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Short Communication

COLD EXPOSURE AND ARSENIC EFFECT ON THE INORGANIC PHOSPHATE CONTENT IN ROOT OF GROWING PADDY

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Abstract

Development of root growth is impaired by environmental and chemical effectors although the mechanism is not known. Pot experiments were carried out to examine the effects of cold acclimation and arsenic on inorganic phosphate (Pi) level in roots of growing paddy (*Oryza sativa*). Whenever, paddy was exposed to cold, the amount of Pi in root was reduced by 50.1%, 18.8% and 27.3% after 24h, 48h and 72h period respectively. Contrary, when the paddies were exposed to Na_2HAsO_4 (1 and 10 mM) along with cold acclimation for the above mentioned time, the Pi level in root extract was enhanced remarkably. Moreover, the Pi content was increased with the increase in arsenic concentration and duration of plant exposure to arsenic.

Key words: Cold acclimation, Plant growth, Pi, Arsenic, Adaptive response.

Root development is impaired either by environmental or chemical factors. Temperature fluctuation is a common phenomenon of the atmosphere and is involved in changes of various metabolic functions. For example, cold acclimation has been recognized as a major environmental sympathetic stimulus and is a stressful event that elicits different thermogenic adaptive responses in endotherms and exotherms (Lowel and Spiegelman, 2000). The roots of plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones and storage functions. However, the development of root is altered by environmental and chemical environment. It has been revealed that roots and other protected parts are less cold hardy than the aerial parts of the plant (Pellet, 1971). Therefore, it is presumably assumed that cold acclimation may have the role in the

development of plant root. Evaluation of the cold hardiness of roots during the winter is difficult due to the frozen soil and the lack of reliable methods for assessing freezing damage.

Arsenic has been identified to be toxic to the living organisms. Prolonged exposure of arsenic has detrimental effects in tissues. It may impair the glycolysis as well as the oxidative processes (Tchounwou *et al.*, 2003) and causes different types of pathogenic syndromes in rodents and other organisms. More than 40 million people worldwide are at risk from drinking As-contaminated ground water and chronic inorganic As poisoning has reached a massive scale in Bangladesh and West Bengal, India (Bhattacharjee, 2007). Arsenic concentration in rice grain are often high enough to cause concern even in uncontaminated soils containing background levels of As, because paddy rice appears to be particularly efficient in As assimilation compared with other cereal crops (Williams, 2007). It is therefore crucial that the mechanism of arsenic accumulation in rice is understood to counteract this widespread contamination of the food chain. Adenosine triphosphate (ATP) and inorganic phosphate (Pi) are very energetic compounds involved in the development of root growth. The diverse stimuli either environmental or chemical cause the degradation of ATP or the formation of ATP from Pi and ADP, the processes catalyzed by the respective enzymes and thereby, the alteration of root rigidity is observed. However, the mechanism underlying the phenomena in root is not well understood. Therefore, to fully understand the morphogenesis of roots along with the rigidity, it is necessary to define the organization of the root meristem whether cold acclimation and arsenic may have the roles on the development of root growth by alteration of tissue Pi, since these stimuli cause the critical environment where the plants survive, the current protocol was undertaken.

Soil collection and pot preparation: The soil was collected from the rice field of Rajshahi University Campus and kept in several plastic pots. The unwanted materials like stones, gravels, pebbles, plant roots etc. were removed from the bulk soil. For this experiment, three plastic pots were used; each pot size was 70 cm in diameter and 24 cm in height. An adequate amount of soil was taken in each plastic pot. Then sufficient amount of water was poured into the each pot and kept for over night and mixed well. Then the pots were ready for seedling of germinated rice.

Seed germination: For the germination of seeds (*Oryza sativa*), the following points were carried out: i) the strongest seeds were selected; the seeds were added to the normal water and floating seeds are discarded; ii) the seeds were kept in normal water with temperature below 37°C in overnight; iii) the seeds were swollen by water absorption and were expected to be effective for germination; iv) the total 154 seeds were seeded in the pots, non germinated seeds were 20 and the efficiency of seed germination was about 90%.

Cold acclimation and arsenic treatment: Four plastic pots were prepared with soil and seeded with Oryza sativa. After 10 days of germination, the four different pots were described as control, cold, arsenic (1 mM) plus cold and arsenic (10 mM) plus cold. Control pot was used for 24h, 48h and 72h treatments in the room temperature without cold acclimation. The second pot was used for 24h, 48h and 72h duration in the cold chamber and given cold exposure (4~8°C) with full aeration. In the third pot, paddies were treated with arsenic (Na_2HAsO_4. $7H_2O$, BDH Chemical Ltd.) (1 mM) and kept similarly in cold for 24h, 48h and 72h in the cold chamber. The fourth pot containing paddy was similarly treated with arsenic (10 mM) and kept in cold for 24h, 48h and 72h in the cold chamber. All seedlings in third and fourth pot were exposed to cold plus arsenic. After 10 days of germination, paddies were ruptured consecutively from each pot for 24h, 48h and 72h duration and the different parts of paddy including root and shoot were sampled carefully.

Assay of inorganic phosphate (Pi): 1.0 g of paddy roots was placed into a mortar with pestle which was kept on ice. It was homogenized with 12 ml of distilled water and centrifuged with 8000 rpm for 10 minutes. The supernatant was collected and used as crude extract. Tissues were homogenized with pre-cooled water and were centrifuged at 8000 rpm for 10 min. The supernatants from each tissue homogenate were used as crude extract for assay of inorganic phosphate (Pi) described by Ramnik (1999). 200 μL tissue extract was diluted to 5 mL with water and was mixed vigorously with 5 mL of 5% TCA (Trichloroacetic acid) and centrifuged at 6000 rpm for 10 min. 5 mL supernatant was transferred to another tube and kept on ice. 1 mL molybdate reagent (10 g of ammonium molybdate in 100 mL water was taken and 100 mL of 5N H_2SO_4 was added to prepare 200 mL solutions) was added and mixed. The solution was

mixed with 0.4 mL aminonaptholsulphonic acid reagent. 3.6 mL water was added and after mixing, the tube was kept standing for 10 min for the complete development of color. For blank, 5 mL of 5% TCA and 5 mL water were mixed only. Absorbance was taken at 690 nm against the blank. The Pi in tissue extract was calculated using standard KH_2PO_4 solution.

Statistical analysis: Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by paired t-test using SPSS software.

Effects of cold and Na_2HAsO_4 (1 and 10 mM) on inorganic phosphate content in root after 24h, 48h and 72h of treatment: As shown in Fig. 1, the inorganic phosphate (Pi) level in roots of treated paddy was recorded to determine the effect of cold on root growth. After 24 hours of treatment, the root Pi was estimated as 4.99 ± 0.21 mg for control, for cold treated paddy, the value was 2.49 ± 0.107 mg/100 g of root. Cold acclimation causes a decrease in Pi by 50.1% when compared to control. The reducing property of Pi in root follows the impairment of root development in such critical situation. However, when paddies were exposed to cold and arsenic (1 mM Na_2HAsO_4) for above mentioned time, the different Pi content was observed and found to be 3.09 ± 0.1 mg/100 g of root. The results demonstrate that the root Pi content of paddy had been similarly decreased by 38.0% when compared to control, however, in comparison to cold acclimated paddy, the increased effect (24.0%) on root Pi was observed in response to arsenic and cold.

To examine the effect of cold on Pi, the plants were also exposed to cold for 48h. As shown in Fig. 2, the Pi levels in root of treated paddy were 3.60 ± 0.09 mg for control and 2.92 ± 0.13 mg for cold treatment. It was found that the amount of Pi in root was reduced by 18.8% for cold acclimation. The paddies exposed to cold and arsenic (1 mM Na_2HAsO_4) had root Pi 5.58 ± 0.13 mg/100 g of root showing the increased effect on Pi (55.0%) when compared to control. However, compared to cold exposed paddy, the amount of Pi was also increased by 91.0%.

Fig. 3 shows the effect of cold acclimation on Pi of paddy root after 72 hours of treatment. Paddies treated with cold had root Pi content of 9.06 ± 0.49 mg whereas 11.00 ± 0.21 mg/100 g of root for arsenic (1 mM) treatment was observed. The amount of Pi in root of

Fig. 1.

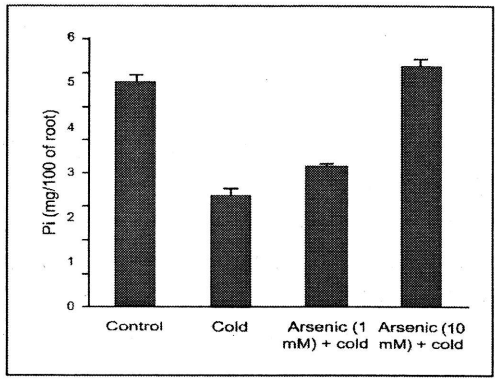

Fig. 1. Effect of cold and arsenic (1 and 10 mM) on inorganic phosphate in roots after 24h of treatment. The paddy was treated with Na_2HAsO_4 and kept for 24h in the cold. The paddies in another pot were exposed to cold for 24h only in the cold chamber. Control paddy was similarly used except giving cold exposure and arsenic treatment. The data are means SE for 3 individual measurements in each group.

Fig. 2.

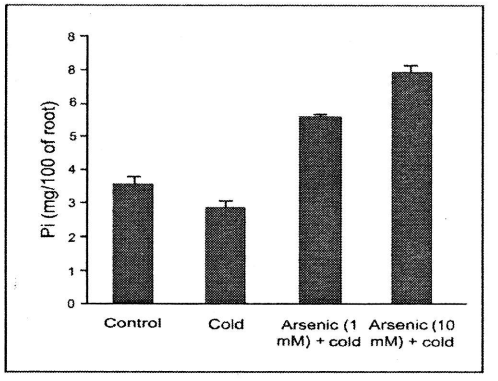

Fig. 2. Effect of cold and arsenic (1 and 10 mM) on inorganic phosphate in roots after 48h of treatment. The paddy was treated with Na_2HAsO_4 and kept for 48h in the cold. The paddies in another pot were exposed to cold for 48h only in the cold chamber. Control paddy was similarly used except giving cold exposure and arsenic treatment. The data are means SE for 3 individual measurements in each group.

paddy for control was found to be 12.47 ± 0.49 mg/100 g of root. These results indicated that the amount of Pi in root of paddy was reduced by 27.3% for cold treatment and 11.7% for arsenic when compared to control. The results also showed that the Pi value was increased by 21.4% when the paddies were exposed to cold and arsenic compared to cold acclimation. Although 72h treatment also caused the mild reduction of Pi in response to arsenic, the enhanced effects compared to cold exposed paddy were observed. Further extension of time is required to verify the test.

As shown in Fig. 1, the Pi levels in roots of treated paddy were recorded to determine the effect of cold and higher concentration of arsenic (10mM Na_2HAsO_4) on root growth. After 24 hours of treatment, the root Pi was estimated as 4.99 ± 0.21 mg for control, and for cold treated paddy the value was 2.49 ± 0.10 mg/100 g of root. Cold acclimation caused a decrease in Pi by 50.1% when compared to control. However, when paddies were exposed to cold and arsenic for above mentioned time, the different Pi value was observed and found to be 5.32 ± 0.24 mg/100 g of root. The results

Fig. 3.

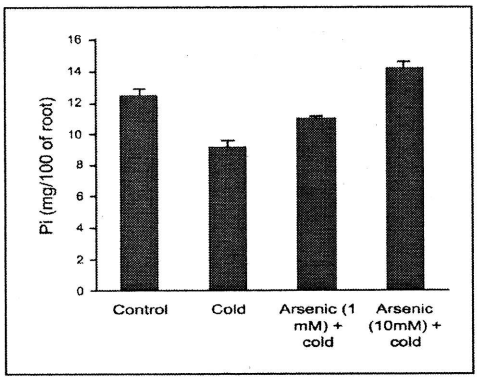

Fig. 3. Effect of cold and arsenic (1 and 10 mM) on root inorganic phosphate after 72h of treatment. The paddy was treated with Na_2HAsO_4 and kept for 72h in the cold. The paddies in another pot were exposed to cold for 72h only in the cold chamber. Control paddy was similarly used except giving cold exposure and arsenic treatment. The data are means SE for 3 individual measurements in each group.

demonstrated that the Pi of paddy root had been similarly increased by 6.6% when compared to control. However, in comparison to cold acclimated paddy, the increased effect (113.6%) on root activity was demonstrated in response to arsenic and cold. Therefore, it is obvious that cold induced Pi release is accelerated in response to arsenic and seems to be higher than 1 mM concentration.

To examine the effect of arsenic on cold induced Pi, plants were exposed to cold for 48h along with the combined effect of cold and arsenic. As shown in Fig. 2, the root Pi of treated paddy were found to be 3.6 ± 0.093 mg for control and 2.92 ± 0.138 mg/100 g of root for cold treatment. It was found that the Pi content of paddy root had been reduced by 18.8% for cold acclimation. The paddies exposed to cold and arsenic (10 mM Na_2HAsO_4) had Pi 6.96 ± 0.19 mg/100g of root showing the increased effect on Pi (93.3%) when compared to control. However, compared to cold exposed paddy, the Pi content was also increased by 138.3% and the value is higher than the previous one (1 mM Na_2HAsO_4).

Fig. 3 shows the effect of cold and 10 mM arsenic on Pi content of paddy after 72 hours of treatment. Paddies treated with cold had root Pi 9.06 ± 0.491 mg whereas 14.17 ± 0.48 mg/100 g of root for Na2HAsO4 and cold treatment was observed. The root Pi content of paddy for control was found 12.47 ± 0.498 mg/100 g of root. These results indicated that the Pi content in root of paddy was reduced by 27.3% for cold treatment while increased by 13.6% for arsenic and cold when compared to the control. However, compared to cold exposed paddy, the amount of Pi in root extract was also increased by 56.4%. The results are higher than the previous records obtained by 1 mM arsenic and cold whenever compared to control and

also compared to cold exposed paddy.

The present study demonstrates the effect of cold and the interaction with arsenic on the regulation of inorganic phosphate in root of paddy. Root development is impaired by various stimuli either by environmental and chemical effectors. When paddies were exposed to cold, formation of root is affected, there by acquisition of nutrients and other biological processes are impaired. For rigidity of root, inorganic phosphate is essential molecule and is integral part for the development of root growth. Variation of temperature has direct interaction with the formation of these molecules. We found that this molecule is profoundly reduced in response to the cold acclimation for 24h or prolonged exposure. Because of the lower concentration of these molecules in root, uptake of nutrient or several ions responsible for the growth of paddy might be impaired. Lower temperatures induce rigidification of membranes, leading to a disturbance of all membrane processes (e.g. opening of ion channels, membrane associated electron transfer reactions, etc.). Low temperatures affect different aspects of photosynthesis. For example, low temperatures inhibit sucrose synthesis in the cytosol, leading to the accumulation of phosphorylated intermediates. This results in the depletion of the available inorganic phosphate and in the decreased cycling of inorganic phosphate between the cytosol and the chloroplast (Furbank *et al.*, 1987; Hurry *et al.*, 2000). This, in turn, impedes synthesis of the ATP necessary for the regeneration of ribulose-1, 5- bisphosphate to support CO_2 fixation. Moreover, alteration of calcium ion signaling in plant is associated with the lowering of temperature. Recent studies reveal that calcium is frequently involved as a secondary messenger in plant responses to external signals (Trewavas and Malho, 1997). There is increasing evidence that chilling causes elevated levels of Active Oxygen Species (AOS), which contribute significantly to chilling damage (Wise and Naylor, 1987a). AOS such as superoxide (O^-_2), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen ($^1O_2$), are present in plants in various at 25°C as a result of normal aerobic metabolism. The adverse environment created by cold acclimation for 24h or prolonged period, therefore may induce nutritional deficiencies and other cellular defects in paddy.

Adenosine triphosphate (ATP), guanosine triphosphate (GTP) and Pi

are energetic compounds and are essential for cellular development of root. Degradation of ATP to ADP and Pi is catalyzed by ATPase, conversely, formation of ATP from ADP + Pi by ATP synthetase enzymes is observed. These biochemical processes are influenced by environmental or chemical effector molecules. Although the mechanism is not understood in root of paddy, we found that cold acclimation severely causes the reduced Pi in root of paddy exposed to 24h, 48h and 72h of period. On the other hand, cold induced effect is altered and enhanced in response to different concentrations of arsenic compound (1 and 10 mM Na_2HAsO_4). It seems that 10 mM concentrations augment the higher effect of arsenic on inorganic phosphate release and might be from the degradation of ATP molecules. It has been demonstrated that arsenic causes the enhancement of degradation of ATP (Kitchin, 2001). It is well known that P deficiency increases the capacity of plant roots to take up phosphate (Lee, 1982; Drew *et al.*, 1984). Expression of the genes encoding phosphate transporters is up-regulated in the roots of P-deficient plants (Liu *et al.*, 1998), which also leads to a concurrent increase in the transporter protein (Muchhal and Raghothama, 1999). This means that plants increase their capacity for phosphate uptake in response to P deficiency by synthesis of additional transporter molecules. If phosphate transporters are responsible for arsenate uptake, then arsenate uptake should be enhanced in P-deficient plants. Such an effect has been reported in barley (*Hordeum vulgare*; Lee, 1982) and in the As-nonresistant population of *H. lanatus* (Meharg and Macnair, 1992), and was demonstrated clearly in this study for the As hyper accumulator .*P. vittata* (Wang *et al.*, 2002). Their results correspond to our findings. However, the formation of Pi and its utilization in root in response to these diverse stimuli plays the critical role for the paddy to survive in such abiotic stresses.

Cold acclimation has been found to be a major abiotic stress causing the alteration of root inorganic phosphate in the growing paddy. Although the mechanism of the alteration in this molecule in root is not clarified, however, it might be linked to a variety of cellular metabolic pathways, either by formation of the ROS, rigidification of root cell membrane causing the exposure of calcium channel and thereby affecting the calcium ion signaling. It was found that during the development root growth, Pi was

reduced in response to cold and higher arsenic potentially was involved to enhance cold induced effect. The alteration of Pi in root might be an index for the formation of ATP or its degradation to release Pi. Both these stimuli cause an adverse environment where the paddies survive however alteration of Pi is caused by the abiotic stresses of these effects.

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