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BRIEF COMMUNICATION

The effect of arsenic on pigment composition and photosynthesis in *Hydrilla verticillata*

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Abstract

The present study evaluated the effects of 100 and 500 μM arsenate (Na_2HAsO_4) on pigment composition and photosynthesis in *Hydrilla verticillata* (L.f.) Royle. Arsenic accumulation increased in concentration and duration dependent manner. The maximum accumulation [568 μg(As) g^{-1}(d.m.)] was observed at 500 μM concentration and 96-h exposure. This concentration led to a significant decline in chlorophyll *a* content and PS II efficiency during the whole experiment, and in chlorophyll *b* and carotenoids after 96 h, but no significant changes in photosynthetic pigments were noticed at 100 μM arsenate. Net photosynthetic rate, electron transport rate, and water use efficiency declined whereas transpiration rate increased, and stomatal conductance and photochemical quenching did not show any effect or increased. The content of reactive oxygen species increased and content of reduced ascorbate declined at 500 μM arsenate in comparison to the control.

Additional key words: ascorbate, chlorophyll, electron transport rate, photosynthetic efficiency, stomatal conductance, superoxide radical, transpiration rate, water use efficiency.

Arsenic (As) is a widely distributed toxic metalloid. Its contamination has emerged as one of the worst environmental calamities in some parts of the world. Arsenate (As^V) and arsenite (As^{III}) are the two prevalent inorganic forms of As in soil and water which are taken up by the plants through phosphate transporters and aquaglyceroporins, respectively. Plants have ability to regulate As entry and accumulation (Srivastava *et al.* 2012), however, when As accumulation reaches beyond tolerable limit, they suffer from As toxicity and show disturbance in the whole metabolism (Finnegan and Chen 2012).

Hydrilla verticillata is a widely distributed invasive aquatic weed and a potential As accumulator (Srivastava *et al.* 2007, Srivastava and D'Souza 2009). The mechanism of As toxicity was evaluated in *Hydrilla* and it was found that As exposure induced the production of reactive oxygen species (ROS) and subsequently enhanced the degradation of proteins, lipids, and DNA and increased cell death (Srivastava *et al.* 2011). Since chloroplast is a major organelle where ROS are produced, it is imperative to decipher the inter-relationship between photosynthetic performance of plants and ROS generation under As stress to gain more knowledge about As toxicity mechanism. The present study thus analyzed the effects of different As concentrations and duration of exposure on the content of photosynthetic pigments and photosynthetic reactions. In addition, the ROS and antioxidants were also estimated.

Hydrilla verticillata (L.f.) Royle plants growing in net house in field at the institute were brought to laboratory and were acclimatized for 5 d in 10 % Hoagland's solution (pH 6.5) at temperature of 25 ± 2 °C, a 14-h photoperiod, and irradiance of 115 μmol m^{-2} s^{-1}. Experiments were set up in triplicate in 250 cm^3 conical flasks containing 150 cm^3 of nutrient solution and each replicate contained 10 plants of 5 cm height (approximately 1.0 g fresh mass in total). Plants were exposed to 100 or 500 μM Na_2HAsO_4 in 10 % Hoagland's solution for 4 to

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Abbreviations: ASC - ascorbate; Car - carotenoids, Chl - chlorophyll; c_i - substomatal CO_2 concentration; E - transpiration rate; ETR - electron transport rate; Fv'/Fm' - efficiency of excitation energy capture by PS II; g_s - stomatal conductance; NPQ - non-photochemical quenching; φ_{PSII} - photosystem II efficienty; P_N - net photosynthetic rate; P_N/E - water use efficiency; PQ - photochemical quenching.
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96 h. Flasks containing no As served as a control. At the time of harvesting, plants were washed with double distilled water, blotted gently and used to determine various parameters.

For the analysis of As content, plants were thoroughly washed with distilled water and then kept in ice-cold deionised water for 30 min for the desorption of extracellular As (Irtelli and Navari-Izzo 2008). Arsenic content in the oven-dried plants was quantified as described previously (Srivastava and D'Souza 2010). The rate of superoxide radical production was measured following the method of Chaitanya and Naithani (1994). The reduced ascorbate (ASC) content was measured following the protocol of Gillespie and Ainsworth (2007). The content of photosynthetic pigments was estimated according to Lichtenthaler and Buschmann (2001a,b).

Three leaves from each of 10 plants per treatment were selected to measure the net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), electron transport rate (ETR), and substomatal CO_2 concentration (c_i) using an *LI-6400XT Portable Photosynthesis System* (*LI-COR*, Lincoln, NE, USA) at saturating irradiance of 500 μmol m^{-2} s^{-1}, vapour pressure deficit (VPD) of 2.5 to 3.0 kPa, CO_2 concentration of 430 - 450 μmol mol^{-1}, and the temperature of 30 °C. Leaf area under consideration was 2 cm^2. Averages of ten readings were recorded when the rate of CO_2 uptake had become steady. The P_N/E ratio was used as a measure of water use efficiency (WUE).

The parameters of chlorophyll (Chl) *a* fluorescence were measured in leaves by *6400-40 Leaf Chamber Fluorometer* fitted with *LI-6400XT*. Maximal fluorescence (F_m) was measured using a 0.8 s saturating pulse (8 000 μmol m^{-2} s^{-1}) after 15 min of dark adaptation. When measuring the induction, a pulse of actinic light (1 500 μmol m^{-2} s^{-1}) was given for 20 s and light-adapted steady-state fluorescence yield (F_s) was averaged over 2.5 s and then a second 0.8 s saturating pulse was provided to determine the maximum fluorescence in the light-adapted state (F_m'). The actinic light was then turned off and the minimal fluorescence in the light-adapted state (F_0') was determined by irradiance for 3 s of far red light. The following parameters were then calculated: *1*) efficiency of excitation energy captured by open PS II reaction centers, $F_v'/F_m' = (F_m' - F_0')/F_m'$; *2*) the photochemical quenching coefficient, $PQ = (F_m' - F_s')/(F_m' - F_0')$; *3*) the actual PS II efficiency, $\varphi_{PSII} = (F_m' - F_s')/F_m'$; and *4*) non-photochemical quenching, $NPQ = F_m/F_m' - 1$ (Luo *et al.* 2011). F_v'/F_m' and NPQ were measured only at 4 d.

Plants showed significant As accumulation in a concentration and duration dependent manner. The maximum As accumulation [568.3 μg g^{-1}(d.m.)] was observed after 96 h at 500 μM arsenate (As^V; Table 1). This confirmed our previous studies demonstrating significant potential of *Hydrilla* plants to take up and tolerate As (Srivastava *et al.* 2007, 2011). The content of Chl *a*, Chl *b*, and carotenoids (Car) in As^V-treated plants tended to be lower than in the control beyond 24 h at both As^V doses. However, a significant decline was noticed only at 500 μM As^V and all As treatment durations in Chl *a* and total Chl, and after 96 h in Chl *b* and Car. The Chl *a*/*b* ratio tended to decline at 500 μM As^V during the whole experiment. The reduction in Chl content might be due to decreased activity of enzyme of Chl biosyntehsis [δ-aminolevulinic acid (ALA) dehydratase] and availability of ALA or due to increase of Chl degradation by chlorophyllase (Jain and Gadre 1997, 2004). The decline in photosynthetic pigments upon As exposure has been observed also in other plant species (Rahman *et al.* 2007, Duman *et al.* 2010).

Net photosynthetic rate declined significantly beyond 24 h at 100 μM As^V and beyond 4 h at 500 μM As^V. Stomatal conductance was higher than in the control until 24 h at both As^V doses with the greater increase occurring at 100 μM As^V (Table 1). The initial increase in g_s was probably important to maintain photosynthesis under As stress. Since this increase in g_s was higher at 100 than at 500 μM As^V, plants could maintain P_N close to control levels until 24 h at 100 μM As^V. Substomatal CO_2 concentration was not changed (data not shown), whereas transpiration rate was significantly higher than in the control at both As^V doses (Table 1). PS II efficiency was not affected until 24 h but showed significant decline beyond 24 h at 500 μM As^V whereas at 100 μM As^V, it showed significant increase at 48 h but a decline at 96 h. Photochemical quenching was higher than in the control until 48 h at 100 μM AsV, whereas it increased significantly only at 96 h at 500 μM As^V. Electron transport rate and water use efficiency declined significantly at both As^V doses in comparison to control except ETR at 4 h and the effect was more severe at 500 μM As^V. At 96 h, F_v'/F_m' declined significantly at both AsV doses whereas NPQ was not significantly affected (Table 1).

Chlorophyll content and g_s are usually positively correlated to P_N. However, chlorophyll content and P_N were not correlated in this study. Hence, the observed decrease in content of chlorophylls and carotenoids may be only partly responsible for the As-induced decline in P_N. Further, g_s was also not positively correlated with P_N in this study indicating that the decrease in P_N was not due to low CO_2 concentration as is evident from c_i data (Table 1). It seems that effect of As on the activity of enzymes involved in photosynthetic metabolism might be responsible for the observed decline in P_N. Ahsan *et al.* (2010) analyzed proteome of rice leaves under As stress and found down-regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large subunit and chloroplast 29 kDa protein. The membrane systems of chloroplasts were found to be severely destroyed by As exposure in *Pteris vittata* (Li *et al.* 2006). Thus, As exposure can induce changes in chloroplastic proteins and enzymes which might affect photosynthetic efficiency of plants. Stoeva *et al.* (2005) found that high dose of As (5 mg dm^{-3}) caused reduction in leaf gas exchange, water potential, and biomass accumulation in *Phaseolus vulgaris*. However, in another study, 32 - 96 μM As did not cause significant change in Chl fluorescence of

Table 1. Accumulation of arsenic by *Hydrilla verticillata* plants upon exposure to 0, 100 or 500 μM arsenate for 4 to 96 h and As effect on the content of chlorophylls and carotenoids, P_N, g_s, E, φ_{PSII}, PQ, ETR, WUE, $O_2^{\cdot-}$, and ASC content. F_v'/F_m' and NPQ were analyzed only at 96 h. No arsenic could be detected in control plants (ND). Means ± SD, n = 3. * - significantly different in As^V-exposed plants with respect to the control.

Parameter	Na_2HAsO_4 [μM]	4 h	24 h	48 h	96 h
As accumulation	0 (control)	ND	ND	ND	ND
[μg g^{-1}(d.m.)]	100	24.7 ±1.9*	66.1 ±18.0*	160.0 ±34.5*	180.9 ±45.6*
	500	113.6 ±15.0*	234.5 ±23.1*	477.0 ±100.0*	568.3 ±121.2*
Chl *a*	0	0.17 ±0.007	0.18 ±0.003	0.17 ±0.016	0.18 ±0.002
[mg g^{-1}(f.m.)]	100	0.19 ±0.009	0.17 ±0.021	0.15 ±0.018	0.17 ±0.011
	500	0.15 ±0.016*	0.14 ±0.029*	0.14 ±0.008*	0.13 ±0.003*
Chl *b*	0	0.072±0.005	0.076±0.002	0.073±0.007	0.080±0.001
[mg g^{-1}(f.m.)]	100	0.076±0.003	0.067±0.005	0.066±0.006	0.073±0.004
	500	0.064±0.006	0.066±0.008	0.062±0.003	0.063±0.002*
Total Chl	0	0.24 ±0.012	0.26 ±0.005	0.24 ±0.023	0.26 ±0.003
[mg g^{-1} (f.m.)]	100	0.26 ±0.011	0.24 ±0.002	0.22 ±0.025	0.24 ±0.015
	500	0.21 ±0.022*	0.21 ±0.001*	0.20 ±0.011*	0.21 ±0.006*
Carotenoids	0	0.064±0.004	0.065±0.002	0.063±0.006	0.068±0.003
[mg g^{-1}(f.m.)]	100	0.070±0.004	0.057±0.006	0.056±0.007	0.063±0.003
	500	0.059±0.005	0.057±0.007	0.052±0.002	0.054±0.001*
Chl *a/b*	0	2.41 ±0.12	2.34 ±0.11	2.33 ±0.06	2..25 ±0.11
	100	2.45 ±0.07	2.51 ±0.09	2.31 ±0.17	2.32 ±0.13
	500	2.36 ±0.03	2.19 ±0.12	2.17 ±0.09	2.12 ±0.05
P_N	0	5.78 ±0.56	5.44 ±0.52	5.03 ±0.59	4.07 ±0.40
[μmol(CO_2) m^{-2} s^{-1}]	100	6.18 ±0.39	6.36 ±0.64	3.37 ±0.18*	2.53 ±0.43*
	500	5.9 5±0.49	4.08 ±0.28*	2.74 ±0.48*	1.50 ±0.34*
g_s	0	1.05 ±0.07	1.15 ±0.13	1.48 ±0.11	1.05 ±0.06
[mol(H_2O) m^{-2} s^{-1}]	100	2.04 ±0.09*	1.85 ±0.16*	1.18 ±0.16	1.23 ±0.21
	500	1.65 ±0.11*	1.29 ±0.28	1.20 ±0.10	1.48 ±0.07
E	0	14.34 ±1.47	13.26 ±0.94	13.73 ±1.21	14.32 ±1.12
[mmol(H_2O) m^{-2} s^{-1}]	100	19.32 ±1.61*	25.26 ±1.27*	18.92 ±1.69*	19.81 ±0.58*
	500	22.84 ±2.92*	20.53 ±1.35*	19.47 ±1.57*	17.19 ±2.93*
φ_{PSII}	0	0.043±0.013	0.040±0.009	0.046±0.015	0.040±0.011
	100	0.047±0.013	0.039±0.008	0.056±0.008*	0.026±0.005*
	500	0.045±0.011	0.046±0.011	0.032±0.008*	0.014±0.004*
PQ	0	0.09 ±0.021	0.09 ±0.015	0.09 ±0.013	0.09 ±0.014
	100	0.12 ±0.013*	0.10 ±0.011	0.13 ±0.011*	0.07 ±0.009
	500	0.08 ±0.009	0.09 ±0.005	0.09 ±0.008	0.17 ±0.011*
ETR	0	8.32 ±1.59	8.73 ±0.74	8.46 ±0.57	8.31 ±0.60
[μmol m^{-2} s^{-1}]	100	8.06 ±0.95	5.88 ±1.55*	5.46 ±1.06*	5.39 ±0.59*
	500	6.39 ±1.22	5.59 ±0.83*	3.58 ±0.59*	2.71 ±0.49*
WUE = P_N/E	0	0.40 ±0.05	0.41 ±0.11	0.37 ±0.05	0.28 ±0.10
	100	0.32 ±0.06*	0.25 ±0.07*	0.18 ±0.03*	0.13 ±0.02*
	500	0.26 ±0.03*	0.20 ±0.01*	0.14 ±0.02*	0.10 ±0.01*
$O_2^{\cdot-}$production	0	0.018±0.001	0.017±0.001	0.020±0.001	0.016±0.001
[ΔA_{540} g^{-1}(f.m.) min^{-1}]	100	0.019±0.002	0.026±0.004*	0.026±0.004*	0.030±0.007*
	500	0.027±0.004*	0.038±0.006*	0.034±0.002*	0.034±0.004*
Reduced ascorbate	0	15.84 ±1.44	18.51 ±3.78	19.64 ±1.77	19.64 ±2.66
[μmol g^{-1}(f.m.)]	100	14.78 ±3.53	14.97 ±2.17	19.30 ±2.43	13.08 ±1.35
	500	13.31 ±0.93	6.29 ±0.71*	12.11 ±1.34*	6.87 ±1.34*
F_v'/F_m'	0	-	-	-	0.47 ±0.06
	100	-	-	-	0.12 ±0.04*
	500	-	-	-	0.04 ±0.01*
NPQ	0	-	-	-	0.78 ±0.16
	100	-	-	-	1.00 ±0.13
	500	-	-	-	0.88 ±0.15

soybean leaves (Milivojevic *et al.* 2006). The inhibition of photosynthesis by As has been attributed to an impairment of electron transport activity (Nwugo and Huerta 2008). In this study, significant decline in ETR was observed upon As exposure. Another parameter negatively affected by As^V exposure was P_N/E which demonstrates that As^V affected the ability of plants to sufficiently regulate the rate of water loss with the decline in P_N. Under As stress, an increase in the production of ROS becomes likely. This was evident in the present study as the rate of $O_2^{\cdot-}$ production increased at 100 μM As^V after 24 h of the treatment, whereas at 500 μM As^V already after 4 h. The increase in $O_2^{\cdot-}$ production could also be associated with decreased content of ASC at 500 μM As^V after 24 h. At 100 μM As^V, ASC content was not significantly affected until 48 h and then declined significantly at 96 h. The decline in ASC content might be attributed to reduction of its biosynthesis which is linked to photosynthetic performance of plants (Yabuta *et al.* 2007) or to reduced rate of conversion of oxidized ASC to reduced ASC. A decline in ASC content and ascorbate peroxidase activity has been found to be associated with oxidative modification of RubisCO and with a decline in its activity (Liu *et al.* 2008).

The present study concludes that As stress negatively affected photosynthetic rate and PS II efficiency and resulted in the increased production of ROS. The photosynthesis thus appears to be a crucial hub point for regulating toxicity responses under As stress.

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