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Estimation of Sugar Content By The Effect of Gibberelline InThe Perisperm of *Euryale Ferox* Salisb. (Makhana) Due To Polynomial Regression Fit Equation

Praween N., Choudhary S.K
Department Of Botany , University Department Of Botany , T.M.B.U .,Bhagalpur – 812007, India

Abstract:- *Euryale Ferox* Salisb. (Also Known As Fox Nut , Makhana , Or Gorgon Plant) is the only species in the genus *Euryale* . It is a flowering plant classified in the Waterlily family , Nymphaeceae . The main edible portion of Makhana is the white perisperm inside the seed which is consumed mainly in its popped form either as snacks or as desserts. Raw Makhana seed powder is an essential ingredient of the baby foods in China. Makhana is high nutritional value . Calorific value of Makhana correspond well with staple food materials and Carbohydrate rich Cereals . The moisture content of Makhana is 12.8% and it is free of cholesterol . Polynomial Regression is a form of Linear Regression in which the relationship between the independent variable X and the dependent variable Y is modelled as an 9th degree Polynomial . Due to this method , the estimation of total sugar content was done by Dubois *et al*. (1956) with the use of Phenol-sulphuric acid reagent . The effect of 0.0001% and 0.001% GA_3 after 1min treatment as well as that of 0.0001% GA_3 after 5 min treatment exhibits palallelism in the pattern of changes in total sugar content .

Introduction : . *Euryale ferox* Salisb, also known as Fox Nut, Makhana Or Gorgon Plant is the member of the family Nymphaeceae. Their seeds may be eaten raw or cooked is extremely nutritious consisting of 77% edible starch. Biochemical analysis of its seed revealed that it contain 15.6%Protein, 61% Carbohydrate, 12.1% Moisture, 7.6% Fiber, 1.8% Ash and 1.35% Fat (Alfasane *et al*., 2008) . Makhana seeds with moderate 10-12% protein content are known for its high essential amino acid index (EAAI)) which constitutes about 90% (Jha *et al.*, 1991a, b).The seed of *E. ferox* has been applied in the treatment of Diarrhea , Spermatorrhea , and the petiols and pedicels in Polidipsia, and mouth dryness and dry throat (Editoriol Committee For Chinese Herbal Medicine 1999). Recently , the search for natural antioxidants originated from plants instead of synthetic antioxidants has been a hot topic (Ningappa , M . B 2010., Sun . J . Yao 2009 ., Deng G . F . Xu 2012.) . Natural antioxidants not only can used for medicinal purposes, but also for food preservation , as dietary supplements or functional foods , and in cosmetics (Helmja, K 2009). Variety of cheap waste products from the food or agricultural industries as potential sources of natural antioxidants for the environmental and economical benefits . Therefore, the *E.ferox* seed coat represents a potentially cheap source of natural antioxidants with a vast range of application. Liang *et al* . (1996) also reported that application of Gibberellins was beneficial to green tea quality . Most of the physiological activities and growth of plants are regulated by hormones such s Gibberellins and enhance root growth , shoot growth , shoot dry weight and accumulation of protein , carotenoids and tissue nitrates in Mangrove Species (Kathireasan & Moorthy . 1994) . Many workers have reported stimulation of endosperm metabolism by the addition of exogenous Gibberellic acid . Paleg (1960 , 1961) has described the dependence of loss of dry weight , starch hydrolysis and protein release in the excised barley endosperm in the presence of added GA_3 . GA_3 application has been reported to accelerate the hydrolysis of starch to soluble sugar by enhancing the hydrolytic enzymes such as α – amylase , β – amylase , maltase and invertase in Maize (Subedi & Bhattarai , 2003) . Salla et al . 1991, have also reported a similar result in Rice . Gibberellic acid is khown to induce the Synthesis Of α – amylase in embryoless Rice seeds (Palmiano & Juliano , 1972) . Gibberellic Acid (GA_3) is used to increase the fruit firmness and the fruit size , and to delay maturity in mostly self – fertile and / or high – cropping cherry varities (Kappel & Mac Donald , 2002 ., Looney , 1996). GA_3 application resulted in variable responces in fruit quality and harvest characteristics of sweet cherry (Canli & Orhan , 2009) and couled be harvested later than non – treated fruits (Andrews & Shulin , 1995 ; Choi Et .Al ; 2002 ;). The purpose of using plant growth substances is to improve flowering , producing maximum yield and fruit quality particularly fruit size , as well as controlling fruit maturation (Fathi *et al*., 2011) .

Material & Method : The fruit samples were collected at eight different stages of their maturation and development . The first collection of fruit samples of Makhana (*Euryale ferox* Salisb .) was done at Immature Stage (152 DAS) in the year 2011 . Subsequent fruit samplings were made at regular interval of 12days . At $1/4^{th}$

Mature Stage (164 DAS) , 1/3rd Mature Stage (200 DAS) , ½ Mature Stage (188 DAS) , 2/3rd Mature Stage (200 DAS) , 3/4th Mature Stage (212 DAS) , Fully Mature Stage (224 DAS) and finally at the Over –Mature Stage (236 DAS) Stage . The fruits were treated with three different concentrations 0.0001% , 0.001% and 0.01% of Kinetin at the six stage of fruit maturation and development . Thereafter , chemical treatment was made at beginning from 1/3rd (176 DAS) to Over Mature Stage (236 DAS).

Table / Figure No.3G: Polynomial Regression Fit on the basis of the Equation Y = a + bX + cX^2 + dX^3

DAA (X)	Perisperm of *Euryale ferox* Salisb.			
	Total Sugar (Mean), µg/mg tissue			
	Control (Y1)	Gibberellin Treatment for 1 Minute		
		0.0001% (Y2)	0.001% (Y3)	0.01% (Y4)
152	7.30			
164	46.23			
176	22.22	14.26	21.73	6.67
188	43.73	25.23	27.31	12.14
200	59.16	29.46	33.28	18.07
212	63.94	35.05	40.30	23.80
224	69.77	41.31	47.35	29.99
236	75.69	48.10	53.16	35.88
Y = a + bX + cX^2 + dX^3				
a	-442.83971	-1721.96628	471.77888	20.53386
b	5.65142	24.43494	-7.34881	-0.90599
c	-0.02245	-0.11510	0.03780	0.00642
d	0.00003	0.00018	-0.00006	-0.00001
DAA (X)	*Predicted Y1*	*Predicted Y2*	*Predicted Y3*	*Predicted Y4*
152	14.027			
164	26.547			
176	37.303	14.591	21.805	6.663
188	46.638	24.162	27.075	12.184
200	54.896	30.419	33.475	17.968
212	62.421	35.268	40.381	23.916
224	69.558	40.612	47.172	29.925
236	76.649	48.357	53.223	35.894
DAA (X)	*Residuals Y1*	*Residuals Y2*	*Residuals Y3*	*Residuals Y4*
152	-6.727			
164	19.683			
176	-15.083	-0.331	-0.075	0.007
188	-2.908	1.068	0.235	-0.044
200	4.264	-0.959	-0.195	0.102
212	1.519	-0.218	-0.081	-0.116
224	0.212	0.698	0.178	0.065
236	-0.959	-0.257	-0.063	-0.014

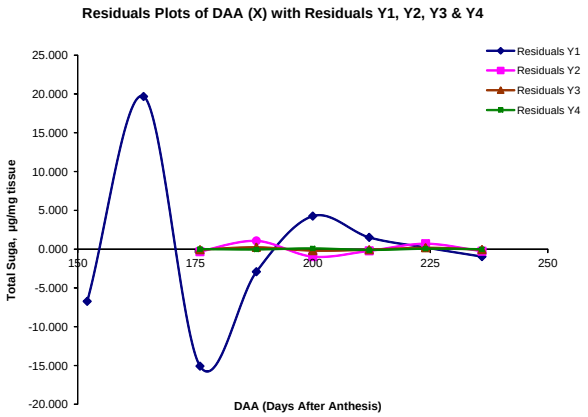
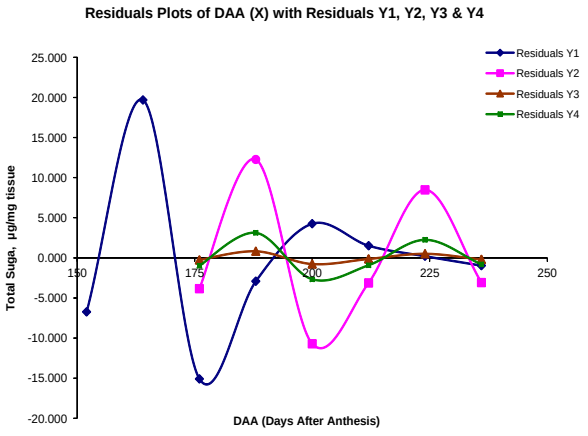


Table / Figure No.3H: Polynomial Regression Fit on the basis of the Equation Y = a + bX + cX^2 + dX^3

DAA (X)	Perisperm of *Euryale ferox* Salisb.			
	Total Sugar (Mean), µg/mg tissue			
	Control (Y1)	Gibberellin Treatment for 5 Minute		
		0.0001% (Y2)	0.001% (Y3)	0.01% (Y4)
152	7.30			
164	46.23			
176	22.22	16.07	19.84	12.37
188	43.73	123.22	32.60	41.05
200	59.16	128.76	39.16	46.29
212	63.94	130.74	45.83	52.01
224	69.77	131.04	51.80	58.56
236	75.69	130.90	57.12	64.78
Y = a + bX + cX^2 + dX^3				
a	-442.83971	-26636.81761	-1453.72111	-6037.23921
b	5.65142	376.83289	19.79171	85.31832
c	-0.02245	-1.76291	-0.08875	-0.39939
d	0.00003	0.00274	0.00014	0.00062
DAA (X)	*Predicted Y1*	*Predicted Y2*	*Predicted Y3*	*Predicted Y4*
152	14.027			
164	26.547			
176	37.303	19.907	20.095	13.353
188	46.638	110.952	31.771	37.925
200	54.896	139.466	39.924	48.957
212	62.421	133.864	45.960	52.926
224	69.558	122.563	51.288	56.311
236	76.649	133.978	57.312	65.588
DAA (X)	*Residuals Y1*	*Residuals Y2*	*Residuals Y3*	*Residuals Y4*
152	-6.727			
164	19.683			
176	-15.083	-3.837	-0.255	-0.983
188	-2.908	12.268	0.829	3.125
200	4.264	-10.706	-0.764	-2.667
212	1.519	-3.124	-0.130	-0.916
224	0.212	8.477	0.512	2.249
236	-0.959	-3.078	-0.192	-0.808



For the purpose of chemical treatment the fruits while intact on the plants were for 1 min and 5 min separately in each of the solutions of three different concentration (0.0001% , 0.001% & 0.01%) of treated hormone . 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 DAS) and 1/4th Mature (164 DAS).

Estimation Of Total Sugar – The estimation of total sugar content was done following the method of Dubois *et al.* (1956) with the use of phenol- sulphuric acid reagent . In a centrifuge tube, 2ml tissue homogenate (100 mg / ml GDW) was taken and 1 ml each of 10% $Znso_4$ and 0.5 N NaoH was added . Thereafter the mixture was centrifuged at 2000 rpm for 20 minutes . Again 2 ml supernatant was taken in a test tube to which 1ml 5% aqueous phenol was added . With the use of ice bath the above tube was kept at 10 C and 5ml of concentrated Sulphuric Acid was added slowly . The colour intensity was recorded at 490 nm against the reagent blank . The amount of total sugar was calculated with the help of standard curve of Glucose and it was expressed as µg Glucose / mg tissue on fresh weight basis.

Result: The Biochemical Investigation in perisperm (Seed) of Makhana (*Euryale ferox* Salisb.) both the treated fruits and control ones were made for the metabolite like Total Sugar . The Experimental value of Total Sugar under conditions of both control and chemical treatment as well as the Predicted / Theoretical values on the basis of Polynomial Regression Fit Equation $Y= a + bX + CX^{\wedge 2} +dX^{\wedge 3}$ in the perisperm during fruit development due to the effect of Gibberelline treatment (0.0001%, 0.001% & 0.01 %) for 1min and 5min have presented in Tables / Figures 3G and 3H Respectively .

Perisperm: Effect Of 1min GA_3 Treatment - In the Perisperm of 0.0001% GA_3 treated fruits for 1min the Total Sugar was low at $1/3^{rd}$ Mature Stage (176 DAS) which increased continuously 3.37- fold in Over- Mature Fruits (236 DAS).

In the Perisperm Of 0.001% Ga_3 Treated Fruits For 1min The Total Sugar Content Increased From $1/3^{rd}$ Mature Stage (176 Das) Continuously 2.44- Fold At Over- Mature Stage (236 Das).

In the Perisperm of 0.01% Of GA_3 treated fruits for 1min the Total Sugar content was low at $1/3^{rd}$ Mature Stage (176 DAS) and thereafter it increased considerably in the continuous manner upto 5.37-fold in Over- Mature Stage (236 DAS).

Perisperm : Effect Of 5min GA_3 Treatment - In the Perisperm of 0.0001% GA_3 treated fruits for 5min the Total Sugar content was low at $1/3^{rd}$ Mature Stage (176 DAS) and thereafter it increased continuously upto Fully Mature Stage (224 DAS) and finally it declined 0.99-fold in Over- Mature Fruits (236 DAS).

In the Perisperm of 0.001% GA_3 treated fruits for 5min the Total Sugar content was low at $1/3^{rd}$ Mature Stage (176 DAS) which increased considerably at successive stages and was 2.87-fold in Over - Mature Fruits (236 DAS).

In the Perisperm of 0.01% GA_3 treated fruits for 5min the Total Sugar Content was low decline at $1/3^{rd}$ Mature Stage (176 DAS) and thereafter it increased considerably in a continuous manner and was 5.23- fold in Over- Mature Fruits .

Discussion : The changes in the content of Total Sugar in the kernel of Makhana during fruit maturation exhibit fluctuations due to the effect of three different concentrations of GA_3 after 1min and 5min treatment . The changes in Total Sugar Content in the kernel of Makhana under both control experimental conditions and due to the effect of 0.01% GA_3 after 1 min treatment as well as under control experimental conditions and due to the effect of 0.001% and 0.01% GA_3 after 5 min treatment exhibit parallel pattern . The effect of 0.0001% and 0.001% GA_3 after 1min treatment as well as that of 0.0001% GA_3 after 5 min treatment exhibits parallelism in the pattern of changes in Total Sugar Content . However , the effect of 0.0001% and 0.001% GA_3 along with the control experimental conditions after 5 min treatment also exhibits parallelism in the changes of Total Sugar Content in the kernel during fruit maturation . Total Sugar profile observed in water chestnut is the general feature of developing seeds (Matheson *et al.*, 1983) Including Sorghum (Subramanian *et al.*,1983), Mung Bean (Tsay *et al* ,1983), Pigeon Pea (Singh *et al* ., 1980) and Chickpea (Singh & Lymbery 1983).

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