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Change in Some Hydrolytic and Oxidative Enzymes Activities of Jute Leaves under Different Foliar Treatments

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Abstract: An experiment was conducted to find out the enzymatic change in jute leaves under different foliar treatments in the experimental land of the Institute of Biological Sciences, Rajshahi University during the period of April-May, 2000-2001. The effect of NPK-fertilizers and cowdung as well as foliar spray of urea and chemicals on the changes in the enzyme contents of matured jute leaves was analyzed. It was found that the activities of all the experimental enzymes in the leaves from treated jute plants were increased significantly as compared to those in control ones. Of the enzymes examined, the activities of protease were found to be increased highest (93.33%), followed by cellulase (88.57%), invertase (87.50%) and so on in decreasing order.

Key words: Matured jute leaves, foliar treatment, oxidative and hydrolytic enzymes content

Introduction

After rice, jute is the most important agriculture crop that grows abundantly in Bangladesh. For the development of diversified use of jute plants and jute fibre as cellulosic raw materials, it is very important to study the agro-chemical effect on whole jute plant. Jute leaf is a lateral outgrowth from a stem and is typically a thin, expanded structure with usually a green colour which is the most important vegetable fibre extensively cultivated in our country and is about 90% of total vegetable product. Although jute is mainly a fibre crop, the young leaves are used as vegetables. These young leaves of jute can occupy major portion of our vegetable diets if we know its food value and role in preventing micronutrients deficiency and malnutritional problem in Bangladesh. The jute leaves are picked several times during the growing phase as also during the thinning of the jute plant for use as vegetable. In Bangladesh vegetables are common as well as cheap source of vitamins and minerals. Therefore, optimum utilization of vegetables rich in nutrients is of paramount importance to minimize the micronutrients gap and thereby improve nutritional status in this country.

For human beings and all animals, trace element as micronutrients are essential constituents in the diet. They play a crucial role virtually in all biological processes to sustain normal life. Majority of the diseases begin when bio-chemical imbalance occurs at the cellular level.

Proteolytic and hydrolytic enzymes play an important role in numerous biological and biochemical processes and were important in human nutrition.

It was found in the present study that the physico-chemical properties of jute leaves are greatly affected by employing NPK-fertilizers in combination with foliar spray of urea and chemicals. In agriculture, interaction between fertilizer and moisture plays a significant role. The choice and the dosages of fertilizers are related to the soil condition and irrigation potential. By application of a balance dosage of fertilizers, jute leaves yield can be increased by 100% under rain-fed conditions and 150% under irrigated conditions (Ullal and Narasimhanna, 1978).

The enzymes are widely distributed throughout the plant kingdom. Proteolytic and hydrolytic enzymes may play some physiological roles during maturation and senescence of fruit (Hasinaga, 1983; Desai and Deshpande, 1978b; Mahadevan and Sriddhar, 1982). Furthermore, foliar spray of urea solution on leaves has been reported to increase leaf yield and leaves nutrients.

In the present study, we first time studied the effects of NPK-fertilizers in combination with foliar spray of urea and chemicals on the content of some hydrolytic and oxidative enzymes in jute leaves at matured stage. The overall experimental data indicated that treatment of jute plant cultivated lands with NPK-fertilizers and cowdung as well as by foliar spray of urea, chemicals and mineral mixtures might be helpful in improving the quality of jute leaves and fibre.

Materials and Methods

The experiment was carried out at the experimental land of the Institute of Biological Sciences, Rajshahi University, Bangladesh during the season April-May, 2000 and 2001. The experiment was laid in a randomised complete block design (RCBD) with ten treatments and one control. The plot sizes being used were 4×5 m with a walking path between the plots of 0.50 m. The treatments being used denoted as T_1 is control while T_2, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} are the experimental. Before sowing of jute seed, cow-dung and NPK-fertilizers were applied at the ground level. Each plot received cow-dung at the rate of 250 kg ha^{-1} and NPK-fertilizers 200 kg ha^{-1}.

Firstly, after 30 days of sowing foliar spray of 0.1% of urea solution and 0.1% KNO_3 solution were performed on jute leaves in different blocks systematically.

Secondly, after 50 days of sowing foliar spray of 0.1% of chemicals were applied on jute leaves. The NPK-fertilizers were applied in the form of urea (N), triple super phosphate (P) and murate of potash (K) in a broadcast method and as chemicals KCl, $Ca_3(PO_4)_2$ and Na_2HPO_4 were used. The experiment was repeated in two seasons.

After 70 days of sowing mature jute leaves were collected from each plot and analyzed the activities of experimental enzymes in triplicate.

The data obtained was statistically analyzed by the method of analysis of variance (ANOVA) and the differences due to treatment means were determined using Dancan's multiple range test (DMRT) by MSTAT programme (Nissen, 1988).

Preparation of crude enzyme extract

At first 10 gm of jute leaves were cut into small pieces and grinded in a mortar with pestle and then homogenized well with cold 0.1 M phosphate buffer of respective pH (for amylase: pH

6.7, for invertase and protease: pH 7.0, for polyphenol oxidase, peroxidase and ascorbic acid oxidase: pH 6.0) while for the measurement of cellulase 0.1 M sodium acetate buffer, pH 5.2 were used. Then the extract was filtered through a double layer of muslin cloth. After centrifugation at 6,000 g for 10 min the supernatant was used as crude enzyme extract.

Amylase activity

Amylase activity was assayed following the method as described in laboratory Manual in Biochemistry (Jayaraman, 1981). One percent starch solution was used as substrate. The amylase activity was measured by estimating the release of maltose. The amount of maltose released was calculated from the standard curve which was prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1 µg of maltose per min at 37^{0}C.

Protease activity

The protease activity was measured following the method of Kunitz (1947) using casein as a substrate. The activity is determined by detecting the release of amino acid (tyrosine). The amount of tyrosine released was calculated from the standard curve which was constructed with tyrosine. One unit of protease activity was defined as the amount required for liberating 1 µg of tyrosine per min at 45^{0}C.

Cellulase activity

The cellulase activity was measured following the procedure as described in Physiological Plant Pathology (Mahadevan and Sridhar, 1982). Cardoxymethyl cellulose was used as substrate. Cellulase activity was measured by estimating the release of reducing sugar by cellulase. The amount of reducing sugar released was determined by dinitrosalicylic acid method (Miller, 1972). One unit of cellulase activity was defined as the amount of enzyme required for liberating 1 µg of reducing sugar per min at 37^{0}C.

Invertase activity

Invertase activity was assayed following the modified method as described in Physiological Plant Pathology (Mahadevan and Sridhar, 1982). Sucrose was used as substrate. The invertase activity was measured by estimating the release of glucose. The amount of glucose released was calculated from the standard curve which was prepared with glucose. One unit of invertase activity was defined as the amount required for liberating 1 µg of glucose per min at 30°C.

Ascorbic acid oxidase activity

Ascorbic acid oxidase activity was measured following the procedure as described in Physiological Plant Pathology (Mahadevan and Sridhar, 1982). In this process ascorbic acid was used as substrate. The enzyme activity was measured by determining the residual ascorbic acid in the reaction mixture. The enzyme activity was described as units min^{-1} gm^{-1} leaf under assay condition. An increase in absorbance by 0.01 at 265 nm in 1 min was taken as one unit of enzyme activity (Kaul and Munjal, 1980).

Polyphenol oxidase activity

The polyphenol oxidase activity was measured following the procedure as described in Physiological Plant Pathology (Mahadevan and Sridhar, 1982). In this method catechol was used as substrate. One unit of enzyme activity was defined as a change in absorbance of 0.01 at 420 nm per min.

Peroxidase activity

The peroxidase activity was measured following the procedure as described in Physiological Plant Pathology (Mahadevan and Sridhar, 1982). In this method pyrogallol is used as substrate. In presence of H_2O_2 pyrogallol is oxidized to coloured derivative. The amount of purpurogallin formed during the reaction can be estimated in a spectrophotometer. One unit of peroxidase is defined as the amount of purpurogallin formed per min under the assay condition.

Results and Discussion

The amount of hydrolytic and oxidative enzymes viz; amylase, cellulase, irotease, invertase, ascorbic acid oxidase and polyphenol oxidase activities present in treated and control jute leaves at matured stage are presented in Table 1-3. The enzymatic change in jute leaf under different treatments analyzed by the method of analysis of variance (ANOVA) and the results obtained showed significant difference among the treatments.

Amylase activity

Amylase is a hydrolytic enzyme which hydorlyses starch to yield monomeric carbohydrate. From the results (Table 1) that the highest amount of amylase activity in jute leaf was found in treatment T_6 and the lowest amount was in T_1 (control). The order of amylase activity in jute leaves was found as $T_6 > T_4 \geq T_5 \geq T_{10} > T_3 = T_{11} > T_8 > T_9 > T_7 = T_2 > T_1$ but T_4, T_5, T_{10} and T_2, T_7 were jointly affected at 5% level of significance by DMRT. The results obtained in the present study clearly demonstrated that the activity of amylase in jute leaf was increased by about 12 to 83% after application of NPK-fertilizers and cow-dung in the cultivated land as well as with the foliar spray of urea and mineral mixtures. Similar result was reported by Desai and Deshpande (1978b), Nabeesa and Unnikrishanan (1988), Mao and Kinsella (1981) and Garcia ***et al.*** (1988) in case of banana.

Protease activity

Protease is a hydrolytic enzyme which acts on proteinaceous substances to produce amino acids and amides. The protease activity in jute leaf showed significantly difference between different treatments. The results (Table 1) clearly indicated that the protease activity in jute leaves increased highly under different treatments. The maximum protease activity was recorded in jute leaves from treatment T_{11} and the minimum amount was found in that from treatment T_1 (control). In this study, protease activity was found to be increased by 50 to 93% due to application of NPK-fertilizers and cow-dung in the soil as well as by foliar spray of urea and mineral mixtures. Hashinaga ***et al.*** (1978) reported that protease activity (KFP-1) increased in

Table 1: Activities of amylase and protease and cellulase in jute leaves under different treatments

	Name of enzymes					
	Amylase activity		Protease activity		Cellulase activity	
Treatments	Unit min^{-1} gm^{-1}	(%) increase	Unit min^{-1} gm^{-1}	(%) increase	Unit min^{-1} gm^{-1}	(%) increase
T_1	10.00±0.001a	-	1.20±0.002a	-	7.00±0.002a	-
T_2	11.24±0.002b	12.40	1.98±0.001c	65.00	9.00±0.003b	28.57
T_3	14.22±0.002e	42.20	1.95±0.001c	62.50	9.25±0.001c	32.14
T_4	16.29±0.003g	62.90	2.00±0.001d	66.66	10.12±0.001d	44.57
T_5	15.52±0.002fg	58.20	2.10±0.002e	75.00	10.38±0.002e	48.29
T_6	18.25±0.002h	82.50	2.20±0.002g	83.33	12.48±0.002h	78.28
T_7	11.55±0.001b	15.50	1.80±0.002b	50.00	8.85±0.001ab	26.43
T_8	13.24±0.001d	32.40	2.12±0.001f	76.66	9.22±0.001bc	31.71
T_9	12.34±0.001c	23.40	1.99±0.002cd	65.83	11.66±0.002g	66.57
T_{10}	15.14±0.002f	51.40	2.12±0.002f	81.66	13.20±0.002l	88.57
T_{11}	14.12±0.003e	41.20	3.00±0.001h	93.33	11.33±0.003f	61.86

In columns, the common letter is not significantly different at 5% level of significance by DMRT, T_1: Control; T_2: Control+Cow-dung; T_3: Control+Cow-dung+KNO_3; T_4: Control+Cow-dung+Urea; T_5: Control+Cow-dung+KNO_3+Minerals* ; T_6: Control+Cow-dung+Urea+Minerals*; T_7: Control+NPK-fertilizers; T_8: Control+NPK-fertilizers+KNO_3; T_9: Control+NPK-fertilizers+Urea; T_{10}: Control+NPK-fertilizers+KNO_3+Minerals* and T_{11}: Control+NPK-fertilizers+Urea+Minerals*; *KCl, $Ca_3(PO_4)_2$, Na_2HPO_4

Kiwifruit flesh during ripening. Increased protease activity was also observed in passion fruit juice during maturation. Increased protease activity during matured stage may be attributed to protein catabolism which is related to leaf senescence (Dilley, 1970).

Cellulase activity

Cellulase is also hydrolytic enzyme, produced by the bacteria in the digestive tracts of animaland is responsible for release of glucose from cellulose. Many plant pathogens are also known to produce either adaptively or non-adaptively proteoalytic, cellulolytic and various polysaccharides (Wood, 1960). The cellulase activity in jute leaf showed significantly different under different condition. As presented in Table 1 the maximum amount of cellulase activity was found in jute leaf from treatment T_{10} and the minimum amount was found in that of T_1 (control). The sequence of order of cellulase activity was found as- $T_{10} > T_6 > T_9 > T_{11} > T_5 > T_4 > T_3 \geq T_8 \geq T_2 \geq T_7 \geq T_1$ but treatments T_1, T_2, T_3, T_7 and T_8 were intercorrelated at 5 % level of significance by DMRT. Strikingly, the content of cellulase activity in jute leaf was increased by about 26 to 89% under different treatments.

Invertase activity

Invertase is a hydrolytic enzyme, which hydrolyzes sucrose to glucose and fructose. Invertase activity in jute leaf under different treatments increased significantly. The present findings (Table 2) indicated that the maximum invertase activity was found in jute leaf from treatment T_{10} and the minimum amount (0.08±0.002 units min^{-1} gm^{-1} leaf) was recorded in that from T_1. Although

jute leaves contained small amount of invertage but present findings clearly demonstrated that the content of invertase in jute leaf was increased by about 13-88% under different treatments. The invertase activity in jute leaf was found in order of- $T_{10} > T_6 > T_5 > T_9 = T_4 = T_3 \geq T_{11} \geq T_8 > T_7 = T_2 > T_1$ but T_2 and T_7 and T_3, T_4, T_8, T_9 and T_{11} were intercorrelated at 5% level of significance by DMRT.

Ascorbic acid oxidase

Ascorbic acid oxidase catalyzes directly the oxidation of ascorbic acid by molecular-oxygen, according to the equation.

$$\text{Ascorbic acid} + \tfrac{1}{2}\, O_2 \longrightarrow \text{Dehydroascorbic acid} + H_2O$$

The ascorbic acid oxidase content in jute leaf under different treatments also increased significantly under different treatments. The maximum activity of ascorbic acid oxidase was found in treatment T_5 and the minimum amount was found (Table 2) in T_1 (control). Further, the contents of ascorbic acid oxidase in jute leaves was increased by about 10-35% under different treatments. The order of ascorbic acid oxidase was found as- $T_5>T_{11}>T_{10}\geq T_3\geq T_9>T_8>T_6>T_4=T_7>T_2>T_1$ but T_4, T_7 and T_3, T_9, T_{10} were jointly affected at 5% level of significance by DMRT.

Table 2: Activities of invertase and ascorbic acid oxidase in jute leaves under different treatments

	Name of enzymes			
	Invertase		Ascorbic acid oxidase	
Treatments	units min^{-1} gm^{-1}	(%) increase	units min^{-1} gm^{-1}	(%) increase
T_1	0.08±0.002a	-	24.00±0.001a	-
T_2	0.09±0.001b	12.50	26.50±0.003b	10.41
T_3	0.11±0.003d	37.50	29.35±0.001ef	22.72
T_4	0.12±0.003d	50.00	27.01±0.002c	13.27
T_5	0.13±0.002e	62.50	32.40±0.005h	35.00
T_6	0.14±0.002f	75.00	28.00±0.003d	16.00
T_7	0.09±0.003d	12.50	27.00±0.004c	12.00
T_8	0.10±0.001c	25.00	28.14±0.001d	19.33
T_9	0.12±0.001d	50.00	29.00±0.002e	24.56
T_{10}	0.15±0.002g	87.50	30.40±0.003f	26.67
T_{11}	0.11±0.001cd	37.50	31.00±0.002g	29.17

In columns, the common letter is not significantly different at 5% level of significance by DMRT See the meaning of T_1, T_2, T_3 etc. under the Table 1.

Polyphenol oxidase

Polyphenol oxidase activity in jute leaf under different treatments showed significantly difference among the treatments. The results (Table 3) revealed that the maximum polyphenol oxidase activity was found in jute leaf from treatment T_{11} and the highest percentage of increase was 75.00% (T_{11}) compared to that of control while the minimum polyphenol oxidase activity was found in that from T_1. The order of polyphenol oxidase activity was found as T_{11} >

Table 3: Activities of polyphenol oxidase and peroxidase in jute leaf under different treatments

	Name of enzymes			
	Ployphenol oxidase		Peroxidase	
Treatments	units min^{-1} gm^{-1}	(%) increase	units min^{-1} gm^{-1}	(%) increase
T_1	16±0.002a	-	40±0.002a	-
T_2	18±0.001b	12.50	50±0.003b	25
T_3	22±0.003e	37.25	56±0.001cd	40
T_4	19±0.003c	21.45	64±0.002f	47
T_5	26±0.001g	62.50	62±0.001e	55
T_6	20±0.002d	25.63	68±0.003h	70
T_7	19±0.005c	18.75	52±0.002c	30
T_8	23±0.004ef	38.26	59±0.002d	46
T_9	25±0.004fg	46.59	64±0.005g	52
T_{10}	25±0.002f	56.25	66±0.004g	65
T_{11}	28±0.003h	75.00	70±0.003i	75

In columns, the common letter is not significantly different at 5% level of significance by DMRT

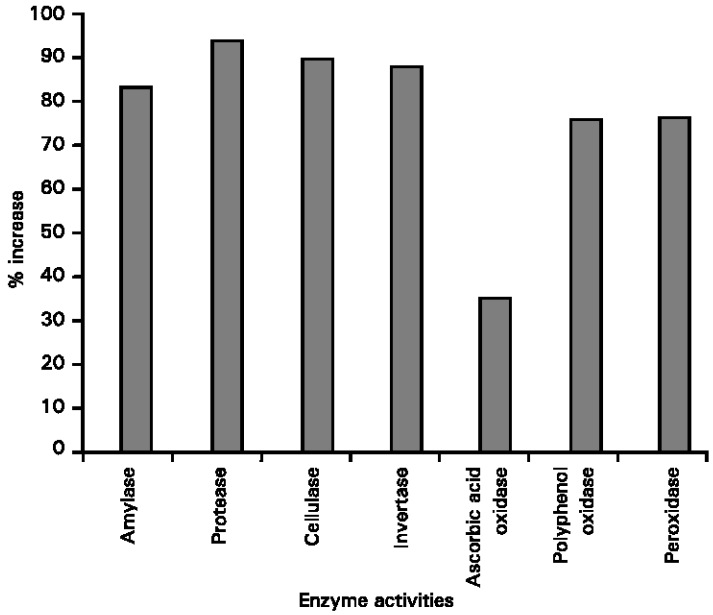

Fig. 1: Comparative study of the activities of both hydrolytic and oxidative enzymes in jute leaves

$T_5 \geq T_9 \geq T_{10} \geq T_8 \geq T_3 > T_6 > T_4 = T_7 > T_2 > T_1$ but T_4, T_7 and T_5, T_9, T_{10}, T_8, T_3 were jointly affected at 5% level of significance by DMRT.

Peroxidase activity

Peroxidases are widely distributed in the plant kingdom. Peroxidase activity in jute leaf under different treatments increased significantly under different treatments. As presented

in Table 3, the maximum amount of peroxidase activity was found in jute leaf from treatment T_{11} and the minimum amount was found in treatment T_1. The highest percentage of increase (75%) of peroxidase activity was observed in jute leaf from T_{11}. The sequence of order of peroxidase activity in jute leaf was found as $T_{11}>T_6>T_{10}\geq T_9\geq T_4>T_5>T_8\geq T_3\geq T_7>T_2>T_1$ but T_8, T_3, T_7 and T_{10}, T_9, T_4 were jointly affected at 5% level of significance by DMRT.

The comparative data in the increase of the activities of both hydrolytic and oxidative enzymes in jute leaves as compared to those of untreated leaves are shown in Fig. 1. The activities of protease were found to be increased highest, followed by cellulase, invertage and so on in decreasing order. In conclusion the present data clearly indicated that the activities of both hydrolytic and oxidative enzymes in jute leaves were increased remarkably after application of NPK-fertilizers and cowdung as well as with the foliar application of urea and chemicals. Strikingly, the activities of hydrolytic enzymes were increased slight more pronouncedly than those of oxidative enzymes.

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