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ANALYSIS OF CERTAIN BIOCHEMICAL CHANGES ASSOCIATED WITH GROWTH AND RIPENING OF PUMPKIN FRUIT IN RELATION TO ITS SEED DEVELOPMENT

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ABSTRACT

A correlative study on biochemical changes during fruit ripening and seed development in *Cucurbita moschata* Duch. (Pumpkin) has been carried out. The pumpkin fruit was collected at its three successive developmental stages viz. mature, pre-ripened and ripened fruit and subjected it for its biochemical analysis. The obtained results showed that with the advancement of fruit maturation, the amount of pigments (total carotenoids, lycopene), total sugars and non-reducing sugars, proteins and phenols increase significantly, while that of reducing sugars decrease. In contrast, a declining trend of total sugars, reducing and non-reducing sugars, proteins and phenols occurs in the maturing seeds. The sequential developmental stages of pumpkin fruit and its seeds show variations in the amount of their minerals. However, the pumpkin fruit with its advancement towards maturation and ripening, show significant accumulation of fibre content, while a large amount of oil get stored in its seeds. This reveals a positive interdependent developmental relationship between the fruit and seed of pumpkin. Moreover, the present study also indicates that the development of seed may possibly have an active role in the ripening of fleshy fruits.

Key words*: composition, Cucurbita moschata, development, fleshy fruit, physiology*.

INTRODUCTION

It is known from the earlier studies that a possible competition occurs between the growth of the fruit and seed development. Moreover, seeds have been reported to have strong influences on the growth of fleshy fruits, ranging from strongly promotive to inhibitory effects, depending upon the stage of fruit. In view of these observations, the present study has been undertaken to elucidate the biochemical relationship between the growth and ripening of a fleshy fruit of *Cucurbita moschata* Duch. with the development of its seed.

Cucurbita moschata is an ancient crop of Americas and its fruits are commonly called pumpkin or squashes, which belongs to the family Cucurbitaceae, also known as the gourd family. In India, the family Cucurbitaceae is represented by nearly 34 genera and 108 species, of which 38 species are endemic. The species is cultivated as an annual or perennial crop, cultivated for its edible fruibts, which are either used as esculents or ornamentals [1]. The fruits of pumpkin are largely used as medicine in various parts of the world for various ailments. It is a gentle and safe remedy for a number of complaints, especially as an effective tapeworm remover for children and pregnant women for whom stronger acting and toxic remedies are unsuitable [2]. The seeds are mildly diuretic and vermifuge; complete seed, together with the husk, is also used to remove tapeworms. The seed, which is known to contain high amount of zinc, has been used in the early stages of prostate problems [3].

The present study has been carried out to understand the biochemical changes associated with the growth and ripening of pumpkin fruit in relation to its seed development.

MATERIALS AND METHODS

The fruits of pumpkin were collected at their three sequential developmental stages i.e. mature, pre-ripened, and ripened (Fig. 1). After recording the measurements (length and diameter) of these fruits (Mature 15.4 cm x 18.9 cm, Pre- ripened 20.3 cm x 23.6 cm, Ripened 25.3cm x 21.7 cm), they were subjected for their biochemical analyses:

Fig. 1 Fruits of pumpkin at their successive stages of growth and ripening

The estimation of pigments such as carotenoids and lycopene were estimated as per the method of Tomes [4]. Following the method of Thimmaiah [5], the amount of total sugars, reducing and non-reducing sugars, protein and phenols were estimated. The oil content of the seeds was measured using Soxhlet apparatus extraction method and crude fibre from fruit pulp was estimated by gravimetric method of Mazumdar and Majumder [6]. The minerals such as Zn, Cu and Mn were estimated by using Atomic absorption spectrophotometer (Nova 400) following the method of Berwal *et al.* [7], while iron, calcium and phosphorous were estimated using Inductive coupled plasma spectrophotometer at SICART, Vallabh Vidyanagar, Gujarat, INDIA. Using HPTLC the profiling of sugars was done following the method of Daniel [8], while that of amino acids, using the method of Jayaraman, [9]. The data obtained from the present study, were subjected for their statistical analysis, using DMRT (Duncan's Multiple Range Test) by IRISTAT software V. 3.0. [10].

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RESULTS AND DISCUSSION

Qualitative and quantitative changes in the pigments of Pumpkin fruit

Color changes in fruit are one of the primary indicators demonstrating the maturity and ripening of fruits. The amount of total carotenoids in the presently studied fruit increased successively by more than one fold from 0.25 mg/100gm at mature stage to 0.35 mg/100gm at pre-repined stage and further increased by more than four fold until ripening (1.50 mg/100gm) (Fig. 2, Table - 1). The amount of lycopene content in the presently investigated pumpkin fruit was recorded to be 0.38 mg/100gm at the mature stage, but it decreased to 0.32 mg/100gm at the pre-ripened stages followed by a sharp increase of more than three fold to the level of 1.01 mg/100gm during ripened stage (Fig. 2, Table - 1). Thus the results of the presently studied pumpkin fruit are in accordance with the results obtained by Dutta *et al.* [11], who reported carotenoid in the pumpkin fruit to be around 0.43 mg/100gm, while Karkleliene *et al.* [12] observed huge variations in the amount of carotenoids among the various pumpkin cultivars.

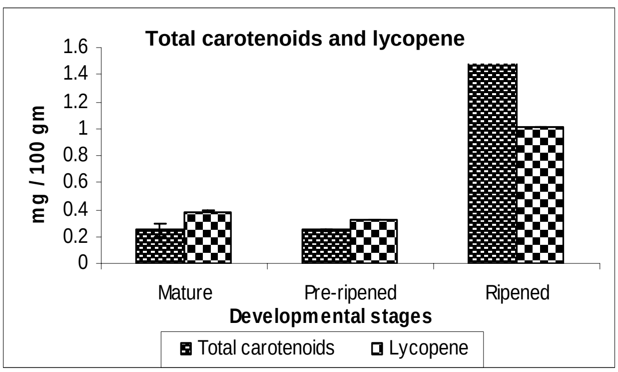

Fig. 2 Total carotenoids and lycopene in fruit

Qualitative and quantitative changes in the sugars of pumpkin's fruit and seed at their successive stages of development

The biochemical analysis of the presently studied pumpkin fruit revealed that the amount of total sugars gradually improved significantly by about two folds in their content from the mature stage to ripened stage (i.e. 6.68 to 12.71 mg/gm) (Fig. 3, Table - 1), while the amount of sugars in the seeds of pumpkin decreased by more than two fold from 22.01 at mature stage to 9.11 mg/gm at the ripened stage (Fig. 4, Table - 1). Also the amount of reducing sugars in the pumpkin fruit increased by more than two folds from mature stage to the ripened stage (i.e. 1.98 to 4.10 mg/gm) (Fig. 3, Table - 1), whereas in case of seeds the amount of reducing sugars decreased by more than two fold from mature stage to ripened stage (10.35 to 4.61 mg/gm) (Fig. 4, Table - 1). Similarly, the amount of non-reducing sugars in the pumpkin fruit showed insignificant increase from mature stage to the pre-ripened stage (4.68 to 4.92 mg/gm) but thereafter showed a significant increase by more than one fold until the onset of ripening measuring 8.59 mg/gm (Fig. 3, Table - 1). On the other hand amount of non-reducing sugars of seed, decreased during their stages i.e. from mature stage to ripened stage (11.65 to 4.45 mg/gm) (Fig. 4, Table - 1). It is apparent from the flavor of different fruits that their sugar content varies widely. The total soluble sugars in the presently worked out fruit of pumpkin showed tremendous increase in their quantity. It was found that the reducing sugars increase during the present study and are in accordance to the results obtained by Pruthi [13]. This could be attributed to the hydrolysis of starch, which gets accumulated during the early stages of growth. But during the later stages of development i.e. ripening, the amounts of sugars tend to increase due to high activity of enzymes such as α-amylase, β-amylase and starch phosphorylase [14]. In contrast the amount of total sugar content decreased in the seed, which may be due to the accumulation of sucrose into starch and such variations, has been observed by Karkleliene *et al.* [12] in various cultivars of pumpkin. Also starch serves as a reserve material and also as a source of nutrition needed by the seed during the germination [15].

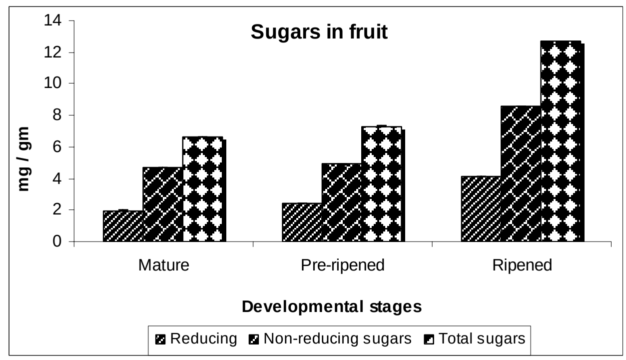

Fig. 3 Changes in sugar content of fruit

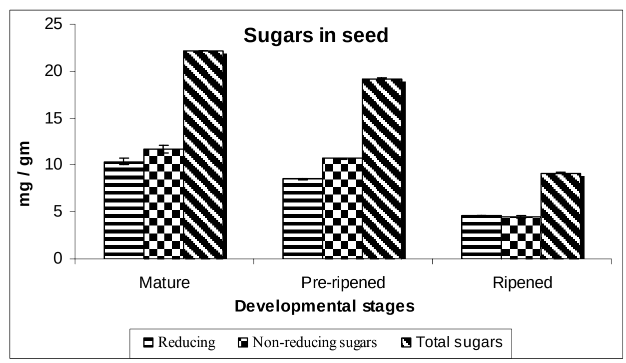

Fig. 4 Amount of sugars in seeds

Proteins alteration in pumpkin fruit and seeds

As per the presently carried out biochemical analysis of pumpkin fruit, the total protein content of it is found to have a gradual increase in their content from 28.72 mg/gm at its mature to 56.68 mg/gm at the ripened stage which is more than one fold (Fig. 5, Table - 1). In contrast, the amount of total protein content of pumpkin seed decreased from 206.97 mg/gm at the mature stage to the tune of 101.72 mg/gm at the ripened stage, which shows the decrease of protein in the developing seed by more than one fold (Fig. 5, Table - 1). Proteins are the principle constituents of cell protoplasm and chiefly function to act as essential component of structural material. The net change in the amount of protein was observed to increase during maturation and ripening of pumpkin fruit. The net increase in the amount of proteins could be due to the metabolic processes within the plant cells, which continues even after harvest by using the free amino acids. Also the reduced activity of enzymes such as protease also helps the fruit in accumulating the proteins during the later stages of ripening [14]. In contrast seeds exhibited a sharp decrease in the amount of total protein. According to Martin [16] pumpkin seeds contain more protein than that present in the fruits. The results of the present study are in agreement with that of Martin [16] who described that the proteins in fruits are principally globulin type and are deficient in lysine but also in sulfur bearing amino acid. In the mature stage high protein content was recorded which indicates that the seeds are metabolically active as it require them for various metabolic activities occurring within the seed. But as the seed of the ripened stage are fully mature and hence are less metabolically active and may be the seeds may undergo the process of dormancy [17].

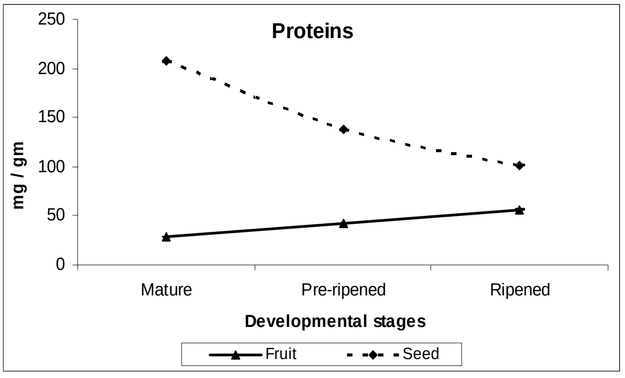

Fig. 5 Amount of proteins in pumpkin fruit and seeds

Changes in Phenols during growth and development of pumpkin fruit and its seeds

Phenolic compounds enjoy a wide distribution in plant kingdom and they are particularly prominent in determining color and flavor of fruits. Total phenol content in pumpkin fruit decreased by 1.5 times as it declined from 0.48 mg/gm at mature stage to 0.31 mg/gm at the pre-ripened stage. But there after it increased by two fold (0.815 mg/gm) at the ripened stage (Fig. 6, Table - 1). In seeds no significant change occurred in the quantity of total phenols except a decline at ripened stage by 5 fold (Fig. 6, Table - 1). The results of the present study are in accordance with the results obtained by Kadam *et al.* [18] who also reported low levels of phenolic compounds in the fruit of pumpkin. The phenolic content of most fruits declines from high levels during early growth to low levels [19], [20]. The fruit of pumpkin shows a gradual increase in phenolics content towards its maturity and ripening. The concentration of phenol decreases as the fruit matures and ripens. The higher levels of phenolic compounds may provide a protection mechanism for the fruit. However, the seed exemplified very low amount of phenolic compounds at all sequential stage of growth and ripening.

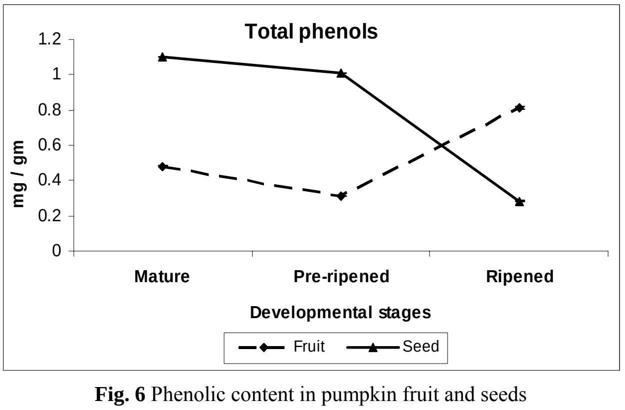

Fig. 6 Phenolic content in pumpkin fruit and seeds

Oil content during development of seed

The oil content increased gradually from 7.75gm/100 gm at the mature fruit stage to 11.15gm/100gm exhibiting more than half fold increase at the pre-ripened stage, while three fold increases in the oil content was observed at the ripened stage (34.40gm/100gm) (Fig. 7, Table - 1). According to Martin [16] the seeds of cucurbits contain up to 50% oil content and most of the oil present is made up of non-saturated fatty acids, thus of high nutritional values. Oils and fats are triglyceride complexes of organic fatty acids. Besides conjugated fatty acids among some cucurbit oils make them highly useful as drying oils [16].

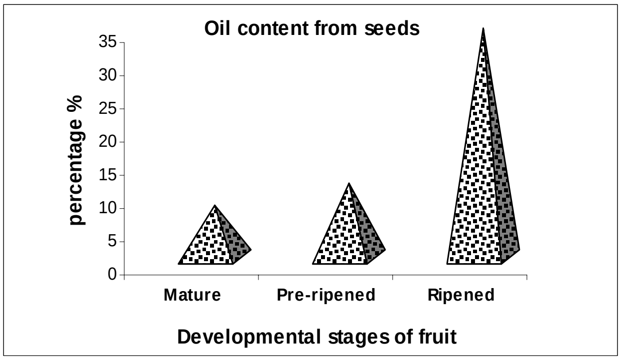

Fig. 7 Changes in oil content during seed development

Fibres

Crude fibre is considered as the material left after digestion of tissues and mainly composed of cellulose, lignin and some minerals [20]. High fibre content of food helps in digestion and prevention of colon cancer [21]. The fibre content of pumpkin fruit pulp increased gradually from 1.62 gm/100gm at mature fruit stage to 1.94gm/100gm at the ripened fruit stage indicating one fold increase in the crude fibre of ripened pumpkin fruit (Fig. 8, Table - 1). The results of the present study show the accumulation of fibre content during successive stages of maturation and ripening. Thus the results of present study are in accordance with the findings of Agostoni *et al.* [22] who stated that non starchy vegetables are the richest sources of dietary fibers. Daniel *et al.* [23] reported that the fibre content has high correlation with solids content of the fruit and carried out detailed observations of the texture content using different pumpkin cultivars. Besides, Saldana [20] stated that these dietary fibers are employed in treatment of diseases such as obesity, diabetes and gastrointestinal disorders. In view of the foregoing account it may be said that the fruit of pumpkin serves as a rich source of dietary fibers.

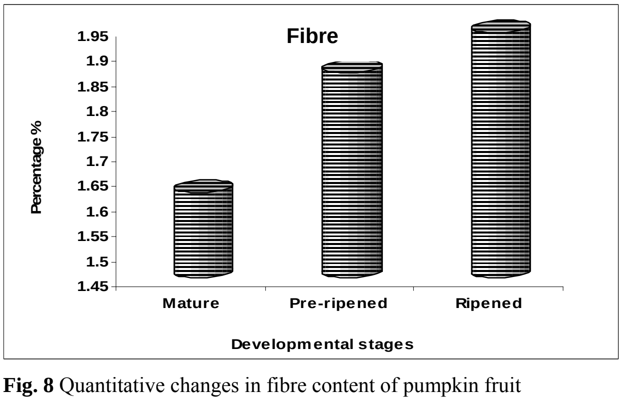

Fig. 8 Quantitative changes in fibre content of pumpkin fruit

Table - 1 Quantitative analysis of biochemical changes during growth and development of pumpkin fruit in relation to its seed development

Biochemical metabolites	Developmental stages of fruit and seed					
	Fruit			Seed		
	Mature	Pre-ripened	Ripened	Mature	Pre-ripened	Ripened
Total Carotenoids (mg/100gm)	0.25 ± 0.051^{a}	0.35 ± 0.005^{a}	1.50 ± 0.005^{b}	-	-	-
Lycopene (mg/100gm)	0.38 ± 0.008^{b}	0.32 ± 0.004^{a}	1.01 ± 0.005^{c}	-	-	-
Total sugars (mg/gm)	6.663 ± 0.017^{a}	7.322 ± 0.047^{b}	12.718 ± 0.017^{c}	22.010 ± 0.160^{c}	19.228 ± 0.018^{b}	9.114 ± 0.065^{a}
Reducing sugars (mg/gm)	1.982 ± 0.006^{c}	2.402 ± 0.011^{b}	4.100 ± 0.013^{a}	10.35 ± 0.342^{c}	8.509 ± 0.071^{b}	4.615 ± 0.057^{a}
Non-reducing sugars (mg/gm)	4.683 ± 0.022^{a}	4.920 ± 0.054^{a}	8.595 ± 0.003^{b}	11.65 ± 0.479^{c}	10.71 ± 0.084^{b}	4.497 ± 0.104^{a}
Proteins (mg/gm)	28.72 ± 0.121^{a}	42.72 ± 0.601^{b}	56.68 ± 0.102^{c}	206.97 ± 0.204^{c}	137.42 ± 0.202^{b}	101.72 ± 0.611^{a}
Phenols (mg/gm)	0.486 ± 0.005^{b}	0.314 ± 0.005^{a}	0.815 ± 0.007^{c}	1.107 ± 0.002^{b}	1.018 ± 0.003^{b}	0.282 ± 0.004^{a}
Oil (%)	-	-	-	7.75	11.15	34.40
Fibre (%)	1.62	1.86	1.94	-	-	-

Table - 2 Quantitative analyses of changes in minerals associated with growth and ripening of pumpkin fruit in relation to its seed development (ppm/gm dry wt.).

Stages of fruit growth and ripening		Fe	P	Ca	Cu	Zn	Mn
Mature	Fruit	54.520	2245.5	2118.1	10.137	N.D	N.D
	Seed	35.72	6030.1	1125.3	N.D	27.17	N.D
Pre-ripened	Fruit	43.320	3106.1	3080.0	N.D	N.D	N.D
	Seed	104.0	7692.6	1491.8	N.D	77.27	N.D
Ripened	Fruit	28.202	3198.5	2278.6	N.D	8.165	N.D
	Seed	85.846	10563.0	1434.8	6.022	5.65	N.D

(N. D. - Not detected)

Minerals during growth of pumpkin fruit and development of it seeds

The analysis of minerals present in pumpkin fruit reveals that iron and copper decreased from the mature fruit stage to ripened stage, while phosphorus, calcium and zinc have been found increasing. In contrast, the amounts of iron and zinc were found to increase in the seeds from mature fruit to its pre-ripened stage but eventually they decline in the seeds of ripened fruit, while gradually phosphorous, calcium and copper increase in their quantity from the fruit of mature stage to its ripened stage (Table – 2). Recently, Hamed *et al.* [24] has observed calcium, potassium, sodium, phosphorous, iron, magnesium, zinc and cobalt to be present in the seeds of pumpkin. Minerals are essential elements, needed for good health. Copper is a necessary element which acts as a co-factor for various oxidative enzymes remained at lower levels during the maturation and ripening of pumpkin fruit and seed. A macro-element, phosphorous, helps in the growth and metabolic process including cell division, cell expansion, respiration and photosynthesis [25]. Calcium, which is known to play an important role in plant growth and development, primarily, because it is required both in cell division and cell elongation, was also observed to increase in the seed as well as fruit with their development [26]. The results of the present study indicate that the fruit of pumpkin contained only reasonable amounts of Cu, Mn and Zn while good sources of some other minerals, especially P, Ca and Fe. Similar observations have been reported earlier by [27, 28, 29].

Characterization of sugars and amino acids

The Characterization of sugars and amino acids with an aid of HPTLC revealed that six sugars and seven amino acids were present at the ripened stage. The sugar include from mature to ripen stage; sucrose (36 - 420 µg/gm F.W.), ribose (500 – 1400 µg/gm F.W.) and fructose (490 - 300 µg/gm F.W.); while three sugars were not identified (Fig. 9). The results of the present study suggest that the main source of these sugars is starch which serves as a reserve material and also as a source of nutrition needed by the seed during the germination [17] and hence the presence of various sugars are observed according to the anabolism or catabolism process that may take place according to the developmental stage of the fruit. In case of amino acids, aspartic acid (800 - 170 µg/gm F.W.), glutamic acid (1200 - 70 µg/gm F.W.) and tyrosine (2100 - 800 µg/gm F.W.) were identified, while presence of four unknown amino acids was recorded (Fig. 10). The amount of free amino acids in relation to metabolic changes during growth and ripening process are fundamentally important in understanding and controlling the ripening behavior of fruits, but has received little attention [30].

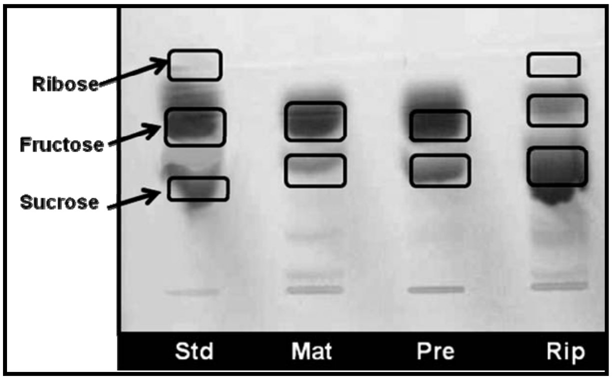

Fig. 9 Characterization of sugars with an aid of HPTLC

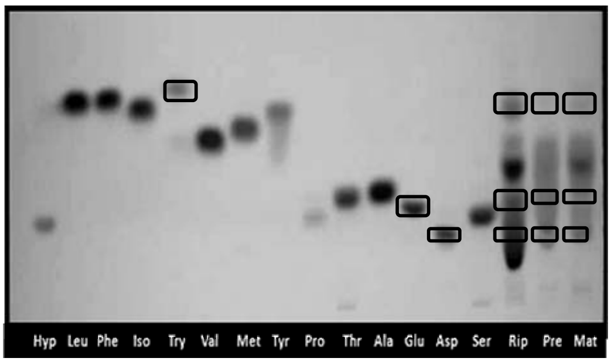

Fig. 10 Characterization of amino acids with an aid of HPTLC

[F. W. - Fresh Weight, Std - Standard, Mat - Mature, Pre - Pre-ripened, Rip - Riened, Hyp - Hydroxy proline, Leu - Leucine, Phe - Phenylalanine, Iso - Isoleucine, Try - Tryptophan, Pro - Proline, Thr = Threonine, Ala - Alanine, Glu - Glutamic acid, Asp - Aspartic acid, Ser – Serine]

CONCLUSION

Looking into the compositional changes associated with the development of pumpkin fruit and its seed, it can be said that fruit serves as a nourishing tissue for the growth and development of seeds. The fruit provides the nutrition to the developing seeds, while seeds accumulate starch / oil as storage reserves to become less metabolically active and enters the dormancy. As fruits show accumulation or synthesis of various storage products, seeds on the other hand show declining trend of their compositional constituents, the present study indicates an inverse relationship between the fruit ripening and seed development. Hence the fruits protect and nurture seeds until they are fully mature and later help the plant in the dispersal of seeds.

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Also announcement of forthcoming Seminars / Conferences / Symposia / Workshops etc. will be considered for publication in PRAJNA.

File format for soft copies:
Texts (should be of Times New Roman with 9 point for Abstract and 11 point for other matter) and Tables, if any, must be saved in *.doc (Word) or *.rtf (rich text) format, graphs in Excel and for illustrations (diagrams, maps, drawings, etc.), the TIF format (300 dpi minimal resolution) is the most appropriate (*.TIF or *.JPEG extension).

Instructions for preparation of manuscripts:
1. The paper should be written in English and neatly typed with double spacing.
2. The title of the paper and the name(s) of the author(s) be in capital letters. The name of the institution be given in small letters below the name (s) of the author(s).
3. The 'Abstract of the paper, in not more than 150 words, should be provided on a separate page along with 4-6 keywords.
4. The sub-titles, e.g. INTRODUCTION, should be written in capital letters.

5. Displayed formulae, mathematical equations and expressions should be numbered serially. Table should be with a title in addition to a serial number for it.
6. Photographs / Figures should be original with good contrast so as to be in a form suitable for direct reproduction / scanning.
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10. All references should be clear and follow the examples below:

Periodical articles

[2] Sadqui, M., Fushman, D. and Munoz, V. (2006) Atom – by – atom analysis of global downhill protein folding. *Nature*, **442:** 317 – 321.

Books

[16] Stebbins, G. L. (1974) *Flowering plants: Evolution above the species level,* Arnold Press, London, pp. 1 – 399.

Chapters from a book

[19] Schafer, H. and Muyzer, G. (2001) Denaturing gradient gel electrophoresis in marine microbial ecology. In *Methods in Microbiology* (Ed. Paul, J. H.), Academic Press, London, Vol. 30, pp. 425 – 468.

Thesis or other diplomas

[21] Nayaka, S. (2004) *The visionary studies on the lichen genus Lecanora sensu lato in India.* Ph. D. Thesis, Dr. R. M. L. Avadh University, Faizabad, India.

Conference proceedings

[4] Mohapatra, G. C. (1981) Environment and culture of early man in the valley of rivers Chenab and Ravi, western sub-Himalayas. In *Proceedings X Congress of IUPPS*, Mexico, pp. 90 – 123.

Online documentation

[9] Koning, R. E. (1994). Home Page for Ross Koning. Retrieved 26-6-2009 from *Plant Physiology Information Website*: http://plantphys.info/index.html.

Note:

Manuscripts prepared faithfully in accordance with the instructions will accelerate their processing towards publication; otherwise it would be delayed in view of their expected re-submission.

For and on behalf of Editorial Board, PRAJNA

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