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RESEARCH ARTICLE

Effect of seasonal variations on the quality and quantity of the flower pigments

■ **S.P. Adhikary**

SUMMARY

The seasonal variations in the different pigment contents indicate the physiological state of the plant and are related to the seasonal water deficiency, high ambient irradiance during summer and very low temperature in winter. The flower constitutes flavonoid pigments. Flavonoids belong to the polyphenol group, which includes so many colouring pigments *i.e.* the anthocyanidin, flavones, flavanones, flavonols, etc. Anthocyanin is among the permitted pigments that can be used for food colourants and having been considered a potential replacement for synthetic dye. Some other flavonoids also enhance the colour of the anthocyanin as co-pigment. The objective of this study was to analyze the flower colouring pigments and their associated phytochemicals qualitatively and quantitatively at developmental stage S-4 (fully opened flower) of *R. indica*, *H. rosasinensis*, *C. ternatea* and *M. jalapa*. Variations in changes in floral pigments (anthocyanin and flavonoids), total sugar and protein content in petals of test flower at S4 stage were analyzed month and season wise. The experimental results noticed at stage 4 the floral pigments (anthocyanin and flavonoids), total sugar and protein content of petal of test flowers were changed their content in different seasons. Regarding seasonal variation, the trend of floral pigment content in petals of test flowers was as follows summer season > winter season > rainy season Among the flowers, the floral pigment content in petal exhibited the following order *Clitoria ternatea* > *Rosa indica* >*Hibiscus rosasinensis* > *Mirabilis jalapa*. The colour pigments *viz*., flavonoids, anthocyanins and phenol derivatives, protein, sugar and other nutrients present in petal of test flower might have act as potent colorant, antioxidant and neutraceuticals in food market. The procurement of colour pigments from petal of the flower at appropriate season is highly essential for further processing and manipulation through screening and evaluation.

Key Words : Seasonal variation, Anthocyanin, Flavonoids, Colour pigment, Petal

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Coloration, in biology is the general appearance of an organism and its parts as determined by the quality and quantity of light that is reflected or emitted from its surfaces. Coloration depends upon several factors: the pigments and its distribution on the organism's body, particularly the relative location of differently coloured areas (*viz*., leave, stem, and flower), latitude, altitude, seasonal variation and different environmental radiative forces and feed-back mechanism. A plant with conspicuous pigmentation draws attention

AUTHOR FOR CORRESPONDENCE
S.P. Adhikary, Department of Botany, Aska Science College, Aska (Odisha) India
Email : adhikarysankarprasad@yahoo.co.in

to itself, with some sort of adaptive mechanism accumulated by seasonal variation and frequent biochemical interaction. Coloration may also play a part in the organism's energy budget, because biochromes create colour by the differential reflection and absorption of solar energy. Energy absorbed as a result of coloration may be used in biochemical reactions, such as photosynthesis, or it may contribute to the thermal equilibrium of the organism. The biochromes in the class of flavonoids, another instance of compound lacking nitrogen, are extensively represented in plants, which rely on plants as sources of these pigments. Flavonoids occur in living tissue mainly in combination with sugar molecules, forming glycosides. Many members of this group, notably an anthoxanthins impart yellow colours, often to flower petals; the class also includes the anthocyanins, which are water-soluble plant pigments exhibiting orange reds, crimson, blue or other colour. The anthocyanins are largely responsible for the red colouring of buds and young shoots and the purple and purple-red colour of autumn leaves. The red colour becomes apparent when the green chlorophyll decomposes with the approach of winter. Intense light and low temperatures favour the development of anthocyanin on reaching maturity; others gain in pigment content during development, often an excess of sugar exists in leaves when anthocyanins are abundant. Injury to individual leaves may be instrumental in causing the sugar excess. Anthocyanins are important pigments from vascular plants. They are responsible for the orange, pink, red, violet and blue colors of the flowers and fruits of some plants (Castañeda *et al.*, 2009) and are mainly used as food colorings in the beverage industry (He and Giusti, 2010). Anthocyanins are flavonoids that have the flavan nucleus as the basic structure, and are characterized by being water - soluble compounds. Their biosynthesis and accumulation depend on factors such as light, temperature, nutritional status, hormones, mechanical damage and pathogen attacks. Light is the most important external factor in their biosynthesis by indirect photoactivation of the involved enzymes through the phytochrome system. The stability of anthocyanins is affected by their chemical structure, concentration and pH, among other factors (Cruz, 2008 and Kong *et al.*, 2003).

The flower constitutes flavonoid pigments. Flavonoids belong to the polyphenol group, which includes so many colouring pigments *i.e.* the anthocyanidin, flavones, flavanones, flavonols, etc. Anthocyanin is among the permitted pigments that can be used for food colourants and having been considered a potential replacement for synthetic dye. Basing on above information and literature, there is very scanty or no information about the effects different seasons on floral pigment of *R. indica*, *H. rosasinensis*, *C. ternatea* and *M. jalapa.* The present investigation was under taken to study the possible effect of different seasons (natural fluctuation in light, temperature, humidity at different months) on total anthocyanin, flavonoid, sugar and protein content in petals of test flowers at S-4 stage was observed.

MATERIAL AND METHODS

South-east Odisha is a vast area constituting some areas of Puri, Khurda and Ganjam districts. As Ganjam district covers major area, some places nearer to Berhampur University were selected as study sites. A brief description of study sites are described below i) Berhampur University campus, a coastal area is situated 3-4 km away from the sea coast of Bay of Bengal (Gopalpur), ii) Berhampur city is also situated at about 12 km away from Gopalpur sea coast and iii) Aska Science College campus which is the central part of south-east Odisha, is at 50 km away from the sea coast.

The above three places of Ganjam district experience to have different soil types such as a mixture of alluvial, laterite and black soil which are slightly acidic and moderately alkaline. Invariably an equable temperature is available all through the year particularly in the closed coastal regions due to high humidity. Winter season in Ganjam ranges from November to February. During this period the mean maximum and minimum temperatures lie between 29.25^{0}C - 19.0^{0}C and its mean monthly relative humidity is 83 %. Summer season in Ganjam ranges from March to June and during this period the monthly mean maximum and minimum temperatures and relative humidity are 31.75^{0}C, 25.75^0 C and 84.5 %, respectively. Rainy season ranges from July to October during which the monthly mean maximum and minimum temperature and relative humidity are 32^{0}C, 26.5^{0}C and 82.75 %, respectively. Its average monthly normal rainfall is 215.4 mm.

Collection and selection of the test flowers :

Flowers of different test plants were collected at S-4 stage in all the months of the year 2014, at an interval of 5 days. The anthocyanins, flavonoids, total sugars and

total protein contents were estimated as per the procedures described below. Basing on the data obtained during monthly analyses, seasonal variation *i.e.* rainy, winter and summer were calculated taking the mean values of July to October, November to February and March to June which were considered as rainy, winter and summer season, respectively. This experiment enables us to know the best season of the year for the extraction of maximum pigments from the studied flowers and make them use in various purposes.

Extraction and estimation of total anthocyanin content :

Extraction :

The total anthocyanin pigments were extracted from petals of each of the individual flowers at stage 4 developmental stage and estimated as per the methods adopted by Harborne (1998). 1gram petal materials of each test flowers collected from different developmental stages were taken in separate conical flasks stoppered with rubber cork and allowed to leach in 10 ml of 0.1% HCl in 80% methanol (v/v), for 3 hours at room temperature in darkness. Then the petals were homogenized thoroughly in a clean sterilized mortar and pestle. The homogenate was then filtered through Whatman filter paper (No.110). The remaining residues were washed with 0.01% HCl in 80% methanol until clear filtrate was obtained. The pooled filtrates were then concentrated using a rotary evaporater at 30 ± 1^0C.Then the concentrated substances were dissolved in acidified water [0.01% HCl in D.W (v/v)] and the solution so obtained was made upto a known volume (10ml) with acidified water and stored at 4 ± 1^0C. The resultant solution served as the floral pigment extract.

Estimation :

The total anthocyanin content in the petal tissues of test flowers was estimated as per the method described by Chirbago and Fransis (1970). The total pigment extracts of different flower at developmental stage 4 obtained and stored at 4 ± 1^0C and were taken out of the fridge and allowed to defrost at the room temperature for 30 minutes.

In one set, to 1ml of each extract taken separately in 10ml conical flask 4 ml of buffer solution of pH 1.0 (0.13M HCl-0.05M KCl) was added and in second set, to 1 ml of extract, 4 ml of buffer solution of pH 4.5 (0.05M HCl-0.05M CH_3COONa) was added. The mixture of buffers and samples were equilibrated in dark for one hour separately and their absorbances were measured at 520nm by spectrophotometer (Elico-digital, Model CL-27). Cyanidin-3-glucoside was used as standard. The data are expressed in mg/g fr. wt of the petal tissues.

The total anthocyanin content was calculated by following the formulae adopted by Fuleki and Francis (1968).

$$\text{Total anthocyanin content } Z \frac{[A \times MW \times DF \times 10^3]}{£ \times I} \text{ mg/g fr. wt.}$$

where A=Absorbance = (A_{520nm} at pH 1.0 – A_{520nm} at pH 4.5),

MW= Molecular weight = (449.2 g/mol) for cyanidin-3-glucoside (cyd-3-glu)

DF = Dilution factor (0.2ml. sample in 2 ml. DW =10)

l = Path length of sample in cuvette in cm =1 cm

ε = 26900 (Molar extinction co-efficient, in $L \cdot mol^{-1} \cdot cm^{-1}$, for Cyd-3-glu.) and

10^3 = Factor for conversion from g to mg.

Extraction and estimation of total flavonoid contents:

Extraction :

Petals of different test flowers at stage 4 developmental stage as mentioned earlier were collected from all the study areas, washed thoroughly in distilled water, soaked on blotting paper under shade in laboratory at 25 ± 2 ºC for a few days, allowed to fully dried and then were ground into fine powders using a sterilized mortar and pestle. There after the floral powders were stored in air tight bottles in cold at 4 ±1°C in the laboratory for further use when required. The flavonoid was extracted as per the method adopted by Khatiwora *et al.* (2010) 0.2 grams of the petals powder of each test flower taken out of the fridge, homogenised with sterilized mortar and pestle in 5-10 ml of 80% methanol and then filtered through Whatman (No.1) filter paper. The left over residues were washed with 80% methanol until clear filtrate was obtained. The filtrates were pooled together and made to 20 ml with 80% methanol which served as flavonoid extract.

Estimation :

The total flavonoid content was estimated by following the aluminum chloride method adopted by Khatiwora *et al.* (2010). For estimation of total flavonoids contents, 1 ml of extract of each test flowers were taken in test tubes and 3 ml of 80% methanol was added to

each test tube separately. Then 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water were added, mixed thoroughly and allowed for reaction at room temperature for 30 minutes. A blank was prepared in a similar way by replacing aluminium chloride with distilled water. The absorbance of the reaction mixtures was measured at 415 nm with UV visible spectro photometer. A standard calibration plot was generated at 415 nm using 0-20 mg/ml concentrations of quercetin in distilled water. The total flavonoid content (TFC) was calculated by using the following formula suggested by Kiranmai *et al*. (2011). The data are expressed in mg/g dry wt. of petal tissues.

$$\text{The total flavonoid content (TFC)} = \frac{\text{R x DF x V}}{\text{W}} \text{ mg/g dry wt.}$$

[where, R–Concentration obtained from the standard curve, D.F (Dilution factor) =10, V= Volume of stock Solution= 20 ml, W = Weight of petal powder used in this experiment= 0.2g].

Extraction and estimation of total sugar contents:

Extraction :

The floral powder of dried petals of stage 4 test flowers obtained during flavonoid extraction as mentioned in the above section, were used for sugar extraction following the method of Mahadevan and Sridhar (1998). 100 mg of petal powder of each test flowers were taken and homogenized with 80% ethanol (v/v) and centrifuged at 4000 rpm at 25 $\pm 1^0$ C for 15 minutes and the supernatants were collected. The process was repeated for three times until a colourless supernatant solution was obtained. All the supernatants were pooled together and allowed to evaporate in a water bath till the volume was reduced to slurry of 1 or 2ml. To this slurry distilled water was added to make a definite volume (10 ml.) which served as extract of total sugar.

Estimation :

For estimation of total sugar, to 1 ml of alcoholic petal extract of each test flower, 4 ml of anthrone reagent (2g / l concentrated H_2SO_4) was added carefully from the side of each test tube separately and mixed thoroughly by shaking. The test tubes were placed in a boiling water bath for 10 minutes with glass marble balls on top to prevent loss of water by evaporation. The reaction mixtures were then cooled and their optical densities (O.D.) were recorded at 625 nm with the help of a spectrophotometer following the method adopted by Mahadevan and Sridhar (1998). D-Glucose was used for the standard curve. For blank, 1 ml of distilled water was used in place of extract. The total sugar content was expressed in mg/g. dry wt.

Extraction and estimation of protein :

Extraction :

The extraction of total protein was performed as per the method suggested by Wasim *et al*. (2011). One g petals of each test flowers of developmental stage 4 were taken separately, after washing with DW and soaking in blotting paper, those were homogenized with 10 ml 80% methanol in a sterilized mortar and pestle for 5 minutes at room temperature 25 $\pm 1^0$ C. The homogenates were then centrifuged at 3000 rpm for 10 minutes at 25 $\pm 1^0$ C. Then to the pallets 5 ml of ethyl acetate (99%) was added and again centrifuged at 4000 rpm for 15 minutes at 25 $\pm 1^0$ C. There after the residues were treated with 5 ml of 5 % sodium sulphite (w/v) and 0.1 g of polyvinylpyrrolidone (PVP) and again centrifuged as mentioned above. There after the pellet was treated with 2 to 3 ml 0.1 N NaOH and centrifuged at 4000 rpm for 15 min at 25 $\pm 1^0$ C. Supernatants so collected were used for protein estimation. 1g of petals after washing with DW and soaking in blotting paper, were homogenized with 80% methanol with mortar and pestle and centrifuged at 3000 rpm for 10 minutes at 25 $\pm 1^0$ C.

Estimation :

To 0.5 ml NaOH extract, 2.5 ml alkaline reagent (mixture of 50 ml of Na_2CO_3 in 0.1 N NaOH and 1 ml of mixture containing equal amount of 1% $CuSO_4$ and 2 % sodium potassium tertarate and 0.5 ml Folin-phenol reagent were added). This reaction mixture were allowed to stand at room temperature 30 $\pm 1^0$ C for 30 minutes and the optical density (O.D) of the reaction mixtures were measured at 620 nm. BSA was used as the reference standard. Equal amount of 0.1 NaOH in place of protein extract and Folin-phenol reagent were run parallel as blank. Proteins content was expressed in mg/g fresh wt.

RESULTS AND DISCUSSION

Influence of different months and seasons on flower pigments (anthocyanin and flavonoids, associated phyto-chemicals (sugars and proteins) were analyzed and the results are described below.

The variations in changes in floral pigments (anthocyanin and flavonoids), total sugar and protein

content in petals of test flower at S-4 stage, analyzed in month and season wise are presented Fig. 1 (a and b) and Fig. 2 (a and b). The period from March-June, July-October and November-February are considered as summer, rainy and winter seasons, respectively basing on temperature, rain fall and rainy days during the periods of experimentation.

Changes in anthocyanin :

Results noticed that the anthocyanin content was found to be highest in all test flowers at stage 4 in month of March and their values were 3.86 ± 0.16, 2.42± 0.13, 1.46 ± 0.01 and least in 0.88 ± 0.05 mg/g fr.wt. in *Mirabilis jalapa*, *Clitoria ternatea*, *Hibiscus rosasinensis* and *Rosa indica*, respectively. It is further noticed that the anthocyanin content gradually increased from December onwards till March and then gradually declined till November where the values were found to be 2.74± 0.12, 1.02± 0.11, 0.74 ± 0.5 and 0.58± 0.04 mg/g fr.wt. in petals of *Mirabilis jalapa*, *Clitoria ternatea*, *Hibiscus rosasinensis* and *Rosa indica*, respectively. Data of intermediate values were recorded in other test flowers in different month during the period of observation. The total anthocyanin content was found to

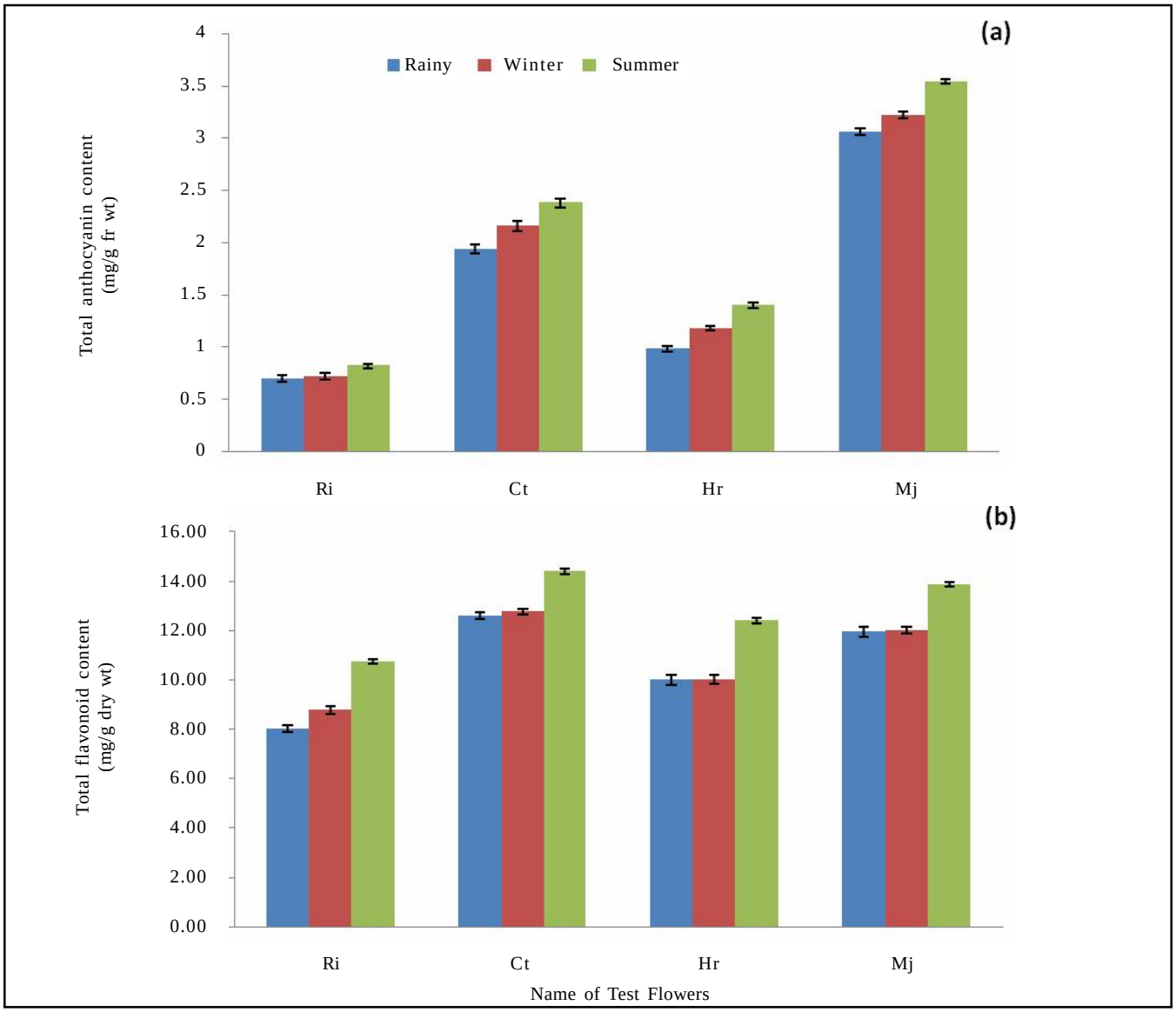

Fig. 1 : Impact of rainy, winter and summer seasons on changes in total anthcyanin (a) and flavonoid (b) contents in petals of the test flowers. *Ri-Rosa indica, Ct-Clitoria ternatea, Hr-Hibiscus rosasinensis, and Mj-Mirabilis jalapa.*Vertical bars represent ± SEM (n=5)

be highest in summer season followed by winter and rainy. Among the flowers, the anthocyanin content in petal of test flowers exhibited the following trend: *Mirabilis jalapa* > *Clitoria ternatea* > *Hibiscus rosasinensis* > *Rosa indica* [Fig. 1(a)].

Changes in flavonoid :

Regarding flavonoid content in the petals of test flowers at S-4 was maximum amount of 14.88 ± 0.19, 14.36± 0.44 and 12.96 ± 0.17 and 11.98± 0.19 mg/g dry wt. were recorded in flowers of *Clitoria ternatea*, *Mirabilis jalapa*, *Hibiscus rosasinensis* and *Rosa indica*, respectively in month of April, whereas the values were reduced to minimum 12.04± 0.28 in *Clitoria ternatea;* 11.08± 0.25 in *Mirabilis jalapa;* 9.12 ± 0.26 in *Hibiscus rosasinensis* and 7.04 ± 0.31 mg/g dry wt. in *Rosa indica*, in the month of November. Petals of test flowers exhibited intermediate values in other months. The change in flavonoid content exhibited more or less same trends in different months and seasons as found in case of total anthocyanin content. Among the test flowers, the flavonoid content was found to be in

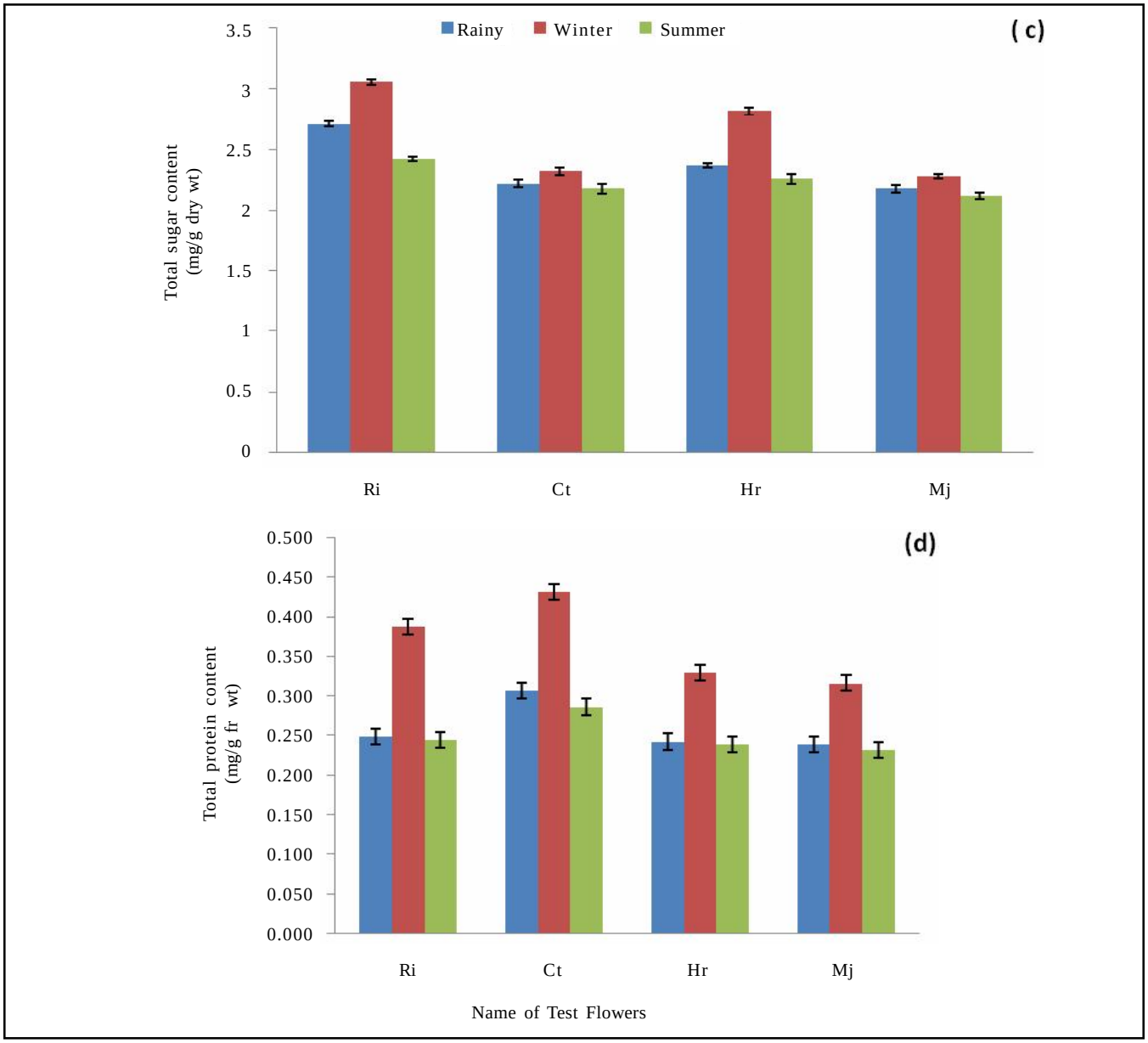

Fig. 2 : Impact of rainy, winter and summer seasons on changes in total sugar (a) and protein content (b) in petals of the test flowers. *Ri- Rosa indica, Ct-Clitoria ternatea, Hr-Hibiscus rosasinensis and Mj-Mirabilis jalapa.* Vertical bars represent ± SEM (n=5)

following order- *Clitoria ternatea* >*Mirabilis jalapa* >*Hibiscus rosasinensis* > *Rosa indica*. Regarding seasonal variation, the trend of flavonoid content in petals of test flowers was as follows: Summer season > winter season > rainy season [Fig. 1(b)]. The flavonoid contents in petals ranged from 14.40 ± 0.11 to 12.58 ± 0.14 in *Clitoria ternatea,* 13.84 ± 0.11 to 11.94 ± 0.19 *in Mirabilis jalapa,* 12.38 ± 0.12 to 09.98 ± 0.18 *in Hibiscus rosasinensis* and 10.74 ± 0.11 to 08.04 ± 0.15 mg/g dry wt. in *Rosa indica*.

Changes in sugar :

From experimental results, it can be observed that the total sugar content in petals of all test flowers exhibited no significant differences throughout the year. The maximum amount of total sugar content of 3.244 ± 0.06, 2.998 ± 0.05, 2.346±0.06 and 2.312 ±0.05 mg/g dry wt. were recorded in the month of December in petals of *R. indica, H. rosasinensis, C. ternatea* and *M. jalapa*, respectively while minimum values of 2.228±0.07, 2.162±0.05, 2.044±0.08 and 2.036±0.04 mg/g dry wt. were noticed in June in *R. indica, H. rosasinensis, C. ternatea* and *M. jalapa*, respectively. Intermediate values were recorded in other months in the petals of four test flowers.

Regarding seasonal variation of total sugar content, it was observed that highest amount of 3.06 ± 0.02 mg/g dry wt. was recorded in petals of *R.indica* in winter season followed by rainy (2.71 ±0.02) and summer (2.42±0.02) seasons. Similar trends were noticed in other flowers too. Among the test flowers, the trend of total sugar content was *R. indica* > *H. rosasinensis* > *C. ternatea* > *M. jalapa* [Fig. 2 (a)].

Changes in protein :

Results of investigated data noticed that the total protein content in petals of all test flowers exhibited the highest values in December and the least in June. Maximum values of 0.460±0.02, 0.422±0.02, 0.342±0.02and 0.328±0.01mg/g fr. wt. were recorded in flowers of *C. ternatea, Rosa. indica, H. rosasinensis* and *M. jalapa*, respectively. Similarly, minimum values of 0.226 ± 0.03, 0.212± 0.01, 0.208± 0.01 and 0.206±0.01 mg/g fr. wt. were noticed during June in *C.ternatea*, *Rosa. indica, H. rosasinensis* and *M. jalapa*, respectively. Intermediate values were recorded in rest of the months in all test flowers.

Regarding seasonal variation of protein content, the petals of test flowers exhibited more or less same trend as was noticed in case of sugar content. Fig. 2 (b) indicates that during winter season higher value of protein were recorded followed by rainy and summer season irrespective of flowers. Maximum values of 0.432 ± 0.01, 0.388 ± 0.01, 0.329 ± 0.01 and 0.316 ± 0.01 mg/g dry wt. of protein was found in petals of *C. ternatea* followed by *R. indica, H. rosasinensis* and *M. jalapa* in winter season, respectively while the least values noticed in summer season were 0.286± 0.01, 0.244± 0.01, 0.238± 0.01 and 0.232± 0.01 mg/g dry wt. in flowers of *C. ternatea, R. indica, H. rosasinensis* and *M. jalapa*, respectively. Data of intermediate values were noticed in different test flowers in different seasons.

Anthocyanin, frequently occurs in various tissues in general and floral tissues in particular, are controlled by different environmental condition (Matile, 2000, Hoch *et al.,* 2001 and Lee, 2002). Further, Hoch *et al*. (2001) reported that anthocyanin production is induced in leaves in response to stresses such as cold, light level, pest and pathogen attack or deficiency of nutrients such as phosphate and nitrogen. The anthocyanin colouration in leaves and flowers can vary with seasons, environment, between individuals of a population and between different leaves on a single plant. It is commonly thought that the anthocyanin have a role in protecting the photosynthetic apparatus from the damage in many of these situations and those tissues that show more anthocyanin accumulation are often at greater photoinhibition risk, *i.e.* during nutrient absorption in senescence leaves of cold-treatment (Hoch *et al.*, 2001). However, the detail of how the anthocyanin achieves this are not determined. Further, Davies (2004 a, b, and c) reviewed on plant pigmentation, functional and economical aspect of specific pigment groups.

The anthocyanin and flavonoid content were found to be maximum during summer season because the biosynthesis of anthocyanin generally depends on carbohydrate (glucose) concentration in floral parts. During the process of photosynthesis, glucose is synthesized in green tissues and is stored as starch inside the plant cells. Onslow (1925) has reported that anthocyanins appear in petals when the carbohydrates concentration is high. Hence, the maximum amount of pigment synthesis can be controlled, somewhat, by starch, right nutrients and optimal light favoring for photosynthesis. The plant uses the starch during both day and night times for the process of respiration, which

are independant on sunlight but is dependent on temperature. The plants utilize more starch when the temperature is warmer and less when it is colder. So, in summer season *i.e.* from the month of March to June, the anthocyanin and flavonoid content in petals reached to maximum because of the production and utilization of higher amount of carbohydrates in the plants.

Though, the temperature during rainy season suits well for the photosynthesis and respiration processes in plants but the weather remains wet due to humid condition. Fall-weather, bright-sunny days, less rain and cold nights are the perfect weather to maximize anthocyanins contents in petals, as a result optimum level of floral pigments are seen in winter season and less in rainy season. Occasionally, during summer season raise of atmospheric temperature ($\geq$ 40°C) and light intensity cause damage to some leaves which in turn check / inhibit the rate of photosynthesis and respiration by disrupting the structural configuration of thyllakoid membrane and affecting negatively on structure and function of chromoplast (Grotewold, 2006 a, b and c). The above factors may disrupt the metabolism of carbohydrates, which directly or indirectly affect anthocyanin synthesis by the denaturation of proteins and the enzyme activities. Some flowers are also very sensitive to the carbohydrate-anthocyanin relation and they may even stop the blooming of flowers all together, if the sugar and protein levels in the cell-sap get too low.

In case of *R.indica* the colour stability reduced from 95% (initial value) to 30%.

The drought resistant plants may produce flowers, but the colours become pale, or some colours may be missed. Hence, the colours of plants and flowers are dependent on anthocyanin pigments, which are affected by extreme summer heat and higher light intensities. Regarding variation in flavonoids, it may be concluded that as the anthocyanin and flavonoids are closely associated with structure and function, the variation might be influenced by different environmental factors pertained in different seasons.

In order to face different environmental stress, both biotic and abiotic, almost every parts of plants such as pollen, flower, buds, fruits, vascular cambium, leaves, roots, shoots etc contain stress-response steroids such as brassimolid (BL) and castasteron (CS) which control the plant from various stresses (Bajguz and Hayat, 2009). Both biotic and abiotic stresses control or influence the morphological, physiological, biochemical and molecular changes that lead to a cellular damage, oxidation steress, which are frequently accompanied by high temperature, salinity, drought, temperature and stress cause denaturation of structure and function of carbohydrates and proteins (Krishna, 2003; Smirnoff, 1995 and Fujita *et al.,* 2006).

Total sugar and protein contents were found to be maximum in winter season, optimum in rainy season and minimum in summer season. This might be due to growth and development of flowers and the net effect of the synthetic processes, especially carbon assimilation and the catabolic process of respiration. The growth phase can be divided into four stages *i.e.* Stage- I, Stage- II, Stage- III and Stage- IV. Stage- I is characterized by a slow accumulation of sugars and acids. Stage- II is the period of starch synthesis, Stage- III is the period of starch hydrolysis and Stage-IV is the period of decomposition of starch and increase in sugar concentration. Hence, Stage-IV is considered as the period of maturation, growth and development of flowers (Steele Catherine, 1949). During winter season total sugar and protein contents were maximum and in summer season the contents were minimum. Intermediate values were noticed in between these two during the rainy season. But anthocyanin and flavonoid concentrations were maximum in summer season and minimum in rainy season. Intermediate values were noticed in between these two values in winter season. Hence, the present experimental results proved that anthocyanin pigments in the flowers of summer season are the best for further use.

In coastal areas, the rainy seasons experiences high levels of relative humidity. The sufficient water and maximum humidity in the atmosphere affect the rate of absorption and transpiration in plants by influencing the DPD gradient between inter-cellular space and outside atmosphere. Hydrolysate solutions are produced during breakdown of peptide bonds of amino acids during hydrolysis of protein. The translocation of hydrolysate may be slow due to low rate of absorption and transpiration to reach the flower. Towards the end of October the percentage of relative humidity lowers down which causes increase in the rate of transpiration as well as increase in the rate of absorption by translocating the hydrolysate to the sink of the flower. Hence, the protein and sugar content were found maximum in winter than rainy season. Though, the rainy season suits well for the metabolic processes of the plant enhancing the growth

and development of the plant, the flower number, quality of the flowers, bio-mass production but the quantity of all the pigment was less in this season compared to the winter season.

Conclusion :

From the above facts and findings, in nutshell, the colour pigments *viz*., flavonoids, anthocyanins and phenol derivatives, protein, sugar and other nutrients present in petal of test flowers might act as potent colorant, antioxidant and neutraceuticals in food market. Hence, the procurement of colour pigments from petal of different test flowers at appropriate level that is a particular period (season) for further processing and manipulation through screening and evaluation.

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