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A Histological Investigation on Graft Formation of Some Nectarine Cultivars Grafted on Pixy Rootstock

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Abstract: This study was conducted between 2006 and 2007 years in Adnan Menderes University, Sultanhisar Vocational College, in Aydin Province in order to observe the graft formation of nectarine varieties Armking, Cherokee, Stark Red Gold and Independence, which were grafted on pixy rootstock. Callus development, necrotic layers, cambial differentiating cambial continuity and vascular tissues formation were examined. In every combination, callus formation, new cambium differentiating, cambial continuity and vascular tissues formation were noted successfully. All combinations were found compatible in histological observations, but combinations should be further observed or followed up in field conditions for some time to determine incompatibility, which may occur in succeeding years.

Key words: Pixy · Nectarine · Grafting

INTRODUCTION

Plants, produced from seeds are commonly used as rootstock in producing nursery plants in many countries. In this production method, many variations occur in development of nursery plants because of genetic heterogeneity of the rootstocks. In fruit gardens, which are established with trees grafted on seed rootstocks, there were some problems such as overheight, strong development and long juvenile period, which can take 3-5 years because of the heterogenity [1].

Clonal dwarf rootstocks grow similarly and most of them propagate easily. Pixy (*Prunus institia*) rootstock also propagate easly by cuttings. It has a %50 dwarfing effect. It is used especially for plums but it also can be used for some peaches and apricots in many countries.

Dwarf rootstocks are very important in fruit culture. Plants, grafted on dwarf rootstocks grow weak and this weak growing gives us some opportunities such as easy pruning, easy harvesting, easy cultural applications and planting of more number plant in a field

On the other hand, weak growing of rootstock may be cause to incompability during the graft formation. Grafting is a troublesome process and waiting for a long time to observe grafting success can cause to money, time and effort loss. Studying on the sections, prepared from the graft samples, gives us opportunity to assess the development of callus tissue, the position of necrotic layers, cambial differentiating, cambial continuity and the development of vascular tissues. In this regard, histological evaluations on graft sections gives us first informations about compabilitiy or incompability of combinations in a short time [2,3,4,5,6].

The successful combinations are not known exactly for some nectarine varieties such as Armking, Cherooke, Stark Red Gold and Independence grafted on pixy. For this reason, it is important to observe histologically the graft formation of pixy rootstock (*P.institia*) with these varieties. This study was conducted in 2006–2007 in Adnan Menderes University, Sultanhisar Vocational College in Aydin Province. It aimed to evaluate graft formation for compatibility/incompability of Armking, Cherokee, Stark Red Gold and Independence nectarine varieties, which grafted on pixy rootstock.

MATERIALS AND METHODS

Materials: Pixy rootstocks were one-year-old potted plants with average diameter one centimeter. The buds were taken from one-year-old shoots of five-year-old nectarine varieteies Armking, Cherokee, Stark Rec Gold and Independence.

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Methods: Grafts were made by using the "T budding" method on 1-cm-diameter rootstocks in late August. 50 grafts were made for each combinations. And 15-30-45 and 60 days graft samples were taken. The samples were kept in 70% ethyl alcohol. Samples were sectioned horizontally into 25-micron thickness with a microtome. The sections were examined with Olympus digital microscope Mic-D and coloring of sections were done by using the digital coloring property of the microscope. In this study, callus development necrotic layers, cambial differentiating, cambial continuity and formation of vascular tissues were evaluated.

RESULTS AND DISCUSSION

In 15 days sections of combinations, low callus producing were observed. Most of the callus were produced from rootstock. Buds produced lower callus. Necrotic layers were scatter and dark brown color. They were located in callus in the side of the grafts. It is not observed a cambial differantiation in callus in this time. In 30 days sections, limited callus producing were observed. Callus tissues were poor especially in thought xylem tissues of rootstock and bud. Callus was poor in the air pockets which located in both sides of graft. Necrotic layers were clear and they were spread into callus. Some of the cells, located in the callus, were start to transform a different formation, but transformation to cambial cells were not clear. Moore and Walker [7] notified that, interdigitation of callus cells at graft interface began in 3 days after grafting in compatible autograph in Sedum. Tekintas and Dolgun [8], reported that, while the callus producing was seen low in 15 days samples, in 28 days samples was satisfactory in Nectarin/Almond combination. Turkoglu [9] reported that the callus formation were satisfactory in 14 days samples of rosa canina / r. centifolia combination. Balta [10] reported that the callus producing were increased in three week after grafting in nut graft combinations. Unal and Ozcagıran [11] notified that callus formation were large in 30 days samples and it was stable in next samples of pyrus communis / eriobotrya japonica combination.

In 45 days sections, callus producing were satisfactory. Callus were fill all spaces between graft members. Callus cells, located on the periphery were transform to a dark brown colored periderm. Some of the newly formed cells were transformed into cambial cells in the callus tissue. The cambial cells were connected as a continuous ring and cambial continuity were occurred successfuly. Cambial continuity was clear but it was curly because of the scattered necrotic layers. Necrotic layers were spread into callus and they were localize as a form of small brown points in callus. It is observed that cambium were produce a limited amount vascular tissue initials.

In 60 days sections, callus tissue filled all spaces. Cambial continuity were observed clearly. Newly formed cambium produced vascular tissues. Vascular tissue elements, xylem and floem, which produced by newly formed cambium were limited but satisfactory. Tekintas and Dolgun [8] notified that cambial continuity and vascular transformation were clearly observed in 45 and 60 days samples of nectarin/almond combination. Tekintas [12] notified that cambial contiunity were occurred in 45 days after grafting in Citrus. Polat and Kaska [13] reported that cambial contiunity were occurred in 40 days after grafting in loquat/quince-c combination.

As we known, There are three important steps in a graft formation. These are callus formation, cambial differentiating and continuity and vascular tissues formations. Callus development is the first important stages of successful grafting. The union of the bud and stock takes places as a result of the formation and commingling of callus on the two components. The callus is produced by the meristematic tissues as a spongy mass of unorganized parenchyma cells placed in cambium, cortex and young xylem and phloem of the rootstock and bud. When the scion placed on the stock, a definite effort is made to have the two cambium layers match at least on one side. If, as a result of poor callus production, scion and stock union poorly, a longer time will be required and the chances of ultimate union are less than if a beter callus production were obtained

Several factors and conditions such as temperature, humidty and plant species influence callus formation. Some species form a large amount of callus and others smaller. Some form callus quickly and these are likely to unite if properly grafted while those that form callus slowly are less likely to unite. It is possible that callus tissue becomes weak when the rootstock is weak or dwarf, In this situation, lower-produced callus tissue has less opportunity to pass water from rootstock to bud. If the callus, produced by the weak rootstock, is weaker than produced by the bud, it is possible that, grafting can not be successful because poor connecting of the newly produced callus by rootstock and bud.

Cambial differentiating is the second and most important stage of grafting. At first, some of the callus cells transform separately to cambium cells in callus. The later, they form to long cords in the callus and at the end, cambium cells or cords union as a continuously ring in

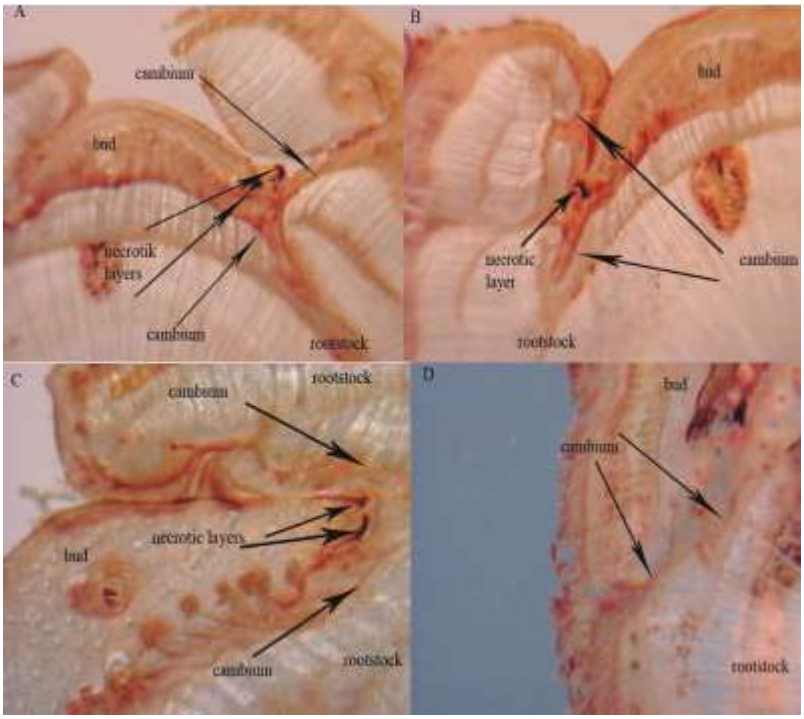

Fig. 1: Horizontal sections from 60 days old samples of nectarine cultivars grafted on pixy rootstock. A. Armking, B. Cherokee, C. Stark Red Gold, D. Independence

callus. The establishment of the cambial continuity is vital for producing of vascular tissues. After this continuity, new cambium cells start to produce new vascular tissues and the grafting process is completed successfully.

Vascular tissue formation is the last stage of the succesful grafting. Formation of a strong union depends on differantiation and amount of new vascular elements. Vascular differentiation begin after establishment of cambial continiuty and the strong connection occur in a short time in the compatible grafts. A good established vascular connection provide a good water and nutrient flow from rootstock to bud [9,11,14,15,16,17,18,19].

In the light of these knowledge, samples were examined carefully. Callus formation, cambial continuity and vascular tissue formation were seen clearly in all combinations. All combinations were compatible in terms of histological observations, but combinations should be observed or followed up in the field after several years to determine incompatibilty, which can subsequently happen.

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