Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
http://ziraatdergi.gop.edu.tr/

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2020) 38(1) 65-72
doi:**10.13002/jafag4762**

The Effects of Fixation and Staining Methods in Histological Investigation on the Grafted Cuttings of Grapevine

Cuma ARIK[1*], Ahmet ALTINDİŞLİ[1]
Horticulture Sciences, Graduate School of Natural and Applied Science, Ege University, İzmir
(orcid.org/0000-0003-1037-7031), (orcid.org/0000-0003-0183-2645)
***e-mail:** cuma.arik@cbu.edu.tr

Alındığı tarih (Received): 24.03.2021 Kabul tarihi (Accepted): 24.04.2021
Online Baskı tarihi (Printed Online): 28.04.2021 Yazılı baskı tarihi (Printed): 30.04.2021

Abstract: For purpose of investigation of graft union and other graft properites in grafted grapevine production, the histological studies are conducted. For this, graft samples are stored in a fixation solution and later sections are stained by using some histological staining methods. Being examined of previously unapplied a fixation method and different staining methods has constituted the importance of this study, in histological investigation of grapevine graft samples. In grapevine graft samples fixation, 70% ethyl alcohol and formaldehyde: glacial acetic acid: alcohol (FAA) fixatives have been used in general. But also, another fixative solution, glycerin: alcohol: water solution is used for lignocellulosic tissue fixation. In this study, observing dissimilar effects between these fixation methods in grapevine graft samples fixation was aimed. As a result, it's thought that, glycerin: alcohol: water fixation method may be suggestible for grapevine graft samples fixatiation. Furthermore, different histological staining methods as toluidine blue, safranin + hematoxylin dual staining and safranin + Lugol's solution dual staining were compared; toluidine blue was forefront and safranin + Lugol's solution was second. And it's seen that, because of staining graft properties (continuity of cambium and vascular differentiation etc.) clearer, toluidine blue is suggestible for graft section staining.

Keywords: Toluidine blue, safranin, lugol, hematoxylin, glycerin

Aşılı Asma Çeliklerinin Histolojik İncelenmesinde Farklı Fiksasyon ve Boyama Yöntemlerinin Etkileri

Öz: Aşılı asma üretiminde aşı kaynaşması ve diğer aşı özelliklerinin incelenmesi amacıyla, histolojik çalışmalar yürütülmektedir. Histolojik inceleme için, aşı örnekleri bir fiksasyon çözeltisinde muhafaza edilmekte ve sonrasında bu aşı örneklerinden alınan aşı kesitleri bazı histolojik boyama yöntemleri ile boyanmaktadır. Asma aşı örneklerinin histolojik incelemesinde daha once uygulanmamış bir fiksasyon yönteminin ve farklı boyama yöntemlerinin çalışılmış olması, bu çalışmanın önemini ortaya koymaktadır. Asma aşı örneklerinin fiksasyonunda genel olarak %70 etil alkol ve FAA (formaldehit: glasiyel asetik asit: alkol) fiksatifleri kullanılmaktadır. Fakat bir diğer fiksatif çözelti, gliserin: alkol:su lignoselülozik doku fiksasyonunda kullanılmaktadır. Bu çalışmada bu fiksasyon yöntemlerinin asma aşı örneklerinin fiksasyonundaki farklı etkilerinin saptanması amaçlanmıştır. Sonuç olarak gliserin:alkol:su fiksasyon metodunun asma aşı örneklerinin fiksasyonu için önerilebilir bulunmuştur. Ayrıca toluidin mavisi, safranin + hematoksilen ikili boyama ve safranin + Lugol ikili boyama gibi farklı histolojik boyama yöntemleri karşılaştırılmıştır; toluidin mavisi en başarılısı olmuş ve bunu safranin + Lugol ikili boyama yöntemi izlemiştir. Aşı özelliklerinin (anaç ve kalem arasındaki kambiyal bağlantı ve vasküler farklılaşma gibi) daha net boyanmasından dolayı, aşı kesitlerinin boyanmasında toluidin mavisi önerilebilir bulunmuştur.

Anahtar kelimeler: Toluidin mavisi, safranin, lugol, hematoksilen, gliserin

1. Introduction

In histological studies on graft union of grapevine, it's seen that formaldehyde: glacial acetic acid: alcohol (FAA) (Cangi, 1996) and %70 alcohol (Cangi et al., 2000; Arık, 2013; Dolgun et al., 2016) fixation methods have used generally. And also, in fixation of graft samples of other species, the most used fixation method is FAA (Kurt, 2000; Olmstead et al., 2006; Bayram, 2013; Azimi et al., 2016; Khotcharat et al., 2016; Lima et al., 2017); %70 alcohol fixation is second (Tekintaş, 1991; Ada, 2008; Polat et al., 2010; Coşkun, 2012); and also only in a few studies, glycerin: alcohol: water (GAW)

method has used (Ljubojević et al., 2017). Furthermore, GAW fixation method is widely used in plant tissue fixation (Brandes et al., 2011; Hernández-Ledesma et al., 2011; Baar et al., 2013; Henderson, 2013; Chong et al., 2014; Martins et al., 2014; Alerico et al., 2016; Chartier et al., 2016). However, GAW hasn't been used in fixation of grapevine graft union tissue before and also in literature it's stated that GAW fixation has important functions. Bozkurt (1973) has recommends that glycerin: ethyl alcohol (1:1) or 1 (glycerin): 2 (ethyl alcohol): 3 (water) fixation to soften hardwood tissue. And in addition, in most studies, it's stated that GAW fixation is able to soften tissues (Brandes et al., 2011; Baar et al., 2013; Chong et al., 2014; Alerico et al., 2016; Chartier et al., 2016). Because of softening tissue by GAW fixatiation, it was thought that, section obtaining could be easier, cambium fractures could become less in graft connection area and also GAW fixative solution could be able to be an alternatife for fixation of grapevine graft samples. And also formaldehyde hardens plant tissues gradually and long term alcohol fixation makes tissues hardener too (Öztürk Çalı and Candan, 2011). Because of these knowledges, in order to reveal the difference between fixation methods, grapevine graft samples were kept in GAW, 70% alcohol and FAA fixatives. Because of facilitating effect of GAW on sectioning by softening the tissue and by decreasing fractures at the graft zone, it's thought that it may be an alternative fixative on fixation of grapevine graft samples. Furthermore, this study has been sole research in using GAW in the fixation of grapevine graft samples.

After taking section from plant tissue samples, the sections are stained by some histological dyes. In literature, safranin (Cangi, 1996; Cangi et al., 2000; Dolgun et al., 2016) and Lugol's solution (I2KI, iodine–potassium iodide) (Arık, 2013) were used in section staining of grapevine graft samples; and safranin:fast green, toluidin blue and safranin are the most used staining methods respectively in section staining of other species graft samples (Tekintaş, 1991; Kurt, 2000; Olmstead et al., 2006; Ada, 2008; Coşkun, 2012; Estay et al., 2016; Khotcharat et al., 2016; Lima et al., 2017; Ljubojević et al., 2017). But also, in literature, in staining of plant tissue sections, toluidine blue, safranin:fast green and safranin are the most used histological staining methods and also Lugol's solution and hematoxylin are remarkable (Begum et al., 2010; Tordable et al., 2012; Baar et al., 2013; Pouzoulet et al., 2014; Nalini et al., 2015; Hanaoka et al., 2016; Harrison et al., 2016; Shtein et al., 2017; Siebers et al., 2017; Song et al., 2018; Navarro et al., 2019; Braga et al., 2019; Falchi et al., 2019). In addition to these knowledges, about histological staining methods, toludin blue is used both in general staining (Uma and Muthukumar, 2014) and also in determining of fungal hypae (Pouzoulet et al., 2014); safranin stains lignin red (Bozkurt, 1973; Öztürk Çalı and Candan, 2011); hematoxylin stains lignified cell walls bluish purple (Bozkurt, 1973; Öztürk Çalı and Candan, 2011) and Lugol's solution stains starch black (Plavcová et al., 2016) (xylem rays parenchyma are stained black because of having abundant starch (Zwart et al., 2017). In this study, safranin-hematoxylin, toluidin blue and safranin-Lugol's solutions staining methods were used for determining the most convenient histological staining method for grapevine graft sections. Toluidin blue was used both mainly for observation of graft union compenents such as callus, cambial continuity and vascular differantiation, and also for observing fungal traces which formed in graft area (hereby, fungal traces was tried to interpret). Furthermore, this study has been the sole in using toluidine blue in the histological investigation of grapevine graft samples. The second staining method used was the safranin + hematoxylin application; in the literature review, it's seen that this staining method hasn't been used before. Last staining method used in this study was safranin + Lugol's solution; in the literature review, it's seen that this method has been applicated in only one study (Ermel et al., 1997).

Being essayed of previously unapplied fixation method and different staining methods

for grapevine graft samples has constituted the importance of this study. The study was performed from PhD thesis of the author (Graduate Faculty of Natural and Applied Science, Ege University).

2. Materials and Methods

The research was carried out in Manisa Celal Bayar University Alaşehir Vocational School (2018). Cuttings of 1103P, 41B and Ramsey rootstocks and Sultani Seedless scions were used as materials in the study. Rootstocks cuttings were obtained commercially and scion cuttings were obtained from the farmer vineyard. Before grafting, for omega grafting, except the bottom bud other buds of rootstocks were shaved off; for chip-budding grafting except bottom and tip buds, other buds of rootstocks were shaved off. At chip-budding grafting, scion was grafted to below side of the tip bud of rootstock cutting. Table omega machine and chip-budding hand machine were used for grafting. After grafting process, paraffin application was done with the commercial grafting paraffin melting at 70-80°C. Grafted cuttings which paraffin applied were placed in boxes with 10-20 cm water height and stored in callusing room for four weeks. In callusing room, conditions were provided by working that temperature was 28-30°C for first two week and 26-28°C following two week and humidity was 80-90%; and for two-week acclimation phase, conditions were provided by working that temperature was 22-24°C and humidity was 50-55% (Cangi, 1996; Arık, 2013). In 14th, 21st, 28th, and 35th days after grafting, the graft samples was taken by cutting with prunning shear below grafting zone and was kept in fixatives (%70 ethyl alcohol, glycerin:alcohol:water (1:1:1) and FAA (5:5:90 formaldehyde: glacial acetic acid: %70 ethyl alcohol)). After the graft samples were taken out from fixation solutions, the sections were obtained. For taking transverse sections, the samples were cut in half by using fretsaw (size no: 3) and then roughness at scions surfaces were smoothed out by using razor blade and / or fine utility knife and / or microtome blade. For obtaining longitudinal sections, outer bark and thin sample layer was removed from samples by cutting with using a utility knife. So, it was strived to obtain as thin as possible thin hand sections as representing the graft area by using microtome blades, razor blades and fine utility knife. After taking the sections, they were placed on the slide and stained by using brushes (Nalini et al., 2015). For removing excess stain solution, the sections were rinsed with 70% alcohol and water; and later excess solution was absorbed to napkins. In section staining, primarily toluidine blue and later safranin (1%) + hematoxylin (1%) and safranin (1%) + lugol staining solutions were applied. To prepare staining solutions, Merck brand Lugol solution (1% iodine potassium iodine, 3.4 g L^{-1} I_2 + 6.8 g L^{-1} KI), Besolab (Histomed) brand Harris' hematoxylin and Zag Kimya branded safranin and toluidine blue histological dyes were used. Stained sections were examined with a light microscope (Olympus, 4x) and photographed. Also in this study, fungi traces which formed at graft area were examined; for this purpose, it was benefited from study of Pouzoulet et al. (2014) which in fungal hypea in vascular tissues was stained with toluidine blue (Figure 1).

To compare fixation methods and staining methods, histological observation and evaluation were done. And also, in order to determine the difference between staining methods, the toluidine blue was chosen as a control and a general evaluation was done according to clarity of graft properties in the sections (clear, unclear and hemi – clear).

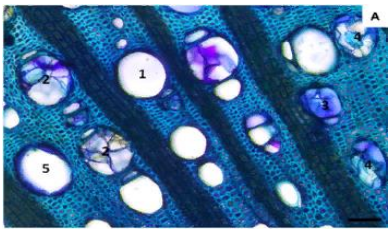

Figure 1. Toluidine blue has stained the fungal occlusion in xylem in *Vitis vinifera* L. (Pouzoulet et al., 2014)

Şekil 1. *Toluidin mavisinin Vitis vinifera L. ksilemlerinde fungal tıkanıklığı boyaması (Pouzoulet et al., 2014)*

3. Result and Discussions

After the grafted samples were stored in the fixatives, the graft sections were taken and stained by histological staining methods (especially toluidine blue). And later, the sections were examined under the light microscope and the histological photographes were taken. Inverstigation results;

a. Fixation methods: In GAW fixation, tissue softening occured slightly at graft samples. Thus, GAW fixation made easier section-taking in generally. Also, FAA fixation caused the tisue degenaration; especially deformity in callus structure was fairly evident (Figure 2). FAA fixation has hardened tissue, so, process of taking section from the graft samples were more diffucult than others. And finally, the taking section process from graft samples, in 70% ethyl alcohol fixatition was easier than in FAA fixation but more difficult than in GAW fixatiton. These results has been confirmed by most researchers' statements (Öztürk Çalı and Candan, 2011; Baar et al., 2013; Chong et al., 2014; Martins et al., 2014; Alerico et al., 2016; Chartier et al., 2016).

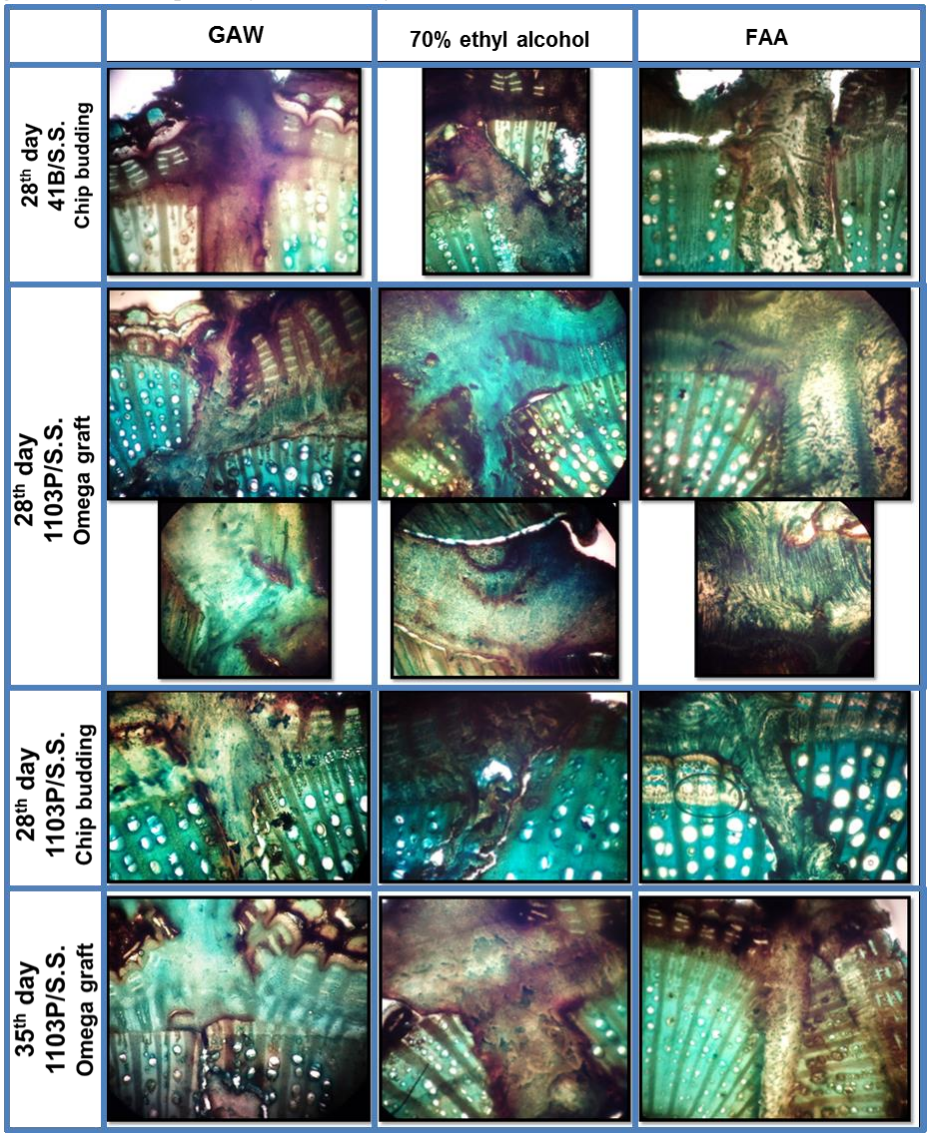

Figure 2. Some sections photographs which belong to graft samples being stored in different fixation methods (S.S.= Sultani Seedless)

Şekil 2. *Farklı fiksasyon methotlarında muhafaza edilen aşı örneklerine ait bazı kesit fotoğrafları (S.S.= Sultani Çekirdeksiz)*

b. Staining methods: To evaluate the staining methods, clarity of firstly cambial continuity and clarity of new cambium, cambial continuity attempt, new xylem and new phloem also was taken into account. Among the applied section staining methods, toluidine blue (1%) was the most effective in terms of being seen clarity of new cambium, cambial continuity and vascular differentiation in the sections. Although in some sections safranin + hematoxylin (%1 + %1) dual staining method was forefront, safranin (%1) + Lugol dual staining method was second after toluidine blue. Both longitudinal and transversa sections were stained with the staining methods (Figure 3. and Figure 4). And also in this study the fungal occlusion was determined by staining with toluidine blue (Figure 5), like statements of Pouzoulet et al. (2014).

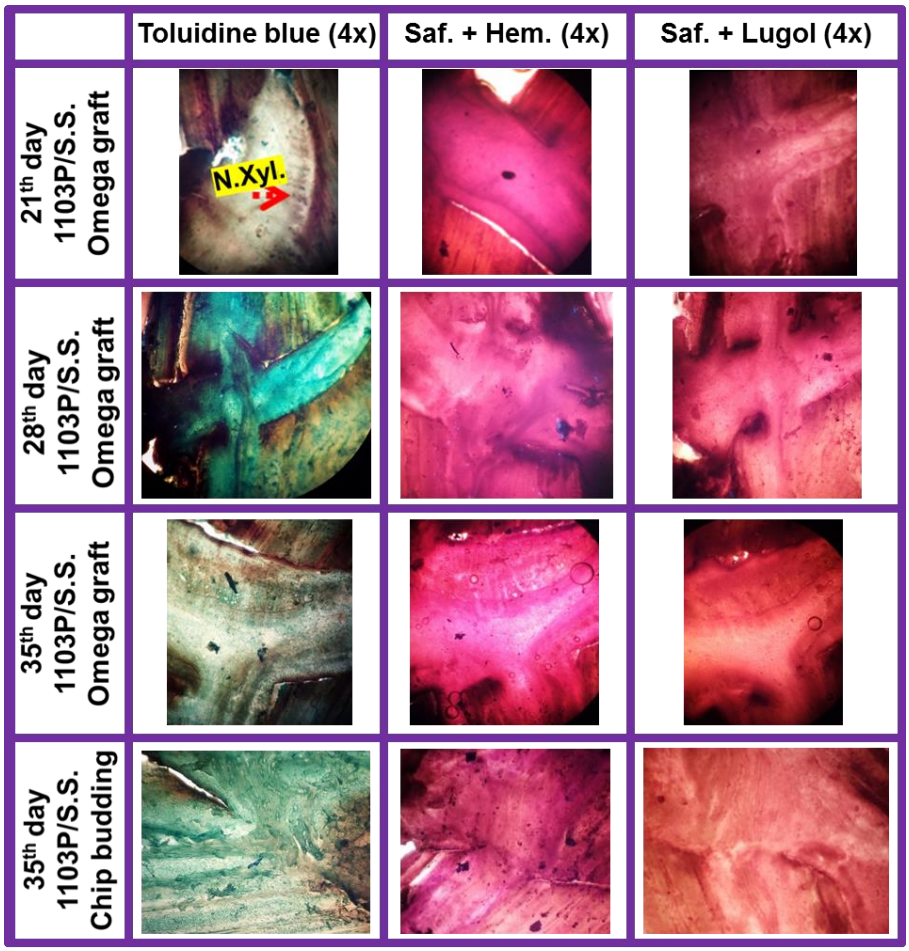

Figure 3. The longitudinal sections being stained by different staining methods (N.Xyl.= new xylem, Saf.= safranin, Hem.= hematoxylin, S.S.= Sultani Seedless)

Şekil 3. *Farklı boyama yöntemleri ile boyanmış boyuna kesitler (N.Xyl.= yeni ksilem, Saf.= safranin, Hem.= hematoksilen, S.S.= Sultani Çekirdeksiz)*

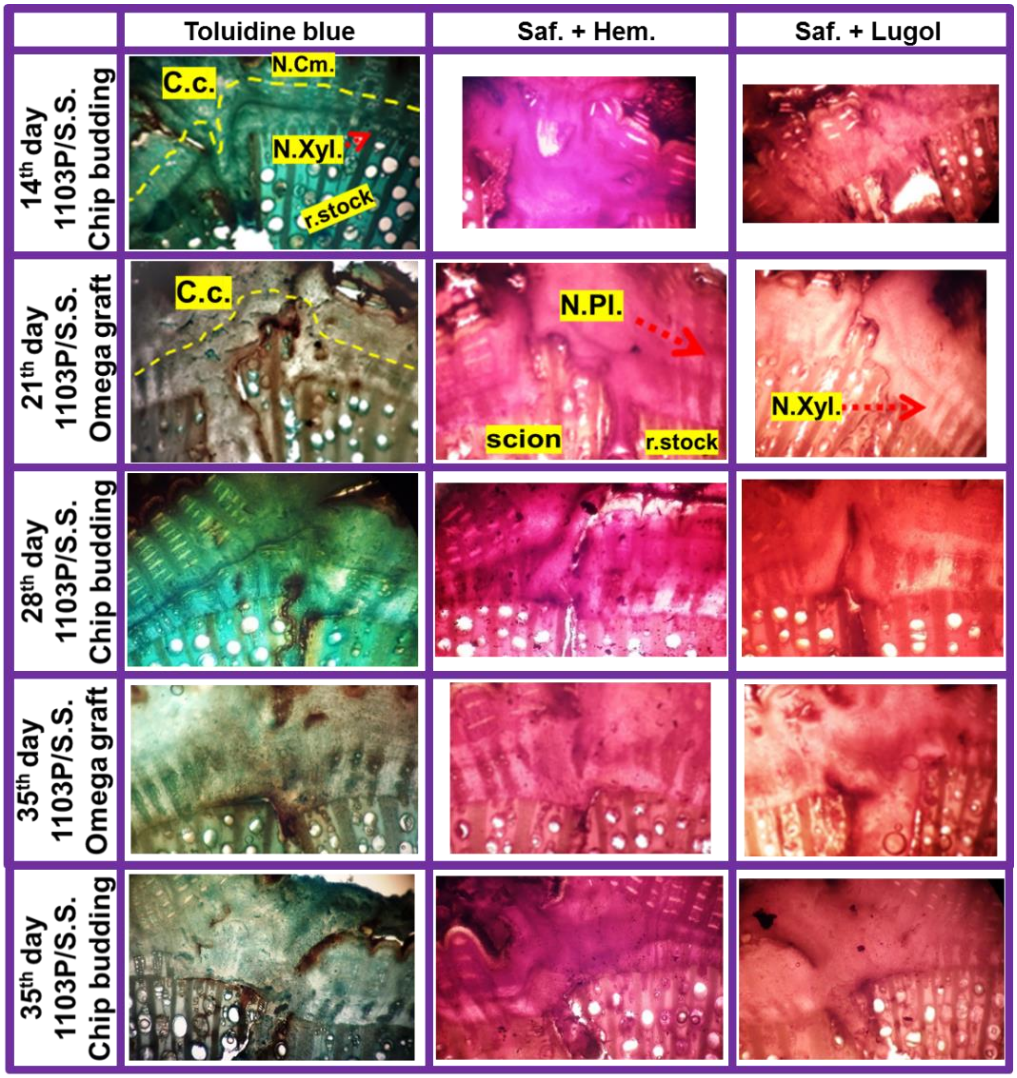

Figure 4. The transversal sections being stained by different staining methods (C.C.= cambial continuity, N.Cm.= new cambium, r.stock= rootstock, N.Pl.= new phloem)

Şekil 4. *Farklı boyama yöntemleri ile boyanmış enine kesitler (C.C.= kambiyal bağlantı, N.Cm.= yeni kambiyum, r.stock= anaç, N.Pl.= yeni floem)*

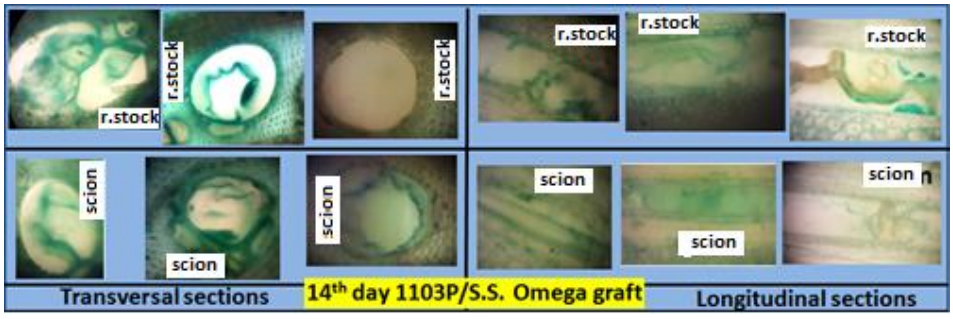

Figure 5. Fungal occlusion which determined by toluidine blue staining

Şekil 5. *Toluidin mavisi ile belirlenmiş fungal tıkanıklar*

4. Conclusions

Grafted grapevine production is very important in viticulture because of especially phyloxera and ecological factors. And also in grafted grapevine production, for histological examination of graft properties as callus fusion, callus differentiation, cambial continuity and graft union, graft samples are stored in a fixation solution. In graft sample fixation, formaldehyde + glacial acetic acid + alcohol and 70% ethyl alcohol fixatives are used generally and in grapevine graft sample fixation too. But also, in literature, another fixative solution has been used for lignocellulosic tissue fixation, and this fixative is glycerin + alcohol + water solution. This solution makes easier section taking by softening tissue and in this study this fixative made too. According to this study, it's understood that using this fixation method may be suggestible. And also, for histological assessment of graft union, sections are staining by some histological staining methods. Safranin:fast green, toluidin blue and safranin have been the most used staining methods in plant tissue staining; and also in grapevine graft section staining, safranin and Lugol's solution have been used in generally. In this study, toluidin blue was forefront and safranin + Lugol's solution was second. Toluidin blue is suggestible for graft section staining because of staining graft properties as cambial continuity and cambial differentiation and fungal hypae prominently also.

References

Ada S (2008). Kestanenin (*Castanea sativa* Mill.) Meşe (*Quercus* sp.) Üzerine Aşılanabilirliği ve Aşı Kaynaşmasının Anatomik Gelişimi ile Toplam Flavan İçeriklerinin Mevsimsel Değişimi. T.C. Adnan Menderes Üniversitesi Fen Bilimler Enstitüsü Bahçe Bitkileri Anabilim Dalı, Yüksek Lisans Tezi, 69 s., Aydın.

Alerico GC, Vignoli-Silva M and Lando V (2016). Morphoanatomical characterization and chemical study of the internal portion of the stem bark of *Sambucus australis* Cham. & Schltdl. Rev. Bras. Pl. Med., Campinas, 18 (1): 223-229.

Arık C (2013). Aşılı Asma Fidanı Üretiminde İndol Butirik Asit (IBA) Uygulamasının Aşı Kaynaşması Üzerine Etkileri. T.C. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Yüksek Lisans Tezi, 92 s., Isparta.

Azimi M, Özkaya MT, Çölgeçen H, Büyükkartal HN (2016). Analysis of phenolic compounds for determination of cambium differentiation and tracheal elements in olive graft combinations. Journal of Experimental Biology and Agricultural Sciences, 4 (6): 714-720.

Baar J, Tippner J, Gryc V (2013). The relation of fibre length and ray dimensions to sound propagation velocity in wood of selected tropical hardwoods. IAWA Journal, 34 (1): 49-60.

Bayram S (2013). Bazı Avokado Çeşitlerinin Anaçlık Özelliklerinin ve Üzerine Aşılı Çeşitlerle Uyuşma Durumlarının İncelenmesi. T.C. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Doktora Tezi, 146 s., Isparta.

Begum S, Nakaba S, Oribe Y, Kubo T, Funada R (2010). Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer *Cryptomeria japonica.* Trees, 24: 43–52.

Bozkurt Y (1973). Odunsu materyalin mikroskop yardımı ile incelenme tekniği. İstanbul Üniversitesi Orman Fakültesi Dergisi, 1(23): 75-94.

Braga ZV, Dos Santos RF, Amorim L, Appezzato-da-Glória B (2019). Histopathology of infection and colonisation of *Elsinoë ampe*lina on grapevine leaves. European Journal of Plant Pathology, 1-11.

Brandes AFN, Lisi CS, Barros CF (2011). Dendrochronology of lianas of the *Leguminosae* family from the Atlantic Forest, Brazil. Trees, 25: 133–144.

Cangi R (1996). Aşılı Asma Fidanı Üretimi ve Aşı Kaynaşmasının Anatomik, Histolojik ve Biyokimyasal Olarak İncelenmesi. T.C. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Doktora Tezi, 111s., Van.

Cangi R, Balta F, Doğan A (2000). Aşılı asma fidanı üretiminde kullanılan katlama ortamlarının fidan randıman ve kalitesi üzerine etkilerinin anatomik ve histolojik olarak incelenmesi. Turk J Agric For, 24: 393–398.

Chartier MP, Giantomasi MA, Renison D, Roig FA (2016). Exposed roots as indicators of geomorphic processes: A case-study from Polylepis mountain woodlands of Central Argentina. Dendrochronologia, 37: 57-63.

Chong SP, Abdul-Rahim K, Awang MR (2014). Histology study of *Aquilaria malaccensis* and the agarwood resin formation under light microscope. J. Agrobiotech., 5: 77-83.

Coşkun AD (2012). Bazı Klon Anaçlarına Aşılı Kayısı Çeşitlerinde Aşı Kaynaşmasının Anatomik-Histolojik Olarak İncelenmesi ve Fidan Gelişimlerinin Belirlenmesi. T.C. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Doktora Tezi, 183 s., Aydın.

Dolgun O, Ulas SS, Teker T (2016). Determination of graft success of grape cultivars grafted on two different roostocks. Acta Sci. Pol. Hortorum Cultus, 15 (4): 135-145.

Ermel FF, Poëssel JL, Faurobert M, Catesson AM (1997). Early scion/stock junction in compatible and incompatible pear/pear and pear/quince grafts: a histo-cytological study. Ann. Bot., 79: 505-515.

Estay C, Cautín R, Neaman A, Castro M (2016). Clonal propagation of the avocado: effects of the rooting step on graft union formation and development. Ciencia e Investigación Agraria, 43 (2): 233-241.

Falchi R, Petrussa E, Zancani M, Casolo V, Beraldo P, Nardini A, Sivilotti P, Calderan A, Herrera JC, Peterlunger E, Braidot E (2019). Summer drought stress: differential effects on cane anatomy and non-structural carbohydrate content in overwintering Cabernet Sauvignon and Syrah vines. BIO Web of Conferences, DOI: https://doi.org/10.1051/bioconf/20191303007 (Date of access: 24.05.2021)

Hanaoka S, Ohiro M, Matsushita M, Kariuki J (2016). Optimizing the size of root cutting in *Melia volkensii* Gürke for improving clonal propagation and production of quality planting stock. African Journal of Biotechnology, 15 (29): 1551-1558.

Harrison N, Harrison RJ, Barber-Perez N, Cascant-Lopez E, Cobo-Medina M, Lipska M, Conde-Ruíz R, Brain P, Gregory PJ, Fernández-Fernández F (2016). A new three-locus model for rootstock-induced dwarfing in apple revealed by genetic mapping of root bark percentage, Journal of Experimental Botany, 67 (6): 1871-1881.

Henderson F (2013). Leaf anatomy of the genus *Leopoldinia* (*Arecaceae*). The Journal of the Torrey Botanical Society, 140 (3): 369-372.

Hernández-Ledesma P, Terrazas T, Flores-Olvera H (2011). Comparative stem anatomy of Mirabilis (*Nyctaginaceae*). Plant Syst Evol, 292: 117-132.

Khotcharat N, Sdoodee S, Meesawat U (2016). Growth performance of clonal rubber rootstocks and combining ability test with the scion of clone RRIM 600. Agriculture and Natural Resources, 50: 98-103.

Kurt H (2000). Fıstık Çamında (*Pinus pinea* L.) Aşı Kaynaşması ve Çelik Köklenmesinin Anatomik ve Histolojik Olarak İncelenmesi Üzerine Bir Araştırma. T.C. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Doktora Tezi, 52 s., Van.

Lima LKS, Soares TL, de Souza EH (2017). Initial vegetative growth and graft region anatomy of yellow passionfruit on *Passiflora* spp. rootstocks. Scientia Horticulturae, 215: 134-141.

Ljubojević M, Zorić L, Maksimović I, Dulić J, Miodragović M, Barać G, Ognjanov V (2017). Anatomically assisted cherry rootstock selection: Scientia Horticulturae 217: 197-208.

Martins AR, Soares MKM, Redher VLG, Bajay MM, Villela PMS, Zucchi MI, Appezato-da-Glória B (2014). Use of anatomical, chemical, and molecular genetic characteristics in the quality control of medicinal species: a case study of sarsaparilla (*Smilax* spp.). Economic Botany, 68 (4): 410-425.

Nalini MS, Shilpa KE, Basavarajappa S (2015). Stem gall of *Michelia champaca* L. *(Magnoliaceae*) induced by *Podothrips* sp.: Identification, histochemical and phytochemical studies. Tropical Plant Research, 2 (2): 90-100.

Navarro BL, Marques JPR, Appezzato-da- Glória B, Spósito MB (2019). Histopathology of *Phakopsora euvitis* on *Vitis vinifera*. European Journal of Plant Pathology, 1-9.

Olmstead MA, Lang NS, Ewers FW, Owens SA (2006). Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing rootstocks. J.Amer.Soc.Hort.Sci., 131 (5): 577-585.

Öztürk Çalı İ, Candan F (2011). Bitki Anatomisi Uygulamaları Kitabı, Nobel Yayınları, 112 s., Ankara.

Plavcová L, Hoch G, Morris H, Ghiasi S, Jansen S (2016). The amount of parenchyma and living fibers affects storage of nonstructural carbohydrates in young stems and roots of temperate trees, American Journal of Botany, 103 (4): 603-612.

Polat M, Dolgun O, Yildirim A, Aşkın MA, Gökbayrak Z (2010). Graft union formation of spur apple varieties grafted on different rootstocks. Journal of Food, Agriculture & Environment, 8 (2): 490-493.

Pouzoulet J, Pivovaroff AL, Santiago LS, Rolshausen P (2014). Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. Frontiers in Plant Science-Plant Physiology, 5 (253): 1-11.

Shtein I, Hayat Y, Munitz S, Harcavi E, Akerman M, Drori E, Schwartz A, Netzer Y (2017). From structural constraints to hydraulic function in three *Vitis* rootstocks. Trees, 31: 851-861.

Siebers T, Catarino B, Agusti J (2017). Identification and expression analyses of new potential regulators of xylem development and cambium activity in cassava (*Manihot esculenta*). Planta, 245: 539-548.

Song W, Yi J, Kurniadinata OF, Wang H, Huang X (2018). Linking fruit Ca uptake capacity to fruit growth and pedicel anatomy, a cross-species study, Front. Plant Sci., 9 (575) DOI: https://doi.org/10.3389/fpls.2018.00575 (Date of access: 24.05.2021)

Tekintaş FE (1991). Farklı anaçlar üzerine aşılanan turunçgil tür ve çeşitlerinde kaynaşmanın anatomik ve histolojik olarak incelenmesi üzerinde araştırmalar. YYÜZF Dergisi, 1(2): 68-81.

Tordable MC, Lax P, Magunacelaya JC, Doucet M (2012). Histopathological analysis of roots of *Vitis vinifera* cultivar Cabernet Sauvignon infected with *Meloidogyne ethiopica*. Nematropica, 42: 276-280.

Uma E, Muthukumar T (2014). Comparative root morphological anatomy of *Zingiberaceae*. Systematics and Biodiversity, 12 (2): 195-209.

Zwart L, Berger DK, Novungayo Moleleki L, Merwe NA, Myburg AA, Naidoo S (2017). Evidence for salicylic acid signalling and histological changes in the defence response of *Eucalyptus grandis* to *Chrysoporthe austroafricana*. Scientific Reports 7, https://www.nature.com/articles/srep45402 (Date of access: 24.05.2021)