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Effect of Fertilizer and Inoculation on Lipase and Urease Activity of Mature Soybean cv. Williams-82 Seeds

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Abstract: A field experiment on soybean cv. Williams-82 was conducted in clay loam soil with six different level (T_2 to T_6) of added N fertilizer @ 23, 25, 50, 75, 100 and 125 kg ha⁻¹ plus a constant dose of P₂O₅ + K₂O fertilizer @ 60 + 30 kg ha⁻¹ respectively, Whereas T_1 with zero level of added NPK was kept control. These six fertilizer treatments were applied to both non-inoculated and inoculated field grown soybean crop. After harvest the mature dry seed of each treatment were analyzed for their lipase and urease activity. Results showed that fertilizer treatments significantly and positively affected the lipase activity in general and urease activity in particular. Statistically maximum activity of both enzymes was recorded in T_3 dose of fertilizer. Results further revealed that by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation significantly increased the urease activity, but reverse was true for lipase activity. Statistically maximum activities of both enzymes were also noted in T_3 dose of inoculated treatment. It was also concluded that lipase activity exhibited significant positive association with starch and soluble sugars, but negative with seed protein and moisture content. On the other hand urease activity showed significant positive correlation with oil and moisture content, but negative with starch content. However, they both were insignificantly correlated with grain yield and remaining biochemical components. Therefore, seed lipase activity in particular and urease activity in general could not be used a suitable selection criteria for predicting the quantity as well as quality of grain yield in soybean cv. Williams-82.

Key words: Soybean, fertilizer, inoculation, lipase, urease and correlation

Introduction

Lipids are important storage compounds of many plant tissues. From a human nutritional point of view the oil storing seeds of most nut crops, castor bean, cucurbits, peanut, soybean, sunflower are the best known among the oil-storing tissues. They are high in unsaturated fatty acids and may contain the essential fatty acids. Fats occur only in small amounts in roots, stems, leaves and fruits, but are abundant in the cotyledonary and endospermic seed (Akhtar *et al.*, 1975; Ting, 1982; Illahi, 1995). Since most of the storage lipids is in the form of triglycerides. While in jojoba (*Symmondsia chinensis* (Link) C.K. Schneid) it is present in the form of wax ester (Kayani *et al.*, 1990).

Lipases (Triacylglycerol acylhydrolase, EC 3.1.1.3) have been used as a biocatalyst for a variety of reactions viz., hydrolysis of fats, synthesis of glycerides & esters and modification of lipids. Lipases are soluble enzymes that bind to lipid/water interfaces where they catalyze hydrolysis of the water-insoluble lipids to more polar products. The high specificity of lipase towards triglyceride substrate with respect to the type and stereospecific position of the fatty acid residue has prompted a number of special applications within the area of food and biosurfactants. With the increased interest in

lipid metabolism, lipase assay in biological samples is getting an attention as a routine test in clinical laboratories (Yaqoob *et al.*, 1994, 1997 and 1999).

Urease, an enzyme which is commonly used in clinical laboratories for the estimation of urea. It is also thought to be involved in ammonia loss from urea fertilizer added in agricultural fields.

As far as we know, a great deal of work has been reported on the lipase activity in particular and urease activity in general in a number of vegetable seeds, fruit seeds, legume seeds and other samples (Akhtar *et al.*, 1975; Masood and Rana, 1989; Kayani *et al.*, 1990; Zonia *et al.*, 1995; Arroyo *et al.*, 1996; Qasim, 1997). But no extensive work has been yet reported on the effects of various level of added N fertilizer on the lipase and urease activity of non-inoculated and inoculated soybean cv. Williams-82 seeds. Therefore an attempt has been made by the authors to purify both enzymes and investigate the specific activities in field grown mature soybean seeds receiving different level of added N fertilizer with and without inoculum.

Materials and Methods

One year field experiment on soybean cv. Williams-82 was conducted during the 1st week of July, 1996 in the

agricultural farm of Agricultural Research Institute (ARI), Quetta. Six different treatments (T) of fertilizer were applied to both non-inoculated and inoculated set of experiments. T₁ was kept control (check), whereas from T₂ to T₇, N-fertilizer was added @ 23, 25, 50, 75, 100 and 125 kg ha⁻¹ along with combination of constant dose of P₂O₅ (60 kg ha⁻¹) and K₂O (30 kg ha⁻¹) respectively. The detailed procedure of sowing, layout plan, inoculation, source & time for application of fertilizer and maturity harvest has been already explained by Achakzai *et al.* (2002).

Lipase and urease assay

Precipitation procedure: 0.3 g of air dried defatted soybean powder was suspended in 50 ml phosphate buffer (0.05 M, pH 8.0), stirred for 45 minutes and the insoluble material was removed by centrifugation at 11000 rpm for 30 minutes. The protein was precipitated with 48% ammonium sulfate at 4 °C with stirring for 30 min., centrifuged at 5000 rpm and the precipitate was then dissolved in minimum volume of phosphate buffer (5.0 ml) and stored it at 4 °C.

a) Protein assay: Protein was determined by using the procedure described by Harris and Angal (1989) and Bovine serum albumin (BSA) was used as a standard.

b) Lipase assay: Lipase were assayed spectrophotometrically using p-nitrophenyl laurate (PNPL) emulsified with polyvinyl alcohol (PVA) as described by Masoom (1989).

Reagents

PNPL-PVA emulsion: PNPL emulsion was prepared by dissolving 1.0 ml PNPL (20 mM in acetone) in a mixture of 8.0 ml acetate buffer (0.1 M, pH 3.8) and 4.0 ml PVA (1%) and emulsified. This emulsion was diluted to 20 ml and was stable for three days when stored at 4 °C.

Procedure: The lipase assay medium was consisting of 2.3 ml Tris-buffer (0.1 M, pH 9.0) and 0.6 ml emulsified substrate was taken in a cuvette. This solution was thoroughly mixed and incubated at 30 °C for 5.0 minutes. At zero time 0.1 ml of unknown enzyme solutions were added to the reactants and were gently mixed. The progress of the reaction was followed at 405 nm. In the blank the enzyme was replaced by 0.1 ml of Tris-buffer (0.1 M, pH 9.0).

c) Urease assay

Reagents:

i) Urea solution (10 mM) was prepared by dissolving 0.015 g urea in 25 ml phosphate buffer (0.01M, pH 8.0).

- ii) Tri-sodium citrate solution (0.36 M) was prepared by dissolving 2.5 g of tri-sodium citrate and 2.5 g of salicylic acid in 9.0 ml NaOH (2.0 M). Heated for complete dissolution, cooled and diluted to 50 ml with water.
- iii) Aqueous sodium-nitroprusside solution (1.0% w/v).
- iv) Sodium hypochlorite solution (0.05 M) was prepared by commercially available sodium hypochlorite solution containing 0.35% available chlorine in 2.0 M NaOH.

Procedure: An aliquot (0.9 ml) of urea solution was mixed with 0.1 ml of enzyme solution (ammonium precipitated) and incubated at 55 °C for 15 minutes. From incubated mixture 0.5 ml sample was mixed with 0.5 ml of tri-sodium citrate solution, 0.05 ml of aqueous sodium nitroprusside solution, 3.0 ml of water and 0.05 ml of sodium hypochlorite solution, respectively. After 1 h, the absorbance was measured at 697 nm against reagent blank (water).

The data obtained were statistically calculated following the procedure described by Steel and Torrie (1980). MSTAT-C Computer software package for statistical analysis was used for calculation of Analysis of Variance (ANOVA) Tables and Least Significance Difference test (LSD) using the experimental model number 8 under factorial Randomized Complete Block Design (RCBD). Simple correlation coefficient (r) studies were also worked out for both enzymes with various chemical components and grain yield of soybean following the procedure reported by Fisher and Yates (1953).

Results and Discussion

Data presented in Table 1 showed that in response to various level of added fertilizer with and without inoculation, the specific activities of lipase and urease were statistically found as highly significant (P<0.01). While the interaction between fertilizer and inoculum was found significant only in case of urease activity, but non-significant in case of lipase activity.

Data regarding mean separation values (Table 2) showed that the specific activity of lipase in response to various level of added fertilizer was only significantly increased up to T₄ level. While they remained unaffected in remaining higher doses of fertilizer. Statistically a maximum activity was recorded in T₃ dose of fertilizer. Results further demonstrated that by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation did significantly but negatively influenced the lipase activity. However on the basis of the basis of marginal mean values, the inoculation effect was recorded as 12.79 units mg⁻¹ lesser

Table 1: Analysis of variance (ANOVA) for the determination of specific activities of lipase and urease (units mg⁻¹) of mature soybean cv. Williams-82 seeds in response to various level of added fertilizer alone (A) and in combination with inoculum (B)

Variables	Mean square for fertilizer (A)	Mean square for inoculum (B)	Mean square for A × B	F-Value of variables at an error of 26		
				Fertilizer (A) (Df = 6)	Inoculum (B) (Df = 1)	A × B (Df = 6)
Lipase activity	374.181	6888.706	72.812	10.360**	190.733**	2.016ns
Urease activity	349.323	2591.915	269.026	9951.6**	73838.9**	7664.1**

* and ** significant at P< 0.05 and P< 0.01 respectively, while ns = non-significant

Table 2: Effect of fertilizer treatments and inoculation on the specific activities of lipase and urease enzymes (units mg⁻¹) of mature soybean cv. Williams-82 seeds

Treatments	Total activity (units)		Total protein (mg)		Specific activity (units mg ⁻¹)	
	Lipase	Urease	Lipase	Urease	*Lipase	**Urease
T ₁ (non-inoc)	2000	1650	27.27	27.18	36.66d	30.34j
(inoc)	1505	2370	29.46	29.38	25.55f	40.34c
T ₂ (non-inoc)	2079	1605	24.35	24.21	42.87bc	33.14g
(inoc)	1505	2445	27.48	27.11	27.39f	45.09b
T ₃ (non-inoc)	2150	1560	21.88	21.88	49.12a	35.66d
(inoc)	1505	2520	25.31	25.32	29.74ef	49.77a
T ₄ (non-inoc)	2105	1616	23.94	25.03	43.96b	33.29g
(inoc)	1505	2279	22.06	28.26	34.13de	45.08b
T ₅ (non-inoc)	2075	1675	27.14	27.12	38.23cd	30.85i
(inoc)	1274	2040	24.88	25.31	25.60f	40.32c
T ₆ (non-inoc)	2047	1822	28.08	27.89	36.45d	32.66h
(inoc)	1376	1859	27.19	26.39	25.30f	35.22e
T ₇ (non-inoc)	2025	1970	28.79	29.13	35.16d	33.81f
(inoc)	1480	1680	29.38	29.05	25.18f	28.92k
Grand Mean	33.945	36.747				
Marginal mean of non-inoculated treatments					40.35	32.82
Marginal mean of inoculated treatments					27.56	40.68
Coefficient of variation (%)					08.85	00.25
LSD (5%)					10.09	00.314

Figures followed by the same letter in right side column are not significantly different from each other at 5% level of significance according to Least Significant Difference (LSD) test. Non-inoc = non-inoculated. Inoc = inoculated. * One unit of lipase activity is defined as the amount of enzyme that liberates 1.0 μmol of p-nitrophenol (PNP) per minute from PNPL at optimum conditions. ** One unit of urease is defined as the amount of enzyme that liberate 1.0 μmol of NH₃ per minute from urea at pH 7.0 and temperature 25 °C under assay conditions.

T₁ = 0+0+0 kg NPK ha⁻¹; T₂ = 23+60+30 kg NPK ha⁻¹; T₃ = 25+60+30 kg NPK ha⁻¹; T₄ = 50+60+30 kg NPK ha⁻¹; T₅ = 75+60+30 kg NPK ha⁻¹; T₆ = 100+60+30 kg NPK ha⁻¹ and T₇ = 125+60+30 kg NPK ha⁻¹.

Table 3: Correlation coefficient (r) studies of enzymes with yield, chemical components and moisture content of mature soybean cv. Williams-82 seeds in response to different level of added nitrogen (with and without inoculation) fertilizer

***Chemical components	1	2	3	4	5	6	7	8
(1)	1.000							
(2)	-0.333NS	1.000						
(3)	-0.143NS	0.334NS	1.000					
(4)	-0.445*	-0.053NS	0.138NS	1.000				
(5)	-0.189NS	0.378*	-0.199NS	0.501**	1.000			
(6)	0.453*	-0.457*	-0.099NS	-0.290NS	-0.272NS	1.000		
(7)	0.677**	0.053NS	-0.108NS	-0.507**	-0.064NS	0.242NS	1.000	
(8)	-0.793**	0.450*	0.264NS	0.230NS	-0.037NS	-0.542**	-0.307NS	1.000

* and ** Significant at P (0.05) and P (0.01), respectively. While NS stands for non-significant. *** (1) Lipase activity, units mg⁻¹. (2) Urease activity, units mg⁻¹. (3) Grain yield/plot, g. (4) Protein, g/kg. (5) Oil, g/kg. (6) Starch, g/kg. (7) Soluble sugars, g/kg. (8) Moisture content, g/kg.

than with non-inoculated treatments. Though no extensive work has been carried out on the effects of fertilizer and inoculum on lipase activity of mature soybean seeds. However, in present findings, the specific activity of lipase in general is far greater than that reported by Akhtar *et al.* (1975) in various vegetable & fruit seeds, nuts, grains and other samples.

Data pertaining to mean values (Table 2) showed that fertilizer treatment significantly and positively influenced the urease activity when compared with their control

treatment (T₁). Statistically a maximum activity was recorded in T₃ dose of fertilizer. Results also revealed that by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation did significantly and positively affected the urease activity (except T₇). Statistically a maximum activity was also noted in T₃ dose of non-inoculated treatment. While on the basis of marginal mean values, the inoculation effect was recorded as 7.86 units mg⁻¹ greater than that of non-inoculated treatments. While on the basis of grand mean

values, the urease activity was found as 2.80 units mg^{-1} greater than that of lipase activity. Though no extensive work has been carried out on the effects of fertilizer and inoculum on urease activity of mature plant seeds. However, in present studies the specific activity of urease in general is far greater than that reported by Qasim (1997) in *Pistachia khinjuk* (viz., 0.017 units mg^{-1}).

Based on aforesaid results it can be safely concluded that N dose of T_3 @ 25 kg ha^{-1} is quite enough to significantly maximize the specific activity of lipase in general and urease in particular for both non-inoculated and inoculated mature soybean seeds.

Results regarding correlation coefficient studies (Table 3) showed that lipase activity exhibited significant positive association with starch and soluble sugars, but negative with seed protein and moisture content. Whereas urease activity showed significant positive association with oil and moisture content, but negative with starch content. While they both were insignificantly correlated with remaining yield and biochemical components. Therefore, lipase activity in particular and urease in general could not be used a suitable selection criteria for predicting the quantity and quality of grain yield in soybean and are not helpful for breeding purposes.

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