SAARC J. Agric., 20(2): 17-30 (2022) *DOI: https://doi.org/10.3329/sja.v20i2. 63448*

COLLECTIVE IMPACTS OF LPGOD PLASMA AND PLASMA ACTIVATED WATER TREATMENT IN RICE (*Oryza sativa L.*)

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ABSTRACT

Combined effects of plasma seed treatment and plasma activated waters (PAW) application on paddy was explored. Low pressure (100 torr) glow oxygen discharge (LPGOD) plasma was used for seed treatment and germination test revealed that 90s seed treatment duration produced highest seed germination of ~96% which is the highest paddy seed germination rate obtained through plasma technology. Plasma activated water (PAW) was prepared with submerged atmospheric pressure air discharge plasma jet. PAW was sprayed 1-5 times to the paddy plants. Field study was divulged that: (a) seed germination percentage was increased by ~10.76% with respect to control; (b) plants growth parameters were enhanced because of combined consequences of plasma seed treatment along with PAW application; (c) defense mechanisms of plants were improved, (d) concentrations of total soluble protein and sugar were increased in the paddy grains, and (d) yield was enhanced by ~16.77%.

Keywords: Plasma agriculture, Plasma activated water, Plant growth, Yield, Enzymatic activities.

INTRODUCTION

From the last decade, application of plasma technology (Junior et al., 2016) is, considered as green technology, fascinating researchers because of its prospective use in the improvement of agricultural yields (Mamunur et al., 2021). Several research groups have applied plasma activated water (PAW) for the improvement of seed germination rate (Kabir et al., 2019, Filatova et al., 2020), plants growth and yields. Reactive oxygen (ROS) and nitrogen species (RNS) play (Zhou et al., 2020) critical roles for enhancement of plant growth and functions as stimulator (Sajib et al., 2020) through generation of different signals. ROS and RNS (RONS) are produced (Rashid et al., 2021) in water because of interactions of plasma species with water.

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Received: 29.05.2021 *Accepted: 17.11.2022*

It is known that, among the RNS, NO_3^- provides one of the most required nutrients (Rashid et al., 2021) to the plants. NO_3^- is also a composition of proteins, amino acids, and chlorophyll along with other cellular components and metabolites (Soriano et al., 2015). Controlled concentrations of H_2O_2 produce signals in activating proteins that plays roles in growth and development of plants (Sajib et al., 2020). Besides, PAW with both H_2O_2, NO_3^- and other species also demonstrated pesticidal effect (Zhao et al., 2020). Previously, Rashid et al. (2021), demonstrated ~92% seed germination of paddy after treated with low pressure (LP) glow air discharge (LPGAD) plasma. This study was designed to explore the effect of plasma seed treatment in combination of foliar spray of PAW on seed germination, plant growth promotion and some biochemical properties of paddy seed at field.

As far as it is known that this work is the first report where the combined, foliar spray of PAW was applied to paddy plants grown from LP glow oxygen discharge (LPGOD) plasma treated seeds, effects on plant growth, yield of rice and major nutrients total soluble protein (TSP) and total soluble sugar (TSS) of yielded paddy grains are studied in field condition.

MATERIALS AND METHODS

Paddy seeds (*Oryza sativa* L,cv. variety BRRI dhan 28), used in this experiment were collected from the Regional Rice Research Institute, Rajshahi.

Paddy seed treatment reactor

A customized seed treatment plasma reactor (Rashid, M. et al., 2021) was used to treat the paddy seed in the present experiment. 100 paddy seeds were placed inside the reactor. Two ends of the reactor were covered with stainless steel circular disk electrodes. These electrodes were connected to the high voltage power supply. The reactor was placed within a bell jar. A rotary pump was used to maintain the inside pressure of the bell jar at 100torr during paddy seed treatment. A mixture of oxygen (99%) and argon (1%) were used as working gas for seed treatment. A sinusoidal bipolar variable power supply (voltage range: 1-10kV, frequency range: 0.5-10kHz) was used for the production of LPGOD plasmas.

PAW preparation

PAW was prepared using a water treatment reactor (Rashid et al., 2021). PAW was prepared with a customized air discharge plasma jet (Rashid et al., 2021). A monopolar pulsed variable power supply (1-10kV, 1-10kHz) was used for operating the plasma jet. Atmospheric compressed air (1lpm) was used as working gas for generating underwater air discharge plasma jet. 250ml distilled water was treated for 10min to prepare PAW in a 500ml glass bottle. The RNS concentrations of the PAW were studied.

Estimation of PAW properties

Distilled water was treated for preparing PAW for 10min and subsequently pH and concentrations of O_3, H_2O_2, NO_2^- and NO_3^- of PAW were measured. pH was measured using a pH meter. Concentrations of O_3, H_2O_2, NO_2^- and NO_3^- of PAW were determined using the methods employed by Rashid et al. (2021). The concentrations of RONS and pH of PAW were $O_3 = 0.43 \pm 0.01 \mathrm{mg.l^{-1}}$, $H_2O_2 = 8.77 \pm 0.06 \mathrm{mg.l^{-1}}$, $NO_2^- = 5.92 \pm 0.05 \mathrm{mg.l^{-1}}$, $NO_3^- = 45 \pm 0.50 \mathrm{mg.l^{-1}}$ and $\mathrm{pH} = 6.43 \pm 0.06$.

Observation of seed surface morphology

After seed treatment with LPGOD plasma, surface morphology of the seeds was studied using scanning electron microscope (SEM). The SEM images of the seed coat are shown in Fig. 1.

Test of water imbibition and germination percentage

100 paddy seeds were treated for the durations of 0 (control), 30, 60, 90, 120 and 150s. Plasma treated seeds were then immersed in tap water for 24h in different petri dishes. Weights of seeds were recorded at 1, 2, 4, 8, 12, 16 and 24h of seed after immersion. Water imbibition (w_i) of wet seeds was estimated employing the following formula (Roy et al., 2018)

$$w_i(\%) = \frac{w_1 - w_0}{w_0} \times 100$$

where w_0 and w_1 are the weights of dry and wet seeds, respectively. The results of water imbibition of seeds are displayed in Fig. 2(a).

Eighteen hundred paddy seeds ($3 \text{ replicates} \times 6 \text{ treatment conditions} \times 100 \text{ seeds} = 1800$) were selected randomly and classified into six groups (for 0 (control), 30, 60, 90, 120, 150s treatment durations) following the method prescribed in ISTA (International Seed Testing Association, Switzerland, 2018) for estimating seed germination percentage. Five groups (30, 60, 90, 120 and 150s) of paddy seeds were treated with LPGOD plasma. The treated seeds were placed in separate petri dishes covered with double-layer moistened filter papers. The seed containing petri dishes were incubated in dark incubator for 3d. Inside temperature and relative humidity of incubator were maintained at 25℃ and 75%, respectively. Required amount of water was supplied daily to filter papers for keeping seeds wet for germination. Seeds with 3.5mm radical length (approximately half of seed length) were considered as germinated seeds. Germinated seeds were counted at 12h interval. Percentage of seed germination (g_r) was calculated using the following formula

$$g_r(\%) = \frac{N_{3d}}{N_t} \times 100$$

where N_{3d} and N_t are the number of seeds germinated within 3 days and the total number of seeds, respectively. Results concerning germination percentage of paddy seeds are displayed in Figure 2(b). On the basis of seed germination result, 90s LPGOD plasma seed treatment condition was selected for field experiment.

Field experiment

90s LPGOD plasma treated and control seeds were submerged in tap water for 24h. Wet paddy seeds were extracted from water and enfolded with wet gunny bags for 72h. 500g.m^{-2} seed was sown in previously prepared nursery bed. After 35d seedlings were transplanted in muddled plots. 500ml PAW was foliar sprayed at a time to each subplot (including 3 replications) and foliar spray of PAW were applied one to five times to subplots as per experimental conditions. PAW was sprayed to paddy plants within 20-50 DAT during their vegetative stage. The experiment was conducted according to RCB design.

The field experiment was performed from February to May 2021 in Agricultural Research Project area, University of Rajshahi. Standard amount (as per instruction of Bangladesh Rice Research Institute, BRRI) of urea (90g) urea, triple super phosphate (24g) and murate of potash (18g) were applied to each subplot of area $4m^2$. Urea was applied thrice: 50% was used at the time of muddled field preparation, 25% was used at 20 DAT, and the remaining 25% was used at 50 DAT.

Estimation of plant growth and yield parameters

The lengths of roots and shoots were measured at 70 DAT with a slide caliper and data were recorded. Roots and shoots were collected at 70 DAT and washed in tap water and dried for 72h at 70℃ and their corresponding dry-weights were registered with balance. Chlorophyll and carotene concentrations were estimated using the method described by Rashid et al. (2021). The yield contributing parameters such as length of panicle was measured and number of grains per panicle was counted randomly from collected panicles and then finally yield was estimated.

Estimation of antioxidant enzymes, protein and sugar

The concentrations of antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX), total soluble protein (TSP) and total soluble sugar (TSS) in roots, leaves and paddy grains were determined as described by Rashid et al. (2021).

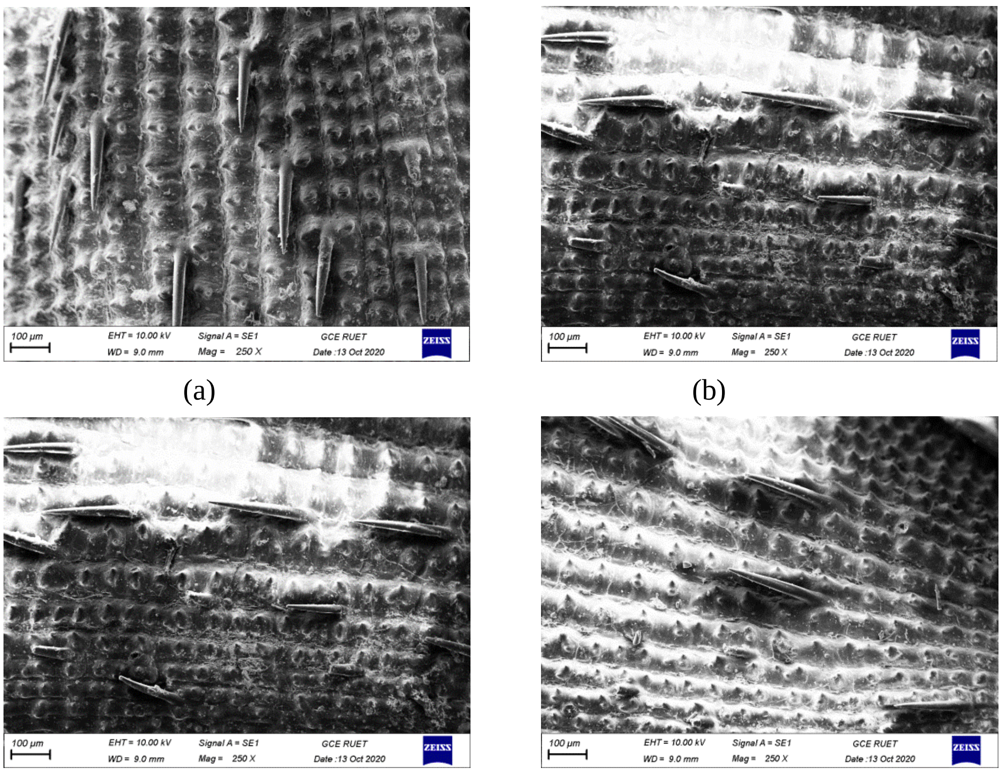
Statistical analyses

Data were analyzed statistically taking the significance at $p \leq 0.05$ using one-way ANOVA using SPSS 16 software under Duncan's Multiple Test Range (DMRT).

RESULTS AND DISCUSSION

Combined effects on water imbibition and germination percentage

LPGOD plasma seed treatment was improved the imbibition and paddy seed germination percentage (Fig. 2(a-b)). It is observed that the water imbibition increased with the increase of plasma treatment time. The maximum water imbibition was obtained for 150s



(d)

Figure 1. Scanning electron microscopic (SEM) images for the inspection of consequences of surface texture of the paddy seeds (a) untreated seed, and seeds treated with low pressure GOD plasma for the durations of: (b) 60s, (c) 90 s, and (d) 150s, respectively.

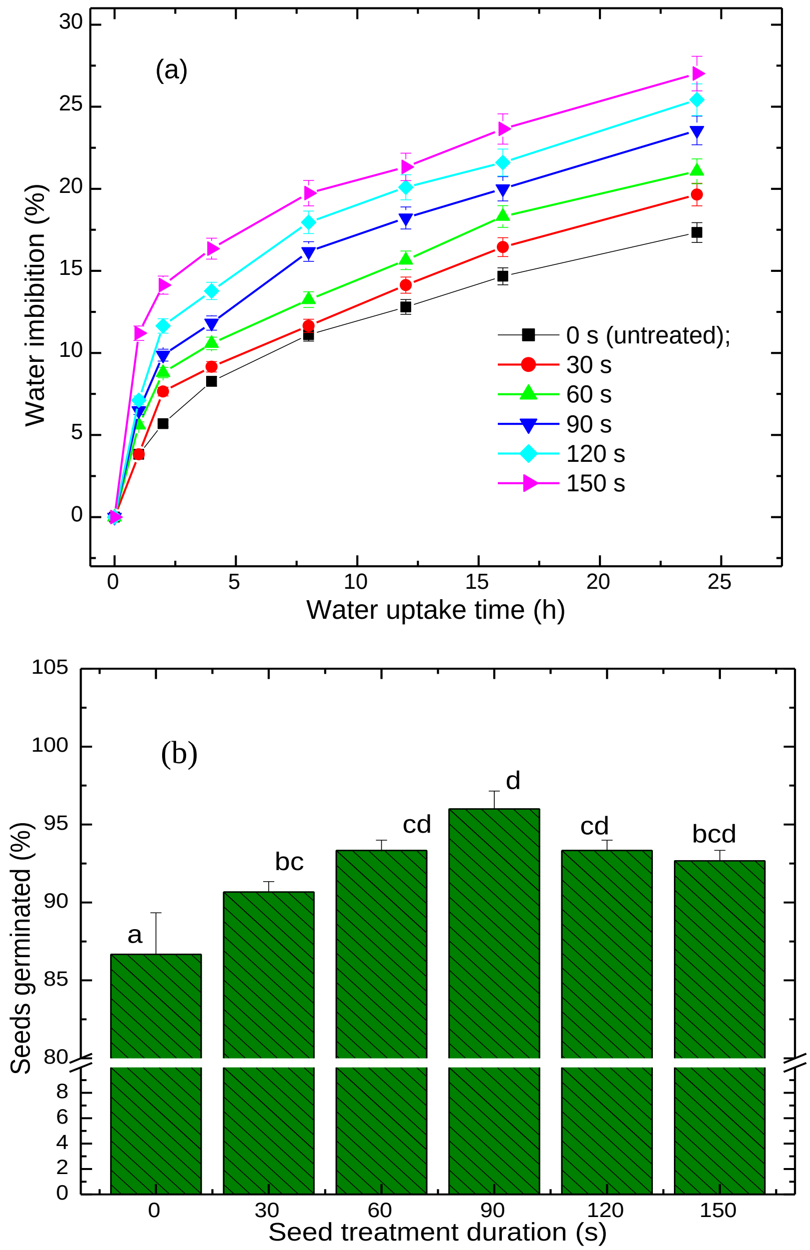


Figure 2. Consequences of low pressure (100 Torr) glow oxygen discharge (GOD) plasma treatment of paddy seeds on (a) water imbibition and (b) seed germination percentage. Errors bars indicate standard errors of three replications. Letters represent statistically significant differences ($p<0.05$).

LPGOD plasma treatment. It can be seen from Fig. 2(a) that 90s treatment period provided the best germination percentage (96%), which was ~10.76% higher than that of untreated seeds. Tanakaran et al. (2022) have investigated jasmine rice (cultivar) seed germination rate treated with glow discharge plasma and found a maximum of 92% germination rate, i.e., 22% increased germination with respect to control for 60s treatment using 30kV DC power supply. But, maximum 96% BRRI 28 (cultivar) paddy seeds were germinated in the present experiment. The SEM images for 0 (untreated), 60, 90 and 150s treated seeds are presented in Fig. 1(a-d), respectively. A large number of trichomes (hair-like structure) were eradicated from seed husk because of plasma etching in compare to control (Fig. 1). Husks that contain lemma and lines of tubercules was partly removed because of plasma treatments most probably due to ROS plasma etching and thereby seed coat becomes smoothed and thinned.

Billah et al. (2020) summarized the mechanism of increased seed water imbibition after plasma treatment as (i) adsorption of RONS onto paddy husk; (ii) diffusion through wider micropyles and finally (iii) trapping the RONS into trichomes, lemma, and irregular shaped paddy husk. Moreover, the thinner seed coat resulted from plasma etching also positively affected the entire process. Therefore, it could be assumed that the higher RONS concentrations plasma treated seeds increased the seed imbibition rate. Previously Hashizume et al., (2020), demonstrated rapid water absorption of seeds with higher RNS concentrations. One may expect that water imbibition as well as higher seed germination percentage can be improved owing to adsorption, diffusion and trapping of RONS in paddy husk.

Combined effects on plant growth

Combined effects of LPGOD plasma seed treatment along with foliar PAW spray improve plant height (PH), stem diameter (SD), dry weight (DW), chlorophyll and total carotene (TC) concentrations (Fig. 3(a-d)), respectively. The highest plant height, stem diameter and dry weight were $\sim$24.33%, $\sim$15.52% and $\sim$9.53%, respectively, produced from the plants where $P + W_5$ treatments were used as compared to that of control plants. Total chlorophyll and carotene concentrations are found $\sim$24.55% and $\sim$52.34% higher, respectively with respect to control, in plants where $P + W_5$ treatments were applied. Interestingly, the growth parameters were improved, with increase of PAW application time. Improved plant growth can be attributed as follows. Plant growth parameters were improved because of LPGOD plasma seeds treatment. Further, as PAW contain higher concentrations of RNS (NO_2^-, NO_3^-) with respect to ROS (H_2O_2, O_3) during their preparations, RNS can transfer from PAW to paddy plants and thereafter relocate into plant tissues. Hence, one may conclude from the above arguments that LPGOD plasma with higher concentrations of RNS can likely be contributed (Hashizume et al., 2020) to increased plant growth. Besides, roots and leaves can intake more amino acids from excess RNS (NO_2^-, NO_3^-) through enzymatic conversion processes. Because NO_3^- actively participate in biochemical and physiological processes through regulating signals (Maniruzzaman et al., 2017) for plant metabolism and developments. It is noted that applications of PAW did not show any negative impacts on plant growth and development. One may infer from the properties of PAW that paddy plants, where PAW were applied five times, are capable of absorbing higher concentrations of RONS containing species.

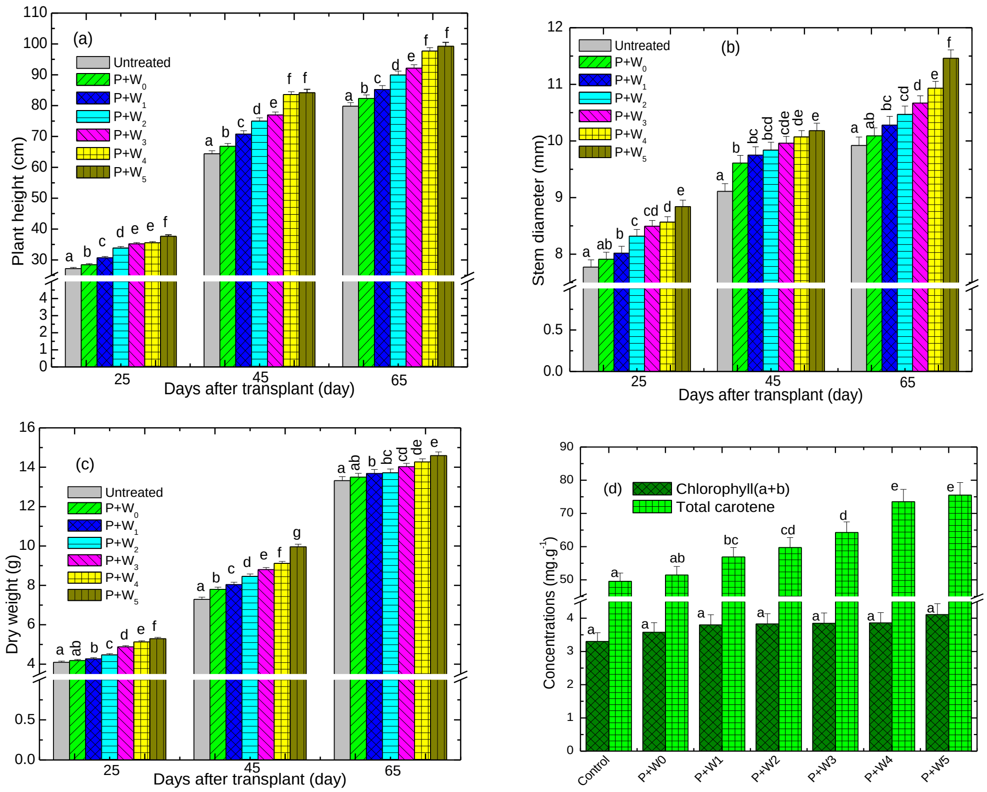


Figure 3. Consequences of low pressure (100 torr) GOD plasma paddy seed treatment and foliar spray of plasma activated water (PAW) on plant (a) height (PH), (b) stem diameter (SD), (c) dry weight (DW) (measured at 25, 45 and 65 days after transplant (DAT)), and (d) chlorophyll and carotene concentrations (measured at 70 DAT). (P and W_x, represent oxygen plasma treated seeds and x (=0, 1, …5) times of PAWs were applied, respectively). Error bars indicate standard errors of three replications. Letters represent statistically significant differences ($p<0.05$).

Combined effects on plant enzymatic activities, protein and sugar

The integrated effects of plasma seed treatment and foliar spray of PAW enhance antioxidant enzymes CAT, APX and SOD, TSP and TSS extracted (at 70 DAT), and grains from the plants grown under different treatment conditions (Fig. 4(a-e)), respectively. The maximum CAT concentration ($8.33 \text{nmol.min}^{-1}(\text{mg protein})^{-1}$)

is found in the $P + W_5$ treated leaves, whereas, it is minimum (2.78 $nmol.min^{-1}(mg\ protein)^{-1}$) in the roots of control plants, respectively. Further, the maximum APX concentrations ($18.67 nmol.min^{-1}(mg\ protein)^{-1}$) is found in roots, whilst the minimum ($13.16\ nmol.min^{-1}(mg\ protein)^{-1}$) is obtained in $P + W_5$ treated leaves (Fig. 4(b)). Furthermore, maximum SOD concentrations of ($37.13 nmol.min^{-1}(mg\ protein)^{-1}$) is determined in the roots of untreated plants, whereas, it is found minimum ($12.38\ nmol.min^{-1}(mg\ protein)^{-1}$) in the leaves of $P + W_5$ treated plants (Fig. 4(c)).

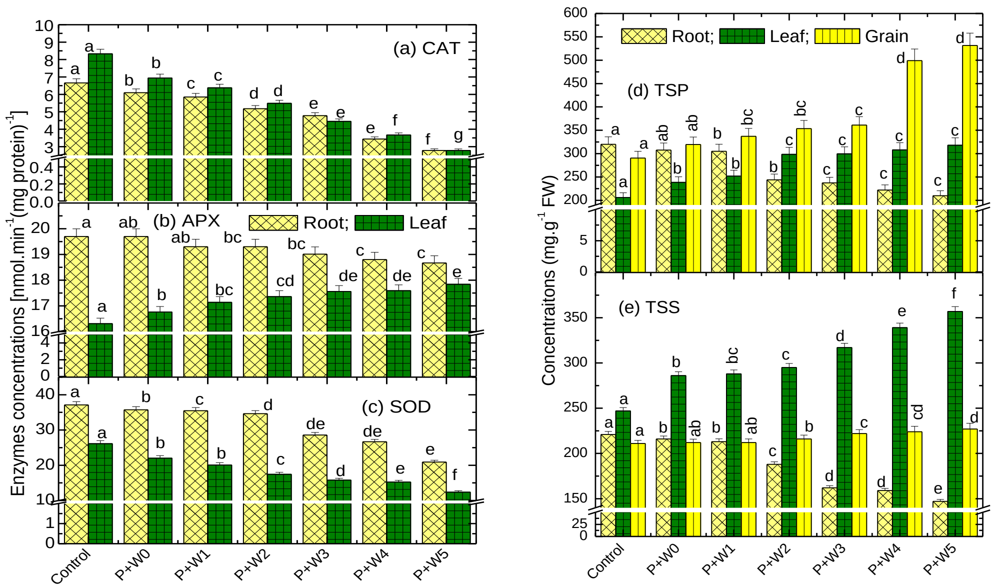


Figure 4. Consequences of LPGOD (100 torr) plasma treatments of paddy seeds and applications of plasma activated water (PAW) as foliar spray on the concentrations of antioxidant enzymes in plant tissues: (a) catalase (CAT), (b) ascorbate peroxidase (APX), (c) superoxide dismutase (SOD) of roots and leaves of the rice plants; and (d) total soluble protein (TSP) and (e) total soluble sugar (TSS) in roots and leaves of rice plants (measured at 70 DAT) and in grains.

Antioxidant enzymes protect plants from environmental stresses (Yodpitaka et al., 2019). Besides, CAT is an imperative enzyme that plays one of the most important roles in initial stage of seed germination and then peroxidase complements seedling growth. The present investigation demonstrated the reduction of CAT activities in roots and leaves with elevated applications of PAW. Kucerova et al. (2018) have also found reduced CAT concentration which is consistent with the result of the present work. Concentration of CAT is found highest in control plants compared to that of PAW treated plants. APX acts as catalyzer to detoxification of ROS through conversion of H_2O_2 into H_2O utilizing ascorbate as electron donor. The concentrations of APX are reduced in roots, while it is enhanced in leaves with increasing number of PAW applications (Fig. 4(b)). Enhanced APX in leaves detoxified elevated concentrations of ROS during vegetative stages (20 to 50 DAT) of paddy plant growth cycle (Kučerová et al., 2018). SOD concentrations are decreased both in roots and leaves of plants with the increase of PAW application time. Besides, higher concentration of SOD are observed in roots compared to leaves. SOD acts as key intermediary enzymes that provides defense against oxidative stress as generated by ROS in plant cells. This finding is in accord with the finding of Kučerová et al. (2018). SOD concentrations are reduced in roots with enhanced applications of PAW; on the contrary it is increased in leaves. It is noted that CAT and SOD are decreasing both in roots and leaves in PAW treated plants due to enhanced concentrations of nitrate and nitrite as compared to control plants. This finding is in agreement with the result obtained by Maniruzzaman et al. (2017). In conclusion, higher concentrations of RNS may provide better defense to plants against both biotic and abiotic stresses.

The combined application of plasma seed treatment and PAW spraying significantly affect the concentrations of TSP and TSS in paddy grain, leaf and root. $\mathrm{P+W_5}$ treatment provided the highest TSP in leaves ($318\mathrm{mg.g^{-1}FW}$) and grains ($531\mathrm{mg.g^{-1}}$) compared to the control. Similarly, the highest TSS also produced in leaves ($357\mathrm{mg.g^{-1}FW}$) and grains ($227\mathrm{mg.g^{-1}}$) in the same treatment applied plot. The untreated control provided the lowest TSP ($206\mathrm{mg.g^{-1}FW}$) and TSS ($247\mathrm{mg.g^{-1}FW}$). Alternatively in root the lowest TSP ($210\mathrm{mg.g^{-1}FW}$) and TSS ($147\mathrm{mg.g^{-1}FW}$) was recorded from P +PAW5 treated plot. Interestingly, the roots of untreated control plot provided the highest amount of TSP ($320\mathrm{mg.g^{-1}FW}$) and TSS ($221\mathrm{mg.g^{-1}FW}$. Therefore, concentrations of TSS are enhanced by $\sim 45\%$ and $\sim 8\%$ in leaves and grains, respectively, for combined consequence of $\mathrm{P+W_5}$.

Both TSP and TSS of leaves and grains are increased when PAW sprayed in the seedling/plant germinated from the plasma treated seeds in field condition. Moreover, the concentrations of both TSP and TSS are increased with increasing number of sprays. It was mentioned earlier that PAW provides a significant amount of RONS such as, NO_2^-, NO_3^- and H_2O_2 directly to the leaves which is one of the significant varieties of nitrogen that plants can absorb. RNS is one of the most indispensable compositions for the creation of protein. Around 50% of total nitrogen present in the leaves and stem tissues are used for the synthesis of amino acids, proteins, metabolites and starch (Perchlik et al. 2018; Li et al. 2018). Later, these synthesized protein and starch could translocate to the grain (Tang et al. 2009). Sucrose synthase is considered as an important enzyme that catalyzes the transformation of sucrose into starch (Kumari et al., 2016). This result is consistent with the finding of Tang et al. (2009). Consequently, one may infer from the above arguments that enhanced PAW application may be responsible for enhancement of sucrose and afterward the paddy grains become enriched with higher concentration of sugar.

TSS concentration is reduced in roots to some extent whilst it is sharply improved in leaves with increasing number of PAW application in contrast to that of control. The level of exogenous sugar concentration (Fig. 4(e)) is improved in paddy plants where $P + W_5$ treatments were applied with respect to that of control. Leaves bear elevated concentration of TSS as compared to that of roots. It is noteworthy that maximum concentration of TSS is accumulated in paddy grains through $P + W_5$ treatments. This result is in agreement with the result of Ozaki et al. (2009). Therefore, depending on this finding concerning enhanced TSS concentration in paddy grains, one may infer that concentration of TSS is increased in paddy grains due to enhanced applications of PAW as it contains high concentration of H_2O_2.

Combined effects on yield

Combined consequences of seed treatment and foliar spray of PAW increase the yield related characters and yield of paddy (Fig. 5(a-b)). The highest panicle length (~24.00cm) and grain/panicle (124) were produced by $P + W_5$ treated plot compared to that of control plot (20.50cm and 90 for panicle length and grain/panicle, respectively) (Fig. 5(a)). The maximum (22.14g) and minimum (18.43g) 1000-grain weights were recorded from $P + W_5$ treated and untreated plants, respectively. Similarly, the maximum and minimum yields (7.22 and $6.17 \mathrm{MT.ha^{-1}}$), were calculated from $P + W_5$ treated and untreated paddy plants, respectively. Since, 1000-grain weight and yield are enhanced by ~20% and~16.77%, respectively, due to combined plasma applications (Fig. 5(b)).

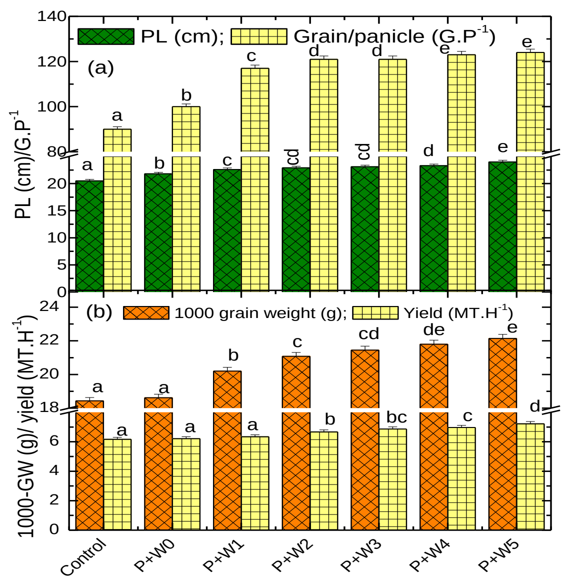


Figure 5. Consequences of LPGOD plasma treatments of paddy seed and applications of plasma activated water (PAW) as foliar spry on (a) length of panicle (PL) and grain per panicle ($G.P^{-1}$), and (b) 1000-grain weight (GW) and yield of paddy.

However, results obtained in this experiment regarding yield can be compared with our previous work (Mamunur et al., 2021). They have studied combined effects: (a) paddy seeds were treated with glow air discharge (LPGAD) plasma and maximum seed germination percentage was ∼92% for the treatment duration of 90s, and (b) PAW were prepared with air discharge plasma jet for treatment duration of 15min and applied to paddy plants. They have found ∼16.67% enhanced yield. But in the present experiment, maximum seed germination percentage was ∼96% for 90s treatment and yield of paddy is increased by ~16.77% where $P + W_5$ treatment was applied with respect to control. Therefore, seed germination rate is 4% higher in LPGOD plasma with respect to LP air plasma. Grain yield is ~0.6% is higher due to combined effects of LPGOD plasma treated seeds along with PAW foliar applications with respect to LP air discharge plasma seed treatment along with PAW application. Further investigations needed to draw conclusive remarks for enhancement of paddy seed germination, growth, enzymatic activities and yield as an alternative of foliar spray of urea.

CONCLUSION

The combined effects of plasma seed treatment and PAW foliar spray enhanced seed germination percentage, enzymatic activities, yield contributing characters and yield of rice. Enhancement of these parameters are likely to be responsible for RONS as produced in LPGOD plasma and PAW. Yield of paddy grains is increased by ~16.77% with respect to control, due to combined applications of plasma seed treatment and PAW foliar spray. This is a resource effective technology that can be applied in Bangladesh. This could increase water use efficiency and reduce urea application. Besides, it could provide high protein content in rice. Finally, field level applications require large scale field investigation along with economic analyses.

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