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Effects of waterlogging and salinity stresses on biochemical changes in tolerant and susceptible varieties of wheat (*Triticum aestivum* L.)

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Abstract
The present investigation was conducted during two consecutive years 2012 and 2013 to understand the possible mechanism of salinity tolerance to wheat (*Triticum aestivum* L.) under waterlogging condition. Fifteen genotypes of wheat were screened on the basis of survival of the seedling kept under waterlogging for 10 days in sodic field. Five centimeter deep waterlogging was created for ten days at 30-day stage of seedling by providing irrigation and at 40 DAS water was drained from field. Soluble sugar and starch content in leaves decreased in all the wheat varieties under water logging condition as compared to non-water logging condition. Potassium and calcium content in leaves significantly decreased with water logging treatment in all the wheat varieties. Greater adverse effect of water logging was observed in susceptible varieties. Manganese content in leaves increased with water logging treatment. Under water logging condition maximum increment was obtained in Susceptible then tolerant varieties.

Keywords: Water logging, sodic soil, soluble sugar

Introduction
Wheat is the most important cereal crop; it is staple diet for more than one third of the world population [1]. Soil salinity is a major abiotic stress which limits plant growth and development, causing yield loss in crops. Salt-affected soils are identified by excessive levels of water-soluble salts, especially sodium chloride (NaCl) NaCl, a major salt contaminant in soil, is a small molecule which when ionized by water, produces sodium (Na^+) and chloride (Cl^-) ions. These toxic ions cause ionic and osmotic stress at the cellular level in higher plants, especially in susceptible [2].
During waterlogging, the gas exchange between soil and air decrease as gas diffusion in water is decreased 10,000 fold slower that than the air. Oxygen in the soil is rapidly depleted and the soil may become hypoxic or anoxic within a few hours. Moreover, some waterlogged soils become rich in Mn^{+2} and Fe^{+2}, devoid of NO_3^- and SO_4^{-2} and anaerobic microbial metabolities may accumulate. These effects become more pronounced during prolonged periods of waterlogging. Oxygen deficiency inhibits aerobic respiration, resulting in severe energy deficiency and eventually death [3] (Greenway & Gibbs, 2003). Carbohydrate which is the fuel for alcoholic fermentation has been recognized as an important factor in submergence tolerance treatments which alter the carbohydrate status of the plants at the time of submergence tolerance [4].
In addition, Waterlogging can also increase the availability of some essential nutrients, e.g. Fe and Mn. Such increases in micronutrients in soil and subsequently in shoots may affect plants both during waterlogging and also after waterlogging during recovery as higher micronutrients concentration in shoot have been reported during recovery period when soils have returned to fully aerated conditions [5]. Barrett-Lennard (2003) [6] reported about 2 times higher Na concentration in shoot of wheat under waterlogging relative to drained condition. Similarly, Fe, Mn, Al & B increase many folds in shoots of wheat under waterlogging relative to drained condition in sodic soil, while waterlogging reduces uptake of N, P, K, Mg & Zn in wheat [7]. Strategies that could be used for short term waterlogging tolerance on the other hand, are high rates of alcoholic fermentation to ensure energy supply during anoxia, high carbohydrate content as substrate for respiration, maintenance of membrane integrity and reduced metabolic leakage, increased efficiency of nutrient uptake and more efficient free radical scavenging system to avoid post waterlogging oxidative damage. Though physiological mechanisms for soil sodicity tolerance are well studied but the interaction between sodicity and waterlogging

tolerance is least reported [8]. In the present study the effects of waterlogging on soluble sugar, starch and uptake of nutrients, were investigated.

Materials and Methods

Field experiments were conducted during two consecutive years of 2012-13 and 2013-14 at the Main Experiment Station, of the Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, (U.P.), India. The experiment was carried out with 15 varieties of wheat, viz DBW-17, Kharchia-65, KRL240, NW 4018, KRL99, BH1146, Krichauff, KRL210, HD2009, Brookton, NW1014, KRL238, HD2851, KRL3-4 and Ducula 4 in factorial randomized block design in three replications under NWL (non-waterlogging) and WL (waterlogging) conditions. The soil of the experimental field was silty clay texture (24% sand, 55% silt and 21% clay), pH 8.9-9.1, EC 2.8 dS m-1 and 210, 22.5 and 231.4 kg of available N, P and K ha^{-1}, respectively. Wheat varieties were collected from Department of Genetics & Plant Breeding of the university. Seeds were sown in the third week of November during both the years. The total phosphorous, potash and half dose of nitrogen were applied @ 120:60:40 (N:P:K) kg/ha as basal dose at the time of sowing and remaining nitrogen was applied in two equaldoses at tillering and at the time of ear emergence, respectively. The waterlogged treatments were given by flooding the field up to 5 cm. depths at 30 days after sowing (DAS) and water depth was maintained for 10 days. After 10 days, water was drained from the field. The total soluble sugar content was determined by the method of Yemm and Willis (1954) [9]. Starch was estimated through following anthrone reagent method, described by Mc Cready *et al.* (1950) [10]. Potassium were determined with the flame photometer. Mn and Ca by atomic absorption spectrophotometer.

Results and Discussion

All the wheat varieties have higher soluble sugar under non-waterlogging condition. Waterlogging decreased the soluble sugar content in all the wheat varieties at all the crop growth stages of observation (Table 1). The tolerant varieties KRL 99 and KRL 3-4 have also maintained higher soluble sugar content during waterlogging relative to susceptible varieties HD 2009 and HD 2851 which showed drastic reduction in soluble sugar content. This was inconformity with the findings of Sharma *et al.* (2005b) [11] in wheat. Reduction in soluble sugar during waterlogging is one of the crucial biochemical events which affect the survival and growth during waterlogging treatments. This reduction is possible due to reduced photosynthesis during waterlogging and utilization of existing soluble sugar in leaf respiration during waterlogging. Loss of chlorophyll reported earlier could be responsible for reduced photosynthesis and soluble sugar under waterlogging condition. Under non-waterlogging condition all wheat varieties have higher starch content in leaves but waterlogging adversely affected the starch content in all the varieties table 1. The effect was more pronounced in susceptible varieties in comparison to tolerant varieties. Sharma *et al.* (2005) [12] also reported the similar trend of starch decreasing in wheat.

Mineral content of wheat plants varied in different varieties showing variable sensitivity of varieties to waterlogging. Tolerant varieties KRL 99 and KRL 3-4 showed higher Ca and K content relative to susceptible varieties HD 2009 and HD 2851 which could be possible due to less adverse effects of waterlogging on metabolic functioning of roots in these varieties (Table 2). Tolerant varieties somehow could maintain higher energy status needed for nutrient uptake. These varieties could also probably maintained appropriate oxygen diffusion rates even in waterlogged soil condition enabling roots to continue their functions without any drastic impairment of nutrient uptake [5]. Manganese content in leaves increased with waterlogging treatment. Under waterlogging condition maximum increment was obtained in HD 2851 followed by HD 2009, while minimum increment recorded in KRL 3-4 (Table 2). Sharma and Swarup (1988) [13], and Stieger and Feller (1994) [14] also reported reduced uptake of N, P, K, Ca, Mg and Zn while increasing Na, Fe and Mn absorption under alkaline soil conditions. Less nitrogen concentrates and accumulates in the upper leaves of waterlogged wheat, probably due to the denitrification of soil nitrogen [15]. Nitrogen remobilization from lower leaves is accelerated on flooded soils and explains their chlorosis [14].

Iron, aluminum and manganese content in leaves increased in waterlogging as compared to non-waterlogged. Khabaz-Saberi *et al.* (2012) [16] also reported that Al, Mn and Fe concentrations increased in wheat shoot by up to 5-, 3- and 9-fold respectively due to waterlogging in various soils. Waterlogging causes reduction of oxidized compounds, *e.g.*, Fe^{3+} and Mn [4+ 17], leading to an increase in concentration of Fe and Mn beyond the plant nutritional requirements, which results in poor plant growth. Gutierez Boem *et al.* (1996) [18] reported that water logging resulted in a decrease of N, P, K and Ca uptake by *Brassica napus* L. On the other hand, water logging changes the available ion concentration of the soil solution. Due to electrons excess, Fe^{2+} and Mn^{4+} are reduced to $Fe^{2+}$and Mn^{2+}, respectively. Anaerobic soil conditions increase oxalate-soluble P and Fe in soils [19]. Also, soil flooding improves the bioavailability of P, Fe, and Mn to rice, which is adapted to water logging. Plants that are not tolerant to water logging, may suffer from Fe or Mn toxicity[20]. Yaduvanshi *et al.* (2007) [21] also reported waterlogging significantly increased the Mn concentration in both the soils compared with drained conditions. Stieger and Feller (1994)[14] reported that waterlogged condition in wheat affected flag leaf and second leaf from top leaves and decreased the potassium, phosphorus and magnesium content in shoots. Morad and Silvestre (1996) [22] reported that under oxygen-deficient conditions the root cell energy pool greatly decreased. It is likely that ATP concentrations in roots decreased because of inhibition of respiration by anoxia [23]. Low ATP concentrations in roots affect the activity of the plasma membrane H^+ ATPase [24].

Tolerant varieties KRL 99 and KRL 3-4 showed higher Ca and K content relative to susceptible varieties HD 2009 and HD 2851 which could be possible due to less adverse effects of waterlogging on metabolic functioning of roots in these varieties. Tolerant varieties somehow could maintain higher energy status needed for nutrient uptake. Thus the characters may be transferred to high yielding varieties for better yield.

Table 1: Effect of waterlogging on total soluble sugar in leaves (mg g^{-1} dry wt.) of wheat varieties under sodic soil

Varieties	40 DAS			80 DAS		
	NWL	WL	Mean	NWL	WL	Mean
KRL210	77.99	57.31 (27)	67.65	75.25	64.97 (14)	70.11
HD2009	75.97	53.28 (30)	64.63	71.00	62.96 (11)	66.98
BROOKTON	76.91	55.22 (28)	66.07	73.03	63.90 (13)	68.46
NW1014	81.66	63.00 (23)	72.33	77.62	68.65 (12)	73.14
KRL238	74.15	50.45 (32)	62.30	68.98	61.14 (11)	65.06
DUCULA4	72.24	47.54 (34)	59.89	70.83	59.49 (16)	65.16
KRL3-4	86.04	70.40 (18)	78.22	85.45	76.05 (11)	80.75
HD2851	62.73	45.41 (28)	54.07	64.43	55.41 (14)	59.92
DBW17	55.33	40.94 (26)	48.14	59.24	51.38 (13)	55.31
KH-65	76.50	64.55 (16)	70.53	79.95	69.20 (13)	74.57
KRL240	58.71	40.72 (31)	49.71	61.03	54.43 (11)	57.73
NW4018	65.94	54.73 (17)	60.33	67.62	59.51 (12)	63.57
KRL99	77.39	66.56 (14)	71.98	82.87	74.59 (10)	78.73
BH1146	79.63	61.31 (23)	70.47	77.26	66.62 (14)	71.94
KRICHAUFF	61.05	40.90 (33)	50.98	58.23	48.04 (18)	53.13
Mean	72.15	54.16	63.15	71.52	62.42	66.97
	V	C	VxC	V	C	VxC
SEm±	1.33	0.49	1.89	1.36	0.50	1.92
CD at 5%	3.74	1.36	NS	3.81	1.39	NS

Values in parenthesis indicate percent decrease in WL over NWL

Table 2: Effect of waterlogging on starch content in leaves (mg g^{-1} dry wt.) of wheat under sodic soil.

Varieties	40 DAS			80 DAS		
	NWL	WL	Mean	NWL	WL	Mean
KRL210	77.56	59.09 (24)	68.32	88.98	74.74 (16)	81.86
HD2009	72.77	54.30 (25)	63.54	84.19	68.19 (19)	76.19
BROOKTON	74.90	56.43 (25)	65.66	86.32	70.78 (18)	78.55
NW1014	77.70	62.25 (20)	69.97	92.14	82.93 (10)	87.53
KRL238	71.40	52.93 (26)	62.17	82.82	66.26 (20)	74.54
DUCULA4	69.50	47.26 (32)	58.38	80.92	61.50 (24)	71.21
KRL3-4	84.33	75.90 (10)	80.11	105.79	99.44 (6)	102.62
HD2851	71.13	47.47 (33)	59.30	77.36	58.02 (25)	67.69
DBW17	72.24	43.64 (40)	57.94	73.53	55.15 (25)	64.34
KH-65	82.37	73.64 (11)	78.01	103.53	92.15 (11)	97.84
KRL240	73.14	44.43 (39)	58.79	74.32	53.51 (28)	63.91
NW4018	74.15	62.29 (16)	68.22	75.65	67.33 (11)	71.49
KRL99	81.81	70.36 (14)	76.08	108.26	94.19 (13)	101.22
BH1146	79.04	60.57 (23)	69.80	90.46	75.08 (17)	82.77
KRICHAUFF	79.79	62.24 (22.0)	71.02	72.30	59.29 (18)	65.79
Mean	76.12	58.19	67.15	86.44	71.90	79.17
	V	C	VxC	V	C	VxC
SEm±	1.44	0.53	2.03	1.65	0.60	2.33
CD at 5%	4.03	1.47	5.70	4.61	1.68	NS

Values in parenthesis indicate percent decrease in WL over NWL.

Table 3: Effect of water logging on shoot Mn, Ca and K concentration of Wheat varieties under sodic conditions

Varieties	Mn			Ca			K		
	NWL	WL	Mean	NWL	WL	Mean	NWL	WL	Mean
KRL210	76	83 (+10)	79.57	7556	2166 (71)	4861.00	3.22	1.24 (61)	2.23
HD2009	56	76 (+34)	66.01	1813	1505 (17)	1659.00	3.02	1.18 (61)	2.10
BROOKTON	64	81 (+25)	72.54	2652	2334 (12)	2493.00	3.56	1.17 (67)	2.37
NW1014	68	89 (+30)	78.40	2854	2569 (10)	2711.00	2.85	1.31 (54)	2.08
KRL238	70	77 (+10)	73.50	2518	2090 (17)	2304.00	3.12	1.09 (65)	2.11
DUCULA4	51	66 (+28)	58.50	2015	1571 (22)	1793.00	2.96	0.89 (70)	1.92
KRL3-4	47	52 (+10)	49.37	2619	2331 (11)	2475.00	3.12	1.34 (57)	2.23
HD2851	42	56 (+35)	48.93	2216	1732 (22)	1974.00	2.72	0.90 (67)	1.81
DBW17	73	90 (+23)	81.50	2149	1793 (17)	1971.00	3.12	1.06 (66)	2.09
KH-65	40	48 (+20)	43.96	2317	1992 (14)	2155.00	3.02	1.31 (57)	2.17
KRL240	68	85 (+25)	76.50	2383	1859 (22)	2121.00	2.35	0.87 (63)	1.61
NW4018	59	74 (+26)	66.41	2048	1597 (22)	1823.00	2.79	1.09 (61)	1.94
KRL99	49	59 (+20)	54.00	2317	1969 (15)	2143.00	3.49	1.45 (58)	2.47
BH1146	43	54 (+27)	48.41	1917	1470 (23)	1694.00	2.99	1.01 (66)	2.00
KRICHAUFF	50	65 (+30)	57.16	1914	1400 (27)	1657.00	2.62	1.02 (61)	1.82
Mean	56.67	69.87	-	2619.0	1892.0	2255.00	3.00	1.13	2.06
	V	C	VxC	V	C	VXC	V	C	VXC
SEm±	1.34	0.49	1.89	52.46	19.07	73.88	0.05	0.02	0.07
CD at 5%	3.74	1.37	NS	146.32	53.43	206.96	0.14	0.05	0.19

Values in parenthesis indicate percent decrease in WL over NWL

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