ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Chromosome Staining with Natural Dyes from *Punica granatum* and *Beta vulgaris*

Huda Elgubbi[1]* and Eman Abobaker Asayh[2]

[1]*Botany Department, Misurata University, Libya*
[2]*Department of chemistry , Faculty of science , Alejelat , Zawia university , Libya*

***Corresponding Author**: Huda Elgubbi, Botany Department, Misurata University, Libya.

Received: September 08, 2017; **Published:** October 10, 2017

Abstract

Most synthetic stains are expensive as they are synthesized from mixtures of various chemicals, there is need to explore other cheaper and natural sources of obtaining dyes used as biological stains. For this purpose, *Punica granatum* peels and *Beta vulgaris* root extracts were used as a source of natural dyes. Plant parts were extracted using three solvents (acetic acid, ethyl acetate and ethanol) indicate that most dyes with different solvent can capable for plant chromosome staining and also define mitotic cell division. Dyes used with staining agent tannic acid or ferrous sulfate were more sufficient to stain the chromosomes. *Punica granatum* ethanol solvent show not clearly plant chromosome staining and unable to define the cell division. *Punica granatum* and *Beta vulgaris* extracts may be used as chromosome dyes.

Keywords: *Natural Dyes; Chromosome; Punica granatum and Beta vulgaris; dye Agents*

Introduction

Synthetic dyes such Giemsa's, Aceto-orcein and Aceto-carmine have been used to stain chromosomes during meiotic and mitotic cell division. Most synthetic stains are expensive, carcinogenic and have a toxic effect. Natural dye is generally used as a dyes because of its advantages, they are less expensive, nontoxic and less health hazardous. Natural dyes can be obtained either from animals, such as the orange from cochineal (*Coccus cacti*) and the purple from Lac (*Laccifer lacca Kerr*), or from plant parts as Vegetal yellow dyes, are largely found in *Reseda luteola*, *Reseda lutea*, Natural henna (*Lawsonia inermis*) and Indian mulberry root (*Morinda citrifolia L.*), the most important source of a red stain; true indigo leaves (*Idigofera tinctoria L.*), which gives blue stain and Bengal almond leaves (*Terminalia catappa L.*), Myrabolan bark (*Terminalia chebula Retz*), they have been used to give a green color of dyes [1-3]. Futragul and Piromthammasiri [4] documented that the chemical components of natural dye are including Flavonoids, Carotenoid, Anthraquinones, Alkaloid, Curcumin, Chlorophyll and Prodigiosin that give colors ranged from yellow, yellow to orange, yellow to red and red, blue or reddish violet, green and red, respectively. Those active component can be extracted from different parts of plant including root, leaves, seeds, flowers and barks. Recently, remarkable interest in natural dyes has been viewed in the scientific researches for chromosomes stain, because they are less expensive, less contaminating, biodegradable and less well being a hazard (Adeel., *et al.* 2009 and Saha and Dutta; Siva, 2007). For this purpose, extracts from Root of and fruit shells of *Punica granatum* were used, in this study, to examine their ability to stain chromosomes of *Allium cepa* meristematic cell, that used as a model plant cell in cytogenetics.

Material and Methods

Preparation of Natural Dyes

Plant samples preparation:

- The fruit shells of *Punica granatum* L. was exposed to air and dried then grinded to the fine powder (Yellow color).
- Root of *Beta vulgaris* L. was peeled and exposed to air and dried then grinded to the fine power (dark red color).

Extracts preparation

- A 20 ml of acetic acid, was used as a solvent, mixed with 10g of dry powder of *Punica granatum* fruit shells and the Root of *Beta vulgaris* (individually). The mixtures were incubated in water bath at 40°C for 6 hours.
- A 20 ml of ethyl acetate, was used as a solvent, mixed with 10g of dry powder of *Punica granatum* fruit shells and the Root of *Beta vulgaris*. The mixtures were incubated in water bath at 40°C for 6 hours.
- A 20 ml of ethanol, was used as a solvent, mixed with 5g of dry powder of *Punica granatum* fruit shells and 6.65g Root of *Beta vulgaris*. The mixtures were incubated in water bath at 40°C for 6 hours.

After incubation the mixtures were centrifuged at 4000 rpm for 5 minutes and the extracts were transferred to dark bottle and kept in fridge at 4°C.

Fixation

With each extract 0.5g of ferrous sulfate or 0.5g of Tannic acid were added to fix the stain.

Preparation of *Allium cepa* root tips

Soaked seeds were germinated on water-moistened filter paper in a Petri dish until the roots reached a length of about 1.5 cm. Young roots were excised and prepared for mitotic study. Excised root tips were fixed in absolute ethanol: glacial acetic acid in proportion of 3:1 for 24h at 4°C. After that rinsed with 70% ethanol for 2 - 3 times and kept in 70% ethanol.

Preparation root tip chromosome slide

The root tips were hydrolysed in 1N hydrochloric acid (HCl) at 60ºC for 4 - 5 minutes. The cell wall has to dissolve by hydrolysis with acid. The roots then were transferred on clean slide.

Detection of chromosome staining

Each natural dye samples obtained from several solvents were used for *Allium cepa* root chromosome staining. chromosomes were accomplished by squash technique [5,6]. Drops of glycerin were added to the prepared slide. Stained slides left for 24h. Chromosomes recognized under a compound light magnifying instrument and were captured.

Result and Discussion

In this study, three organic solvents (acetic acid, ethanol and acetic acid) were used to extract *Punica granatum* and *Beta vulgaris*; our results revealed that ethanol extract of *Punica granatum* is not capable to stain chromosomes. It is more appropriate for nuclei stain (Figure 3). In contrast, ethanol extract of *Beta vulgaris* was able to stain chromosomes and show obvious mitotic cell division. Acetic acid and ethyl acetic extract of *Punica granatum* and *Beta vulgaris* are considered an optimum solvents for natural dyes extraction. This outcome in concurrence with [6,7].

Natural dyes from *Punica granatum* with pH 3 need staining agent to stain chromosomes. Mordants play very important role in imparting color to the chromosome [8]. ferrous sulfate and tannic acid have been utilized, as a staining agents, to enhance staining quality. Accordingly, *Punica granatum* dye with acetic acid and ferrous sulfate stained chromosome and well defined mitotic cell division. Additionally, *Punica granatum* dye with ethyl acetate and tannic acid showed well-defined results, similar to acetic acid (Figure 1, 2). Mordants play very important role in imparting color to the chromosome. *Punica granatum* dye with ethanol extract pH 3 only defined the nucleus and cytoplasm only (Figure 3).

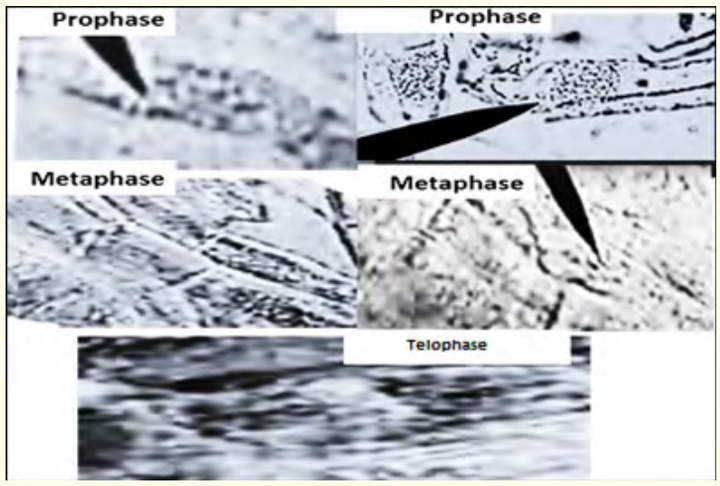

Figure 1: *Alluim cepa chromosome staining with natural dyes from Punica Granatum in concentrate ethyl acetate with tannic acid.*

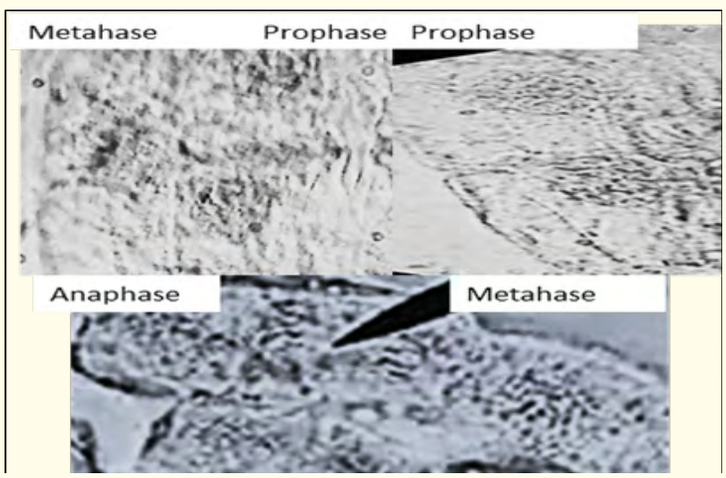

Figure 2: *Alluim cepa chromosome staining with natural dyes from Punica Granatum in concentrate acetic acid with ferrous sulfate.*

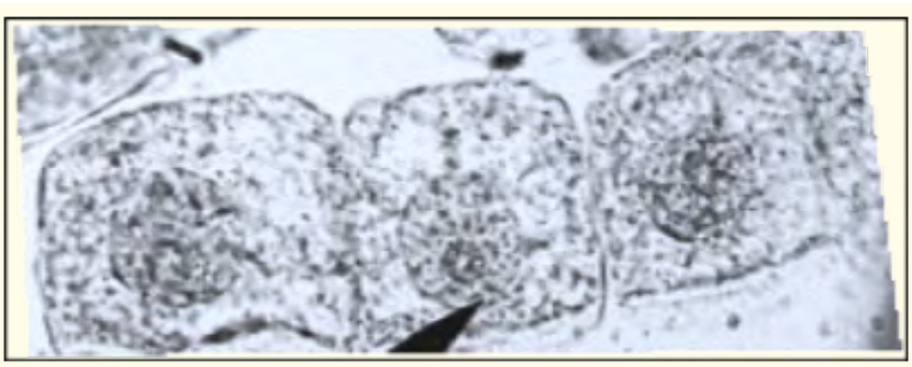

Figure 3: *Alluim cepa chromosome staining with natural dyes from Punica Granatum in concentrate ethanol.*

Natural dye from *Beta vulgaris* with various solvent (ethyl acetate, acetic acid and ethanol) stained chromosomes and characterized mitotic cell division. The stains quality were improved by using chemical called mordants such ferrous sulfate and tannic acid [9,10]. From the results, it can be seen that acetic acid solvent used as a solvent for *Punica granatum* dye defined both nuclei and chromosome during cell division. ethanol solvent used for *Punica granatum* dye showed clear mitotic division (Figure 4-6).

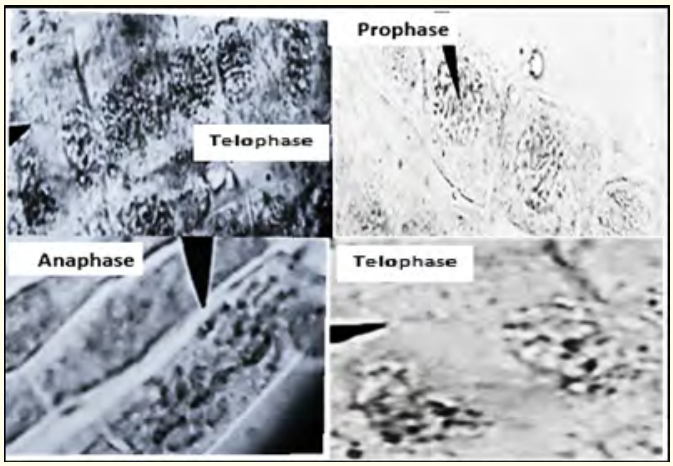

Figure 4: *Alluim cepa chromosome staining with natural dyes from Beta vulgaris in concentrate ethyl acetate with tannic acid.*

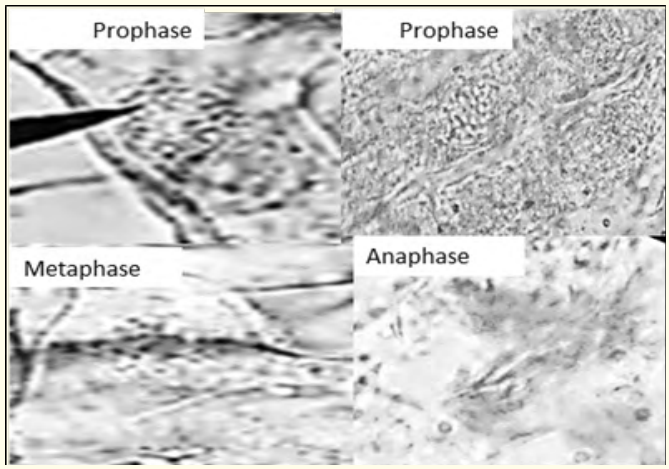

Figure 5: *Alluim cepa chromosome staining with natural dyes from Beta vulgaris in concentrate acetic acid with ferrous sulfate.*

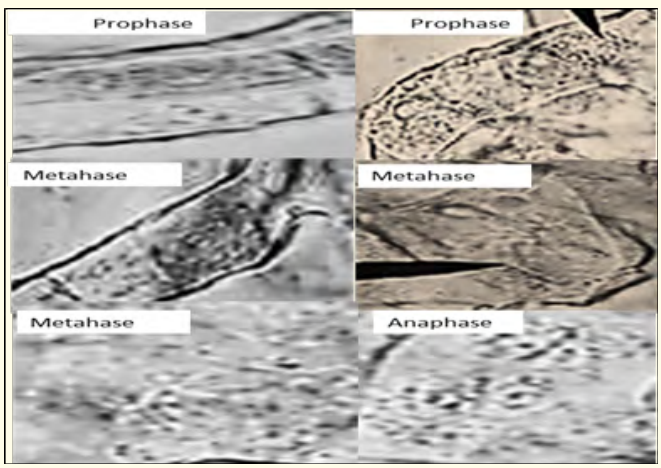

Figure 6: *Alluim cepa chromosome staining with natural dyes from Beta vulgaris in concentrate ethanol.*

Conclusion

In conclusion, the obtained results have shown the dyeing potential of *Punica granatum* and *Beta vulgaris* as source for chromosome dyeing. Good fastness exhibited by the dyed chromosome is because of the mordants used and pH adjustment. There were several optimum condition for a good staining of plant chromosomes, Firstly, the concertation of solvents. Secondly, the adjustment of pH. Thirdly, using dye agents such tannic acid and ferrous sulfate. Fourthly, staining time should take 24 hours.

Bibliography

1. Gilbert GK and Cookke TD. "Dyes from plants: past usage, present understanding and potential". *Plant Growth Regulator* 34.1 (2001): 57-69.

2. Chengaiah B and Rao K. "Medicinal importance of dyes". International Journal of Pharmacological Research 2.1 (2006): 144-154.

3. Bechtold T. "In Handbook of Natural Colorants". T. Bechtold, R. Mussak, Eds., Wiley, New York (2009): 151.

4. Futragul D and Piromthammasiri K. "Development of natural dyes in the upper of Thailand". Natural Dyes Project Thailand Research Fund, Bangkok, Thailand (1996).

5. Chaiyasut K. "Cytogenetics and cytotaxonomy of the family Zephyranthes". *Cytologia* 75.1 (1989).

6. Campiranon A. "Cytogenetics. 2nd edition". Department of Genetics, Faculty of Science, Kasetsart University, Bangkok (2003).

7. Sikhruadong S., *et al*. "Chromosome Staining of Crinum Lily (Crinum asiaticum L.) Using Natural Dyes". *Cytologia* 74.1 (2009): 17-22.

8. Kulkarni SS., *et al*. "Cotton Dyeing with Natural Dye Extracted from Pomegranate (Punica granatum) Peel". *Universal Journal of Environmental Research and Technology* 1.2 (2011): 135-139.

9. Vankar PS., *et al*. "Dyeing Cotton, Silk and Wool Yarn with Extract of Garnicia mangostana Pericarp". *Journal of Textile and Apparel Technology and Management* 6.1 (2009): 1-10.

10. Samanta AK and Agarwal P. "Application of Natural Dyes on Textiles". *Indian Journal of Fibre and Textile Research* 34 (2009): 384-399.

Volume 11 Issue 4 October 2017