https://doi.org/10.46341/PI2021005
UDC 58.086

SHORT COMMUNICATION

Modified staining protocol with Safranin O and Astra Blue for the plant histology

Andriy Novikov [1,*], **Mariia Sup-Novikova** [2]

[1] State Natural History Museum, National Academy of Sciences of Ukraine, Teatralna str. 18, 79008 Lviv, Ukraine; * novikoffav@gmail.com

[2] Ukrainian Catholic University, Stryiska str. 29A, 79000 Lviv, Ukraine

Received: 19.05.2021 | **Accepted**: 31.05.2021 | **Published online**: 16.06.2021

Abstract

Many staining protocols are widely applied in botanical microtechniques and serve specific histological purposes. In particular, some dyes are used simultaneously to receive contrasting colorations of different chemical structures, e.g., lignin and cellulose. One of the most popular differential staining protocols is based on the Safranin O / Astra Blue dyes combination. Safranin O is a water-soluble basic dye that stains lignin in red. Astra Blue is also a water-soluble dye but having an acidic reaction, which stains cellulose in blue. Usually, a 1–2 % solution of Safranin O in distilled water or 50–70 % ethanol is applied in combination with the 0.5–1 % water solution of Astra Blue to detect lignified structures and obtain contrasting pictures convenient for the light microscopy. For a long time, Astra Blue was used exclusively with water solutions, and such recommendation without additional options is indicated on producers' web sites. However, in 2002 it was proposed to use 1 % Astra Blue solution in 95 % ethanol to identify the lignified tissues. Later, such an ethanol solution of Astra Blue was also successfully applied by other researchers for different experimental purposes.

We tested the modified staining protocol with the application of both 1 % Safranin O and Astra Blue solutions in a slightly lower concentration of ethanol (70 %) on the flower buds of *Gagea lutea* (Liliaceae) and found it working well. We believe that such a modified protocol with the solutions of these two dyes in 70 % ethanol allows simplifying the procedure of the plant material staining due to application of the same concentrations of dissolvent and reducing the difference in solvent concentration between two following contrasting staining solutions. Such differential staining can be effectively applied for plant histology purposes, especially where there is a need to distinguish lignified structures and secretory tissues.

Keywords: Astra Blue, Safranin O, histological dyes, botanical microtechnique, lignin detection

Authors' contributions: A. Novikov designed an experiment and conducted the staining tests. A. Novikov prepared the draft of the manuscript. M. Sup-Novikova assisted during the tests, verified protocols, and contributed to the manuscript edition.

Funding: This investigation was fully covered by A. Novikov personal funds.

Competing Interests: The authors declared that they have no conflict of interests.

Many different staining protocols are widely applied in botanical microtechniques and serve specific histological purposes (Berlyn & Miksche, 1976; Gerlach, 1984; Barykina et al., 2004; Soukup & Tylova, 2019). In particular, some dyes are used simultaneously to receive contrasting colorations of different chemical structures, e.g., lignin and cellulose. One of the most popular and convenient differential staining protocols is based on the application

of Safranin O and Astra Blue dyes by turns. Safranin O is a water-soluble basic dye (pH=10.0) that stains lignin in red. Astra Blue is a water-soluble acidic dye (pH=4.9) that stains cellulose in blue but only in the absence of lignin (Srebotnik & Messner, 1994). Usually, a 1–2% solution of Safranin O in distilled water or 50–70% ethanol is applied in combination with 0.5–1% water solution of Astra Blue (Srebotnik & Messner, 1994; Goujon et al., 2003; Barykina et al., 2004; Feio et al., 2016). For a long time, Astra Blue was used exclusively with water solutions. However, Vazquez-Cooz & Meyer (2002) proposed to use approx. 1% Astra Blue dissolved in 95% ethanol. Later, such an ethanolic solution of Astra Blue was also successfully applied by other researchers (De Micco & Aronne, 2007; Richet et al., 2011, and others).

We tested the modified staining protocol with the application of both 1% Safranin O (C.I. 50240) and Astra Blue (C.I. 48048) solutions in 70% ethanol. The flower buds of *Gagea lutea* (L.) Ker Gawl. (Liliaceae Juss.) were fixed in 70% ethanol, embedded in Paraplast Plus (McCormick Scientific), and later cut on the rotary microtome MPS-2 onto 20 µm thick cross-sections. Cross-sections were mounted on glass slides and later processed the following protocol:

A. Deparaffinization
1. Xylene, 25 min.
2. Xylene, 25 min.

B. Rehydration
3. Absolute ethanol, 10 min.
4. 96% ethanol, 10 min.
5. 70% ethanol, 10 min.

C. Staining
6. 1% Safranin O in 70% ethanol, h.
7. Rinse in the extra volume of 70% ethanol
8. Contrasting by 70% ethanol, 5–10 min.
9. Contrasting by 70% ethanol, 5–10 min.
10. 1% Astra Blue in 70% ethanol, 10 min.
11. Rinse in the extra volume of 96% ethanol

D. Dehydration and further contrasting
12. 96% ethanol, 5–10 min.
13. Absolute ethanol, 10 min.
14. Absolute ethanol : xylene (1:1), 15 min.

E. Infiltration by resin solvent
15. Xylene, 15 min.
16. Xylene, 15 min.

After that, slides were embedded in Roti Histokit II (Carl Roth GmbH) and left to dry for one day. On the next day, they were examined under Amplival (Carl Zeiss Jena) light microscope (Fig. 1). Pictures were taken by Canon EOS 650 camera with microscope adapter.

We believe that such protocol with the solutions of these two dyes in 70% ethanol allows simplifying the staining procedure due to using the same concentrations of dissolvent and reducing the difference in solvent concentration between two contrasting staining solutions. Such differential staining can be effectively applied for plant histology purposes, particularly where there is a need to distinguish lignified structures and secretory tissues. Astra Blue stains cellulose and unlignified cell walls in light blue color, while lignified structures obtain different shades of red or brown color as a result of incorporating Safranin O in tissues. Safranin O also stains in dark red or pink color the nuclei and protoplasm of secretory tissues. However, the success and degree of coloration by Safranin O significantly depends on lignification level, quality of material, and staining exposure. Even after two hours of staining by Safranin O, it was dramatically washed out from tissues after five minutes of exposure in each of the following ethanols. It is crucial to control the time of exposure of slides in ethanols carefully or to stain the slides by Astra Blue before putting them in Safranin O. Therefore, this protocol must be checked and adopted for each kind of material (e.g., flower, bark, stem or root tissues, or other specific tissues and purposes).

References

Barykina, R. P., Veselova, T. D., Deviatov, A. G., Djalilova, H. H., Iljina, G. M., & Chubatova, N. V. (2004). *Handbook of the botanical microtechniques.* Moscow University Press. (In Russian)

Berlyn, P.G., & Miksche, J.P. (1976). *Botanical microtechnique and cytochemistry.* The Iowa State University Press.

De Micco, V., & Aronne, G. (2007). Combined histochemistry and autofluorescence for identifying lignin distribution in cell walls. *Biotechnic & Histochemistry, 82*(4–5): 209–216. https://doi.org/10.1080/10520290701713981

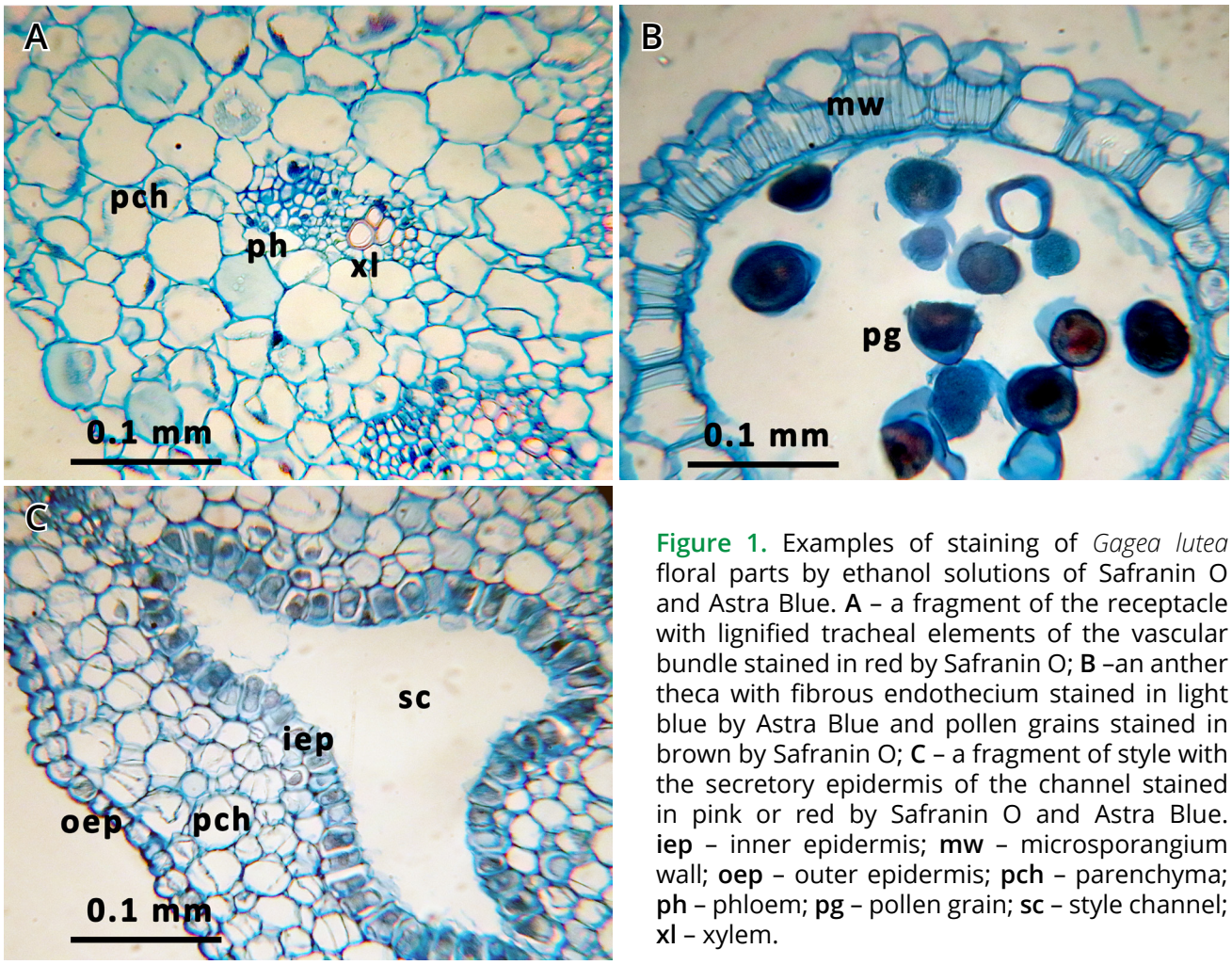

Figure 1. Examples of staining of *Gagea lutea* floral parts by ethanol solutions of Safranin O and Astra Blue. **A** – a fragment of the receptacle with lignified tracheal elements of the vascular bundle stained in red by Safranin O; **B** –an anther theca with fibrous endothecium stained in light blue by Astra Blue and pollen grains stained in brown by Safranin O; **C** – a fragment of style with the secretory epidermis of the channel stained in pink or red by Safranin O and Astra Blue. **iep** – inner epidermis; **mw** – microsporangium wall; **oep** – outer epidermis; **pch** – parenchyma; **ph** – phloem; **pg** – pollen grain; **sc** – style channel; **xl** – xylem.

Feio, A.C., Riina, R., & Meira, R. (2016). Secretory structures in leaves and flowers of two dragon's blood croton (Euphorbiaceae): new evidence and interpretations. *International Journal of Plant Sciences, 177*(6), 511–522. https://doi.org/10.1086/685705

Gerlach, D. (1984). *Botanische Mikrotechnik – Eine Einführung. 3.* Thieme.

Goujon, T., Ferret, V., Mila, I., Pollet, B., Ruel, K., Burlat, V., Joseleau, J., Barrière, Y., Lapierre, C., & Jouanin, L. (2003). Down-regulation of the AtCCR1 gene in *Arabidopsis thaliana*: effects on phenotype, lignins and cell wall degradability. *Planta, 217*, 218–228. https://doi.org/10.1007/s00425-003-0987-6

Richet, N., Afif, D., Huber, F., Pollet, B., Banvoy, J., Zein, R.E., Lapierre, C., Dizengremel, P., Perré, P., & Cabané, M. (2011). Cellulose and lignin biosynthesis is altered by ozone in wood of hybrid poplar (*Populus tremula×alba*). *Journal of Experimental Botany, 62*(10), 3575–3586. https://doi.org/10.1093/jxb/err047

Soukup, A., & Tylová, E. (2019). Essential methods of plant sample preparation for light microscopy. In: F. Cvrčková & V. Žárský (Eds.), *Plant cell morphogenesis. Methods in Molecular Biology, 1992*, 1–26. https://doi.org/10.1007/978-1-4939-9469-4_1

Srebotnik, E., & Messner, K. (1994). A simple method that uses differential staining and light microscopy to assess the selectivity of wood delignification by white rot fungi. *Applied and Environmental Microbiology, 60*(4), 1383–1386. https://doi.org/10.1128/aem.60.4.1383-1386.1994

Vazquez-Cooz, I., & Meyer, R. W. (2002). A differential staining method to identify lignified and unlignified tissues. *Biotechnic & Histochemistry, 77*, 277–282. https://doi.org/10.1080/bih.77.5-6.277.282

Модифікований протокол фарбування сафраніном О та астра синім для цілей гістології рослин

Андрій Новіков [1,*], Марія Суп-Новікова [2]

[1] Державний природознавчий музей НАН України, вул. Театральна, 18, Львів, 79008, Україна; * novikoffav@gmail.com

[2] Український католицький університет, вул. Стрийська, 29А, Львів, 79000, Україна

Різноманітні протоколи фарбування використовуються у ботанічній мікротехніці відповідно до специфічних гістологічних задач. Зокрема, деякі барвники використовуються суміжно для отримання контрастного забарвлення тих чи інших хімічних структур (наприклад, лігніну і целюлози). Один з найбільш популярних протоколів комбінованого фарбування передбачає використання сафраніну О та астра синього. Сафранін О – це водорозчинний основний барвник, що забарвлює лігнін у червоний колір. Астра синій – це також водорозчинний барвник, який, однак, є кислим, і забарвлює целюлозу у синій колір. Зазвичай використовують 1–2 % розчин сафраніну О у дистильованій воді або ж у 50–70 % етанолі у комбінації з 0.5–1 % розчином астра синього у дистильованій воді. Ця комбінація барвників дозволяє виявити лігніфіковані структури та отримати якісне контрастне зображення, що чудово підходить для цілей світлової мікроскопії. Довший час, астра синій використовувався виключно у водних розчинах, і саме така рекомендація без жодних додаткових опцій розміщена на сайтах виробників. Однак, у 2002 році для виявлення лігніфікованих структур у рослин було запропоновано використовувати 1 % розчин астра синього у 95 % етанолі. Згодом, аналогічні спиртові розчини астра синього були успішно використані рядом дослідників для різноманітних дослідних задач.

Ми розробили новий модифікований протокол фарбування з використанням 1 % розчинів обидвох барвників (сафраніну О та астра синього) у дещо нижчій концентрації етанолу (70 %) і випробували його на квіткових бутонах *Gagea lutea* (Liliaceae). Новий модифікований протокол підтвердив свою ефективність. Ми сподіваємося, що новий протокол дозволить спростити процес фарбування рослинних препаратів завдяки використанню однакових розчинників однакової концентрації та усуненню різниці в концентрації між послідовними розчинами в серії. Таке комбіноване фарбування може бути ефективно застосоване для цілей гістології рослин, особливо коли необхідно виокремити лігніфіковані структури і залозисті тканини.

Ключові слова: астра синій, сафранін О, гістологічні барвники, ботанічна мікротехніка, виявлення лігніну