Research Paper :

Production of lipase enzyme by seed borne fungi of groundnut

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International Journal of Plant Protection, Vol. 4 No. 1 (April, 2011) : 103-106

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SUMMARY

Fungal association in deterioration of seeds can better be correlated with the production of extra cellular hydrolytic enzymes. Therefore, seed borne fungi of groundnut were screened for their ability to produce lipase enzyme. Experiments on the influence of physico-nutritional conditions on lipase production were carried out extensively in *Aspergillus flavus*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria alternata*, *Rhizopus nigricans* and *Sclerotium rolfsii*. The degree of enzyme production was found to be variable among the mycoflora. *A. flavus* and *A. niger* were found to be highly amylolytic. Effect of different substrates on lipase production was also studied. Different substrates used were edible oils, fats, carbohydrates and nitrogen sources. Oils of some crops proved stimulatory while at the same time oils of other crops exhibited inhibitory nature for lipase production. At the same time, in case of carbohydrates, most of them except a few were found to be stimulatory for lipase production.

Kadam, R.M., Biradar, R.P. and Reddy, N.J.M. (2011). Production of lipase enzyme by seed borne fungi of groundnu. *Internat. J. Pl. Protec.*, **4**(1): 103-106.

Key words : Groundnut seeds, Lipase, Triacetin, Phenolphthalein

Received : *October, 2010*
Accepted : *December, 2010*

The chief groundnut growing regions are India, China, Indonesia, West Africa, USA and France. India occupies the top position in the world with regard to acreage and production of groundnut.

Seeds are generally associated with certain saprophytic or parasitic microorganisms which perpetuate in the seed lots on the advent of favorable conditions. Fungal association in deterioration of seeds can better be correlated with the production of extracellular hydrolytic enzymes (Agrawal and Kharlukhi, 1987). Therefore, 12 pathogenic moulds were screened for their ability to produce lipase in particular. Groundnut seeds are rich source of oils hence, the vigour of pathogenic fungi in the process of biodeterioration of these seeds may be related to the degree of lipase production.

MATERIALS AND METHODS

Selected seed moulds were studied for their ability to produce lipase enzymes.

Lipase production:

Lipase production was studied by using liquid medium containing 1 % Oil, KNO_3 0.25 %, KH_2Po_4 0.1 % and $MgSo_4.7H_2O$ 0.05 %, pH 5.0. Rest of the details were same as in case of amylase.

Enzyme assay (Titration):

The activity was assayed as described by Schneider (1957). The reaction mixture contained 2 ml of glycerol triacetate (Triacetin), 5 ml of 0.2 M Citrate phosphate buffer at pH 8.0 and 2 ml of enzyme source, incubated at $36^0C \pm 1^0C$ for 3 hours. The reaction was terminated by adding 10 ml of absolute alcohol. The amount of acids produced by the activity of enzyme was estimated by titrating against 0.05 N NaOH using 1 % phenolphthalein (1 ml) as an indicator till the development of pink coloration. Reaction mixture soon after the addition of enzyme served as blank. The enzyme activity was expressed in units, one unit is defined as 0.1 ml of 0.05 N NaoH required to neutralize the fatty acids liberated during incubation.

RESULTS AND DISCUSSION

The results obtained from the present

investigation as well as relevant discussion have been presented under following heads:

Effect of substrate and non-substrate media on lipase production in mycoflora of groundnut seeds :

Biodeterioration of groundnut seeds has been attributed mainly due to lipase activity in addition to other hydrolytic enzymes of the moulds associated with the seeds. Therefore, extensive studies were made on the production of lipase. Totally, 12 moulds isolated from different cultivars of groundnut seeds were screened for their ability to lipase production in substrate containing and non-substrate containing media.

It is clear from the data that with more or less degree, all the moulds produced lipase both in substrate and non-substrate media. Maximum production of lipase on GN medium was seen in case of followed by *A. terreus, R. nigricans* followed by *S. solfsii, M. phaseolina.* While poor lipase production was seen in *A. flavus*, *A. niger*, *A. alternata* and *F. oxysporum.* It was seen that mycelial weight did not correlate with that of enzyme production in most of the cases (Table 1).

Effect of different edible oils on Lipase production :

Ten different edible oils were used as substrate against lipase production in six selected fungi. It is understood from the results that, *A. flavus* produced maximum lipase in the presence of oils of sunflower, sesamum and olive while, in the presence of mustard oil it could not produce lipase. Similarly, very poor enzyme production was seen in the presence of coconut, cotton and linseed oils. Similarly, *A. alternata* produced maximum lipase on groundnut, cotton and hemp seed oil while, it did not produce on safflower oil. *R. nigricans* and *S. rolfsii* produced maximum lipase on hemp seed oil, mustard and sesamum oils while, poor production took place on cotton, safflower and groundnut oils. *M. phaseolina* produced maximum lipase on groundnut and sesamum oils but not on mustard oil (Table 2).

Effect of fats on lipase production :

Five different fats were tested against lipase production of fungi (Table 3). It was found that triacetin favoured lipase production in *S. rolfsii*, *F. oxysporum*, *R. nigricans* and *A. flavus* but not in *M. phaseolina* and *A. alternata*. Tripalmatin was found to be a good source for lipase production only for *M. phaseolina* and *S. rolfsii*. Tween-20 and Tween-80 supported lipase production to all except *A. flavus*.

Effect of carbohydrates on lipase production :

Eleven different carbohydrates at 1 % concentration were tested against lipase production in fungi (Table 4). In case of *A. flavus*, rhamnose, sorbose, pectin and mannitol were found to be stimulatory for lipase production over the control while six carbohydrates for *A. alternata*, three for *F. oxysporum*, six for *M. phaseolina*, seven for *R. nigricans* and eight for *S. rolfsii* were found to be stimulatory for lipase production. It was interesting to observe that sorbitol proved stimulatory for most of the fungi but inhibitory only for *F. oxysporum*. Similarly, glycogen stimulated only while rhamnose to *A. flavus* and *S. rolfsii*.

Effect of nitrogen sources on lipase production :

Eleven different nitrogen sources were tested against

Table 1 : Lipase production in seed moulds of groundnut seed cultivars

Fungi	Lipase production in			
	GN medium		Substrate medium	
	Growth (dry wt. mg)	Enzyme activity (units)*	Growth (dry wt. mg)	Enzyme activity (units)*
A. flavus	16	04	15	12
A. niger	11	06	14	17
A. fumigatus	15	22	21	15
A. nidulans	05	21	08	14
A. terreus	11	32	16	19
A. alternata	13	03	05	08
F. oxysporum	07	02	15	15
F. semitectum	09	02	11	14
M. phaseolina	04	09	04	10
P. citrinum	06	20	07	21
S. rolfsii	08	16	11	12
R. nigricans	08	23	16	11

*one unit is equivalent to 0.05 N NaOH required to neutralise fatty acid liberated

Table 2 : Effect of different edible oils on lipase production in seed borne fungi

Seed oils (1 %)	Enzyme activity (Units)*					
	A. flavus	*A. alternata*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*S. rolfsii*
Safflower	02	-	19	03	03	01
Sunflower	08	04	09	03	07	14
Seasamum	12	02	07	08	09	19
Groundnut	04	08	06	09	02	03
Mustard	-	03	12	-	09	08
Linseed	03	02	-	06	05	03
Hemp	07	05	02	03	10	10
Cotton	04	04	02	04	03	03
Olive oil	09	03	05	14	07	13
Coconut	02	02	22	03	04	04

Table 3: Effect of fats on lipase production in seed borne fungi

Fats (1 %)	Enzyme activity (units)*					
	A. flavus	*A. alternata*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*S. rolfsii*
Triacetin	12	04	09	02	09	20
Tripalmatin	05	02	04	10	04	16
Tween – 20	03	09	12	15	16	15
Tween – 80	06	15	13	13	09	14
Amsule	03	20	02	02	08	03

* one unit is equivalent to 0.1 ml of 0.05 N NaOH required to neutralise fatty acids liberated

Table 4: Effect of carbohydrates on lipase production by seed borne fungi

Carbohydrates (0.5%)	Enzyme activity (units)*					
	A. flavus	*A. alternata*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*S. rolfsii*
Monosaccharides						
1. Fructose	04	06	07	07	07	11
2. Rhamnose	12	09	09	09	09	13
3. Sorbose	12	08	07	06	13	09
Disaccharide						
1. Lactose	08	11	08	10	07	09
2. Maltose	03	10	04	10	09	10
3. Sucrose	06	09	06	07	14	14
Polysaccharides						
1. Glycogen	09	21	09	07	13	11
2. Pectin	12	13	06	10	13	14
3. Starch	05	13	08	11	11	14
Sugar ald						
1. Mannitol	09	09	12	10	12	11
2. Sorbitol	08	14	05	16	11	15
Control	06	08	08	07	07	10

lipase production of the seed moulds (Table 5). It is clear from the data that, in case of *A. flavus*, calcium nitrate, sodium nitrate, ammonium oxalate and urea and proved to be inferior for lipase production. Similarly, sodium nitrate, sodium nitrite, ammonium oxalate, urea, gelatin and casein proved inferior for *A. alternata*. It was interesting to observe that urea was found to be stimulatory in *M. phaseolina*, *R. nigricans* and *S. rolfsii* but inhibitory to *A. flavus* and *A. alternata*. Chander *et al.* (1981) conducted the studies on factors affecting lipase production in *R. nigricans* and Haq *et al.* (1998) also carried out investigations on the production of lipase

Table 5 : Effect of nitrogen sources on lipase production by seed borne fungi

Nitrogen source (0.25 %)	Enzyme activity (units)*					
	A. flavus	*A. altarnata*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*S. rolfsii*
Nitrates						
1. Cal. nitrate	02	09	01	07	14	08
2. Sod. nitrate	02	04	02	06	05	06
Nitrites						
1. Sod. nitrite	07	04	04	04	04	05
Ammonia forms						
1. Amm. oxalate	01	05	03	07	10	09
2. Amm. chloride	12	15	13	14	15	17
3. Amm. nitrate	13	16	08	13	14	19
4. Amm. sulphate	14	19	16	28	15	28
Amids						
1. Urea	04	04	06	16	15	24
Organic forms						
1. Casein	07	04	07	08	08	13
2. Gelatin	07	03	05	14	15	17
3. Peptone	08	09	02	09	14	18

* one unit is equivalent to 0.1 ml of 0.05 N NaOH required to neutralise fatty acids liberated.

by different mould cultures.

Acknowledgement:

Our sincere thanks to Dr. N.J.M. Reddy, Associate Professor, P.G. Dept. of Botany, Shri Shivaji College, Kandhar for his guidance and encouragement. We extend our special thanks to Principal, Dr. G.D. Bagde and Head, Shri R.K. Kalme, Aerobiology Research Centre, Mahatma Gandhi Mahavidyalaya, Ahmedpur for inspiration and providing facilities to carry out these investigations.

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