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EFFECT OF KINETIN ON PROTEIN CONTENT OF *Euryale ferox* Salisb (*Makhana*) DURING FRUIT DEVELOPMENT

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ABSTRACT : *Euryale ferox* Salisb, also known as fox nut, Makhana or gorgon nut, is the member of the family Nympheaceae. Fresh weight and dry weight as well quantitative and protein changes in the developing Makhana seeds and pericarp were described from 12 days after flowering until maturity. The results revealed that in immature fruit, the seeds were more proteinaceous in the control condition reaching up to an average of 25.03days followed by 0.0001% kinetin in the perisperm. In the controlled conditions, the development of pericarp and seeds were normal and gradually increased in the mature stage, while in the over- mature stage it just multiplied twice the rate of protein development. Of all the stages, it was observed that 0.01% Kinetin was more ideal for seed as well as for pericarp development as there is no drop out in the increasing protein concentration during each successive stage of development.

Keywords: *Euryale ferox, kinetin, seed, kernel, pericarp, protein content.*

Euryale ferox Salisb, also known as fox nut, Makhana or Gorgon plant, is the member of the family Nympheaceae. The seeds of Makhana, eaten raw or cooked, are very nutritious containing 77% edible starch. Biochemical analysis of its seed revealed that it contain 15.6% protein, 61% carbohydrate, 12.1% moisture, 7.6% fibre, 1.8% ash and 1.35% fat (Alfasane *et al.,* 2). Makhana seeds with 10-12% protein content are known for its high essential amino acid index (EAAI) which constitutes about 90% (Jha *et al.,* 6 & 7). The seeds of *E. ferox* are medicinally useful. Makhana is recommended for treatment of diseases related to respiratory, circulatory, digestive, excretory and reproductive system. Its seeds are applied in the treatment of diarrhea, spermatorrhea, and the petioles and pedicels in polidipsia, and mouth dryness and dry throat (Anon., 1). The *E. ferox* seed coat represents a potentially cheap source of natural antioxidants with a vast range of application. Large quantities of protein are accumulated over a short period of time during seed development in many legumes (Beevers and Poulson, 4; Millerd *et al.,* 9). These accumulated proteins are few in kind but constitute a high proportion of the total protein of the mature seed (Altschul, 3). Phytohormones play an essential role in regulating plant growth and development. Cytokinins have been implicated in many developmental processes and environmental responses of plants, such as regulation of cell division, apical dominance, chloroplast development, anthocyanin production and maintenance of source-sink relationship (Nieto and Frankenberger, 10). Their exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins (Wingler *et al.,* 12), to cause induction of flower/pod set, to reverse leaf and fruit abscission, to release seed dormancy (Pospisilova *et al.,* 11) and to modify substantially plant responses to a variety of environmental stresses (El-Shihaby *et al.,* 5). Only few studies have critically analysed the physiological effects of kinetin application and their overall impact on plant productivity of *Euryale sp.* Thus, the present study deals with the synthesis or accumulation of the seed proteins of Makhana due to kinetin application.

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MATERIALS AND METHODS

Collection of material

The seeds of *Euryale ferox* Salisb. were sown in the pond of P.G. Department of Botany, Bhagalpur University for the present study. Fruits were collected between May to August, 2010 and 2011. On the accomplishment of anthesis, several flower bearing pedicels were marked and tagged with nylon threads and a small plastic card mentioning the date of opening of flower buds and different concentration of Kinetin (0.0001%, 0.001%, 0.01%) and control were marked. The opening of flower bud was considered as accomplishment of anthesis. The fruit samples were collected at eight different staged of their maturation. The first collection of fruits was made at the immature stage (152 DAA) and thereafter, fruits were subsequently picked for analysis at ¼ mature stage (164 DAA) to over mature stage (230 DAA).

Treatment of Kinetin during fruit development

During maturity and development, the fruits were treated for 1minute and 5 minute with three different concentrations of Kinetin (0.0001%, 0.001%, 0.01%) and at each of the eight stage separately (Figure 1) for the period of a week (7 days duration) in each concentration.

Treatment of Kinetin during seed maturation

The mature fruits were soaked for 1minute and 5 minute at three different levels of Kinetin (0.0001%, 0.001% and 0.01%) along with control (in distilled water) on 480 days (16 weeks) after sowing for the period of 7 days interval.

Preparation of homogenate

At all the stages of fruit development and seed maturation, six apparently identical healthy samples were selected and weighed. All the weighed amount of kernel in six different replicates was homogenized in a pre-chilled glass mortar and pestle separately. These were diluted with glass distilled water (GDW) or specific buffer solution in a desired ratio. All the chemicals used were of analytical grade.

Colorimetric estimation

The calorimetric estimation of the content of protein was made in the kernel of Makhana fruits at the different stages of their maturation. The soluble protein content was estimated by FC (Folin- Cioalteace reagent (Lowry *et al.,* 8). One ml tissue homogenate with 3ml 30% trichloro-acetic acid was centrifuged for 15 min 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 to thoroughly dissolve protein the proceeding day. Further 1ml of the protein suspension was taken to add 5ml alkaline Copper Sulphate reagent (50 part 10% Na_2CO_3 in 0.5N NaOH and 1 part of 0.5% $CuSO_4$. $5H_2O$ in 1% Potassium tartarate). The solution was allowed to stand for 10 min and then was treated with 0.5 ml of 1N FC reagent. Optimum colour 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 of fresh weight basis. The protein of seed and pericarp were separated by the method of Lowry *et al.* (8) into three major concentrations of Kinetin (0.0001%, 0.001% and 0.01%) and a controlled (untreated) condition. Eight different stages of development were chosen for calculating the day after anthesis (DAA). These were described 12 days after flowering until maturity.

RESULTS AND DISCUSSION

Effect of Kinetin on Protein Content during Fruit Maturation

The changes in protein content in kernel due to the effect of different concentrations (0.0001%, 0.001%, 0.01%) of kinetin after 7 days treatment separately at each of the eight stages of fruit development and maturation in Makhana are

Fig. 1: Effect of kinetin (kn) on protein content (µg/mg tissue fresh wt) in the pericarp of *Euryale ferox* Salisb. during pericarp development

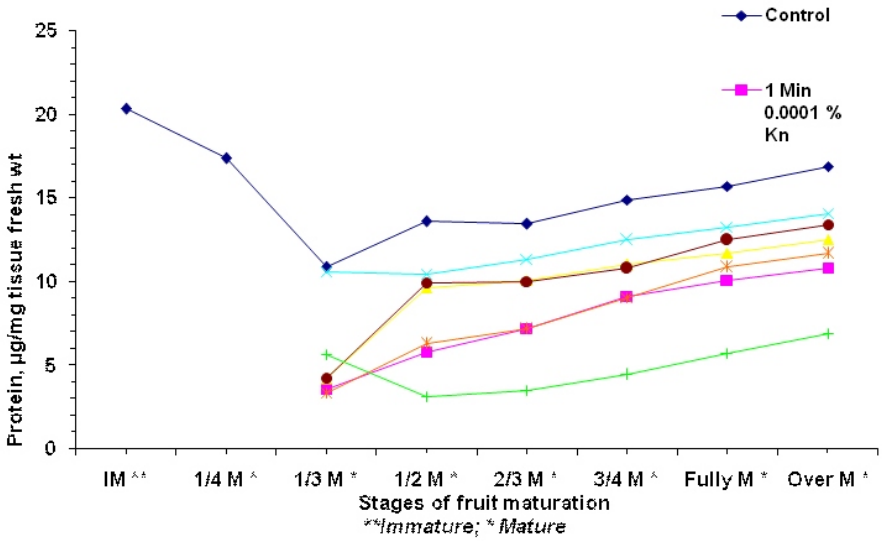
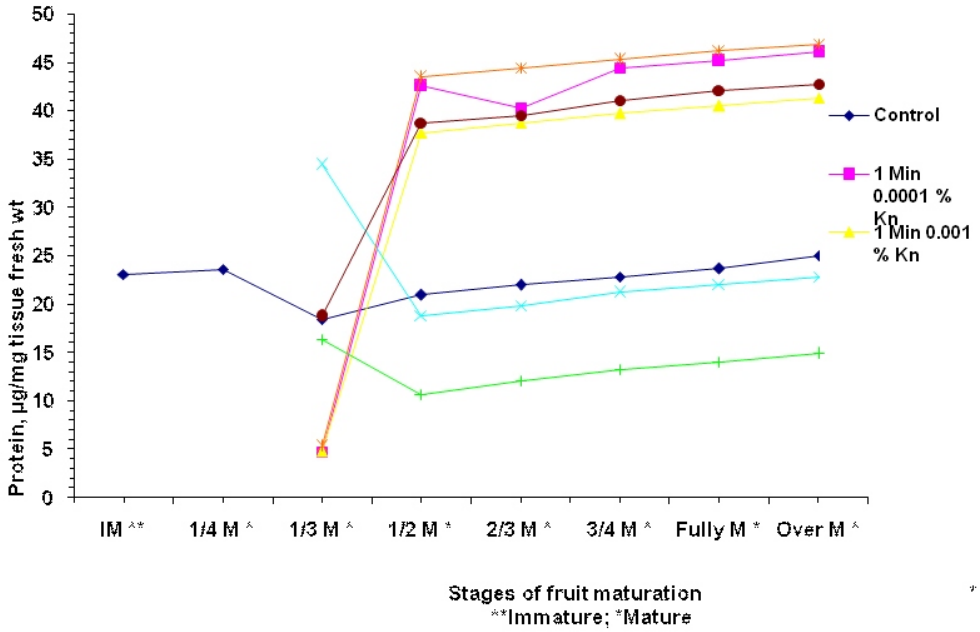


Fig. 2 : Effect of kinetin (kn) on protein content (µg/mg tissue fresh wt) in the perisperm of *Euryale ferox Salisb.* during fruit maturation



described in Figure 1 and 2. All the calculations were made on the fresh weight basis.

1. Protein content in pericarp of the fruit (1 minute treatment)

In the pericarp of control (untreated) fruits, the amount of protein ranged in between 20.29 ±2.63, 17.33 ±0.38, 10.38 ± 0.36, 13.55 ± 0.29, 13.40 ± 0.17 , 14.84 ± .10, 15.69 ± 0.08, 16.83 ± 0.21 µg/mg tissue in the kernel during the development of fruits. At 1/3rd mature stage, the content of protein in the kernel of control fruits was minimum and increased considerably at the over mature stage to reach its maximum level.

In fruits treated with 0.0001% Kinetin, the protein content ranged between 3.56 ± 0.51, 5.75± 0.22, 7.16 ± 0.13, 9.07 ± 0.27, 10.03 ± 0.11 and 10.81± 0.08 µg/ mg tissue during fruit development. Under the condition of 0.0001% treatment, protein content was minimum (3.56± 0.51) at 1/3rd mature stage and there after it undergone a significant increase at the over mature stage. In 0.001% kinetin treated fruits, the protein content ranged between 4.16 ± 0.62 and 12.47± 0.08 µg/mg of tissues. In this stage there was a large increase in protein content in 1/3rd mature stage to over mature stage which further increased at fully mature stage.

In 0.01% kinetin treated fruits, the amount of protein ranged between 10.56 ± 0.47 and 14.03 ± 0.09 µg/mg of tissues. In this stage there was a sharp decrease in protein content during IV & V stages of development (i.e. 1/3rd mature to ½ mature stages and 2/3rd mature stage). From 2/3rd mature stage the protein content was increased more during V to VIII stage (*i.e* from 2/3rd to over mature stage).

2. Protein content in pericarp of the fruit (5 minute treatment)

In 0.0001% kinetin treated fruits, the protein content ranged between 3.31 ±0.46, 6.25± 0.23, 7.12 ± 0.16, 9.03 ± .49, 10.83 ± 0.11 and 11.64 ± 0.07 µg /mg tissue during fruit development. Under the condition of 0.0001% kinetin level, protein content was minimum (3.31 ± 0.46) at 1/3rd mature stage and there after it undergone to a significant increase at the over mature stage. In 0.001% kinetin treated fruits, the protein content ranged in between 4.19 ±0.54 and 13.39 ± 0.14 µg / mg of tissues. In this stage there was a large increase in protein content in 1/3rd mature stage to over mature stage which further decreased at fully mature stage.

In 0.01% kinetin treated fruits, the amount of protein ranged between 5.61± 0.88 ±to 6.85 ±0.09 µg /mg of tissues. In this concentratin also, there was a sharp increase in protein content during III to VIII stages of development.

3. Protein content in seeds of fruit (1 minute kinetin treatment)

In the control fruits the amount of protein ranged in between 23.11 ± 2.95 and 25.03 ± 0.17 µg/mg tissue in the kernel during the development of fruits. There was a slight (unnoticeable) change up to ¾ mature stage. A sudden increase of 23.64± 0.10 µg/mg tissue in the kernel of the fully mature stage was observed which considerably at over mature stage reached 25.03± 0.17 µg/mg tissue. In 0.0001% kinetin treated fruits, the protein content ranged between 4.73± 0.24 and 46.00 ± 0.09 µg /mg in the kernel during the development of fruits. In this concentration, the protein content increased much from 1/3rd mature to ½ mature stage and then sharp decrease from ½ mature stage 2/3rd mature stage was noticed, and then increased to over mature stage. Under this condition protein content was observed as ideal of all the stages in seeds of the kernel.

In 0.001% kinetin concentration, protein content ranged between 4.79 ±0.45 and 41.29 ± 0.10 µg/mg tissue on fresh weight basis during the development of fruits. In this concentration, the result was one of the most unstable because of several ups and down in each subsequent stages of development which finally in the over mature stage reached in the maximum value of 41.29 ± 0.10 µg/mg as compared to all studied stages.

In 0.01% kinetin treated fruits, the protein content ranged between 34.39 ±0.47 and 22.83 ± 0.09µg /mg in the kernel during the development of fruits. This stage was also one of the most unstable because of several ups and down in each subsequent stages of development which in the over mature stage reached up to 22.83 ± 0.09 µg/mg.

4. Protein content in seeds of fruit (5 minute kinetin treatment)

In 0.0001% kinetin treated fruits the protein content ranged between 5.28 ± 0.23 and 46.89 ± 0.08 µg/mg in the kernel during the development of fruits. In this concentration, the protein content increased much from 1/3rd mature to 1/2 mature stage and then sharp increase from ½ mature stage to over mature stage was observed. In 0.001% concentration of kinetin, protein content ranged between 18.72 ± 2.41 and 42.76 ± 0.08 µg / mg tissue on fresh weight basis during the development of fruits. In 0.01% kinetin treated fruits, the protein content ranged in between 16.36 ± 0.82 and 14.87 ± 0.09 µg / mg in the kernel during the development of fruits . This stage was found one of the most unstable because of several ups and down in each subsequent stages of development which in the over mature stage reaches 14.87± 0.09 µg /mg of tissue.

The present study deals with the protein content in seeds and pericarp tissue (µg/ mg) of *Euryale ferox* during different stages of development. Six replicates were taken in four condition of kinetin saturation. Although kinetin is known to have a direct influence on photosynthesis. Eight different stages (Figure 1 and 2) of development were chosen by calculating the day after anthesis. Out of two stages i.e. immature stage to 1/4th mature was without treatment i.e. untreated (control). The four different saturation points taken were 0.01%, 0.001%, 0.0001% of kinetin and the control condition of the six replicates (Figure 1). The mean value of each replicates was calculated and the standard error was derived. Figure 1 shows the protein content during the pericarp development and the protein content during seed development while Fig. 2 shows the protein content during seed development

It was observed that of all the eight stages studied the maximum protein content is found in the seed in the over mature stage in 0.001% kinetin which is up to 41.29 ± 0.10 µg / mg followed by 0.01% and the 0.0001% kinetin concentrations (Fig. 2). Results showed that in 0.01% conc., the seeds contained nearly the equal amount of protein in the mature stages. In the early stages of development, the seeds was more proteinaceous as compared to pericarps which gradually increased up to an average of 41.29 ± 0.10 µg / mg. In 0.0001% conc., the protein content of seeds increased up to 10% as compared to pericarp.

On the basis of present study it can be concluded that in immature fruit, the seeds are more proteinaceous in the control condition reaching up to an average of 25.03 ± 0.17 µg / mg followed by 0.0001% kinetin level. In the controlled conditions, the development of pericarp and seeds were normal and gradually increased in the mature stage, while in the over-mature stage it just multiplied twice the rate of protein development. In other words we can say that there was a drop out in 0.01% and 0.0001% kinetin condition between 1/3 maturity and mature stage. While in all the stages i.e., 0.01%, and 0.0001% of Kinetin and controlled condition, there was an unbalance in protein concentration in pericarp. In seeds, the protein content was more constant in full mature to over mature stage at 0.01% concentration. While the pericarp showed variability in protein content as calculated in full mature to over mature stage in

0.01% of kinetin saturation. This drop out was calculated up to 20%.

It can be concluded that of all the stages we studied, the mature pericarp along with seeds have a drop out of approximately 20%, in all the taken concentrations of kinetin, which is a matter of further consideration. Of all the stages, we observed 0.001% conc. Kinetin is more ideal for seed development and 0.0001% conc. for pericarp as there was no drop out in the increasing protein concentration during each successive stage of development. It was that in these two kinetin concentrations (*i.e.* 0.001% and 0.0001%), the protein content increased up to 16 and 32%, respectively for pericarp and seeds in the over mature stage. But as we discussed earlier that overall protein content was maximum in 0.001% kinetin in seeds and 0.0001% Kinetin in pericarp. Thus, 0.001% concentration was found ideal for the growth of *Euryale ferox* from the view point of protein content for both pericarp and seeds development.

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