SHORT COMMUNICATION

USE OF INK FOR STAINING AM STRUCTURES IN HEPATICS

Anu Sharma* and Eshan Sharma

Dept of Botany, University of Jammu, Jammu 180 006.

Received-02.12.2017, Revised-19.12.2017

Abstract: Commonly available inks were used to stain AM fungal structures in rhizoids of three liverwort species so as to find a suitable replacement for carcinogenic stains like trypan blue. None of the inks were found to be suitable.

Keywords: Ink, Stain, Chemicals, AM fungi

INTRODUCTION

Staining is an essential step in studying various AM fungal structures in roots. Philips and Hayman (1970) developed a method of staining by using trypan blue. Trypan blue is listed by the International Agency for Research on Cancer as a possible carcinogen (International Agency for Research on Cancer, 1975.). Such hazardous chemicals may cause skin irritation (Arena, 1986) and their vapours may irritate the eyes, nose,throat, and lungs thus, use of such chemicals should be reduced for health and safety reasons. In an attempt to replace the use of these hazardous chemicals, literature was surveyed so as to search for some substitutes. Vierheilig *et al.,* 1998 has developed a technique to replace the use of trypan blue with ink and vinegar to study AM associations of three fungal species (*G. mosseae*, *G. intraradices*, and *G. margarita*) in families having different root characteristics (bean [*Phaseolus vulgaris* L.], soybean [*Glycine max* L.], cucumber [*Cucumis sativus* L.], maize [*Zea mays* L.], wheat [*Tritium aestivum* L.], barley [*Hordeum vulgare* L.], and ryegrass [*Lolium perenne* L.]) and found out that staining of all three AM fungi by the black ink-vinegar solutions gave excellent results. Another worker, (Walker, 2005) replaced the use of vinegar by dilute HCl. Cao *et al.,* (2013) found only blue ink to be suitable for staining roots of *Citrus*. He got a bad color contrast in the roots stained by black and red ink-acetic acid solution. Therefore, he suggested the use of blue ink-acetic acid solution to stain mycorrhiza in *Citrus* roots. He pointed out that staining time is vital for the staining procedure. Chhetri and Maharjan (2012) tested two locally available inks in Nepal (Chelpark permanent black and Chelpark washable royal blue) for staining arbuscular mycorrhiza and found both of them suitable for staining the fungal structures (arbuscules, vesicles and internal hyphae).

Our objective was to determine whether this technique can be adapted for staining of AM fungi in hepatic rhizoids or not, thus replacing these harmful chemicals with non-toxic yet equally effective products.

MATERIAL AND METHOD

For staining process, rhizoids were detached from the thallus and boiled in 0.01% Potassium hydroxide (KOH) for 2-3 hours and then kept at room temperature for 1 hour followed by 3-4 washings in order to remove KOH. These rhizoids were then stained in ink-vinegar solution for 5 minutes so as to assess the effectiveness of the both. After destaining in water, rhizoids were mounted in glycerine.

Rhizoids of three liverwort species *Plagiochasma appendiculatum, Marchantia paleacea* and *Marchantia papillata* were used for testing the effectiveness of the staining process.

RESULT AND DISCUSSION

Results obtained were quite different from those obtained on higher plants (Table 1). After destaining in water, red and blue inks gave totally zero result (Figs. 1a & b) . Black ink was able to stain fungal hyphae but the results were not upto the mark (Fig. c). Moreover, the contrast developed was also very poor.

Vierheilig *et al.,* 1998 found that not all inks of different colours were effective in staining. Purple and green ink gave zero result whereas one of the blue and red ink gave good contrast. Black inks of four different companies gave good results of which black ink of Schaffer make gave excellent results.

CONCLUSION

None of the inks included in the present study were suitable for studying AM associations in rhizoids of liverworts. Some more inks of different brands need to be tested so as to find suitable substitutes for hazardous stains like trypan blue

Table 1. Comparison of different inks for staining of AM fungi in roots.

Color of the ink	Company	Staining time	Staining result	Comments
Red	Camlin	5 min	Fungus not stained	Not suitable

*Corresponding Author

	Chelpark	”	Fungus not stained	”
Blue	Camlin	”	Fungus not stained	”
	Chelpark	”	Fungus not stained	”
	Parker	”		
Black	Camlin	”	Fungus stained	”
	Chelpark	”	Fungus stained	”

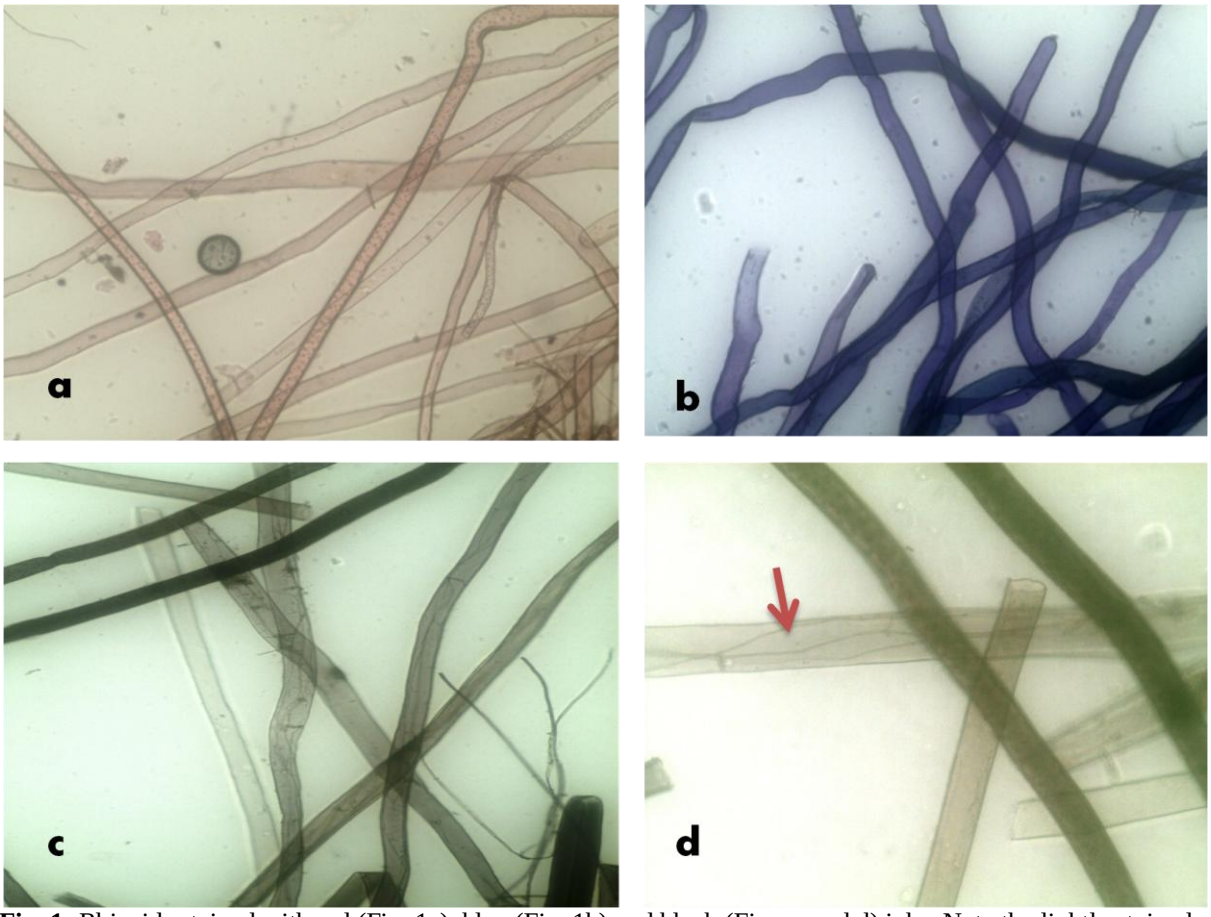

Fig. 1: Rhizoids stained with red (Fig. 1a), blue (Fig. 1b) and black (Figs. c and d) inks. Note the lightly stained fungal hyphae (arrow).

REFERENCES

Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55,158–161.

International Agency for Research on Cancer. (1975). IARC Monogr. Eval. Carcinog. Risks Man 29:295.

Arena, J.M. (1986). Poisoning, 5th ed. Charles C. Thomas, Springfield, Ill.

Vierheilig, H., Coughlan, A.P., Wyss, U. and Piche, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology*. 64(12), 5004-5007.

Walker, C. (2005). A Simple Blue Staining Technique for Arbuscular Mycorrhizal and Other Root-Inhabiting Fungi. *Inoculum* 56(4), 68-69.

Cao, M.Q., Qiang-Sheng, W. and Ying-Ning, Z. (2013). An Improved Ink-acetic Acid Technique for Staining Arbuscular Mycorrhizas of *Citrus. Int. J. Agric. Biol.* 15(2), 386-88.

Chhetri, B.K. and Maharjan, S. (2012). Evaluation of some locally available inks in Nepal for staining arbuscular mycorrhiza. *Kathmandu University Journal of Science, Engineering and Technology*.8, 33-35.