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EFFECT OF KINETIN ON PROTEIN CONCENTRATION OF MAKHANA (EURYALE FEROX SALISB.) DURING FRUIT DEVELOPMENT

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ABSTRACT :

Makhana (*Euryale ferox* Salisb.) known as fox nut or Gorgon nut is the member of the family Nympheaceae. Effect of different concentration *i.e.*, 0.1%, 0.01%, 0.001% and 0.0001% of Kinetin on protein concentration of makhana (*Euryale ferox* Salisb.) during fruit development was analyzed from flowering to maturity. On the basis of this study, in immature fruit (Normal control) the seeds were more proteinases in the control condition reaching up to an average of 20.29µg/mg as fruit and seed matured protein concentration was gradually decreased. After giving the treatment with kinetin in different concentration it was observed that at after treatment of 0.01% kinetin, seed and fruit had higher concentration protein.

KEY WORDS : *Makhana, Pericarp, Kinetin, Protein, Antioxidants*

INTRODUCTION:

Makhana (*Euryale ferox* Salisb.) is an aquatic cash crop (Jha, *et al.*, 1991a,). It is a seasonal annual crop having floating leaves. After fruits maturation and dryout the leaf makhan seeds are cultivated. Along with Makhana cultivation fish culture is also done simultaneously. It is cultivated in different districts of North Bihar, where it is a main support for livelihood of the poor people (Jha, *et al.*, 1991b). The plant grows in fallow wetlands of standing shallow water of about 2.5m depth and has rhizomatous stem. Makhana cultivation in India, is covering from Kashmir to Manipur alongside the Himalayan stretch from Northwest to East. Now, its distribution has been confined within Bihar, along with adjacent states like Orissa, West Bengal, and Assam. It prefers tropical and sub-tropical climate, temperature between 20°C - 35°C, humidity between 50% - 90%, and rainfall between 100 - 250cm. Makhana is either eaten as raw puff or blended with vegetables, dal, *etc.*, The seeds become only edible after being processed and are highly nutritious (Boyd, 1968; Sikka, 1978; and Eggum, 1977). It falls under one of the superior food qualities, which is reflected in its high amino acid index (89% - 93%) and arginine + lysine/proline

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ratio (4.74 - 7.6) (Nath, *et al.,* 1985). Calorific value (3.62 kcal/gm) is also remarkable as compared to staple foods. It has a prominent place in Indian dietary chart with medicinal values for respiratory, circulatory, digestive, renal, and reproductive diseases (Nath, *et al.,* 1985). It contains 15.6% protein, 61% Carbohydrate, 12.1% moisture, 7.6% fiber, 1.8% ash and 1.35% fat (Alfasane, *et al.,* 2008). Makhana seeds have moderate 10 - 12% protein and high levels of essential amino acid index (EAAI) which constitutes about 90% (Jha, *et al.,* 1991). The seeds of *E. ferox* have medicinal properties *i.e.,* in the treatment of diarrhoea, spermatorrhoea, mouth dryness and throat dryness. This is a natural antioxidants plants (Ningappa, *et al.,* 2008; Sun, *et al.,* 2009; Deng, *et al.,* 2012 and Lonni, *et al.,* 2012). Natural antioxidants are used not only for medicinal purposes but also for food preservation, diet supplements and cosmetics (Helmja, *et al.,* 2009). Large amount of proteins are accumulated over a short period of time during seed development in many plants (Beever, *et al.,* 1972; Millerd, *et al.,* 1971). The accumulation of proteins in immature stage is high and gradually decreased in mature stage (Altschul, *et al.,* 1966). Different phytohormones play an essential role in regulating plant growth and development. Cytokines have been implicated in many developmental processes, environmental responses of plants, regulation of cell division, apical dominance, chloroplast development, anthocyanin production and maintenance of source-sink relationship (Kappel, *et al.,* 2002). It is also considered as the most important senescence-retarding hormones, exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins to cause induction of flower reverse leaf, fruit abscission, release seed dormancy and modify substantially plant responses to a variety of environmental stresses (Andrews, *et al.,* 1995).

Kinetin is a type of cytokinin plant hormone that promotes cell division. Kinetin was originally isolated as a compound from autoclaved herring sperm DNA and having cell division-promoting activity (Livne, *et al.,* 1965). Kinetin is often used in plant tissue culture for inducing, formation of callus and regenerates the shoot tissues from callus (Tal, *et al.,* 1970). This work was designed to investigate "Effect of kinetin on protein concentration of Makhana (*Euryale ferox* Salisb.) during fruit development".

MATERIAL AND METHODS :

Treatment Protocol :

The seeds of *Euryale ferox* Salisb. were sown in the pond of University Department of Botany, T. M. Bhagalpur University for the present study. Fruits were collected between May to August, 2010 and 2011 in two successive seasons. Kinetin was purchased from Sigma Aldrich. Four different concentrations of Kinetin *i.e.,* 0.0001%, 0.001%, 0.01% & 0.1% exposure was made in seed and fruit of *Euryale ferox* Salisb. The fruit samples were collected at five different stage of their maturation

i.e., immature stage (DAA 152), ¼ mature stages (DAA 164), 1/3 mature stages (DAA 176), 1/2 mature stages (DAA 188) and mature stage (236).

Protein Estimation:

At all the maturation stages of seed and fruit development six identical healthy samples were collected from each maturation stage and weighed. All samples were homogenized in a pre-chilled glass mortar and pestle separately. These were diluted with Phosphate buffer saline. Protein concentration was made in the kernel of Makhana fruits at the different stages of their maturation by Folin- Cioalteace Reagent (Lowry, *et al.*, 1951). 1gm tissue homogenate with 3ml of 30% tri-chloroacetic acid was centrifuged for 15min at 3000rpm. The supernatant was discarded and the tubes were left inverted over night. 5ml of 0.1N Sodium hydroxide (NaOH) was mixed in the tubes and 5ml alkaline Copper sulphate reagent (50 part 10% of Na_2CO_3 in 0.5N NaOH and 1 part of 0.5% $CuSO_4$, $5H_2O$ in 1% Potassium tartarate) was added. The solution was allowed to stand for 10 min and then was treated with 0.5ml of FC reagent. Optimum color was developed in 30 min duration. The absorbance was recorded at 600nm against the reagent blank. The amount of protein was calculated with the help of standard curve of Bovine serum albumin (BSA) and expressed as µg protein per mg tissue.

RESULTS:

In this study, it was analyzed that the changes in protein concentration in kernel due to the effect of different concentrations *i.e.*, 0.0001%, 0.001%, 0.01% and 0.1% of kinetin for one minute after 7 days treatment separately at each of the five stages of fruit development in Makhana. All the calculations were made on the fresh weight basis.

Table 1: Effect of Kinetin protein concentration (µg/mg) on seed and fruit pericarp of *Euryale ferox* Salisb. during fruit maturation.

Group	DDA	Protein concentration (µg/mg) ± S.E.								
		Control	1 min kinetin treatment (seed)				1 min kinetin treatment (fruit)			
			0.0001%	0.001%	0.01%	0.1%	0.0001%	0.001%	0.01%	0.1%
Group-I (Immature)	152	20.29 ± 2.63	-	-	-	-	-	-	-	-
Group-II (1/4 mature)	164	17.33 ± 0.38	-	-	-	-	-	-	-	-
Group-III (1/3 mature)	176	10.83 ± 0.36	3.56 ± 0.51	4.16 ± 0.62	10.56 ± 0.47	6.78 ± 0.23	3.31 ± 0.46	4.19 ± 0.54	8.61 ± 0.88	6.43 ± 0.42
Group- IV (1/2 mature)	188	13.55 ± 0.29	5.75 ± 0.22	9.60 ± 0.21	10.41 ± 0.18	6.35 ± 0.51	6.25 ± 0.23	9.91 ± 0.16	11.31 ± 0.15	7.03 ± 0.52
Group-V (mature)	236	13.40 ± 0.17	7.16 ± 0.13	10.03 ± 0.10	11.28 ± 0.17	6.11 ± 0.52	7.12 ± 0.16	9.99 ± 0.11	12.45 ± 0.18	7.82 ± 0.62

In the seed control groups (untreated) concentration of protein was 20.29 ± 2.63, 17.33 ± 0.38, 10.83 ± 0.36, 13.55 ± 0.29 and 13.40 ± 0.17 µg/mg. In 0.0001% kinetin treated group the protein concentration was analyzed 3.56 ± 0.51, 5.75 ± 0.22 and 7.16 ± 0.13µg/ mg tissue during fruit development. In the 0.0001% kinetin treatment group protein content was analyzed minimum 3.56 ± 0.5µg/mg. In the 0.001% kinetin treated group the protein concentration was analyzed 4.16 ± 0.62, 9.62 ± 0.21 and 11.28 ± 0.17µg/mg. In the 1/3rd stage group, large increase in protein concentration with respect to immature stage was analyzed. In 0.01% Kinetin treated group, the concentration of protein was analyzed 10.56 ± 0.47, 10.41 ± 0.18 and 11.28 ± 0.17µg/mg. In this stage huge increase in protein concentration was noticed among all the development stages. In 0.1% kinetin treatment group the protein concentration was analyzed 6.78 ± 0.23, 6.35 ± 0.51 and 6.11 ± 0.52µg/mg. The high concentration treatment showed drastic decrease in the protein concentration beyond 0.01% of kinetin.

In pericarp of the fruit after 1minute treatment 0.0001% Kinetin treated group the protein concentration was analyzed between 3.31 ± 0.46, 6.25 ± 0.23 and 7.12 ± 0.16µg/mg. In 0.0001% treatment group protein concentration was minimum 3.31 ± 0.46µg/mg. In 0.001% Kinetin treated group the protein concentration was analyzed 4.19 ± 0.54, 9.91 ± 0.16 and 9.99 ± 0.11µg / mg. In this stage there was a large increase in protein content in 1/ 3rd mature stage to over mature stage which further decreases at fully mature stage. In 0.01% Kinetin treated fruit groups; the concentration of protein analyzed 8.61 ± 0.88, 11.33 ± 0.15 and 12.45 ± 0.18 µg/mg. In this stage there is a highly increase in protein concentration among all the development stages.

CONCLUSION:

During five different groups of developmental stage of seed and fruit pericarp of *Euryale ferox* Salisb. fruit maturation were analyzed. The protein concentration was obtained that increased in the kernel of over mature stage as compared to immature stage during fruit maturation in both Control and Kinetin treated groups (0.0001%, 0.001%, 0.01% and 0.1%). It can be concluded that the concentration of protein in both seed and pericarp fruit group's increased linearly along with the concentration of kinetin up to the 0.01%. The concentration of kinetin at 0.1% analyzed in the decrease in protein concentration in both seed and pericarp fruit groups. Thus, 0.01% concentration of kinetin was ideal for the enrichment of protein concentration for both pericarp and seeds of *Euryale ferox* Salisb.

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