Article

Unraveling morphophysiological and biochemical responses of *Triticum aestivum* L. to extreme pH: Coordinated actions of antioxidant defense and glyoxalase systems

MHM Borhannuddin Bhuyan [1, 2], Mirza Hasanuzzaman [3] , Jubayer Al Mahmud[4], Md. Shahadat Hossain[1], Tasnim Farha Bhuiyan[5] and Masayuki Fujita[1,*]

[1] Laboratory of Plant Stress Response, Department of Applied Biological Sciences, Faculty of Agriculture, Kagawa University, Japan; razon_sau@yahoo.com (M.H.M.B.B.); shahadatsau24@gmail.com (M.S.H.); fujita@ag.kagawa-u.ac.jp (M.F.)
[2] Citrus Research Station, Bangladesh Agricultural Research Institute, Jaintapur, Sylhet-3156, Bangladesh
[3] Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka1207, Bangladesh; mhzsauag@yahoo.com (M.H.)
[4] Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka1207, Bangladesh; jamahmud_bd@yahoo.com (J.A.M.)
[5] Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka1207, Bangladesh; farhatasnim28@gmail.com (T.F.B.)
* Correspondence: fujita@ag.kagawa-u.ac.jp; Tel.: +81-87-891-3033 (M.F.)

Abstract: Soil pH, either low (acidity) or high (alkalinity) is one of the major constraints that affect many biochemical and biological processes within the cell. The present study was carried out to understand the oxidative damage and antioxidant defense in wheat (*Triticum aestivum* L. cv. BARI Gom-25) grown under different pH regimes. Eight-day-old seedlings were exposed to growing media with different pH levels (4.0, 5.5, 7.0 and 8.5). Seedlings grown in pH 4.0 and in pH 8.5 showed reductions in biomass, water, and chlorophyll contents; whereas plants grown at pH 7.0 (neutral) exhibited better performance. Extremely acidic (pH 4.0) and/or strongly alkaline (pH 8.5)-stress also increased oxidative damages in wheat by excess reactive oxygen species (ROS) generation and methylglyoxal (MG) production, which increased lipid peroxidation and disrupted the redox state. In contrary, the lowest oxidative damage was observed at neutral condition followed by strong acidic condition (pH 5.5), which was attributed mainly due to better performance of the antioxidant defense and glyoxalase systems. Interestingly, seedlings grown at pH 5.5 showed a significant increase in morphophysiological attributes compared with extreme acidic (pH 4.0)- and strong alkaline (pH 8.5)-stress treatments, which indicates the tolerance of wheat to the acidic condition.

Keywords: Acidity; Alkalinity; Antioxidant defense; Methylglyoxal; Phytotoxicity; Reactive oxygen species

1. Introduction

Abiotic stresses hampering crop production and challenging farmers to grow food for the enormous world community, which will attain 10.9 billion by 2050 [1]. Among the abiotic stressors, extreme pH both acidity and alkalinity cover about 60 percent of the global land surface with spatial variability [2]. Soil pH is the indicator to describe acidity or alkalinity of the soils. The Soil Survey Division Staff, USDA [3], , proposed 9 classes based on pH; ultra acidic (pH <3.5), extremely acidic

(3.5-4.4), very strongly acidic (4.5-5.0), strongly acidic (5.1-5.5), moderately acidic (5.6-6.0), slightly acidic (6.1-6.5), neutral (6.6-7.3), slightly alkaline (7.4-7.8), moderately alkaline (7.9-8.4), strongly alkaline (8.5-9.0), Very strongly alkaline (>9.0). From the agricultural point of view, pH between 6.0 and 7.3 is best. However, some plants prefer pH 4.0 to 5.5 (e.g., potato, rye, blueberry, cranberry), some grow better in pH 5.5 to 6.5 (e.g., barley, rice, carrot, eggplant, cauliflower), while some prefer pH 6.0 to 7.0 (e.g., maize, cabbage, mustard, kohlrabi), and again some crops (garlic, pepper, winter squash etc.) can grow in a wide range of soil pH (5.0-7.5) [4]. Geographic, geological, meteorological biological and anthropogenic reasons are responsible for developing either acidity or alkalinity [2], thus hampered the productivity of crops by exerting stresses [5].

Plant cells need a range of pH 7.0–7.5 in cytoplasm to maintain normal physiological function [6]. The pH of the external growing media exerts negative impact on the cellular pH. Besides the enzymes accomplish the normal physiological activities are also pH dependent. Reports suggested that 1 decrease in pH of the external growing media reduce cytoplasmic pH by around 0.1 units [7]. On the other hand when the pH of the external growing media increases; it causes precipitation of phosphorus and metal ions, increases the absorption of inorganic anions, and disrupts the ion balance [8]. Therefore, both acidic and alkaline pH of growing media alters pH homeostasis in the tissues. Yet, to combat with extreme pH stress induced cellular pH alteration; proton pumps play an important role in the adaptation to acidic or alkaline stress by mediating proton efflux and influx [9, 10]. But their effectiveness to balance the cytoplasmic pH reduced gradually [6], eventually disrupts many cellular activities, including enzyme activities. Moreover excess energy dissipation may raise toxic reactive oxygen species (ROS) [11,12] including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). Reports suggested that a reduction of 0.5 units in pH tend to increase the ROS accumulation to many folds in *Hordium vulgare* L. [11], and *Pinus sylvestris* L.[13], whereas the increase in pH also generate oxidative stress in *Malus* spp rootstocks [12].

These toxic ROS can oxidize important cellular ultrastructures leading to oxidative damage and destruction of cellular organelles [14]. Fortunately plant cells are coupled with the antioxidative system to minimize the oxidative stress comprised of the non-enzyme (ascorbic acid, AsA; glutathione, GSH; phenolic compounds; alkaloids; α-tocopherol; non-protein amino acids) and enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; and glutathione S-transferase, GST), which works coordinately to detoxify the toxic ROS [15,16,17]. Another cytotoxic compound, a glycolysis byproduct, methylglyoxal (MG), also reported being overproduced under abiotic stress [16], which can damage cell by creating oxidative stress. The MG detoxification system comprising of glyoxalase I (Gly I) and glyoxalase II (Gly II) enzyme helps to detoxify MG [18]. Reports suggested that metabolomic modification of antioxidant and glyoxalase system can improve abiotic stress tolerance [14,17,19,20,21]. But, to mechanize the antioxidant defense and glyoxalase system under extreme pH, the first requirement is to elucidate different attributes that are disturbed, and which can further be targeted to modulate by phytoprotectants and/or genetic manipulation. But, there are a few reports that demonstrated the involvement of oxidative damage generation by acidity or alkalinity, yet their comparative physiological study is unavailable. Moreover, there is no information available on the extreme pH-induced regulation of the antioxidative and glyoxalase systems.

Wheat (*Triticum aestivum* L.) ranks first among the food grain crops and its productivity is negatively affected by the acidic or alkaline condition. Therefore, we examined the growth and biomass, water status, photosynthetic pigments, oxidative damage, and performance of the antioxidant and glyoxalase systems of wheat under extreme pH-stress during the early seedling stage. To the best of our knowledge, this is the first report to elucidate the negative impact of extreme-pH stress on wheat seedling, in which the co-ordinated actions of the antioxidant and glyoxalase systems have been investigated.

2. Results

2.1. Growth and biomass accumulation

Extreme pH of the growing media influenced the growth and biomass accumulation of the wheat seedlings. Upon exposure to extremely acidic (pH 4.0)-stress the plant height was reduced by 33% compared with control, where, 14% reduction in shoot length was resulted in strongly acidic (pH 5.5)-stress. Similarly, strongly alkaline (pH 8.5)-stress reduced the shoot height by 31% compared to control (Table 1; Figure 1).

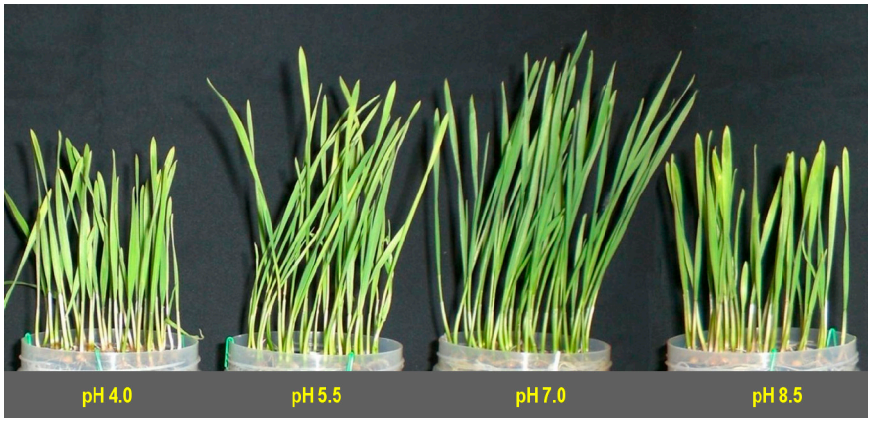


Figure 1. Visual images of morphological differences among wheat (*Triticum aestivum* L. cv. BARI Gom-25) seedlings grown under different pH

The root length was also influenced by extreme pH-stress. Compared to control, upon exposure to acidic pH-stress (pH 4.0 and pH 5.5) root length reduced by 18% and 5% respectively, whereas 18% reduction in root length was observed in seedlings grown in strongly alkaline (pH 8.5)-stress compared with control (Table 1).

Extreme pH-stress again hampered the biomass accumulation of wheat seedlings. The fresh weight of shoot and root was reduced by 12 and 27% upon exposure to extremely acidic (pH 4.0) while strongly acidic (pH 5.5)-stress condition reduced the shoot and root fresh weight by 5 and 12% respectively. On the other hand strongly alkaline (pH 8.5)-stress reduced the shoot and root fresh weight by 17 and 26%, respectively (Table 1).

The dry weight of shoot and root was declined by 7 and 35% upon exposure to extremely acidic (pH 4.0) while strongly acidic (pH 5.5)-stress didn't reduced the shoot dry weight but reduced the root dry weight by 6%. Similarly, strongly alkaline (pH 8.5)-stress reduced the shoot and root dry weight by 6 and 32%, respectively (Table 1).

Table 1 Shoot and root fresh weight, shoot and root dry weight, leaf RWC, and Pro content in leaves of wheat (*Triticum aestivum* L) seedlings under different levels of pH. Means (±SD) were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at P ≤ 0.05 applying Fisher's LSD test

Treatments	Plant height (cm)	Root length (cm)	Shoot FW (g plant^{-1})	Root FWt (g plant^{-1})	Shoot DW (g plant^{-1})	Root DW (%)	Leaf RWC (%)	Pro (nmol g^{-1} FW)
pH 4.0	10.32±0.23c	5.34±0.04c	0.98±0.003b	0.17±0.001d	0.15±0.002b	0.05±0.001c	82.31±0.53c	5.53±0.09b
pH 5.5	13.17±0.37b	6.18±0.08b	1.06±0.056a	0.21±0.002b	0.16±0.002a	0.07±0.002b	88.67±1.23b	1.08±0.04c
pH 7.0	15.33±0.57a	6.52±0.10a	1.12±0.014a	0.24±0.001a	0.16±0.002a	0.07±0.001a	94.31±0.44a	0.30±0.01d

pH 8.5	10.52±0.13c	5.32±0.02c	0.93±0.003b	0.18±0.001c	0.15±0.001b	0.05±0.001c	81.12±0.21c	6.57±0.10a

2.2. Relative water and proline content

Extreme pH-stress alters the leaf RWC of the wheat seedlings. Compared to control, leaf RWC was reduced by 13 and 6% at acidic-stress (pH 4.0 and pH 5.5, respectively) condition; while 14% reduction in leaf RWC was observed from the alkaline-stressed (pH 8.5) seedlings (Table 1).

The contents of Pro were boosted up upon exposure to extreme pH condition in wheat seedlings, and compared to control 19- and 4-fold increase in Pro content was found in acid-stressed seedlings (pH 4.0 and pH 5.5, respectively). Alkaline-stress (pH 8.5) also gave rise to Pro content by 22-fold compared to control (Table 1).

2.3. Oxidative stress markers

Malondialdehyde—a stress indicator produce from lipid peroxidation in leaf tissues, was determined and illustrated in (Figure 2a). Compared to control (pH 7.0) seedlings, MDA content increased by 199 and 194% in pH 4.0 and pH 8.5 exposed seedlings respectively. However, seedlings grown on pH 5.5 showed lower increases in MDA content (95%) compared with control seedlings.

In line with MDA, a remarkable raise in H_2O_2 content was noticed in leaf tissue upon exposure to varying rhizosphere pH (Figure 2b). Compared with control a sharp increase in H_2O_2 content (134, and 90%) was observed both at extremely acidic (pH 4.0) and strongly alkaline (pH 8.5) pH. However, seedlings grown under strongly acidic (pH 5.5)-stress condition was found with slightly increased H_2O_2 content (30%) compared to control.

Similarly varying rhizosphere pH increased the activity of lipoxygenase (LOX) drastically. Upon exposure to extreme pH, LOX activity increased by 73 and 67% (compared with control) in extremely acidic (pH 4.0) and strongly alkaline (pH 8.5) stressed seedlings, respectively. But compared with control, seedlings grown under strongly acidic (pH 5.5)-stress were found with a slight increase (23%) in LOX activity (Figure 2c).

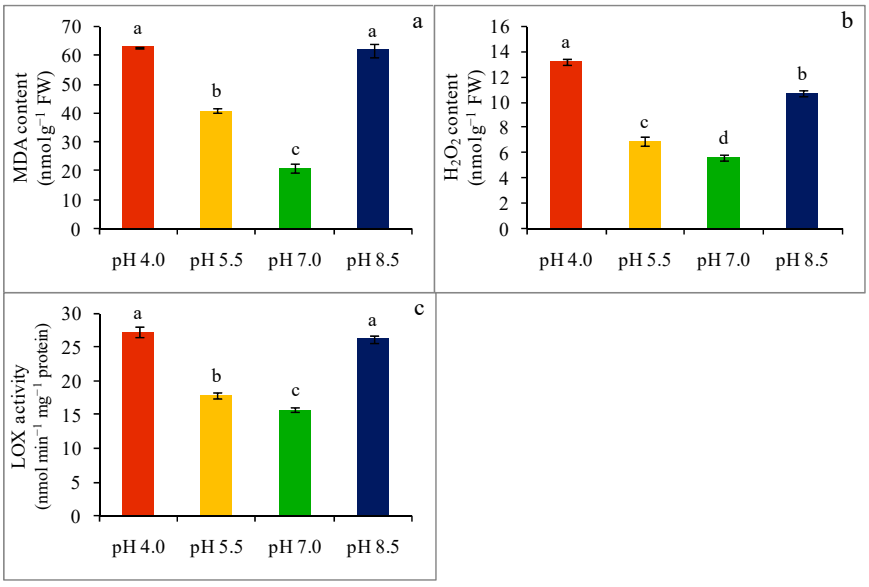


Figure 2. Malondialdehyde (MDA) content (a), H_2O_2 content (b), and LOX activity (c), of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

2.4. Photosynthetic pigment contents

Extreme rhizosphere pH destroyed chl in leaf tissues. Chlorophyll *a* content was decreased in acid-stressed wheat seedlings in a dose-dependent manner by 13 and 8% at pH 4.0 and pH 5.5, respectively (Figure 3a), while alkaline rhizospheric pH (8.5) also diminished chl *a* content by 17% compared with control. Extreme pH-stress also altered chl *b* content in a similar fashion. Compared to control 31 and 15% decrease in chl *b* content was found in acidic condition pH 4.0 and pH 5.5 respectively, while compared to control 28% reduction in chl *b* content was found in alkaline (pH 8.5) condition (Figure 3b). Extreme pH-stress once more reduced chl (*a*+*b*) content; in comparison with the control seedlings. Hence, the chl (*a*+*b*) values were decreased by 19 and 10% compared with control in acid-stress, pH 4.0 and pH 5.5 treated seedlings, respectively (Figure 3c), whereas compared to control 20% decrease in chl (*a*+*b*) content was found in alkaline (pH 8.5) treated seedlings. Another photosynthetic leaf pigment car was also reduced by the negative effect of extreme pH, and compared to control 30 and 16% reduction was observed in acidic (pH 4.0 and pH 5.5 respectively) condition, while 28% reduction in car content was found at in alkaline (pH 8.5) condition (Figure 3d). The chlorotic symptoms were also visible in the leaves in seedlings exposed to extreme pH (Figure 1).

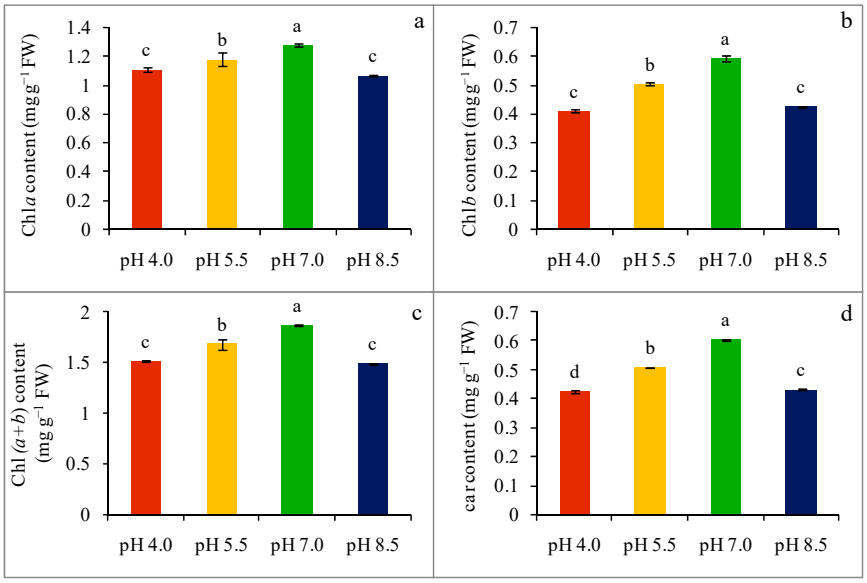


Figure 3. Chlorophyll *a* (a), chl *b* (b), total chl (*a* + *b*) (c), and car (d) contents of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

2.5. Nonenzymatic antioxidant content

The non-enzymatic antioxidant contents of wheat seedlings were affected greatly upon exposure to extreme pH. A sharp decrease in AsA content was noticed in respect of acidity-stress, which was 46 and 31% in seedlings subjected to pH 4.0 and pH 5.5, respectively, compared to the control (Figure 4a), while strongly alkaline-stressed (pH 8.5) seedlings were found with 76% decrease in AsA content compared to the control seedlings. Noticeable increase in DHA content (94 and 45 and 89% in pH 4.0, pH 5.5 and pH 8.5, respectively) was observed compared to control (Figure 4b). Extreme pH stress lessened the AsA/DHA ratio in wheat seedlings. Compared to control, 72 and 52% decrease in AsA/DHA ratio was observed in pH 4.0 and pH 5.5 acidity-stresses respectively, whereas, AsA/DHA ratio was decreased by 70% in alkaline-stressed seedlings, compared with the control (Figure 4c).

Glutathione content was also altered by the effect of extreme pH. It was observed that 16% of the decrease in GSH content occurred upon exposure to extreme acidic condition, whereas 15% decrease in GSH content was found in strong alkaline-stress (pH 8.5) compared with the control (Figure 4d). But strong acidic condition didn't alter GSH content (Figure 4d). On the other hand, GSSG content was increased regarding extreme pH-stress. The GSSG content increased by 74 and 31% in acidity-stress (pH 4.0 and pH 5.5, respectively), while strong alkaline-stress (pH 8.5) increased GSSG content by 106% compared control (Figure 4e). The ratio of GSH and GSSG was also changed due to extreme pH-stress. In response to acidity-stress, 52 and 25% decrease was observed in GSH/GSSG ratio under pH 4.0 and pH 5.5, respectively. Whereas, 58% decrease in GSH/GSSG ratio was observed in strongly alkaline (pH 8.5)-stress (Figure 4f).

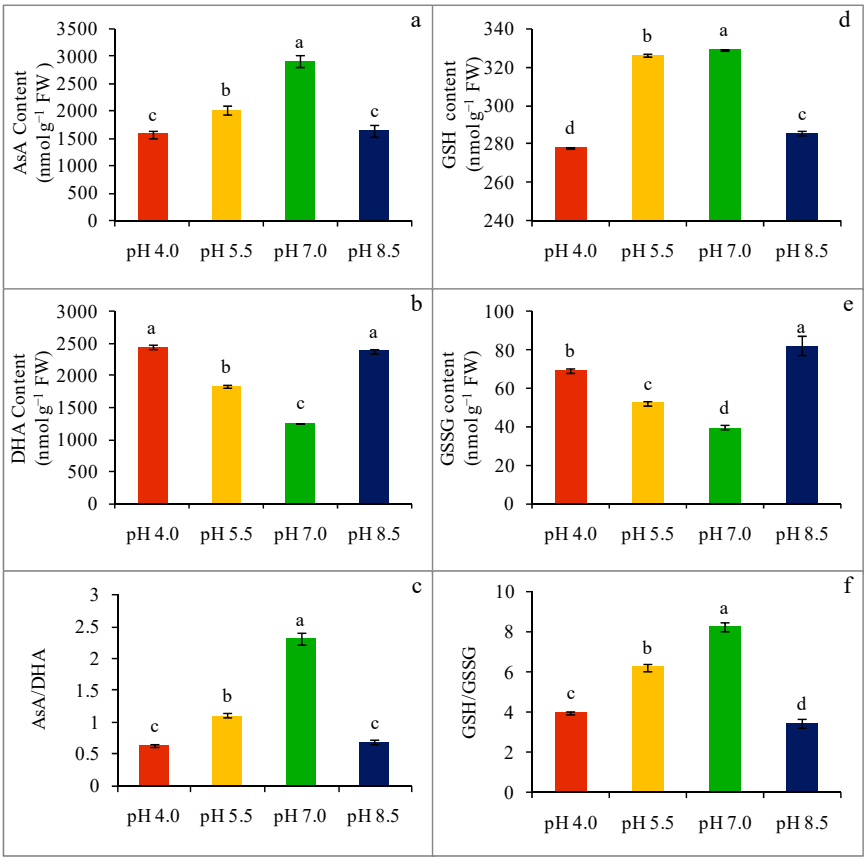


Figure 4. AsA (a) and DHA (b) contents, AsA/DHA ratio (c), GSH (d) and GSSG (e) contents, and GSH/GSSG ratio (f) of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

2.6. Enzyme activity

Compared to control, acidity-stressed (pH 4.0 and pH 5.5) wheat seedlings were found with 43 and 17% upregulated APX activity, whereas alkaline stressed (pH 8.5) seedlings were also showed upregulation of APX activity by 30% (Figure 5a). Strongly acidic (pH 5.5) condition reduced the activity of MDHAR enzyme by 15% in comparison to untreated seedlings while extremely acidic (pH 4.0) condition and strongly alkaline condition (pH 8.5) favored upregulation of MDHAR activity by 19 and 35%, respectively compared with control (Figure 5b). Unlike MDHAR, the DHAR enzyme activity was down-regulated by 17 and 3% in acidity-stress (pH 4.0 and pH 5.5 respectively) compared with the control seedlings (Figure 5c), whereas DHAR activity reduced by 7% in alkaline-stressed (pH 8.5) seedlings. On the other hand, in contrast to the control seedlings, extreme pH exposure didn't alter GR activity significantly (Figure 5d).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 January 2019 doi:10.20944/preprints201901.0143.v1

Peer-reviewed version available at Plants 2019, 8, 24; doi:10.3390/plants8010024

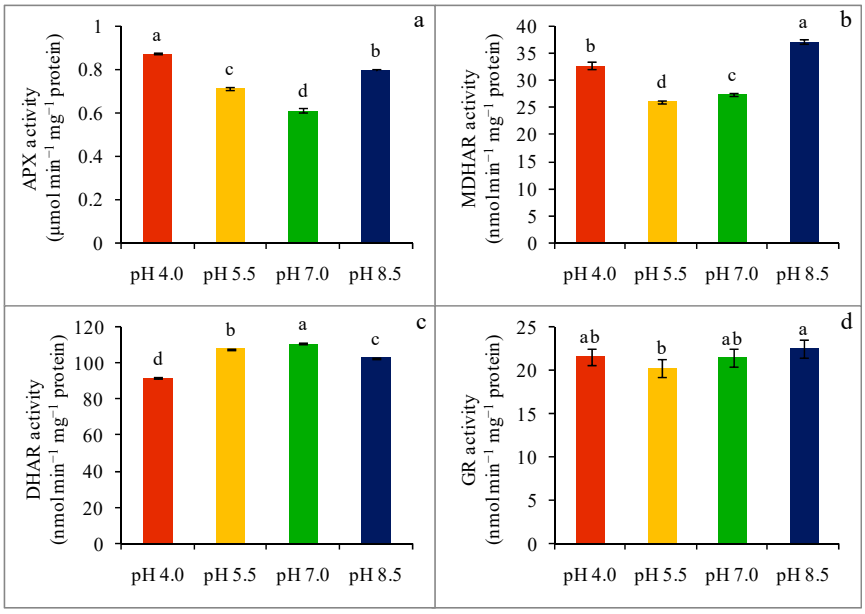
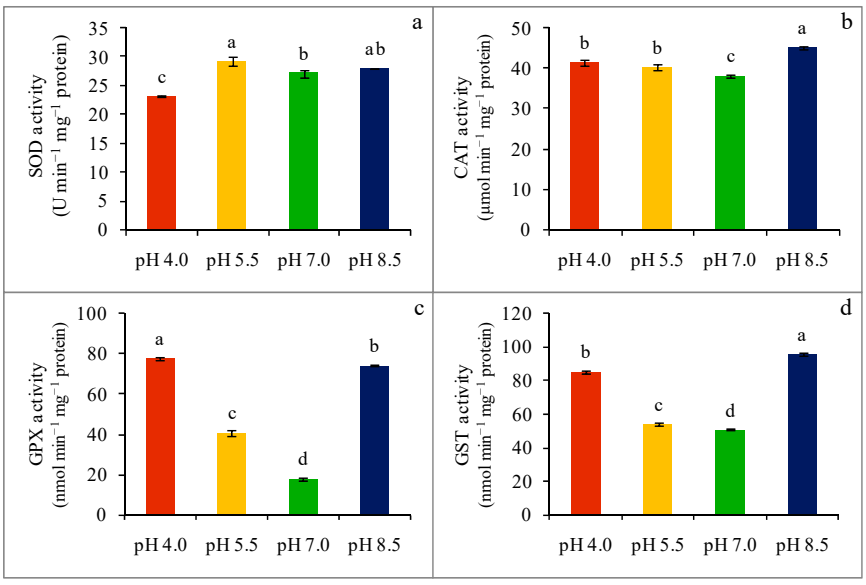


Figure 5 Activities of APX (a), MDHAR (b), DHAR (c), and GR (d) of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

The activity of SOD was down-regulated by 15% in response to extremely acidic-stress pH 4.0 (Figure 6a). On the other hand, SOD activity remained statistically indifferent in strongly acidic (pH 5.5)- and strongly alkaline (pH 8.5)-stressed seedlings (Figure 6c). The activity of CAT was also up-regulated in response to extreme pH stress (Figure 6b). Catalase activity increased by 9 and 6% in pH 4.0 and pH 5.5 acidic-stressed wheat seedlings respectively, whereas compared to control, strongly alkaline (pH 8.5)-stressed seedlings were found with 18% upregulation of CAT activity (Figure 6b). A considerable increase in GPX activity was observed in wheat seedlings upon exposure to extreme pH-stress which was 332 and 128% higher in acidity-stressed (pH 4.0 and pH 5.5 respectively) seedlings, while 313% increase in GPX activity was found in strongly alkaline (pH 8.5)-stressed seedlings (Figure 6c). The GST activity was also upregulated at any level of extreme pH-stress. Compared to control GST activity was increased by 68, 6 and 89% in extreme pH-stressed (acidic pH 4.0 and pH 5.5, and alkaline pH 8.5, respectively) seedlings (Figure 6d).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 January 2019 doi:10.20944/preprints201901.0143.v1

Peer-reviewed version available at Plants 2019, 8, 24; doi:10.3390/plants8010024

Figure 6. Activities of SOD (a), CAT (b) GPX (c) and GST (d) of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

2.7 Glyoxalase enzymes activity and methylglyoxal content

Glyoxalase system comprised of enzyme Gly I and Gly II activity was distorted due to extreme pH of the growing media, as a result, MG detoxification also hampered. Acidity (pH 4.0 and pH 5.5) of growing media decreased Gly I activity by 36 and 5%, respectively (Figure 7a), whereas 27% reduction in Gly I activity was attributed to alkaline (pH 8.5) growing condition compared to control. Meanwhile, in respect to control, Gly II activity was reduced by 12, 5 and 27% in extreme pH condition (pH 4.0, pH 5.5 and pH 8.5, respectively) of the growing media (Figure 7b).

Because of down-regulation of these two vital enzymes, MG content increased notably. Compared with the control seedlings MG content was increased by 35 and 8% in acidity-stressed (pH 4.0 and pH 5.5) seedlings, while a sharp increase (78%) in MG content was observed when the seedlings were exposed to strong alkaline condition (Figure 7c).

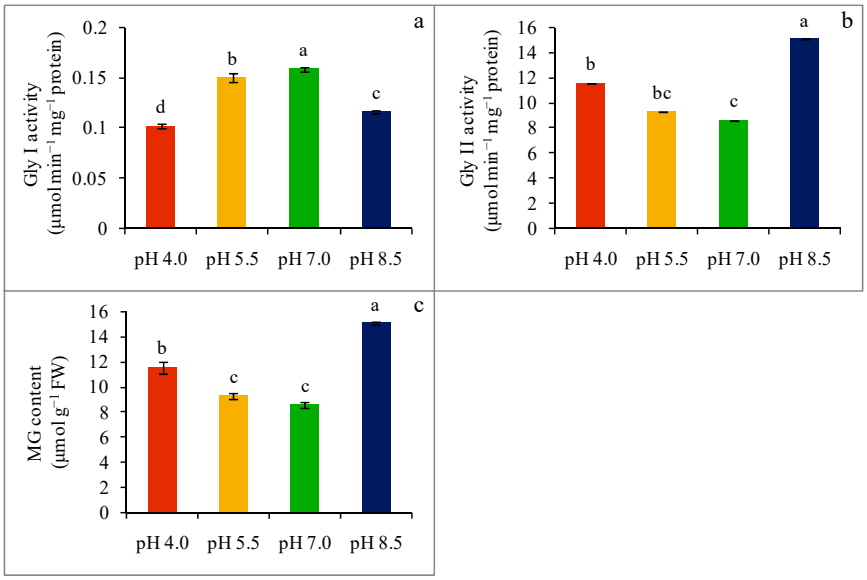


Figure 7. Activities of Gly I (a) and Gly II (b) and MG contents (c) of wheat leaves under different levels of pH. Mean (±SD) was computed from three replications of each treatment. Bars with dissimilar letters are significantly different at $P \leq 0.05$ from Fisher's LSD test

3. Discussion

Crop productivity can be hampered by the extreme (both low and high) pH of the growing media. Both acid and alkaline soils, are one of the most important limitations to agricultural production worldwide [22]. It has been reported that alkali stress involves the same stress factors like salt stress with the added involvement of high pH and the injurious effects on plants are more severe than salt stress, where root growth reduction is much greater than shoot [23].. Similar growth reduction was also reported in *Solanum lycopersicum* [24]. Contrary, due to H^+ rhizooxicity, acidity stress inhibits root growth directs to shallow root systems, hamper water and nutrient uptake [25]. Hence both acidity and alkalinity increase the risk of drought stress.

In the present study, we found the negative effects of extreme pH both acidity- and alkalinity-stress on the morphophysiological attributes of wheat seedlings. Exposure to both acidity and alkalinity, a significant decrease in the seedling growth in terms of seedling height, root length, fresh weight, and dry weight of both shoot and root were observed, which corroborates with previous studies [23,24, 25, 26]. Significant reduction in root elongation is reported under low pH (pH 4.0) due to higher H^+ toxicity in extreme acidic soil to crop plants such as alfalfa, wheat,

spinach, common bean, barley etc. [23,24]. This reduction in root growth might be due to the decrease of root cell division and enlargement [22]. Similarly, Silva et al. [26] reported the reduction in root length and both shoot and root biomass under alkaline stress (> pH 7.0) due to higher pH and metal(loids) toxicity.

As a sessile organism, plants cannot avoid the occurrence of environmental stresses and one of its obvious consequence i.e. overgeneration of ROS ($O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$). In line with other abiotic stress factors, under extreme pH, both acidic and alkaline-stress, we observed elevated lipid peroxidation (measured as MDA), H_2O_2 overgeneration and increased LOX activity in the leaf tissue of wheat seedlings, which keep in with other studies [11,13,27,28]. Alkali stress induced greater cellular structural damage and higher lipid peroxidation was found in tomato plants [27]. Similarly, acidic stress (pH 4.5) induced lipid peroxidation (higher MDA content) was also observed in *H. vulgare* seedlings [11]. Parallel, rising ROS content and lipid peroxidation was also found in *P. sylvestris* and *Lotus corniculatus, Oryza sativa* and citrus following exposure towards acidity [13,28, 29, 30].

Extreme pH-induced ROS production might also be involved in the breakdown of photosynthetic pigments, which was evident in the present study. We found the lower of photosynthetic pigments content viz. chl *a*, chl *b*, chl (*a*+*b*) and car, which corroborates with the previous studies. Yet, ionic imbalance and disturbance in pH homeostasis within plant tissue under alkalinity-stress cause the precipitation of metal ions, consequently responsible for photosynthetic pigments breakdown and subsequent leaf chlorosis, reduced photosynthetic rate and consequently stunted growth [9] . Similar, decreased photochemical efficiency, chl and car content were reported also under higher pH (alkali stress) in *Cucumis sativus*, *Medicago sativa*, and *O. sativa* [28]. Thus, this reduction in photosynthesis leads to lower biomass accumulation. On the other hand Long et al. [30] found low pH (2.5) induced alteration of chl pigments contents, which further reduce the photosynthetic capacity as well as CO_2 assimilation in Citrus. But they didn't find any changes regarding the photosynthetic efficiency at pH ≥2.5. However, the differential response to same stress may happen due to the genetic makeup, which indicate more tolerance of citrus to acidity stress.

We found reduced RWC in leaf tissue of wheat seedlings due to extreme pH, both acidity and alkalinity, which might be attributed to the reduce root length due to alteration in growing media pH, which subsequently caused water unavailability in the growing shoot, and induces artificial drought to the plants. Many of the research reports suggested that abiotic stresses are responsible for reducing the RWC in the plants [14,16,17,19]. Yang et al. [9] reportedthat higher rhizosphere pH caused lowered water content in *T. aestivum* seedlings, alike reduction was also found in *Helianthus annus* , *Aneurolepidium chinense*, and *T. aestivum* seedlings [9]. Similarly, low pH induced root damage and reduced RWC was also observed under acidity stress [29, 30].

Proline is a low molecular weighted amino acid, well known as osmoregulator and ROS scavenger [15]. The adjacent relation between Pro accumulation and dehydration tolerance is the basic strategy to avoid detrimental effect on many major physiological processes, such as leaf expansion, retention of cell osmotic potential, stomatal conductance, and photosynthesis. Therefore, higher Pro accumulation in our study, due to acidity or alkalinity-stress might be because of the increase in Pro biosynthesis together with a decrease in its oxidation [32]. Abiotic stress induced overaccumulation of Pro was reported under various abiotic stressors in many previous studies, which further gave protection to stress induced dehydration and oxidative damage [14,16,17,19]. Therefore, the elevated Pro content in the present study under both acidic and alkaline stress m might have given protection against oxidative injury as well as maintaining water balance.

Extreme pH exposure reduced the photosynthetic pigments; hence, the energy converted from the sunlight couldn't be consumed. This excess energy activates the triplet oxygen ($^3O_2$) to singlet oxygen ($^1O_2$) and subsequently produces reactive $O_2^{\bullet-}$ [33]. Superoxide dismutase enzyme is the first line defense that dismutase $O_2^{\bullet-}$ and convert it to H_2O_2 [34]. In present study, SOD activity slightly increased under mild acidic-and strong alkaline-stress. It was reported that SOD activity gradually but significantly increased in plants under acidity [13], but in our study SOD activity further decreased under extreme acidity stress. This might be due to the overproduction of $O_2^{\bullet-}$ and H_2O_2 at extreme acidity stress, hence the enzyme molecules might be unable to release their product due to

higher concentration of H_2O_2 at releasing pool. On the other hand higher CAT activity is vital to reduce the H_2O_2 content [16]. Although we observed a remarkable upregulation of CAT activity under extreme pH-stress, the H_2O_2 content was not reduced, hence it can be mentioned that the upregulation of CAT activity was not enough to scavenge the overproduced H_2O_2, which indicates the necessity of AsA-GSH pathway in scavenging overgenerated H_2O_2. At AsA-GSH pool we observed a decreased in AsA content and increased APX activity during extreme pH-stress, which is probably due to scavenging H_2O_2. This reduction of AsA content is also resulted from reduced DHAR activities, which increased the DHA content. Although the MDHAR activity increased in both strongly acidic and alkaline condition, however, the AsA/DHA ratio could not be maintained. Alternatively, H_2O_2 scavenging lowered and seedlings suffer from severed oxidative load. The result of our study is in line with previous studies [12,35]. The activity of the ROS detoxification enzymes were significantly increased in plants under acidified [35] and alkaline media [8] indicating that the anti-oxidative defense mechanism is directly involved in the response of seedlings to extreme pH-stress. Previous reports have shown that low pH stress also decreases the activities of SOD and CAT in *Cucumis sativus* [36]. Decreased SOD and CAT activities indicate that the ability to scavenge $O_2^{\bullet-}$ and H_2O_2 is weakened by low pH stress, which may result in ROS induced damage, including lipid peroxidation in membranes [37]. Generally, an appropriate intracellular balance between ROS generation and scavenging exists in cells. The redox homeostasis requires the efficient coordination of an array of antioxidants that can scavenge ROS and protect cells from oxidative damages. Beside AsA, the S-containing potent antioxidant—GSH, also boosts up ROS scavenging together with other enzymes GPX/GST, while GSH also contributes to xenobiotics detoxification coordinating with enzyme GST. In our study, strong acidity and alkalinity stress reduced the content of GSH, consequently increased GSSG content. Although the DHAR activity decreased, but at the same time the GR activity didn't increased noticeably. Therefore, the over generated GSSG content in the stress affected seedlings were observed. On the other hand, the GSH costing two enzymes GPX and GST activities increased in extreme pH-stress; as a result the GSH content decreased, which corroborates with previous studies [16,19]. It was reported that GPX play vital role for scavenging overgenerated H_2O_2 and lipid hydroperoxides with the help of GSH, which prevents ROS induced oxidative damages and confer stress tolerance [115,16]. On the other hand, increased GST activity is also important for ROS detoxification and abiotic stress tolerance [16,19]. Therefore, the alteration in the antioxidant activity (both enzymatic and nonenzymatic) in wheat seedlings is the defense mechanism to avoid oxidative damage [38], and boosting up redox homeostasis to get tolerance [19, 39]. However, the differential response to same stress might be due to the genetic makeup of a species, where the tolerant individuals may show a higher antioxidative capacity, and might get more protection against severe oxidative damage [40].

Another potent cytotoxic glycolytic byproduct MG—over-produced at abiotic stress condition [41]. Overproduction MG hampered the normal morphophysiological processes like growth, root elongation and photosynthetic process in *Arabidopsis* [41]. Furthermore, a higher accumulation of MG results in the inhibition of various physiological processes like the disruption of the antioxidant defense, and other metabolic dysfunctions like inhibition of protein biosynthesis and functions, and nucleic acid biosynthesis, as well as glycation of proteins [42]. In this process, Gly I and Gly II, two vital enzymes, works together with GSH to eliminate MG, hence, this MG detoxification pathway is also termed as GSH-dependent glyoxalase pathway [43]. In present study, we found higher MG content under extreme pH-stresses, which is corroborating with decreased activities of Gly I and Gly II enzymes. This result is coherent with previous research reports, which suggested, ROS induced enhanced MG production, while MG induced ROS production vice versa [41]. Therefore, reduction of activity of any of these two vital enzymes might be attributed to severe oxidative stress [17, 19]. Modulation of the MG detoxification system is also reported as an effective way to reduce the overproduced MG under various abiotic stresses [18, 43].

Therefore, the alteration of the wheat seedlings physiology due to extreme pH can be anticipated based on the present study as, the root growth inhibition followed by the reduction in water uptake and dehydration stress, subsequent ROS and MG generation and destruction of

photosynthetic pigments due to oxidative damage. Meanwhile the antioxidative and glyoxalase systems activated to detoxify the overgenerated ROS and MG, but fails after a certain extent. Consequently, the plant died. But interestingly, the seedlings grown under strong acidic condition (pH 5.5) performed better compared with the seedlings grown under extreme acidic (pH 4.0) and strong alkaline (pH 8.5) condition, which indicate that wheat can tolerate strong acidic (pH 5.5) condition by regulating the antioxidant and glyoxalase system.

4. Materials and Methods

4.1. Plant materials and stress treatments

Wheat (*Triticum aestivum* L. cv. BARI Gom-25) seeds were manually sorted by hands for any dirt, debris, small sized or dead seeds. Separated quality seeds were then surface sterilized with 1% sodium hypochlorite for 10 minutes and washed a number of times with deionized water. Plastic vessels of 8 cm diameter and volume of 25° mL were used for growing the seedlings. The vessels were prepared with a plastic net on top of it and pored with 210 mL of distilled water and 45 seeds were planted in each vessel on top of the net. The vessels were then kept incubation for 40 h. After that, the vessels were transferred to the growth chamber after keeping 25 best seedlings and grown under managed conditions of light 350 µmol photon m^{-1} s^{-2}, temperature 25±2°C; relative humidity 65~70% in cultivation chamber for 6-d. During this tenure 5,000-fold diluted Hyponex solution (Hyponex, Japan) was supplied as a nutrient to the seedlings controlling the pH value at 6.8~7.0 and changed every alternate day. At 8-d, The seedlings were grouped in 4 and exposed to a nutrient solution of 3 different level of acidic and alkaline pH viz. 4.0, 5.5, 8.5 along with a neutral pH as a control for 72 h. The pH of the nutrient solution was checked daily and adjusted to the desirable pH value again. Hence, the experiment consisted of 4 treatments viz. a) extremely acidic (pH 4.0), b) strongly acidic (pH 5.5), c) neutral (pH 7.0) or control, d) strongly alkaline (pH 8.5) fitted in a completely randomized design (CRD) with three repetitions. After 72 h of extreme pH stress leaves from different treatments were harvested separately and data were collected following standard methodologies described later. The experimental outline has been presented in Figure S1.

4.2. Growth and biomass accumulation

The length of the seedlings was recorded after 72 h of stress treatment. The height was measured from base to the leaf tip of 10 randomly selected plants and the mean value was expressed in cm for shoot length. Similarly, the root length also measured for those selected plants from the base to the root tip of the longest root and the average value was expressed in cm as root length.

Ten randomly selected fresh seedlings from each treatment; incised at the joint of root and shoot and weighed separately, recorded and considered as fresh weight of root and shoot. Dry weights were found after drying the seedlings at 80°C in an oven for 48 h. Both fresh weight and dry weight of root and shoot were expressed as g $seedling^{-1}$.

4.3 Determination of stress markers

Malondialdehyde (MDA) and H_2O_2, two vital stress markers were determined according to Health and packer [44] and Yu et al. [45] respectively. Malondialdehyde was measured as thiobarbituric acid-reacting substances (TBARS) after extracted 0.5 g fresh leaf sample with 5% trichloroacetic acid (TCA) and centrifuged at 11500×g. Resultant supernatant (1 mL) was mixed with 4 mL of 0.5% TBA (in 20% TCA) and incubated at` 95°C for 30 min, then quickly cooled in ice to terminate the reaction, and the absorbance was read at 532 and 600 nm, calculated using extinction coefficient (155 $mM^{-1}cm^{-1}$) and expressed as nmol g^{-1}FW [44].

The amount of H_2O_2 accumulation was determined using 5.5 mM $TiCl_4$ (in 20% H_2SO_4) after extracting the 0.5 g plant sample with 3 ml of potassium-phosphate (K-P) buffer (50 mM, pH 6.5) and centrifuged at 11500×g for 15 min. The supernatant (2 mL) was mixed with the reaction mixture and centrifuged again at 11,500×g for 12 min after incubating at room temperature for 10 min. The

absorbance of the final supernatant was read at 410 nm spectrophotometrically, calculated using 0.28 $\mu M^{-1}cm^{-1}$ as extinction coefficient and expressed as nmol $g^{-1}FW$ [45].

4.4 Photosynthetic pigment contents

Photosynthetic pigments were determined using the method described by Arnon [46], and Wellburn [47], homogenizing 0.25g of fresh leaf in 10 mL of 80% Acetone on an ice cold mortar, centrifuged at 2000×g for 10 minutes and the of supernatant was then read/observed spectrophotometrically at 663, 645 and 470 nm and the value of chlorophyll (chl) *a*, *b*, chl (*a*+*b*) and carotenoids (car) were then calculated.

4.5 Relative water content (RWC) and proline content

Relative water content (RWC) was measured according to Barrs and Weatherly [48]. Randomly selected 5 fully developed leaves were weighed, recorded as FW, was sunk into deionized water water in a petri dish for 12 h. Then, the leaves were removed from the water and excess surface water was blotted with paper towels, weighed and recorded as turgid weights (TW). The leaves were dried at 80°C for 48 h for achieving DW and RWC was calculated with the following formula—

$$RWC\ (\%) = [(FW-DW)/ (TW-DW)] \times 100$$

For determining proline (Pro), 0.25 g fresh leaves were homogenized in 3% sulfo-salicylic acid and centrifuged at 11,500×g. The supernatant was further mixed with acid ninhydrin solution (ninhydrin in glacial acetic acid mixed with 6 M phosphoric acid) and glacial acetic acid in an equal proportion and incubated at 100°C for an hour. The mixture is then cooled and toluene was added, and mixed thoroughly for separating the Pro chromophore, read in 520 nm in a spectrophotometer calculated comparing with a standard curve [49].

4.6 Nonenzymatic antioxidant assay

Nonenzymatic antioxidants AsA and GSH were assayed after extracting 0.5 g of leaf sample in 3 ml 5% meta-phosphoric acid containing 1 mM Ethylenediaminetetraacetic acid (EDTA) and centrifuged at 11,500×g for 12 min to remove all plant debris and to get the clear supernatant. The supernatant was neutralized using K-P buffer (0.5 M, pH 7.0) and used to measure AsA and GSH. For measuring total AsA, 0.1 M dithiothretitol was added to convert the DHA to AsA. Than AsA (total and reduced) was assayed at 265 nm spectrophotometrically using a standard curve and DHA was calculated after subtracting reduced AsA from total AsA [14]. Glutathione was determined by enzymatic recycling and the rate of absorption change was read by spectrophotometer at 412 nm and plotted against standard curves with known concentrations of GSH and GSSG. Yet, 2-vinylpyridine dependent removal of GSH was used to determine GSSG content. Finally, the content of GSH was calculated by subtracting GSSG from total GSH [14].

4.7 Protein determination and enzyme activity assay

For assaying of soluble protein and enzymatic activity leaf samples (0.5 g) were homogenized with 1 ml of ice-cold extraction reagent containing 1 mM AsA, 50 mM K-P buffer (pH 7.0), 100 mM KCl, 5 mM β-mercaptoethanol and 10% (w/v) glycerol and followed by centrifugation at 11500×g for 10 min, which were further used for assay maintaining a temperature 0~4°C.

The protein determination involves binding of Coomassie brilliant blue dye with soluble protein, which is thus read under 595 nm and calculated using a standard curve [50].

Lipoxygenase (LOX; EC: 1.13.11.12) activity was assayed according to Doderer et al. [51] using linoleic acid as a substrate at 234 nm spectrophotometrically.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was estimated based on the xanthine–xanthine oxidase system [52]. The reaction mixture contained K-P buffer (50 mM), NBT (2.24 mM), CAT (0.1 units), xanthine oxidase (0.1 units), xanthine (2.36 mM), and expressed as U min^{-1} mg^{-1} protein.

Catalase (CAT; EC: 1.11.1.6) activity was assayed following Hasanuzzaman et al. [53] by monitoring the decrease in absorbance at 240 nm, activity was calculated using 39.4 $M^{-1}cm^{-1}$ as extinction coefficient.

Glutathione peroxidase (GPX; EC: 1.11.1.9) activity was enumerated according to Elia et al. [54], using reaction buffer containing 100 mM K-P buffer (pH 7.0), 1 mM EDTA, 1mM sodium azide (NaN_3), 0.12 mM NADPH, 2 mM GSH, and 1 unit of GR, using 0.6 mM H_2O_2 as substrate, and the activity was expressed as nmol $min^{-1}mg^{-1}$ of protein.

Glutathione S-transferase (GST; EC: 2.5.1.18) activity was measured spectrophotometrically according to Hasanuzzaman et al. [16], using reaction mixture contained 1.5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The increase of absorbance was occurred by the conjugation of CDNB with GSH which read at 340 nm for a min. The enzyme activity was calculated using the extinction coefficient of 9.6 $mM^{-1}cm^{-1}$.

Ascorbate peroxidase (APX; EC: 1.11.1.11) activity was determined as stated by Nakano and Asada [55], where the assay mixture included K-P buffer (50 mM, pH 7.0), EDTA (0.1 mM), AsA (0.5 mM), and H_2O_2 (0.1 mM). The activity of APX was computed using 2.8 $mM^{-1}cm^{-1}$ as extinction coefficient.

Monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4) activity was assayed following Hossain et al. [56], using the 703.4 µL of reaction mixture consisted of Tris-HCl buffer (50 mM, pH 7.5), AsA (2.5 mM), NADPH (0.2 mM), and AO (0.5 unit) and read at 340 nm and calculated using 6.2 $mM^{-1}cm^{-1}$ as extinction coefficient.

Dehydroascorbate reductase (EC: 1.8.5.1) activity was assayed with the mixture contained K-P buffer (50 mM, pH 7.0), GSH (2.5 mM), EDTA (0.1 mM) and DHA (0.1 mM). The activity of DHAR was observed from the increase in absorbance at 265 nm and calculated using 14 $mM^{-1}cm^{-1}$ as extinction coefficient [55]

Glutathione reductase (EC: 1.6.4.2) activity was measured following Hasanuzzaman et al. [16] by observing the decline in absorbance at 340 nm, where reaction mixture consisted of K-P buffer (0.1 M, pH 7.0) and EDTA (1 mM), calculated using 6.2 $mM^{-1}cm^{-1}$ as extinction coefficient.

4.8 Glyoxalase activity assay and methylglyoxal content

Glyoxalase I (Gly I; EC: 4.4.1.5) activity was recorded according to Hasanuzzaman et al. [16], where reaction mixture contained K-P buffer (100 mM, pH 7.0), magnesium sulfate (15 mM), GSH (1.7 mM) and MG (3.5 mM). After adding MG, the reaction was started and the increase in absorbance was obtained at 240 nm for 1 min. The activity of Gly I was computed using 3.37 mM-1cm-1 as extinction coefficient.

Glyoxalase II (EC: 3.1.2.6) activity was assayed by Principato et al. [57], where 500 µL of the reaction mixture contained Tris-HCl buffer (100 mM, pH 7.2), DTNB (0.2 mM) and S-D-lactoylglutathione (SLG, 1 mM). The increase of absorbance was recorded at 412 nm spectrophotometrically and counted using 13.6 $mM^{-1}cm^{-1}$ as extinction coefficient.

Methylglyoxal was determined after extracting 0.25 g leaf in 5% perchloric acid and centrifuged at 11,000×g; decolorize using charcoal and subsequent neutralization by Na_2CO_3. The neutralized supernatant was then used for N-acetyl-L-cysteine assay for MG estimation at a wavelength of 288 nm using a standard curve [58].

4.9 Statistical analysis

The data obtained for various morphophysiological and biochemical parameters were then subject to statistical analysis using XLSTAT 2017 [59] software and the mean differences were separated using Fisher's LSD test ($P \leq 0.05$).

5. Conclusions

Apart from the optimal pH, when plants exposed to extreme pH conditions; higher H_2O_2 and MG production created subsequent oxidative stress, produced MDA together with enhanced LOX

activity, disturbed plant antioxidant defense and glyoxalase systems. Moreover, reduction in water balance and photosynthetic pigments content leads to poor growth and development. However, modulating the coordinated actions of antioxidant defense and glyoxalase system could be an important strategy in improving the performance of wheat seedlings under extreme pH-stress. In sum, our findings provide information regarding the points of damage in antioxidant defense and glyoxalase systems. Thus, these findings might further assist in selecting extreme pH (acidity or alkalinity) tolerate genotypes. Moreover, researchers might get information discovering suitable phytoprotectants to tinker the antioxidant defense and glyoxalase system under extreme pH-stress.

Supplementary Materials: The following is available online at www.mdpi.com/xxx/S1, Figure S1: Work flow scheme of the conducted experiment

Author Contributions: M.H.M.B.B. with the help of M.H. and M.F. designed the experiment. M.H.M.B.B. performed the experiment, J.A.M.; M.S.H. and T.F.B. helped in the executing the experiment. M.H. did the statistical analysis. M.H.M.B.B. has written the manuscript. M.H.; J.A.M.; M.S.H. and T.F.B critically reviewed and edited the manuscript. M.F. supported the laboratory and other technical facilities. All the authors read and approved the final manuscript.

Acknowledgments: Author M.H.M.B.B. is grateful to the the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan for bestowing scholarship for Ph.D. study. Special thanks to Khursheda Parvin, Assistant professor, Department of Horticulture, Sher-e-Bangla Agricultural University and Sayed Mohammad Mohsin, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Bangladesh, for their critical review and editing of the manuscript.

Conflicts of Interest: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Fraire-Velázquez, S.; Balderas-Hernández, V.E. Abiotic stress in plants and metabolic responses, In *Abiotic Stress-Plant Responses and Applications in Agriculture*, Vahdati, K., Leslie C., Eds., InTech: London, UK, 2013; pp. 25–48.
2. Merry, R.H. Acidity and alkalinity of soils, In *Environmental and Ecological Chemistry-volume II*; Sabljic, A., Ed., Eolss Publications: Oxford, UK, 2009; pp. 115–131.
3. Soil Survey Division Staff, Examination and Description of Soil Profiles. In *Soil survey manual, US Department of Agriculture Handbook 18;* Soil Survey Division Staff, Eds., US Department of Agriculture: Washington DC, USA, 2017; pp. 83–230.
4. Albert, S. *The Kitchen Garden Grower's Guide: A Practical Vegetable and Herb Garden Encyclopedia*, Create Space Independent Pub: USA; 2008.
5. Zheng, S.J. Crop production on acidic soils: overcoming aluminium toxicity and phosphorus deficiency. *Ann. Bot.* **2010,** 106, 183–184.
6. Shavrukov Y.; Hirai Y. Good and bad protons: genetic aspects of acidity stress responses in plants. *J. Exp. Bot.* **2015,** 67, 15–30.
7. Wilkinson, R.E.; Duncan, R.R. Sorghum seedling growth as influenced by H^+, Ca^{2+}, and Mn^{2+} concentrations. *J. Plant Nutr.* **1989**,12, 1379–1394.
8. Yang, C.W. Wang, P.; Li, C.Y.; Shi, D.C.; Wang, D.L. Comparison of effects of salt and alkali stresses on the growth and photosynthesis of wheat. *Photosynthetica* **2008,** 46, 107–114.
9. Yang, Y.; Qin, Y.; Xie, C.; Zhao, F.; Zhao, J.; Liu, D.; Chen, S.; Fuglsang, A.T.; Palmgren, M.G.; Schumaker, K.S.; Deng, X.W. The *Arabidopsis* chaperone J3 regulates the plasma membrane H^+-ATPase through interaction with the PKS5 kinase. Plant Cell **2010,** 22, 1313–1332.
10. Joliot, P.; Johnson, G.N. Regulation of cyclic and linear electron flow in higher plants. Proc. Nat. Acad. Sci. U.S.A. **2011** 108, 13317–13322.
11. Song, H.; Xu, X.; Wang, H.; Tao, Y. Protein carbonylation in barley seedling roots caused by aluminum and proton toxicity is suppressed by salicylic acid. *Russ. J. Plant Physiol.* **2011**, 58, 653–659.
12. Zhang, K.X.; Wen, T.; Dong, J.; Ma, F.W.; Bai, T.H.; Wang, K.; LI, C.Y. Comprehensive evaluation of tolerance to alkali stress by 17 genotypes of apple rootstocks. *J. Integr. Agric.* **2016,** 15, 1499–1509.
13. Ivanov, Y.; Savochkin, Y.; Kuznetsov, V. Effect of mineral composition and medium pH on Scots pine tolerance to toxic effect of zinc ions. *Russ. J. Plant Physiol.* **2013,** 60, 260–269.

14. Hasanuzzaman, M.; Nahar, K.; Hossain, M.S.; Anee, T.I.; Parvin, K.; Fujita, M. Nitric oxide pretreatment enhances antioxidant defense and glyoxalase systems to confer PEG-induced oxidative stress in rapeseed, *J. Plant Interact.* **2017a,** 12, 323–331.
15. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010,** 48, 909–930.
16. Hasanuzzaman, M.; Hossain, M.A.; Fujita, M. Exogenous selenium pretreatment protects rapeseed seedlings from cadmium-induced oxidative stress by upregulating antioxidant defense and methylglyoxal detoxification systems; *Biol. Trace Elem. Res.* **2012,** 149, 248–261.
17. Hasanuzzaman, M.; Nahar, K.; Gill, S.S.; Alharby, H.F.; Razafindrabe, B.H.; Fujita, M. Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in *Brassica napus* L.: An intrinsic study on antioxidant defense and glyoxalase systems. *Front. Plant Sci.* **2017b**, 8, 115.
18. Hasanuzzaman, M.; Nahar, K.; Hossain, M.S.; Mahmud, J.A.; Rahman, A.; Inafuku, M.; Oku, H.; Fujita, M. Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. *Int. J. Mol. Sci.* **2017c**, 18, 200.
19. Nahar, K.; Hasanuzzaman, M.; Suzuki, T.; Fujita, M. Polyamines-induced aluminum tolerance in mung bean: A study on antioxidant defense and methylglyoxal detoxification systems. *Ecotoxicology* **2017,** 26, 58–73.
20. Viveros, M.F.Á.; Inostroza-Blancheteau, C.; Timmermann, T.; González, M.; Arce-Johnson, P. Overexpression of GlyI and GlyII genes in transgenic tomato (*Solanum lycopersicum* Mill.) plants confers salt tolerance by decreasing oxidative stress. *Mol. Biol. Rep.* **2013,** 40, 3281–3290.
21. Mahmud, J.A.; Hasanuzzaman, M.; Nahar, K.; Rahman, A.; Hossain, M.S.; Fujita, M. Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in *Brassica juncea* L.. *Ecotoxicol. Environ. Saf.* **2017,** 144, 216–226.
22. Kochian, L.V.; Piñeros, M.A.; Liu, J.; Magalhães, J.V. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annu. Rev. Plant Biol.* **2015,** 66, 571–598.
23. Yang, C.; Xu, H.; Wang, L.; Liu, J.; Shi, D.; Wang, D. Comparative effects of salt stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. *Photosynthetica* **2009,** 47, 79–86.
24. Wang, X.; Geng, S.; Ri, Y.J.; Cao, D.; Liu, J.; Shi, D.; Yang, C. Physiological responses and adaptive strategies of tomato plants to salt and alkali stresses. *Sci. Hort.* **2011,** 130, 248–255.
25. Marschner, H. Mechanisms of adaptation of plants to acid soils. *Plant Soil* **1991,** 134, 1–20.
26. Silva, C.M.S.; Zhang, C.; Habermann, G.; Delhaize, E.; Ryan, P.R. Does the major aluminium-resistance gene in wheat, *TaALMT1*, also confer tolerance to alkaline soils? *Plant Soil* (**2018**) doi:10.1007/s11104-017-3549-6.
27. Gong, B.; Li, X.; Vanden Langenberg, K.M.; Wen, D.; Sun, S.; Wei, M.; Li, Y.; Yang, F.; Shi, Q.; Wang, X. Overexpression of S-adenosyl-l-methionine synthetase increased tomato tolerance to alkali stress through polyamine metabolism, *Plant Biotechnol. J* **2014**, 12, 694–708.
28. Pal'ove-Balang, P.; Čiamporová, M.; Zelinová, V.; Pavlovkin, J.; Gurinová, E.; Mistrík, I. Cellular responses of two Latin-American cultivars of *Lotus corniculatus* to low pH and Al stress. *Cent. Eur. J. Biol.* **2012,** 7, 1046–1054.
29. Zhang, Y.K., Zhu, D.F., Zhang, Y.P., Chen, H.Z., Xiang, J., Lin, X.Q. Low pH-induced changes of antioxidant enzyme and ATPase activities in the roots of rice (*Oryza sativa* L.) seedlings. *PloS One* **2015,** 10, p.e0116971.
30. Long. A., Zhang, J., Yang, L.T., Ye, X., Lai, N.W., Tan, L.L., Lin, D., Chen, L.S. Effects of ow pH on photosynthesis, related physiological parameters, and nutrient profiles of *Citrus*. *Front Plant Sci* **2017**, 8, 185.
31. Wu, Z.H. Yang, C.W. Yang, M.Y. Photosynthesis, photosystem II efficiency, amino acid metabolism and ion distribution in rice (*Oryza sativa* L.) in response to alkaline stress, *Photosynthetica* 52 (2014) 157–160.
32. Amist, N.; Singh, N.B. Responses of enzymes involved in proline biosynthesis and degradation in w*heat seedlin*gs under stress, *Allelopathy J*.**2017,** 42, 195–205.
33. Hasanuzzaman, M.; Nahar, K.; Fujita, M. Alteration in chlorophylls and carotenoids in higher plants under abiotic stress condition. In *Photosynthetic Pigments: Chemical Structure, Biological Function And Ecology* Golovko, T.K., Gruszecki, W.I., Prasad, M.N.V., Strzałka K. Eds.; Syktyvkar: Russia, 2014; pp. 218–264.

34. Gill, S.S.; Anjum, N.A.; Gill, R.; Yadav, S.; Hasanuzzaman, M.; Fujita, M.; Mishra, P.; Sabat, S.C.; Tuteja, N. Superoxide dismutase—mentor of abiotic stress tolerance in crop plants, *Environ. Sci. Pollut. Res.* **2015**, 22, 10375–10394.
35. Martins N., Goncalves S., Romano A., Metabolism and aluminum accumulation in *Plantago almogravensis* and *P. algarbiensis* in response to low pH and aluminum stress. *Biol. Plant* **2013**, 57, 325–331.
36. Shi, Q.H., Zhu, Z.J., Li, J., Qian, Q.Q. Combined effects of excess Mn and low pH on oxidative stress and antioxidant enzymes in cucumber roots. *J Integr Agri* **2006,** 5, 767–772.
37. Mead, J.F. Free radical mechanisms of lipid damage and consequences for cellular membranes. In *Free radicals in biology*; Pryor, W.A. Ed.; Academic Press:New York, USA, 1976, Volume 1, pp. 51–68.
38. Xu, C.; Liu, Z.; Zhang, L.; Zhao, C.; Yuan, S.; Zhang, F. Organization of actin cytoskeleton during meiosis I in a wheat thermo-sensitive genic male sterile line. *Protoplasma* **2013**. 250, 415–422.
39. Varga, B.; Janda, T.; László, E.; Veisz, O. Influence of abiotic stresses on the antioxidant enzyme activity of cereals. *Acta Physiol. Plant* **2012**, 34, 849–858.
40. Shah, Z.H.; Rehman, H.M.; Akhtar, T.; Daur, I.; Nawaz, M.A.; Ahmad, M.Q.; Rana, I.A.; Atif, R.M.; Yang, S.H.; Chung, G. Redox and ionic homeostasis regulations against oxidative, salinity and drought stress in wheat (A Systems Biology Approach). *Front. Genet.* **2017**, 8, 141.
41. Kaur, C.; Kushwaha, H.R.; Mustafiz, A.; Pareek, A.; Sopory, S.K.; Singla-Pareek, S.L. Analysis of global gene expression profile of rice in response to methylglyoxal indicates its possible role as a stress signal molecule. *Front. Plant Sci.* **2015**, 6, 682.
42. Hoque, T.S.; Hossain, M.A.; Mostofa, M.G.; Burritt, D.J.; Fujita, M.; Tran, L.S.P. Methylglyoxal: An emerging signaling molecule in plant abiotic stress responses and tolerance. *Front. Plant Sci.* **2016,** 7, 1341.
43. Kaur, C.; Singla-Pareek, S.L.; Sopory, S.K. Glyoxalase and methylglyoxal as biomarkers for plant stress tolerance. *Crit. Rev. Plant Sci.* **2014**, 33, 429–456.
44. Heath, R.L.; Packer, L. Photo peroxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968,** 125, 189–198.
45. Yu, C.W.; Murphy, T.M.; Lin, C.H. Hydrogen peroxide induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Func. Plant Biol.* **2003,** 30, 955–963.
46. Arnon, D.T. Copper enzymes in isolated chloroplasts polyphenal oxidase in *Beta vulgaris*, *Plant Physiol.* **1949,** 24, 1–15.
47. Wellburn, A.R. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **1994,** 144, 307–313.
48. Barrs, H.D.; Weatherley, P.E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **1962,** 15, 413–428.
49. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973,** 39, 205–207.
50. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, 72, 248–254.
51. Doderer, A.; Kokkelink, I.; van der Veen, S.; Valk, B. Schram,; A. Douma, A.Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim. Biophys. Acta* **1992,** 112, 97–104.
52. El-Shabrawi, H.; Kumar, B.; Kaul, T.; Reddy, M.K.; Singla-Pareek, S.L.; Sopory, S.K. Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma* **2010,** 245, 85–96.
53. Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Bhuyan, M.H.M.B.; Oku, H.; Fujita, M. Exogenous nitric oxide pretreatment protects *Brassica napus* L. seedlings from paraquat toxicity through the modulation of antioxidant defense and glyoxalase systems. *Plant Physiol. Biochem.* **2018,** 126, 173–186.
54. Elia, A.C.; Galarini, R.; Taticchi, M.I.; Dorr, A.J.M.; Mantilacci, L. Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicol. Environ. Saf.* **2003,** 55, 162–167.
55. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **1981,** 22, 867–880.
56. Hossain, M.A.; Nakano, Y.; Asada, K. Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol.* **1984**, 25, 385–395.

659 57. Principato, G.B.; Rosi, G.; Talesa, V.; Giovanni, E.; Uotila, L. Purification and characterization of two forms
660 of glyoxalase II from the liver and brain of Wistar rats. *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.*
661 **1987**, 911, 349–355.
662 58. Wild, R.; Ooi, L.; Srikanth, V.; Münch, G. A quick, convenient and economical method for the reliable
663 determination of methylglyoxal in millimolar concentrations: The N-acetyl-L-cysteine assay. *Anal. Bioanal.*
664 *Chem.* **2012,** 403, 2577–2581.
665 59. Addinsoft, XLSTAT V. 2017.1.01: Data Analysis and Statistics Software for Microsoft Excel, Paris:
666 Addinsoft, 2017.