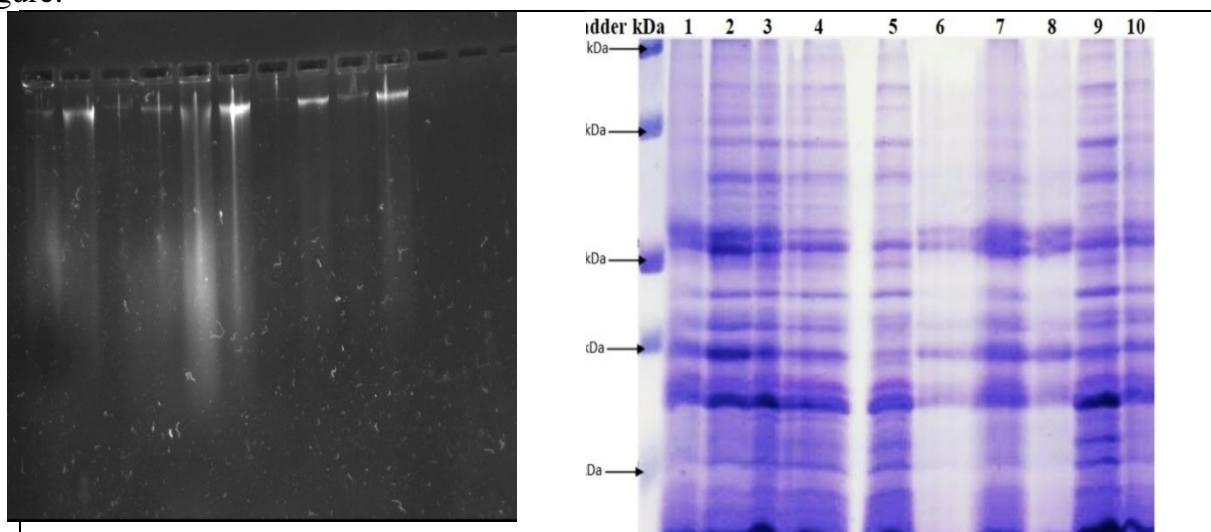
**3.14) Nitrite reductase activity**

The estimation of nitrite reductase activity was done by wheat leaves, from ten different wheat genotypes. There was maximum nitrite concentration was found in KH65 and KRL3-4 in treatment and least in FLW11 and HD2851, while it was also observed that the nitrite reductase activity was maximum in FLW11 (596.21 nmol) in control.

Nitrite reductase				
No.	Genotype	Control	Treatment	Mean
	FLW11	21		605
	V303	14		707
	V71	92	79	855
	V129	51	28	395
	73	24	85	7045
	V187	91	71	81
	78	17	44	805
	65	79	34	565
	851	37		185
	3-4	24	47	86
	n	35	688	
	ors	)	n)	
	ment(T)			
	ety(V)			8
	r			7

### 3.15) Estimation of proline

Proline was measured in both root and shoot at vegetative stage and result are presented in figure.



Initial proline content was found to be higher in leaf than in root. Salinity treatment was result in increase in proline concentration in both root and shoot. However increase was more in leaf than root. The maximum accumulation of proline was recorded in KH 65 and followed by KRL3-4, DBW187 in treatment. And least in FLW 11, HD2851, followed by DBW303.

Sample	Proline type	Proline concentration (µg/ml control)	Proline concentration (µg/ml treatment)	Change (%)
FLW 11				
DBW303			5	
FLW71				
FLW129			5	35
FLW73				75
DBW187				5
FLW78				75
KH65				
HD2851				
KRL3-4				
FLW11			2	
Control	Control	Control	Control	
Treatment (T)	9	9	5	1
Salinity (V)	7	7	5	0
Control	0	0	7	9

### Conclusion

Soil salinity has become of the limiting environmental factors for crop productivity in many parts of India. It severely hampers the response of standing crops by altering its physiological attributes. Hence, for sustaining crop production, it is imperative to understand the physiological and biochemical adaptations, imparting tolerance to crops towards abiotic stress like salt. Salt stress negatively affects seed germination, plant growth, photosynthesis, ATP production, water relationships, nutrient uptake and yield because of a salt-induced oxidative stress and ionic and hormonal imbalances. Wheat crop shows a wide range of morphological, physiological, and molecular responses under salinity stress. The physiological and molecular mechanisms are very important because they can help the breeders to develop salt tolerance in wheat. These mechanisms against salinity stress are well understood in wheat. However, a better understanding is still needed in many fields, especially in understanding the physiological basis of assimilate partitioning from plant sources to sinks. Additionally, more studies are needed to study the response of roots to salinity stress involving the root-shoot signaling and corresponding impacts on the nutrient and water uptake. Genetic manipulation of salt-tolerant traits is also an important approach to improve salinity tolerance in wheat crops.

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