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The Comparative Study of Three Chemical Fixatives on Tomato

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Cytological study is an important basis to analyze tomato grafting body healing. However, sample fixed is a key step to begin cytology study. Fixative type, concentration, fixed time and its temperature will be affected by the result of tissue fixation, so further influence the subsequent experiments. This paper observes different fixatives on tomato stem cells and cell inclusions of fixed effects using a simple\direct hand sectioning with spontaneous fluorescence. The fixed effect of 2.5% glutaraldehyde fixation fluid of tomato stem is the best method in the three that the cellular structure of all tissues remained intact under light microscopic and complete chloroplast aglow phenomenon, a spontaneous fluorescence, can be observe in cells. The fixed effect of 50% alcohol fixed fluid to stem is poorer, and there is no cell fluorescence phenomenon through spontaneous fluorescence, and the autofluorescence of cuticular layer and epidermal hair also decreased significantly. The chloroplasts in cells can be fixed by the FAA fixed liquid, and through the cuticular layer and epidermal hair spontaneous fluorescence observation were not weakened, but the fixed effects of cell wall are not better compared with glutaraldehyde fixation fluid. When fixed plant stem, therefore, consider using 2.5% glutaraldehyde fixation fluid is very necessary.

Key words: Tomato; Fixative; Spontaneous fluorescence.

Tomato grafting is widely used in agricultural production of asexual propagation technology. Developmental mechanism of graft is an important research topic, especially the changes in different tissue interface fusion process and physical. It involves many plant issues, such as regeneration of interface integration process in tissues and organs, the genetic material in the scion and rootstock intercellular transfer etc. A sequence of distinct steps can be described in herbaceous and woody plants during the development of a graft union (McCully,1983; Hartmann *et al*., 2002; Pina and Errea, 2005). Anatomical studies of graft union formation have shown that the processes involved are similar in different fruit tree species such as apricot, peach, pear and apple (Eyre *et al*., 1994a; Soumelidou *et al*.,1994; Elmer *et al*., 1997; Zarrouk *et al*., 2010). These processes include adhesion between grant partners, callus formation, establishment of new vascular tissue, and the formation of a functional vascular system across the graft. The adhesion of parenchymatous tissues is an early event in the formation of a graft union between graft partners. The presence of cell wall projections at the graft interface has been proposed as leading to a mutual cellular recognition (Yeoman *et al*., 1978; Jefree and Yeoman,1983).Cytological study is an basis for process analysis of tomato graft healing. The fixed sample is a prerequisite for cytological study. If the fixed liquid improper selection, uneven

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density, unsuitable fixed time and temperature or little fixed liquid, it will make the tissue fixation adverse detection and observation. As a consequence, a better understanding of the early physiological nature of graft formation could lead to possible diagnostic for compatible or incompatible graft unions before the establishment of vascular connectivity .In the long process of previous experiments, they may accumulate the fixed effects of differnent fixatives on plant material, but the range is too large, it is difficult to select the fixed fluid. FAA called standardly fixed liquid, is universal stationary liquid and is applicable to the general root, stem, leaf, anther culture, ovary tissue slices. It is widely use on research in plant anatomy. It has the biggest benefit of both stationary liquid and preservative effect. However, the observed effect on chromosome is poor.

2.5% glutaraldehyde has advantage on glycogen, glycoproteins, microtubules, endoplasmic reticulum and the cell matrix, which is better fixation. It has strong penetration on tissues and cells and some enzymes also save vitality. Long fixed (one to two weeks or even months) does not make the organization become brittle. The disadvantage is that it cannot save the fat and poor display on the cell membrane. 50% of the alcohol fixation of both the fixed action, but also dehydration, it is a common organic solvent, can dissolve many organic compounds.

The tomato stem has obvious secondary growth and is high lignification, then the tomato grafted cells in the healing process cannot easily be fixed. The research studied the FAA, 50% ethanol and 2.5% glutaraldehyde fixation effect on tomato stems. Plant samples with endogenous fluorescent compounds are Aromatic amino acids, pyridine nucleotide, pyridoxal, vitamin A, flavins, porphyrins, chlorophyll and so on. They can emit faint blue fluorescence under UV irradiation. Cell wall due to the presence of phenolic compounds can producea blue autofluorescence under UV excitation. Different types of plants have different cell wall composition of phenolic compounds. For the majority of gymnosperms and dicots, the phenol in cell wall is lignin. These type plant cell walls are mainly due to the presence of lignin to produce autofluorescence lignin. Chlorophyll and other compounds conjugated system and also has a rigid planar structure of the present study is more mature, Blu-ray excitation of chlorophyll that can be issued in a strong red fluorescence. It can help observe the different cells and cell fixative inclusions fixed effects by autofluorescence further..

MATERIALS AND METHODS

Test Materials

Tomato (*Lycopersicon esculents*) cv Castlemart was used as the test material. Tomato seedlings were grown in autoclaved mixture of peat and vermiculite in a growth chamber maintained under 17 h of light at 28°C and 7 h of dark at 18°C. With daily watering and use of nutrient solution once a week. Watering and use of nutrients were conducted so that differences among plants were minimal. Select 4-5 true leaves tomato seedlings into 0.5cm stems following three fixative, FAA fixative(100 mL, 38% formaldehyde 5 ml, Glacial acetic acid 5ml,50% Alcohol 90ml), 0.1mol/L Phosphate buffer(pH 7.2)and 2.5% Glutaraldehyde, Alcohol 50%. Pumping to the material sinking and fixed at room temperature for one night.

Fluorescent microscopy

To avoid damaging the material in the subsequent processing and on cells during slicing, the most simple and direct way, hand sliced legal piece, is used in this study. The prepared tissue section is seen by Olympus BX51 fluorescence microscope. Excitation light source with a green light (peak 536 nm), image detection and green in color RGB monochromatic. Photographed parts of the fluorescence and changes in organizational structures. Meanwhile, the bright-field image is taken as a reference to determine the fluorescence changes in the organizational structure.

Grafting experiment

In this study, autograft of CM/ CM is made for the follow-on experiment. Splice grafting was carried out as follows. Rootstock seedlings have three true leaves, and scion seedlings have one or two true leaves. With a singly angled cut, remove one cotyledon with the growing point attached. Cut the scion and match the two cut surfaces, rootstock and scion. Hold in place with a grafting clip. Place the grafted seedling in a chamber with high humidity at about 77°F and discard the unused parts. After grafted plants from pots, the rootstock/

scion sections of 10 mM in length from fresh samples, cut by a razor blade on a clean and rigid support, were used for imaging experiments. The cuts were made transversely in respect to the grafting junction, as shown in Fig.5.

RESULTS

Histological localization

We can see leaf blade green fluorescence mainly located in the upper and lower epidermis cuticle, epidermal glands and bundle sheath cells in Fig. 1.

Green fluorescence in upper and lower epidermis and mesophyll cells is weak. The chloroplast particle in the mesophyll cell is clear, at the same time red fluorescence is intensity. However, There is no red fluorescence in the upper and lower epidermis is observed. A large number of green fluorescent cells close together in the vascular bundles around and can be judged bundle sheath cells from the morphology and anatomy.

Different fixative fixed effects on tomato stems

2.5% glutaraldehyde fixed stems of tomato make good results: Clear epidermis, cortex and

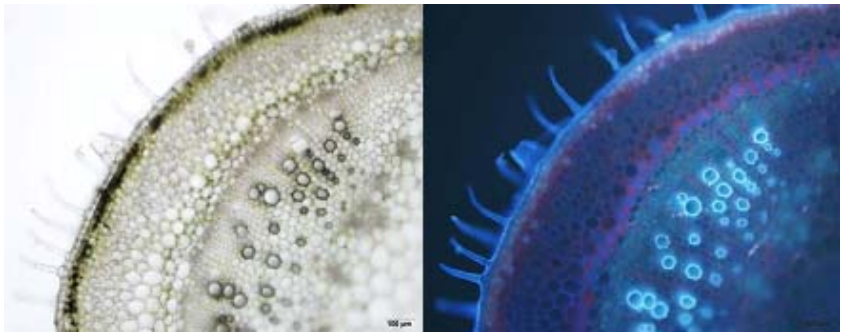

Fig. 1. Tomato stem autofluorescence. The prepared tissue section is observed by Olympus BX51 fluorescence microscope. Excitation light source with a green light (peak 536 nm), image detection and green in color RGB monochromatic

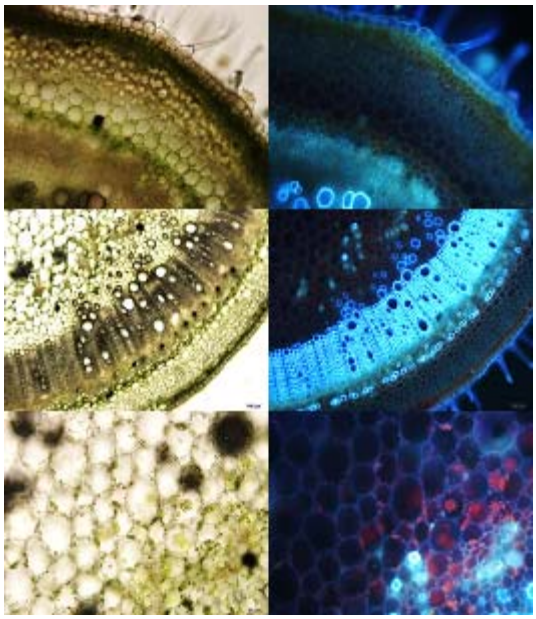

Fig. 2. 2.5% Glutaraldehyde fixation effect on tomato stem

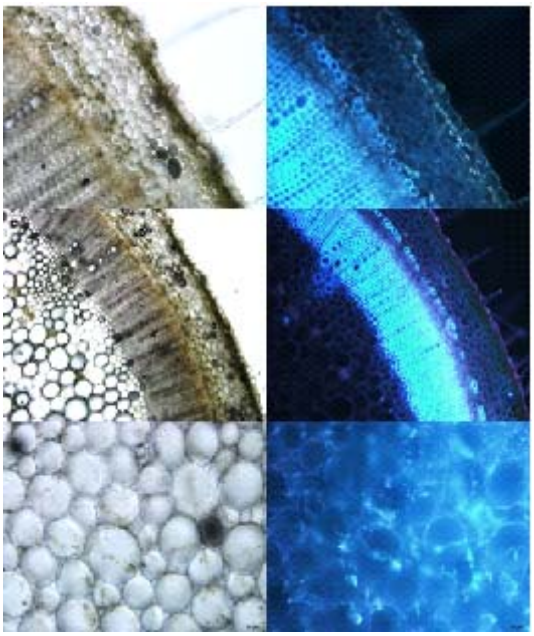

Fig. 3. 50% alcohol fixation effect on tomato stem

medullary parenchyma cell structure. Cell inclusions such as chloroplast present autofluorescence significantly. It is well fixed in the cell.

Tomato stem fixed by 50% ethanol results in cortical and medullary parenchyma cells leads to cell gap significantly due to the loss of water and this decreases the autofluorescence. Autofluorescence in epidermis and trichomes also significantly reduced. 50% of the alcohol has capable of dissolving the cell autofluorescence substance significantly and makes changes in cell morphology because of dehydration (Fig.3).

FAA fixative fixed effects on tomato stems, Cell inclusions such as red fluorescence and chloroplast epidermis and trichomes clear blue fluorescent. For fluorescent cell contents have a good fixed effects, but for cortical and medullary parenchyma cells fixed with glutaraldehyde result is not clear.

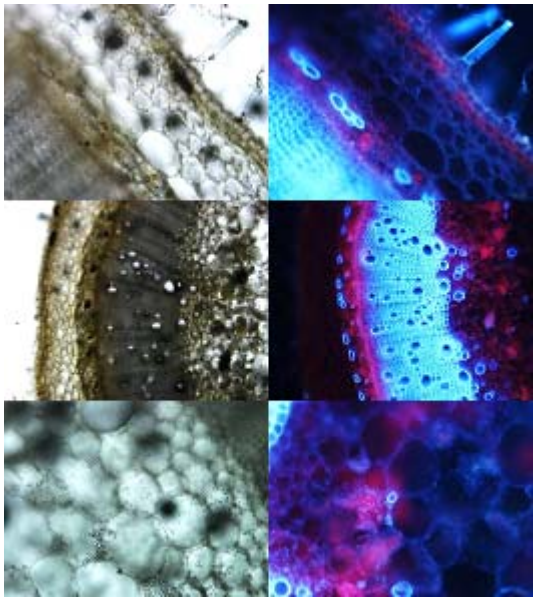

Fig. 4. FAA fixation effect on tomato stem

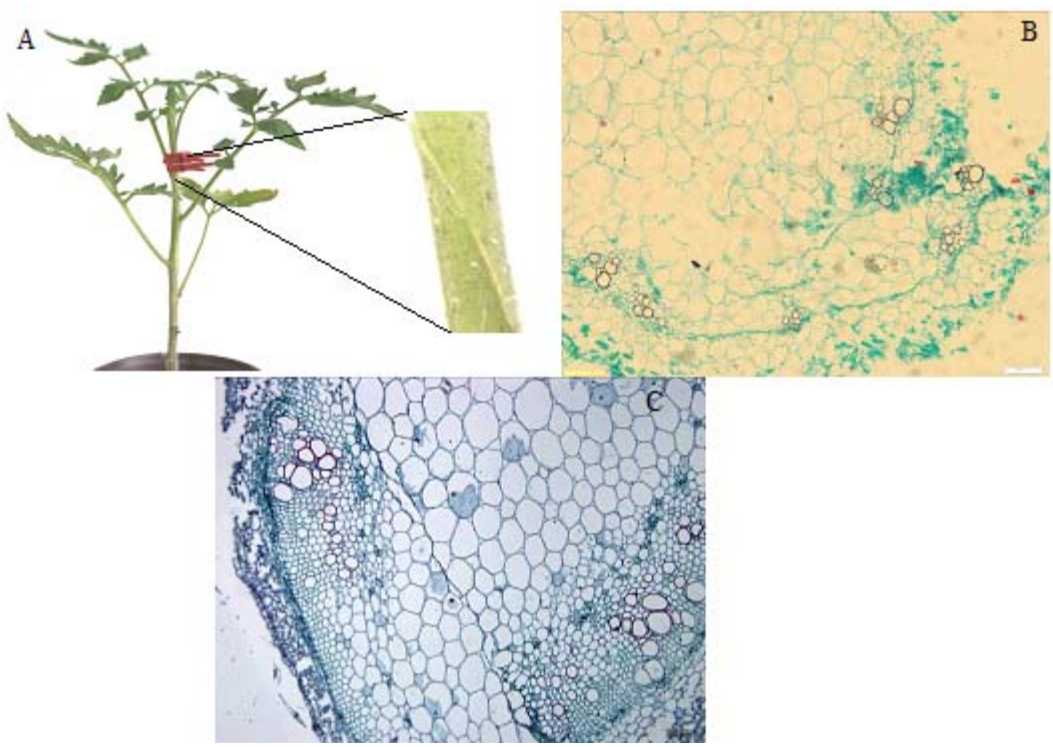

Fig.5 Photograph of a typical grafted tomato plant. A The arrow indicates the position of the graft junction between the stock and scion. It is fixed in FAA fixative and 2.5% glutaraldehyde quickly. B) Graft union fixed in FAA fixative and C) in 2.5% glutaraldehyde .Paraffin sectioning with optical microscopy was adopted to examine the stem wound healing morphological structures of tomato

The result of FAA and 2.5% glutaraldehyde fixing solution on tomato stem paraffin sectioning Fig.5C

In order to further study what kind of stationary liquid is more suitable for tomato stem paraffin section, FAA and 2.5% glutaraldehyde fixation were used to fix the grafting tomato stem. Analysis results show that the organization has no obvious contraction and expansion and could observe the cell structure clearly and satiation fixed by 2.5% glutaraldehyde fixation (Fig.5C). On the contrary, The FAA fixed tomato stem cells, can't keep the clear structure of cells. (Fig.5B)

DISCUSSION

Organizations in certain chemical reagent to make the material inside the cell as far as possible close to the life state of form and structure, Called fixed. Biopsy after fixed liquid precipitation can make proteins, enzymes, such as sugar or solidified into insoluble substances to keep the tissue cell morphology similar to that of a normal life. Choose the best fixed liquid can narrow the difference of cell morphology under a microscope. Tomato stem has the secondary growth makes the larger lignification degree and a hard material. 50% ethanol has the ability of high penetrability, But it makes surface pyknosis, tissue contraction significantly, harden, and morphological changes, also it can dissolve partly cell inclusions that make spontaneous fluorescence. The FAA fixed fluid inclusions have very good fixed effect on fluoresce cells, But the parenchyma cells of cortex and pith is not clear. Liquid 2.5% glutaraldehyde fixation is stronger for cell penetration, Can fix plant cells and inclusions in a relatively short period. In fixed plant, therefore, consider using glutaraldehyde fixation fluid is very necessary.

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REFERENCES

1. Aloni B, Karni L, Deventurero G, Levin Z, Cohen R, Katzir N, Lotan Company M,Edelstein M, Aktas H, Turhan E, Joel DM, Horev C, Kapulnic Y. Physiological and biochemical changes at the rootstock–scion interface in graft combinations between Cucurbita rootstocks and a melon scion. *J Hort Sci Biotechnol* 2008; **83**: 777-83.
2. Hake S and Char B., Cell-cell interactions during plant development. *Genes Dev* 1997; **11**:1087-1097.
3. Gutierrez C., The Arabidopsis Cell Division Cycle. The Arabidopsis Book (012), 2009
4. Andrews PK, Marquez CS. Graft incompatibility. *Hort Rev* 1993;**15**:183–218.
5. Davis AR, Perkins-Veazie P, Hassell R, Levi A, King SR, Zhang X. Grafting effects on vegetable quality. *Hort Sci* 2008;**43**(6):1670–2.
6. Edelstein M, Plaut Z, Ben-Hur M. Sodium and chloride exclusion and retention by non-grafted and grafted melon and Cucurbita plants. *J Exp Bot* 2011;**62**(1):177–84.
7. King SR, Davis AR, Liu W, Levi A. Grafting for disease resistance. *Hort Sci* 2008;**43**(6):1673–6.
8. Kollmann R, Glockmann C. Studies on graft unions. III. On the mechanism ofsecondary formation of plasmodesmata at the graft interface. *Protoplasma* 1991; **165**: 71–85.