Original Research Article Website : www.ijbasr.org ISSN : 2349-1965
International Journal of Basic & Applied Science Research
2015; 2 (2); 169-174

ESTIMATION OF PROTEIN IN GIBBERLLIN TREATED PERISPERM OF EURYALE FEROX SALISB. (MAKHANA) BY POLYNOMIAL REGRESSION

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Praween N*, A. K Singh and J. Aparajita. "ESTIMATION OF PROTEIN IN GIBBERLLIN TREATED PERISPERM OF EURYALE FEROX SALISB. (MAKHANA) BY POLYNOMIAL REGRESSION". IJBASR; 2015: 2(2);169-174.

ABSTRACT

In the present study, a correlation has been made by using polynomial regression between the protein productions against the treatment of different concentration of Gibberllin in perisperm of *Euryale ferox* Salisb. After 0.001% Gibberellin treatment on perisperm for 1min and 5min leads to higher concentration *i.e.*, 50.08µg/mg protein was observed in content of the kernel on mature fruits as compared to 1/3rd mature fruits after of treatment with respect to lower protein production at 0.0001% and 0.01% Gibberellin treatment. The highest concentration was observed after 236 day after showing (DAS).

KEY WORDS: Makhana, Gibberellin, Perisperm, Protein and Day after showing (DAS).

INTRODUCTION:

Euryale ferox Salisb. (fox nut / makhana / gorgon plant) belonging to Nymphaeaceae family and generally cultivated in the different wet lands of North Bihar, mainly in the districts of Madhubani, Darbhanga, Purnea, Saharsa and Katihar. It is one of the cash crops and related to wet land cultivation. The edible portion is mainly white perisperm of the mature seed which is used as popped form as snacks or as milk desserts (kheer). The protein content in *E. ferox* is usually lower than some other fruits *i.e.*, ficus, pipal figs and dates etc present in normal Indian diet. It is best culti-

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Date of Acceptance : 20.09.2015
Date of Publication : 30.10.2015

vated in permanent wet lands and perenial water bodies with shallow bottoms. Growth of plants is not proper in freshly excavated ponds or water area because they lack the highly nutritive mucky bottom (Thakur, 1978). Fish farmers of the banper subcaste are skilled in harvesting makhana seeds from the pond bottom (Jha, 2002). Read (1946) reported biochemical composition of *E. ferox* containing carbohydrate (75.7%), Protein (9.9%), fat (0.3%), and ash (0.6%). According to Phang (2002), protein of Arthrospira (Spirulina), a non conventional aquatic source of nutrition, contains isoleucine (3.5- 4.1%), leucine (5.4- 5.8%), lysine (2.9- 4.0%), methionine (3.5- 4.1%), phenylalanine (2.8- 4.0%), threonine (3.2- 4.2%), tryptophan (0.91- 1.1%) and valine (4.0- 6.0%). The seeds are mostly used as stomachic, for articular pains, micturition and for seminal loss (Roi, 1950). Because of its less fat contents it is ideal for invalids. Also, these are used as tonic for seminal organs (Crevost, *et al.,* 1920) as well as remedy for diseases of the spleen and gonorrhea. Jha (1987) reported that net protein utilization (NPU 49.3), true digestibility (TD 89.6) and apparent digestibility (AD 69.1) of makhana were comparable to the values of most cereals. The above value were lower when compared to soyabean, egg and human and cow milk (Jha, 1991).

MATERIALS AND METHODS:

Sample collection: The fruit samples were collected at eight different stages of their maturation and development. The first collection of makhana (*Euryale ferox* Salisb.) fruit samples was done at immature stage *i.e.,* 152 Days. Subsequent fruit samplings were made at regular interval of 12 days *i.e.,* at 1/4th mature stage (164 days), 1/3rd mature stage (176 days), ½ mature stage (188 days), 2/3rd mature stage (200 days), 3/4th mature stage (212 days), fully mature stage (224 days) and finally at the over mature stage (236 days) stage. The fruits were treated with three different concentrations *i.e.,* 0.0001%, 0.001% and 0.01% of GA_3 at the six stages of fruit maturation and development. Thereafter, chemical treatment was made at beginning from 1/3rd mature (176 days) to over mature stage (236 days). For the purpose of chemical treatment the intact fruits were dipped for one minute and five minute separately in each of the solutions of three different concentrations *i.e.,* 0.0001%, 0.001% and 0.01% of GA_3. All such treated fruits were properly tagged mentioning the concentration of treated hormone with date of chemical treatment and the fruits were picked after 12 days of chemical application. However, no chemical treatment was made in the fruits at immature (152 days) and 1/4th

mature (164 days). The experiments were repeated thrice and the mean was taken as final data.

Estimation of Protein:

The estimation of soluble protein content was made using F.C (Folin-Ciocalteau) reagent (Lowry *et al.*, 1951). The 5 gm sample fruits and macerated in phosphate buffer (pH 7.4) with course material. The thin slurry was centrifuge (5000rpm, 15 min) to isolate supernatant and taking 1 ml supernatant was treated with 30% trichloroacetic acid (TCA) and left for protein precipitation and was centrifuged at 3000rpm for 20 minutes. After discarding the supernatant the centrifuge tube was left in a inverted position in over night for 24 hours. Next day, addition of 5ml 0.1N sodium hydroxide (NaOH) was made to the above centrifuge tube and it was mixed thoroughly to dissolve. Then, 0.5ml of above protein suspension was taken in a test tube and 5 ml alkaline copper sulphate reagent (10 parts of 10% Na_2CO_3 in 0.5 NaOH and 1 part of 0.5% CuSO4, 5H2O in 1% potassium tartarate) was added. Again, 0.5ml of FC reagent was mixed and left for 30 minutes for optimum colour development. The absorbance was recorded at 600nm against the reagent blank. The quantitative analysis of protein was analysed by using double beam UV-Vis Specrtonic spectrophotometer (Model- 350). The protein amount was calculated with the help of standard curve of Bovine Serum Albumin (BSA). Polynomial regression was performed by using Sigma plotter statistical software tool.

RESULT AND CONCLUSION:

The biochemical investigations in perisperm (seed) of makhana (*Euryale ferox* Salisb.) for treated fruits and control ones were made for the metabolites like protein. The proteins present in different stages of both 1 min and 5 min treatment groups for all 6 maturing stages were depicted in Table 1.0 and 2.0. The experimental value of protein under conditions of both control and chemical treatment as well as the predicted / theoretical values on the basis of Polynomial Regression Equation

Y= a+ bX+ Cx^2 + dX^3 in the perisperm during fruit development due to the effect of GA_3 treatment (0.0001%, 0.001%, 0.01%) for 1min and 5 min have been presented in Fig 1 respectively.

Perisperm:

Effect of 1min GA_3 treatment - In the perisperm of 0.0001% GA_3 treated fruits for 1min, it was revealed that the amount of protein gradually increases from 1/3rd mature stage (176 days) to the fully mature differentiation stage and a sharp decline within over mature stage. In the perisperm of 0.001%

GA_3 treated fruits for 1min the protein content increased continuously from 1/3rd mature stage (176 days) from a minimum content 23.97mg and shows gradual increase upto fully mature stage 43.11 mg and down-fall in overmature stage. The similar pattern was observed in the treatment group of 0.01% with maximum protein content (32.12mg) at fully mature stage and minimum protein content (23.35 mg) at 1/3rd stage.

Perisperm:

Effect of 5min GA_3 treatment - In the perisperm of different treated group for 5 min the minimum protein concertrations were recorded as 25.45mg, 25.61mg and 24.89 mg for the treatment concentration of 0.0001,0.001 and 0.01% GA_3 respectively. The maximum protein contents were recorded as 47.60mg, 47.65 and 38.84 mg for the treatment group of 0.0001, 0.001 and 0.01% GA_3 respectively.

Table: 1 Data of protein production for 3 different treatment of GA_3 for 6 develop

DAS	Immature	¼ th	GA Treatment	1/3rd		1/2nd		2/3rd		3/4th		Fully mature		Over mature	
	Control groups			Treatment time in minutes											
				1	5	1	5	1	5	1	5	1	5	1	5
152	23.11	23.19	0.0001%	-	-	-	-	-	-	-	-	-	-	-	-
			0.001%	-	-	-	-	-	-	-	-	-	-	-	-
			0.01%	-	-	-	-	-	-	-	-	-	-	-	
164	23.61	23.69	0.0001%	-	-	-	-	-	-	-	-	-	-	-	-
			0.001%	-	-	-	-	-	-	-	-	-	-	-	-
			0.01%	-	-	-	-	-	-	-	-	-	-	-	-
176	23.73	23.78	0.0001%	23.93	25.45	24.53	30.14	25.93	35.81	27.74	41.76	**37.98**	**46.37**	35.15	43.21
			0.001%	23.97	25.61	26.82	27.53	28.14	30.93	29.46	33.04	**30.21**	**37.75**	28.62	35.71
			0.01%	23.35	24.89	25.63	26.91	27.84	30.69	29.54	34.96	**32.12**	**38.84**	30.64	35.61
188	26.04	26.14	0.0001%	26.08	30.54	28.84	35.69	30.27	37.45	34.65	40.58	**38.31**	**43.92**	36.87	39.37
			0.001%	26.05	30.86	29.15	33.53	31.72	37.68	35.23	41.37	**39.43**	**45.40**	37.61	40.51
			0.01%	26.04	26.16	26.56	27.19	26.91	28.21	27.35	28.65	**27.62**	**29.11**	25.87	25.17
200	27.35	27.48	0.0001%	27.36	27.51	27.63	27.94	28.31	28.67	28.91	29.32	**29.53**	**45.17**	27.45	41.23
			0.001%	27.28	29.55	27.68	34.98	28.41	38.71	28.87	42.42	**29.91**	**46.69**	27.85	43.42
			0.01%	25.02	25.13	25.43	25.64	25.79	26.97	26.13	28.45	**26.78**	**31.72**	24.14	28.18
212	28.28	28.38	0.0001%	28.19	29.42	30.45	35.74	32.76	38.89	36.27	42.98	**39.32**	**45.81**	36.76	40.13
			0.001%	28.34	30.54	31.74	34.23	35.47	38.83	38.93	42.46	**40.10**	**47.65**	37.65	43.21
			0.01%	28.02	28.04	28.06	29.08	28.09	30.10	28.11	31.12	**28.12**	**33.04**	26.36	32.68
224	29.25	29.35	0.0001%	29.26	33.43	34.65	35.84	36.76	37.56	38.12	39.63	**40.22**	**46.84**	38.65	45.73
			0.001%	29.32	30.43	35.86	34.58	38.65	37.24	41.96	39.74	**43.11**	**48.61**	38.52	46.78
			0.01%	29.04	30.86	29.06	32.64	29.09	34.83	29.12	36.71	**29.23**	**37.99**	27.94	35.63
236	30.07	30.17	0.0001%	30.05	31.84	30.56	34.83	33.47	38.43	36.86	42.56	**41.76**	**47.60**	36.56	45.69
			0.001%	27.04	30.63	32.61	33.53	35.58	36.83	37.86	38.56	**40.02**	**41.08**	38.43	36.86
			0.01%	25.07	31.84	26.12	33.48	27.27	35.53	28.31	37.37	**28.43**	**38.79**	26.68	36.51

Tables: 2 a, b Shown Protein concentrations after treatment of GA in *Euryale ferox* Salisb.

a. Protein concentration after 1 min G_A treatment			
DAS	0.0001%	0.001%	0.01%
152	-	-	-
164	-	-	-
176	29.21	27.80	28.18
188	32.50	33.19	26.72
200	28.19	28.33	25.54
212	33.95	35.37	27.79
224	36.27	37.90	28.91
236	34.87	35.25	26.98

b. Protein Concentration of after 5 min G_A treatment			
DAS	0.0001%	0.001%	0.01%
152	-	-	-
164	-	-	-
176	36.95	31.71	31.91
188	37.92	38.22	27.76
200	33.30	39.29	27.58
212	38.82	39.48	30.67
224	39.84	41.06	34.83
236	34.77	36.24	29.50

Fig: 1 a, b; Show polynomial regression of different treatment group of GA_3 *Euryale ferox* Salisb

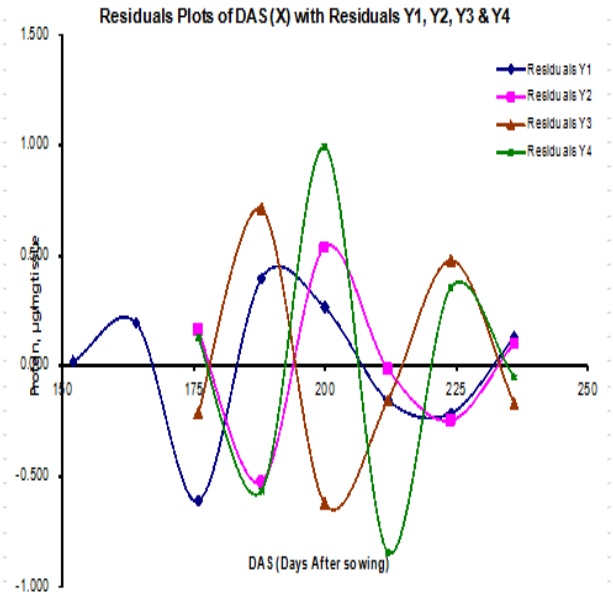

Fig 1; a. Polynomial regression for 1min treatment

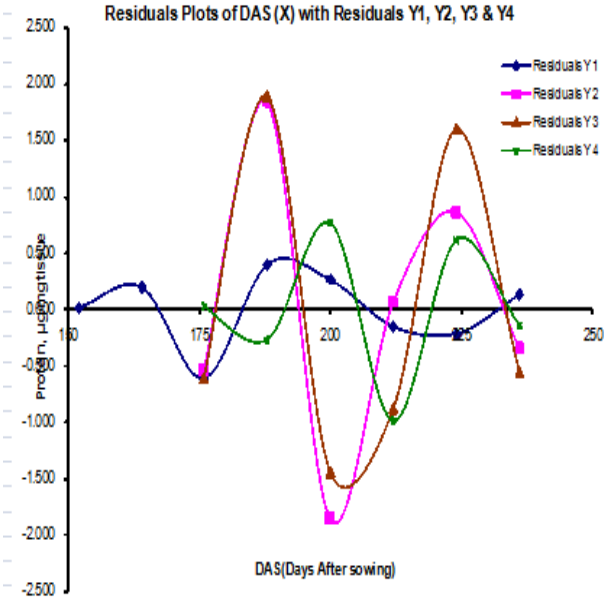

Fig. 1; b. Polynomial regression for 5 min treatment

CONCLUSION:

The effect of 0.001% GA_3 after 5min treatment was analysed to have maximum effect upon protein enhancement with respect to 1 min treatment of same experimentation. The effect of other concentrations was not satisfactory this may be due to the optimal signalling caused by 0.01% treatment. This may be further correlated with the optical signalling mechanism of GA_3 upon protein synthesis coinciding with the 0.01% of GA_3. The higher as well as lower concentration may not produce maximal transcriptional and translational potential of the makhana cells for the same protein enhancement. The linearity in protein synthesis in gradual stages which is not continuous as the experiment shows can be correlated with differential expression of protein synthesis at different stages under similar treatment of GA_3 causing slight decrease in protein concentration.

ACKNOWLEDGEMENT:

Authors are thankful to HOD, University Department of Botany, T M Bhagalpur University, Bhagalpur (India) for providing laboratory assistance for this marvellous type of work.

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