Investigations on anatomical and histological development of chip budding and tongue grafts in loquat (Eriobotrya japonica Lindl.)

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in

Llácer G. (ed.), Badenes M.L. (ed.).
First international symposium on loquat

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 58

2003
pages 75-78

Investigations on anatomical and histological development of chip budding and tongue grafts in loquat (*Eriobotrya japonica* Lindl.)

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SUMMARY – This piece of research was carried out at Karadeniz Technical University, Faculty of Agriculture, Department of Horticulture in Ordu, Turkey. The aim was to observe the anatomical and histological developments of chip budding and tongue grafts on loquat (*Eriobotrya japonica* Lindl.). Trials were carried out under controlled conditions, 25±2°C temperature and 75-80% humidity. The graft union and development were studied at the cross and longitudinal graft sections after 15, 22, 30, 45, 60 and 120 days from grafting by means of a microscope. The cambial continuity on the callus bridge can be observed 22 days after grafting in both graft techniques. Results showed that the graft union and growth of loquat were excellent, consequently grafting of loquat was similar to the other fruit species.

Key words: Loquat, graft union, tongue, chip-budding, anatomical and histological developments.

RESUME – *"Investigations sur le développement anatomique et histologique de greffes en écusson et de greffes en fente anglaise chez le néflier (*Eriobotrya japonica *Lindl.)". Cette recherche a été menée à l'Université Technique de Karadeniz, Faculté d'Agriculture, Département d'Horticulture à Ordu, en Turquie. Le but était d'observer les développements anatomiques et histologiques de greffes en écusson et en fente anglaise sur néflier (*Eriobotrya japonica *Lindl.). Les essais ont été menés en conditions contrôlées, à 25±2ºC de température et 75-80% d'humidité. La soudure de la greffe et le développement ont été étudiés sur des sections de greffage transversales et longitudinales à 15, 22, 30, 45, 60 et 120 jours après greffage, par microscope. La continuité du cambium sur le bourrelet du callus peut être observée 22 jours après greffage pour les deux techniques de greffe. Les résultats ont montré que la soudure de greffage et la croissance du néflier ont été excellentes, et par conséquent le greffage du néflier est semblable aux autres espèces fruitières.*

Mots-clés : *Néflier, soudure de greffage, greffage en fente anglaise, greffage en écusson, développements anatomiques et histologiques.*

Introduction

Loquat is one of the evergreen fruit species that can be easily grown in subtropical clime. It is estimated that loquat was brought to Turkey from Algeria and Lebanon about 150-200 years ago (Demir, 1987). Mediterranean Turkish region has more suitable ecological conditions for loquat species than other areas. Therefore, 97.5% of the total production is from Mediterranean region. Aegean region supplies 1.46% and Eastern Black Sea region produce the rest (Anonymous, 1999).

In the recent years loquat production is increasing speedy. From 3000 tonnes produced in 1980, it was increased to 12,000 tonnes in 1999 (Anonymous, 1999), which implied an increase of the demand of grafted trees. This demand is not being covered because of the difficulties of the graft technique on loquat species. The vegetative propagation by grafting is the most convenient method (Hartman and Kester, 1975). However, in some species the graft method can result unsuccesful. Some authors studied the reasons of this fact by means of observation of the anatomic and histologic structure of the tissue that is to be effective at the union of graft (Ashurov, 1977; Poincelot, 1979; Lagerstedt, 1981; Moore, 1982; Moore, 1984; Ünal and Özçagiran, 1986).

In dicotyledons, cambium tissue produces callus cells in the graft union. These paranchymatic callus cells have important roles between cambial and vascular connection in graft union (Hartman and Kester, 1975; Poincelot, 1979; Lagerstedt, 1981; Watanable and Watatsuki, 1988).

While the graft is being made, phenolic compounds from the cells in the cut surface oxidize and produces necrotic layers on them (Simons and Chu, 1985). Young xylem of which don't specialize from the cambium, callus cells of which form from the xylem medullar ray and seconder shell cells destroy the necrotic layers on the cut surface. Then, the cavity between rootstock and scion is filled and provides the connection between them. So, transportation of water and nutrients throught the graft is provided (Hartman and Kester, 1975; Simons and Chu, 1985). It is important for successfully grafting, to know the anatomical development between tissue of scion and rootstock after grafting. This study was carried out to study the graft union using two graft techniques: tongue and chip-budding on loquat under controlled conditions.

Material and methods

This study was carried out at Karadeniz Technical University, Faculty of Agriculture, Horticulture Department, during 2001-2002 with one-year-old loquat seedlings as rootstock and Tanaka cultivar as scion.

They were grafted using two techniques, tongue and chip-budding on November, 10th, 2001. The grafted plants were held at temperature 25±2°C and 75-80% humidity, during 60 days. Cuttings of 20-40 micro in cross section were taken at 15, 22, 30, 45, 60 and 120 days after grafting. They were dyed with fast green and saffron solution. Observations of the callus tissue between rootstock and scion, the necrotic layers formed, formation of new cambium tissue between rootstock and scion, cambial continuity and formation of new vascular tissues on cambium were made.

Results and discussion

Observation of the cross sections of grafts from tongue and chip-budding showed that callus cells between graft component were formed at a satisfactory level. First contact between scion and rootstock was supplied by the bridge that formed from callus tissue in both grafting techniques which is the first step for the success union of the grafting (Hartman and Kester, 1975; Tekintas, 1988).

In both graft techniques, the callus tissue had higher density at the rootstock than at the scion, which agrees with previous studies (Tekintas, 1988; Polat and Kaska, 1992). However, the amount of the callus tissue formed was higher in chip-budding than tongue on samples taken after 15 days from grafting.

Necrotic layers were observed at high density on cut surfaces especially cortex areas. Loquat fruit contain high concentrations of polyphenols and high activity of polyphenol oxides (Ding *et al*., 2002), which could explain the amount of the necrotic layers at cutting area.

Dense necrotic layers were formed, and these layers were destroyed by callus tissues especially from rootstock, and were pushed towards the scion in both grafting method. In chip-budding, cambial continuity was observed between rootstock and scion whereas tongue had this continuity place to place. Callus between rootstock and scion was produced in chip-budding, however in tongue the callus showed an irregular distribution.

The development of callus between rootstock and scion was formed at a better level in chip-budding than tongue in those cutting from 22 days after grafting.

A study by Polat and Kaska (1991) showed that callus tissue is absent in chip-budding and so, the union was not satisfactory, which differs from our results. On the other hand, Soule (1971) and Simons *et al*. (1978) stated that callus tissue of chip-budding grafts were less abundant and had a crisp structure. However, our findings showed that callus tissue could be produced and the humidity of piece of bud could be responded at a satisfactory level.

The space between rootstock and scion was filled with callus tissues especially from rootstock in chip-budding, but this situation was weak in tongue grafting. Callus tissue began to produce regular structure in chip-budding. Necrotic layers were formed both on cut surfaces and in callus tissue especially at side unions in both graft methods.

The cambial differentiation of callus tissue was the second stage of graft union (Hartman and Kester, 1975; Poincelot, 1979; Lagerstedt, 1981). But, the first cambial differentiation was observed 22 days after grafting in both graft methods, especially chip-budding. It was observed that the cambial differentiation began to form from the cells of original cambiums of scion and rootstock in the callus cells which were formed between graft components. The studies that were performed at same topic provided the same results (Kozlowski, 1971; Balta *et al*., 1993).

Observation from cuttings from 30 days after grafting showed that the space between rootstock and scion was filled in chip-budding but it was poor filled in tongue grafting. Cambial continuity was assembled in both graft methods. Callus cell began to be regular.

Thick necrotic layers were observed at early period of grafting in both graft techniques. These necrotic layers were destroyed by callus cells which were formed by dividing of cells that acquired living and meristematic characters at late periods. In addition, necrotic layers were not destroyed in many areas at early periods in tongue. However, forming callus developed poorly and for this reason this tissues couldn't destroy the necrotic layers at satisfactory level. In late periods of chip-budding forming the callus tissue increased and this layers hadn't affected the graft union negatively at 45 days and later. On the other hand, while we were observing the necrotic layers in this period these layers didn't loss and kept existence. A lot of researcher gave same information of necrotic layers (Simons and Chu, 1985; Tekintas, 1988; Polat and Kaska, 1992; Balta, 1993).

Cutting from 60 days after grafting showed that the cambial continuity was set up and new vascular system were produced. Cutting from 120 days after grafting showed that the cambial continuity that was set up between rootstock and scion had continued successfully. Spaces were observed place to place between rootstock and scion in chip-budding, these spaces were more clear in tongue grafting. Rootstock and scion were developed and grown as a normal plant. While the anatomical investigation were being done, all stages of graft union were formed. The graft union of loquat had similar properties to other fruits.

Conclusions

Chip-budding has some advantages to tongue graft. It has higher density of callus and earlier assembling of the vascular system. It has less necrotic layers. However, both techniques can be used on loquat.

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