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Effect of nitric oxide on some morphological and physiological parameters in maize exposed to waterlogging stress

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Out of 28 genotypes (inbred lines) of maize (*Zea mays* L.) one waterlogging resistant (HUZM-265) and a susceptible (HUZM-55) were identified on the basis of waterlogging tolerant coefficient (WTC) by imposing waterlogging stress during early growth phase. Selected genotypes were further grown in pots and after 20 days subjected to root zone waterlogging with or without 50, 500 and 2000 µmol L^{-1} sodium nitroprusside (SNP) as a donor of NO in the flooding water. Waterlogging caused reduction in leaf number, leaf area and dry weights of plants in both genotypes. Flooding root zone with 50 µmol L^{-1} SNP, alleviated the stress effects or sensitivity (not tolerance), but to a greater magnitude in susceptible genotype. Stomatal conductance, transpiration rate, chlorophyll decreased as the waterlogging duration increased. Nitrogen content in roots and shoot of waterlogged plants also declined significantly. 500 µmol L^{-1}SNP treatment tend to alleviate the deleterious effect of waterlogging. Cell membrane injury in roots of waterlogged plant was higher in genotype HUZM-55 than in HUZM-265 and 500 µmol L^{-1}SNP were found to have mitigating role in combating it. 500 µmol L^{-1}SNP was found effective for alleviating transpiration rate, chlorophyll content and nitrogen content in both genotypes while 50 and 2000 µmol L^{-1}SNP increased stomatal conductance in HUZM-265 and HUZM-55, respectively. It is concluded that SNP mitigates the deleterious effect of waterlogging in maize. However, the effective concentration varies for different parameters and the different genotypes.

Key words: *Zea mays* L., waterlogging, nitric oxide, sodium nitroprusside, waterlogging tolerance coefficient.

INTRODUCTION

Maize (*Zea mays* L.) is widely grown in temperate to tropical regions of the world. In 2013-14 worldwide production of maize was more than 960 million tons. Global production of maize has grown at a compound annual growth rate (CAGR) of 3.40% over the last ten years; from 717 million tons in 2004-05 to 967 million

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Abbreviations: CAGR, Compound annual growth rate; **c-PTIO,** 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoneline-1-oxyl-3-oxide; **NO,** nitric oxide; **PTIO,** 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; **ROS,** reactive oxygen species; **SNP,** sodium nitroprusside;

tons in 2013-14. The area under maize cultivation has grown at CAGR of 2.20% from 146 Mha in 2004-05 to 177 Mha in 2013-14. Productivity of maize has increased at CAGR of 1.20%, from 4.90 tons h^{-1} in 2004-05 to 5.50 tons h^{-1} in 2013-14 (Anonymous, 2014). Excess soil moisture stress caused by waterlogging or high water table or heavy soil texture, is one of the most serious constraints lowering the production and productivity of maize. The extent of damage depends upon the crop growth stage and on environmental conditions at the time of waterlogging. It has been seen that the submergence of maize roots for only one day may restrict the optimum production of the crop (Singh and Ghildyal, 1980). Waterlogging stress at an early vegetative stage is detrimental for the maize crop (Shah and Srivastava, 2007) and is one of the important abiotic stresses affecting plants growth and productivity. Waterlogging causes various consequences like leaf wilting, epinasty, chlorosis, stomatal closure, reduced photosynthesis and altered carbohydrate partitioning, reduced growth rate, disruption of cell membranes, adverse effects on mineral uptake, altered growth regulator relationships and altered respiration. In recent years, nitric oxide (NO) has gained considerable importance in abiotic stresses of plants. NO is a highly reactive, membrane-permeable free radical and a highly toxic compound. Research on NO in plants has gained attention mainly due to its function in plant growth and development and also as a key signaling molecule in different intracellular processes in plants. Nitric oxide plays a vital role in diverse physiological functions in plants like regulation of plant metabolism and senescence (Guo and Crawford, 2005), induction of cell death (Pedroso and Durzan, 2000), regulation of stomatal movement (Garcia Mata and Lamattina, 2001; Bright et al., 2006), photosynthesis regulation (Takahashi and Yamasaki, 2002) and mitochondria functionality (Zottini et al., 2002). It has been seen that high levels of NO have the capacity to damage membranes and cause DNA fragmentation (Romero et al., 2004). Reduced stomatal conductance is among the earliest response to waterlogging in maize followed by leaf yellowing, inhibition of root growth, alteration in root and shoots morphology, leaf senescence and brace root development from above ground parts. Yordanova and Popova (2001) reported that flooding of barley plants for 72 h led to noticeable decrease in photosynthesis, leaf chlorophyll, and protein contents. However, the effects of NO on different types of cells have been proved to be either protective or toxic, depending on the situation and concentration. The crucial signaling role of NO in plant responses to pathogens is well established. Still knowledge about its protective function in plants exposed to abiotic stress is rudimentary. Nevertheless, there is increasing evidence indicating the involvement of NO in alleviation of harmful effects of many abiotic stresses (Del Rio et al., 2004). Not much research has been done to find out the exact mechanism of NO action when exogenously supplied during various abiotic stresses, particularly under waterlogging stress. Under waterlogging, plants suffer from nitrogen deficiency. During flooding, nutrient uptake is greatly influenced. According to Manai et al. (2012) exogenous NO is involved in prevention of Na^+ accumulation, and the increase of K^+ concentrations, also NO influence Ca^{++} absorption and increase nitrate uptake. It was hypothesized that significant genotypic differences exist in physiological and morphological processes of maize inbred lines to waterlogging stress and nitric oxide has significant role in ameliorating deleterious effect of waterlogging in maize. Therefore; in present investigation maize genotypes were screened for their relative resistance/susceptibility to waterlogging stress at early growth phase and effect of different levels of SNP (a donor of NO) on morpho-physiological process was visualized taking relatively resistant and susceptible genotypes.

MATERIALS AND METHODS

Plant materials and treatments

Twenty eight genotypes (inbred lines) of maize, *viz.* HUZM-69, HUZM-175-1, HUZM-65-1, HUZM-63, HUZM-60, HUZM-59, HUZM-58, HUZM-55, HUZM-47, HUZM-36, HUZM-46, HUZM-85-1, HUZM-184, HUZM-81, HUZM-53, HUZM-80-1, HUZM-211-1, HUZM-148, HUZM-78, HUZM-242, HUZM-71, HUZM-147, HUZM-121, HUZM-107, HUZM-265, HUZM-97-1, HUZM-355 and HUZM-88 were sown in plastic pots containing 750 g sand. Five seeds were sown in each pot. After germination thinning was done to maintain three seedlings of uniform growth. After one week of growth each pot was supplied with 100 mL normal Hoagland's nutrient solution. Waterlogging stress was imposed 20 days after sowing (DAS) by placing half set of pots of each genotype in water filled plastic containers in such a way that pots were completely submerged and water level in the containers was maintained 4 to 5 cm above the sand surface in the pots. This water level was maintained daily by adding tap water in the morning and evening. This treatment is referred to as "waterlogged" of early seedling stage. Normal plants were maintained at optimal supply of soil moisture in pots. After 7 days of waterlogging, sampling for various morphological parameters *viz.* shoot length, root length, shoot dry weight, root dry weight and leaf area were done to calculate waterlogging tolerant coefficient (WTC) of each genotype Waterlogging tolerant coefficient (WTC) was determined using the formulae given by Liu et al. (2010) as:

WTC = Mean value of a parameter in waterlogged condition/mean value of the same parameter in normal condition

The WTC values were calculated by taking parameters *viz*. shoot length, root length, shoot dry weight (SDW), root dry weight (RDW) and leaf area. Shoot and root lengths were measured using scale and dry weights of samples were determined by oven drying at 105°C for one h and then at 65°C till constant weight. Leaf area was measured by leaf area meter (CI-202, CID Bioscience, U.S.A).

Out of 28 genotypes, 2 genotypes, one relatively most resistant and the other most sensitive to waterlogging stress, were identified and sown in plastic pots containing 750 g sand. 20 days after sowing plants were subjected to waterlogging stress by putting pots in plastic containers containing water or 50, 500 and 2000 µmol L^{-1}

SNP (as a donor of NO) solution. Water/SNP solutions in containers were maintained 4 to 5 cm above the sand surface in the pots. Normal plants were maintained at optimum supply of soil moisture in pots. Various parameters were studied after 0, 3 and 7 days of imposing stress in normal and stressed plants.

Morphological parameters

These parameters were measured after 7 days of imposing stress. Plants were harvested with roots intact, washed carefully and roots and shoots were separated. Leaves, (green and dead) were counted manually. Area of green leaves was determined by leaf area meter (CI-202, CID Bioscience, USA). Dry weights of roots and shoots were taken after oven drying.

Cell membrane injury

Cell membrane injury of terminal portion of roots was done at 0, 3 and 7 days after imposing stress by the method described by Zhu et al. (2000). Freshly sampled 100 mg plant material (roots) was taken. It was washed thoroughly with glass distilled water then placed in test tubes containing 10 ml of double distilled water. These were divided in two sets. One set of test tubes were incubated at 40°C in a water bath. After 30 min of incubation test tubes were brought to room temperature and electrical conductivity of the solution (C_1) was recorded with the help of conductivity meter (Systronics model 304). Another set was boiled at 100°C for 10 min and its conductivity was also measured (C_2). Membrane injury was calculated as %:

$$\% \text{ membrane injury} = [1-(1-C_1/C_2)] \times 100$$

Stomatal parameters

Stomatal parameters including transpiration rate (E) and stomatal conductance (gs) were recorded at 0, 3 and 7 days after imposing waterlogging stress on first fully expanded leaf from top by infra-red gas analyzer; IRGA (ADC Biosynthetic Ltd.). Observations were made between 10 to 12 h. Principle and methodology involved in the operation of IRGA is elaborated by Bansal and Srivastava (2015).

Chlorophyll content

Changes in chlorophyll content in leaves of both genotypes were measured at 0, 3 and 7 days after imposing waterlogging stress with the help of SPAD meter (Minolta). The instrument directly measures chlorophyll content in intact leaves with expression unit as SPAD units. First fully expanded leaf from top was tagged initially and observations were recorded on this leaf only till the end. Amount of chlorophyll was expressed in terms of SPAD units.

Nitrogen content

Total nitrogen content in root and shoot was determined at 0, 3 and 7 days after imposing waterlogging stress by Semi-automatic Nitrogen Analyzer (Pelicon, Model, KEL 20L) adopting Kjeldahl method.

Sample digestion

Plant sample (100 mg) was taken in a Kjeldahl digestion tube containing 3 g of catalyst mixture (1:5 ratio of $CuSO_4$ and K_2SO_4) and 10 ml concentrated sulfuric acid. Tubes were put in the digestion block, fitted with manifolds and scrubber. The temperature was gradually raised to 350°C. The digestion continued till the solution became colorless. After completion, samples were brought to room temperature.

Distillation

Distillation of digested samples was done by auto distillation system (Pelicon Distil EM). Kjeldahl tubes containing digested plant samples were fitted in the assembly. Sufficient amount (20 to 30 ml) 40 % NaOH was added till the colour of the solution becomes brown. At the collection end a conical flask containing 24 ml 4% boric acid and 0.5 ml mixed indicator (0.3 g bromocresol green and 0.2 g methyl red dissolved in 400 ml of 90 % ethanol) was put. The sample was allowed to steam distilled for 9 min.

Titration procedure

The boric acid solution of the conical flask was titrated by 0.1 N HCl with the help of micro titration unit. At the end point light brown colour appeared. The amount of nitrogen in the sample was calculated as:

$$\text{N (mg g}^{-1}\text{ dry weight)} = \frac{14 \times \text{Titrant value} \times \text{normality of acid} \times 100}{\text{Sample weight (g)} \times 1000}$$

Statistical analysis

All data were taken in triplicates. For comparing within genotypes for WTC, least significant differences (LSD) were calculated at probability level ≤ 0.05 of significance by SAS software using Duncan's multiple range test (DMRT). To draw the statistically valid and significant conclusions, the data obtained by various other observations were analyzed statistically by adopting method of "Analysis of Variance" for completely randomized design factorial. Critical differences were calculated at 1% level of significance in order to compare treatment means as described by Gomez and Gomez (1984).

RESULTS

Screening of genotypes

In this experiment we attempted to identify relatively resistant and susceptible genotypes of maize to waterlogging stress at early stage of growth on the basis of WTC. The WTC was calculated on the basis of per plant shoot dry weight (SDW), root dry weight (RDW), shoot length, root length and leaf area (Table 1). High value of WTC indicates relatively resistant and low value relatively susceptible natures of genotypes to waterlogging stress. When WTC was calculated on the basis of shoot dry weight, it was the maximum for HUZM-265 (3.2503) and the minimum for HUZM-55 (0.2393) (Table 1). Different genotypes followed a similar trend when WTC was calculated on the basis of leaf area plant^{-1}. Nevertheless, WTC in studied genotypes followed

Table 1. Screening of 28 genotypes of maize under waterlogged condition on the basis of waterlogging tolerant coefficient (WTC) for different parameters.

S/N	Genotype	WTC for parameters				
		Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)
1	HUZM-55	0.2393^{d}	0.5592^{ecd}	0.8057^{c}	0.5873^{h}	0.5635^{f}
2	HUZM-175-1	0.5803^{dc}	0.3269^{e}	0.8273^{c}	0.6881^{edfhg}	1.3233^{efcd}
3	HUZM-65-1	1.4399^{bdc}	0.9950^{ecd}	1.1073^{bac}	0.7058^{edfhcg}	0.9311^{efcd}
4	HUZM-63	0.4702^{d}	0.7783^{ecd}	$0.8690^{\mathbf{c}}$	$0.7389^{ebdfhcg}$	1.0661^{efcd}
5	HUZM-60	0.5317^{dc}	0.4272^{e}	$0.9063^{\mathbf{bc}}$	0.5503^{h}	2.1059^{bc}
6	HUZM-59	0.6400^{dc}	0.6604^{ecd}	$0.9170^{\mathbf{bc}}$	0.9499^{ebdfc}	1.6306^{efcd}
7	HUZM-58	0.5897^{dc}	0.4404^{e}	$0.9927^{\mathbf{bc}}$	$0.7792^{ebdfhcg}$	0.9167^{efcd}
8	HUZM-69	0.5375^{dc}	0.7428^{ecd}	0.9117^{bc}	0.6447^{efgh}	0.7540^{f}
9	HUZM-47	0.6115^{dc}	0.6216^{ecd}	$0.8690^{\mathbf{c}}$	0.8974^{ebdfcg}	0.8128^{ef}
10	HUZM-36	0.5843^{dc}	0.6042^{ecd}	1.0553^{bac}	$0.8032^{ebdfhcg}$	1.3265^{efcd}
11	HUZM-46	0.8502^{dc}	0.5670^{ecd}	0.9983^{bc}	0.5860^{h}	0.9960^{efcd}
12	HUZM-85-1	0.5904^{dc}	0.8542^{ecd}	0.9147^{bc}	$0.8354^{ebdfhcg}$	0.8940^{efd}
13	HUZM-184	0.7236^{dc}	1.4073^{bcd}	0.8517^{c}	$0.8726^{ebdfhcg}$	0.9928^{efcd}
14	HUZM-81	0.6015^{dc}	0.4273^{e}	0.9753^{bc}	0.6954^{edfhcg}	2.0710^{bcd}
15	HUZM 53	0.8009^{dc}	0.8760^{ecd}	0.9780^{bc}	$0.8858^{ebdfhcg}$	2.0517^{bcd}
16	HUZM-80-1	2.6910^{ba}	1.4377^{bc}	1.1650^{bac}	0.6336^{fhg}	1.3583^{efcd}
17	HUZM-211-1	0.8643^{dc}	0.6722^{ecd}	1.0500^{bac}	$0.7708^{ebdfhcg}$	0.8175^{ef}
18	HUZM-148	1.3400^{bdc}	0.9138^{ecd}	0.9763^{bc}	1.0055^{bdac}	1.1753^{efcd}
19	HUZM-78	2.1670^{bac}	0.7682^{ecd}	1.0023^{bc}	1.0680^{ba}	1.4080^{efcd}
20	HUZM-242	0.8439^{dc}	0.4658^{ecd}	0.9350^{bc}	0.9820^{ebdac}	0.7892^{ef}
21	HUZM-71	1.0033^{dc}	0.6717^{ed}	1.0683^{bac}	$0.8457^{ebdfhcg}$	3.6472^{a}
22	HUZM-147	0.5009^{d}	0.4840^{ecd}	0.8823^{c}	$0.7901^{ebdfhcg}$	0.9161^{efcd}
23	HUZM-121	0.6143^{dc}	0.6635^{ecd}	0.8360^{c}	1.2825^{a}	0.7528^{ef}
24	HUZM-107	0.8463^{dc}	0.9437^{ecd}	0.9923^{bc}	0.9285^{ebdfcg}	0.8666^{efd}
25	HUZM-88	0.9207^{dc}	1.4419^{bc}	1.1500^{bac}	1.0347^{bac}	0.6392^{f}
26	HUZM-97-1	1.4623^{bdc}	2.4715^{a}	1.4783^{a}	$0.8044^{ebdfhcg}$	1.8748^{ecd}
27	HUZM-355	0.7062^{dc}	0.4020^{e}	0.7740^{c}	$0.7318^{ebdfhcg}$	0.8706^{efd}
28	HUZM-265	3.2503^{a}	1.9172^{ba}	1.3887^{ab}	$0.8439^{ebdfhcg}$	3.0290^{ba}

WTC=Waterlogging tolerant coefficient; Means followed by same letters in a column are not significantly different but different letters are significantly different (P≤ 0.05) using Duncan's multiple range test (DMRT).

variable trends when calculated on the basis of RDW, shoot length and root length, but values were always relatively higher for HUZM-265 and lower for HUZM-55.

Morphological parameters

As compared to normal plants, number of green leaves per plant in waterlogged plants decreased and number of dead leaves per plant increased, however; genotypic differences were not significant (Table 2).

Also leaf area per plant declined under waterlogged condition in both genotypes. Reduction % under waterlogged condition over normal was more in HUZM-265. SNP at 50 µmol L^{-1} tend to ameliorate the deleterious effects of waterlogging stress on leaf area; while 500 and 2000 µmol L^{-1} SNP concentrations appeared to be deleterious for both genotypes. Under waterlogged condition root and shoot dry weights per plant also declined, however; SNP at 50 µmol L^{-1} caused a marginal increase in above parameters in both genotypes as compared to other two concentrations of SNP.

It was evident that waterlogging induced membrane injury of root cells (Figure 1). Susceptible genotype HUZM-55, registered more cell injury than the resistant one and 500 µmol L^{-1} concentration of SNP was effective in ameliorating this damage. Concentration of SNP, effective in ameliorating harmful effects of waterlogging on cell membrane integrity varied in both genotypes; HUZM-265 and HUZM-55 (Figure 1).

Table 2. Different morphological parameters in maize genotypes under normal and waterlogging stress and different levels of sodium nitroprusside.

Treatment	Total leaves (plant^{-1})		Green leaves (plant $^{-1}$)		Dead leaves (plant^{-1})		Leaf area (cm^2)		Shoot dry weight (g)		Root dry weight (g)	
	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55
N	4.00	4.00	2.66	2.66	1.00	1.00	41.52	32.17	0.25	0.11	0.14	0.11
W	3.66	4.00	2.33	2.33	2.33	2.00	37.58	31.76	0.20	0.10	0.09	0.11
W1	4.00	4.00	3.00	2.00	1.00	2.00	34.25	26.44	0.22	0.22	0.14	0.12
W2	3.66	4.00	2.00	1.66	1.66	2.33	19.22	18.24	0.20	0.10	0.06	0.07
W3	4.00	4.00	2.00	2.00	2.00	2.00	18.90	13.50	0.25	0.19	0.11	0.08
Mean	3.86	4.00	2.46	2.06	1.40	1.86	30.29	24.42	0.22	0.14	0.11	0.10
Annova	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD ≤ 0.01**
G	0.05	NS	0.09	0.35	0.08	0.33	0.28	1.14	0.01	0.03	0.01	NS
T	0.07	NS	0.14	0.56	0.13	0.52	0.45	1.80	0.01	0.04	0.01	0.04
G×T	0.06	NS	0.11	NS	0.11	NS	0.36	1.47	0.01	0.04	0.01	NS

Plants were grown under normal condition in plastic pots. After 20 days of sowing waterlogging stress was imposed. Different parameters were analyzed after 7 days of imposing waterlogging stress. #N=normal, W=waterlogged,W_1=waterlogged with 50µm L^{-1} SNP,W_2=waterlogged with 500µm L^{-1} SNP,W_3=waterlogged with 2000µm L^{-1} SNP.

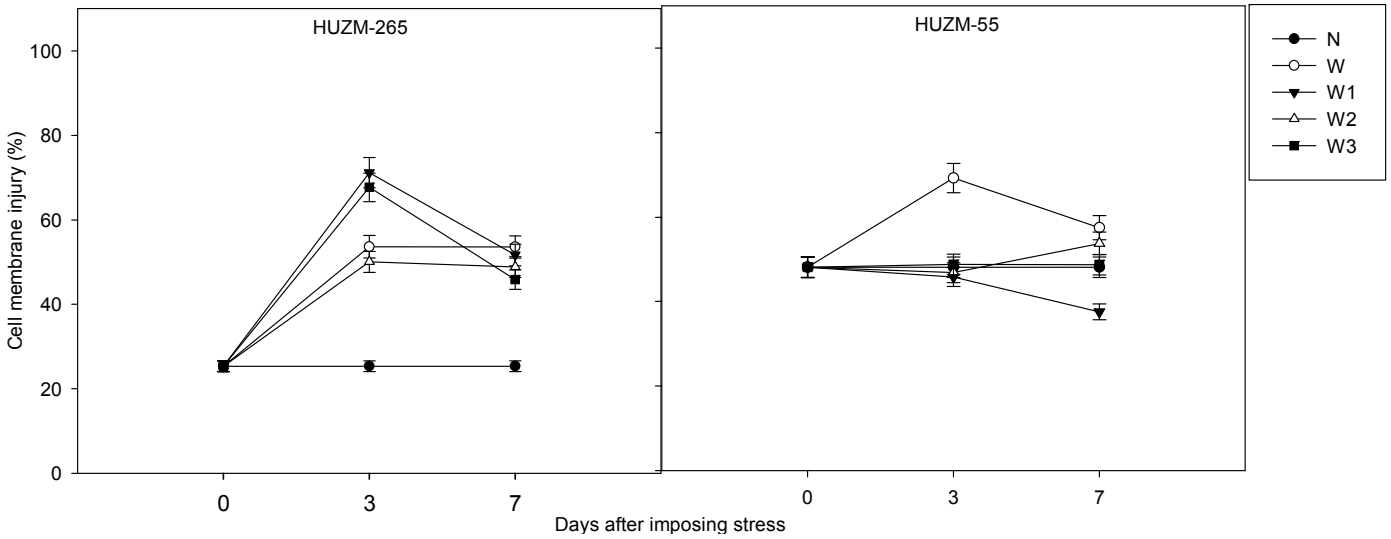

Figure 1. Changes in cell membrane injury (%) in roots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

Physiological parameters

Waterlogging caused a marked decline in stomatal conductance (gs), transpiration rate (E) and chlorophyll content in maize crop under this study. In both genotypes, exposure of waterlogged plants to SNP treatment ameliorated the deleterious effects of waterlogging at a concentration of 50 µmol L^{-1} for stomatal conductance in resistant genotype and 2000 µmol L^{-1} in susceptible one (Figure 2). Transpiration rate and chlorophyll content was found higher at SNP concentration of 500 µmol L^{-1} (Figures 3 and 4). Here, waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. Reduction in stomatal conductance (gs) was more in susceptible genotype than in resistant one.

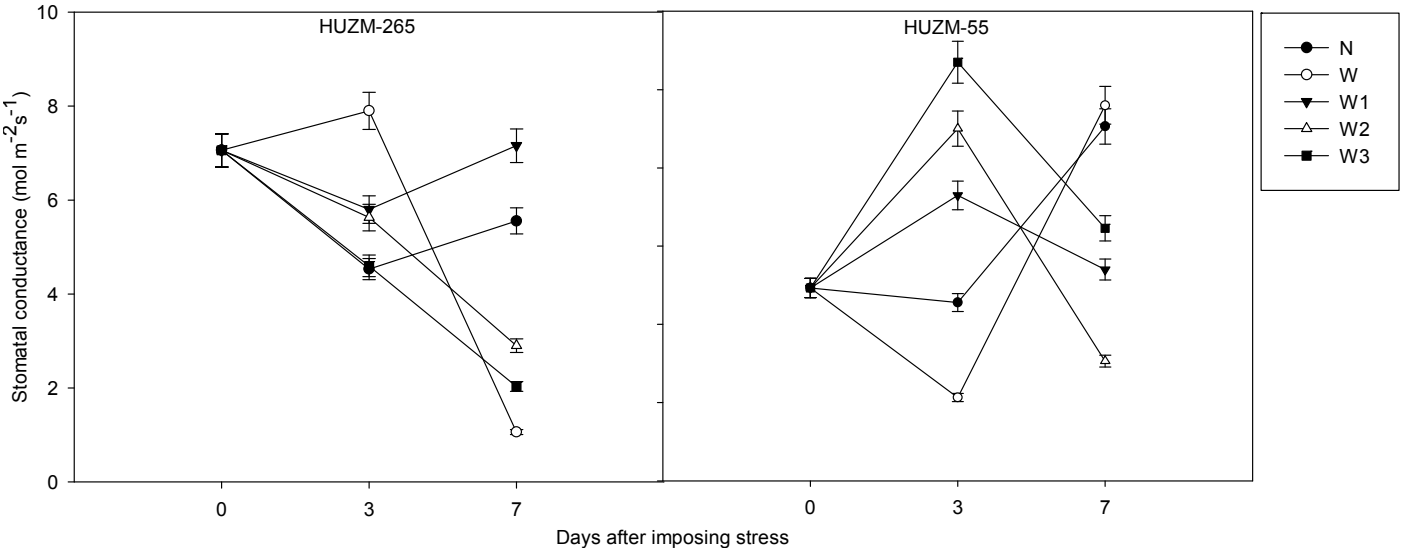

Figure 2. Changes in stomatal conductance (mol $m^{-2}s^{-1}$) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

Nitrogen content of roots and shoots decreased under waterlogging stress and the reduction was more in susceptible genotype than in the resistant. This level further declined with advancement in waterlogging duration, that is, at 7th day of stress (Figure 5). SNP treatments at 500 µmol L^{-1} ameliorated the deleterious effects of waterlogging on root nitrogen in both genotypes (Figure 5).

DISCUSSION

Screening of genotypes

Cornelious et al. (2005) reported waterlogging injury scores for the identification of quantitative trait loci (QTLs) underlying waterlogging tolerance in soybean. Qiu et al. (2007) performed QTL mapping associated with waterlogging tolerance during seedling stage in maize using traits of root length, root dry weight, plant height, shoot dry weight and total dry weight. Similar work has been carried out in wheat by Yu and Chen (2013). It is advocated that determination of WTC is a suitable parameter to identify waterlogging resistant and susceptible genotypes of maize (Liu et al., 2010). High values of WTC are associated with relatively more waterlogging resistance. Though WTC was calculated on the basis of per plant shoot dry weight, root dry weight, shoot length, root length and leaf area, but as in most crop species, plant dry matter is directly linked with their vigour and yield, therefore; WTC on the basis of shoot dry weight was chosen as the basis for identification of relatively resistant and susceptible genotypes of maize. Among studied genotypes, therefore; HUZM-265 was identified as relatively resistant (as it exhibited highest WTC value) and HUZM-55 as relatively susceptible (as it registered the lowest WTC value) to waterlogging stress (Table 1). Further experiments were carried out by taking these two genotypes.

Morphological parameters

Data indicated that waterlogging induced senescence of existing leaves more than the appearance of new leaves as in waterlogged plants leaf number per plant decreased lesser than leaf area per plant (Table 2). Such results are also reported by Yordanova and Popova (2001). SNP at 50 µmol L^{-1} tend to ameliorate the deleterious effects of waterlogging stress on leaf area; while 500 and 2000 µmol L^{-1} SNP concentrations appeared to be deleterious for both genotypes. Under waterlogged condition root and shoot dry weights per plant also declined (Table 2), however; SNP at 50 µmol L^{-1} caused a marginal increase in these parameters in both genotypes. Thus, it was evident that treatment of waterlogged maize plants with 50 µmol L^{-1} SNP ameliorated the harmful effects of waterlogging stress, but higher concentrations were detrimental to growth in maize. As the genotypic differences with respect to SNP levels were not significant, therefore; it was considered that 50 µmol L^{-1} SNP as NO donor could be used for ameliorating harmful effects of waterlogging in maize. Similar observations have been reported by Wang et al. (2011) in maize. Fan et al. (2014) also reported that spraying with 100 µM SNP markedly improved the plant height, fresh and dry

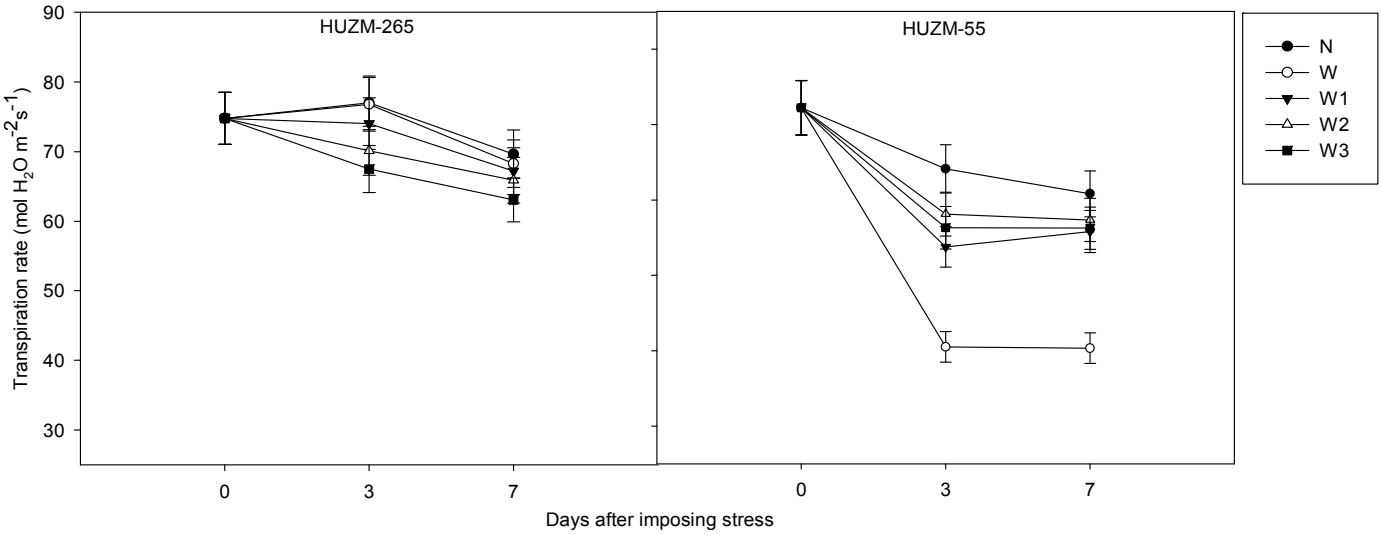

Figure 3. Changes in transpiration rate (mol $H_2Om^{-2}s^{-1}$) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

weights in cucumber seedlings exposed to waterlogging stress.

Physiological parameters

In this investigation it was evident that waterlogging induced membrane injury of root cells (Figure 1) in maize. Severity of injury increased with increased stress duration. Magnitude of NO-induced reduction of damage varied in resistant and susceptible genotypes, susceptible genotype being more protected. Therefore, genotypic differences in membrane damage under stress were found to be associated with their relative resistance and susceptibility to waterlogging. These results indicated that perhaps waterlogging susceptible maize genotypes require relatively higher levels of SNP to overcome deleterious effects of waterlogging as for as cell membrane integrity was concerned. These results are in accordance with those of Wang et al. (2011) who observed that cell membrane injury in both SNP treated and non-SNP treated maize plants under waterlogging increased rapidly to higher levels.

In this investigation we observed decline in stomatal conductance and transpiration rate in both genotypes under waterlogged condition (Figures 2 and 3). Similar observations were made by Baranwal and Singh (2002) in maize and Bansal and Srivastava (2015) in pigeonpea under waterlogging stress. Treatment of waterlogged plants to SNP ameliorated the deleterious effects of waterlogging. Effective SNP concentration in rehabilitation of stomatal conductance was 50 µmol L^{-1} in resistant genotype and 2000 µmol L^{-1} in susceptible genotype (Figure 2). Transpiration rate was higher at SNP concentration of 500 µmol L^{-1} (Figure 3). Waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. It has been proposed that waterlogging results in reduced water absorption by plants leading to derangement in plant-water relation parameters, closure of stomata and reduction in transpiration rate (Bansal and Srivastava, 2015). Contrary, Garcia-Mata and Lamattina (2001) showed that exogenous NO reduced transpiration and induced stomatal closure in several species such as *Vicia faba*, *Salpichroa* and *Tradescantia* species. Many different NO donors induce stomatal closure in a dose and time dependent manner and their effects was reversed by simultaneous co-incubation with the NO scavengers; 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) or 2-(4-carboxyphenyl)- 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (Bright et al., 2006). Chlorophyll content also decreased in waterlogged plants of both genotypes (Figure 4). Such results are reported by other workers like Rai et al. (2004). SNP treatment of waterlogged plants increased leaf chlorophyll content. Here, waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. Decreased chlorophyll content per unit fresh weight of leaf as well as reduction in leaf area per plant are the proposed to be the major causes for reduction in plant dry weight under waterlogged condition. Nevertheless, reduction in bio-chemical processes associated with photosynthesis is also decreased under waterlogging stress in plants. Takahashi and Yamasaki (2002) showed that SNP did not modify the maximal quantum efficiency of PSII, but inhibited the photosynthetic linear electron transfer rate, Δ pH formation across the thylakoid membrane and

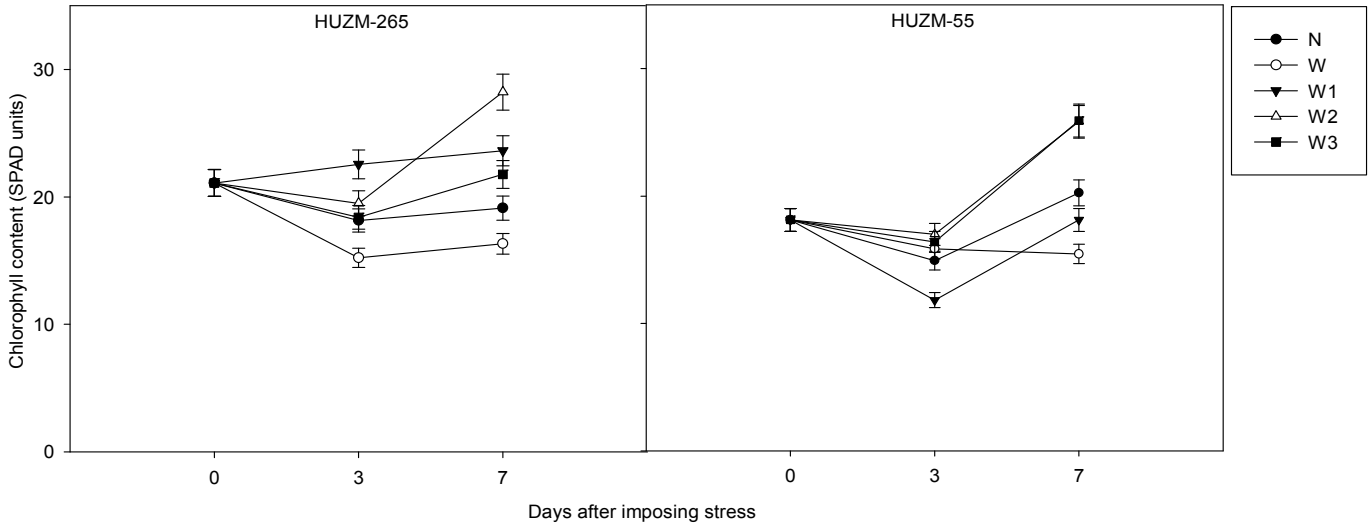

Figure 4. Changes in chlorophyll content (SPAD units) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP.

decreased the rate of ATP synthesis. A moderate decrease in Fv/Fm was observed by SNP treatment in pea leaves (Wodala et al., 2005) and one possible reason for the observed changes in the rates of photosynthesis and transpiration was attributed due to the effect of NO on altered stomatal behavior. Many plant studies have used the NO donor SNP that generates cyanide. However, neither cyanide itself nor light-inactivated SNP induced stomatal closure (Takahashi and Yamasaki, 2002), the effects mediated by NO donors SNP are indeed due to the release and biological activity of NO).

Under waterlogged condition, soil redox potential decreases, which results in increase or decrease in availability of essential mineral elements (Purvis and Williamson, 1972). Waterlogging induced nitrogen deficiency leading to chlorosis and finally death of leaves, particularly of older leaves. Leaves showing yellowness under flooding stress is attributed mainly due to N deficiency (Rai et al., 2004; Srivastava et al., 2007; Steffens et al., 2005). It was interesting to note that N content in roots of SNP treated waterlogged plants was more than in roots of normal plants. Similar trend was seen for shoot N content (Figure 6). As SNP is also a source of N, therefore; it has probably supplemented additional N to waterlogged plants. This inference was further supported with the observation that SNP supplied plants generally had more chlorophyll content in leaves (Figure 4). Similar observations were made by Fan et al. (2014) who observed that chlorophyll content of waterlogged cucumber seedlings improved when plants were treated with varying concentrations of SNP. SNP treatments at 500 µmol L^{-1} ameliorated the deleterious effects of waterlogging on root nitrogen in both genotypes (Figure 5). Results are in agreement with those of Wang et al. (2011) who reported that SNP at 50 and 500 µmol L^{-1} could keep chlorophyll to a relatively higher level in maize plants. Waterlogging resistant genotype; HUZM-265, responded more to SNP application than the susceptible genotype. Shoot nitrogen declined under waterlogging and was ameliorated by using exogenous supply of SNP in rhizosphere, particularly at 500 µmol L^{-1} concentration (Figure 6). Maize roots are the organ initially damaged during initial phase of waterlogging. Longer waterlogging duration induces yellowing and decline in leaf chlorophyll content due to induction in nitrogen deficiency leading to senescence of older leaves. Waterlogging also leads to derangement in water relation parameters and other physiological and biochemical processes of plants.

Present investigation indicated genotypic differences in waterlogging resistance in maize. WTC was found to be a suitable parameter to distinguish waterlogging resistant and susceptible genotype in this crop. Differential responses in morphological, physiological and biochemical parameters in resistant and susceptible genotypes were also evident. SNP, as a donor of NO, ameliorated the harmful effect of waterlogging on plant processes; however, effective SNP concentration varied with respect to genotype and the studied plant process. Much work is still needed to visualize the potential role of SNP, a NO donor, in waterlogging stress resistance in plants.

Conflict of Interest

The authors have not declared any conflict of interest.

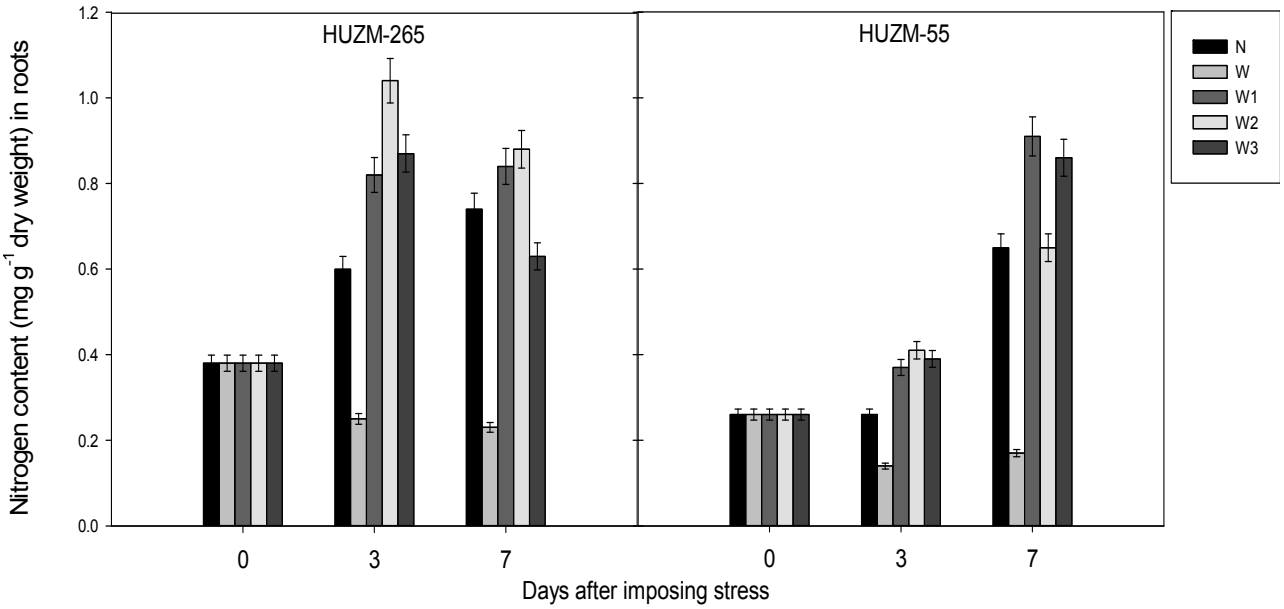

Figure 5. Changes in nitrogen content (mg g^{-1} dry weight) in roots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP.

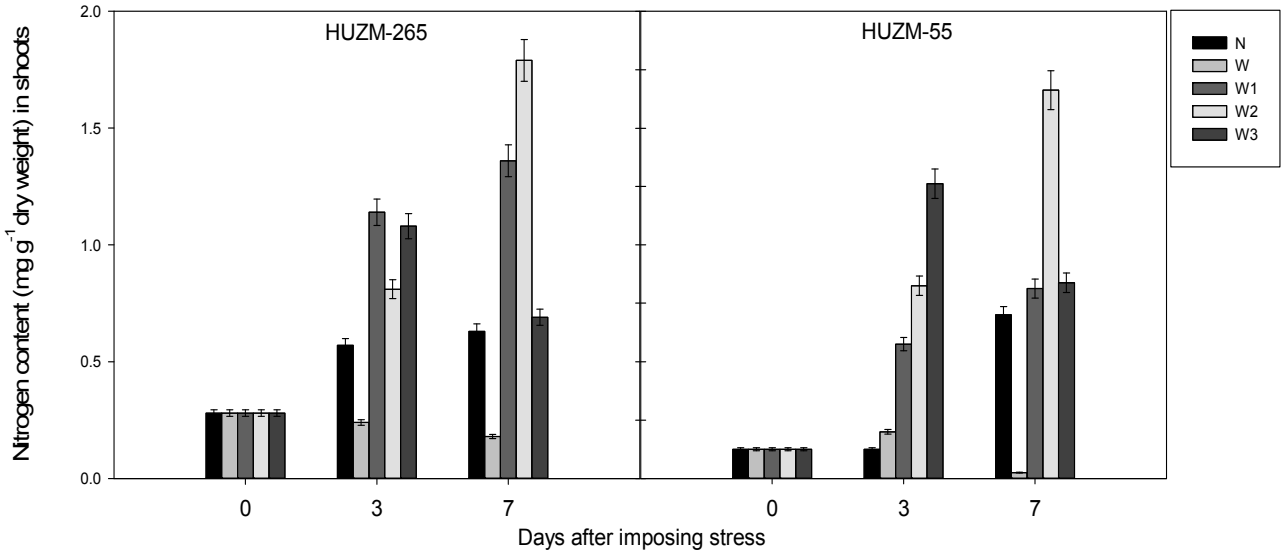

Figure 6. Changes in nitrogen content (mg g^{-1} dry weight) in shoots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W_1= waterlogged with 50 µmol L^{-1} SNP, W_2= waterlogged with 500 µmol L^{-1} SNP, W_3= waterlogged with 2000 µmol L^{-1} SNP.

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