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An Impairment of Urea Induced Root Development of Paddy (*Oryza Sativa*): An Adverse Effect of Cold Acclimation

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Abstract Root growth and development is affected by different ways although the mechanism is not clarified. To identify the factors involved in impaired growth, we used pot experiment for cultivation of rice (*Oryza sativa*) and examined the effects of cold acclimation on urea induced root number, root length and shoot length. The root numbers and root length were severely impaired whenever exposed to cold for 48h and 72h of the treatment while mild effect was observed after 24h duration when compared to the control. Acclimation of cold in presence of urea also reduces the root numbers and root length effectively however, enhanced values were found after 24h of the treatment. To clarify whether, plant growth is affected by these two effectors; we examined the effects of cold and urea exposure on shoot length and were found to be impaired for prolonged time while the stimulatory effects on shoot length had been found after 24h of the treatment. Our findings demonstrate that prolonged cold acclimation causes the adverse effects on the development of urea induced plant and root growth, however short term acclimation may not impair the urea induced growth of roots.

Keywords: adaptive response, cold acclimation, metabolic effects, root growth, urea

Introduction

Root development is impaired either by environmental or chemical effectors. Temperature fluctuation is a common phenomenon of the atmosphere and is involved in changes of various metabolic functions (Janska *et al.*, 2010). 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. In mammals, including humans, the physiological responses involve changes in energy expenditure, heat production and dissipation, physical activity and appetite (Lowel and

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Spiegelman, 2000). Changes in environmental temperature affect the plant kingdom either by suppression of their total growth and development or by augmenting diverse both physiological and superficial changes. It has been revealed that roots and other protected parts are less cold hardy than the aerial parts of the plant (Havis, 1976; 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. The roots of plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones and storage functions. Urea has been recently used as a potent fertilizer inducing fertility and plant growth. For higher production of rice, urea is applied to the soil. It is reported that urea enhances the synthesis of proteins, enzymes (Claus-Peter *et al*., 2002; El-Shora, 2001) and other cellular functions (Nishi *et al*., 2001). Cell proliferation and differentiation in response to urea has been observed. Plants optimize and regulate nitrogen acquisition by regulated expression of specific transport proteins. Expression and activity of such transporters depend on the environment, nutrient availability, competitors, stress and metabolism. Therefore, to fully understand the morphogenesis of roots, it is necessary to define the organization of the root meristem and determine the fate of cells that emerge from the meristem. The present investigation has been carried out to clarify the role of cold acclimation on root number, root length and shoot length of paddy induced by urea to identify root development and plant growth.

Materials and methods

Soil collection and pot preparation

The soil was collected from the rice field of Rajshahi University Campus, Bangladesh 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 of rice (*Oryza sativa*) (BRRI-28), the following points were carried out: i) the strongest seeds were selected; the seeds were added to the normal water and floating seeds were discarded; ii) the seeds were kept in normal water with temperature below 37 ^{o}C in overnight; iii) the seeds were swollen by water absorption and were expected to be effective for germination; iv) the seeds were seeded in the pots prepared with soil and the efficiency of seed germination was about 90%.

Cold acclimation and urea treatment

Three plastic pots were prepared with soil and seeded with *Oryza sativa*. After 10 days of germination, the three different pots were described as control, cold and urea 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 ^{o}C) with full aeration. In the third pot, paddies were treated with urea (10 mM) and kept similarly in cold for 24h, 48h and 72h in the cold chamber. 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.

Determination of root number, root length and shoot length

After 10 days of germination and treatment, the paddy with roots were collected from the pots carefully and cleaned with water. In case of cold and urea treatment, paddies with roots were collected from the pots after 24, 48 and 72 hours. The root numbers were immediately measured by counting visually. The root length and shoot length were estimated very carefully by using the measuring scale and then paddy was stored in refrigerator at -20 ^{o}C.

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.

Results

Effect of cold acclimation and urea on the root number of paddy after 24h, 48h and 72h of treatment

To examine the role of cold exposure and urea treatment on the regulation of root number of paddy, the plants in the pot were exposed to cold for 24h in the cold chamber. As shown in Table-1, the average root numbers of paddy for control were 8.40 ± 0.34 while for cold treatment, the value was 9.60 ± 0.47.

The root numbers of paddy were increased by 14.28% ($P<0.1$) in response to cold. Whenever, the paddy was exposed to urea and cold, the different root numbers were observed. For example, the paddy exposed to urea and cold had the root numbers 8.50 ± 0.30 after 24 hours of treatment. The results indicated that the roots number of paddy had been found to be influenced by urea when compared to control, however, compared to cold acclimated paddy, the root numbers were decreased by 11.45% ($P<0.05$). Therefore, short term cold exposure may not be involved in the impairment of urea induced root growth and development.

The root numbers of different types of treated paddy were estimated as 10.50 ± 0.42 for control, 9.00 ± 0.25 for cold treatment and 9.80 ± 0.44 for urea and cold treatment after 48h of treatment (Table-1). The results showed that the root numbers of paddy had been decreased by 14.28% ($P<0.05$) when they were exposed to cold for 48h compared to control while urea treated paddy showed the increased root numbers when compared to cold acclimated paddy (8.88%). The results appeared to indicate that the root numbers of paddy were affected by both cold acclimation and urea; however, cold acclimation was critically involved to reduce the root number.

After 72 hours of treatment, the root numbers of different types of treated paddy were estimated as 9.30 ± 0.42 for control, 7.80 ± 0.32 for cold treatment and 8.40 ± 0.49 for urea and cold treatment (Table-1). Similar reducing effects on root growth were observed whenever the plants were exposed to cold as well as urea. The experimental results indicated that the root numbers of paddy had been reduced by 16.12% ($P<0.05$) for cold treatment and 9.67% ($P<0.05$) for urea and cold when compared to control, however, urea in presence of cold was found to be involved to enhance the root number compared to cold acclimated paddy.

Effect of cold acclimation and urea on the root length of paddy after 24h, 48h and 72h of treatment

The average root lengths of different types of treated paddy were recorded to determine the effect of cold on urea induced root growth of paddy. After 24 hours of treatment, the root lengths were estimated as 6.37 ± 0.32 cm for control, 7.35 ± 0.26 cm for cold treatment and 6.98 ± 0.17 cm for urea (10 mM) and cold treatment (Table-2). The root length of paddy had been increased by 15.38% ($P<0.1$) and 9.57% for cold treatment and urea and cold respectively when compared to control, however, the reduced effect on root length was observed by 10 mM urea and cold compared to cold acclimated paddy.

To examine the effect of cold on urea induced root length, plants were exposed to cold for 48h along with the combined effect of cold and urea. The reduced root lengths were observed whenever the plants were exposed to cold and urea for 48 h. As shown in Table 2, the root length of treated paddy were found to be 7.73 ± 0.10 cm for control and 7.41 ± 0.44 cm for cold treatment while 7.25 ± 0.18 cm for urea and cold treatment. It was found that the root length of paddy had been reduced by 4.13% for cold treatment and 6.20% for urea and cold treatment compared to control. Therefore, urea induced root growth is impaired in such an adverse environment created by cold acclimation showing its potency to prevent further extension of root length.

Table-2 shows the effect of cold on urea induced root length of paddy after 72 hours of treatment. Paddies treated with cold had root length 5.95 ± 0.17 cm where as 5.85 ± 0.18 cm root length for urea and cold treatment were observed. The root length of paddy for control was found to be 6.27 ± 0.11 cm. These results indicated that the root length of paddy had been reduced by 5.10% ($P<0.1$) for cold treatment and 6.69% ($P<0.01$) for urea and cold treatment compared to control however the effects were severe than the effect of 48h treatment showing the higher efficiency of cold acclimation on reducing urea induced root length after 72h of exposure.

Effect of cold acclimation and urea on the shoot length of paddy after 24h, 48h and 72h of treatment

To clarify whether cold acclimation and urea exposure are involved not only to root development but also to the plant growth of paddy, we examined the effect of these two effectors on the development of shoot length. The average shoot length of treated paddy were estimated as 6.59 ± 0.17 cm for control and 6.48 ± 0.29 cm for cold treatment while 7.01 ± 0.19 cm for urea and cold treatment after 24 hours of treatment (Fig. 1.). The results indicated that the shoot length of paddy had been found to be reduced slightly (1.66%) for cold treatment while increased by 6.37% for urea and cold treatment when compared to control suggesting that urea is potentially involved to promote the growth of root in this short exposure of cold.

As shown in Fig. 2., the shoot lengths of different types of treated paddy after 48 hours of treatment were estimated as 6.32 ± 0.25 cm for cold treatment and 6.93 ± 0.19 cm for urea and cold treatment while for control paddy, the value was 7.36 ± 0.17 cm. The shoot length of paddy had been severely affected and decreased by 14.13% ($P<0.01$) when they were exposed to cold for 48h compared to control. Similar reducing shoot lengths (5.84%) ($P<0.05$) were observed whenever they were treated with urea and cold, however, the result was lower than the cold acclimation alone. The results argued that prolonged cold acclimation gives an adverse environment where they survive and could not recovery the urea induced plant growth.

After 72 hours of treatment, the shoot lengths of treated paddy were found to be 6.23 ± 0.19 cm for control and 5.27 ± 0.09 cm for cold treatment. On the other hand, the paddy exposed to cold as well as 10 mM urea had shoot lengths 5.84 ± 0.15 cm (Fig. 3.). The results indicated that the shoot length of paddy had been decreased by 15.40% ($P<0.01$) for cold treatment and 6.26% ($P<0.05$) for urea and cold treatment compared to control. The results demonstrate that prolonged exposure of cold had profound effects on the impairment of paddy growth even in presence of urea.

Table 1. Effect of cold and urea on the root number of paddy after 24h, 48h and 72h of treatment

Treatment	Root number after 24h (n = 10)	Root number after 48h (n = 10)	Root number after 72 h (n = 10)
Control	8.40 ± 0.34	10.50 ± 0.42	9.30 ± 0.42
Cold	9.60 ± 0.47	9.00 ± 0.25	7.80 ± 0.32
Urea (10 mM) + cold	8.50 ± 0.30	9.80 ± 0.44	8.40 ± 0.49

The data are means ± SE for 10 paddies in each group.

Table 2. Effect of cold and urea on the root length of paddy after 24h, 48h and 72h of treatment

Treatment	Root length (cm) after 24h (n = 10)	Root length (cm) after 48h (n = 10)	Root length (cm) after 72h (n = 10)
Control	6.37 ± 0.32	7.73 ± 0.10	6.27 ± 0.11
Cold	7.35 ± 0.26	7.41 ± 0.44	5.95 ± 0.17
Urea (10 mM) + cold	6.98 ± 0.17	7.25 ± 0.18	5.85 ± 0.18

The data are means ± SE for 10 paddies in each group.

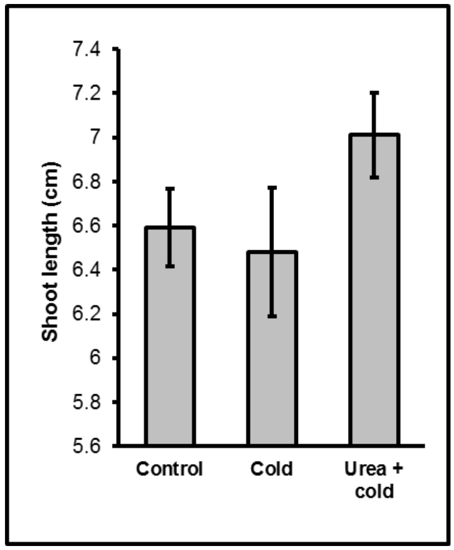

Fig. 1. Effect of cold and urea on the shoot length of paddy after 24h of treatment. The groups of paddy were treated with urea (10 mM) and kept for 24h in the cold. The paddies in another pot were exposed to cold for 24h only in the cold chamber. After the treatment, the paddy was immediately removed from the pot and sampling of shoot was performed. Control paddy was similarly used except giving cold exposure and urea treatment. The data are means ± SE for 10 paddies in each group

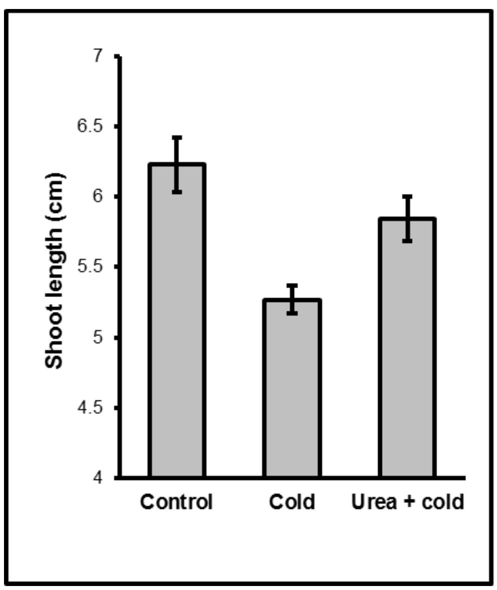

Fig. 2. Effect of cold and urea on the shoot length of paddy after 48h of treatment. The groups of paddy were treated with urea (10 mM) and kept for 48h in the cold. The paddies in another pot were exposed to cold for 48h only in the cold chamber. After the treatment, the paddy was immediately removed from the pot and sampling of shoot was performed. Control paddy was similarly used except giving cold exposure and urea treatment. The data are means ± SE for 10 paddies in each group

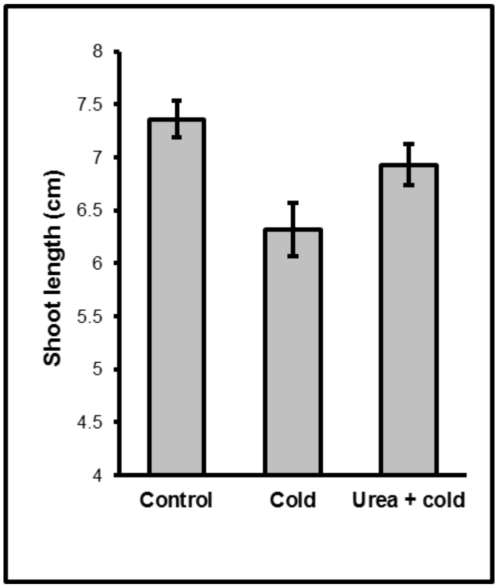

Fig. 3. Effect of cold and urea on the shoot length of paddy after 72h of treatment. The groups of paddy were treated with urea (10 mM) and kept for 72h in the cold. The paddies in another pot were exposed to cold for 72h only in the cold chamber. After the treatment, the paddy was immediately removed from the pot and sampling of shoot was performed. Control paddy was similarly used except giving cold exposure and urea treatment. The data are means ± SE for 10 paddies in each group

Discussion

Although root morphology is guided by a genetic program, the ultimate configuration of a root system under natural conditions is largely determined by environmental factors. The effects of gravity on root growth have been explored most extensively; roots generally respond in a positive fashion to gravity, with the root cap cells playing a major role in perception. Roots also respond to chemical gradients; they proliferate in regions of the soil that contain high concentrations of certain ions, such as nitrate or phosphate (Drew and Saker, 1975; Fitter *et al.*, 1988). In addition, root growth can be influenced by the soil moisture content, with roots penetrating deeper when soil moisture is low and developing air spaces (aerenchyma) when the soil is waterlogged. Although roots usually grow in a subterranean environment, light has been shown to affect root extension, gravitropism, and lateral root production in some species (Wilkins and Wain, 1974).

In our study, we found that cold acclimation causes an adverse environment where the paddies survive for their growth; therefore, the impaired root and shoot growth is possible. Recent study reveals that in woody species, cold hardening of roots is determined by genotype, soil temperature, and moisture (Wildung *et al*., 1973). However, little information on root cold hardiness and development following freezing is available for winter cereals. It has been suggested that roots and the lower portions of the crown of cereals are more susceptible to freezing injury than the leaves and upper crown tissue (Olien, 1981; Olien and Marchetti, 1976). There was a reduction in shoot and root growth in wheat plants frozen from -10 to -20 ^{o}C when transplanted to soil. The reduction in shoot growth was probably due to the effect of the lower temperatures on root regeneration. The growth of roots can be influenced by temperature gradients (Fortin and Poff 1990), mechanical impedence (Barley and Greacen, 1967), aeration (Cannell, 1977) and the roots of adjacent plants (Mahall and Callaway, 1991). Recent investigation reveals that urea promotes paddy growth; therefore, roots and shoots of paddy might be altered and influenced by urea treatment. The present study reveals that prolonged cold acclimation also prevents urea induced root and shoot growth. Therefore, cold acclimation is recognized to be a potent antagonist to yield an adverse effect for root growth and development.

All aspects of root development are profoundly affected by plant hormones, with the strongest effects attributed to auxin, cytokinins, and ethylene (Torrey, 1976; Feldman, 1984). Because of the difficulty in interpreting the effect of exogenously applied hormones on interna1 hormone ratios, there is considerable controversy in the literature as to the relative importance of various growth regulators on root development (Feldman, 1984). Alternative approaches, such as the analysis of transgenic plants in which hormone ratios have been modified in vivo by expression of hormone biosynthetic enzymes (Klee *et al*., 1987; Medford *et al*., 1989) and the characterization of mutants with reduced hormone biosynthesis or altered sensitivity, may help resolve many of the outstanding questions.

Conclusion

Cold acclimation has been found to be involved in the impairment of root development and plant growth. Our study reveals that urea has stimulatory effect on root and shoot growth and the rate of uptake of amino nitrogen in the plant is also related to the availability of urea in the soil as well as with the disappearance of soil urea. Although several factors might be involved in this respect, however, these two both environmental and chemical effectors are found to be predominantly involved. Our investigations will give the concept to find the new strategy for survival of the plant species in the critical situation like cold. It is assumed from the experimental results that the impairment of root growth is partially recovered by the availability of soil urea.

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