STANDARDIZATION OF GRAFTING TECHNIQUES IN AFRICAN MARIGOLD (*Tagetes erecta* L.) FOR COMBATING BACTERIAL WILT

BY

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(2016-12-010)

THESIS

Submitted in partial fulfilment of the
requirements for the degree of

Master of Science in Horticulture

(FLORICULTURE AND LANDSCAPING)

Faculty of Agriculture
Kerala Agricultural University

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2018

DECLARATION

I, hereby declare that this thesis entitled **"Standardization of grafting techniques in African marigold (*Tagetes erecta* L)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara,
11-09-2018

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CERTIFICATE

Certified that this thesis entitled **"Standardization of grafting techniques in African marigold (*Tagetes erecta* L.) for combating bacterial wilt"** is a record of research work done independently by **Athira Baburaj (2016-12-010)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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ACKNOWLEDGEMENT

First and foremost, I bow my head before the ***Almighty God*** *for all the blessing showered up on me to complete the thesis work successfully.*

With immense pleasure I avail this opportunity to express my deep sense of gratitude and indebtedness to my major advisor ***Dr. Sreelatha U****. Professor and Head, Department of Floriculture and Landscaping, chairperson of my advisory committee for her inspiring guidance, practical suggestions, extreme patience, pains taking scrutiny of the manuscript, friendly approach, kind advice and timely help at various stages of my work and thesis preparation. She has been a support to me during each step of this venture. I really consider myself being greatest fortunate in having her guidance for my research work and will be remembered forever.*

I would like to express my extreme indebtedness to ***Dr. C. Narayanankutty,*** *ADR (Vegetable Mission & seeds) member of my advisory committee for his meticulous help, unwavering encouragement, timely support and critical evaluation.*

Words are inadequate to express my sincere gratitude to ***Dr. Anoop E.V.*** *Professor and Head, Dept. Of Wood Science, member of my advisory committee for his valuable suggestions, criticisms, critical scrutiny and well timed support throughout the course of study.*

I express my heartiest gratitude to ***Mrs. Anupama T.V,*** *Assistant Professor, AICRP on Floriculture, member of my advisory committee for her overwhelming help, valuable guidance and creative suggestions throughout the period of my study.*

I am highly thankful to ***Dr. Mini Sankar****, Assistant Professor, AICRP on Floriculture for her support, relevant suggestions guidance and friendly approach rendered throughout my study period.*

I sincerely thank ***Dr. T. Pradeep Kumar****, Professor, Department of Olericulture, and Director Planning, KAU, for his support by rendering the lab facilities for my studies .*

It is my privilege to express my deepest gratitude to ***Dr. S . Krihnan****, Professor and Head, Department of Agricultural Statistics for his support towards the statistical analysis of data .*

I am highly indebted to ***Dr. C. K. Geetha*** *(Retd.)Professor and Head, Department of Floriculture and Landscaping, and* ***Dr. P. K. Sudhadevi*** *(Retd.) Professor and Head, Department of Floriculture and Landscaping for offering all possible help and support during the period of pursuance of my post-graduation.*

I wish to extend my heartfelt thanks to my beloved teachers, ***Dr.Suma A, Dr. Jyothi Bhaskar, Dr. K. Ajith kumar*** *of Department of Fruit Science for their encouragement, valuable help, and friendly suggestions rendered during, the course of study.*

I am thankful to ***Dincy chechi, Rajani chechi*** *and all other non-teaching staff of the Department of Floriculture and Department of Fruit Science for their sincere help and valuable cooperation during investigation period.*

With pleasure I express my heartfelt gratitude to my friends ***Lakshmi, Nayana, Dhanyasree, Priya, Reshma, Swathi, Sreelaja, Amal, Murthala, Manju, Kitty, Megha, Hasi, Ammu, Niveditha, Navya, Aswath, my seniors Umesh, Shilpa, Deepa and Sameer****,* ***Jeevan****, my juniors and all my batch mates whose constant support and encouragement could never be forgotten.*

I wish to express my sincere thanks to our librarian ***Dr. A. T. Francis*** *and other staffs of the college and university library for their patience, guidance, consideration and immense help rendered to me.*

I wish to express my thanks to ***Kerala Agricultural University*** *for the financial attention offered during the study period.*

On my personal ground, I cannot forget the fondness, constant support and encouragement showered by my loving family. I am deeply indebted to my loving parents, my dearest brother and all my family members for their everlasting support, sacrifice, prayers and blessings, without them, this would not have been a success. I convey my affection and heartfelt gratitude to them who supported me a lot in my long journey of research.It would be impossible to list out all those who have helped me in one way or another, for the completion of my work. I once again express my heart felt thanks to all those who helped me in completing my work on time.

- Athira Baburaj

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Introduction

1. INTRODUCTION

Floriculture is an emerging industry with great potential both at domestic as well as at export level. Owing to steady increase in demand for flowers, floriculture has become one of the important commercial trades in Agriculture. It has been reported that commercial floriculture has higher potential per unit area than majority of the field crops and is therefore a lucrative business. Total area under floriculture in India is estimated to be 3.09 lakh ha with production of 22.4 million tonnes of loose flowers in 2016-17 (NHB, 2017). The diverse agro climatic conditions of our country provides a great scope for the cultivation of almost all major cut and loose flower crops.

African marigold (*Tagetes erecta* L.) is an important flower crop belonging to the family Asteraceae, commercially grown in different parts of our country. The adaptability of this flower crop to varied soil and agro climatic conditions in addition to its prolonged blooming period has attracted the attention of both producers and traders. Marigold flowers are highly attractive with wide spectrum of colour, shape, size and keeping quality. It is mainly grown as a loose flower crop used for making garlands, decorations and for religious offerings. Due to its varied uses, marigold cultivation is found tobe quiet remunerative.

Marigold has also emerged asone of the major source of carotenoid pigment, which is widely used as a dietary supplement in poultry feeds that enhances the yolk pigmentation and chicken skin colour (Narsude *et al.*, 2010). Tagetes oil has been found to be valuable and precious for use in high grade perfumes and cosmetics in the recent past. The oil is also having insect repellent, bronchodilatory, tranquillizing and anti- inflammatory properties, and is also used in traditional Mexican medicines as well. Both leaves and flowers were found to have medicinal properties. The leaf paste is used to cure boils and carbuncles. Marigold flower extract is considered as a blood purifier and is also used forulcer treatments. Marigold is well known to reduce the plant parasitic nematode (PPN) population in soil by several mechanisms, such as acting as non host or poor host producing allelopathic compounds that are toxic or inhibit PPN development,

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creating an environment that favours nematode antagonistic flora and fauna (Wang *et al.*, 2001). Hence, it can also be grown as a trap crop to control nematode population in vegetable field.

There is high demand for marigold flowers in Kerala especially during festival seasons. Neighbouring states serves as the major source of the flowers. The agro climatic conditions of Kerala is suitable for successful cultivation of African marigold throughout the year. However cultivation of marigold has only taken up in the near past in Kerala. Nowaday farmers are using F_1 hybrids of marigold that produces attractive large flowers. But, recently many of farmers are finding it difficult to grow F_1 hybrids in our soils due to sudden wilting of plants caused by soil borne bacteria *Ralstonia solanacearum.* Every year more cases of bacterial wilt incidence are being reported from different parts of Kerala.

Bacterial wilt caused by *Ralstonia solanacearum*is a major devastating disease of solanaceous vegetables in Kerala. Infestation by the same pathogen was found to cause wilt in African marigold in observational trials conducted at Agricultural Research Station, Mannuthy (unpublished data) and Nimisha (2016) also reported the incidence of bacterial wilt in African marigold. Wide spread incidence of bacterial wilt in marigold has been reported from West Bengal (Mondal *et al* 2011, 2014). Bacterial wilt is a devastating disease that is very difficult to control asthe pathogen has wide host range and high survival.

Grafting on wilt resistant root stocks have been successful to overcome bacterial wilt in solanaceous and cucurbitaceous vegetables. Hundred per cent control of bacterial wilt through grafting was reported by Narayanankutty *et al.* (2015). Grafting of susceptible genotypes on resistant rootstock was also found to be an effective tool for controlling bacterial wilt in African marigold (Umesh, 2017).

However, there is a need to standardise the grafting methods as well as age of root stock and scion. This will help to popularise the marigold cultivation in Kerala. Keeping this in view, the present study was conducted with the following objectives:

- To standardize the age of root stock, scion and grafting methods in African marigold.

Review of Literature

2. REVIEW OF LITERATURE

African marigold is an important commercial loose flower. Marigold flowers are highly attractive with a prolonged blooming period and comparatively long shelf life. Though this crop can be successfully raised throughout the year in Kerala, it is found to be highly susceptible to the bacterial wilt caused by *Ralstonia solanacearum.* Many farmers are facing heavy crop loss due to this soil borne disease.

Management of the disease is very difficult in the field. Alternative methods suggested are crop rotation, disinfection of field equipments, removal of weeds, use of soil amendments, host resistance and grafting on resistant rootstocks, of which the most feasible management practices are either cultivation of resistant genotypes or grafting susceptible genotypes on resistant rootstocks.

Grafting can be referred as creation of a new plant by joining of two different plantparts. Though this technique has been practised in fruit trees from years back, this has been started as a commercial method in herbaceous vegetables only recently. Rivero *et al.* (2003) reported that grafting serves a spectrum of purposes such as to boost plant growth and development, to control wilt caused by pathogens, to reduce viral, fungal and bacterial infection, to strengthen tolerance to thermal or saline stress, to increase nutrient and mineral uptake to the shoot.

Grafting methods vary considerably with the type of crops being grafted, and the sowing time of scion and stock (Lee, 1994). The success level of grafting in any crop depends up on grafting methods and age of root stock and scion.It is a low cost, more flexible and short term method for controlling soil borne pathogens. Since no scientific study has been carried out on grafting in marigold, attempts have been made to review similar kinds of works in other herbaceous crops especially vegetables.

2.1 Multi dimensional aspects of grafting

Grafting of herbaceous seedlings is a unique horticultural technology practiced for many years in East Asia to overcome issues associated with intensive cultivation.The self-grafting technique to produce a large gourd fruit by increasing root-to-shoot ratio through multiple grafting was described in an ancient book written in China in the 5th century and in Korea in the 17th century (Lee and Oda, 2003). Growing of grafted vegetables was first started in Japan and Korea in late 1920's. At present, the cultivated area of grafted vegetable, as well as the kind of vegetable being grafted has increased consistently.

Research on herbaceous vegetable grafting began in the 1920's with watermelon (*Citrullus lanatus)* grafted onto squash rootstocks (*Cucurbita moschata* Duch.) to overcome yield loss caused by fusarium wilt (Sato and Takamatsu, 1930).

The purpose of grafting has been expanded greatly, from reducing infection by soil borne diseases, increasing tolerance to low temperature, salt and wet soil conditions and enhancing nutrient and water uptake (Marukawa and Takatsu, 1969; Choi *et al.*, 1980; Gomi and Masuda, 1981; Park 1987)

Grafting methods vary considerably with the type of crops being grafted, and the sowing time for scion and stock seeds vary with grafting method and crop. Plants with vigorous root systems efficiently absorb water and inorganic nutrients, this being one of the main motives for the widespread use of grafted rootstocks (Lee, 1994).

Romero *et al.* (1997) compared the effects of salinity on two varieties of melon grafted onto three hybrids of squash with its effects on non-grafted melons. It was found that the grafted melons were more tolerant to salinity than non-grafted ones, grafted plants developed various mechanisms to prevent the physiological damage caused by excessive accumulation of Cl^- and Na^+ in the leaves.

According to Ruiz and Romero (1999), the efficiency of grafting was significantly evident for fruit yield, in which fruit weight per plant was two times higher in the majority of grafted plants than in their controls.

The foliar nitrate concentrations were lower in grafted watermelons and melons accompanied by higher nitrate reductase activities in comparison with self-rooted plants, which suggests a lower level of nitrogen assimilation in grafted plants (Pulgar *et al.*, 2000).

Grafting can influence the firmness in a highly significant way. Watermelon fruits obtained from plants grafted onto *Lageneria* rootstocks, and *Cucurbita maxima* × *Cucurbita moschata* were firmer by 24% and 27%, respectively than the fruits from the ungrafted plants independent of cultivar, rootstock and growing conditions (Yetisir *et al.*, 2003).

Fernandez-Garcia *et al.* (2003) showed that under saline conditions (60mMNaCl) the Cl− and Na+ uptakes by grafted tomato were significantly lower than those by non-grafted plants, indicating that the grafted plants had higher salinity tolerance than the non grafts.

The selection of rootstocks resistant to soil pathogens implies an advantage in terms of reduced use of chemical agents (Rivero *et al.*, 2003).

Passam *et al.* (2005) reported that grafting can increase fruit size in eggplant and tomato, respectively. It was also found that the average fruit weight and size of plants of solanaceae and cucurbitaceae are often influenced by grafting.

Phosphorus is a macronutrient that often limits plant growth as a result of its low mobility in the soil. Phosphorus uptake can be enhanced by grafting with vigorous rootstocks as confirmed by analyzing phosphorus concentrations in plant tissues of grafted brinjal plants (Leonardi and Giuffrida, 2006).

Vegetable grafting has been practised in the United States as an alternative to soil fumigants and as an integrated pest management practice in various crop production systems (Kubota *et al.*, 2008).

According to Lee *et al.* (2010) there are enormous benefits from using grafted seedlings. These include high income from higher yield and offseason growing, lower input of fertilizers and irrigation water due to the wide root systems of the rootstocks, considerable saving in agrochemicals due to high resistance of the rootstocks, extension of the harvest period, efficient maintenance of popular cultivars against diseases and other physiological disorders, no need for long-term crop rotations, overcoming problems due to saline soils and thermal stress, ease of producing organically grown vegetables.

Studies on cucumbers grafted onto *Cucurbita moschata* indicated that grafting increased the population of bacteria and actinomycetes while reducing the total number of fungi in the rhizosphere (Dong *et al.*, 2010).

Research on grafted peppers also showed that actinomycetes populations in the rhizosphere were higher in the resistant rootstock and grafted plants compared with the self-rooted scion (control) plants when plants were inoculated with *Fusarium solani* (Jiang *et al.*, 2010).

Colla *et al.* (2010) reported that the efficiency of nitrogen uptake and use can be improved by grafting vegetables onto vigorous rootstocks.

Salehi *et al.* (2010) found that *Cucurbita moschata* rootstocks exhibited higher nitrate concentration in xylem sap than that of self-rooted melon plants.

2.2. Grafting for combating biotic stress

Plants are constantly being exposed to a number of adverse conditions in environment. Biotic stress can be defined as the stress produced in the plants due to damage caused by living organisms such as fungi, bacteria, virus, weeds, insects and other native and cultivated plants. The stressful conditions imposed by these biotic factors will affect the growth and development of plants. Deciphering the mechanism involved in the plant defence against these biotic factors will help to develop biotechnological strategies for crop protection. The studies indicated that the grafting can be employed in plants to combat these biotic stress to a great extend.

Chatterjee *et al.* (1997) reported the occurrence of bacterial wilt in various crops including marigold in West Bengal.Wide spread incidence of bacterial wilt caused by *Ralstonia solanacearum* was reported in West Bengal in marigold and in many solanaceous crops was reported by Mondal *et al.* (2011,2014). Severe crop loss was reported during the season when temperature was high and they also reported that isolates of *Ralstonia solanacearum* from marigold was pathogenic on tomato and brinjal.

Nimisha (2016) has observed the incidence of bacterial wilt in African marigold. Umesh (2017) also reported severe incidence of bacterial wilt caused by *Ralstonia solanacearum* in African marigold genotypes and the pathogenecity was also confirmed. He also observed that grafting of susceptible genotypes on to resistant rootstock (M-1) was found to be an effective tool for controlling bacterial wilt in African marigold in wilt sick field.

Studies on grafting as a management technique to combat bacterial wilt in solanaceous crops have been conducted by several scientists.

Control of bacterial wilt in tomato by grafting on resistant root stock has been reported by Lum and Wong (1976). Two major soil borne diseases viz., bacterial wilt caused by *Ralstonia solanacearum* and Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* were effectively controlled by grafting

susceptible heirloom tomato scions onto resistant root stocks in the south-eastern United States (Rivard and Louws, 2008).

Lin *et al.* (2008) found that grafting scions of susceptible tomato cultivars onto resistant rootstocks has been successful for managing tomato bacterial wilt.Complete control of bacterial wilt and better yield in grafted tomato, chilli and capsicum hybrids has been reported by Narayanankutty *et al.* (2015).
Colla *et al.* (2008) observed an increase in yield for grafted *Capsicum annum* hybrid cultivars onto commercial rootstocks. They also reported that grafting on resistant rootstock became popular for control of different soil-borne pathogens

Corky root disease which was caused by *Pyrenochaeta lycopersici* is a severe problem affecting crops of Solanaceae family. Hasna *et al.* (2009) found lower incidence of corky root disease intomatoes grafted onto 'Beaufort' rootstocks. They had also observed higher yield, and larger fruit than the non grafted plants. Similar results were also found in grafted brinjal (*Solanum melongena* L.) by Iouannou (2001).

Grafting of susceptible tomato cultivars on root knot nematode resistant rootstocks was effective in controlling root knot nematode in fields naturally infested with root knot nematode (Rivard *et al.*, 2010).

Various types of viral diseases such as tomato yellow leaf curl virus, tomato spotted wilt virus, and *pepino mosaic virus* were also reported to be controlled by grafting (Louws *et al.*, 2010).

According to Yin *et al.* (2008) incidence of Verticillium wilt was reduced when a susceptible eggplant scion was grafted onto *Solanum torvum* rootstock accompanied by enhanced ratios of bacteria and actinomycetes to fungi in the rhizosphere of grafted plants.

In a study conducted by Edelstein *et al.* (1999) grafting scions of susceptible melon cultivars onto *Cucurbita maxima* Duch. and *Cucurbita moschata* rootstocks improved resistance of melons to the wilt.

Verticillium wilt, primarily caused by *Verticillium dahliae*, is another vascular wilt disease that often affects Solanaceae and Cucurbitaceae. Studies with plants grafted onto commercial rootstocks and subjected to infection with *Verticillium dahliae* indicated that both scions and rootstocks contributed to disease resistance of the grafted combinations in watermelons, melons, cucumbers, and tomatoes (Paplomatas *et al.*, 2002).

Wang *et al.* (2004) reported that in *Phytophthora capsici* infested fields there was a significant increase in the yield of cucumbers grafted on bottle gourd *Cucurbita moschata* and wax gourd rootstocks, and vegetative vigour was also found to be high.

Zhang *et al.* (2006) claimed that the cucumbers grafted on the bur cucumberrootstock exhibited increased root knot nematode resistance.

According to Cohen *et al.* (2007) the use of resistant rootstocks for controlling the soil borne melon necrotic spot virus in cucurbits had a significant advantage over soil fumigation with methyl bromide, which did not control the viral disease.

Crino (2007) reported that grafting as an effective tool for controlling the race 1,2 of *Fusarium oxysporum* f. sp. *melonis* (FOM) and *Didymellabryoniae* in melon (*Cucumis melo* L).

According to Kousik and Thies (2010) watermelons grafted onto selected bottle gourd rootstocks exhibited resistance to *Phytophthora capsici.*

The foliar fungal and viral diseases were suppressed when susceptible scions were grafted onto specific rootstocks. Commonly used cucurbitaceous rootstocks

were non-hosts to most species of *Fusarium oxysporum*, and thus grafting has been successfully used to control fusarium wilt in cucurbit production.Complete control of bacterial wilt was observed in tomato grafted on selected rootstock (Louws, 2010, 2011).

Guan *et al.* (2012) reported that grafting with resistant rootstocks is an effective strategy to manage a variety of soil borne diseases and root-knot nematodes in solanaceous and cucurbitaceous vegetables. In addition, improved resistance to some foliar diseases and viruses has also been reported in grafted plants. Hence, grafting technology is considered an important and innovative practice of integrated pest management and a promising alternative for soil fumigants in vegetable production.

Grafted plants were resistant to soilborne diseasessinceit has strong root systems, and increased photosynthesis resulting in higher yield and improved fruit size (Xu *et al.*, 2005).

Grafting can be successfully followed to reduce the soil borne diseases, and is a replacement strategy against the use of chemicals to control soil borne pathogens (Miguel *et al.*, 2004).

Pradhan *et al.*, (2017) reported that grafting with suitable rootstocks is the best alternative for controlling the soil borne disease such as fusarium wilt and bacterial wilt, root knot nematodes, parasitic pests and weeds.

2.3. Effect of method of grafting on graft success

Grafting is an age old horticultural practice, where a new plant is created by joining of two different plant parts. There are different types of grafting methods, but the grafting methods vary considerably with the type of crops being grafted, and the sowing time of scion and stock (Lee, 1994).In herbaceous crops, such as in vegetables the most commonly followed methods of grafting are tongue approach grafting, splice grafting, cleft grafting, hole insertion grafting, tube grafting and one cotyledon grafting.

Hole insertion grafting and one cotyledon grafting were preferred when scion and rootstock have hollow hypocotyl (Hang *et al.*, 2005). This grafting techniques requires highly experienced labour and healing chamber for higher survival rates. Cleft grafting, hole insertion grafting and one cotyledon grafting owing to their high grafting positions decrease the chance of scion adventitious roots contradicting soil borne diseases.

Sakata *et al.* (2007) studied the percent share of different grafting methods employed for production of grafted water melon seedlings in Japan. Hole insertion grafting (53%) was most commonly adopted in case of water melon, as it does not require special grafting gadgets. In melons (56%) and cucumber (89%) tongue approach grafting was adopted, even though it required more operation and longer time for grafting and healing. In contrary nursery growers adopted splice grafting in cucumber (39%) and hole insertion grafting for melons (38%) and watermelon (35%).

According to Lee *et al.* (2010) a high survival rate was observed in the case of tongue approach grafting,even though it required high space and labour compared to other methods.

The savings and efficiency of labour in three different grafting methods viz., pin grafting, hole insertion grafting and tongue approach grafting were evaluated by Davis *et al.* (2008). It was observed that the efficiency was highest for pin grafting (46.0 min/100 plants) followed by hole insertion grafting (60.3 min/100 plants)

and tongue approach grafting (71.4 min/100 plants). Pin grafting saved 35.8% labour, while in hole insertion grafting it was 15.6%.

Bekhradi *et al.* (2011) compared hole insertion grafting and tube grafting in water melon cv. "Charlestone Grey" with bottle gourd as rootstock. It was reported that hole insertion grafting showed highest survival rate (90%) than that of tube grafting.

A study was conducted by Abd El-Wanis *et al.*(2013) to identify the best grafting method for producing grafted water melon seedlings. Watermelon (*Citrullus lanatus* Thunb.) Cv. Aswan was grafted on to bottle gourd rootstock (*Lageneria siceraria*). Different types of grafting methods such as splice grafting, hole insertion grafting and tongue approach grafting were done. The survival percentage was found to be 99%, 98% and 97% in tongue approach grafting, hole insertion grafting and splice grafting respectively. In terms of survival rate, vegetative characters, yield and yield components, splice grafting was the best followed by hole insertion grafting.

Punithaveni *et al.* (2014) found that hole insertion grafting was found to be more successful in cucurbits with high survival rate as compared to side grafting.

In a comparative study on yield of bitter gourd (*Momordica charantia* L.) grafted on to bottle gourd (*Lageneria siceraria*) rootstock using hole insertion and splice grafting revealed that the average yield of hole insertion grafting per plant (607.84 g) was found to be higher than that of splice grafted plants (571.79 g) (Uap, 2014).

Marsic and Osvald (2004) carried out a study on the effect of grafting in tomato grown under protected cultivation. Two types of grafting namely splice and tube grafting were done. It was observed that there was high percent survival of grafts

in both the methods,which indicated that both these methods can be followed in tomato.

Higher survival rate was observed in case of tongue approach grafting as the root system of the scion remained alive until the formation of graft union (Pandey and Rai, 2003).

Tongue approach grafting was found to be the best grafting method in bittergourd in terms of survival percentage. It was observed that 89% plants survived in tongue approach method, whereas in wedge grafting method survival percent was observed to be only 67% (Akhila and George, 2017).

2.4.Grafting success and Enzyme activity

For successful graft union formation the vascular connection between the root stock and scion is to be restored. Xylem differentiation as well as lignification is the prerequisite for successful development of new vascular system. Lignification is associated with activities of enzymes like peroxidase and catalase.

2.4.1. Catalase activity

Catalase, a haem containing enzyme catalyses the dismutation of H_2O_2 into water and oxygen. This enzyme helps in the removal of H_2O_2 produced in the cells. It was the first enzyme to be isolated in the purified state. In grafted plants as a result of xylem differentiation and followed by lignifications there will be production of high level of H_2O_2, which induces the activity of catalase.

Fernandaz-Garcia *et al.*, (2004) reported that grafted tomato plants showed a significant increase in H_2O_2 at eighth day of grafting, and catalase activity increased in parallel. Thus it was considered that catalase could mainly be

involved in cellular defence against the high level of production of H_2O_2 observed at this stage. This H_2O_2 might have originated from the high level of lignification observed in the graft union as xylem cells are actively lignifying their cell walls.

Rivero *et al.* (2003) reported that there was a higher catalase activity in grafted tomato plants under thermal stress condition than that of non grafted plants. Therefore, the use of grafted plants under excessively high temperatures may offer an advantage over nongrafted plants in terms of resistance against thermal shock.

Grafting with salt tolerant rootstock improved the photosynthesis performance with higher stomatal conductance (*Gs*) and water use efficiency (WUE).The activity of catalase enzyme was found to be high in grafted plant, which enhanced the growth of tomato under saline stress condition .There was a higher catalase activity in grafted tomato plants which reduces the oxidative damage in plant tissue, when compared to that of non grafted and self grafted plants (He *et al.*, 2009)

In tomato it was found that grafting can completely prevent the chill-induced accumulation of H_2O_2 in leaves (Rivero *et al.*, 2003).

Drought tolerance in tomato plants, by grafting it with two different cultivars namely cv. Zarina and cv. Josephina. It was observed that there was a higher catalase activity in plants grafted on to drought tolerant cv. Zarina than that grafted on to cv. Josephina and non graftedplants.A significant increase in the activity of catalase enzyme was observed in grafted tomato plants under water stress condition, when compared with non-grafted plants (Rodriguez *et al.*, 2011, 2012).

The activities of catalase in the leaves of grafted water melon seedlings were significantly higher than that of non-grafted plants (Zhu *et al.*, 2008).

Kun *et al.* (2010) reported that in grafted cucumber plants the activity of catalase enzyme was higher, which in turn reduced the reactive oxygen species in the roots.

Liu *et al.* (2014) reported that the tobacco plants grafted on to drought tolerant cultivar Nongda 202, showed high catalase activity than non-grafted and self-grafted plants under drought stressed condition. The activity of catalase in grafted plants increased significantly at 6^{th}day after grafting. It indicated that high level of catalase in rootstock grafted plants might help to scavenge toxic levels of reactive oxygen species induced by drought stress and to improve drought tolerance.

According to Peter *et al.* (1989) hydrogen peroxide can be detoxified from the different cell compartmentsby different enzymes, either on the one hand by the activity of guaicol peroxidase (GPX) or catalase (CAT), which bring about the automatic catalysis of hydrogen peroxide to water and oxygen.

2.4.2.Peroxidase activity

Peroxidase enzyme is a glycoprotein with the hematin compounds as cofactor that typically catalyses a reaction where the substrate is hydrogen peroxide. Peroxidase plays an important role in increasing the defense mechanism of the plants. In grafted plants, xylem differentiation in graft union begins as small areas of lignifications, which is catalysed by the enzyme peroxidase.

In grafted plants guaicol peroxidase showed higher activity than the non-grafted plants, therefore grafted plants showed no massive accumulation of phenolic compounds, this being directly reflected in greater biomass production and better development than non-grafted plants.It was also found that under thermal stress condition there was a higher peroxidase activity in grafted tomato plants than that of non grafted plants. Therefore, the use of grafted plants under

thermal stress condition might offer an advantage over non-grafted plants in terms of resistance against thermal shock (Rivero *et al.*, 2002).

Increased peroxidase activity in relation with lignin synthesis in grafted tomato plants has been reported by Fernandaz- Garcia *et al.* (2004). They observed that after the graft assemblage, between the rootstock and scion the cells were developed, differentiated and the new vascular system begins to develop. For this to occur, xylem differentiation and lignification were necessary, The new xylem strands developed in the scion as result of graft union exhibit a radial pattern with a high degree of lignification. This was correlated with a large increase of peroxidase activity in the grafts. They also observed that peroxidase activity increased during plant development in both control and grafted plants, however it was always higher in grafted plants than in control. Four days after grafting, peroxidase activity was mainly localised in the graft union between the rootstock and scion, at eighth day after grafting, peroxidase activity was higher than that of fourth day.

The difference in the level of peroxidase was noticed over time after grafting in tomato plants (Silva *et al.*, 2016).

The activity of antioxidant enzyme peroxidase was found to be higher in grafted brinjal under Ca $(NO_3)_2$ stress condition (Wei *et al.*, 2008). Similar finding was reported in brinjal by Zhang *et al.* (2008).

Lignification is a process that requires hydrogen peroxide and cell wall peroxidases to bring about polymerization of lignin. In addition, hydrogen peroxide may serve as an immediate mechanism for disease resistance in response to pathogens and may play an important role in wound-response and cell apoptosis (Pellinen *et al.*, 2002)

According to Nicholson and Hammerschmidt (1992) peroxidase is one of the enzyme involved in the last stage of lignification.

Role of peroxidase in lignin synthesis was reported by Whetten *et al.* (1998). They reported that the last catalytic step in the synthesis of lignin is the oxidation of cinnamyl alcohols which is catalysed by peroxidase enzyme thus peroxidase plays important role in lignification.

Some genetic evidence for the roles of peroxidases were wound-induced lignin polymerization and suberinization (Bernards and Razem, 2001)

The level of peroxidase activity in grafted plants can determine the graft compatibility, higher the difference in enzyme activity lower will be the compatibility (Liu and Wang, 2011).

Schmid and Feucht (1985) reported that proteins and peroxidases in the phloem of grafted cherry plants confirmed the possibility of good union formation by morphological characters and biochemical methods.

2.5. Histological studies of grafts

As a result of grafting there will be formation of proliferation or necrotic layer, followed by the generation of rapidly dividing parenchymatous cells. A callus bridge made of vascular tissue will beformed by the multiplication of callus cells which promotes the movement of water and nutrients between the rootstock and scion thus ensuring the success of the grafting.

The re-establishment of vascular continuity through interface zone is the critical event that determines the compatibility on the stock and scion on development of graft union formation (Moore, 1984).

Celik (2002) reported that the degree of callus formation at graft union after grafting operation is the main determinant of graft-compatibility.

According to Estrada *et al.* (2002) the histology of the union formation includes five general stages; formation of graft union, development of necrotic

layer and proliferation of callus bridge at graft interface, differentiation of new vascular cambium, restoration of continuity of new vascular tissue and restoration of the continuity of epidermis at union zone.

According to Lilieth *et al.* (2012) there is an establishment of wound repair xylem across the graft union, which allowsthe movement of water and mineral nutrients between the scion and rootstock in grafted chilli plants.

Abd El-Wanis *et al.* (2013) carried out histological analysis of water melon grafted on to bottlegourd. Three grafting methods namely splice, hole and tongue approached grafting were experimented and the results showed that there was a strong connection between the stock and scion in splice grafting compared to other two methods. The parenchymatous cells formed cambial region which was capable of meristamatic activity resulting in better vascular connection, vigorous growth and yield of splice grafted plants.

Priyadarshini and Ventamoni (2015) conducted the studies on grafting of annual moringa and observed histological development during graft union formation. Fifteen days after grafting a necrotic layer was formed followed by the enlargement of callus cells between the scion and the rootstock. Thirty days after grafting, linkage of cambium and healing of graft union were observed which results in successful graft union formation.

Anatomical studies were conducted by Tamilselvi and Pugalendhi (2017) to determine the graft compatibility in bittergourd with two different rootstock namely pumpkin and fig leaf gourd. Seven days after grafting transverse section of graft union were studied. Dark strands of proliferation or necrotic layer were seen in outer sides and interfaces of stock and scion in case of pumpkin rootstock, and a discontinuous xylem elements were observed in the case of fig leaf gourd root stock.

The anatomy of successful graft union in cucumber plants grafted on fig leaf gourd, bottle gourd, pumpkin and squash was studied by Shehata *et al.* (2000). The cell division in the scion and rootstock at graft union

(dedifferentiation), redifferentiation of callus tissue, connection between vascular bundles of rootstock and scion and secondary growth of rootstock and scion, resulted in graft success. Incompatible grafts lacked vascular connection between rootstock and scion.

Histological observation of bitter gourd at graftinterface revealed the formation of necrotic layer in response to woundrepair and proliferation of cells of rootstock andscion which is the stage prior to callus formation (Akhila and George, 2017)

In egg plants phenolic and lignin contents in leaves and roots of grafted plants were also higher than that of self-rooted plants after inoculation with *Fusarium solani* (Jiang *et al.*, 2010)

The histo chemical staining for lignin was based on the reaction of phloroglucinol with aromatic aldehyde fraction present in lignin, which yields a pink stain. The intensity of pink stain shows the progress of lignification during graft development.

Fernandaz-Garcia *et al.* (2004) reported that at fourth day of grafting a small spots of pink stain was observed in the graft union of grafted tomato plants. There was a significant increase in staining on eighth day after grafting and at fifteenth day of grafting the graft union exhibited an intense pink colour.

Materials and Methods

3. MATERIALS AND METHODS

The study on Standardization of grafting techniques in African marigold (*Tagetes erecta* L.) for combating bacterial wilt was conducted at Department of Floriculture and Landscaping during the year 2017-18. The details regarding the experimental materials, methodology of experiments and analytical techniques followed are presented in this chapter.

3.1. Experimental materials

The local type of marigold M-1, which was already screened as resistant to bacterial wilt was used as the root stock. Bacterial wilt susceptible variety of marigold, Maria-91 was used as the scion (Plate 1). The requirements of the grafting operation includes razor blade, grafting clips and mist chamber.

The study were conducted out in three experiment viz.,

1. Standardization of grafting methods, age of rootstocks and scion

2.Assay of enzyme activities

3.Histological studies

3.1.1. Standardization of grafting methods

The experiment was conducted from June 2017 to May 2018. Three types ofgrafting methods viz.,cleft ,splice and hole insertion was evaluated and under each method of grafting combinations of three stages of rootstock and three stages of scion were carried out to optimise the age of rootstock and scion .

Grafting methods – cleft, splice and hole insertion

Age of rootstock – 4, 5 and 6 weeks after sowing

Age of scion - 3, 4 and 5 weeks after sowing

Design - CRD

No.of .treatments -27 + control

No. of. Replication – 2

No. of grafts/ treatment/replication – 100

3.1.1.1.Raising of rootstocks and scion for grafting

Seeds of root stock (M-1) and scion (Maria 91) were sown in pro-trays filled with soilless media comprising of coco peat, vermiculite and perlite in the ratio 3:1:1. Seeds of rootstock and scion were sown at required time intervals to ensure seedling availability for different root stock and scion combinations. Grafting was done once the seedlings reached the specified age.

3.1.1.2. Grafting methods

Three types of grafting namely cleft, splice and hole insertion were done. Grafting was carried out in shade houses preferably during morning and evening hours. The plants after grafting were immediately transferred to the mistchambers, where they were maintained at high relative humidity for successful graft union.

For cleft grafting the growing tip of root stock was removed by providing a straight horizontal cut, followed by a downward vertical cut was made on the rootstock. A matching incision in a wedge form was made on the lower portion of the scion. The scion was inserted in to the vertical slit of the rootstock. The stock and scion were fixed in the position using the grafting clip (plate 2). The grafted plants were kept inside the mistchambers for to attain successful graft union (Plate 3)

Splice method of grafting was done by providing a slant cut on the rootstock and a matching slant cut at the base of the scion and both the cut ends were placed in position using grafting clips (Plate 4). The grafts were kept in mist chamber (Plate 5)

Plate 1: Rootstock – M-1 and Scion – Maria-91

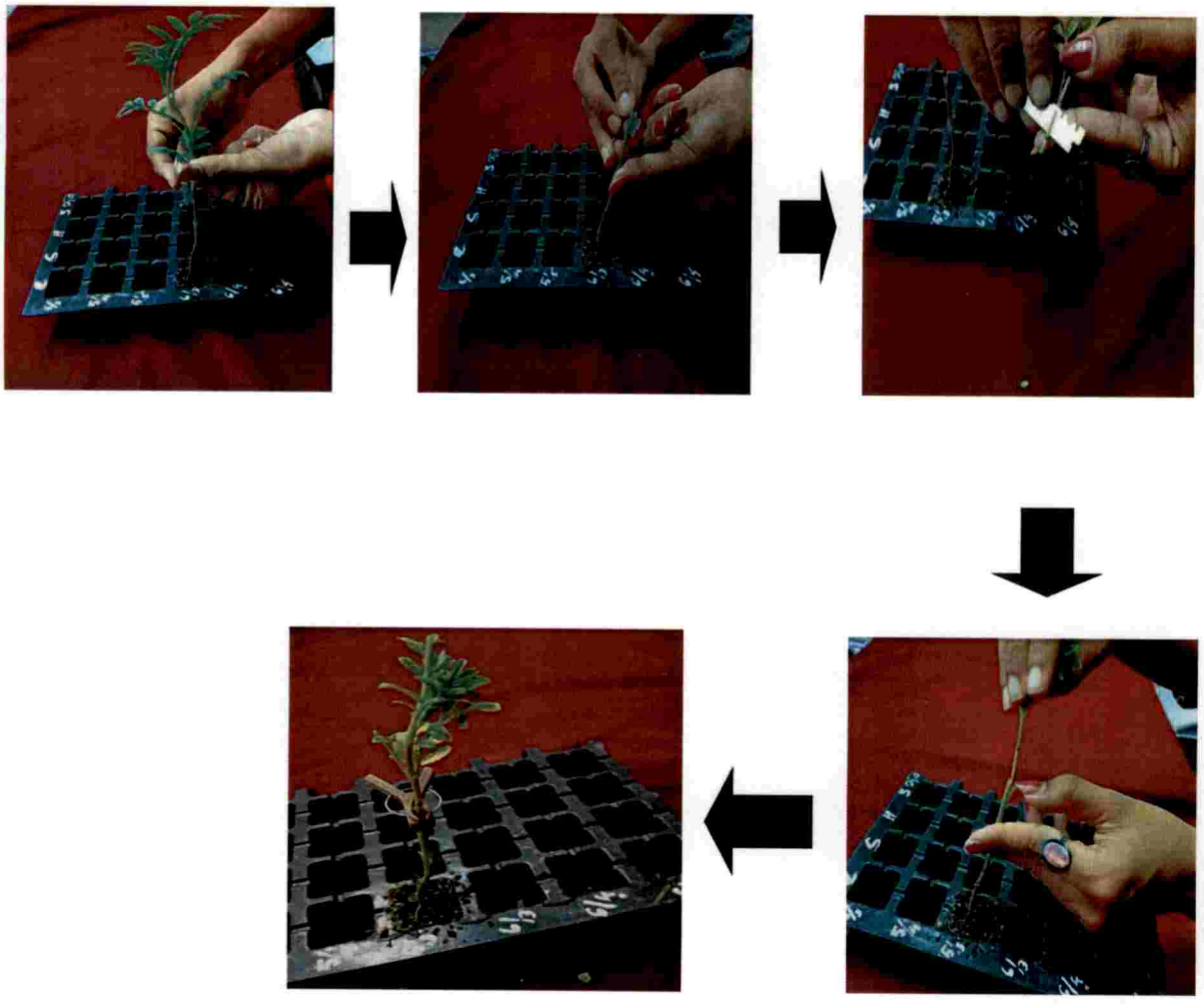

Plate 2. Procedure of cleft grafting

T1

T2

T3

T4

T5

T6

Plate 3. Cleft grafted marigold plants

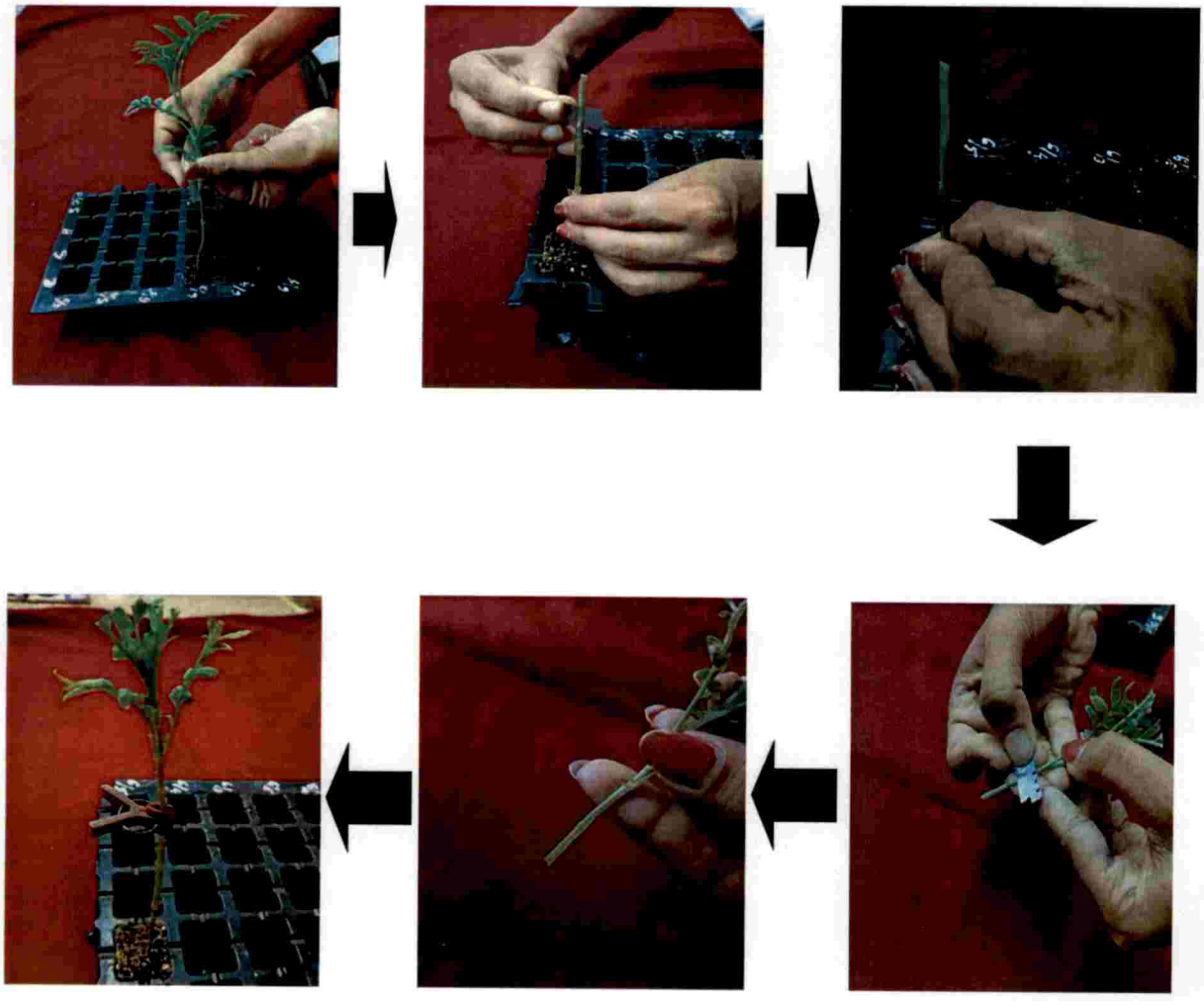

Plate 4. Procedure of splice grafting

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Plate 5. Splice grafted marigold plants

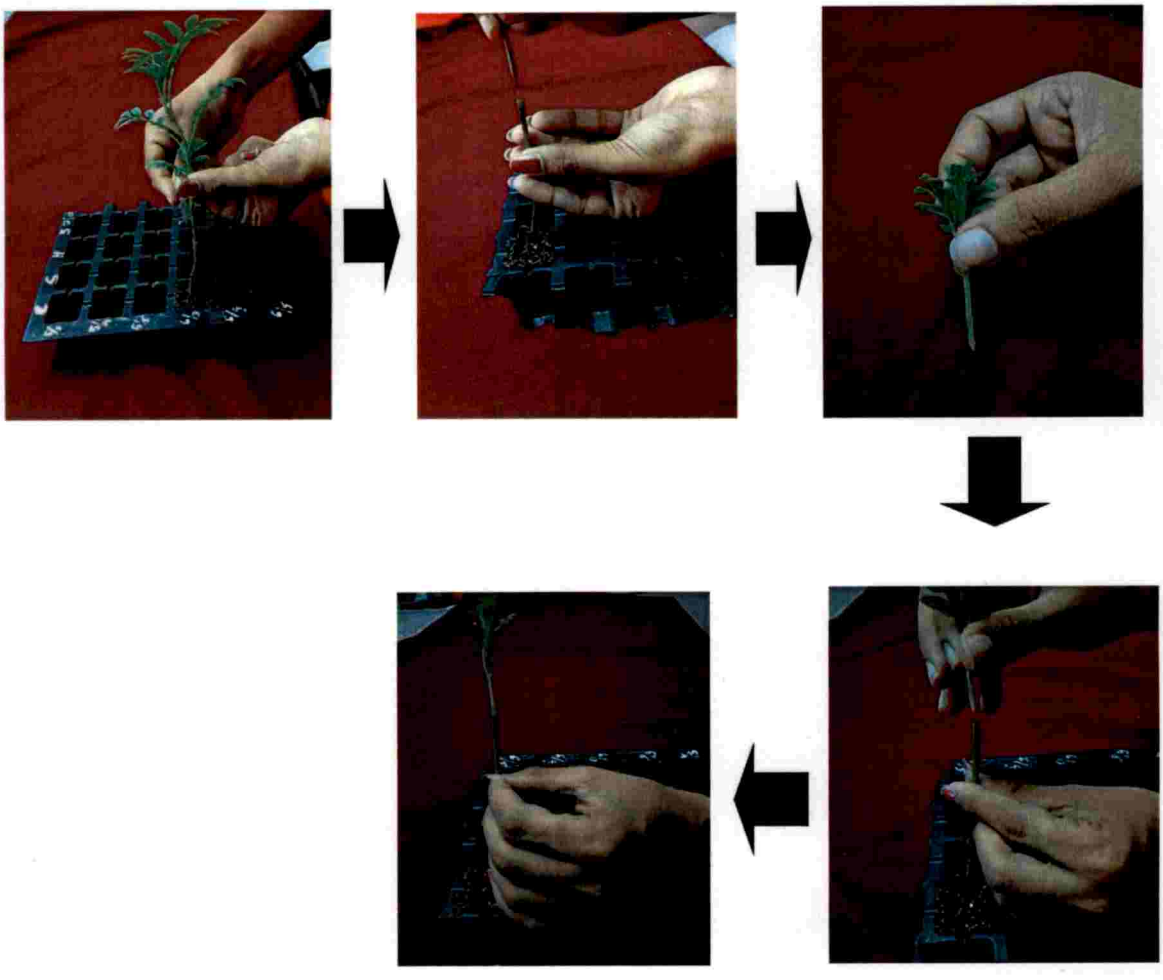

Plate 6. Procedure of hole insertion grafting

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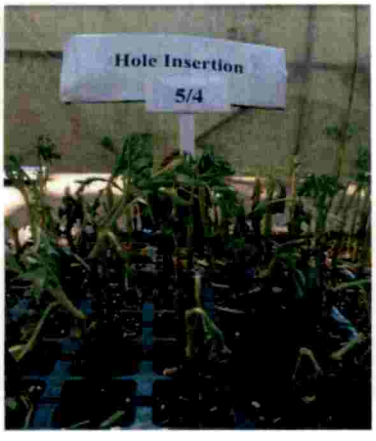
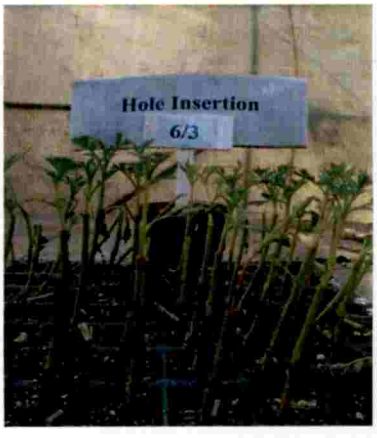
T20

T21



T22

T23



T24

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T26

T27

Plate 7. Hole insertion grafted marigold plants

In hole insertion method of grafting, the top of the rootstock was given a straight horizontal cut. Using tooth pick a hole was made on the root stock. The basal end of the scion was given a sharp pointed cut and then the scion was inserted to the hole made in the root stock stem (Plate 6). The grafts were kept inside mist chambers (Plate 7).

The grafts were regularly observed and watered with fine spray of water based on the requirement. In case of cleft and splice grafted plants, the grafting clips were removed on fifth day after grafting. All the grafted plants were transferred to a hardening structure which was cladded with 200 micron UV stabilised polythene sheet and 75% shade net.

Observations on percent graft survival and days to graft union formation were recorded.

3.2. Assay of enzymes

Estimation of catalase and peroxidise enzymes were carried out in order to study their role and influence in grafting success.The enzyme assays were carried out in both grafted as well as non-grafted plantsusing at specific sampling intervals.

Sampling interval –4,8& 15 days after grafting (DAG).

No. of plants analysed per treatment – 5

3.2.1. Estimation of catalase

The catalase activity was estimated by the method described by Luck (1974). A homogenate of the plant sample (20%) was prepared in 0.1M phosphate buffer (pH 7). The homogenate was centrifuged (1500 rpm) at 4°C for 15 minutes, and the supernatant was used for assay. Assay mixture excluding enzyme solution served as the control. In an experimental cuvette 3ml ofH_2O_2-phosphate buffer was taken, followed by the rapid addition of enzyme extract and was mixed thoroughly. The time required for decrease in absorbance by 0.05 units was

43

recorded at 240 nm in spectrophotometer (Eppendorf230V/50-60Hz). One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

3.2.2 Estimation of peroxidase

The peroxidase activity was assessed by the method followed by Reddy *et al.* (1995). A 20 percent homogenate of plant sample was prepared in 0.1M phosphate buffer (pH 6.5). The supernatant from the samplewhich was clarified by centrifugation was used for the assay.To 0.1 ml of enzyme extract, 3.0 ml of 0.05M pyrogallol solutionwas added and at 430 nm spectrophotometer was adjusted to read zero. To the test cuvette 0.05 ml H_2O_2 was added. The change in absorbance was recorded every 30 seconds up to 3 minutes in spectrophotometer. One unit of peroxidase was defined as the change in absorbance/minute at 430 nm.

3.3. Histological studies

Histochemical staining for lignin was carried out according to the method described by Ros-Barcelo (1998). The middle of graft union was sectioned with a scalpel. Fresh cut sections were obtained using a hand-microtome. The sections were soaked in 1.0% (w/v) phloroglucinol in 25:75 (v/v) HCl-ethanol for 10-15 minutes. The stained sections were observed and photographed under a stereomicroscope (MZ8, Leica 10X). The sections were also observed for their anatomical details like formation of necrotic layer between rootstock and scion.

Sampling interval – 4, 8 & 15 days after grafting

No. of plants analysed per treatment – 5

3.4. Main items of observation

The following observations were recorded for studies on standardization of grafting methods, enzyme assay and histological studies

3.4.1. Per cent survival of grafts

Number of plants survived at 15 days after grafting in each treatment per replicationwas counted and expressed in terms of per cent survival.

3.4.2. No.of days taken for graft union

The number of days taken for successful graft union was counted and expressed as number of days taken for graft union.

3.4.3. Enzyme activities

The assay of enzymes such as peroxidase and catalase were carried at 4^{th}, 8^{th} and 15^{th} days after grafting and expressed in the terms of enzyme units/g of tissue (EU/g).

3.4.4. Histological studies

Anatomical details of formation of necrotic layer and intensity of histochemical stain for lignin was observed at 4^{th}, 8^{th} and 15^{th} days after grafting.

3.5. Statistical analysis

Data collected with respect to different observations were subjected to an analysis of variance using OP-STAT (HAU, Hissar).

Results

4. RESULTS

The present study was conducted at Department of Floriculture and Landscaping, College of Horticulture, during the year 2017-2018. The work was carried out under three experiments namely 1.Standardisation of grafting techniques, 2. Assay of enzyme activity 3.Histological studies. Results of the studies were statistically analysed and presented in this chapter.

4.1. Standardisation of grafting techniques, age of rootstock and scion

The experiment was conducted from June 2017 to May 2018. Three grafting methods such as cleft, splice and hole insertion were done and observed for grafting success.

4.1.1. Percent survival of grafts

Significant differences were observed with respect to percent survival of grafts (Table 4.1) in different methods of grafting at different stages of rootstock and scion. Cleft grafting four week old scion onto six week old rootstock showed maximum survival percent (61%) which was on par with cleft grafting five week old scion onto six week old rootstock (56%) and cleft grafting three week old scion onto seven week old rootstock (45%). Lowest survival of grafts(13%) in cleft grafting method was observed when five week old scion was grafted on to five week old rootstock. There was no survival percent when grafting was done on four week old rootstock irrespective of the grafting methods. In splice grafting method maximum survival of grafts (37%) was also observed when four week old scion was grafted onto six week old root stock. From the data given in the table, it could also be inferred that irrespective of the age of root stock and scion, hole insertion method was not successful.

Table 4.1. Percent survival of African marigold grafts

Grafting methods	Age of root stock (weeks)	Age of scion (weeks)	Percent survival (%)
Cleft	4	3	0 (1)
”	4	4	0 (1)
”	5	3	31 (5.59)
”	5	4	19 (4.43)
”	5	5	13 (3.73)
”	6	3	33 (5.67)
”	6	4	61 (7.86)
”	6	5	56 (7.54)
”	7	3	45 (6.78)
Splice	4	3	0 (1)
”	4	4	0 (1)
”	5	3	29 (5.43)
”	5	4	14 (3.76)
”	5	5	13 (3.73)
”	6	3	24 (4.84)
”	6	4	37 (6..09)
”	6	5	25 (4.92)
”	7	3	34 (5.90)
Hole insertion	4	3	0 (1)
”	4	4	0 (1)
”	5	3	0 (1)
”	5	4	0 (1)
”	5	5	0 (1)
”	6	3	0 (1)
”	6	4	0 (1)
”	6	5	0 (1)
”	7	3	0 (1)
”	4	3	0 (1)
”	4	4	0 (1)
CD (5%)			16.67 (1.57)
SE(m±)			5.71 (0.539)

Values in parentheses are transformed value

4.1.2. Number of days taken for graft union

Irrespective of the grafting methods and the age of rootstock and scion, visual graft union could be observed on 4th day after grafting in all the treatments where there was survival of grafts after 15 days. In all other treatments which failed to survive, there was nograft union observed.

4.2. Assay of enzyme activity

Estimation of catalase and peroxidase was carried out to study the activity of the enzymes in graft union formation

4.2.1 Catalase assay

Significant differences were noticed among the treatments for catalase enzyme activity at different intervals of assay of the enzyme after grafting (Table 4.2). At 4DAG, the highest activity of catalase (0.85EU/g) was recorded in cleft grafting four week old scion onto six week old rootstock. This was followed by cleft grafting three week old scion onto seven weeks old root stock with a catalase activity of 0.56 EU/g and splice grafting four week old scion onto six week old rootstock with catalase activity of 0.46 EU/g. For all other treatments except the control, in which there was no survival of grafts, no catalase activity (0 EU/g) was observed.

At 8DAG, maximum activity of catalase (1.39 EU/g) was observed in cleft grafting with six week old rootstock and four week old scion and also in splice grafting with seven week old rootstock and three week old scion (1.38 EU/g).

At 15DAG maximum catalase activity was observed in cleft grafting four week old scion on to six week old rootstock (1.60 EU/g) followed by splice grafting seven week old scion on to three week old rootstock (1.54 EU/g).

From the data shown in the Table 4.2, it was also observed that, there was a general increase in the enzyme activity from 4DAG to 15DAG in both the cleft and splice grafting methods. In cleft grafting methods four week old scion grafted on to six week old rootstock and splice grafting three week old scion to seven

week old rootstock recorded highest catalase activity at 4, 8 and 15DAG.From the observations recorded it can also be inferred that in hole insertion grafting irrespective of age of rootstock and scion there is no activity of catalase due to its failure to form graft union.

For control *i.e.* seedlings in which the activity of catalase was the minimum at all the sampling intervals and the activity was almost the same at different intervals

4.2.2. Peroxidase activity

Significant differences were noticed among the treatments for peroxidase enzyme activity at different intervals of assay of the enzyme after grafting (Table 4.3). At 4 DAG the highest peroxidase activity (1.26 EU/g) was observed in splice grafting with seven week old rootstock and three week old scion. It was followed by cleft grafting with seven week old rootstock and three week old scion (0.82 EU/g) as well as cleft grafting with six week old rootstock and four week old scion with a peroxidase activity of 0.75 EU/g.

At 8DAG also the maximum activity of peroxidise (1.44 EU/g) was observed in splice grafting six week old rootstock and five week old scion and splice grafting using seven week old rootstock and three week old scion with a peroxidise activity of 1.37 EU/g and these were on par also.

However, at 15 DAG the highest activity of peroxidase (1.55 EU/g) was observed in cleft grafting six week old root stock with four week old scion followed by splice grafting six week old rootstock with four week old scion (1.54 EU/g) and these were on par also.

In all the treatments where there was no graft survival the peroxidase activity could not be traced. As in the case of catalase, the activity of peroxidsae was also minimum in control at all sampling intervals.

Table 4.2. Catalase activity (EU/g) in African marigold grafts

Grafting methods	Age of root stock (weeks)	Age of scion (weeks)	4 DAG	8 DAG	15 DAG
Cleft grafting	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0.32 (1.15)	0.88 (1.36)	1.36 (1.53)
"	5	4	0.40 (1.18)	0.89 (1.37)	1.24 (1.49)
"	5	5	0.26 (1.23)	0.94 (1.39)	0.99 (1.41)
"	6	3	0.40 (1.18)	0.52 (1.23)	0.58 (1.25)
"	6	4	0.85(1.35)	1.39 (1.54)	1.60 (1.61)
"	6	5	0.41 (1.19)	0.89 (1.37)	1.44 (1.56)
"	7	3	0.56 (1.25)	0.99 (1.41)	1.11 (1.45)
Splice grafting	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0.29 (1.13)	0.45 (1.20)	0.52 (1.23)
"	5	4	0.36 (1.16)	0.49 (1.22)	0.64 (1.28)
"	5	5	0.38 (1.17)	0.75 (1.32)	0.69 (1.30)
"	6	3	0.38 (1.17)	0.51(1.23)	0.53 (1.24)
"	6	4	0.46(1.20)	0.89 (1.37)	1.10 (1.45)
"	6	5	0.34 (1.15)	0.79 (1.34)	1.27 (1.50)
"	7	3	0.41 (1.18)	1.38 (1.54)	1.54 (1.60)
Hole insertion	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0(1)	0(1)	0(1)
"	5	4	0(1)	0(1)	0(1)
"	5	5	0(1)	0(1)	0(1)
"	6	3	0(1)	0(1)	0(1)
"	6	4	0(1)	0(1)	0(1)
"	6	5	0(1)	0(1)	0(1)
"	7	3	0(1)	0(1)	0(1)
Control			0.19 (1.09)	0.18 (1.08)	0.20(1.10)
CD (5%)			0.04 (0.01)	0.21 (0.07)	0.11(0.38)
SE(m±)			0.01(0.006)	0.07(0.026)	0.057(0.01)

Values in parentheses are transformed value

Table 4.3. Peroxidase activity (EU/g) in African marigold grafts

Grafting methods	Age of root stock (weeks)	Age of scion (weeks)	4 DAG	8 DAG	15 DAG
Cleft grafting	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0.49 (1.22)	0.72 (1.31)	0.98 (1.41)
"	5	4	0.51 (1.23)	0.75 (1.32)	0.81 (1.34)
"	5	5	0.51 (1.23)	0.82 (1.35)	0.89 (1.37)
"	6	3	0.48 (1.21)	0.52 (1.23)	0.63 (1.28)
"	6	4	0.75 (1.32)	1.10 (1.44)	1.55 (1.59)
"	6	5	0.33 (1.15)	1.01 (1.41)	1.45 (1.56)
"	7	3	0.82 (1.34)	1.09 (1.44)	1.31 (1.52)
Splice grafting	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0.40 (1.18)	0.65 (1.28)	.82 (1.35)
"	5	4	0.35 (1.16)	0.55 (1.24)	0.76 (1.32)
"	5	5	0.49 (1.22)	0.54 (1.24)	0.70 (1.30)
"	6	3	0.57 (1.25)	0.54 (1.24)	0.60 (1.26)
"	6	4	0.52 (1.23)	1.22 (1.49)	1.54 (1.59)
"	6	5	0.26 (1.12)	1.44 (1.56)	1.26 (1.50)
"	7	3	1.26 (1.50)	1.37 (1.54)	1.43 (1.56)
Hole insertion	4	3	0(1)	0(1)	0(1)
"	4	4	0(1)	0(1)	0(1)
"	5	3	0(1)	0(1)	0(1)
"	5	4	0(1)	0(1)	0(1)
"	5	5	0(1)	0(1)	0(1)
"	6	3	0(1)	0(1)	0(1)
"	6	4	0(1)	0(1)	0(1)
"	6	5	0(1)	0(1)	0(1)
"	7	3	0(1)	0(1)	0(1)
Control			0.18 (1.08)	0.2 (1.09)	0.19(1.09)
CD (5%)			0.07 (0.02)	0.15 (0.05)	0.05(0.01)
SE(m±)			0.025(0.01)	0.053(0.019)	0.026(0.006)

Values in parentheses are transformed value

4.2.3. Correlation between enzyme activity and graft survival

In order to understand the influence of enzyme (catalase and peroxidase) activity on graft survival, correlation analysis was done between the activity of these enzymes at 4, 8 and 15 DAG with per cent survival of grafts at 15 DAG and the data revealing the correlation is given in table 4.4.

4.2.3.1. Correlation between catalase activity and graft survival

From the data given in the Table 4.4 it was observed that there was a significant positive correlation between catalase activity and percent survival of grafts in marigold.

4.2.3.2. Correlation between peroxidase activity and graft survival

Significant and positive correlation was also observed between peroxidise activity and graft survival (Table 4.4).

Table 4.4 Correlation of catalase and peroxidase activity with survival of African marigold grafts

Enzyme	4 DAG	8 DAG	15 DAG
Catalase	0.926^{**}	0.872^{**}	0.874^{**}
Peroxidase	0.784^{**}	0.857^{**}	0.926^{**}

4.3. Histological studies of grafts

The study consisted of both anatomical as well as histo chemical staining for lignin in the graft union. Histo chemical staining for lignin is based on the reaction of phloroglucinol with aromatic aldehyde fraction present in lignin, which yields a pink stain. The results ofthe study are given in plates 8, 9, 10, 11 & 12. It is clear from the plates that at fourth day of grafting, a slight pink colour was observed in graft union in both cleft and splice method and the intensity of the stain was more at 8 and 15 DAG. On eighth day the graft union showed a notable intensity in the pink stain compared to that on 4^{th} day and the maximum intensity of the stain could be noticed on fifteenth day of grafting.Hence it could be inferred that in the all the grafts survived in cleft and splice method, there was an intensification of the pink colour during graft union development. The result of staining revealed that, there was biosynthesis of lignin in a slow rate on 4^{th} day of grafting followed by active lignin synthesis on 8^{th} and 15^{th} day of grafting. In hole insertion method there was no staining at the mechanical contact portion of rootstock and scion and only a greenish yellow colour was observed at this region (Plate 13).

From the plates above mentioned the anatomical details were also observed. It was also observed that there was development of a necrotic layer between the stock and scion at the graft union in all rootstock and scion combinations that showed success in both cleft and splice method. This necrotic layer was formed in response to wound repair and proliferation of cells of rootstock and scion, which is the prior stage to callus formation. The necrotic layer disintegrates as the corticular parenchyma of the stock and scion joins. When the anatomical sections of hole insertion grafted plant was taken at 4 DAG, it was observed that there was no development of cell to cell contact between stock and scion and a wide gap could be observed and this might be the reason for graft failure in this method.

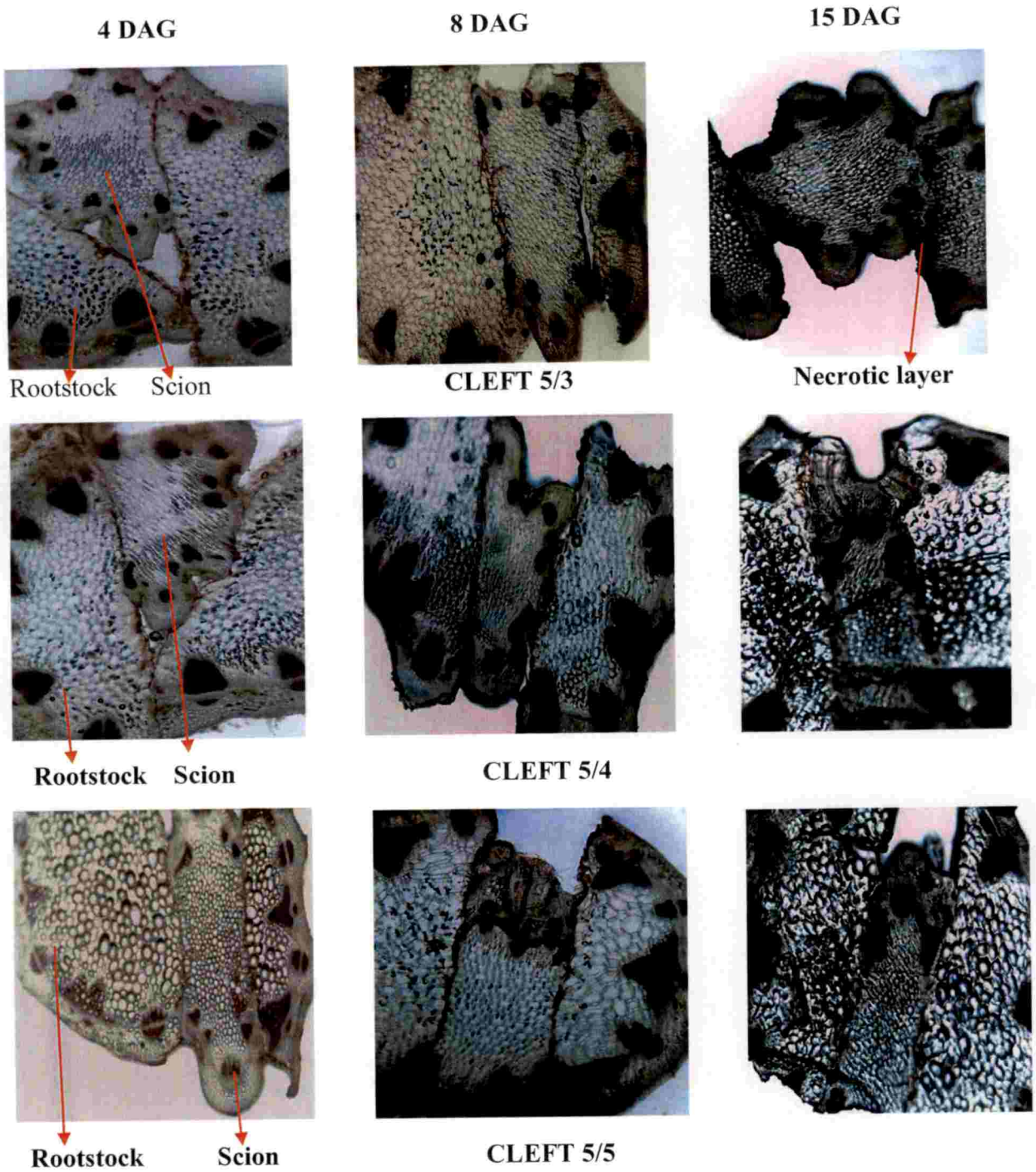

Plate 8. Anatomical sections of graft union in African marigold

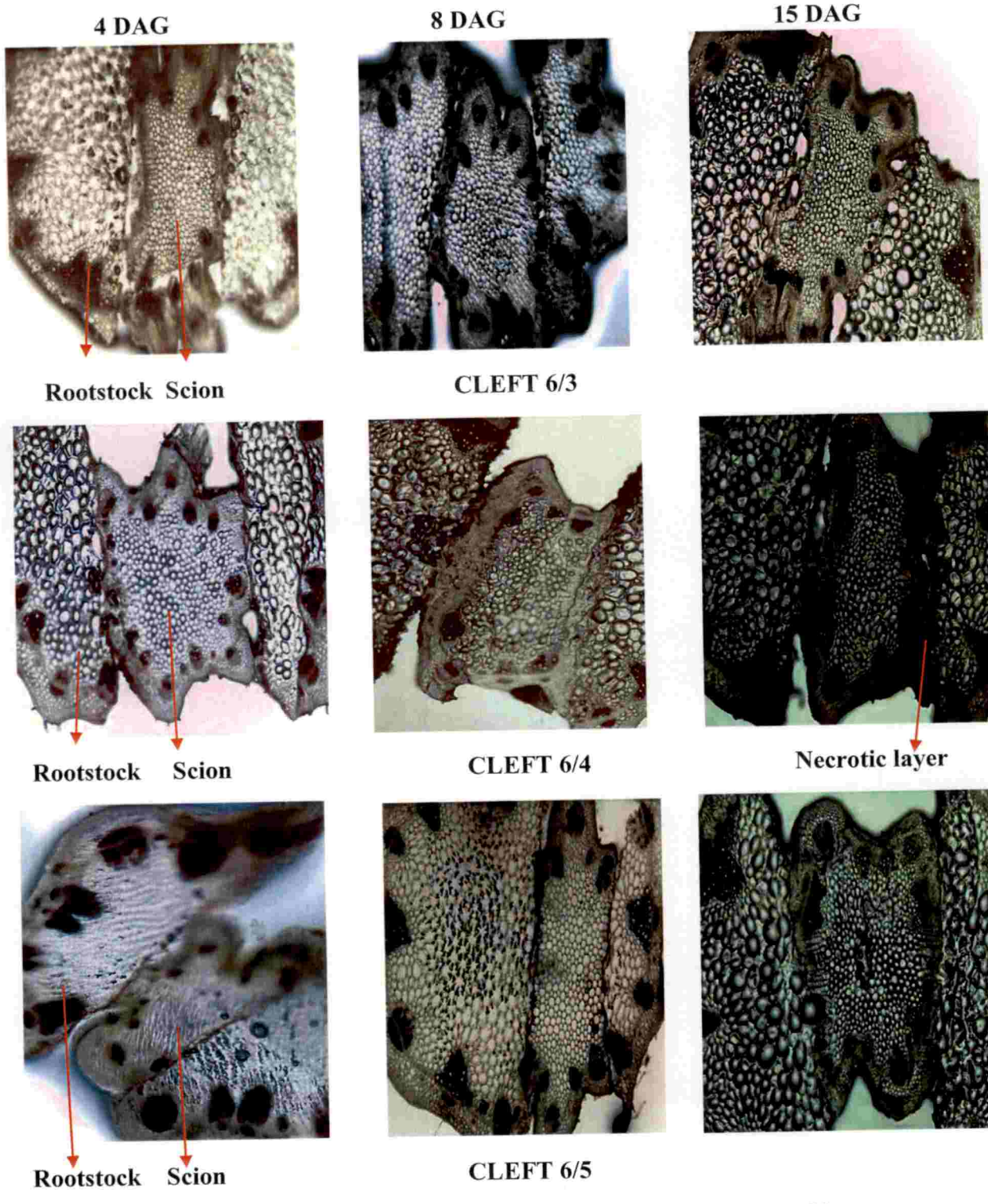

Plate 9. Anatomical sections of graft union in African marigold

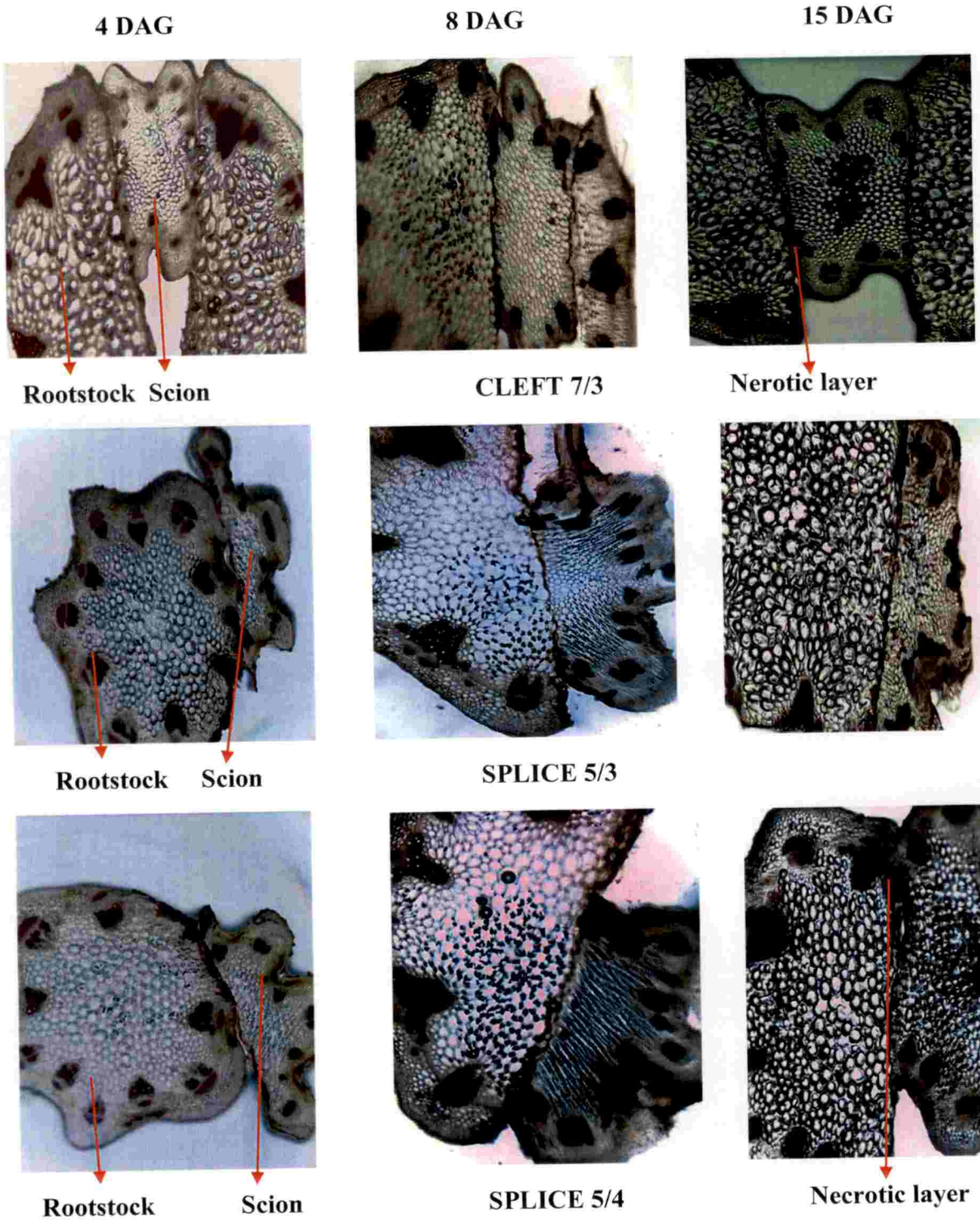

Plate 10. Anatomical sections of graft union in African marigold

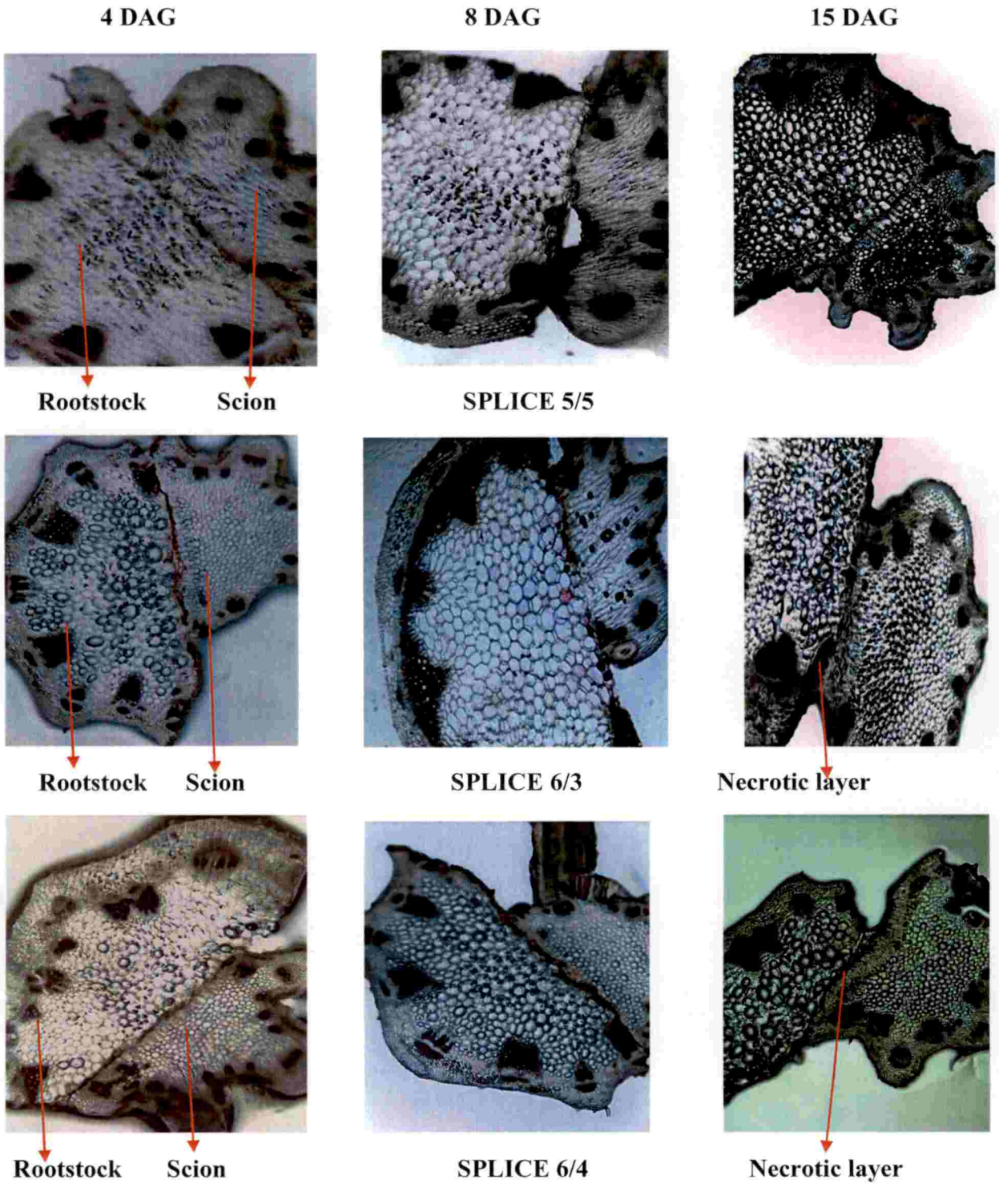

Plate 11. Anatomical sections of graft union in African marigold

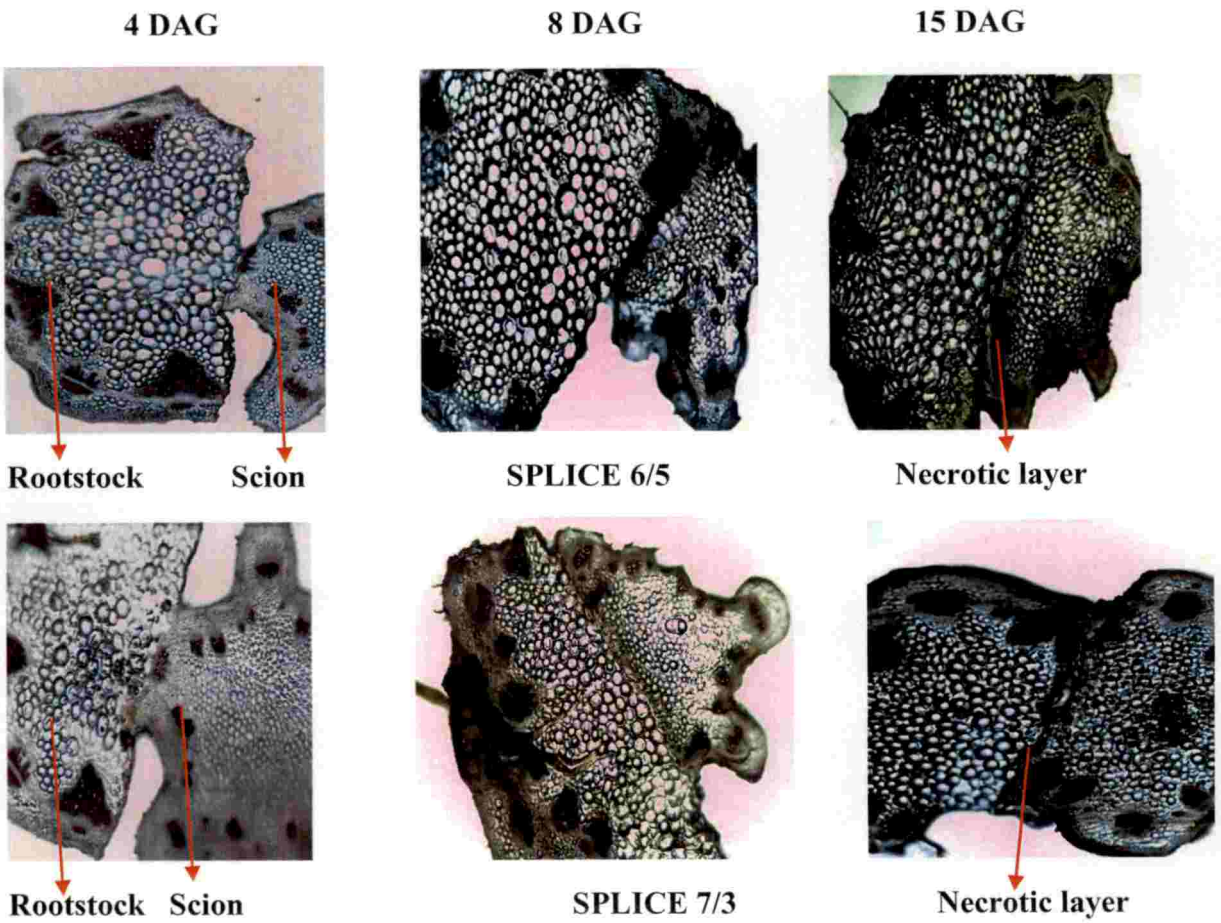

Plate 12. Anatomical sections of graft union in African marigold

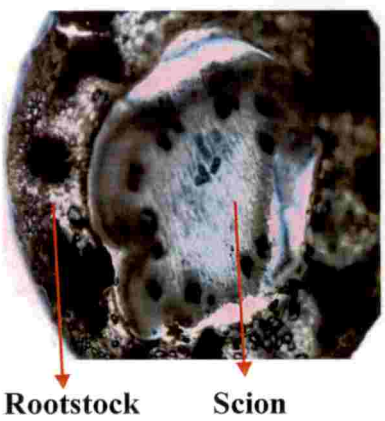

Plate 13. Anatomical section of Hole insertion grafting 4 DAG

Discussion

5. Discussion

African marigold (*Tagetes erecta* L.) is one of the important loose flower crop belonging to Asteraceae family. Due to its wide adaptability and easy cultivation practices, it has gained popularity among the flower growers. Recently in Kerala, cultivationof this flower has gained momentum due to increasing demand for marigold flowers. One of the major problem faced in marigold cultivation in Kerala is the incidence of bacterial wilt caused by *Ralstonia solanacearum* and the incidence of the disease is severe in most of the F1 hybrids/varieties tested in Kerala.One of the management strategies to control bacterial wilt in susceptible genotypes is by grafting these types onto wilt resistant genotypes. Field trial conducted at Agricultural Research Station Mannuthy showed that there was 100 percent control of bacterial wilt in grafted solanaceous vegetables (Narayanankutty *et al.*, 2015). Umesh (2017) has identified the local collection 'M-1' as bacterial wilt resistant genotype in marigold and he also reported that grafting susceptible genotypes on this resistant rootstock was found to be an effective tool for controlling bacterial wilt in African marigold. However, the study had reported the need for standardization of grafting methods as well as age of root stock and scion in African marigold for best results.

Hence the present investigation was carried out with the objective "To standardize the age of root stock, scion and grafting methods in African marigold for combating bacterial wilt".

5.1. Standardisation of grafting techniques, age of rootstock and age of scion.

Standardisation of grafting techniques in African marigold (*Tagetes erecta* L.) was carried out using three methods of grafting at different ages of rootstock and scion.

The present study revealed that,cleft grafting four week old scion onto six week old rootstock was found to be best which recorded a maximum survival of 61 percent at 15 DAG (Fig 5.1). Cleft grafting as the best method has been reported in tomato by Vuruskan and Yanmaz (1991) and Silva *et al.* (2016). In brinjal also cleft grafting has been reported as the best method by Honma (1977) and Trentini and Maioli (1989). Suitability of cleft grafting in solanaceous crops has been reported by Goto *et al.* (2003). Khankahdani *et al.* (2012) reported that difference in graft success depends on the method of grafting followed, irrespective of rootstock and scion in watermelon. Lee (1994) reported that grafting methods vary considerably with type of crop being grafted and sowing time of rootstock and scion.

Age of rootstock was found to have pronounced effect on successful graft union. Among the different agesof rootstock used in this study, six week old rootstock was found to be more suitable and successful in terms of survival of graft in both cleft and splice grafting method. Traka-Mavrona *et al.* (2000) also reported that even by adopting the same grafting method, survival ratio may be different in relation to both rootstock and scion.

Under various treatments of different scion ages studied to determine the suitable age of scion, it was observed that four weeks old scion was the best for survival in both cleftand splicemethod of grafting. This result is in agreement with the findings of Oda *et al.*, (2000). As the case of suitability of different grafting methods in different crops, the optimum age of rootstock and scion would also vary with the crops. This is justified by the findings of Tamilselvi and Pugalendhi (2016) in bitter gourd where they have observed best results with rootstock and scion having the same diameter.

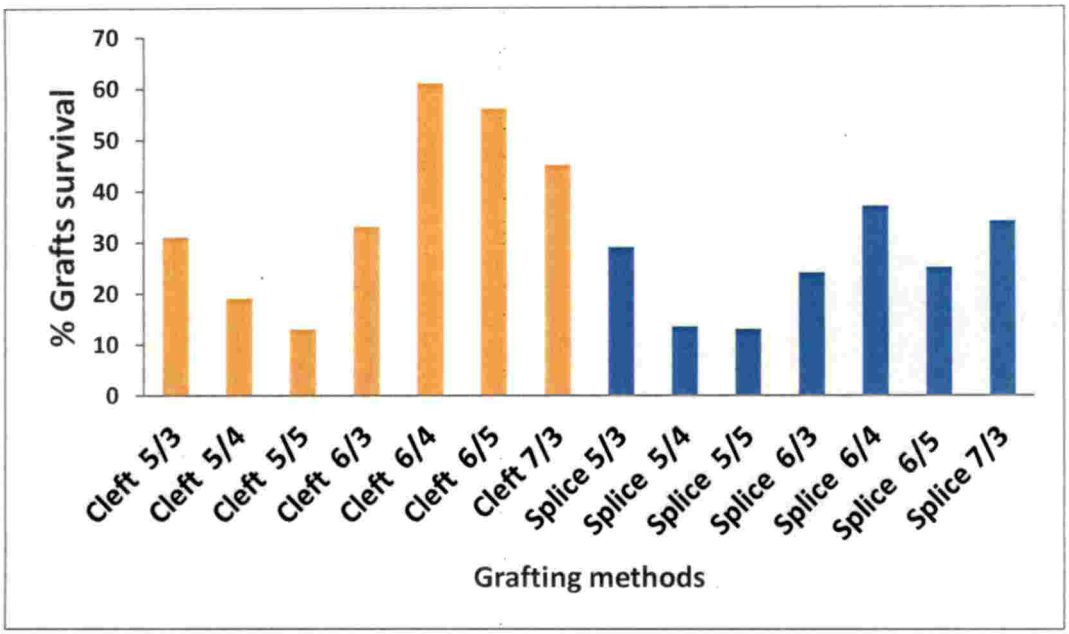

Fig 5.1. Percent survival of African marigold grafts

- Cleft 5/3 – 5 week old rootstock and 3 week old scion
- Cleft 5/4 – 5 week old rootstock and 4 week old scion
- Cleft 6/3 – 6 week old rootstock and 3 week old scion
- Cleft 6/4 – 6 week old rootstock and 4 week old scion
- Cleft 6/5 – 6 week old rootstock and 5 week old scion
- Cleft 7/3 – 7 week old rootstock and 3 week old scion
- Splice 5/3 – 5 week old rootstock and 3 week old scion
- Splice 5/4 – 5 week old rootstock and 4 week old scion
- Splice 6/3 – 6 week old rootstock and 3 week old scion
- Splice 6/4 – 6 week old rootstock and 4 week old scion
- Splice 6/5 – 6 week old rootstock and 5 week old scion
- Splice 7/3 – 7 week old rootstock and 3 week old scion

5.2. Number of days taken for graft union

No significant difference was observed for the number of days taken for graft union.Wherever grafting was successful, irrespective of the methods and, the age of rootstock and scion, visual union of rootstock and scion was observed on fourth day after grafting. In all other treatments where grafting was not successful,no visual graft union was observed. Similar results was reported by Fernandaz-Garcia *et al.* (2004). They observed that xylem differentiation begins as small areas of lignification fourth day after grafting in tomato.

5.3. Effect of enzyme activity on graft union formation

Enzyme assay of catalase and peroxidase were carried out at specified time interval, in order to study its influence and role in grafting success.

5.3.1. Catalase activity at 4^{th}, 8^{th} and 15^{th} DAG

Significant differences were noticed among the treatments for catalase activity 4DAG. Cleft grafted plants of six week old rootstock with four week old scion showed maximum catalase enzyme activity at 4^{th}(0.85 EU/g), 8^{th}(1.39 EU/g) and 15 DAG (1.60 EU/g). From this study it was observed that the activity of catalase was increased at 15^{th} DAG, being about eight times higher than that of control plants (0.21 EU/g) (Fig 5. 2). Catalase activity was also found to be positively correlated with the graft success. Similar results were also observed by Fernandaz-Garcia *et al.*, (2004). They observed that in grafted tomato plantsthe catalase enzyme activity increased significantly at 8^{th} DAG, being about three times greater than that of non grafted plants.The result was also found to be in accordance with the reports of He *et al.* (2009). They reported that there was a higher catalase activity in grafted tomato plants which reduced the oxidative damage in plant tissue caused by H_2O_2, when compared to that of non-grafted and self-grafted plants.

5.3.2. Peroxidase activity

Activity of peroxidase enzyme showed significant differences at 4th, 8th and 15th DAG. At 4th DAG treatment combination splice grafted plants of seven week old rootstock with three week old scion showed maximum enzyme activity(1.26 EU/g)followed by cleft grafted plant of seven week old rootstock with three week old scion(0.82 EU/g) and. The maximum enzyme activity was observed in treatment combination of splice grafted plant of six week old rootstock with five week old scion (1.44 EU/g) at 8th DAG, whereas at 15th DAG treatment combination cleft grafted plant of six week old rootstock with four week old scion (1.55 EU/g) showed maximum peroxidase activity (Fig 5.3).Correlation studies of peroxidise activity with graft survival revealed that there was a significant and positive correlation and this suggests an advancement of lignification in the graft union. Role of peroxidase enzyme in lignification process inthe graft union of tomato plants has been reported by Fernandaz-Garcia *et al.*, (2004). They reported that four days after grafting peroxidase activity was found to be low in graft union whereas, at 8th and 15th DAG peroxidase activity increases, this was correlated with high degree of lignification in the graft union. Pellinen *et al.* (2002) also reported that lignification process required H_2O_2 and cell wall peroxidases to bring about polymerization of lignin.

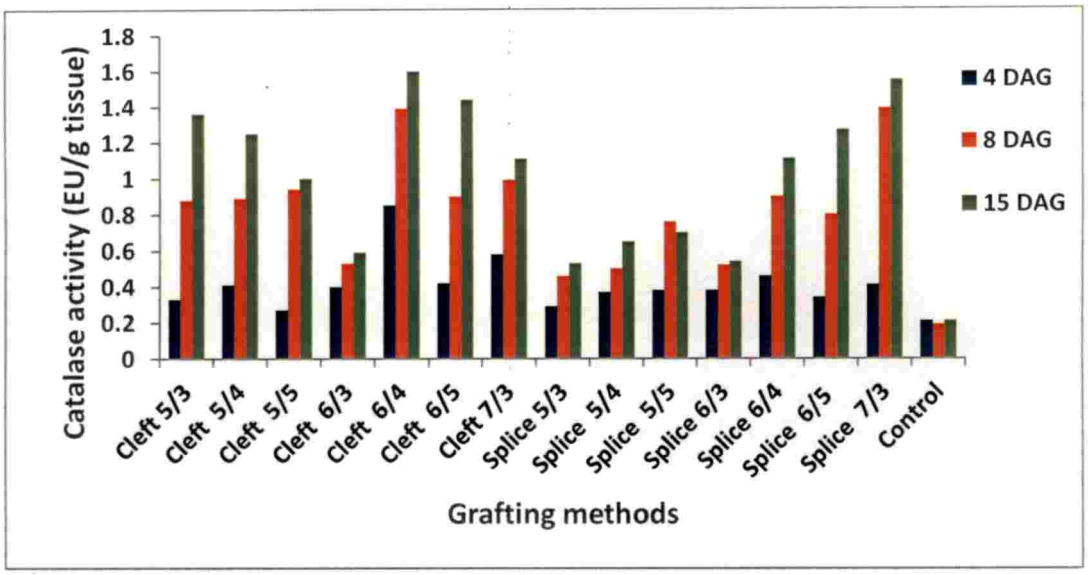

Fig 5.2. Catalase activity

- Cleft 5/3 – 5 week old rootstock and 3 week old scion
- Cleft 5/4 – 5 week old rootstock and 4 week old scion
- Cleft 6/3 – 6 week old rootstock and 3 week old scion
- Cleft 6/4 – 6 week old rootstock and 4 week old scion
- Cleft 6/5 – 6 week old rootstock and 5 week old scion
- Cleft 7/3 – 7 week old rootstock and 3 week old scion
- Splice 5/3 – 5 week old rootstock and 3 week old scion
- Splice 5/4 – 5 week old rootstock and 4 week old scion
- Splice 6/3 – 6 week old rootstock and 3 week old scion
- Splice 6/4 – 6 week old rootstock and 4 week old scion
- Splice 6/5 – 6 week old rootstock and 5 week old scion
- Splice 7/3 – 7 week old rootstock and 3 week old scion
- Control – Non grafted plants

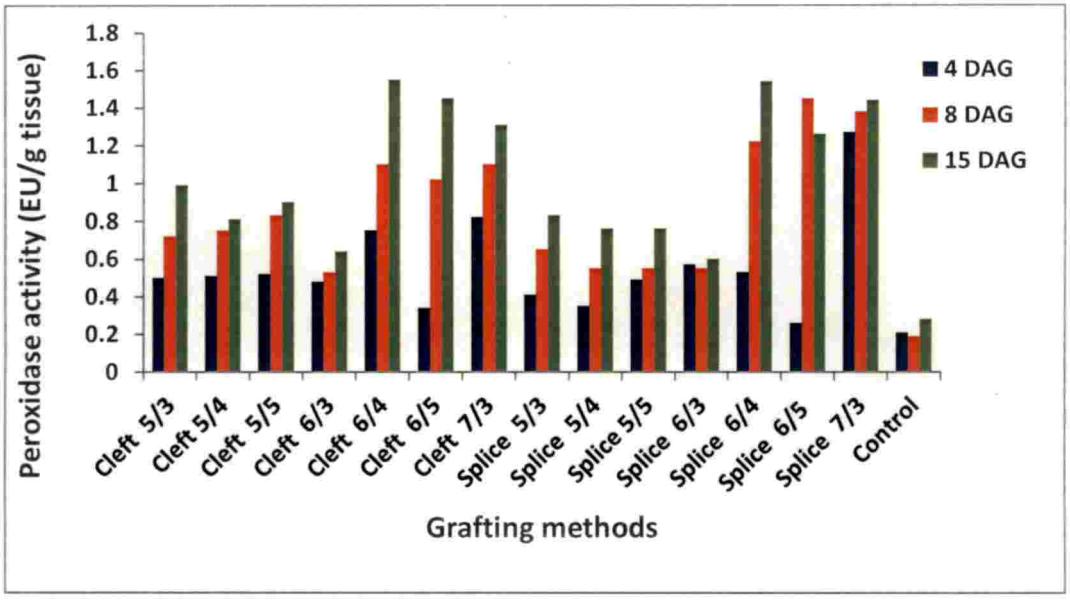

Fig 5.3. Peroxidase activity

- Cleft 5/3 – 5 week old rootstock and 3 week old scion
- Cleft 5/4 – 5 week old rootstock and 4 week old scion
- Cleft 6/3 – 6 week old rootstock and 3 week old scion
- Cleft 6/4 – 6 week old rootstock and 4 week old scion
- Cleft 6/5 – 6 week old rootstock and 5 week old scion
- Cleft 7/3 – 7 week old rootstock and 3 week old scion
- Splice 5/3 – 5 week old rootstock and 3 week old scion
- Splice 5/4 – 5 week old rootstock and 4 week old scion
- Splice 6/3 – 6 week old rootstock and 3 week old scion
- Splice 6/4 – 6 week old rootstock and 4 week old scion
- Splice 6/5 – 6 week old rootstock and 5 week old scion
- Splice 7/3 – 7 week old rootstock and 3 week old scion
- Control – Non grafted plants

5.3. Histological studies

In order to study the anatomical changes in grafted marigold plants, transverse section of graft union was made and micro graphed at 4th, 8th and 15th DAG.Lignin synthesis in grafted plants were analysed using Weisner staining. This can be used for specific detection of aldehyde end units in lignin, which was formed during the early stages of lignification in the graft union. The results showed that in all the survived treatment combinations of cleft and splice method, there was an intensification of pink colour during graft union development.A slight pink colour was observed in graft union during the 4th day of grafting. On 8th day the graft union showed a notable increase in staining and graft union attained an intense pink colour 15th day after grafting. Histological studies also revealed that the formation of necrotic layer in the graft union, which occurred in response to wound repair and proliferation of cells of rootstock and scion. This necrotic layer formation is a prior stage to callus formation. -The necrotic layer disintegrates as the corticular parenchyma of the stock and scion joins. The results of staining were found to be similar to the staining results of Fernandaz-Garcia *et al.* (2004). They reported that at fourth day after grafting small spots of pink stain was observed in the graft union of tomato plants. There was a significant increase in staining on eighth day after grafting and at fifteenth day of grafting the graft union exhibited an intense pink colour. Necrotic layer formation in graft union has been reported by Akhila and George (2017). They reported that the histological observation of bitter gourd at graft union interface revealed the formation of necrotic layer in response to wound repair and proliferation of cells of rootstock and scion in grafted bitter gourd. Tamilselvi and Pugalendhi (2017) also observed dark strands of proliferation or necrotic layer in outer sides and interfaces of stock and scion in case of bittergourd grafted on to pumpkin rootstock.

6. SUMMARY

The experiment was conducted at the Department of Floriculture and Landscaping, College of Horticulture, Vellanikkara, during the year 2017-18. The objective of the study was to "Standardize the grafting techniques, age of root stock and age of scion in African marigold (*Tagetes erecta* L.) to combat bacterial wilt". The findings of the study are summarized in this chapter.

- Among the three grafting methods studied, percent survival of grafts was found to be high in cleft grafting method.
- Cleft grafting four week old scion on to six week old rootstock showed maximum survival percentage (61%).
- There was no survival when grafting was done on four week old rootstock irrespective of the grafting methods.
- Irrespective of the age of root stock and scion, hole insertion method was not successful.
- In all the survived method of grafting, visual union of rootstock and scion was observed on fourth day after grafting.
- Highest activity of catalase was recorded in cleft grafting four week old scion onto six week old rootstock at 4, 8 & 15 days after grafting.
- At 15 days after grafting the highest activity of peroxidise was observed in cleft grafting six week old root stock with four week old scion.
- Catalase and peroxidase activities are positively correlated with graft survival grafts survival.

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STANDARDIZATION OF GRAFTING TECHNIQUES IN AFRICAN MARIGOLD (*Tagetes erecta* L.) FOR COMBATING BACTERIAL WILT

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(2016-12-010)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

(FLORICULTURE AND LANDSCAPING)

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2018

ABSTRACT

The present study entitled "Standardization of grafting techniques in African marigold (*Tagetes erecta* L.) for combating bacterial wilt" was taken up in the Department of Floriculture and Landscaping, College of Horticulture, during the year 2017-2018. The work was carried out under three experiments viz.,standardisation of grafting techniques, assay of enzyme activity and histological studies. The rootstock used in the study was the bacterial wilt resistant local collection ' M-1' and the wilt susceptible F_1 hybrid ' Maria 91' was used as scion. Grafting was done in the month of January.

Three methods of grafting viz., cleft, splice and hole insertion were tried at different ages of rootstock (4, 5, 6 and 7 weeks after sowing) and different ages of scion (3, 4 and 5 weeks after sowing). Observation of graft survival was taken at 15 DAG (Days after grafting). It was observed that cleft grafting four week old scion on to six week old rootstock was found to be the best with a graft survival of 61 percent. It was also observed that there was no survival when grafting was done on four week old rootstock irrespective of age of scion and grafting method followed.

Estimation of catalase and peroxidase was carried out to study the activity of these enzymes in graft union formation. Sampling was done at 4,8 and 15DAG. It was observed that, activities of both the enzymes increased from 4DAG to 15DAG. Highest catalase activity at 4^{th} (0.85 EU/g), 8^{th} (1.39 EU/g) and 15^{th} (1.60 EU/g) DAG was observed in cleft grafting of four week old scion on to six week old rootstock. Similar results were also observed in peroxidase activity. Correlation of catalase and peroxidase activity with survival of grafts indicated that both the enzymes are positively correlated with graft survival.

Histological studies of the grafts consisted of both anatomical studies and histochemical staining for lignin. It was observed that there was an intensification of the stain from 4DAG to 15DAG with advancement of lignification process in graft union. Histological studies also revealed the formation of necrotic layer in the graft union, as a result of wound repair and cell proliferation.

Cleft grafting of four week old scion on six week old wilt resistant rootstock is the best method in African marigold for combating bacterial wilt.