MITIGATION OF ARSENIC TOXICITY BY FOLIAR APPLICATION OF SALICYLIC ACID IN WHEAT

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Abstract: Pot experiment was conducted to assess the contribution of Salicylic acid (SA) on mitigation of Arsenic (As) toxicity in wheat during November 2015 to March 2016. In this experiment, the treatment consisted of four different As levels *viz.* As_0 = without As (control), As_1 = 30 mg As kg^{-1} soil, As_2 = 60 mg As kg^{-1} soil, AS_3 = 90 mg As kg^{-1} soil and three different levels of SA *viz.* S_0 = 0 mM, S_1= 0.2 mM and S_2 = 0.4 mM.The total treatment combinations were 12 (4x3).The morphological characters: plant height, number of leaf, tillers and effective tiller $plant^{-1}$, yield contributing characters: number of spikelet, effective spikelet $spike^{-1}$, spike length, number of grains $spike^{-1}$and grain yield $plant^{-1}$ of wheat were noticeably decreased due to As whereas exogenous application of SA improved the morphological, yield contributing characters and yield of wheat. The physiological character, SPAD value which indicates chlorophyll content was not significantly changed due to salicylic acid. The interaction between different levels of As and SA has significant effect on almost all the morpho-physiological parameters including yield contributing characters and yield of wheat. The highest grain yield $plant^{-1}$ (2.4 g) was recorded at As_0S_2 (Without As + 0.4 mM SA) treatment combination. But the grain yield was gradually decreased with the increasing level of As toxicity. The application of SA increased the grain yield differently according to the levels of As. The minimum grain yield $plant^{-1}$ were found 1.8 g, 1.5 g and 1.00 g at 30mg, 60mg, and 90mg As kg^{1} soil, respectively. These yields were increased with SA (0.4 mM) from 1.8 to 2.2 g, 1.5 to 1.8 g and 1.0 to 1.20 g at 30mg, 60mg, and 90mg As kg^{-1} soil, respectively. These results suggest that SA mitigate the adverse effects of As toxicity and thereby increase the grain yield of wheat.

Keywords: Wheat; Salicylic acid; Arsenic; Yield

1. Introduction

Agriculture is the source of food and one of the largest sectors of economy all over the world. Wheat (*Triticum aestivium* L.) is one of the most important cereal crops under Poaceae family grown throughout the world including Bangladesh. It is one of the most important winter crops which is sensitive to temperature. It was reported in 2016 that the world production of wheat was 749 million tons, making it the second most-produced cereal after maize (1.03 billion tons) (FAO, 2016; FAOSTAT, 2016). In Bangladesh, average yield and total production of wheat has been estimated 3.086 m t/ha & 13, 47,926 metric tons, respectively (BBS 2015). It is being a staple food of millions of people which contains large amount carbohydrates and protein.In Bangladesh, specially north and north-western zone is more suitable for wheat cultivation. Bangladesh has become highly dependent on wheat imports as dietary preferences are changing and wheat is becoming a highly desirable food supplement to rice. Wheat production is very sensitive to environmental conditions and management practices. It has been reported that, abiotic stresses such as drought, heat, cold, salinity, heavy metals cause broad losses in agricultural production worldwide. There are numerous heavy metals, such as cadmium (Cd), lead (Pb), chromium (cr), and arsenic (As) are increasing gradually into the agricultural land or soil. Therefore the crop production and yield are decreasing in trend. Arsenic (As) is a heavy metal, environmental toxicant and a ubiquitous trace element is found in ground water and agricultural land. It has been reported that Bangladesh is extremely high As contaminated regions where As concentration in water has been reported upto 3200 $\mu g L^{-1}$ against the safe limit of 10 $\mu g L^{-1}$ recommended by WHO (McCarty *et al.,* 2011).In addition, the rate of arsenic contamination increasing day by day due to As contamination from ground water by excess use of pesticides, Physiological activities of wheat seedlings were also changed under As stress (Li *et al.*, 2007). As exposure induces reactive oxygen species (ROS) synthesis which leads to cellular membrane damage (Kumar *et al.*, 2013, 2014b).Many plant physiologist have developed several techniques to decrease the detrimental effect of arsenic toxicity in wheat. Plant biosynthesis different biomolecules such as PGRs, salicylic acid (SA), jasmonic acid, amino acid, sugar etc. can moderate the adverse effects of arsenic. SA is a well-known naturally occurring signaling molecule that affects various physiological and biochemical activities of plants.

The exogenous application of SA enhanced the photosynthetic rate and also maintain the stability of membranes thereby improved the growth of barley plants (El-Tayeb, 2005). The pre-sowing soaking treatment of seeds with SA positively affected the osmotic potential, shoot and root dry mass, Na+/K+ ratio and contents of photosynthetic pigments in wheat seedling under stress condition (Kaydan *et al.,* 2007). SA could be used as a potential growth regulator for improving growth and development under abiotic stress (Shing and Usha, 2003). SA not only helps in establishing and signaling defense response against various pathogenic infection, but also play an important role in mediating plant response to some abiotic stresses such as salinity, temperature, UV radiation, ozone and heavy metal stress (Hayat *et al* ., 2010). SA has been reported to provide protection against heavy metal stress such as, against mercury in Medicago sativa (Zhou et al., 2009), cadmium stress in barley and rice (Metwally et al., 2003; Guo et al., 2007). The dry mass of wheat shoots and roots increased by SA to about 1.5–3 folds of SA-untreated plants. In perspective to this scenario, the present investigation was carried out to evaluate the effectiveness of SA on improving wheat As tolerance in order to spread sustainable agriculture through wheat production with climate change.

2. Method and Materials

This was a pot experiment conducted in the net house at the field laboratory of Agricultural Botany Department, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, and Dhaka. The location of the site is 23º74´N latitude and 90º35´E longitude with an elevation of 8.2 meter from sea level under the agro-ecological zone of Modhupur tract, AEZ-28 during November 2015 to March 2016 to examine the influence of different levels of SA on As contaminated area on morphological, yield contributing character and yield of wheat variety BARI Gom 24 is known as variety, Prodip. The experiment was laid out in two factors in Completely Randomized Design (CRD) with 4 replications.Total 12 treatment combinations were as followed:

As_0S_0: Control (Without As and SA) ,

As_0S_1: Zero As + 0.2 mM SA ,

As_0S_2: Zero As + 0.4 mM SA

As_1S_0: 30 mg As kg^{-1} soil + No SA

As_1S_1: 30 mg As kg^{-1} soil + 0.2 mM SA

As_1S_2: 30 mg As kg^{-1} soil + 0.4 mM SA

As_2S_0: 60 mg As kg^{-1} soil + without SA

As_2S_1: 60 mg As kg^{-1} soil + 0.2 mM SA

As_2S_2: 60 mg As kg^{-1} soil + 0.4 mM SA

As_3S_0: 90 mg As kg^{-1} soil + No SA

As_3S_1:90 mg As kg^{-1} soil + 0.2 mM SA

As_3S_2: 90 mg As kg^{-1} soil + 0.4 mM SA

The total pot number was 12*4=48. Each earthen pot was 35 cm in diameter and 30 cm in height.Wheat plants were treated with 0, 30, 60 and 90 mg of As per kg soil @ 0, 6.24, 12.48, and 18.73 g sodium arsenite salt, respectively. The arsenic used in the form of $NaAsO_2$ of sodium arsenite and SA used in the form of C_6H_4 (OH) COOH of Merck India which collected from local market. According to treatment, the total amounts of arsenic were mixed in soil and covered with polythene sheet for three (3) days to mix with the soil correctly. The soil was fully rotated once to mixture the arsenic well. Then the treated soil was put into the pot which contains 10 kg soil per pot. All 48 pots were filled on 30 November 2015. Weeds and stubbles were completely removed from the soil. As a Arsenic stress mitigation agent, salicylic acid (SA) was sprayed exogenously at 0, 0.2 mM and 0.4 mM concentrations which were maintained by adding 0, 0.03 g and 0.06 g SA respectively per liter of water and 0.1% of Tween-20 was used as an adhesive material. At 35, 45 and 55 day after sowing (DAS) the SA solution was sprayed by a hand sprayer at 10.00 -12.00 am.

After the germination of seeds, various intercultural operations such as irrigation, weeding, top dressing of fertilizer and plant protection measures were accomplished for better growth and development of the wheat seedlings.

3. Results and Discussion

Plant height (cm)

Among the heavy metals Arsenic (As) is a serious problem for crop production in all over the world including Bangladesh. As is absorbed by plants, especially cereals, entering the grains and thus the food chain which is harmful for animal body.It is well known that, Salicylic acid (SA) is an endogenous plant growth regulator which participates the regulation of physiological process of plants as well as defense mechanism. Numerous author stated that SA treatment enhances the plant growth in terms of root, shoot, length and biomass production. Growth stimulating effects of SA has been previously reported in soybean (Gutierrez-coronado *et al.*, 1998), wheat (Shakirova *et al.*, 2003). However little is known about the influence of SA in As contaminated to change the morphological characters of wheat including plant height in our climatic and edaphic condition. Therefore, I used different concentrations of SA to find the contribution of SA on change in plant height of wheat.The highest plant height was observed 69.00 cm from the treatment As_0S_2 and the lowest plant height 55.30 cm from As_3S_0treatment (Table 1).

Number of leaves per plant

Significant variation was observed on the number of leaves plant^{-1} at 60 DAS (table 1) under different stress condition. The maximum number of leaves plant^{-1} was observed 14.40 from As_0S_2 treatment while minimum number of leaves per plant was observed 6.50 from As_3S_0. Sharuhan (2012) reported that higher concentration of As inhibit leaf formation and reduces leaves number. Higher levels of arsenic in soils have been phytotoxic in plants: decreases in plant growth, discolored and stunted roots; withered and yellow leaves (Meloni, 1941). Zhou *et al.* (1999) indicate that SA increases the leaf number in sugarcane plants. These result also consistent with the result of other morphological character including plant height. Therefore it is suggests that SA increase the number of leaves per plant in wheat to different concentration of As contamination.

Number of Tiller per plant

Significant variation had been seen on the combine effect of As and SA on the number of tillers plant^{-1} at 60 DAS (Table 1). The maximum number of tillers per plant was observed 13.05 from As_0S_2 treatment which is statistically similar with As_0S_1 (2.98) and As_0S_0 (2.90) while the minimum number of tillers per plant was observed 1.92 from As_3S_0 treatment which is statistically similar with As_3S_1 (2.01) and As_3S_2 (2.11). when plants are exposed to excess arsenic either in soil or in solution culture, they exhibits toxicity symptoms such as: depress in tillering (Kang *et al.,* 1996; Rahman *et al.*, 2004); reduction in root growth (Abedin *et al.*, 2002a). Azimi *et al* (2013) also reported that SA had significant effects on number of tiller per m^2 and maximum tiller was observed at 1.5mM across SA treatments. These result also consistent with the result of other morphological character including plant height (Table 1), leaf number (Table 1) etc. Therefore it is suggests that SA increase the number of Tiller per plant in wheat to different concentration of As contamination.

Number of Effective Tiller plant^{-1}

Significant variation had been seen on the interaction effect of As and SA on the number of effective tillers plant^{-1} at 60 DAS (Table 1). The maximum number of effective tillers per plant was observed 2.68 from As_0S_2 treatment and minimum number of effective tillers per plant was observed 1.40 from As_3S_0 treatment. Certainly, the reduction of wheat plant growth, in terms of tillering, plant height and shoot biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Rahman *et al.*, 2008).

SPAD Value

The combine effect of Arsenic and Salicylic Acid had no significant effect on SPAD values. The maximum SPAD value was observed 51.00 from As_0S_2 treatment and minimum SPAD value was observed 49.30 from As_3S_0 treatment.This result was disagreed with Meharg *et al.,* (2002) reported that arsenic concentrations of 25 mg kg^{-1} soil did not have negative effect on the photosynthetic process in bean plants (Phaseolus vulgaris L.), while the higher doses (50 and 100 mg of As kg^{-1} soil) inhibit the photosynthesis by 42 and 32. It was reported that SA enhanced chlorophyll content and the activity of antioxidant enzymes that eventually activated the photosynthetic process and alleviated oxidative stress (Li *et al.*, 2014). These result also not consistent with the result of other morphological character including plant height, leaf number, tiller number etc.

Spike Length (cm)

The combine effect of As and SA had significant effect on Spike Length (Table 1). The maximum Spike Length was observed 11.9 cm from As_0S_2 and minimum Spike Length was observed 7.2 cm from As_3S_0 treatment. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (Garg and Singla, 2011). SA treatment enhances the plant growth in terms of root, shoot length and biomass. Co-application of SA and As, partially restored the plant growth in As exposed plants. Some studies have indicated that salicylic acid can enhance the plant growth, yield and quality (Khodary, 2004). Therefore it is suggests

Treatment	Plant height	Leaf number	Tiller number	Effective tiller number	Spike length
As_0S_0	67.3c	13.2bc	2.90ab	2.39ab	11.6bc
As_0S_1	68.43b	13.8ab	2.98ab	2.51ab	11.8ab
As_0S_2	69.0a	14.4a	3.05a	2.68a	11.9a
As_1S_0	64.3e	10.3d	2.02de	1.95cd	11.0d
As_1S_1	66.8d	12.4c	2.50c	2.15bc	11.2d
As_1S_2	67.3c	12.7c	3.02a	2.55a	11.5c
As_2S_0	59.3h	8.4ef	2.02be	1.72de	9.1g
As_2S_1	60.2g	9.0e	2.32cd	1.84cd	9.5f
As_2S_2	60.8f	8.45ef	2.64bc	1.95cd	10.0e
As_3S_0	55.3k	6.5g	1.92e	1.40e	7.2j
As_3S_1	56.2j	7.5f	2.01e	1.52e	8.1i

As_3S_2	57.3i	8.2ef	2.11de	1.68de	8.4h
LSD (0.01)	0.14	0.91	0.15	0.47	0.23
Significant level	**	**	**	**	**
CV%	7.11	4.51	8.50	9.58	5.19

Table 1. Interaction effect of different levels Arsenic and Salicylic Acid on Plant height, Leaf number, Tiller number, Effective tiller number and Spike length plant^{-1} at 60 DAS of wheat.

As_0 = without Arsenic (control), As_1 = 30 mg Arsenic kg^{-1} soil, As_2 = 60 mg Arsenic kg^{-1} soil, As_3 = 90 mg Arsenic kg^{-1} soil.

S_0 = no Salicylic acid, S_1 = 0.2 mM SA, S_2 = 0.4 mM SA ** = Significant at 1% level of probability.

Number of Spikelet per spike

The combine effect of Arsenic and Salicylic Acid had significant effect on the Number of Spikelet spike^{-1} (Table 2). The maximum Number of Spikelet spike^{-1} was observed 20.20 from As_0S_2 treatment and minimum Number of Spikelet spike^{-1} was observed 15.10 from As_3S_0 treatment which is statistically similar to As_3S_1 (15.50). There are several reports regarding the loss of fresh and dry biomass of roots as well as shoots, loss of yield and fruit production, morphological changes; when the plants are grown in As-treated soils (Carbonell-Barrachina *et al.* 1998). The Number of Spikelet per spike significantly increased with the increase of Salicylic Acid. Aldesuquy *et al.* (2012) stated that mechanism of SA induced yield enhancement might be an increase in the number of spikelets because SA has the capacity to both directly or indirectly regulate yield. Therefore it is suggests that SA increase the Number of Spikelet spike^{-1}in wheat to different concentration of As contamination.

Number of Effective Spikelet per spike

The combine effect of Arsenic and Salicylic Acid had significant effect on the Number of Effective Spikelet spike^{-1} (Table 2). The maximum number of Effective Spikelet spike^{-1} was observed 16.50 from As_0S_2 treatment and minimum number of Effective Spikelet spike^{-1} was observed 10.20 from As_3S_0 treatment which is statistically similar to As_3S_1 (10.50). In bean plants foliar mass (leaf dry weight) had an average reduction of 50% and fruit production or yield (fruit dry weight) showed even a higher reduction of 84% compared with controls when As was present in the growing solutions (Carbonell-Barrachina *et al.* 1998). Some studies have indicated that salicylic acid can enhance the plant growth, yield and quality (Khodary, 2004). Therefore it is suggests that SA increase the Number of effective Spikelet spike^{-1} in wheat to different concentration of As contamination.

Number of Grain spike^{-1}

The combine effect of Arsenic and Salicylic Acid had significant effect on the Number of Grain spike^{-1} (Table 2). The maximum number of Grain spike^{-1} was observed 42.30 from As_0S_2 treatment and minimum number of Grain spike^{-1} was observed 21.50 from As_3S_0 treatment. High levels of arsenic in soils have been phytotoxic in plants: decreases in plant growth and fruit yields (Carbonell-Barrachina et al., 1995).

Grain Yield Plant^{-1} (gm)

The combine effect of Arsenic and Salicylic Acid had significant effect on the amount of Grain yield Plant^{-1} (Table 25). The maximum amount of Grain yield plant^{-1} was observed 2.40 gm from As_0S_2 treatment which is statistically similar with As_0S_1 (2.1) and As_0S_0 (2.0) while minimum amount of Grain yield plant^{-1} was observed 1.10 gm from As_3S_0 treatment which is statistically similar with As_3S_1 (1.1) and As_3S_2 (1.2). These results are consistent with the other morpho-physiological and yield contributing characters such as plant height, number of leaves Plant^{-1}, tillers Plant^{-1}, effective tillers Plant^{-1}, number of spikelet spike^{-1}, number of effective spikelet spike^{-1} and number of grains spike^{-1}. Therefore it is suggested that SA increase the Grain Yield Plant^{-1} in wheat to different concentration of As contamination.

Treatment	Number of Spikelet per spike	Number of Effective Spikelet per spike	Number of Grain Per spike	Grain Yield Plant^{-1} (gm)
As_0S_0	17.5d	13.5c	36.3d	2.0bc
As_0S_1	18.8b	15.2b	40.5b	2.1b
As_0S_2	20.2a	16.5a	42.3a	2.4a
As_1S_0	16.2f	14.4b	34.3e	1.8cd
As_1S_1	18.2c	15.2b	38.4c	2.0bc
As_1S_2	19.1b	16.0a	40.5b	2.2ab
As_2S_0	15.1h	13.5c	30.2h	1.5e
As_2S_1	16.1f	14.5b	31.1g	1.6de
As_2S_2	17.1e	15.0b	32.5f	1.8cd
As_3S_0	15.1h	10.2e	21.5k	1.0f
As_3S_1	15.5g	10.5de	25.3j	1.1f
As_3S_2	16.0f	11.0d	27.8i	1.2f

LSD (0.01)	1.50	1.10	1.97	0.23
Significant level	**	**	**	**
CV%	4.07	4.8	6.74	6.81

Table 2. Interaction effect of different levels Arsenic and Salicylic Acid on Number of Spikelet per spike,Number of Effective Spikelet per spike, Number of Grain per Spike, Grain Yield Plant^{-1} (gm) of wheat.

As_0 = without Arsenic (control), As_1 = 30 mg Arsenic kg^{-1} soil, As_2 = 60 mg Arsenic kg^{-1} soil, As_3 = 90 mg Arsenic kg^{-1} soil.

S_0 = no Salicylic acid, S_1 = 0.2 mM SA, S_2 = 0.4 mM SA ** = Significant at 1% level of probability.

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