



## RESEARCH PAPER

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## Regulatory mechanism of enhancing fertilization of mango flower in response to urea application

Uttam Kumar Roy, Md. Shahidul Haque\*, Sohel Hasan, Swapan Kumar Roy, Narayan Roy

*Laboratory of Protein and Enzyme Research, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi 6205, Bangladesh*

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### Abstract

Urea is a potent N fertilizer inducing biological process of plants. However the regulatory mechanism of enhancing fertilization in mango flower in response to urea is not clarified. In this respect, flower of Langra variety of mango were used and the enzyme urease in flower extract when treated with 100 mM urea were maximally increased. For the regulation of urease activity, role of heavy elements like arsenic (As) and mercury (Hg) and light elements like zinc (Zn) and calcium (Ca) was investigated. 10 mM  $\text{Na}_2\text{HAsO}_4$  potentially prohibited the urea induced urease activity in flower than the effect of 1 mM concentration. Similar inhibitory effects were observed whenever the extract was treated with different doses of mercuric chloride (1, 10, 50, and 100 mM) and the effective concentration was 10 mM which reduces 36% of activity. On the other hand, the effects of 1, 10 and 100 mM  $\text{Zn}(\text{NO}_3)_2$  were examined on urea induced urease activity where 1 and 10 mM concentrations were found to enhance 30 and 58.4% enzyme activity respectively. We also examined the effect of different doses of  $\text{CaCl}_2$  (1, 10 and 100 mM) on urea induced urease activity and declined by increasing concentration of  $\text{CaCl}_2$ . However, the effective concentration of  $\text{CaCl}_2$  was 1 mM showing 103.2% increased activity. The findings indicate that calcium is more potent than zinc on increasing urease activity. To clarify the regulatory mechanism of urea induced primary amine synthesis, the flower was treated with 10 mM  $\text{Na}_2\text{HAsO}_4$  which potentially reduced color pigmentation for primary amine demonstrating clearly that arsenic is involved in prevention of primary monoamine during flowering.

\* **Corresponding Author:** Md. Shahidul Haque ✉ [haque\\_drshahidul@yahoo.co.in](mailto:haque_drshahidul@yahoo.co.in)

## Introduction

Mango is a delicious and nutritious fruit in Bangladesh and other countries; however its yield is retarded by different ways although the mechanism of this reason is yet to be identified. Nitrogen is the most limiting element in plant nutrition. Efficient recycling of reduced nitrogen present in the form of urea is important for plant growth since urea contains a significant amount of this element. In addition to internally generated urea, externally applied urea can also be utilized by plants. Urea is a widely used fertilizer because of its low cost, easy in handling and high nitrogen content. In plant, urease is the only enzyme that is able to recapture nitrogen from urea. Fertilization with urea through leaves could be an efficient method of plant feeding and any modifications leading to increased urease activity in leaves could result in more effective assimilation of this fertilizer. Such an increase might have positive impact on the nitrogen metabolism in plants since more ammonia would be available for assimilation. There is a possibility that the reduced activity of the enzyme may impair the fertilization process. Moreover, recent investigation suggests that deficiency of amino nitrogen causes pathogenic syndromes, pale appearance associated with other physiological disorders in plants (Zhao *et al.*, 2005). The disappearance of amino nitrogen from urea is an important aspect in nitrogen metabolism and an essential step for utilization to promote growth.

Primary monoamine has been recognized to be stimulatory factor for fertilization of mango flower and protective functions (Dey and Harborne, 1997). It is well known that even after havoc flowering in mango tree, percentage and numbers of mango fruits are very low, of course, adverse environmental factors are responsible for this effect. The flowers can not survive in that circumstances or lack of proper fertilization process is involved. However, other factors might be involved in this respect. Higher production of mango fruit after flowering might be retarded because of the deficiency of primary monoamine. Therefore, it is presumably assumed that if monoamine concentration is induced by any ways,

mango production will be accelerated. Urea has been considered as promoting agent and N-fertilizer for plant growth. Urea can induce the synthesis of primary monoamine and may augment the development of fruiting. The enhancement of fertilization in flower is an important aspect in mango production and the utilization of urea particularly the role of urease in this respect could be the subject matter in the current research and the regulation of this enzyme in presence of different modulators may give a new insight in the development of mango production. Therefore, the current study has been undertaken to involve the effect of urea on the synthesis of primary monoamine in mango flower. To examine whether urea induces the synthesis of primary monoamine, extract of flower was used for the test of primary monoamine. It might be assumed that augmentation of urease activity in response to urea is coupled to the synthesis of primary monoamine.

Environment is the major stimulus involved in metabolic regulation in plants and other organisms. Adverse effects caused by changing of the environmental stimuli might be involved in impairment of the fruit yield. Basic stresses such as drought, salinity, temperature and chemical pollutants are simultaneously acting on the plants causing cell injury and producing secondary stresses such as osmotic and oxidative ones (Wang *et al.*, 2003; Abu-Khadejeh *et al.*, 2012). Plants could not change their sites to avoid such stresses but have different ways and morphological adaptations to tolerate these stresses. Environmental stress can disrupt cellular structures and impair key physiological functions of plants. Changes in environmental temperature affect the plant kingdom either by suppression of their total growth and development or by augmenting diverse physiological, metabolic and superficial changes. Moreover, high temperature has been recognized to be involved in metabolic regulation and has been shown to cause the synthesis of ROS in plants (Mahajan and Tuteja, 2005). Therefore, it is assumed that variation of temperature may affect both metabolic activities as

well as its biological importance of this species of plant. Arsenic and other heavy metals are 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 the organisms. Exposure of higher concentration of arsenic in soil may also cause severe effects in plants and might be involved in producing diseases or other cellular effects. However, the mechanism underlying the effects of acute arsenic and mercury exposure on the regulation of oxidative and glycolytic processes in tissues of mango flower is not known. Although the identification of urease from several sources has been performed, its regulation is yet to be done. For the regulation of urease activity, role of arsenic and other heavy elements together with light elements like calcium (Ca) and zinc (Zn) has been investigated. These molecules can prohibit or induce the activity of enzyme. Moreover, these heavy metals have been found to accumulate in water spinach (Gothberg *et al.*, 2002). Therefore, the regulation of urease in mango flower is an important aspect in metabolism and may augment the clarification of urea induced fertilization in mango flower. The results are good agreement of the fertilization of mango flower and its regulation in the biosphere.

## Materials and methods

### *Plant material*

Mango flower (*Mangifera indica*) (Langra) was collected from the mango garden located to the northern side of the University Campus during February-March. The flowers were quickly stored at  $-80^{\circ}\text{C}$  refrigerator. About 5–6 g of flower were homogenized with mortar and pestle with 10 mM phosphate buffer (pH 7.0) and centrifuged at 6000 rpm for 10 min. After centrifugation, the supernatant was collected and used as crude extract while the sediment was discarded.

### *Assay of urease activity*

5.0 g of mango flower were placed into a mortar with pestle and were homogenized with 30 ml of 0.2 M

phosphate buffer (pH 7.0) and centrifuged at 6000 rpm for 10 min. The supernatant was collected and used as crude extract. The urease activity in crude extract of flower was assayed by the method as described by Jayaraman (1981). For assay of urease activity, 0.4 ml of the crude extract was used. To examine the effect of urea on urease activity, 50, 100 and 200 mM of urea were used as substrate of the enzyme. The enzyme activity was expressed as  $\mu\text{mole}/\text{min}/\text{mg}$  of protein.

### *Treatment with $\text{Na}_2\text{HAsO}_4$ , $\text{HgCl}_2$ , $\text{Zn}(\text{NO}_3)_2$ and $\text{CaCl}_2$*

To examine the role of different modulators (heavy and light elements) on the activity of urease in mango flower extract, the effect of arsenic compound (1 and 10 mM  $\text{Na}_2\text{HAsO}_4$ ) was performed. Different concentrations of mercuric chloride (1, 10, 50 and 100 mM) were used to examine the role of Hg on urease activity. Similarly  $\text{Zn}(\text{NO}_3)_2$  solutions (1, 10 and 100 mM) was used to find the role of Zn on the activity of urease in the flower extract. Another compound  $\text{CaCl}_2$  (1, 10 and 100 mM) was also used to examine its effects on the enzyme activity in the extract. The flower sample extracts obtained by the homogenization procedure were treated with the different concentrations of the above modulators and the urease enzyme activity was determined accordingly by the conventional procedure as described above.

### *Test of primary monoamine*

400  $\mu\text{L}$  samples (5 g of flower in 30 mL solution) in a test tube was taken, mixed well with 0.5 mL urea (100 mM) and incubated at  $55^{\circ}\text{C}$  for 15 min. After incubation, 1.0 mL diluted HCl and 4 drops of 10%  $\text{NaNO}_2$  were added and cooled. In another test tube, a few drops of alkaline  $\beta$ -naphthanol were taken and the mixture of the previous test tube was added slowly to the alkaline  $\beta$ -naphthanol. An orange deep color was appeared showing the synthesis of primary monoamine in the flower sample. Control tube was similarly used for identification of primary amine where no urea was used and 1.5 mL diluted HCl were used and the color pigmentation was different from

the urea induced sample tube. To examine the role of arsenic on the regulation of primary amine synthesis in flower, similar procedure was done however the flower extract was treated with 0.5 mL  $\text{Na}_2\text{HAsO}_4$  (10 mM) instead of urea. Deep orange color was disappeared in response to arsenic.

#### 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 different doses of urea on urease activity

Urea is a potent N-fertilizer inducing the growth of plant and is involved in inducing diverse metabolic and physiological functions. To analyze whether urea is involved in stimulation of urease activity in flower of mango, different doses of urea (50, 100 and 200 mM) were used as substrate for the enzyme. Urease activity was examined by using 400  $\mu\text{L}$  flower extract where

the effects of 50, 100 and 200 mM urea were done. The urease activity in response to 50 mM urea was  $0.0178 \pm 0.0006$   $\mu\text{mole}$  while  $0.0244 \pm 0.00008$   $\mu\text{mole}$  for 100 mM and  $0.0196 \pm 0.0006$   $\mu\text{mole/mg}$  of protein/min for 200 mM were found. On the contrary, flower extract without any urea treatment contained the urease activity  $0.0130 \pm 0.0006$   $\mu\text{mol}$ ,  $0.0166 \pm 0.0006$   $\mu\text{mol}$  and  $0.0142 \pm 0.0006$   $\mu\text{mol/mg}$  of protein/min respectively. As shown in Table 1, urea causes an increase in enzyme activity significantly by 36.92% ( $p < 0.001$ ), 46.98% ( $p < 0.01$ ) and 38.02% ( $p < 0.05$ ) for 50, 100 and 200 mM concentrations when compared to the respective controls. However, 100 mM urea predominantly stimulated the urease activity compared to other doses thereby the dose might be an optimum for the urease activity. The urease activity might be regulated by the variation of temperature and be strictly followed by the availability of urea in the soil. Therefore, it is reasonable that the growth of mango tree along with the production of flower and other necessary development might be improved because of the higher uptake of urea in the soil.

**Table 1.** Effect of different doses of urea in flower extract (400  $\mu\text{L}$ ) on urease activity.

Substrate concentration (mM)	Urease activity ( $\mu\text{mole/mg}$ of protein/min)	
	Control	Urea
50	$0.0130 \pm 0.0006$	$0.0178 \pm 0.0006^{\text{B}}$
100	$0.0166 \pm 0.0006$	$0.0244 \pm 0.00008^{\text{C}}$
200	$0.0142 \pm 0.0006$	$0.0196 \pm 0.0006^{\text{A}}$

The data are means  $\pm$  SE. <sup>A</sup> $p < 0.05$  versus respective control. <sup>B</sup> $p < 0.001$  and <sup>C</sup> $p < 0.01$  versus respective control.

#### Effect of arsenic compound on urea induced urease activity in flower extract

Arsenic has been well recognized to be the potent and toxic compound causing the impairment of biological and biochemical processes in plants and other organisms. Therefore, to find the regulatory mechanism of urea induced urease activity in mango flower, the extracts were treated with 1 and 10 mM  $\text{Na}_2\text{HAsO}_4$  solution. As shown in Table 2, the urease activity in response to 1 mM  $\text{Na}_2\text{HAsO}_4$  was  $0.0250 \pm 0.0006$   $\mu\text{mole}$  while for the control, the value was  $0.0250 \pm 0.0006$   $\mu\text{mole/mg}$  of protein/min. Whenever, the extract was treated with 10 mM

arsenic, the enzyme activity in 400  $\mu\text{L}$  extract was  $0.0220 \pm 0.0003$   $\mu\text{mole}$  and for the respective control, the activity was  $0.0250 \pm 0.0006$   $\mu\text{mole/mg}$  of protein/min. 1 mM  $\text{Na}_2\text{HAsO}_4$  does not have any effect on urease activity however the activity was reduced significantly by 12% ( $p < 0.05$ ) in response to 10 mM  $\text{Na}_2\text{HAsO}_4$  when compared to the respective controls. Therefore, arsenic might be involved in prevention of urease activity thereby prevention of the total growth of flower and other functions.

#### Effect of $\text{HgCl}_2$ on urea induced urease activity in flower extract

As shown in Table 2, different concentrations of HgCl<sub>2</sub> (1, 10, 50 and 100 mM) were used to examine the urease activity in extract (400 µL) of mango flower induced by 100 mM urea. The urease activity in response to 1 mM HgCl<sub>2</sub> was 0.0202 ± 0.0006 µmole while for the control; the value was 0.0250 ± 0.0006 µmole/mg of protein/min. In response to 10 mM HgCl<sub>2</sub>, the enzyme activity in 400 µL extract was 0.0160 ± 0.0006 µmole and for the respective control, the activity was 0.0250 ± 0.0006 µmole/mg of protein/min. Whenever, the extract was treated with 50 mM HgCl<sub>2</sub>, 0.2918 ± 0.00008 µmole urease activity was observed. On the contrary, flower extract without HgCl<sub>2</sub> contained the urease activity 0.0250 ± 0.0006 µmol/mg of protein/min. Similarly, for 100 mM dose of HgCl<sub>2</sub>, the activity in the extract was 0.2441 ± 0.0006 µmol while for the control, the value

was 0.0250 ± 0.0006 µmol/mg of protein/min. HgCl<sub>2</sub> was found to reduce the enzyme activity significantly by 19.2% (p<0.001) and 36.0% (p<0.01) respectively for 1 and 10 mM concentration of HgCl<sub>2</sub> when compared to the respective controls. However, 10 mM concentration was found to be more potent in reducing the activity than 1 mM concentration. Accordingly, 1 and 10 mM HgCl<sub>2</sub> were found to be involved in prevention of urease activity specifically however, 50 and 100 mM concentrations did not show these effects. It is assumed that lower concentrations rather than higher concentrations were effective in this phenomenon. Of course, higher concentrations sometimes produce adverse effects in the biological system and also the effects of these concentrations might be non specific. Therefore, in the current study, 1 and 10 mM were considered to be the optimum in this respect.

**Table 2.** Effect of different doses of arsenic and mercury in flower extract (400 µL) on urease activity.

Treatments	Urease activity (µmole/mg of protein/min)
Control	0.0250 ± 0.0006
Na <sub>2</sub> HAsO <sub>4</sub> (mM)	
1	0.0250 ± 0.0006
10	0.0220 ± 0.0003 <sup>A</sup>
HgCl <sub>2</sub> (mM)	
1	0.0202 ± 0.0006 <sup>B</sup>
10	0.0160 ± 0.0006 <sup>C</sup>
50	0.2918 ± 0.00008
100	0.2441 ± 0.0006

The data are means ± SE. <sup>A</sup>p<0.05 versus control. <sup>B</sup>p<0.001 and <sup>C</sup>p<0.01 versus control.

#### *Effect of Zn(NO<sub>3</sub>)<sub>2</sub> on urea induced urease activity in mango flower extract*

To clarify the regulatory mechanism of urea induced urease activity in extract of flower, we examined the effect of 1, 10 and 100 mM Zn(NO<sub>3</sub>)<sub>2</sub> on urease activity in 400 µL flower extract. The urease activity in response to 1 mM salt was 0.0325 ± 0.00008 µmole while 0.0396 ± 0.00008 µmole for 10 mM and 0.0207 ± 0.00008 µmole/mg of protein/min for 100 mM were found. On the contrary, flower extract without treatment of Zn(NO<sub>3</sub>)<sub>2</sub> contained the urease activity 0.0250 ± 0.0006 µmol, 0.0250 ± 0.0006 µmol and 0.0250 ± 0.0006 µmol/mg of protein/min respectively. As shown in Table 3, Zn(NO<sub>3</sub>)<sub>2</sub> causes an increase in enzyme activity significantly by 30%

(p<0.05) and 58.4% (p < 0.05) respectively by 1 and 10 mM concentrations, while the activity was reduced by 17.2% for 100 mM concentration when compared to the respective controls. However, 10 mM concentration predominantly stimulated the urease activity compared to other dose (1 mM) of Zn(NO<sub>3</sub>)<sub>2</sub> thereby the dose might be an optimum for the urease activity. The urease activity might be regulated by the variation of temperature and be strictly followed by the availability of urea as well as Zn<sup>2+</sup> as cofactor for the enzyme in the soil. Therefore, it is reasonable that the growth of mango tree along with the production of flower and other necessary development might be improved because of the presence of Zn in the soil.

*Effect of CaCl<sub>2</sub> on urea induced urease activity in mango flower extract*

For the regulatory mechanism of urea induced urease activity in extract of flower, the effects of 1, 10 and 100 mM CaCl<sub>2</sub> on urease activity in 400 µL extract were examined. As demonstrated in Table 3, the urease activity in response to 1 mM CaCl<sub>2</sub> was 0.0508 ± 0.00008 µmole while 0.0407 ± 0.00008 µmole for 10 mM and 0.0349 ± 0.00008 µmole/mg of protein/min for 100 mM were found. On the contrary, flower extract without treatment of CaCl<sub>2</sub> contained the urease activity 0.0250 ± 0.0006

µmol/mg of protein/min for the respective doses. CaCl<sub>2</sub> treatment was found to cause an increase in enzyme activity significantly by 103.2% (p<0.01), 62.8% (p<0.01) and 39.6% (p<0.01) respectively compared to the respective controls. However, 1 mM CaCl<sub>2</sub> was more effective on increasing the urease activity than 10 and 100 mM concentrations. Therefore, it is reasonable that the growth of mango flower induced by urea might be linked to the higher activity of urease enhanced by the availability of Ca<sup>2+</sup> as the activator in the soil.

**Table 3.** Effect of different doses of zinc nitrate and calcium chloride in flower extract (400 µL) on urease activity.

Treatments	Urease activity (µmole/mg of protein/min)
Control	0.0250 ± 0.0006
Zn(NO <sub>3</sub> ) <sub>2</sub> (mM)	
1	0.0325 ± 0.00008 <sup>A</sup>
10	0.0396 ± 0.00008 <sup>A</sup>
100	0.0207 ± 0.00008
CaCl <sub>2</sub> (mM)	
1	0.0508 ± 0.00008 <sup>B</sup>
10	0.0407 ± 0.00008 <sup>B</sup>
100	0.0349 ± 0.00008 <sup>B</sup>

The data are means ± SE. <sup>A</sup>p<0.05 and <sup>B</sup>p<0.01 versus control.

*Effect of Na<sub>2</sub>HAsO<sub>4</sub> on primary mono amine content in flower*

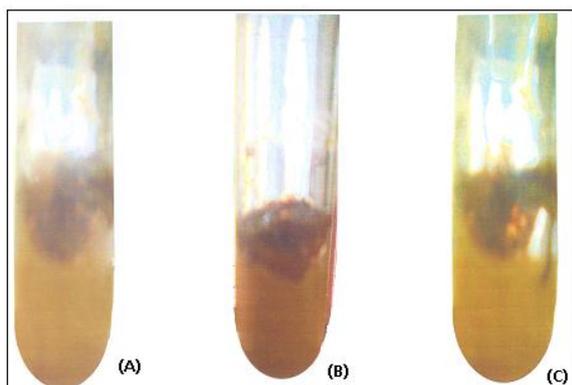
Primary monoamine has been recognized to be stimulatory factor for fertilization of mango flower and protective functions. To examine whether arsenic prevents the synthesis of primary monoamine, extract of flower was used for the test of primary monoamine. The color pigmentation induced by urea was prevented whenever the extract was treated with Na<sub>2</sub>HAsO<sub>4</sub> (10 mM) compared to the non-treated extract of flower (Fig. 1). The results agree that 10 mM arsenic is involved in prevention of the synthesis of primary monoamine. The results conclude that augmentation of urease activity in response to urea is coupled to the synthesis of primary monoamine and arsenic shows the toxic effect in the prevention of this biological process.

### Discussion

Due to change of climate and other environmental factors, insufficient growth and production of mango

are observed. Therefore, it is substantial to take the measure and make a plan to develop the strategy for identifying the causes and remedy for the development of higher mango production. Although few studies involved in mango research were done in Bangladesh, the current research project might be a significant aspect in higher yielding of mango fruit. Urea fertilization in the soil or foliar application may induce the growth of flower or fruiting and might be an essential approach in this respect. The previous investigation reveals that urea is a potent organic N fertilizer causing augmentation of the growth of plant (Abd and Faten, 2009). The enhanced urea degradation is because of the higher activity of urease in the biological system. Our current study reveals that urea administration stimulates the urease activity in the flower. The higher activity of urease in presence of different doses of urea (50, 100 and 200 mM) was observed however, 100 mM urea was found to be involved in enhancing the enzyme activity greatly and therefore, the dose is optimum for higher

activity of urease. The results also indicate that 100 mM urea induces the higher uptake of urea caused by the increased activity of urease. The previous investigations reveal that foliar spraying of urea and  $\text{KNO}_3$  significantly increased the flowering percentage of mango and generally  $\text{KNO}_3$  has been shown to give better results in flowering and fruiting (Khattab *et al.*, 2006). Jain (2006) indicated that single and double spray treatment with 4% urea gave maximum yield in Madhya Pradesh, India. The product of urease activity, ammonia is incorporated into organic compounds mainly by glutamine synthetase. Urease catalyzes the conversion of urea into ammonia, which is subsequently assimilated by the plant via glutamine synthesis. The main function of plant ureases is thought to be related to nitrogen recycling from urea either formed endogenously or derived from external sources (Sirko and Brodzik, 2000; Follmer, 2008). Urease also has a fundamental role in recycling exogenous urea used as fertilizer (Witte *et al.*, 2002). The findings from the current investigation reveal that the flower extract is a good source of urease which uses urea as a substrate for its catalytic activity. The enhanced activity in response to urea may induce the growth of flower along with other necessary parameters regarding fruiting and fertilization process.



**Fig. 1.** Primary monoamine synthesis in mango flower (control) (A), induced by 100 mM urea (B) and role of arsenic on primary monoamine synthesis in mango flower induced by 100 mM urea (C).

The regulatory mechanism of enhancing urease activity was clarified. The urea induced urease activity in extract has been found to be regulated in response to arsenic or mercury compounds. These

elements have been recognized to be heavy and toxic to the living organisms. Recent investigation reveals that arsenic impairs various metabolic activities in plants (Verbruggen *et al.*, 2009). Similarly, the heavy element Hg has been associated to involve the impairment of growth, physiology and other biological functions of plants (Azevedo and Rodriguez, 2012). Therefore, in the current study, these compounds have been considered as inhibitory molecules and have been found to impair the urease activity in flower extract. It is reasonable that, for mango production, these heavy elements will produce an adverse effect to the environment. Among the different doses of arsenic, 10 mM concentrations were found to inhibit the enzyme activity maximally rather than the other doses. Similarly,  $\text{HgCl}_2$  in different doses has been also found to involve the impairment of the urease activity however 1 and 10 mM concentrations were maximally associated in this respect. However, other doses did not show this inhibitory effect on urease activity and might be non specifically produce the effect. Of course, higher concentration sometimes may produce the adverse effect in the biological system. The effects of  $\text{ZnNO}_3$  and  $\text{CaCl}_2$  have been adopted to examine the role of Zn and Ca on urease activity in flower. The enzyme activity was up regulated in response to Zn and Ca salt and was recognized to be the positive modulators for this enzyme. It is speculated that these elements act as cofactors for the enzyme urease. Zn is involved in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis and degradation. Similarly,  $\text{Ca}^{+2}$  has been found to be involved in diverse metabolic and biological processes. Therefore, both  $\text{Zn}^{+2}$  and  $\text{Ca}^{+2}$  might be involved in metabolic regulation and act as cofactors in these metabolic processes. It has been demonstrated that magnesium, zinc and boron have promising effect on plant metabolism. They are responsible for producing the natural hormones IAA, activating some enzymes, biosynthesis of chlorophylls, enhancing germination of pollens and regulating water uptake by plants (Nijjar, 1985). Foliar application of nutrients especially magnesium, boron and zinc is essential for producing healthy

mango trees as well as producing productive trees. The regulatory mechanism of fertilization of flower was investigated in the current research project. Primary monoamine was recognized to be the potent compound responsible for fertilization process. Application of urea was found to enhance urease activity as well as the higher color pigmentation of primary monoamine. Therefore, it is assumed that 100 mM urea may induce the fertilization process. In separate investigation,  $\text{Na}_2\text{HAsO}_4$  was found to be involved in reducing the color pigmentation showing the impairment of synthesis of primary monoamine and fertilization process. The fertilization of mango flower might be affected by other environmental factors. Environmental stress can disrupt cellular structures and impair key physiological functions of plants. Drought, salinity and low temperature stress impose an osmotic stress that can lead to turgor loss. Membranes may become disorganized, proteins may undergo loss of activity or be denatured and often excess levels of reactive oxygen species (ROS) are produced leading to oxidative damage. Recent investigations reveal that high temperature induced injury is associated with the formation of oxidative stress which leads to activation of enzymes involved in the production of reactive oxygen species (ROS) (Zhu, 2002). To prevent the oxidative damage caused by such abiotic stress, plants generate the different mechanism by which they survive in such critical environment. Anti oxidative enzymes like super oxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are the most important components in the scavenging system of ROS. Several lines of evidences reveal that anti oxidative enzymes and anti oxidant molecules can neutralize ROS (Oidaira *et al.*, 2000; Lee and Lee, 2000). Polyphenol oxidase (PPO) and peroxidase (POD) have been widely recognized to be an anti oxidative causing the biosynthesis of diverse metabolites essential for diagnosis and other purposes and have been found to be involved in scavenging system of reactive oxygen species synthesized in the biological system. During drought or high temperature in the environment, these two enzymes might be involved in the prevention of oxidative damage in plant and therefore could be an

essential index for the adaptive mechanism in adverse circumstances. It has been demonstrated that mango is a rich source of various polyphenolic compounds. The amounts of the different polyphenolic compounds in the mango vary from part to part (pulp, peel, seed, bark, leaf and flower) (Masibo and He, 2008). The extracts from mango leaves, bark and flowers have been found to exhibit a wide range of pharmacological effects: antioxidant, anticancer, antimicrobial, antiatherosclerotic, antiallergenic, anti-inflammatory, analgesic and immunomodulatory etc. Therefore, it is assumed that the antioxidative enzymes like polyphenol oxidase and peroxidase could play the critical role on fertilization of mango flower during the adverse environmental circumstances.

### Conclusion

Although different doses of urea were used in this study, however, the results reveal that 100 mM concentration plays the critical role on enhancing urease activity in the mango flower extract. The increase activity of urease shows the higher uptake of urea in the flower because higher concentration of  $\text{NH}_4^+$  is formed in response to urea. To clarify the regulatory mechanism of enhancing the urease activity in extract of flower, the effects of different modulators have been performed. Among them, As and Hg have been found to be involved in the prevention of urease activity thereby these compounds might be involved in the prevention of mango production. However, the activity of urease has the higher specificity for the specific doses of these compounds. In separate studies, Zn and Ca were found to be involved in enhancing the urea induced urease activity in the extract. Therefore, these molecules play the vital role on the regulation of the urease activity, there by the augmentation of the flowering in the environment. Primary monoamine has been believed to be involved in fertilization of mango flower and other protective functions. To examine whether arsenic prevents the synthesis of primary monoamine, extract of flower was used for the test of primary monoamine. The color pigmentation induced by urea was prevented

whenever the extract was treated with Na<sub>2</sub>HAsO<sub>4</sub> (10 mM) compared to the non-treated extract of flower. Therefore, it is reasonable that arsenic might be a toxic substance for plant fertilization and might be a potent inhibitor of the synthesis of primary monoamine which would have severe effects on the impairment of mango fruit production.

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