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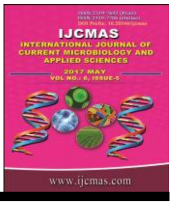

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Effect of Water Logging and Salinity Stress on Physiological and Biochemical Changes in Tolerant and Susceptible Varieties of *Triticum aestivum* L.

Mubeen[1]*, A.H. Khan[2], S.P. Singh[3], A.K. Singh[2], A.R. Gautam[2],
Mohd. Meraj Khan[4] and Nadeem Khan[5]

[1]Mohammad Ali Jauhar University, Rampur, (U.P.) India
[3]Indian Institute of Sugarcane Research, (IISR) Lucknow, (U.P.) India
[2]Department of Crop Physiology, [4]Department of Vegetable Science, Narendra Deva University of Agriculture and Technology Kumarganj, Faizabad- 224 229 (U.P.), India
[5]Integral University, Lucknow, India
**Corresponding author:*

ABSTRACT

The present investigation was conducted during two consecutive years 2012 and 2013 to understand the possible mechanism of salinity tolerance to wheat under water logging condition. Fifteen genotypes of wheat were screened on the basis of survival of the seedling kept under water logging for 10 days in sodic field. Five centimeter deep water logging was created for ten days at 30-day stage of seedling by providing irrigation and at 40 DAS water was drained from field. The results revealed that water logging treatment reduced chlorophyll content in leaves in all the genotypes. Sodium and iron content increased in leaves under water logged condition in all the varieties while reverse trend was observed under non waterlogged condition. Antioxidant enzymes (superoxide dismutase, catalase and peroxidase) and nitrate reductase activity increased under waterlogged condition in all the varieties as compared to non waterlogged but drastic increase was noted in case of tolerant than susceptible varieties.

Keywords

Sodic soil,
Water logging,
Antioxidant enzyme, Na and Fe.

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Introduction

Wheat is the most important cereal crop; it is staple diet for more than one third of the world population (Abd-El-Haleem *et al.*, 2009). 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), 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 (Chinnusamy *et al.*, 2005).

Waterlogging changes plant metabolic activity. One of the root metabolic features affected by waterlogging condition is the antioxidant system. Waterlogging generates oxidative stress and promotes the production of reactive oxygen species (ROS) including superoxide (O_2^-), singlet oxygen hydroxyl anion (OH^-), and hydrogen peroxide(H_2O_2)

which can be detrimental to proteins, lipids and nucleic acid. In plants, enzymatic and non enzymatic defense systems are involved in ROS scavenging and detoxification. In enzymatic defense system, superoxide dismutase (SOD) constitutes the first line of defense against ROS by dismutating O_2^- to H_2O_2. When plant roots are subjected to waterlogging condition SOD activity increases in barley roots Kalashnikov *et al*., (1994) and remain unaffected in tomato Lin *et al*., (2004). H_2O_2 is decomposed by peroxidase (POX) and catalase (CAT).

Waterlogging is a serious problem, which affects crop growth and yield in low lying rainfed areas. The main cause of damage under waterlogging is oxygen deprivation, which affect nutrient and water uptake, so the plants show wilting even when surrounded by excess of water. Lack of oxygen shift the energy metabolism from aerobic mode to anaerobic mode. Plants adapted to waterlogged conditions have involvement of antioxidant defense mechanism to cope with the post hypoxia/anoxia oxidative stress. Gaseous plant hormone ethylene plays an important role in modifying plant response to oxygen deficiency. Waterlogged plants are affected by various stresses, such as limitations to gas, and mineral nutrient deficiencies and microelement toxicities (Setter *et al*., 2009). In addition, waterlogging can also reduce the availability of some essential nutrients, e.g. Fe and Mn (Ponnamperuma, 1972). Such increase in micronutrients in soil and subsequently in shoots may affect plants both during waterlogging and during recovery as higher micronutrients concentrations in shoots have been reported during recovery period when soils have returned to fully aerated conditions (Setter and Waters, 2003).The above effect of waterlogging is more aggravated in sodic soils. Barrett and Lennard (2003) reported about 2 folds higher Na concentration in shoot of wheat under waterlogging relative to drained condition. Similarly, Fe and Mn increase many folds in shoots of wheat under waterlogging relative to drained condition in sodic soil (Setter *et al*., 2004), Growing wheat genotypes tolerant to waterlogging and element toxicities may be desirable in sodic soil but there is no much literature about the extent of variability in waterlogging tolerance in wheat genotypes. Some wheat varieties may adopt better or have greater tolerance to waterlogging in sodic soil than others.

In the present study the effects of waterlogging on chlorophyll content, carbohydrate, uptake of nutrients, activity of nitrate reductase and antioxidant enzymes 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, KH-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 equal doses 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 and chlorophyll, total soluble sugar, antioxidant enzymes and nitrate reductase activity were determined. The total chlorophyll content was determined by the method of Arnon (1949) in fresh leaves. Nitrate reductase activity was assayed according to the method of Jaworski (1971). Catalase activity by Sinha (1972), Peroxidase by Curne and Galston (1959) and SOD by Giannopolitis and Ries (1977), in fresh leaves. Sodium were determined with the flame photometer and iron by atomic absorption of spectrophotometer.

Results and Discussion

Waterlogging and sodic condition produced reduction in chlorophyll content in all wheat varieties. The effect was more pronounced in HD 2009, KRICHAUFF, KRL-240, DACULA 4, BROKTON, DBW17 and HD2851 as compared to tolerant wheat genotypes NW1014, NW4018, BH1146, KRL-3-4, KH-65 and KRL 99 (Table 1). Similar results were also reported by Sharma *et al.*, (2005 b) in wheat and Prasad *et al.*, (2004) in maize genotypes. Decreased in leaf chlorophyll under waterlogging condition may also be directly related to nitrogen deficiency caused by leaching and increased denitrification of the applied nitrogen as reported by Tsai *et al.*, (1997) in corn. In addition it could also be due to increase in enzyme of chlorophyll degradation. The loss of chlorophyll could be high due to ethylene content in soil and its transport to leaves or imbalance in nitrogen metabolism which induces chlorosis of leaves. A perusal of data presented in (Table 2) clearly indicates that nitrate reductase activity significantly decreased in all the wheat varieties at the end of waterlogging period. Highest activity of enzyme was recorded in KRL3-4 and KRL 99 were higher than rest of the varieties KRL 240, NW 4018, HD 2009, KRICHAUFF, DBW 17, and DUCULA-4 showed the lowest enzyme activity at 40 DAS. Highest reduction due to waterlogging treatment was observed in KRL 238 followed by DUCULA-4, HD 2851, HD 2009, KRL 240 and NW 4018. While KH-65 was least affected due to waterlogging and varieties like KRL 3-4, KRL 99, NW 1014 and KRICHAUFF recorded less reduction due to waterlogging Nitrate reductase plays a vital role in the regulation of assimilation of nitrate in plants. Soil moisture saturation adversely affects the nitrate reductase activity Nelson *et al.*, (1996). The results are in accordance with Prasad *et al.*, (2004) in maize.

The catalase and peroxidase activity significantly increased under waterlogging in all wheat genotypes. Maximum enzyme activity was found in varieties KRL 99, KH-65 and KRL 3-4 (Table 3) under water logging condition. However, minimum enzyme activity was observed in HD-2009, DBW-17 and KRL-240. The oxidative damage to cellular component is limited under control condition due to efficient processing of reactive oxygen species (ROS) through a well coordinated and rapidly responsive antioxidant system consisting of several enzymes and redox metabolites. Zhou and Lin (1995) reported reduction in leaf catalase activity in *Brassica napus*. Superoxide dismutase activity significantly increased under water logged condition in comparison to control in all the genotypes (Table 2) but maximum increase was noted in KRL 3-4, KR 99, NW 1014 for DBW17, HD 2009 and this enhancement was one and half fold more than non waterlogged. It is also evident that plants with higher constitutive active oxygen scavenging system (AOS) and ability to synthesize them more rapidly and efficiently during post-anoxia, presumably suffer less damage (Bokhina *et al*., 2003) and had better growth during recovery phase (Jackson and Ram, 2003).

Results observed on various antioxidant enzymes like SOD, APX, GR and CAT under waterlogged condition in tolerant and susceptible wheat genotypes reveal an increase in all the three enzymes. It has been suggested by various workers that the reason for the increase in antioxidant enzyme activities during waterlogging is primarily to take care of post hypoxia oxidative stress. Monk *et al*., (1987) observed a continuous increase in SOD activity in rhizomes of *Iris pseudacorus* under waterlogging stress. The results obtained by Blokhina *et al*., (2001) suggested that there indeed is an increase in oxidative stress during waterlogging, and the increase in antioxidant enzymes were to scavenge build up ROS. The plants can also suffer by ROS production when they are returned to aerobic condition and this explains overall higher antioxidant enzymes activity in tolerant genotype not only during waterlogging but also during recovery as compared to control plants. Waterlogging significantly increased Na (Table 1) in the leaves of all the varieties as compared to non waterlogged condition.

Table.1 Effect of water logging on total chlorophyll content and nitrate reductase activity of different wheat varieties in sodic soil

Varieties	**Total chlorophyll content (mg g^{-1} fresh weight)**			**NR Activity (µg nitrate produced g^{-1} fresh weight)**		
	WL	**NWL**	Mean	**WL**	**NWL**	**Mean**
KRL210	2.86	2.06(28)	2.46	94.70	51.38(46)	73.04
HD2009	2.76	1.74(37)	2.25	84.28	36.93(56)	60.60
BROOKTON	2.82	1.84(35)	2.33	91.58	46.24(50)	68.91
NW1014	2.95	2.21(25)	2.58	96.48	60.01(38)	78.24
KRL238	2.72	2.02(26)	2.37	86.43	37.06(67)	61.74
DUCULA4	2.66	1.84(31)	2.25	85.40	34.02(60)	59.71
KRL3-4	2.83	2.21(22)	2.52	97.83	71.20(27)	84.52
HD2851	2.62	2.02(23)	2.32	89.54	36.14(60)	62.84
DBW17	2.57	2.00(22)	2.29	87.25	44.50(49)	65.87
KH-65	2.83	2.18(23)	2.51	86.14	66.48(23)	76.31
KRL240	2.52	1.79(29)	2.16	81.81	36.82(55)	59.31
NW4018	2.90	2.17(25)	2.53	82.18	39.44(52)	60.81
KRL99	2.82	2.17(23)	2.50	93.90	67.61(28)	80.75
BH1146	2.89	2.23(23)	2.56	96.48	55.17(43)	75.83
KRICHAUFF	2.41	1.78(26)	2.09	85.94	50.70(41)	68.32
Mean	2.75	2.02	2.38	89.33	48.91	69.12
	V	C	VxC	V	C	VxC
SEm±	0.05	0.02	0.07	1.44	0.53	2.03
CD at 5%	0.13	0.05	NS	4.03	1.47	5.70

Values in parenthesis indicate percent decrease in WL over NWL.

Table.2 Effect of waterlogging and salinity stresses on biochemical changes in tolerant and susceptible varieties of wheat

Varieties	**Catalase activity (units/g fresh wt.)**			**Peroxidase activity (unit/g fresh weight/ min.)**			**SOD (enzyme unit g^{-1} fresh weight)**		
	NWL	**WL**	**Mean**	**NWL**	**WL**	**Mean**	**NWL**	**WL**	**Mean**
KRL210	92.69	254.23(174)	173.46	170.27	257.61(51)	213.94	114.18	295.5 (159)	204.86
HD2009	82.62	218.10(164)	150.36	160.19	246.58(54)	203.38	103.77	254.24(145)	179.01
BROOKTON	90.68	252.21(178)	171.44	168.25	255.41(52)	211.83	111.83	293.18 (162)	202.51
NW1014	91.68	262.21(186)	176.95	174.30	278.88(60)	226.59	117.88	327.70(178)	222.79
KRL238	86.65	248.18(186)	167.41	164.22	251.00(53)	207.61	107.80	289.15(168)	198.48
DUCULA4	84.63	222.58(163)	153.60	162.21	248.79(53)	205.50	105.79	270.82(156)	188.30
KRL3-4	96.72	266.95(176)	181.83	165.23	275.93(67)	220.58	119.89	335.70(180)	227.80
HD2851	88.66	236.72(167)	162.69	166.24	253.21(52)	209.72	109.82	291.17(165)	200.49
DBW17	76.57	214.40(180)	145.48	154.15	239.91(56)	197.03	97.73	261.91(168)	179.82
KH-65	98.74	296.21(200)	197.47	176.31	290.92(65)	233.61	107.80	306.16(184)	206.98
KRL240	78.59	225.54(187)	152.06	156.16	242.13(55)	199.15	99.74	266.31(167)	183.03
NW4018	80.60	229.71(185)	155.16	158.18	244.36(54)	201.27	101.76	283.11(178)	192.43
KRL99	105.79	303.61(187)	204.70	175.31	282.24(61)	228.77	114.86	331.93(189)	223.39
BH1146	94.71	256.24(171)	175.47	172.28	259.81(51)	216.05	115.86	297.21(157)	206.54
KRICHAUFF	84.63	236.09(179)	160.36	153.14	232.77(52)	192.96	94.71	265.98(181)	180.34
Mean	88.93	248.20	168.56	165.10	257.30	211.20	108.23	291.34	199.78
	V	C	VxC	V	C	VxC	V	C	VxC
SEm±	3.97	1.45	5.61	4.63	1.69	6.54	4.50	1.64	6.37
CD at 5%	11.11	4.06	15.72	12.96	4.73	NS	12.61	4.60	17.83

Values in parenthesis indicate percent decrease in WL over NWL.

Table.3 Effect of waterlogging and salinity stresses on uptake of Na and Fe in tolerant and susceptible varieties of wheat

Varieties	**Na(ppm)**			**Fe(ppm)**		
	NWL	**WL**	**Mean**	**NWL**	**WL**	**Mean**
KRL210	11083	14740(+33)	12911.50	117	470(+302)	293.50
HD2009	7556	11259(+49)	9407.50	136	612(+350)	374.00
BROOKTON	8564	12503(+46)	10533.50	120	556(+360)	338.00
NW1014	9168	12102(+32)	10635.00	99	385(+290)	241.90
KRL238	9571	12825(+34)	11198.00	88	368(+320)	227.90
DUCULA4	9840	14661(+49)	12250.50	101	474(+370)	287.14
KRL3-4	9471	12122(+28)	10796.50	87	329(+280)	207.95
HD2851	12090	18135(+50)	15112.50	111	521(+370)	315.85
DBW17	10075	13601(+35)	11838.00	104	457(+340)	280.19
KH-65	9269	11772(+27)	10520.50	90	350(+290)	219.69
KRL240	9612	13552(+41)	11582.00	91	389(+330)	239.50
NW4018	9706	13297(+37)	11501.50	89	395(+340)	242.10
KRL99	7590	9715(+28)	8652.50	90	362(+300)	226.00
BH1146	9571	12930(+35)	11250.50	111	488(+340)	299.23
KRICHAUFF	10075	13433(+33)	11754.00	131	563(+330)	347.08
Mean	9549.40	13109.80	11329.60	103.67	447.53	276.02
	V	C	VxC	V	C	VxC
SEm±	221.68	80.95	313.51	6.63	2.42	9.38
CD at 5%	620.94	226.74	NS	18.58	6.79	26.28

Values in parenthesis indicate percent decrease and decrease (-)/increase (+) in WL over NWL

Mineral content of wheat plants varied in different varieties showing variable sensitivity. Susceptible varieties HD2851, and KRL 210 always showed higher Na content than tolerant varieties HD 2009 and KRL 99 which could be possible due to less adverse affects of WL on metabolic functioning of roots in these varieties. These findings are in corroborated to Setter *et al*., (2009). Tolerant varieties somehow could maintain higher energy status needed for nutrient uptake. These varieties could also probably maintain appropriate oxygen diffusion rates even in waterlogged soil conditions enabling roots to continue their functions without any drastic impairment of nutrient uptake (Setter and Water, 2003). Sodium content in shoot increased with waterlogging treatments. Maximum sodium content was found in waterlogging treatments in all varieties. Though the accumulation of sodium increased due to water stagnation treatments but it did not reach the toxic range. Similar findings were also reported by Sharma *et al.*, (2005a) in pigeon pea and Kong *et al*., (2001) in wheat. Waterlogging significantly increased the percentage of Fe concentration in varieties HD 2009, KRICHAUFF, BROOKTON and HD 2851comparatively to tolerant varieties viz, KRL 3-4, KRL238, NW4018 and KRL 99 and for sodic soil (pH 8.9-9.1) (Table 1). Patrick (1964) found that soluble iron begins to increase when the redox potential decreased to about 150 mV or less, and it continued to increase with further decreases in redox potential. This observation suggests that the transformation of iron is mainly caused by the reduction of ferric compounds to the more soluble ferrous forms.

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