BIOCELL
2022 46(7): 1613-1627

Tech Science Press

Screening and evaluation of chilli (*Capsicum annuum* L.) genotypes for waterlogging tolerance at seedling stage

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Key words: Abiotic stress, Anoxia, Flooding, Hypoxia, Pepper, Photosynthetic pigments, Proline

Abstract: Waterlogging is an illustrious abiotic stress and the constrictions it enforces on plant roots have negative effects on growth and development. This study was undertaken to investigate waterlogging stress tolerant potential in chilli (*Capsicum annum* L.) genotypes through evaluating morphological, physiological, biochemical and anatomical parameters. Thirty-five days old seedlings of 10 chilli genotypes were exposed to waterlogging stress maintaining water height 3–5 cm over the soil surface artificially for three days. This duration (36–38 DAE) was termed as waterlogging period, and subsequent withdrawal of waterlogging condition (39–45 DAE) was regarded as a recovery phase. Based on their survival performance, two tolerant genotypes viz., SRC-517 and BARI morich-2 and two susceptible genotypes viz., AHM-206 and RI-1(6) were selected for studying stress tolerance mechanism. Under waterlogging, however, both genotypes (tolerant and susceptible) exhibited reduced root shoot length, dry weight ratio, petiole weight and leaf area, and noticeable reduction regarding these parameters was observed in susceptible genotypes. Moreover, tolerant genotypes displayed a higher recovery than susceptible genotypes after removal of waterlogging stress. Lower reduction of leaf area and photosynthetic pigments as well as higher reduction of relative water content (RWC) were noticed in susceptible genotypes. Higher accumulation of proline and total antioxidant capacity (TAC) during waterlogging condition in tolerant genotypes suggested lower oxidative damage. Although both genotypes lost total soluble sugar (TSS) relative to control at waterlogging stress, better performance was recorded in tolerant genotypes. During the period after the removal of extra water, a similar genotypic response in terms of TSS gain was seen. Undoubtedly, under flooding conditions, the development of aerenchyma cells in tolerant genotypes is a means of tolerance mechanism for long-term survival. Thus, the morpho-physiological and biochemical changes help to understand the tolerance mechanism in chilli under waterlogging stress.

Introduction

Waterlogging or flooding is one of the most threatening abiotic stresses for agricultural crop production and desired yield owing to the fickle pattern of precipitation and that fascinated with extreme events of other climatic conditions. Excessive rainfall and poor drainage are the main causes for waterlogging (Hasanuzzaman *et al.*, 2012a). Under waterlogging condition plants suffer from the scarcity of available O_2 (Capon *et al.*, 2009). Hypoxia (lack of O_2) is the primary phase of waterlogging that limits the mitochondrial respiration, followed by anoxia (inhibited respiration results from O_2 deficiency) (Wegner, 2010). Evidential reports in several crop species suggested that, along with altered morpho-physiological and growth processes, waterlogging brutally hampers the reproductive development, leading to ultimate yield loss. Chilli (*Capsicum annum* L.) belongs to the family of Solanaceae is very sensitive to waterlogging which may be due to shallowly distributed root systems and water stress may cause a dramatic reduction in their desired production. Irrigation and water management is critical in chilli cultivation. Flooded fields should be drained within 48 h. Otherwise, the chilli plants lead to death (Rob, 2010). In that situation, tolerant germplasm will play a pivotal role in combating the most crucial natural hazard. In Bangladesh context, chilli is not only a valuable spice, but also a treasured cash crop. Statistically, cultivated area

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Received: 11 September 2021; Accepted: 17 November 2021

Doi: 10.32604/biocell.2022.019243

and the production of chilli in summer (April to September) season is 19,000 ha and 36,000 t, respectively. The area and production is 83,000 ha and 105,000 t in winter (October to March) season, respectively. The yield was around 1.42 t ha^{-1} (BBS, 2019). However, several researchers suggested the growth parameters like root-shoot length, ratio and dry weight as practical attributes at early stages in the selection of tolerant genotypes to waterlogging stress such as soybean (Kim *et al*., 2015), sesame (Saha *et al*., 2016) and tomato (Gotame, 2006). However, there has been limited research that has observed at chilli under waterlogging stress. Under the circumstances, the current study was carried out to examine the morphological, physiological, stomatal and biochemical response of tolerant and susceptible chilli seedlings under waterlogging stress which may ultimately help to select chilli genotype(s) with potential tolerance to waterlogging stress at the seedling stage.

Materials and Methods

Study materials and stress application

The experiment was conducted in Plant Breeding Division of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during March to September 2019. Thirty seedlings per treatment (10 seedlings per replication) of 10 summer chilli genotypes were evaluated to find out their survival capacity under waterlogging stress (Table 1). Germinated seeds in the pot were properly watered for 35 days. Waterlogging area was prepared by enclosed polythene sheets which were fixed firmly into the ground and outspreading 15 cm directly above the ground to confine the water. After 35 days of seedling emergence (DAE) the waterlogging environment was executed and retained (water height was 3–5 cm above the soil surface) for three (36–38 DAE) days, and subsequent withdrawal of waterlogging, i.e., 39–45 DAE was designated as the recovery period. Finally, the death and recovery percentage were calculated from the number of alive and dead seedlings from each genotype. These experiments were repeated two times under the same condition. According to the results of survival capacity, the most two tolerant and susceptible genotypes were used in morphological, physiological, anatomical and biochemical study. Seedlings under control, waterlogging stress and recovery period were evaluated with three replications in this study. Treatments consisted of control, waterlogging stress and recovery (after removal of waterlogging).

Determination of growth parameters

To measure root length and shoot length from each treatment three seedlings were selected erratically, and measurement was performed by using a scale in cm. Similarly, three seedlings from each treatment were taken to quantify the dry weight (DW) in gram (g) and were dried at a temperature of 80°C for 48 h. The root-shoot weight ratio was calculated from the dry weights. An area meter (LI-3100, LI-COR Environmental, USA) were used to determine leaf area (LA) in cm^2. The average weight of three leaf petioles was recorded on precision balance or the determination of petiole weight (PW).

Measurement of leaf relative water content

Relative water content (RWC) in leaves of 10 seedlings in replication in each treatment was measured and calculated following the procedure of Weatherley (1950). The following formula was used to compute the RWC and expressed in percentage.

RWC = [(FW–DW)/(TW–DW)] × 100. Where, FW, DW and TW mean fresh weight, dry weight and turgid weight, respectively.

Measurement of chlorophyll pigments, carotenoids and proline content

Spectrophotometrically photosynthetic pigments, e.g., chlorophyll (Chl) *a*, *b*, and total Chl (*a*+*b*) contents in the chilli leaves collected from 10 seedlings were measured and calculated comprehending the procedure of Lichtenthaler (1987) and Arnon (1949), respectively. Content of carotenoids (Car) were quantified following the equation proposed by Kirk and Allen (1965). Colorimetric determination of proline (Pro) was preceded applying the process of Bates *et al*. (1973).

Determination of total soluble sugar (TSS) and total antioxidant capacity

Procedure of Yemm and Willis (1954) were adopted to determine the total soluble sugar (TSS). Leaf sample

TABLE 1

List of summer growing chilli genotypes used in this study with their collection sites in Bangladesh

Sl. No.	Genotypes	Location of collecting site	Latitude (N)	Latitude (E)
01	SRC-517	RSRC, BARI, Gazipur	24° 22.8′	88° 39.42′
02	BARI morich-2	RSRC, BARI, Gazipur	24° 22.8′	88° 39.42′
03	AC-312	Kaliganj, Gazipur	23° 55.30′	90° 34.01′
04	IAH-160	Sadar, Gazipur	24° 0′	90° 25.30′
05	IAH-164	Kaliakoir, Gazipur	24° 4.30′	90° 13.0′
06	AHM-217	Dhamrai, Dhaka	23° 54.30′	90° 13.0′
07	RAI-67	Hathazari, Chittagong	22° 30.13′	91° 48.27′
08	AC-63	Shakhipur, Tangail	24° 19.9′	90° 10.20′
09	RI-1(6)	Ramgarh, Khagrachori	22° 58.0′	91° 42.0′
10	AHM-206	Dhamrai, Dhaka	23° 54.30′	90° 13.0′

Note: RSRC: Regional Spices Research Centre; BARI: Bangladesh Agricultural Research Institute.

collected from 10 seedlings was extracted with 10 ml 80% ethanol and ethanol extract (0.1 to 0.2 ml) was evaporated to dryness in a test tube. Anthrone reagent was added to the wall of the test tube and gently combined with distilled water for absorbance calculation with the reagent blank at 620 nm. A standard curve was prepared from glucose-graded concentration calculates the TSS in the extract and the data are expressed as mg g^{-1} DW (dry weight).

Diphenyl-picrylhydrazyl (DPPH) radical degradation method proposed by Sarker and Oba (2019a, b) was used to estimate the total antioxidant activity (TAC). Absorbance was taken against methanol at 734 nm by using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). Trolox was used as the reference standard, and the results were expressed as µg Trolox equivalent g^{-1} DW.

Aerenchyma observation

After six days of imposing waterlogging treatment (water level for the first three days was maintained at 3 cm above the ground and last three days at saturated field capacity), root anatomy was examined in lateral roots of flooded and control plants. However, root tissue fixation and cross section were done, repeating the method described by Pardales *et al.* (1991), and the quantity of root cortex aerenchyma was scored visually applying the technique of Mano *et al.* (2006).

Experimental design

The current study was arranged in two factors completely randomized design. Each experiment was repeated twice independently with three replications.

Statistical analysis

Data of three separate replications were reported as the mean ± SE. Data was analyzed statistically by analysis of variance (ANOVA) using Statistix (version 10) software. Least significance difference (LSD) at 5% level of significance was used to equate the mean values. Differences with $P \leq 0.05$ were assessed as significant difference.

Results

Estimation of survival performance based on death and recovery percentage

Waterlogging stress caused significant variation in seedling death and recovery percentage which enabled to select waterlogging tolerant and responsive genotypes in seedling stage (Figs. 1a and 1b).

The highest seedling death percentage was recorded in the genotypes RI-1(6) (76%) and AHM-206 (75%) with least recovery rate (5% and 4%, respectively) while, the genotypes SRC-517 and BARI morich-2 showed the lowest seedling death (12% and 16%, respectively) with higher recovery rate (73% and 66%, respectively). Based on these results, the most waterlogging tolerant genotypes were SRC-517 and BARI morich-2, contrarily; RI-1(6) and AHM-206 were the most susceptible. A comparative phenotypic response to waterlogging stress in one of the most tolerant genotype (SRC-517) and one of the most susceptible genotype (AHM-206) was shown in Fig. 1c.

Evaluation of morphological parameters in selected genotypes

Root length

Significant variation in root length due to waterlogging stress was observed (Fig. 2a). Intra-genotypic differences showed that tolerant genotypes had significantly higher root length than the susceptible genotypes. Waterlogging stress reduced the root length, and the root further increased in recovery period. Interaction effect showed that BARI morich-2 had the longest

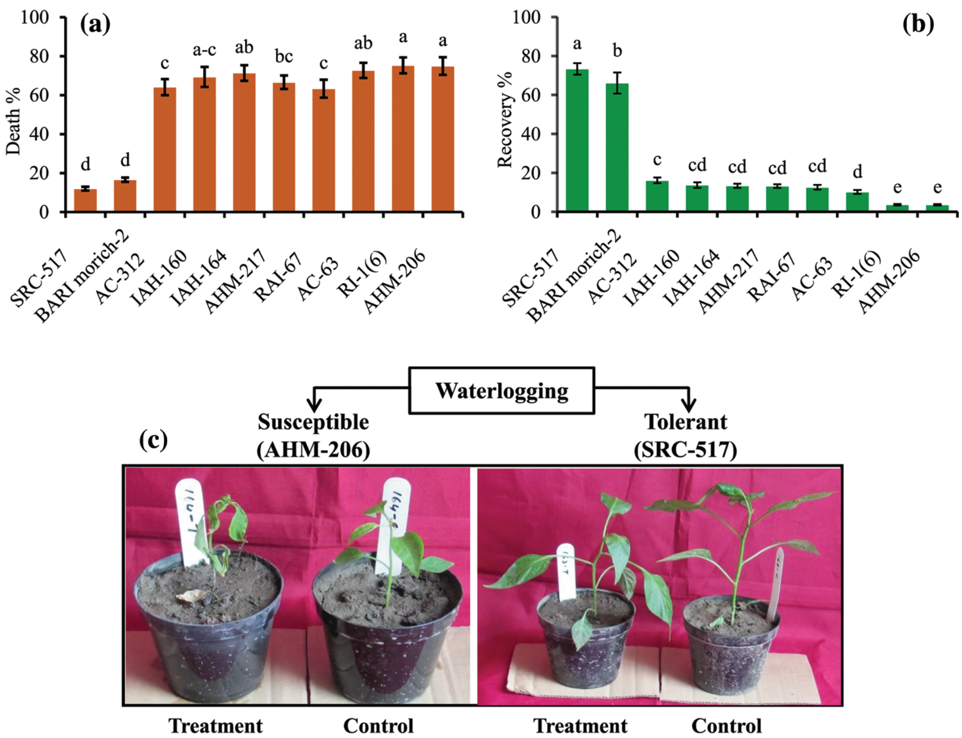

FIGURE 1. Death (a) and recovery (b) percentage of chilli seedlings under waterlogging stress. Comparative phenotype of the most waterlogging tolerant (SRC-517) and susceptible (AHM-206) genotypes (c). Values in bar are mean ± SE of three replications and various letters on bars are significantly different at $P \leq 0.05$.

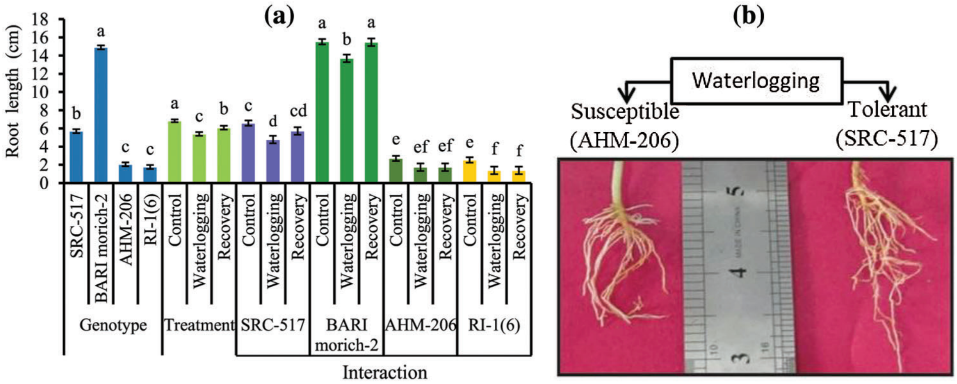

FIGURE 2. Effect of waterlogging stress on root length in chilli seedlings (a). The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. Values in bar are mean ± SE of three replications and various letters on bars are significantly different at $P \leq 0.05$. Control (no stress); waterlogging (impose excess water for three days, 36–38 DAE), recovery (removal of excess water for seven days, 39–45 DAE). Comparative root length between one tolerant and one susceptible chilli genotype in waterlogging (b).

roots followed by SRC-517 whereas, the sensitive genotypes significantly lower roots in waterlogging conditions. A comparative length and density of root under waterlogging stress in one tolerant and one susceptible genotype were also shown in Fig. 2b. It is also noticed that tolerant genotypes recovered the root length in recovery period.

Shoot length

Interaction effect of waterlogging stress showed that it reduced the shoot length by 18% and 17% relative to control in tolerant genotypes SRC-517 and BARI morich-2, respectively while, 13% and 12% reduction was observed in susceptible genotypes (RI-1(6) and AHM-106, respectively). Tolerant genotypes SRC-517 and BARI morich-2 showed 7% and 14% increment in recovery, respectively. However, no increment was recorded in susceptible genotypes in recovery (Fig. 3).

Root dry weight

The interaction effects waterlogging stress showed that reduction of root DW was observed in both tolerant and susceptible genotypes as compared to respective control where, susceptible genotype RI-1(6) showed the highest (47%) reduction (Fig. 4a). Importantly, higher recovery was observed in tolerant genotypes. The genotypes SRC-517 and BARI morich-2 showed 41% and 36% recovery, respectively over waterlogging stress. On the other hand, no recovery was found in susceptible genotypes, and further reduction was observed in susceptible genotypes (2% and 18% in AHM-106 and RI-1(6), respectively) (Fig. 4a).

Shoot dry weight

Waterlogging significantly reduced the shoot DW in all the studied genotypes as compared to control. The interaction effect showed that comparatively lower reduction was observed in tolerant genotypes (36% and 21% in SRC-517 and BARI morich-2, respectively), whereas the susceptible genotypes AHM-106 and RI-1(6) showed 45% and 59% reduction, respectively (Fig. 4b). However, as compared to waterlogging stress only tolerant genotypes SRC-517 and BARI morich-2 recovered SDW by 4% and 5%, respectively. Notably, instead of recovery, further reduction in Shoot DW was noticed in susceptible genotypes (15% and 14% in AHM-106 and RI-1(6), respectively) (Fig. 4b).

Root-shoot ratio

Significant variation was found in root shoot ratio (RSR) among genotype, treatment and interaction by waterlogging (Fig. 4c). Interaction effect showed that significant reduction in RSR by

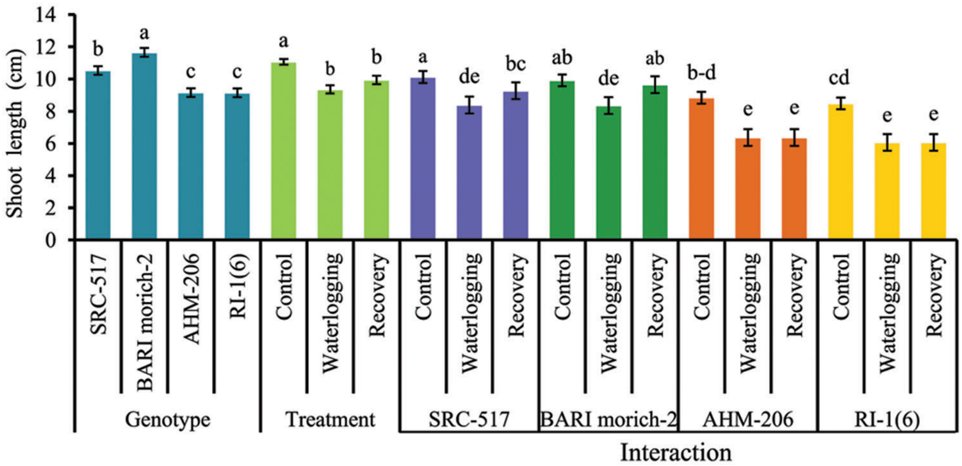

FIGURE 3. Effect of waterlogging stress on shoot length in chilli seedling. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

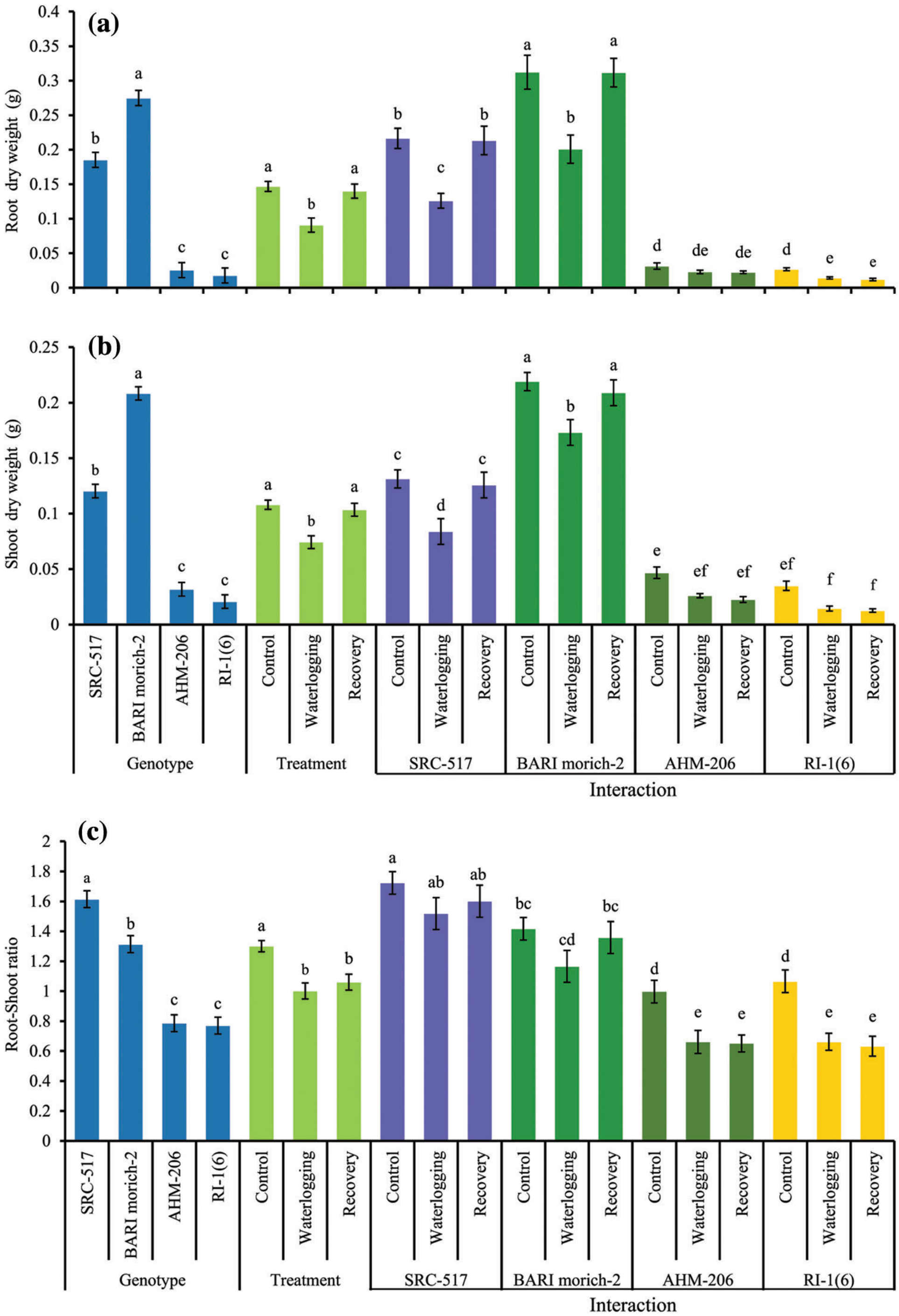

FIGURE 4. Effect of waterlogging stress on root dry weight (a), shoot dry weight (b) and root-shoot ratio (c) in chilli seedling. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

18% and 17% relative to control in tolerant genotypes SRC-517 and BARI morich-2, respectively while, 34% and 38% reduction was observed in susceptible genotypes RI-1(6) and AHM-106, respectively. Interestingly, tolerant genotypes showed 14% and 5% increment in recovery. However, no recovery was noticed in susceptible genotypes (Fig. 4c).

Leaf area

Although waterlogging stress decreased LA by 28% and 30% in tolerant genotypes SRC-517 and BARI morich-2, respectively, the values were significantly higher in tolerant genotypes than the susceptible genotypes (Fig. 5a). The reduced LA in tolerant genotypes by waterlogging was further increased significantly in the recovery period. However, in susceptible genotypes, the increment was not remarkable (Fig. 5a).

Petiole weight

Loss of PW of susceptible chilli genotypes was higher as compared to tolerant genotypes in waterlogging stress (Fig. 5b). As compared to control waterlogging stress decreased petiole weight by 21% and 25% in tolerant genotypes SRC-517 and BARI morich-2, respectively. In contrast, 36% and 32% reduction were found in AHM-206 and RI-1(6), respectively. In recovery, PW increased in only tolerant genotypes SRC-517 and BARI morich-2 by 17% and 24%, respectively (Fig. 5b).

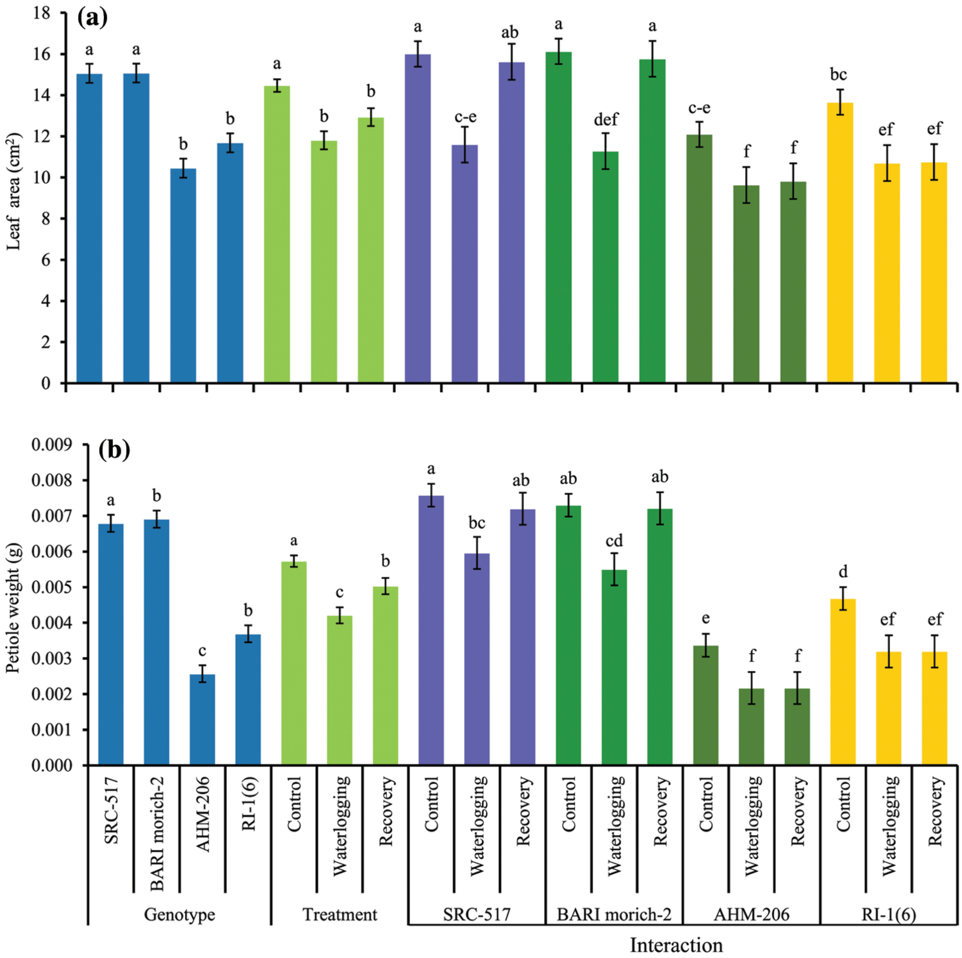

FIGURE 5. Effect of waterlogging stress on Proline (a), TSS (b) and TAC (c) in leaf of chili seedlings. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

Evaluation of physiological and biochemical parameters

Relative water content in leaf

The relative water content (RWC) of leaf signifies the water content of the plant. The data on RWC in leaf in waterlogging stress showed that genotypic, treatment and interaction effect had significantly different in RWC (Fig. 6). Notably, loss of RWC by waterlogging was higher in susceptible genotypes where, 12% and 4% loss were found in tolerant genotypes SRC-517 and BARI morich-2, respectively while, 16% and 29% were exhibited in susceptible genotypes AHM-206 and RI-1(6), respectively. At the same time, RWC improved in tolerant genotypes during recovery (Fig. 6).

Photosynthetic pigment contents

Although the loss of Chl *a* was noticed by waterlogging in both tolerant and susceptible genotypes as compared to respective control, the degradation was substantially higher in susceptible genotypes (Fig. 7a). The interaction effect showed that 62% and 63% reduction of Chl *a* over control was observed in tolerant genotypes SRC-517 and BARI morich-2, respectively whereas, the susceptible genotypes AHM-106 and RI-1(6) showed 64% and 63% reduction, respectively. However, when recover of Chl *a* was calculated over waterlogging stress, the values were higher in tolerant genotypes SRC-517 and BARI morich-2 (36% and 21%, respectively) as compared to those in susceptible genotypes AHM-206 and RI-1(6) (15% and 19%, respectively) (Fig. 7a).

Like Chl *a*, higher loss in Chl *b* was found in susceptible genotypes (Fig. 7b). Waterlogging caused loss in Chl *b* by 45% and 50% relative to control in tolerant genotypes SRC-517 and BARI morich-2, respectively while, 63% and 68% in susceptible genotypes RI-1(6) and AHM-106, respectively. Importantly, better maintenance in Chl *b* was also found in tolerant genotypes (Fig. 7b). As compared to waterlogging stress tolerant genotypes SRC-517 and BARI morich-2 showed higher (22% and 18%, respectively) recovery of Chl *b* than susceptible genotypes (14% and 13% in AHM-206 and RI-1(6), respectively).

Total Chl (*a*+*b*) content of tolerant and susceptible chilli genotypes was significantly affected by waterlogging stress, but the degradation was higher in susceptible genotypes (Fig. 7c). Total Chl (*a*+*b*) content was decreased by 55% and 57% relative to control in tolerant genotypes SRC-517 and BARI morich-2, respectively while, the values were 64% and 69% in susceptible genotypes AHM-106 and RI-1(6), respectively (Fig. 7c). At the same time, higher recovery in total Chl was also found in the tolerant genotypes (Fig. 7c). In recovery, total Chl content increased by 27% and 21% over waterlogging stress in SRC-517 and BARI morich-2, respectively while, 14% and 17% in AHM-106 and RI-1(6), respectively (Fig. 7c).

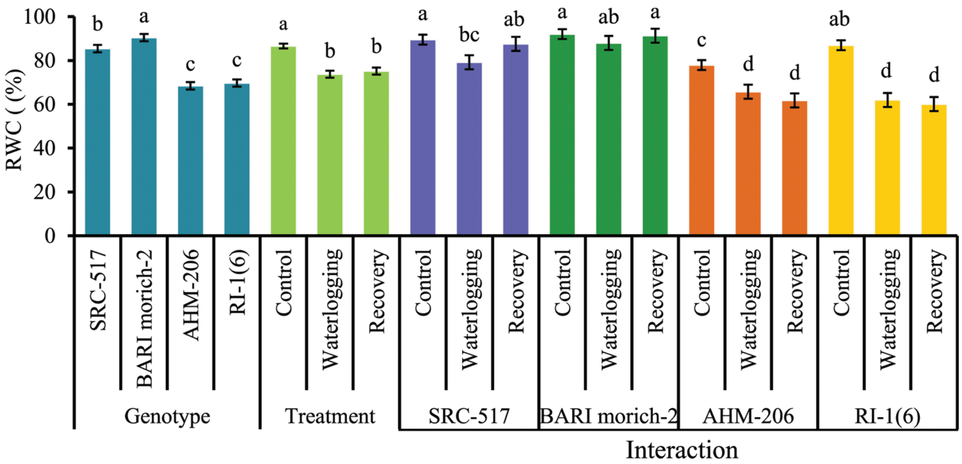

FIGURE 6. Effect of waterlogging stress on leaf relative water content (RWC) at seedling stage. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

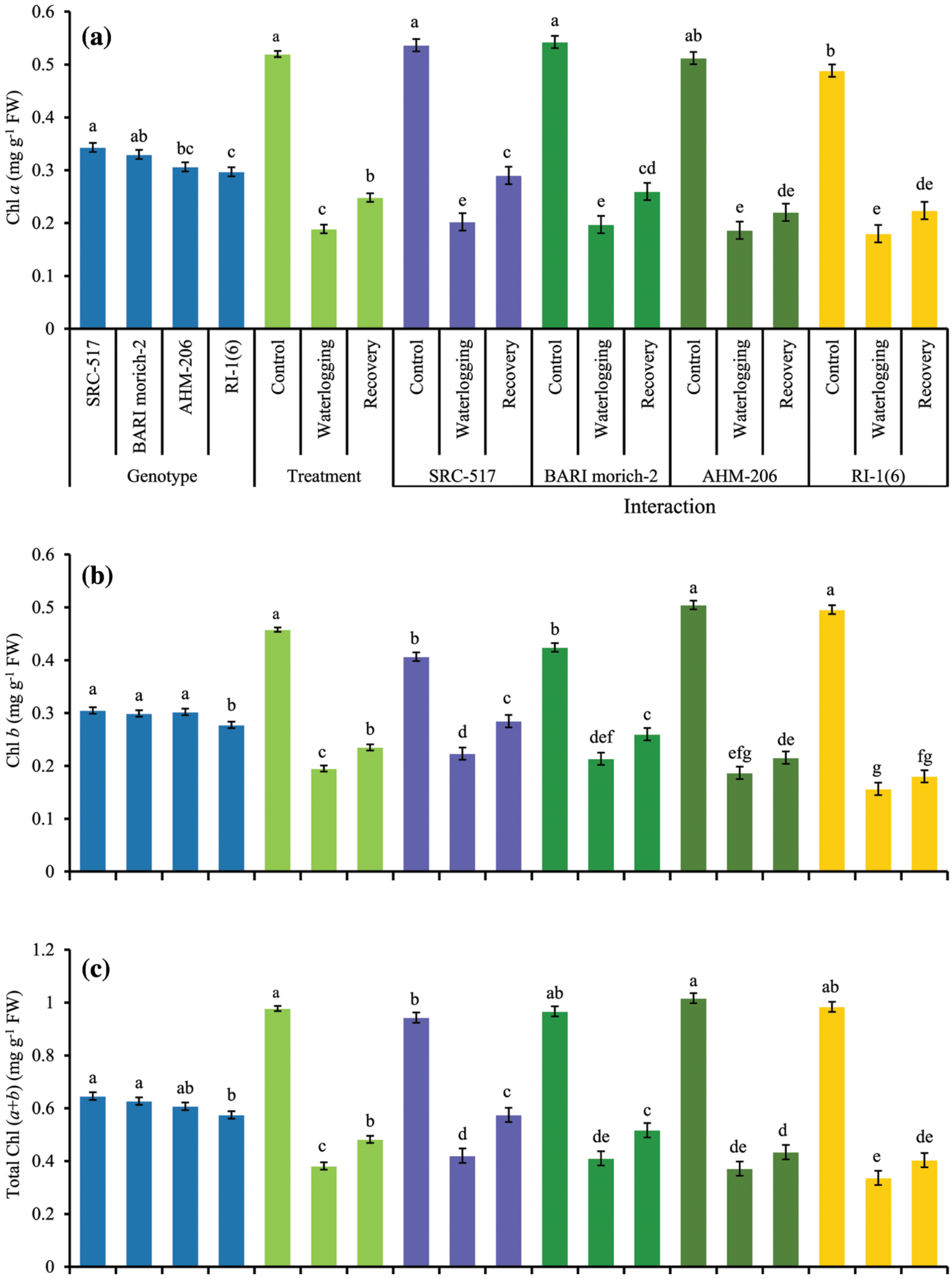

FIGURE 7. (Continued)

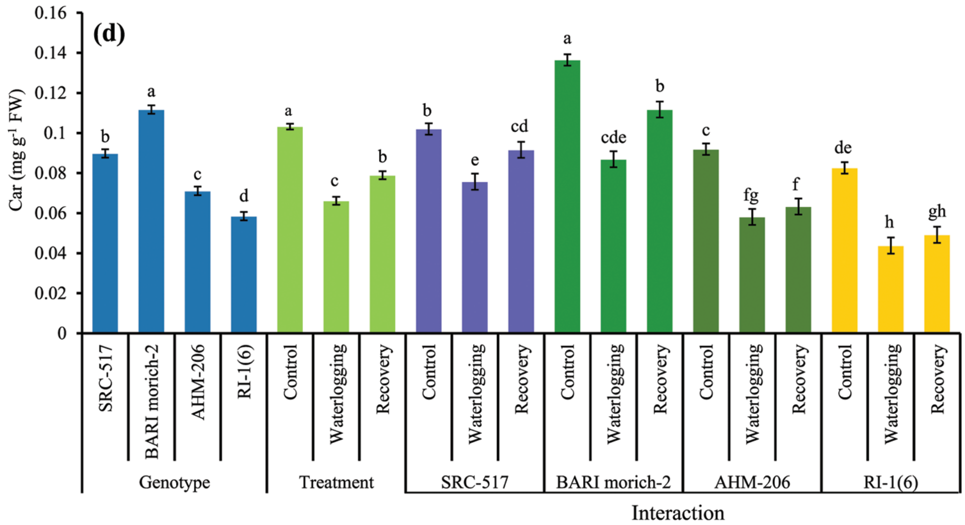

FIGURE 7. Effect of waterlogging stress on chlorophyll *a* (a), chlorophyll *b* (b), total Chl (*a*+*b*) (c) and carotenoids (d) at seedling stage. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

Results depicted that the Car content was significantly affected by waterlogging stress, and the content was significantly higher in tolerant genotypes than susceptible genotypes. Relative to control, waterlogging reduced the Car content by 26% and 36% in tolerant genotypes SRC-517 and BARI morich-2, respectively while, 37% and 46% reduction in susceptible genotypes RI-1(6) and AHM-106, respectively. In recovery, Car content increased by 17% and 22% over waterlogging stress in respective tolerant genotypes SRC-517 and BARI morich-2, respectively while, 8% and 11% in susceptible genotypes AHM-106 and RI-1(6), respectively (Fig. 7d).

Proline content

Significant variation was found in proline (Pro) content in genotypes, treatments and interactions in waterlogging condition (Fig. 8a). Interaction effects showed that both tolerant and susceptible genotypes accumulated more Pro as compared to control. However, the accumulation in tolerant genotypes was significantly higher. In retrieval, both tolerant and sensitive genotypes decreased the Pro content (Fig. 8a).

Total soluble sugar content

Significant variation was observed in genotypes, treatments and interaction for TSS in leaf (Fig. 8b). Interaction results show that stress decreased the TSS content in both tolerant and sensitive genotype, and the content was significantly higher in tolerant genotypes. In recovery, TSS was higher in tolerant genotypes only (Fig. 8b).

Estimation of total antioxidant capacity by DPPH radical scavenging

Although the loss of TAC (DPPH) was noticed by waterlogging in both tolerant and susceptible genotypes, the TAC was higher in tolerant genotypes in control, stress conditions and recovery (Fig. 8c). Waterlogging mediated reduced TAC was further increased significantly during recovery period in tolerant genotypes only (Fig. 8c).

Evaluation of anatomical features

Aerenchyma formation

Plant roots alter internal anatomical structures in order to adapt to flooding conditions. Under anoxic or hypoxic soil conditions, enzymes such as cellulase destroy the root cell of the plant's walls to produce aerenchymatic cells. In this study, in the control plant, there was no aerenchyma formation (Figs. 9a and 9c), but under waterlogging condition, lateral roots developed in tolerant genotypes (SRC-517 and BARI morich-2, data not shown) and formed a higher number of aerenchymatic cells (Fig. 9b, shown only for tolerant genotype SRC-517) than those in susceptible genotypes (Fig. 9d, shown only for susceptible genotype AHM-206).

Discussion

In the present investigation, among the 10 genotypes of chilli, acute decreasing of survival and increasing of dead percentage was observed in AHM-206 and RI-1(6) under waterlogging and recovery (Figs. 1a and 1b). The genotypes SRC-517 and BARI morich-2, on the other hand, showed the opposite trend in survivable ability. Kumutha *et al.* (2008a) reported in green gram that MH96-1, a tolerant genotype, did not show mortality whereas, a susceptible genotype MH 1K-24 had more than 60% mortality after eight-day recovery.

Meena *et al.* (2017) used a similar technique for the screening of waterlogging tolerant and susceptible genotypes of pigeon pea and reported that survival percentage was high in relatively tolerant genotypes ICPB 2039 and KPBR 80-2-1, and minimum in susceptible genotype ICPL 20128 followed by RG 188. Thus, genotypes SRC-517 and BARI morich-2 with minimum death and maximum recovery percentage can be selected as tolerant genotypes under waterlogging stress at seedling stages. On the other hand, genotypes AHM-206 and RI-1(6) confirmed their susceptibility to waterlogging stress.

Plants show different kinds of morphological, anatomical, biochemical and physiological adaptation in response to excess

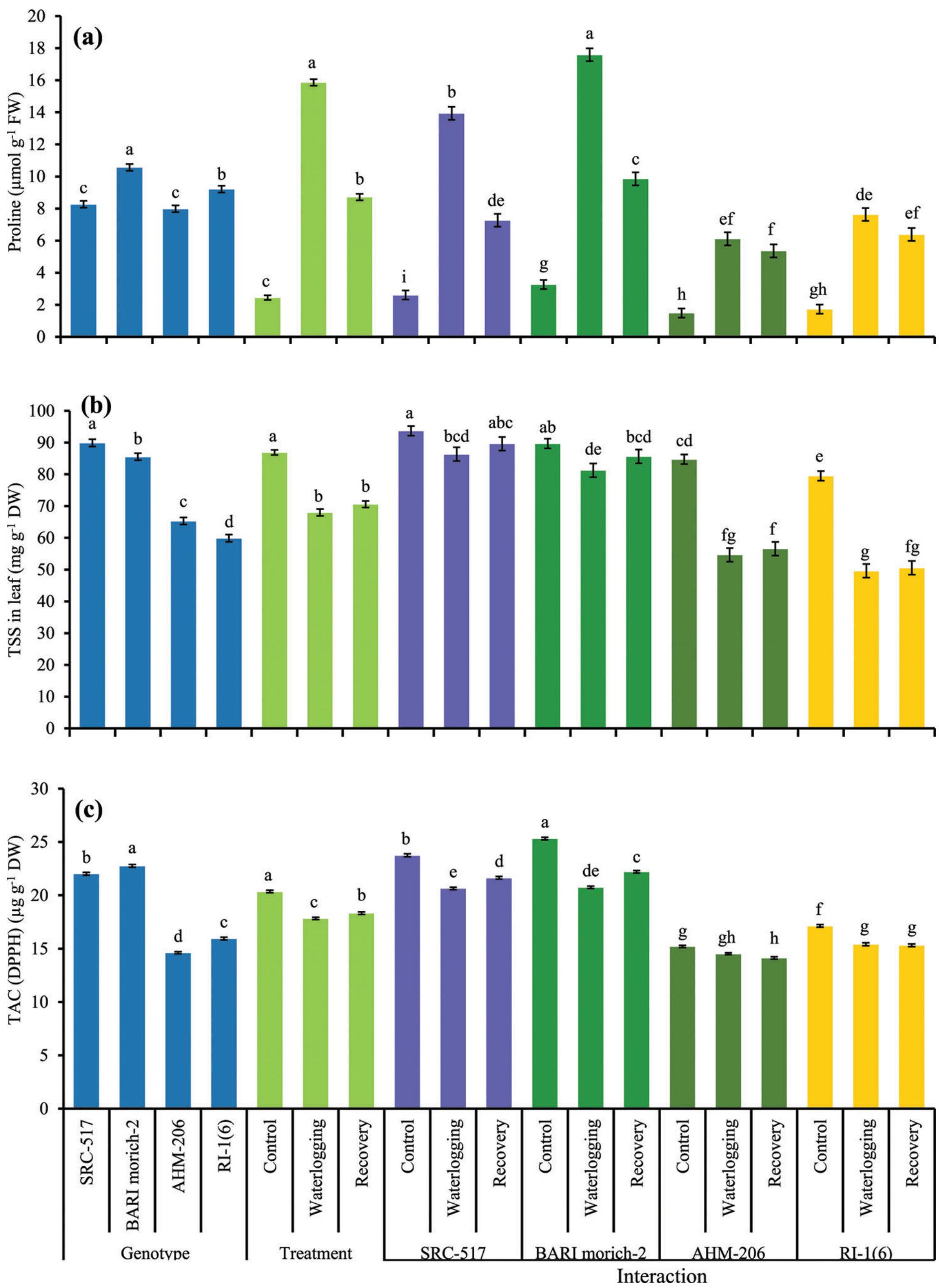

FIGURE 8. Effect of waterlogging stress on proline (a), TSS and TAC in leaf of chili seedlings. The first four bars represent genotypic effect, next three bars treatment effect and remaining bars interaction effect of genotype and treatment. The mean values are ± SE of three replicates and various letters on bars are significantly different at $P \leq 0.05$.

water stress (Adhikari and Paje, 1993; Meena *et al.*, 2017). These adaptations manifested by tolerant/resistant plants could further serve as selection indices for waterlogging condition during screening or selection of genotypes. Hence, this experiment was conducted to assess the effect of excess water on morphological and physiological parameters of tolerant and susceptible chilli genotypes which was involved in their survival and death mechanism.

In the findings presented here, waterlogging stress has, in general, modified the growth of the plants. In excess of water, the growth of the root system seemed to be a very visible criterion for waterlogging (Figs. 2a and 2b). As the root moisturizes the lower soil layers and suffers more than any others (Morgan *et al.*, 1986; Dhanda *et al.*, 1995; Baloch *et al.*, 2012). Root length and volume are therefore the most important features to combat water stress in varieties of plants and genotypes with longer and longer roots (Leishman and Westoby, 1994; Cardoso *et al.*, 2014; Saha *et al.*, 2016). In this investigation, excess water treated plants had lower length and DW of root than did the controls. Additionally, waterlogging susceptible genotypes AHM-206 and RI-1(6) showed higher reduction in length and dry weight of root than the tolerant genotypes during water stress and recovery periods (Figs. 2a and 4a). Waterlogging stress reduced the length and dry weight of root in all genotypes, while in recovery, tolerant genotypes recovered the root length and DW but it remained similar or further reduced in susceptible genotypes (Figs. 2a and 4a). Similar results were noticed in shoot length and dry weight (Figs. 3 and 4b). Transient waterlogging was tolerated by tolerant genotypes with strong root and shoot growth more effectively (Hartley *et al.*, 1993). The reduction of the root-shoot length could lead to cell splitting and elongation impediment (Raziuddin *et al.*, 2010; Khakwani *et al.*, 2011),

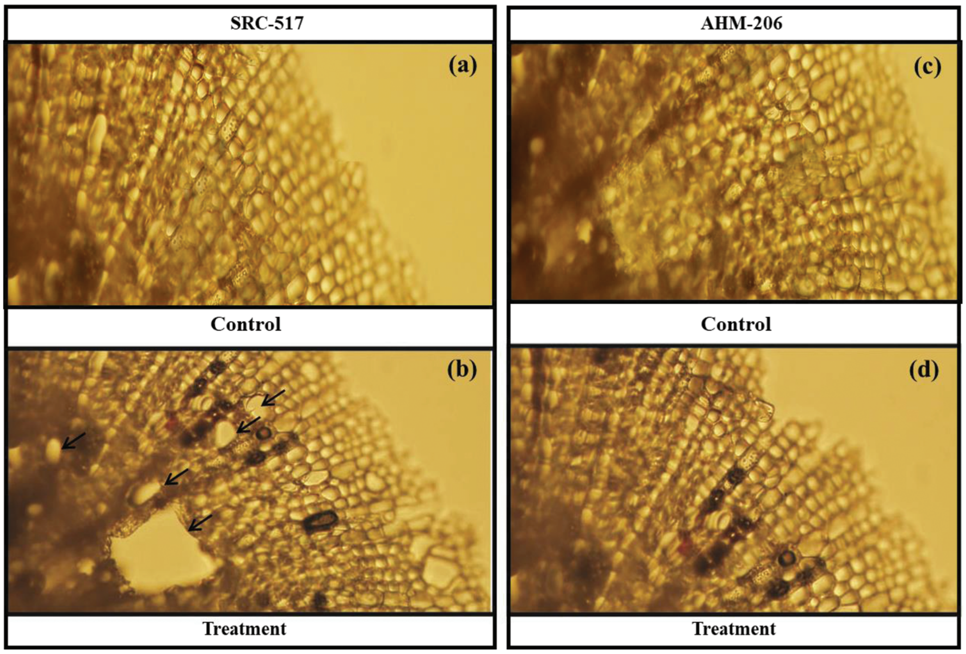

FIGURE 9. Effect of waterlogging stress on aerenchyma formation in tolerant genotype (SRC-517) (a,b) and susceptible genotype (AHM-206) (c,d).

a cell division stoppage and cell elongation ultimately result in a root shoot reduction and a DW reduction (Molla *et al.*, 2019). This is consistent with earlier studies conducted by Kim *et al.* (2015) who examined between the resistant and susceptible waterlogging soybean lines and concluded that DW of roots and shoots reduction were higher in susceptible than the tolerant genotypes. A higher reduction in root-shoot ratio of susceptible genotypes under waterlogging (Fig. 4c) indicates that the effect of flooding was more on root than shoot growth and thus, affects the availability of ions to root zone (Crane and Davies, 1988). In another study, Adhikari and Paje (1993) reported that effects of excessive moisture on the roots include slower root elongation, suppressed root hair formation and rotting and drying of roots and eventually death of the plant. Besides, tolerant genotypes were able to revive again by improving the root:shoot ratio after the recovery period (Fig. 4c). The findings indicate that tolerant genotype with a higher root proportion could be more successful in competing with soil nutrients while those with a higher shooting rate could gather more light energy. The results of this study are consistent with Gotame (2006) and Saha *et al.* (2016). However, several studies have found the growth parameters of seedlings, such as root and shoot length ratio and DW, used as useful features in selecting resistant genotypes for water stress such as tomatoes (Gotame, 2006) and sesame (Saha *et al.*, 2016).

In this experiment, the detrimental effects of waterlogging stress on LA and PW were determined in all tested genotypes where the effects of stress were higher in sensitive genotypes than the tolerant, and the sensitive genotypes could not retain increasing LA and PW even after the recovery period (Figs. 5a and 5b). Saha *et al.* (2016) observed that LA reduction was higher in sesame when susceptible plants were subjected to waterlogging and removal of excess water. This reduction may be due to restricted nitrogen supply and increased ethylene production is attributed to cause leaf growth reduction (Adhikari and Paje, 1993).

The relatively higher leaf RWC percentage was present in a saturated leaf (Tanentzap *et al.*, 2015). Leaf RWC is considered as the best criterion for plant water conditions from the mid-1980s onwards. As RWC covers the cell volume, the balance between the water consumed by plant and the water lost during transpiration can be accurately indicated (Hassanzadeh *et al.*, 2009; Lugojan and Ciulca, 2011) hence, it is considered as an important marker for selecting tolerant plants to drought and waterlogging (Kumutha *et al.*, 2008b; Sairam *et al.*, 2009; Prasanna and Ramarao, 2014). In the present investigation, a decrease in RWC was observed with waterlogging treatment, and the susceptible genotypes had more detrimental effects and still decreased until the end of the recovery period. However, there was increasing on RWC in tolerant plants over control at the end of recovery period (Fig. 6). Similar results in RWC due to water stress were reported earlier (Kumutha *et al.*, 2008b) experimented with four genotypes (two tolerant and two susceptible) of pigeon pea (*Cajanus cajan*) and confirmed that even after six days of flood tolerant generosity higher RWCs had been maintained while the sensitive genotypes were experiencing a sharp decline in RWC beyond the recovery stage. The lower loss of leaf water in tolerant genotypes might be attributed to better stomatal regulation (Jones, 1998). The difference in RWC between tolerant and sensitive genotypes also indicated that the waterlogging periods used in this study was effective at simulating water deficiency and abundance in plants of *C. annuum*. Lobato *et al.* (2009) investigated *C. annuum* plants under water deficiency, and Sairam *et al.* (2009) and Kumutha *et al.* (2008b) studied *C. cajan* plants under abundance of water and reported similar results.

Photosynthetic pigments continue to be important drivers of photosynthetic abilities of a plant, since they play a key role in absorption (Chl) and dissipation (Car) light energy (Kamanga *et al.*, 2018). The loss of photosynthetic pigment levels in abiotic stress including waterlogging stress, reduces the photosynthesis capacity (Montagu and Woo, 1999) where numerous studies have shown that the higher destruction of photosynthetic pigments is related with ROS higher production, mainly H_2O_2, in plants under water stress (Montagu and Woo, 1999; Anjum *et al.*, 2011; Saraswathi and Paliwal, 2011; Chakraborty and Pradhan, 2012; Rohman *et al.*, 2016a), because it provokes the inactivation/oxidation of the pigments pre-existing in chloroplasts (Almási *et al.*, 2000). In this study, waterlogging stress caused higher reduction in the contents of Chl and Car contents in susceptible AHM-206 and RI-1(6) genotypes compared to tolerant SRC-517 and BARI morich-2 and (Figs. 7a–7d) might be due to higher production of H_2O_2 as we found higher concentration H_2O_2 in leaves of susceptible genotypes. During waterlogging, relatively higher level of Chl and Car in tolerant genotypes indicated that they are capable to continue their phosynthetic role even under prolonged waterlogging stress (Kumar *et al.*, 2013). Moreover, higher Chl and Car is also a genetical tolerant mechanism in tolerant genotypes that avoids degradation of Chl in tolerant genotypes, than those susceptible to excess water stress (Kumutha *et al.*, 2008b). Similar results of Chl contents were observed in previous studies of waterlogging genotypes of green gram and pigeon pea (Kumutha *et al.*, 2008a; Kumutha *et al.*, 2009). In recovery, removal of water stress led to increase concentration of Chl and Car contents in tolerant genotypes (Figs. 7a–7d). It might be due to restoration of photosynthetic machinery for normal aerobic environment (Kumutha *et al.*, 2009). Kumar *et al.* (2013) also reported faster recovery in tolerant genotypes of mung bean after waterlogging termination probably due to lesser damage to photosynthetic machinery by waterlogging treatment. Moreover, lower reactive oxygen species (ROS) like hydrogperoxide (H_2O_2) in tolerant genotypes might have comparatively lower adverse effect on stomatal regulation along with balance of relative water content (Menconi *et al.*, 1995; Alam *et al.*, 2014; Rohman *et al.*, 2016b; Yuan *et al.*, 2016).

Proline, a vital amino acid, is known to participate during growth and development in biosynthesis of primary metabolism (Hare *et al.*, 1999; Funck *et al.*, 2012). In response to the imposition of a broad range of stress responses in plants, Pro often accumulates. It was well known as an osmotic regulator for osmotic damage reduction (Slama *et al.*, 2008; Reddy *et al.*, 2015). The aggregation of compatible solutes, such as amino acid and Pro, is one mechanism for osmotic adaptation. The results of this study showed Pro highly accumulate in waterlogging stress tolerant chilli leaves and that the elimination of stress in waterlogging led to an increased accumulation of Pro in sensitive genotype (Fig. 8a). These findings were accepted in other earlier studies that Pro is highly accumulated in the waterlogging stress situation of the tolerant plant. Waterlogging caused a maximum increase of Pro content in tolerant genotypes of green gram (Prasanna and Ramarao, 2014) and pigeon pea (Singh *et al.*, 2017) compared to susceptible genotypes. At the end of recovery period, the tolerant genotype Pro content quickly declined to a level comparable to those under control condition (Fig. 9). Several studies showed that when the water deficit or abundance is removed Pro accumulated in episodes of water stress is quickly lost (Ranganayakulu *et al.*, 2015; Dien *et al.*, 2019). Pro, also known as Pro oxidase in the Pro degradation pathway is oxidized into 1-pyrroline-5-carboxylate (P5C) until water stress is taken away. The enzyme P5C dehydrogenase is then transformed back into glutamate (Hare *et al.*, 2002).

Induced stress from water suppression induces a range of metabolic responses in the anaerobic environment. It helps plants to survive a waterlogging environment, by fermenting sugars available for correct metabolic function, including rice (Mustroph and Albrecht, 2003), green gram (Kumutha *et al.*, 2008a) and pigeon pea (Singh and Srivastava, 2021). Carbohydrate starvation has been shown as one potential cause of injuries caused by hypoxia/anoxia (Schluter and Crawford, 2001). In the present investigation, waterlogging tolerant genotypes SRC-517 and BARI morich-2 showed higher TSS content in leaf among all interaction effects (Fig. 8b). The recovery studied seven days after removal of waterlogged condition, plants showed that the TSS in leaf of tolerant genotypes has been increased (Fig. 8b). Genotypes which can preserve their carbon content are more likely to survive than those which are deficient on this count. Osmolytes and compatible solutes under osmotic stress are overproduced in order to make osmotic adjustments possible (Kameli and Lösel, 1995; Shao *et al.*, 2005). In the present study, significantly greater accumulation of major compound like soluble sugars can provide vital osmoprotection in tolerant chilli genotypes under waterlogging stress, which is in the agreement with the results found by Khan and Naqvi (2012) and Kumari *et al.* (2014).

Antioxidant constituents of plant origin are very important substances that have the ability to defend the body from injuries caused by free radical induced oxidative stress (Ahmad *et al.*, 2010). Plant-based antioxidant components are essential substances which can protect the body from injuries caused by free oxidative stress induced by radical activity (Ahmad *et al.*, 2010). SRC-517 and BARI morich-2 are possible antioxidant activity candidates as they are higher than the sensitive genotypes RI-1(6) and AHM-106 compared to their scavenging percentage. In addition, SRC-517 and BARI morich-2 showed clear tolerance, with the elimination of waterlogging stress, their scavenging percentage increased further (Fig. 8c). The high degree of operation of the DPPH has been linked to tolerance of various stress conditions (Kang and Salveit, 2002).

Several plant species have evolved flood stress-control mechanisms that enable them to even grow and reproduce under water or wet soil. The first technique, to prevent oxygen failure in the flooded areas of the plant, involves mainly anatomic and morphological changes that enhance the exchange of gas with the environment (Voesenek and Bailey-Serres, 2015; Yamauchi *et al.*, 2018). Flooding mainly limits the diffusion of gas between the facility and its environment because of physical properties (Armstrong, 1980; Yamauchi *et al.*, 2018). The stomata and cell walls under water are not a simple means of exchanging both O_2

and CO_2. This is caused by a lack of O_2 in flooded areas of the plant and primarily reduces mitochondrial heterotrophic power generation. In addition, poor availability of carbon-dioxide in flooding leaves limits photosynthesis. Therefore, flooding in plant cells triggers an energy crisis. Aerenchyma cells can be used as a tolerance mechanism for long-term survival under flood conditions (Visser *et al.*, 1996; Shimamura *et al.*, 2014). Aerenchyma is a special tissue consisting of channels filled with continuous gas or much enlarged gas spaces. Enhanced formation of aerenchyma is one of plants' most common responses to soil hypoxia and anoxia (Vartapetian and Jackson, 1997; Jackson and Armstrong, 1999; Colmer, 2003). The main reason in the present study is the growth of the intercellular gas space, the so-called development of aerenchyma (Fig. 9a). In the present investigation, SRC-517 and BARI morich-2 which was tolerant to flooding environment and this may be an increase in inter-cellular gas space, the so-called formation of aerenchymas (Fig. 9a), is the cause of the most significant modifications to improve gas transport and distribution in submerged tissue of the plant. The aerenchyma formation was less in sensitive genotypes AHM-206 and RI-01(6) as compared to tolerant genotypes (Fig. 9b). Similar observations were found by Kim *et al.* (2015), where aerenchymatic cells were well formed in the root of tolerant line and significantly reduced abscisic acid (ABA) content compared with susceptible line, which may explain the better aerenchymatic cells development in the tolerant than susceptible line after flood care. To survive flooding conditions, plants need morphological changes in their roots, and in response, the aerenchyma cells are formed. For aerenchyma cells development, a root cell must be unsuberized, and therefore, the biosynthesis of suberin must be suppressed by the down regulation of ABA (Shimamura *et al.*, 2014). This down regulation of ABA after flooding treatment might suggest some explanation for better development of aerenchyma cells in tolerant genotypes than in susceptible genotypes. Lizaso *et al.* (2001) observed formation of more than 30% aerenchyma production in maize cv. Venezuelan which was tolerant to flooding environment. Intensive production of aerenchyma was also observed due to waterlogging in maize cultivars such as cv. Seneca Horizon (Enstone and Peterson, 2005) and cv. single cross 704 (Pourabdal *et al.*, 2008). Benz *et al.* (2007) noticed the occurrence of aerenchyma in the waterlogging tolerant variety of *Piriqueta caroliniana* while the waterlogging susceptible variety did not show any aerenchyma tissue. Huang *et al.* (1994) also reported that in waterlogging conditions, tolerant wheat cultivars had significantly higher root porosity than sensitive cultivars. Hossain and Uddin (2011) noticed the formation of aerenchyma in root tissue of tolerant variety of wheat in response to anoxia.

Conclusions

The role of the morphological, physiological, biochemical and anatomical parameters of resistant genotype or tolerant in their adaptation to waterlogging stress conditions have been discussed in the present investigation. Our findings reveal that, genotypes having vigorous root and shoot growth, performed better to stand brief waterlogging, maintaining turgor and osmotic adjustment of tolerant genotypes in the seedling stage. Moreover, higher amount of photosynthetic pigments in expanded leaf of tolerant genotypes might be maintained proper photosynthetic activity under excess water stress. Survival rate of seedlings were higher which showed higher accumulation of osmolytes (Pro, soluble sugar) under waterlogging stress than the osmolytes lacking ones. Formation of aerenchyma increased of intercellular gas spaces which might optimistically contributed to flood tolerance.

Acknowledgement: The authors thank Dr. Rumman Ara and Mahmuda Binte Monsur for supply seeds and technical support while conducting the experiments and assessments.

Ethics Approval: Not applicable.

Author Contribution: The authors confirm contribution to the paper as follows: Study conceptualization, M.R.M. and M.M.R.; Data curation, M.R.M. M.R.I. and M.M.R.; Funding acquisition, M.R.M and M.M.R.; Investigation, M.R.M, M. M.R and L.H.; Project administration, L.H.; Supervision, M. M.R and L.H.; Visualization, M.H.; Writing-original draft, M.R.M; Writing-review and editing, M.R.I., M.M.R, M.H. and L.H. All authors have read and approved the manuscript.

Availability of Data and Materials: All data are available in the manuscript.

Funding Statement: This work was supported by the R&D Projects of Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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