EFFECTS OF CUTTING POSITION OF ROOTSTOCK AND EXOGENOUSLY APPLIED AUXINS ON ROOTING ABILITY, GROWTH AND YIELD OF ROSE (*Rosa hybrida*).

BY

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DECLARATION

Declaration by the student

I declare that this thesis has not been previously presented for a degree in Maseno University, or in any other University. The work reported herein has been carried out by me and all sources of information have been acknowledged by means of references.

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DEDICATION

I dedicate this work to my Parents; Meshack Otiende and Mary Juma Otiende, brothers and sisters.

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ABSTRACT

Rose, is the leading cut flower crop in Kenya and enjoys high demand in both national and international markets. Low rooting and survival of the cuttings increases cost of production and may limit the use of some rose rootstocks, quality or yield virtues notwithstanding. This study was conducted to evaluate the effects of cutting position of rootstocks and exogenously applied auxins on rooting ability, growth and yield of *Rosa hybrida*. The studies included 1) self-rooting; the treatments involved three cutting positions (top, middle and bottom) on vertical shoots of two rootstock cultivars ('Rosa progress' and 'Natal briar') and treatment of the basal ends of the cuttings with i) IBA at concentrations of 0%, 0.2%, 0.4% and 0.6% and ii) NAA at concentrations of 0%, 0.2% and 1%, before planting; 2) Top grafting of scions of the variety 'Inca' onto the three cutting positions of the two rootstocks above and treating with 0.2%NAA and 0.4% IBA. The rooted grafts in the second experiment were later transplanted in the field to evaluate the flower yield. Both experiments were factorial laid out in a randomized complete block design. The rootstock cuttings were analysed for IAA (Indole-3-acetic acid) and cytokinins in the stem base and bud region. Carbohydrates and mineral nutrients were analysed in the stem base of cuttings. Cutting position significantly influenced the shoot and root growth parameters measured in both experiments. In the self-rooting experiment, the root and shoot growth was promoted acropetally and corresponded to higher endogenous carbohydrate, IAA concentrations, zinc and nitrogen levels and IAA: cytokinin in the stem base of cuttings and also high IAA and IAA: cytokinin in the bud region of top position cuttings than bottom position cuttings. The growth of grafts on the rootstock 'Rosa progress' was promoted basipetally and those grafted on 'Natal briar' acropetally and was attributed to high sucrose content in the stem bases of bottom and top position cuttings respectively. The grafts on 'Natal briar' produced higher rooting percentage and flowering stem weight but lower total number of harvestable stems and stem length than grafts on 'Rosa progress'. Exogenously applied auxins promoted rooting and concentrations of 0.4% IBA and 0.2% NAA recorded higher rooting responses than the other concentrations in the self-rooting experiment. In the grafting experiment, 0.4% IBA exhibited higher root number than 0.2%NAA. The top position cuttings should be used to multiply the two rootstock cultivars, but in grafting, the variety 'Inca' should be grafted on the bottom position cuttings of 'Rosa progress' and on top position cuttings of 'Natal briar' to achieve higher grafting take. The cuttings should be treated with auxins for enhanced rooting.

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LIST OF ACRONYMS/ABBREVIATIONS

%	Percentage
2, 4, 5-T	2, 4, 5-trichlorophenoxyacetic acid
2, 4-D	2, 4-dichlorophenoxyacetic acid
ABA	Abscisic acid
ABP1	Auxin-Binding Protein 1
ANOVA	Analysis of Variance
cis-ZR	cis-Zeatin Riboside
DAAD	German Academic Exchange Service
DHZ	Dihydrozeatin
DHZR	Dihydrozeatin Riboside
E.C	Electrical conductivity
GC-MS/MS	Gas chromatography-Mass spectrometry/Mass spectrometry
GDP	Gross Domestic Product
HCDA	Horticultural Crop Development Authority
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
IAA: tr-Z	ratio of concentration of Indole-3-acetic acid to trans-Zeatin

IAA: tr-ZR	ratio of concentration of Indole-3-acetic acid to trans-Zeatin Riboside
IBA	Indole-3-Butyric Acid
ILRI	International Livestock Research Institute
IMF	International Monetary Fund
IPR	Isopentenylaldenide Riboside
KFC	Kenya Flower Council
KNBS	Kenya National Bureau of Statistics
LSD	Least Significant Difference
NAA	Naphthalene Acetic Acid
NACOSTI	National Commission for Science and Technology
NPA	l-Naphthylphthalamic acid
NS	Not Significant
^{o}C	Degrees Celcius
PAT	Polar Auxin Transport
RCBD	Randomized Complete Block Design
RDW	Root dry weight
RDW: SDW	Root dry weight to shoot dry weight ratio

RFW	Root fresh weight
RFW: SFW	Root fresh weight to shoot fresh weight ratio
rpm	revolutions per minute
SDW	Shoot dry weight
SFW	Shoot fresh weight
tr-Z	trans-Zeatin
tr-ZR	trans-Zeatin Riboside
UPLC-MS/MS	Ultra Performance Liquid Chromatography-Mass Spectrometry/Mass spectrometry
Pmol/gFM	Picamoles per gram of fresh mass
ZR	Zeatin riboside

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CHAPTER ONE: INTRODUCTION

1.1 Background

Kenya's economy relies heavily on the Agriculture sector, which contributes 22% of Gross Domestic Product (GDP). Three percent of the GDP is from the horticulture sub-sector of which 1.6% is from the flower industry. Horticultural exports are a leading source of foreign exchange in Kenya. The industry exports increased from 916 million US dollars in the year 2013 to 924 million US dollars in the year 2014 (KNBS, 2014) due at least partly to great improvement in production. The floriculture sub-sector contributed 49% of horticulture exports by value in 2013 and it is today the fastest-growing sector of the Kenyan economy. During the last two decades the production and export of Kenyan flowers has experienced phenomenal growth interms of volume and value from 1980 (7422 tons) to 2014 (138,601 tons) (Table 1) (KNBS, 2014).

Table 1: Export Volume and Value: 1980-2014 (Selected Years)

Year	Volume (Tonnes)	Value (Kshs. Billions)
1980	7422	227
1985	10,000	463
1990	14,425	940
1995	29,374	3,642
2000	38,757	8,650
2005	81,217	22,896
2010	120,221	36.50
2014	138,601	54.6

Sources: KNBS, (2014), KFC, (2014)

The industry is an important source of foreign exchange which is often invested in local food production. Its employment potential also continues to grow. For example, over 100,000 people are directly employed and approximately 1.2 million indirectly employed in floriculture sub-

sector (KFC, 2008). About three quarters of this work force is female making it one of the sectors where feminization of labour is most explicitly manifested. More than half of the Kenyan population lives below the absolute poverty line, in this respect the cut flower industry takes on a pivotal role in poverty alleviation considering that the majority of the poor are women. The poverty incidence, for instance, around Naivasha where most flower farms are concentrated was between 30-40 % whereas at a national level it was well above 50 % (IMF, 2005).

Rose (*Rosa spp*.) is one of the most important flower crops in the world and has economic values in ornamental, pharmaceutical and cosmetic trade. Rose is the foremost flower in the international cut flower trade and the demand for rose as modern cut flower is growing enormously due to the development of tourism, hotel industry and urbanization. Roses contribute 74% of total cut flowers revenue in Kenya (HCDA, 2014; KNBS, 2014; KFC, 2014). Rose, the queen of flowers is favored for its beauty and many other uses like production of crystallized petals, making rose oil, water, wine, marmalade, jam, honey, extraction of perfumes, extraction of vitamin C from hips, for medicinal uses and sale as cut flowers (Khan and Khan, 1991).

Production of roses is mainly under greenhouses where it is propagated vegetatively mostly by budding and grafting. In either budding or grafting method, selection of the proper type of rootstock not only influences the rooting but also the growth, yield and quality of the scion grafted or budded on the rootstock. Rootstock-scion relationship is important in grafting as it influences rooting and growth of the grafts. For instance, De Vries and Dubois (1990) observed that in grafted plants, vigor of the genotype used as a rootstock is transferred to the scion and thus influences growth and productivity. Other researchers have attributed the increased vigour to several factors such as increased synthesis of cytokinins (Salehi *et al*., 2010; Yetisir and Sari,

2003) and enhanced uptake, synthesis and translocation of water, minerals and hormones (Pheasant and Clarke, 1991).

Some of the rootstock cultivars being used are 'Natal briar', 'Indica major', 'Multiflora', 'Canina' and 'Manneti' (Gerardo, 2007). The flower yield (stems/m^2) and quality (stem length and stem weight) of grafted plants have been found to vary with the type of rootstock used, for instance, the scion cultivars of rose grafted on the rootstock 'Multiflora' produced higher yields than 'Manetti', 'Indica major', and 'Canina Inermis' (Ohkawa, 1986). However, rooting potential and yield of rose variety 'Pink Aurora' grafted onto the rootstock 'Multiflora' was lower than that in plants grafted on the rootstock 'Indica major' (Park and Jeong, 2015) due to higher rootstock activity (Salehi *et al*., 2009). Other studies have also indicated the influence of the scion on the performance of the rootstock for instance, Cardinal *et al*. (2007) showed that the vigorous scion had a major influence on the yield of *Hevea brasiliensis*, than the rootstock. De Vries and Dubois (1994) and De Vries (1993) also found that plant vigour of different clones grafted on seedlings of *Rosa amino* was mainly determined by the vigorous scion and to a lesser extent by the rootstock.

The cultivar 'Natal briar' represents 60-70% of the world-wide cultivated surface of grafted roses (Gerardo, 2007) and is commonly used for rootstock due to its ability to root faster than the others. The rootstock also gives high production and resistance to root-borne diseases, however, it is susceptible to nematodes (de Vries, 2003; Gerardo, 2007). Some of the *Rosa hybrida* cultivars used for rootstocks are difficult-to-root though they can impart great improvement on the performance of the scions of higher demand cultivars during the peak periods. In particular, the rootstock cultivar 'Rosa progress' is difficult to root, however, it provides more production per unit area than 'Natal briar' and 'Indica major (personal communication with rose grower) but

limited documented information is available on the growth and yield performance of scions grafted on it. The rootstock-scion relationship may vary among the rootstock cultivars and their positions with regard to compatibility, rooting ability and subsequent flower yield and therefore establishing the best cutting position of the rootstock cultivars for grafting the variety 'Inca' is essential for maximum rooting and yield.

Multiplication of the rootstocks can be done by use of seeds or vegetative means. The seeds are mainly used in breeding new cultivars. Cultivars are usually not seed propagated because germination is often problematic and the seed derived progeny will segregate widely from traits and therefore have characteristics that differ from the parents (Zlesak, 2006). Vegetative propagation is the most vital and sole method to reproduce roses to maintain desirable traits. They are propagated through cutting, budding, grafting, layering and tissue culture. Among these methods, the use of stem cuttings is the easiest and most common method for propagating roses (Anderson and Woods, 1999). The cuttings are mostly used to maintain clones and genetic uniformity. The stem cuttings are currently obtained from the horizontal or vertical shoots on the plant irrespective of the position yet adventitious root formation of these cuttings may be influenced by a number of endogenous and external factors (Hartmann *et al*., 1997) which may vary with cutting positions.

The cutting position on the parent shoot may influence adventitious root formation (Bredmose *et al*., 2004: Hansen and Kristensen, 2006). The endogenous auxins, carbohydrate content, mineral nutrients and other biochemical components, such as phenolics that could act as rooting co-factors or auxin transport modulators are usually transferred from the stock plants to the propagules when the cutting is severed and their concentrations may therefore vary with the cutting position along the parent shoot. Suxia *et al*. (2009) and Hartman and Kester (1983)

reported that the variation in rooting ability with cutting positions could be due to high concentration of endogenous root promoting substances such as auxins in the apical cuttings which arise from the terminal buds or the presence of juvenile tissues which are actively differentiating (Tchoundjeu and Leaky, 2001; Chaummaravong, 1998; Ling, 1993). It has been reported that there are cells in younger parts of shoots (upper cuttings) which in view point of metabolic activities are more active than mature tissues and their cell walls are less ligneous and this possibly leads to absorption of more auxins for enhanced rooting compared to lower cuttings (Taiz and Zeiger, 2006; Suxia *et al*., 2009). However, apical cuttings may have low storage carbohydrates that could limit rooting of such cuttings (Lyon and Kimuir, 1997).

On the other hand, Keeley *et al*. (2004) and Soonhuae and Limpiyaprapart (1996) attributed higher rooting percentage from the bottom position cuttings of 'Norton' grape vine rootstock and *Dipterocarpus alatus* respectively to high storage carbohydrates. The relationship between carbohydrates and adventitious root formation remains controversial. Some literature reports no effect of carbohydrates (Veierskov and Andersen, 1982) or even a negative effect on rooting (Treeby and Cosidine, 1982). However, there is strong evidence demonstrating that carbohydrates do influence rooting of cuttings positively (Pellicer *et al*., 2000; Husen, 2008; Druege *et al*., 2009 and Ruedell *et al*., 2013). Carbohydrates contribute to the formation of adventitious roots by supplying energy and carbon necessary for cell divisions, establishment of the new root meristems and root formation itself (da Costa *et al*., 2013).

Other endogenous rooting co-factors such as mineral nutrient composition of the cuttings play key positive roles in determining root morphogenesis (Assis, 2001) especially the micronutrients such as zinc, iron, manganese and boron (Rejman *et al*., 2002). They may act synergistically or additively with hormones and especially auxins by protecting auxins against oxidation, affect

certain enzymes that are active during root formation (Jankiewicz, 1997) or stimulate the transport of exogenous auxins in plant tissues (Basu, 1971). The mechanism of their action may be different, even within the same species depending on the differences in their contents in plant parts at the moment of their harvest for propagation (Szydlo and Pacholczak, 2004). Zinc stimulation on rooting has been reported in mother vines of Dog ridge rootstock (Somkuwar *et al.*, 2013). Zinc is required for the biosynthesis of the main auxin precursor, the amino acid tryptophan (Marschner, 1995). Manganese and iron are co-factors and structural components of peroxidases and can therefore affect auxin (Indole-acetic acid) catabolism enzymes (Campa, 1991; Fang and Kao, 2000). The relationship between nitrogen and adventitious root formation remain controversial, for instance, the work on bitter almond (Kasim *et al*., 2009) showed a negative relationship between nitrogen concentration and rooting percentage. On the other hand, high nitrogen supply to stock plants in herbaceous cuttings has been shown to strongly promote adventitious root formation (Zerche and Druege, 2009). Nitrogen is necessary for root initiation because it is essential in synthesis of nucleic acid and protein which are necessary for root differentiation (Hambrick *et al*., 1985).

The cuttings of the rose rootstocks for propagation are commonly obtained along the shoot irrespective of the position on the shoot yet it is evident that rooting ability and rooting determinants greatly vary along the positions on the shoot. It is therefore imperative that for each rootstock cultivar, and particularly for ‘Rosa progress’ and ‘Natal briar’**,** that the cutting position with optimum rooting ability be established. In addition, correlating cutting position of the shoots to juvenility factors and biochemical constituents along the shoot would greatly enhance the scientific understanding of the roles of the rooting determinants and any interactions among them on rooting of cuttings.

Auxins are effective inducers of adventitious root formation but at higher concentrations can inhibit differentiation and outgrowth of roots (da Costa *et al.,* 2013), cause yellowing and dropping of leaves (abscission), blackening of the stem (necrosis) and eventual death of the cutting (Hartmann *et al*., 2011). The promotive effect of auxin on rooting could be attributed to its role in stimulating cell division in the vascular cambium which leads to the formation of root primordial (Rahman *et al*., 2002) or by stimulating redistribution and mobilization of some auxin co-factors towards the base of the cuttings. Lack of competency in difficult to root cultivars may be due to sub-optimal level of endogenous auxin, excessively high concentration of exogenous auxin (Kochhar *et al*., 2005) or high concentration of rooting inhibitors. Raju and Prasad (2010) reported that rooting percentage is changed significantly depending on the types and concentrations of hormone used. 0.6% IBA has been shown to enhance rooting of the rootstock 'Rosa progress', however, some may fail to form roots or form fewer roots (personal communication with rose grower) and establishing the appropriate exogenous auxin concentration for such rootstocks may improve their rooting and survival. The influence of cutting position and interaction between cutting position and exogenously applied auxins on rooting of rootstock cultivars 'Rosa progress' and 'Natal briar' has not been documented, however, the use of synthetic hormones such as IBA has been reported to stimulate adventitious root formation in cut rose cuttings (Bredmose *et al*., 2004).

1.2 Statement of the problem

Roses are propagated vegetatively by use of stem cuttings, grafting or budding. In either budding or grafting method, selection of the proper type of rootstock not only influences the rooting but also the flower yield (stems/m^2) and quality (stem length and weight) of the grafted or budded variety of rose flower. The flower yield and quality of the grafted rose varieties may vary with

the type of rootstock used due to variations in vigour and rootstock activity. The rootstock 'Natal briar' represents 60-70% of the world-wide cultivated surface of grafted roses and in Kenya, it is the main rootstock used in grafting cut roses due to its ability to root faster. Other promising rootstocks have been used due to their ability to impart desirable traits on the scion however, their use has been hindered due to poor rooting ability. The rootstock 'Rosa progress' is difficult to root however, it gives more stems per unit area and higher production of grafted cultivar per season than the cultivar 'Natal briar' and this limits its use in cut rose propagation.

The rootstock-scion relationship may vary among the rootstock cultivars and their positions with regard to compatibility, rooting ability and subsequent flower yield and therefore establishing the best cutting position of the rootstock cultivars for grafting the variety 'Inca' is essential for maximum rooting and yield. The cuttings of the rose rootstock cultivars for propagation are commonly obtained along the shoot irrespective of the position on the shoot, however, the variation with regard to juvenility factors and biochemical constituents along the shoot from the top to the bottom may affect their rooting. Therefore establishing the right cutting position is essential for prompt rooting, enhanced growth and possibly yield.

The internal auxin concentration among the rootstock cultivars and their cutting positions are known to vary and despite this variation, the general tendency in the industry is to apply a standard auxin concentration at the stem bases before rooting. The exogenously applied type of auxin and its concentration may interact with the endogenous ones leading to improved or retarded rooting. It is therefore crucial to establish the appropriate type of exogenous auxin and its concentration for best rooting of each rootstock cultivar and cutting positions. It is also important to analyse the endogenous rooting determinants of the cuttings from the different positions on the shoot of these two rootstock cultivars to understand the roles of these intrinsic

factors on rooting ability. Establishing endogenous auxin concentration is also important in determining the concentration of exogenous auxin to be used since high internal auxin concentration as well as externally applied auxins can retard rooting of cuttings by inducing ethylene production and inhibition of bud break.This study therefore aims to examine some of the internal and external factors which may influence rooting ability of 'Rosa progress' and 'Natal briar' and yield especially of 'Rosa 'progress' cultivar with the goal to increase the use of this rootstock in the rose flower industry.

1.4 Objectives

1.4.1 General objective

To study the effects of cutting position of rootstocks and exogenously applied auxins on rooting ability, growth and yield of rose (*Rosa hybrida*).

1.4.2 Specific objectives

i. To evaluate the effects of different rootstock cutting positions (top, middle and bottom) on rooting and growth of cuttings of 'Rosa progress' and 'Natal briar'.
ii. To determine the effects of exogenously applied Indole-3-butyric acid and 1-naphthaleneacetic acid at different concentrations on rooting and growth of rose rootstock cultivars 'Rosa progress' and 'Natal briar'.
iii. To monitor the levels of auxins, cytokinins, mineral nutrients and photoassimilates in the rootstock cultivars and their relationship to rooting and outgrowth of buds of cuttings.
iv. To evaluate the growth performance and yield of the rose variety 'Inca' as influenced by the cutting positions of rootstock cultivars and auxins.

1.5 Hypothesis

i. Different rootstock cutting positions (top, middle and bottom) have no significant effect on rooting of 'Rosa progress' and 'Natal briar'.

ii. Exogenously applied Indole-3-butyric acid and 1-naphthaleneacetic acid at different concentrations have no significant effect on rooting of the rootstock cultivars 'Rosa progress' and 'Natal briar'.

iii. The levels of auxins, cytokinins, mineral nutrients and photoassimilates of 'Rosa progress' and 'Natal briar' have no significant relationship to their rooting and outgrowth of buds.

iv. The cutting positions of the rootstock cultivars and auxins have no significant effect on growth performance and yield of cut rose variety 'Inca'.

1.6 Justification

Cut rose (*Rosa spp*.) is one of the most important flower crops in the world and has economic values in ornamental, pharmaceutical and cosmetic trade. The demand for rose as modern cut flower is growing enormously due to the development of tourism, hotel industry and urbanization. However, rose flower propagation poses challenges in terms of rootstock –scion relations. Different rootstocks of cut roses have been shown to differently affect either top grafted or budded cut rose cultivars in terms of yield, quality, compatibility, life cycle, and resistance to diseases and to other environmental stresses such as salinity. The rootstock 'Rosa progress' grows faster and ~~its~~ production interms of number of harvestable stems per unit area is higher than 'Natal briar' however, it is difficult to root. Improving rooting ability of the rootstock 'Rosa progress' by establishing the right position and auxin type and its concentration will reduce the cost of propagation, increase productivity and may result into its adoption as an alternative rootstock in the rose flower industry in Kenya which has been dominated by the

rootstock ‘Natal briar’ due to its ability to root faster and also its compatibility with the scions of many rose flowers. Increased productivity will increase the foreign exchange earning derived from the floriculture industry since roses contribute 74% of total cut flowers revenue in Kenya (HCDA, 2014; KFC, 2014) thus improving the economic status of the country. Increased income from the industry will improve the livelihood of many employees in the floriculture industry especially the women who constitute three quarters of this work force and who are also considered as drivers of rural economy hence hastening rural development in Kenya’. Cut flower production does not require a large area and in some areas this can be seen as a solution to the shrinking land sizes due to increasing population and limited productive agricultural land.

CHAPTER TWO: LITERATURE REVIEW

2.1 Roses (*Rosa hybrida*)

Roses are deciduous woody perennials that can be used for their blossoms (for both fresh and dried arrangements) and for landscaping. Cut rose (*Rosa hybrida*) belongs to the family Rosaceae. It has a determinate inflorescence that may assume paniculate, solitary or corymbose form. Flowers are developed on upright prickly shoot tips. They have varied shapes in leaf, axillary buds and in the rates at which the axillary buds grow (Larson, 1992; Pertwee, 2000). The rose shoot at maturity has from 12-20 nodes including those with scales and compound leaves (Larson, 1992). The number of compound leaflet leaves per node ranges from three to seven on vigorous shoots. Flower color ranges from red, white, pink, yellow, orange to lavender with many shades, hues and tints between. The rose fruit is called hips and is a major source of vitamin C and believed to be 10 times richer in vitamin C than oranges. The roses favourite ornamental plants armed with prickles, are among most important floricultural crops in the world.

Roses perform well between lower highland and lower midland zones. In Kenya, areas with an altitude between 1400-2500M above sea level such as Naivasha, Athi river, Karen, Kiambu, Thika, Nyeri, Nanyuki, Kericho among others are ideal for growing roses. The greenhouse rose is self-inductive, meaning the flowers are initiated autonomously in extending shoots (Halevy, 1972; Sarkka, 2004). Roses are generally classified as day neutral and flowering is recurrent throughout the year provided the growing conditions are suitable (Zieslin and Moe, 1985; Sarkka, 2004). The most commercially important types of roses are sweetheart, hybrid tea, and spray roses. Sweetheart roses have one small bloom per stem, generally 3.5cm to 5cm in diameter, and are typically used in bridal bouquets. Hybrid tea roses also have one bloom per

stem but with a much larger flower head, ranging from 7.5cm to 15cm in diameter. Spray roses are relatively newer varieties with multiple blooms, 3.5 cm to 5 cm in diameter, growing on a single stem (Pertwee, 2000; Robert *et al.*, 2001). At least 100 species and thousands of varieties of roses are known to exist. The choice of a variety is determined by market and ease of production in terms of yields and disease resistance. Although the most typical roses are red, they may be almost any color except true blue or black. As fresh cut flowers, roses may last 3 to 7 days in the home without the use of floral preservatives, depending on the variety of the rose and environmental factors such as temperature and care. However, the vase life of a rose can be doubled when floral preservatives are used (Ichimura and Shimizu-Yumoto, 2007). Roses are mainly produced under greenhouses and multiplication is by use of seeds or vegetative means. The vegetative methods include stem cuttings, grafting, budding and tissue culture.

2.2 Grafting/ Stenting

Grafting is a propagation procedure that entails joining of two suitably selected cuttings (scion and rootsock) by first making a slant cut at each end and securing them together with a cloth peg or any other material that would ensure continued intimate contact. The scion should have one five-leaflet leaf. This guarantees the survival of the cuttings as it is the source of energy, nutrients and rooting auxins (Gerardo, 2007). The scion is grafted on a single internode of unrooted rootstock and the formation of the graft union and of adventitious roots on the rootstock occurs simultaneously (Hartmann *et al*., 2011; Mathew and Karikari, 1995). For a successful graft union, there must be a close matching of the callus producing tissues near the cambium layers. In most cases, the more closely the plants are related botanically the better the chances are for the graft union to be successful, though there are numerous exceptions to this rule (Hartmann *et al*., 2011). Grafting may serve many different purposes; perpetuating clones that cannot be

readily maintained by other asexual means, changing cultivars of established plants, repairing damaged parts of trees, hastening reproductive maturity, studying viral diseases (Hartmann *et al*., 2011) and obtaining the benefits of certain rootstocks for instance, higher percentage survival and growth has been reported in rose cultivars 'Milva' and 'Shocking vasilla' grafted on 'Natal briar' (Otiende *et al*., 2011) than own rooted cuttings. Stenting, where cutting and grafting are performed simultaneously and the formation of the graft union and adventitious root development occurs concurrently, by far is the most commonly used propagation method for roses around the world (Nazari *et al*., 2009) and is also an efficient technique in propagating species of conifers, rhododendron, apple, pears and plums (Hartmann *et al*., 2011). Studies have demonstrated that grafted plant roots are capable of producing proteins called shock proteins which assist the plant to survive in conditions where there may be water stress, oxygen deficiency, excess or lack of nutrients which frequently occur in soil cultivation and in environments that are not entirely adapted to rose cultivation (Gerardo, 2007; Nazari *et al*., 2009). Selection of the appropriate rootstock cultivar adapted to the environment or region, compatible with scions and high in production is therefore essential in rose production.

2.3 Rootstocks

Rootstocks have been used in floriculture, pomology, forestry and even tomatoes to improve the performance of the scion. They may affect (either directly or indirectly) scion characteristics such as plant architecture, vigor, nutrient status, flower yield and quality, disease resistance and response to environmental conditions (Pertwee, 2000; Carbera, 2002; Solis-Perez and Carbera, 2007). In today's production of cut roses, choice of the rootstock type essentially depends on vigour, capacity to increase the leaf area, yield and quality of the grafted variety, phytosanitary status and compatibility with the varieties to propagate (Gerardo, 2007). The effect of rootstock

on vigour of the scion has been reported in *Malus spp* (Moore, 1975), *Protea spp* (Ben-Jaacov *et al*., 1991) and *Rosa canina* 'Inermis' (Fuchs, 1994). The cultivars used for rose rootstocks include 'Natal briar', Rosa 'progress', 'Indica major', 'Multiflora' and 'Mannetti'.

The cultivar 'Natal Briar' is most commonly used as rootstock because it is easy to root (de Vries, 2003), gives good stem length and head size, and is moderately resistant to root borne diseases. However it is susceptible to nematodes and also sensitive to high boron level in the soil (Gerardo, 2007). 'Natal briar' represents 60-70% of the world-wide cultivated surface of grafted roses (Table 2). Its introduction as a rootstock is quite recent. This is demonstrated by the fact that, in Colombia until 1996-1997, 'Rosa indica', 'Rosa mannetti' and 'Rosa canina' were the main rootstocks used. Its origin is not clearly known but it is thought to be from South Africa. The rootstock 'Rosa Progress' is a difficult to root rootstock however, it provides more production per unit area than 'Natal briar' and 'Indica major' (personal communication with rose grower).

The rootstock Rosa 'Indica major', 'Rosa odorata', 'Rosa chinensis' belong to the Chinensis group. 'Indica major' rootstock is difficult to root compared to 'Natal Briar'. It prefers warm climates, with slightly sandy soils and a pH of 6.5-7.5 and is the predominant rootstock in Mediterranean areas (Italy, France, Spain, Turkey and Morocco). It gives more stems per unit area and season than 'Natal briar', gives a more vivid and intense color to the rose variety, but has the tendency to die back after pruning (Gerardo, 2007) and is moderately sensitive to nematodes as well as root borne diseases. Due to their difficulty to root, 'Rosa progress' and 'Indica major' are often not used in propagation. The rootstock cultivars 'Manetti' and 'Inermis' are resistant to nematodes and are salinity hardy (Solis–Perez, 2009) but are rarely used due to lower production per unit area compared to 'Natal briar'.

Table 2: Types of rootstocks in major countries

Country	Natal briar	Indica major	Multiflora	Canina	Manneti
China	5%	-	95%	-	-
Ecuador	100%	-	-	-	-
Colombia	100%	-	-	-	-
Holland	98%	2%	-	-	-
Japan	35%	35%	30%	-	-
South Korea	5%	-	-	95%	-
Kenya	100%	-	-	-	-
Uganda	100%	-	-	-	-
Ethiopia	100%	-	-	-	-
Zimbabwe	100%	-	-	-	-
Zambia	100%	-	-	-	-
Italy	5%	90%	-	5%	-
Poland	20%	-	40%	40%	-
Mexico	10%	-	-	-	90%
Iran	100%	-	-	-	-
Spain	5%	5%	-	-	90%
India	40%	30%	30%	-	-
Uzbekistan	-	-	-	100%	-
Kazakhastan	-	-	-	100%	-
France	20%	80%	-	-	-
Russia	80%	-	10%	10%	-

Source: Gerardo, (2007)

The rootstock *Rosa Multiflora* (Synstylae group) is a native wild rose of Japan, Korea and China. It is easier to root and use during budding as its bark lifts very easily for an inserted bud. However, it is more susceptible to chlorosis and produces many bullheads (distorted flowers buds) on some varieties (Gerardo, 2007). The rootstock can be propagated by seed due to ease of germination (Gerardo, 2007; Rowley, 1956).

Several other rootstocks are recommended in various areas in the world based on regional differences in climate and soil conditions, in addition to the consideration of rootstock and scion compatibility. For example, *R. multiflora* is used in the South-Central United States, Canada, and

Japan, whereas ‘Dr. Huey’ is used in the Western United States (Pemberton, 2003). *R. fortuniana* is used in areas with year-round temperate climate (Morrell, 1983). In the United States, *R. fortuniana* is mainly used in Florida and in the South Western region (Martin, 2008). *R. odorata* is one of the most popular rose rootstock for greenhouse cut flowers, but the species is also valued for garden roses (Cabrera, 2002; Singh and Chitkara, 1987). The type of rootstock used in top grafting or budding therefore depends on their adaptability and desirable traits required to improve the growth and survival of the scion (Pertwee, 2000; Robert *et al.*, 2001).

For the multiplication of the rootstocks, the stem cuttings of the rootstock cultivars are rooted in the propagation unit for 4-5weeks after which they are transferred to the field. Six weeks later, the vigorous and robust stems are broken at the extremity (pinching) on the part where the stem turns color from green to violet-red (where the tissue is tender and less consistent) to harden the stems which are then harvested ten days later. The harvesting cycle is between 2-3 months which translates to 7-8 productive phases within a period of 18 months (Gerardo, 2007).

2.4 Stock-scion relations in roses

After grafting, the union of the rootstock and the scion needs to heal. The healing process of the graft union involves lining up of the vascular cambium, wound healing response by producing necrotic material from the damaged cells, callus bridge formation where the undamaged layer of cells produces a large number of parenchyma cells that form a callus providing a mechanical link between the scion and the stock. The callus differentiates to form cambium cells from which the secondary xylem and phloem cells are formed (Barnett and Asante, 2000; Hartmann *et al*., 2011). The vascular connection transports assimilates and storage products, as well as endogenous growth regulators and other substances both upwards and downwards through the grafted plant (Barnett and Asante, 2000; Hartmann *et al.*, 2011). Excellent grafting take helps

auxin and carbohydrate transport to the base of the cuttings that influences the rooting of roses (Izadi *et al*., 2014). Earlier and recent comparative studies on rose rootstock performance in terms of rooting and growth have shown variable results due to various factors including a multitude of scion-rootstock combinations, rootstock clonal effects, type and depth of growing media, length of experimental period, geographic region, environmental parameters and cultural practices (Cabrera, 2002). The scion–rootstock interaction influences water relations, leaf gas exchange, mineral uptake, plant size, blossoming and yield (Gonçalves *et al*., 2003). Younis and Riaz (2005) found that rose cultivars 'Gold medal', 'Whisky mac' and 'Kardinal' showed maximum growth and flowering when budded on Grussanteplitz as compared to *Rosa bourboniana*. *Rosa chinensis* 'Indica Major' was found to be a better rootstock for grafting the cultivar 'Cocktail 80' than Rosa canina 'Inermis', while 'Inermis' was better for "Motrea" (Van de Pol and Breukelaar, 1982). De Vries and Dubois (1990) observed that in grafted plants, vigor of the genotype used as a rootstock is transferred to the scion and thus influences growth and productivity.

Incompatibility reactions between the stock-scion have been observed within some species. They may show immediately or after many years. Some of the incompatibility symptoms include; failure to form a successful graft union, early defoliation of deciduous plants, decline in vegetative growth due to shoot die-back, premature death of trees after a few years or while still in the nursery, marked differences in growth rate or vigour between scion and rootstock; overgrowth above, or below the graft union and graft components break apart cleanly at the graft union (Hartmann *et al*., 2011). The graft incompatibilities can be avoided by the use of a mutually compatible interstock, which is an insertion between the intended rootstock and scion

of a third cultivar. This interstock then provides a bridge but still allows the characteristics of both scion and rootstock to be expressed (Hartmann *et al*., 2011).

2.5 Effects of rose rootstocks on quality and yield of flowers

In cut roses, the flower quality and yield are separate but dependent parameters, and are usually expressed in an inversely proportional pattern when the total photosynthate is the same. Therefore, high yield is not always accompanied with better quality when the total photosynthate is the same (Park and Jeong, 2015) due to competition of soil resources for growth and development. Hu (2001) observed a strong negative correlation between flower quality (either shoot length or shoot weight) and shoot or plant density, especially at high densities where there is high competition for nutrients, space and light. Robert *et al*. (2001) also reported that rose variety 'Escimo' grafted on 'Multiflora' and 'Indica major' gave higher yields but slightly poorer quality.

Rootstocks have been shown to enhance quality and yield of roses (Pertwee, 2000). Safi and Sawwan (2004) found that the yield and other growth parameters measured for the three *Rosa hybrida* L. cultivars grafted on the rootstocks 'Indica major', 'Canina L. inermis', and 'Natal briar' were higher than the own rooted plants. The rootstocks differ in their effects on yield and quality of grafted flower varieties. The rootstock 'Multiflora' produced higher yields than 'Manetti', 'Indica Major' and 'Canina inermis' on which scion cultivars were grafted (Ohkawa, 1986). Park and Jeong (2015) found that the yield of rose variety 'Pink Aurora' grafted onto the rootstock 'Multiflora' was lower than that in plants grafted on the rootstock 'Indica major'. Increased yield of grafted scions could be due to the rootstocks vigorous root system which increases the efficiency of water and nutrient consumption resulting in enhanced growth and yields of the scion of grafted vegetables (Lee, 1994). Lee and Oda (2003) reported different

responses of vegetative growth of the grafted vegetables and ornamental plants to be related to the vigour and compatibility of rootstocks and scion. Other researchers attributed increased vigour to several factors such as higher rootstock activity (Salehi *et al*., 2009), extensive rootstock root system (Edelstein, 2004), increased synthesis of plant growth substances (Salehi *et al*., 2010; Yetisir and Sari, 2003) and enhanced uptake, synthesis and translocation of water, minerals and hormones (Pheasant and Clarke, 1991).

Cut flowers of roses are graded into 1st, 2nd, 3rd, 4th, and 5th grades according to their stem length as ≥ 80, 79-70, 69-60, 59-50, and ≤ 50 cm, respectively. Stem length has been used as a determinant of stem quality and rose rootstocks have been reported to increase flower stem length (Park and Jeong, 2015; Gerardo, 2007) due to the rootstock effect on nutrient uptake and accumulation patterns particularly under conditions of poor water quality (Cabrera, 2001), for instance, high tissue concentrations of calcium and chloride, and boron and chloride have been reported on scions growing on 'Indica major' (Johansson, 1979) and 'Multiflora' (Byrne and Furuta, 1967), respectively. The variety 'Pink Aurora' grafted on the rootstock 'Indica major' yielded significantly taller shoots (69-60cm) than on the rootstock 'Multiflora' (Park and Jeong, 2015). Rootstock effect on stem length has also been reported in *Pinus sylvestris* L. (Hansen *et al*., 1978) and could be due to rootstock effect on vigour of the scion that has also been reported in *Malus spp* (Moore, 1975), *Protea spp* (Ben-Jaacov *et al*., 1991) and *Rosa canina* 'Inermis' (Fuchs, 1994) and has been attributed to high rootstock activity.

Other studies have also indicated the influence of the scion on the performance of the rootstock for instance, Park and Jeong (2015) reported that the yield, stem length, stem fresh weight and stem diameter of grafted rose variety 'Yellow King' was not affected by the rootstocks 'Multiflora' and 'Indica major'. De Vries and Dubois (1994) and De Vries (1993) also found

that plant vigour of different clones grafted on seedlings of *Rosa amino* was mainly determined by the vigorous scion and to a lesser extent by the rootstock.

2.6 Adventitious root formation

Adventitious rooting is a multifactorial response leading to new roots at the base of stem cuttings, and the establishment of a complete and independent plant. Adventitious roots may also be formed in non-pericycle tissue in older roots, differing from primary roots, of embryonic origin, and lateral roots, which are derived from the pericycle layer (Li *et al*., 2009). There are two main patterns of adventitious root development: direct and indirect. The tissues involved in the process of root development are most frequently the cambium and vascular tissues, which undergo the first mitotic divisions, leading directly to root primordia in the first pattern. In the indirect pattern of adventitious root formation, albeit the same tissues often take part, the formation of a callus is observed prior to differentiation of root primordia. In both cases, before root primordia become distinguishable, clusters of usually isodiametric cells are formed (meristemoids) (Altamura, 1996). In the indirect pattern of adventitious root formation a bottleneck is frequently observed, that is, the difficulty in establishment of an effective vascular connection between the newly formed root primordia and the stem. Poorly connected vasculature with the stem leads to non-functional roots, with negative consequences for cutting survival (Fleck *et al*., 2009).

A large body of evidence has supported the existence of successive physiological phases in the process of adventitious root development, each with specific requirements that can even be antagonistic, but operate in complementary fashion. The most widely recognized adventitious root formation phases are induction, initiation, and expression (Kevers *et al*., 1997). The induction phase in cuttings or detached organs, such as leaves, is generally marked by the

immediate consequences of the wounding response caused by severance. It encompasses the first hours after cutting removal, with a local increase in jasmonate, phenolic compounds and auxin at the cutting base, often associated with a transiently lower peroxidase activity, and the establishment of a sink for carbohydrates in the same zone (Schwambach *et al*., 2008; Ahkami *et al*., 2009). Peroxidases are heme-containing enzymes with oxidative catalytic action on diverse organic compounds, including indole-3-acetic acid (IAA), and their activity has been used as a biochemical marker of the rooting phases (Corrêa *et al*., 2012a). The induction phase is devoid of visible cell divisions and involves reprogramming of target cells for the establishment of meristemoids, which takes place in the initiation phase. Studying adventitious root formation in apple, De Klerk *et al*. (1999) initiated the concept of an early phase of dedifferentiation (0– 24 h), taking place before the induction phase.

During initiation phase, besides cell divisions, meristemoids and development of root primordia, often a lower auxin and phenolic concentration and higher peroxidase activity are observed. The expression phase corresponds to the growth of root primordia through the stem tissues and the establishment of vascular connections between the newly formed root and the original stem cutting. For simplification purposes, it is not uncommon to join the initiation and expression phases under a single denomination of formation phase (Fett-Neto *et al*., 1992). Adventitious rooting is therefore a complex process that can be affected by numerous variables, both internal and external.

2.7 Some of the factors influencing rooting and survival of cuttings

2.7.1 Cutting position

Cuttings within a single tree are confounded by different chronological ages and topohysis resulting in exposure to different environmental conditions such as light and humidity

(Matsuzaki *et al*., 2005). Genetically determined processes of, bud growth and adventitious rooting may also be influenced by the ontogenetical age of the plant part from which the cutting originates (cyclophysis), by the environment of the parent shoot (periphysis), and by the position on the parent shoot of the cutting in relation with its axillary bud (topophysis). Together these phenomena determine the phenotype of the resulting plant (Büsgen and Münch, 1927). Cyclophysis, is the process of ontogenetic ageing (Olesen, 1978) and is most advanced in the apical meristem as this meristem has produced the majority of the total growth (Olesen, 1978). Cyclophysis is genetically programmed, localized in the meristems, accelerated by improved growth conditions, and difficult to reverse (Fortanier and Jonkers, 1976). Periphysis, introduced by Büsgen and Münch (1927), is comprised of the effects of environment and is manifested as a certain carry-over effect on the progeny plants (Schaffalitzky de Muckadell, 1959; Olesen, 1978), without causing a permanent effect on the genetic nature of the propagule (Hartmann *et al*., 1997). Topophysis is the persistent growth and differentiation without genetic change of a cutting depending on the tissue of source (Kenneth, 1963).

The position of the cutting on the parent shoot may be particularly important (Wilson, 1993) in adventitious root formation and the ability to form roots may increase with distance from the apex of the shoot (Hansen, 1986; Leakey and Mohammed, 1985) due to changes in endogenous rooting ingredients. According to Tukey and Green (1934), the woodiness of the shoot is reduced towards the tip of the cutting compared with that of the basal and medial portions. This indicates that the structure and degree of differenciation vary from the tip to the base and the rooting response varies with the degree of hardiness (Jensen, 1967; Tukey and Green, 1934). This could be due to reduced polar auxin transport (Davies, 1995) or increased peroxidise activity and less

IAA biosynthesis (Woodward and Bartel, 2005). Zimmerman (1925) found that for Weigelia and 'American Pillar' rose, there is a gradient in rooting response from the tip to the base of cutting. In many cases, the highest rooting has been produced in the cuttings which have been made from lower parts of the shoot (Satisha *et al*., 2007) but in some species where soft wood cuttings are used, the upper shoots have better rooting. Soonhuae and Limpiyaprapart (1996) reported that cuttings of *Dipterocarpus alatus* taken from bottom position produced better rooting percentage and higher root number because it was larger in size with a better storage of carbohydrates. The medial and basal cuttings of cabbage rose (*Rosa* ×*centifolia* L.) (Al-Saqri and Alderson, 1996) rooted better than the apical cuttings. Bressan *et al*. (1982) found that *in vitro* cultured axillary rose buds from the medial section of the parent shoot developed more rapidly than either apical or basal buds. Lyon and Kimuir (1997) attributed low rooting percentage from the apical cuttings of *Acacia mangium* to low storage carbohydrates. Poor rooting of apical cuttings could also be due to less favourable water relations of these young, relatively succulent cuttings as they tend to transpire faster (Leakey, 1993).

In contrast, Zieslin *et al*. (1976) found that shoot growth from axillary buds of roses increased acropetally. Shoot growth is essential in the supply of rooting ingredients such as auxins and photoassimilates. Choummaravong (1998); Ling (1993) and Tchoundjeu and Leakey (2001) reported that top position cuttings of *Azadirachta excelsa, Shorea spp* and *Lovoa trichiliodes* respectively produced good rooting and survival response compared to the bottom and middle positions due to the presence of juvenile tissues which are actively differentiating. It has been reported that there are cells in younger part of shoots (upper cuttings) which are metabolically more active than mature tissues and their cell walls have fewer lignin and this possibly leads to absorption of more auxin, water and nutrient required for growth. Therefore rooting potential in

upper cuttings is higher than lower cuttings (Taiz and Zeiger, 2006; Suxia *et al.*, 2009). Low rooting ability of cuttings from older tissues has been attributed to anatomical barriers such as thickening of sclerenchymatous cells which become a barrier to root initiation (Darus, 1989) and reduced polar auxin transport (Davies, 1995).

2.7.2 Phytohormones

Plant hormones are naturally occurring secondary metabolites that play key roles in most physiological processes, including cell division, enlargement and differentiation, organ development, seed dormancy and germination, leaf and organ senescence, and abscission. The structurally diverse compounds that act usually at nanomolar levels include five groups of the so-called "classic" hormones, comprising auxins, cytokinins, gibberellins, abscisic acid and ethylene, and several other plant growth regulators, including jasmonates, salicylates, brassinosteroids, polyamines or the very recently discovered strigolactones, which fit several of the criteria to be considered hormones (Pan *et al.*, 2008; Dun *et al.*, 2009).

Recent studies support the contention that hormone actions build a signaling network and mutually regulate several signaling and metabolic systems in interactive ways. Such interactions have been reported for auxins and gibberellins in growth regulation (Ross *et al.*, 2003), for cytokinins and auxins, for abscisic acid and strigolactones in apical dominance (Dun *et al.*, 2009; Tanaka *et al.*, 2006), for auxins and brassinosteroids in cell expansion (Goda *et al.*, 2004; Nemhauser *et al.*, 2004), for ethylene and cytokinins in the inhibition of root and hypocotyl elongation (Cary *et al.*, 1995), for ethylene, abscisic acid and gibberellins in some plant stress responses (Gazzarrini and McCourt, 2001), and for salicylates, jasmonates and auxins in plant responses to pathogens (Wang *et al.*, 2007; Vlot *et al.*, 2009).

2.7.2.1 Auxins

Auxins were the first class of plant hormones to be identified. They are produced in young parts of the shoot (apex, bud, leaves) and small amounts in roots (Ljung, *et al*., 2001). The auxins always are transported downward in the plant from shoot to root. This polar movement of auxin requires calcium ions and also involves special carriers in cell membranes (Peer *et al.*, 2011; da Costa *et al*., 2013). The naturally occurring auxins (IAA) play an essential role in coordination of many growth and behavioral process in the plant life cycle. They stimulate cambium cells to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds lower down the stems (apical dominance), and also to promote lateral and adventitious root development and growth (Hartmann *et al.*, 2011). They also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots, and fruits, and convert stems into flowers.

Auxins play a significant role in stimulating adventitious rooting from stem cuttings of tree species (Poupard *et al.*, 1994; Tchoundjeu *et al*., 2001). Removal of the presumed auxin sources (apex, leaves, bud) by decapitation, debudding or defoliation inhibited rooting in carnations (Eliasson and Areblad 1984, Garrido *et al.*, 2002). Specific inhibitors of polar auxin transport (PAT) such as l-N-naphthylphthalamic acid (NPA) also inhibits rooting (Liu and Reid, 1992, Garrido *et al*., 2002) and accumulation of auxin during the induction phase of rooting (Ahkami *et al*., 2013). All the above suggest that PAT might play a decisive role in providing the IAA from the sources to the sink (rooting zone) where rooting occurs. It has been repeatedly confirmed that auxin is required for adventitious root formation on stems and that the divisions of the first root initials are dependent on exogenous and endogenous levels of auxins (Ludwig-Müller, 2000; Kochhar *et al*., 2005). In addition to enhancing the rate of adventitious root development, auxin

application has been found to increase the number of roots initiated per rooted cutting in a variety of species (Mesen *et al*., 1997; Palanisamy *et al*., 1998).

Auxin treatment has been shown to enhance the movement of boron, nitrogen, Zinc and potassium from the leaves and buds of the cuttings to the rooting zone (Blazich *et al*., 1983). Jarvis and Ali (1984) explained that the nutrients boron, nitrogen, zinc and potassium sustain cell division, auxin biosynthesis and organization leading to root initiation and growth. Auxin stimulates the mobilization of carbohydrates in leaves and the upper stem and increases the translocation of assimilates toward the rooting zone (Veierskov and Anderson, 1982; Haissig, 1986; Husen and Pal 2007; Agullo-Anton *et al*., 2011). Exogenously applied auxin activates sugar metabolism to release energy and to provide carbon skeletons for the synthesis of new compounds such as proteins (Haissig, 1974), that are essential for rooting.

The most important, naturally occuring physiological active auxin is Indole-3-Acetic Acid (IAA). Synthetic auxins such Naphthalene Acetic Acid (NAA) and Indole-3-Butyric Acid (IBA) are available commercially and are used as rooting hormones. Compared to these synthetic auxins, IAA is not stable and is degraded rapidly by light, plant enzymes and microbes hence it is not popular in plant propagation. IBA and NAA are more stable, more effective in promoting rooting than IAA and hence they are very popular with plant propagators. They can be applied with liquid (liquid formulation) or in talc (powder formulation) for rooting and sprouting of stem cuttings (Ercisli, 1999; Hopkins, 1999). Auxins are however toxic to plants in large concentrations as it can cause cell membrane damage (Hąc-Wydro and Flasiński, 2015). Because of this property, synthetic auxin herbicides including 2, 4-D (2, 4-dichlorophenoxyacetic acid) and 2, 4, 5-T (2, 4, 5-trichlorophenoxyacetic acid) have been developed and used as weed killers (Hartmann *et al*., 2011).

a. Role of endogenous auxins (IAA)

In bioassays, IAA is generally the most biologically active indole compound (Woodward and Bartel, 2005). IAA levels are regulated by a balance between biosynthesis, conjugation, degradation and transport (Sztein *et al*., 1999). In vascular plants, there are several tryptophan-dependent biosynthetic pathways where tryptophan is converted to IAA via various intermediates such as indole-3-pyruvic acid, indole-3-acetaldehyde, or indole-3-acetonitrile. A tryptophan-independent pathway for IAA synthesis has also been elucidated (Woodward and Bartel, 2005). IAA conjugation facilitates storage, transport and protection from peroxidation and catabolism and free IAA is formed by the hydrolysis of these conjugates (Woodward and Bartel, 2005). IAA links either with sugars to form ester conjugates, or with amino acids and small peptides to form amide conjugates (Sztein *et al*., 1999). IAA is more sensitive to light (Hartmann *et al*., 2011) as light inhibits IAA transport thus reducing the availability or effectiveness of IAA for rooting (Jones *et al*., 1991) High concentration of IAA can stimulate ethylene synthesis which is known to inhibit root production (Mullins, 1972) .

Conflicting information is available on changes in the levels of naturally occurring indole-3-acetic acid (IAA) during the rooting process. In some cases, IAA levels in cuttings increase substantially either immediately after removal from the mother plants or after a short delay (Schwambach *et al*., 2008). In other cases, IAA decreases during the induction phase of adventitious rooting and then increases during the initiation phase (Noiton *et al*., 1992, Wiesman and Epstein, 1987). On the overall rooting is related to the presence or accumulation of free auxins in bases of cuttings. Free auxin increased in the rooting zone prior to rooting in olive (Pio *et al*., 2005; Ayoub and Qrunfleh, 2006) *Petunia hybrida* (Ahkami *et al*., 2013) and *Pisum sativum* (Rasmussen *et al*., 2015) cuttings. Endogenous auxin levels have been reported to be

positively correlated to rooting ability of cuttings of *Abeliophylum distichum*, Nakai (Yong Kweon and Kisun, 1996), of *Acerrubrum* (Smalley *et al*., 1991) and of herbaceous cuttings (Ayoub and Qrunfleh, 2006). Endogenous auxin increased in grapevine cuttings when the root initiation began but decreased when the root appeared (Kawai, 1996). The promotive effect of auxin on rooting could be attributed to its role in stimulating cell division in the vascular cambium which leads to the formation of root primordial (Rahman *et al*., 2002).

b. Effects of exogenous auxins

In many recalcitrant species application of exogenous auxin is required to achieve satisfactory rooting responses (Diaz-Sala *et al*., 1996; Fett-Neto *et al*., 2001). Exogenous auxins (as do also endogenous auxins) normally act by stimulating cell divisison in the vascular cambium resulting in the initiation of numerous lateral roots (Hameed *et al*., 2004; Durbak *et al*., 2012). In these cases, endogenous auxin produced in the shoot apex and transported basipetally to the cut surface may be complemented by exogenously applied auxin aiming at improving the rooting response (Pop *et al*., 2011). IBA application has been found to be critical for rooting of both softwood and hardwood cuttings (Ofori *et al*., 1999; Polat *et al*., 2000; Sebastiani and Tognetti, 2004; Tworkoski and Takeda, 2007). Whereas all the three common auxins IAA, NAA, and IBA can be exogenously applied to enhance rooting, there are differences in their efficacy in causing rooting. The differences in rooting response among auxins could also be related to their attributes such as stability and a rate of conjugation. IBA is the best for general use because it is non toxic to plants over a wide concentration range than NAA or IAA (Hartmann *et al*., 2011) and also effective in promoting rooting of a large number of plant species (Teklehaimanot *et al*., 1996; Henrique *et al*., 2006). IBA has higher stability and a slower rate of conjugation so that the free IBA required to induce rooting will be available over a longer period of time than IAA or NAA

(Krisantini *et al*., 2006). Moreover, Butola and Badola (2004, 2007) have recommended IBA as promising treatment to improve rooting, growth and biomass in *Angelica glauca* and *Heracleum candicans*.

Increased number of roots in IBA treated cuttings may be due to its effect on cell wall turgidity, which accelerates cell division (Rahman *et al*., 2002). The effectiveness of auxin to raise rooting percentage of the cuttings could be through increasing cambial activity and differentiation of root primordial (Davies and Joiner, 1980) or by stimulating redistribution and mobilization of some auxin cofactors towards base of the cuttings. Auxins in excess concentration can inhibit bud development and outgrowth of roots, cause yellowing and dropping of leaves (abscission), blackening of the stem (necrosis) and eventual death of the cutting (da Costa *et al*., 2013; Hartmann *et al*., 2011).

2.7.2.2 Cytokinin and auxin/cytokinin ratio

Cytokinins are a class of plant hormones known as key regulators of plant growth and development, including cell division, chloroplast biogenesis, bud and root differentiation, shoot meristem initiation and growth, flowering, stress tolerance, nutrient metabolism and organ senescence (Argueso *et al*., 2009; Kuroha *et al*., 2009; Muller and Sheen, 2007; Dodd, 2005). Cytokinins are thought to be synthesized in the roots and transported through the xylem to the shoot during transpiration (Van Staden and Davey, 1976). The discovery of cytokinins by Folke Skoog and colleagues in the 1950s focused on kinetin, a synthetic compound derived from autoclaved salmon sperm DNA (Miller *et al*., 1955). All native cytokinins are derivatives of adenine with a side chain at the N6 position. Depending upon this N6-substituent, these compounds may be classed into isoprenoid (zeatin-type), isoprenoid-derived (dihydrozeatin-type) and aromatic cytokinins (benzyladenine-type) (Zazımalova *et al*., 1999), and all can occur

as free bases, as well as conjugates with sugars and amino acids (Letham and Palni, 1993). Isoprenoid cytokinins are divided into four groups; Isopentenylaldenide (iP), trans-zeatin (tZ), cis-zeatin (cZ) and Dihydrozeatin (DHZ), with aromatic cytokinins falling into two groups, benzyladenine (BA) and topolins. Conjugates of these free-bases include ribosides, ribotides (riboside- 5'-phosphates), O-glycosides, N-glycoside and amino-acid conjugates. Free-bases and ribosides are the most active cytokinin forms in vascular plants, binding with high affinity to different receptors to elicit a physiological response (Spichal *et al*., 2004; Yonekura-Sakakibara *et al*., 2004). However, both forms are susceptible to degradation by cytokinin oxidase (Armstrong, 1994).

In vascular plants, free-bases and ribosides can be converted to O-glucosides by O-glycosyltransferases which, are considered storage forms (Sakakibara, 2006). When required, they are easily hydrolysed by β- glucosidase to their free-bases and ribosides. These cytokinins have no or low activity in bioassays, are not readily converted to other cytokinin forms and are extremely resistant to enzymatic degradation (Sakakibara, 2006). Thus they are considered as deactivation products. The tr-Z-type cytokinin and its metabolites, including DHZ are the most prevalent cytokinins in vascular plants while IP-type cytokinins are generally minor components; however in non-vascular plants such as mosses and algae, iP is generally the principle free cytokinin (Auer, 1997). Zeatin riboside (ZR) is a riboside of Z and is thought to be one of the most biologically active compounds influencing adventitious root formation, along with isopentenyladenosine (iPA) and dihydrozeatin riboside (DZR), ribosides of iP and DZ. IP may be a weak cytokinin with respect to inhibition of root meristemoid formation (Palni and Horgan, 1983). This may be due to an increased instability of IP or it is not active in itself but is actually a

precursor to Z (Palni and Horgan, 1983) and that the activity of the enzyme responsible for the conversion of IP to Z also decreases some time after excision.

The influence of cytokinins on plant morphogenesis cannot be considered without mentioning their antagonistic interaction with auxins. Studies with Arabidopsis callus *in vitro* indicated that both auxins and cytokinins are required for organogenesis; auxins act as a triggering factor, while cytokinins modulate this process (Pernisova *et al*., 2009). The auxins and cytokinins are also responsible for maintainance of meristem activity, depending on the balance between cell divisions and cell differentiation (Dello *et al*., 2007). Due to antagonistic effects of auxins and cytokinins on shoot and root meristem development, the direction of plantmorphogenesis depends on the ratio of auxin to cytokinin. Pijut *et al*. (2011) reported that cytokinins are involved in regulation of polar auxin transport as well as induction of auxin gradient and the subsequent coordination of cell division and differenciation. Cytokinins stimulate cell differenciation into vascular tissues through spatially selective expression of cytokinin dehydrogenase gene in the transition zone (Dello Ioio *et al*., 2007; da Costa *et al*., 2013).

Among the three interdependent phases of adventitious root formation; during the induction phase when there are no visible anatomical changes, hormone levels and tissue sensitivity to hormone signals are the most significant indicators of initiation of rhizogenesis (De Klerk *et al*., 1995; Pop *et al*., 2011). Quantification of the endogenous levels of cytokinin and auxins in the basal region of the cuttings from diverse woody and herbaceous plants, including *Populus, Malus, Solanum* and *Phaseolus*, reveals that the concentrations of these two hormones follow opposite patterns during the initial 48 hours of rooting (Ramirez-Carvajal *et al*., 2009). The induction and expression phases are characterized by high levels of IAA at the base of the cutting and high sensitivity to exogenous auxins (Gasper *et al*., 2003) and relatively constant and low

levels of cytokinins (Taylor and Van Staden, 1997). Application of exogenous cytokinins at a higher concentration during these phases increases the concentration of endogenous zeatin and this inhibits rhizogenesis (Laplaze *et al*., 2007) by distrupting primordial initiation and regular pattern of cell divisions. This leads to reduced root meristem size due to progressive decrease in the number of meristematic cells (Laplaze *et al*., 2007; Werner and Schmulling, (2009). High auxin to cytokinin ratio is therefore required during the induction and expression phases of adventitious root formation while high cytokinin to auxin ratio is required during root initiation for cell division (Hartmann *et al*., 2011).

2.7.3 Mineral nutrients

Mineral nutrients have essential and specific roles in plant metabolism; they can function as constituents of organic structures, activators of enzymatic reactions or as charge carriers and osmoregulators (Marschner, 1995). Nutrition is therefore a key factor determining root morphogenesis (Assis, 2001). The micronutrients such as zinc, iron, manganese and boron are the most active rooting cofactors (Rejman *et al*., 2002) that may act synergistically or additively with auxins. They may protect auxins against oxidation, affect the functioning of certain enzymes that are active during root formation (Jankiewicz, 1997) or stimulate the transport of exogenous auxins in plant tissues (Basu, 1971). The mechanism of their action may be different, even within the same species depending on the differences in their contents in plant parts at the moment of their harvest for propagation (Szydlo and Pacholczak, 2004).

Zinc stimulation on rooting has been widely reported (Pacholczak and Szydlo, 2008; Somkuwar *et al*., 2013). Zinc is required for the biosynthesis of the main auxin precursor, the amino acid tryptophan (Blazich, 1988; Marschner 1995) and also a structural component of the auxin

receptor Auxin-Binding Protein 1 (ABP1) (Tromas *et al*., 2010). Decreased Fe in the induction phase showed higher root number and root lengths in *Eucalyptus globulus* (Schwambach *et al*., 2005). Manganese and iron are co-factor and structural components of peroxidases and can therefore affect auxin (Indole-acetic acid) catabolism enzymes (Campa, 1991; Fang and Kao, 2000).

The initial nitrogen and carbohydrate status of the cuttings affects adventitious root formation (Blazich, 1988; Veierskov, 1988). High nitrogen supply to stock plants meeting or surpassing the level necessary for maximum growth has been observed to decrease subsequent rooting of cuttings (Ling and Zhong, 2012). Optimum nitrogen concentration is necessary for root initiation because nitrogen is essential in synthesis of nucleic acid and protein which are necessary for root differentiation (Kim *et al*., 1977; Hambrick *et al*., 1985). The relationship between nitrogen and adventitious root formation remain controversial. The works on bitter almond (Kasim *et al*., 2009) and soft wood cuttings of white Forsythia (Yong Kweon and Kisun, 1996) showed a negative relationship between nitrogen concentration and rooting percentage. On the other hand, high nitrogen supply to stock plants and the resulting elevated nitrogen content in herbaceous cuttings have been shown to strongly promote adventitious root formation (Druege *et al*., 2000; 2004; Zerche and Druege, 2009). The promotive effect of nitrogen in root formation may be manifested by the manner in which it relates to carbohydrate content and metabolism (Blazich, 1988). For instance, the findings in Tetraploid locust (Ling and Zhong, 2012) and *Thunbergia grandiflora* (Hussein, 2008) revealed a positive correlation of high carbon to nitrogen ratio to high rooting percentage.

2.7.4 Carbohydrates

The relationship between carbohydrates and adventitious root formation remains controversial. The carbohydrate pools of sugars (soluble carbohydrates) and storage carbohydrates (starches or insoluble carbohydrates) are important to rooting as building blocks of complex macromolecules, structural elements, and energy sources. Some literature reports no effect of carbohydrates (Ali and Westwood, 1966; Veierskov and Andersen, 1982) or even a negative effect on rooting (Treeby and Cosidine, 1982). However, there is strong evidence demonstrating that carbohydrates do influence rooting of cuttings positively (Caboni *et al*., 1992: Wiesman and Lavee, 1995; Druege *et al*., 2000; Pellicer *et al*., 2000). Weigel *et al*. (1984) reported that carbohydrates help in auxin transport as well as growth of shoots and roots. Sucrose can enhance the sensitivity to auxin (Anonymous, 2008). On the other hand, high simple sugar levels such as glucose may reduce auxin signaling (Agullo-Anton *et al*., 2011). The accumulation of assimilates in the rooting zone of cuttings has been suggested to be a necessary triggering factor for root initiation in woody and non-woody species (Lovell *et al*., 1972; Veierskov *et al*., 1982; Haissig, 1984; Rodriguez *et al*., 1988), although the critical concentration required to induce root initiation is apparently difficult to establish (Welander, 1994).

Root initiation includes the processes of induction, cell dedifferentiation, division and differentiation, whereas root growth should include the processes of cell division, expansion and differentiation (Taylor, 1997) which gives rise to the root primordial through the cortex till emergence and further elongation of the functional root. Root initiation has been commonly reported to be less dependent on carbohydrates than root growth. Middelton *et al*. (1980) showed that root initiation (occurring on the first day following severance) in stem cuttings of *Phaseaolus aureaus* was not limited by carbohydrates whereas further root primordia growth

depended on sugar supply. Likewise, Veierskov *et al*. (1982) found no relation between carbohydrate concentrations and the number of roots formed, but they suggested that carbohydrates could affect root growth. Furthermore, indirect evidence that root initiation depends less on the supply of current carbohydrates than root growth is the fact that leaf removal decreased more the dry weight than the number of roots (Fournioux, 1997).

In contrast with the view that carbohydrates do not influence root initiation, Lovell *et al*. (1972) showed that current photosynthesis and the accumulation of photosynthates in the lower petiole are pre-requisites for root initiation in *Synapsis* cotyledons. Welander (1994) and Haissig (1984) described the same for woody cuttings. Low availability of carbohydrates may have a negative regulatory effect on mitotic activity (Moritz and Sundberg, 1996; Borisjuk *et al*., 1998; Muller *et al*., 1998). The mitotic activity of the cambium was shown to be influenced by sugars and hormones in *Pinus sylvestris* (Moritz and Sundberg, 1996). Also Muller *et al*. (1998) found that both cell division and elongation were limited by low contents of glucose and fructose. Pallardy and Kozlowski (2007) and Li and Leung (2000) also observed that application of sugars to the rooting medium often improved rooting response by increasing the carbohydrate level and facilitating growth. This implies that low carbohydrates, e.g. as a consequence of reduced leaf photosynthesis, can decrease rooting, especially when root initiation occurs within the callus tissue.

Overproduction of carbohydrates may inhibit rooting. For instance, Hansen and Eriksen (1974) have shown that the inhibition of rooting of pea cuttings observed under high irradiance was related to the over production of carbohydrates that turned out to be too high when compared to the amount of endogenous auxin or may be due to inhibited auxin signaling (Agullo-Anton *et al*., 2011). The establishment of a carbohydrate sink in the rooting zone is considered as an important

metabolic event (Ahkami *et al*., 2009) and that auxin accumulation at the rooting zone contributes to this process (Ahkami *et al*., 2013).

The relationship between carbohydrate content of tissues and their susceptibility to disease has been reported (Sabet and Hassan, 1961; Schonenweiss, 1967; Kiyomoto and Bruehel, 1977) and sugars such as sucrose and glucose are known to induce the resistance to diseases in plants. Druege *et al*. (2004) reported that the survival rate of pelargonium cuttings was positively correlated with sugar level in the stems. Howard and Harrison-Murray (1995) reported that stem rot, a non-pathogenic disorder, is primarily due to carbohydrate starvation of tissues in *Syringa vulgaris* cuttings. The susceptibility of leafless cuttings of rose to stem black rot, a disease caused by various soil fungi is related to the energy status of cuttings (Ypema *et al*., 1987) and keeping the original leaf or part of it enables the accumulation of carbohydrates and dry weight at the rooting zone of the cuttings which is known to prevent dieback, enhance resistence to soil born fungi (Ypema *et al*., 1987) and to promote rooting (Okoro and Grace, 1976; Haissig, 1984; Hoad and Leakey, 1996; Howard and Harrison-Murray, 1995; Pellicer *et al*., 2001).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experimental sites

Two studies namely; i) Self rooting and ii) Top grafting were conducted under two greenhouse conditions; i) In the propagation unit to evaluate the rooting and survival of rose rootstock cultivars as influenced by cutting position and auxin concentrations. The self rooting study and part of the grafting study involving the rooting of the grafts were done in the propagation unit. ii) In the production greenhouse to evaluate the yield of cut rose variety 'Inca' (scion) as influenced by the cutting position on the shoot of the rootstock cultivars 'Rosa progress' and 'Natal briar'. The propagation experiments were conducted at Harvest Flower Limited in Athi river, Machakos County, Kenya. The site lies on a latitude 0^0 35' South, longitude 30^0 23' East. The production greenhouse experiment was done at Finlay Flowers Company Limited in Kericho County, Kenya. The site lies on a latitude 0^0 35' South, longitude 30^0 23' East. The experiments were conducted between December 2012 and August 2014.

3.2 Experimental Materials

i) The effects of auxin (NAA and IBA) concentrations and cutting positions on rooting and survival of the rootstock cultivars 'Rosa progress' and 'Natal briar' (Self rooting study).

The stem cuttings of the rootstock cultivars 'Rosa progress' and 'Natal briar' were selected from 3 months old mother stock at James Finlays Flowers Company in Kericho. The cuttings were taken from the vertical shoots on the rootstock mother plants based on the height, presence of a bud and 5-leaflet leaves then stored at 2-4^{o}C for one hour to remove the field heat. Medium sized vertical shoots, each measuring 120-150cm long, were selected and divided into three equal positions consisting of the bottom (first 3rd from bottom), the middle (second 3rd from bottom) and the top (last 3rd). Each stem cutting was 5-6cm long and consisted of a node, one five-

compound leaflet leaves and a bud. The stem cuttings were thereafter stored in the cold store at 2-4^{o}C for 12 hours before planting.

During planting, the basal ends of each of the cuttings from the above three positions were dipped in powdered form of indole-3-butyric acid (IBA) (w/W-Indole-3-butyric acid, Rhizophon, Chryzotek) at concentrations of 0%, 0.2%, 0.4% and 0.6% in the first experiment and 1-naphthaleneacetic acid (NAA) at concentrations of 0%, 0.2% and 1% for 5 seconds in the second experiment then immediately planted in jiffy bags containing clean sterile coccus with a pH of 6.5-7.5 and electrical conductivity (EC) of 0.18-0.24mS/cm, inside a greenhouse equipped with the misting and heating systems. The NAA powdered formulations were commercially available at 0.2% and 1% concentrations only.

After planting a relative humidity of ≥90%, temperature of 30-35^{o}C (day time) and 22-24^{o}C (night time) and misting cycles of 10-30 minutes (day time) and 1-2hrs (night time) were maintained in the first 2 weeks thereafter, the temperature, relative humidity and misting cycles were gradually reduced to harden the plants. Using shade netting, the light intensity was maintained at 300 watts/m^2 throughout the propagation period. Fertigation started 15 days after planting and continued every 4 days thereafter depending on measured EC. Each experimental unit had 20 potted plants. A total of 2880 rootstock cuttings from 240-288 shoots were used. The experiments were factorial in a randomized complete block design (RCBD) with 24 treatment combinations in the first experiment and 18 treatment combinations in the second experiment each replicated 6 times.

ii) The effects of cutting position of the rootstock cultivars and auxins on rooting and subsequent flower yield of top grafted rose variety 'Inca' (Top grafting study).

This study involved top grafting the scions of the variety 'Inca' on each of the above three cutting positions (top, middle and bottom) of the two rootstock cultivars. The source of the rootstock cultivars and the choice of the cuttings was the same as in the self rooting study. The cuttings for the rootstock had no leaves and where the buds appeared they were de-eyed especially in 'Natal briar' that has shorter internodes than 'Rosa progress'. The scions were obtained from the shoots of 'Inca' at full bloom stage at portions where five-leaflet leaves occurred. The scion and the rootstock were then joined together and the union held in place with a silicon material. The basal ends of each of the rootstock cutting positions were dipped in 0.4%IBA and 0.2%NAA concentrations. These auxin concentrations, 0.4%IBA and 0.2%NAA, were used because they showed higher percentage survival in the first study. The treated grafts were raised in the propagation unit under similar conditions as in the first study until transplanting time (30 days after planting) in the production greenhouse. Each experimental unit had 20 potted plants. A total of 2,880 rootstock cuttings and 2,880 scion cultivar cuttings were used. The experiment was factorial in a randomized complete block design.

Six rooted grafts from each of the 18 treatments in the propagation unit were transplanted in the production greenhouse at 35 days after planting. Planting was done on raised beds filled with pumice as a media and at a spacing of 20cm by 30cm. The cultural practices like fertigation, irrigation, pest and disease management and disbudding commenced after transplanting. The first bending of the main shoot was done at 5 weeks after transplanting and subsequent bending was done on crooked and weak stems. Crop protection was provided against diseases and pests by foliar spray using appropriate insecticides and fungicides, as and when required. Harvesting

started 3 months after transplanting and a flush lasted for 45 days. Harvesting the lateral shoots took place just above the second 5-leaflet set counted from the base. The field experiment ran from December 2013 to August 2014.

3.3 Treatments

3.3.1 Treatments for the self rooting study

a. IBA experiment

The treatments included two rootstock cultivars ('Rosa progress' and 'Natal briar'), four IBA concentrations (0%, 0.2%, 0.4%, and 0.6%) and three cutting positions (Top, middle and bottom) factorially combined.

b. NAA experiment

The treatments included two rootstock cultivars ('Rosa progress' and 'Natal briar'), three NAA concentrations (0%, 0.2% and 1%) and three cutting positions (Top, middle and bottom) factorially combined.

3.3.2 Treatments for the top grafting study

The treatments consisted two rootstock cultivars ('Rosa progress' and 'Natal briar'), three auxin concentrations (0%, 0.2%NAA and 0.4%IBA) and three cutting positions (Top, middle and bottom) factorially combined.

3. 4 Growth parameters measured.

a) In the propagation unit (Self rooting study and rooting of the grafts of the top grafting study)

To determine both fresh and dry shoot and root weights, 5 plants were sampled randomly from each treatment separated into roots and shoots and their weights were determined at 30 days after

planting using a digital electronic weighing scale. To determine root fresh weight (RFW), the roots were gently released from the media, washed under running tap water, scraped from stem using a sterilized budding knife and then wiped with a tissue paper to absorb surface moisture before being weighed using a digital electronic weighing scale. Samples were then oven dried to remove the moisture before being weighed again to obtain root dry weight. Ratios of RDW to SDW were also determined. Root number was determined by counting the number of primary roots. Root length was determined by measuring the length of the main roots. Five plants per treatment were sampled for shoot heights at the 21 and 28 days after planting. The shoot heights were measured using a centimeter ruler. The percentage rooting was determined by counting the cuttings that had roots while percentage shooted was determined by the number of cuttings that had shoots at 30 days after planting relative to all planted cuttings. Percentage survival and grafting take were obtained by counting the cuttings and grafts respectively with both shoot and roots at 30 days after planting compared to all planted cuttings and expressed in percentage. Percentage mortality was determined by counting the grafts that died in relation to the total number of grafts planted.

b) In the production greenhouse (Top grafting study)

Number of bud breaks after bending was obtained by counting. Time to first harvest was determined by counting the number of days from the time of planting to the time the flower started opening. Stem diameter (3 cm above the base) was measured using a caliper and length (from the stem base to uppermost flower part) was measured using a meter rule at the time of harvesting. Number of harvested stems per flush per meter square was obtained by counting at 3 months after planting, and thereafter after every 45 days upto the third harvest/flush. The flush interval was obtained by determining the number of days between one flush and the next. Vase

life was determined by computing the numbers of days the harvested flowers take in the preservative solution till the leaves and petals start fading under normal room temperature.

3.5 Biochemical analysis

3.5 .1 Analysis of hormones for the self rooting study

Eighty (80) extra non-auxin treated cuttings from the same two rootstock cultivars and two cutting positions (top and bottom) above were planted alongside the main experiments above to provide samples for hormone analysis. They were replicated 3 times. Samples from the stem base and bud region (1cm each, Figure 1) of the unrooted and rooted stem cuttings of the rootstock cultivars 'Natal briar' and 'Rosa progress' were collected between 10.00-10.30 a.m by shock freezing under liquid nitrogen, stored in a freezer at -80^{o}C at International Livestock Research Institute (ILRI) for 10 days then transported in dry ice to Leibniz Institute for vegetables and ornamental crops in Germany, for analysis of IAA, and cytokinins. The samples were collected on days 0, 1, 2, 4 and 8 after sticking the cuttings. The samples for each treatment were packed in labelled aluminium foils which were later transfered into Eppendorf vials during extraction.

Figure 1. Sections of the stem sampled for hormone analysis.

3.5.1.1 Analysis of IAA on the rootstock cuttings.

a. Homogenization

The frozen stem cuttings for each treatment were transferred to a frozen stainless steel container with 1 stainless steel ball of diameter 6mm then immediately placed to a vibrating-ball micromill (Retsch MM301, Haan, Germany) for 5 minutes at a vibration frequency of 30 vibrations s^{-1}. After homogenization, each sample was divided into four portions and each portion was placed into 2ml Epperndorf tube, cooled under liquid nitrogen then stored at -80^{o}C until extraction.

b. Extraction

Extraction, clean-up and analysis of IAA were carried out according to a modified protocol as described by Muller *et al.* (2002). The homogenized frozen plant material weighing 50-150mg from the stem base and bud region of the unrooted and rooted cuttings were transferred into a 1.5ml Eppendorf vial and the following were added to the sample: 990µl methanol, 10µl of internal standard (0.26 pmol/µl (2H) 2-IAA and 2.06 pmol/µl (2H) 6-ABA and Jasmonic acid) and 5 stainless steel balls with diameter of 3 mm each. The samples were heated for 20 min at 60°C then immediately shook in a shaker for 20 mins at a vibration frequency of 30 vibrations s^{-1} and then vortexed. After incubation for 15 min at room temperature the extract was centrifuged for 10 min at 13000 rpm and then the supernatant was pipetted into a new 1.5 ml Eppendorf vial. The residue was re-suspended in 300 µl methanol, vortexed and after incubation for 15 min at room temperature centrifuged again for 10 min at 13000 rpm. The pooled supernatants were centrifuged, transferred into the new Eppendorf vial and reduced to dryness in a vacuum centrifuge (Savant SPD 111 V, Fischer Scientific, Schwerte, Germany) at 40°C for 30 min at 320 mbar and thereafter at 200 mbar.

c. Solid phase extraction and methylation

The dried sample was dissolved in 50µl methanol by using a vortex and a subsequent ultrasonic treatment for 5 min. After centrifugation for 2-5 minutes, 200µl diethyl ether was added, followed by vortexing, ultrasonic treatment for 5 min and centrifuging again for 10 min at 13000 rpm. The aminopropyl solid phase extraction column (Chromabond NH2 shorty 10 mg, Macherey-Nagel GmbH &Co.KG, Duren, Germany) was first equilibrated with 200 µl diethyl ether before application of the dissolved sample. The column was then washed twice with 200 µl diethyl ether, three times with 200µl of a mixture of chloroform/2-propanol (2:1, v/v), three times with 200µl chloroform and lastly with 100µl diethyl ether. The IAA fractionwas eluted three times with 200µl diethyl ether containing 4% acetic acid. Combined eluates were reduced to dryness in a stream of nitrogen at room temperature re-dissolved in 20µl methanol, methylated with 200µl ethereal diazomethane, dried again in a stream of nitrogen and dissolved in 10µl ethyl acetate.

d. Analysis using Gas chromatography-Mass spectrometry/Mass spectrometry (GC-MS/MS)

Separation and mass fragment analysis were conducted using a Varian Saturn 2200 ion-trap mass spectrometer (MS) connected to a CP-3800 gas chromatograph (GC) fitted with a Combial auto-injector (Agilent, Santa Clara, California, USA). The GC settings were as follows: splitless injection (1 µl) with 1min pressure pulse at 24 psi; splitter opening 1:100 after 1min; columns: Phe-Sil retention gap 10 m x 0.32 mm ID, ZB-50 50% Phenyl-50% Dimethylpolysiloxane 30 m x 0.25 mm ID x 0.25µm film thickness, Phenomenex;carrier gas: He, 1 ml min^{-1}, constant flow; temperature program: 1 min isothermally at 60^{o}C, followed by a linear ramp at a rate of 40^{o}C min-1 to 150^{o}C, isothermally for 6 min at 150^{o}C, followed by linear ramp of 20^{o}C min-1 at

250^{o}C; transfer line temperature 230^{o}C. The MS settings were as follows: CI-MRM mode; positive ion detection; reactant gas methanol; temperatures of manifold and ion trap 60^{o}C and 200^{o}C, respectively; axial modulation 4V; scan time 0.4s scan^{-1}; multiplier offset 300V; emission current 50µA; maximum ionisation time 2 ms; maximum reaction time 128 ms; wave form: resonant. Settings for endogenous IAA were chosen as follows: parent ion (m/z)=190(M+H)+, diagnostic production ion=130, excitation amplitude 0.5V. A second channel analysing the isotopically labelled standard (2H)2-IAA used the parent ion (m/z)=192 (M+H)+ and the diagnostic daughter ion (m/z)=132. The amount of endogenous compound was calculated from the signal ratio of the unlabelled over the corresponding stable isotope-containing mass fragments. Recovery of the isotopically labeled standard was close to 50% (Ahkami *et al*., 2013).

3.5.1.2 Analysis of cytokinins

a. Extraction of cytokinins

The extraction was done according to the procedure of Kojima *et al*. (2009). 1 ml of ice-cold solution (extraction buffer) of methanol: formic acid: water in a ratio of 15:1:4 was added to 100-140mg of frozen homogenized sample. The homogenate was thoroughly mixed and stored at -20°C for 16 h. After incubation the homogenate was centrifuged at 4ºC for 20 mins at 13000 rpm. The supernatant was transferred to 2 ml Eppendorf tube and the remaining pellet re-extracted with 300µl of extraction buffer, centrifuged, the resultant supernatant added to the first epperndorf tube and reduced to dryness in a vacuum centrifuge at 40°C for 3½ hours at 200 mbars. The dried samples were first re-suspended in 100µl 80 % methanol then 900µl of 1 M formic acid was added prior to separation with MCX columns.

b. Separation and elution of cytokinins

Separation of the different hormones was done using MCX column (Oasis® MCX Sorbent).The MCX columns were equilibrated first with 1 ml acetonitrile, followed by addition of 1 ml methanol (MeOH) then 1 ml of 1 M formic acid and lastly 1 ml 0.1 M HCl was added. The sample was loaded onto the column and after all the samples had passed through the column, 1 ml of 1 M formic acid was added to the column and the eluent discarded. Elution was done with 1 ml 0.35 M ammonia (NH_3) dissolved in 60 % MeOH to collect cytokinins. The eluents were dried under vacuum at 38ºC for about 3½ hours. The dried eluents were re-dissolved in 50-100µl 25 % MeOH.

c. Analysis using UPLC-ESI-MS/MS

The UPLC-ESI-MS/MS analysis were carried out using an Agilent 1290 infinity system connected to an Agilent triple quadruple mass spectrometer QQQ6490 (Agilent Germany). Separated compounds were ionized at atmospheric pressure via electrospray and directed to the mass spectrometer. The control of the complete system and recording of the spectra were performed with the MassHunter, release B.04.00 (B4038). To separate the individual cytokinins a UPLC system was used including a gradient pump, an autosampler, and a column compartment. Separation was carried out using a high-capacity column (Eclipse Plus C18, RRHD 1.8µm, 2.1 × 50 mm). Gradient was accomplished with LCMS grade water (Chem solute, Th. Geyer, Germany) containing 0.1% formic acid (Fluka, Germany) as buffer A and LCMS grade methanol (Chem solute, Th. Geyer, Germany) including 0.1% formic acid as buffer B. The column was equilibrated with a mixture of buffer A (86.5%) and buffer B (13.5%) at a flow rate of 0.4 ml min^{-1} and heated at 40 °C during the whole measurement. The gradient was produced

by changes of the buffer B as follows: 0–5 min at 18%, 5–6 min at 70%, 6–7 min at 99%, 7–7.1 min at 13.5%, and kept up to 9 min at 13.5%. The whole duration of the run was 9.0 min.

The MS/MS analysis was performed using a triple quadruple 6490 of the Agilent Company. The following parameters were employed: desolvation temperature 350 °C, desolvation nitrogen gas of 720 l hr^{-1}, capillary voltage 2.0 KV, detection in positive ion mode and different dwell times between 20 and 200s. Collision energy differed among the compounds. Protonated ions [M–H]+ were monitored with a span of 1 amu. Multiple reactions monitoring (MRM) was performed to identify individual compounds accurately. A mixture of [15N4]–cis-zeatin and [2H5]–trans-zeatin riboside was used as internal standard for testing the stability of the instruments and the retention times.

3.5.2 Carbohydrates analysis.

Two hundred and fourty (240) extra non-auxin treated cuttings from the same two rootstock cultivars and three cutting positions above were planted alongside the main experiments above to provide samples for carbohydrate and mineral nutrient analysis. They were replicated 3 times.

Five samples from the stem base of stem cuttings (1 cm in length from the basal end) from each of the different cutting positions of the rootstock cultivars (‘Natal briar’ and Rosa ‘progress’) were collected on the day of planting (0 day) and during the first week of adventitious root formation on days 3 and 7 after planting for carbohydrate analysis. Five fresh leaf samples with one- five leaflet leaf of the same cuttings where stem base samples were obtained were also collected on the day of planting (0 day) for carbohydrate analysis. Soluble carbohydrate content (fructose, glucose and sucrose) was determined from chromatographic separation of sugars and their retention time using high performance liquid chromatography (HPLC) method (Rybak-Chmielewska, 2007; Dimins *et al*., 2008). Five grams of fresh leaf tissues and stem base cuttings

from the three cutting positions of the rootstock cultivars ('Natal briar' and Rosa 'progress') were ground in a Wiley mill to homogenize them. The samples were then extracted in 40ml of 96% ethanol followed by centrifugation at 13,800rpm for 20 minutes. The extracts were then filtered through Whatman No. 2 filter paper then reconstituted with 50% v/v HPLC-grade acetonitrile in water. The analysis by HPLC was performed at 25±1 °C under isocratic conditions. The mobile phase consisted of A – acetonitrile and B – water (75:25). Flow rate was 1.4 ml min^{-1} and injection volume was 5 µL. The aminopropylsilyl column (Knauer Eurospher 100-5 NH2) and refractive index detector (RI Knauer S.No 102129) were used for identification of sugars in the stem and leaf samples for each position by comparing retention times of individual carbohydrates in the reference vs. tested solution (qualitative analysis). The quantitative assays were made by the following carbohydrates: fructose, glucose and sucrose. The content of these compounds were assessed based on comparing peak areas obtained from the reference analysis.

3.5.3 Mineral nutrient analysis

Total nitrogen in fresh samples of stem base of cuttings (1 cm in length from the basal end) were determined by digesting 0.3 g of the sample in a mixture of Selenium, Lithium sulphate, Hydrogen peroxide and concentrated Sulphuric acid (Anderson and Ingram, 1993). The Nitrogen content in the digests was determined colorimetrically (Cline *et al*., 1986). Digestion was done at 550^0C for 5 hours in a block digester. For determination of the micronutrients, 0.3 g of fresh samples of stem cuttings (1 cm of the stem base) was placed in a 50 m1 Erlenmeyer flasks then 17 ml of DTPA extracting solution added and shaken for 2 hours. Extractable zinc, iron and manganese were determined with the Atomic absorption spectrophotometry method (Okalebo *et al*., 2002).

3.6 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using GENSTAT statistical package. Significant differences among treatment means were separated using Least significant difference (LSD) at 5%. Correlation analyses were done to determine the relationship between carbohydrate level and mineral nutrient levels of the various cutting positions and rooting percentage.

CHAPTER FOUR: RESULTS

4.1 SELF ROOTING STUDY

4.1.1. Effects of rootstock cultivars, cutting position and auxin concentrations on rooting percentage (%) at 30 days after planting.

The main factor of cutting position significantly ($p \leq 0.05$) influenced the % rooting in both IBA and NAA experiments (Tables 3a and 3b). Irrespective of IBA and NAA concentrations and rootstock cultivars, bottom position cuttings had significantly lower rooting percentage than the middle and top position cuttings.

The interaction between the rootstock cultivars and IBA treatment significantly ($p \leq 0.05$) influenced the % rooting in the IBA experiment (Table 3a). In 'Natal briar', 0.2%IBA treated cuttings had significantly ($p \leq 0.05$) lower (75.9%) rooting percentage than 0.4%IBA (93.3%) but was not significantly different from the control (82.4%) and 0.6%IBA (87.0%) treated cuttings. Though, IBA treatment had no significant ($p \leq 0.05$) effect on rooting percentage in 'Rosa progress', the control had higher rooting percentage than 0.4% IBA treated cuttings. NAA treatment had no significant ($p \leq 0.05$) effect on rooting percentage (Table 3b).

Table 3a: Effects of rootstock cultivars, cutting position and IBA concentrations on rooting percentage (%) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	94.4	97.2	93.3	94.4	94.9	85.0	Top	IBA concentration	NS
	Middle	86.1	90.0	86.7	93.3	89.0		91.2	Rootstocks	NS
	Bottom	86.7	68.3	60.0	68.9	71.0			Position	6.85*
	Means	89.1	85.2	80.0	85.6				IBA x rootstock	11.18*
								Middle	IBA x position	NS
'Natal briar'	Top	88.9	86.7	97.8	76.7	87.5	84.7	89.7	Rootstocks x position	NS
	Middle	83.3	84.4	95.6	97.8	90.3			Rootstock x position x IBA	NS
	Bottom	75.0	56.7	86.7	86.7	76.2		Bottom		
	Means	82.4	75.9	93.3	87.0			73.6		
Grand IBA means		85.7	80.6	86.7	86.3					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 3b: Effects rootstock cultivars, cutting position and NAA concentrations on rooting percentage (%) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	94.4	97.8	95.6	95.9	88.3	Top	NAA concentration	NS
	Middle	86.1	91.7	93.3	90.4			Rootstocks	NS
	Bottom	86.7	82.2	66.7	78.5		90.9	Position	7.28*
	Means	89.1	90.6	85.2				NAA x rootstock	NS
							Middle	NAA x position	NS
'Natal briar'	Top	88.9	77.8	91.1	85.9	85.5	89.8	Rootstocks x position	NS
	Middle	83.3	86.7	97.8	89.3			Rootstock x position x NAA	NS
	Bottom	75.0	86.8	82.2	81.3		Bottom		
	Means	82.4	83.8	90.4			79.9		
Grand NAA means		85.7	87.2	87.8					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; ** significant at $p \leq 0.001$, NS= Not significant*

4.1.2. Effects of rootstock cultivars, cutting position and auxin concentrations on root number at 30 days after planting.

The main factors of rootstock cultivars, cutting position and the interaction between the two had significant ($p \leq 0.05$) effect on root number in the IBA and NAA experiments (Tables 4a and 4b). Irrespective of IBA and NAA concentrations and cutting position, significantly higher root number was obtained from the rootstock 'Rosa progress' than the rootstock 'Natal briar'. Averaged across the rootstock cultivars and IBA and NAA concentrations, the bottom position cuttings had significantly lower root number than the top and middle position cuttings, however, the root number of the latter two positions were not significantly different from each other. In the IBA experiment, bottom position cuttings had significantly lower (14.88) root number than the middle position (24.04) cuttings in 'Rosa progress' (Table 4a). The root number of the latter position was not significantly different from those of the top position (21.25) cuttings. In 'Natal briar', the cutting position had no significant effect on the root number in the IBA experiment, however, the trend was similar to that observed in 'Rosa progress'. In the NAA experiment, for 'Rosa progress'the trend was similar to that recorded in the IBA experiment (Table 4b). In 'Natal briar' the top position cuttings had significantly higher root number than the middle and bottom position cuttings. However, the root number of the latter two positions was not significantly different from each other (Table 4b).

The main factors of IBA and NAA concetrations had significant ($p \leq 0.05$) effect on root number in both experiments (Tables 4a and 4b). Averaged across the cutting position and the rootstock cultivars, the number of roots significantly increased when the auxins were used and it ranged from 10.67 (control) to 21.36 (0.6%IBA). Among the auxin treated cuttings, significantly lower (17.94) root number was obtained from 0.2%IBA than 0.6%IBA treated cuttings. The root

number of 0.4%IBA treated cuttings (20.62%) was not significantly different from those of 0.2%IBA and 0.6%IBA treated cuttings (Table 4a). Averaged across the cutting position and the rootstock cultivars, number of roots of the NAA treated cuttings was significantly higher than those cuttings not treated with NAA, however, the root number of the 1%NAA and 0.2%NAA treated cuttings were not significantly different from each other (Table 4b). The interaction of NAA concentration and the rootstock cultivars was significant for the root number and the control had significantly lower (11.32) root number than 1%NAA (16.45) and 0.2%NAA (19.31) in 'Rosa progress'. In 'Natal briar', the control had significantly lower root number than 0.2% treated cuttings, however, the root number of 1%NAA treated cuttings was not significantly different from the control and 0.2%NAA treated cuttings.

Table 4a: Effects of rootstock cultivars, cutting position and IBA concentrations on root number at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	12.30	20.95	23.95	27.58	21.25	20.06	Top	IBA concentration	3.2**
	Middle	14.48	26.88	26.30	28.48	24.04		19.08	Rootstocks	2.3**
	Bottom	6.98	16.38	20.37	15.80	14.88			Position	2.81**
	Means	11.32	21.41	23.54	23.96				IBA x rootstock	NS
								Middle	IBA x position	NS
'Natal briar'	Top	13.43	16.67	17.90	19.67	16.92	15.24	19.95	Rootstocks x position	4.0*
	Middle	9.13	15.47	19.90	18.98	15.87			Rootstock x position x IBA	NS
	Bottom	7.47	11.28	15.33	17.63	12.93		Bottom		
	Means	10.01	14.47	17.71	18.76			13.91		
Grand IBA means		10.67	17.94	20.62	21.36					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 4b: Effects of rootstock cultivars, cutting position and NAA concentrations on root number at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	12.50	25.37	18.47	18.79	15.69	Top	NAA concentration	2.45**
	Middle	14.48	19.83	17.53	17.28		16.35	Rootstocks	2.14**
	Bottom	6.98	12.73	13.35	11.02			Position	2.45**
	Means	11.32	19.31	16.45			Middle	NAA x rootstock	2.60*
							14.18	NAA x position	NS
'Natal briar'	Top	13.43	14.87	13.40	13.90	11.45		Rootstocks x position	2.60*
	Middle	9.13	13.23	10.87	11.08			Rootstock x position x NAA	NS
	Bottom	7.47	11.02	9.70	9.39		Bottom		
	Means	10.01	13.04	11.32			10.21		
Grand NAA means		10.67	16.18	13.89					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.3. Effects of rootstock cultivars, cutting position and auxin concentrations on total root length (cm) at 30 days after planting.

The main factors of cutting position and rootstock cultivars significantly ($p \leq 0.05$) influenced the total root length in both the IBA and NAA experiments (Table 5a and 5b). Averaged across the rootstock cultivars and IBA treatment, the bottom position cuttings had significantly lower (122.6cm) total root length than the middle (190.9cm) and top (175cm) position cuttings (Table 5a). The total root length of the latter two positions were however, not significantly different from each other. Averaged across the cutting position and IBA treatment, 'Rosa progress' had significantly higher (182.9cm) total root length than 'Natal briar' (142.8cm). The effect of cutting position was dependent on the rootstock cultivars in the NAA experiment, in 'Rosa progress', bottom position cuttings yielded significantly ($p \leq 0.05$) lower total root length than the top and middle position cuttings, however, the total root length of the latter two positions were not significantly different from each other (Table 5b). In 'Natal briar', cutting position had no significant effect on total root length.

The total root length was significantly ($p \leq 0.05$) affected by the main factor of auxin in both IBA and NAA treated cuttings (Tables 5a and 5b). Averaged across the rootstock and cutting position, the total root length significantly increased with IBA application and the control had significantly lower total root length than the IBA treated cuttings, however the total root length of the IBA treated cuttings were not significantly different from each other (Tables 5a). In the NAA experiment, the control had significantly lower total root length than the 0.2%NAA treated cuttings. The total root length of 1%NAA treated cuttings was however not significantly different from the control and 0.2%NAA treated cuttings (Table 5b).

Table 5a: Effects of rootstock cultivars, cutting position and IBA concentrations on total root length (cm) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	133.9	180.9	219.9	233.6	192.1	182.9	Top	IBA concentration	51.56**
	Middle	139.1	236.4	244.7	273.6	223.4		175.0	Rootstocks	36.46*
	Bottom	45.0	143.5	204.6	139.6	133.2			Position	44.65*
	Means	106.0	186.9	223.1	215.6				IBA x rootstocks	NS
								Middle	IBA x position	NS
'Natal briar'	Top	111.8	161.8	166.6	191.5	157.9	142.8	190.9	Rootstocks x position	NS
	Middle	79.6	159.0	222.7	171.9	158.3			Rootstock x position x IBA	NS
	Bottom	55.4	90.3	163.8	138.9	112.1		Bottom		
	Means	82.3	137.0	184.3	167.4			122.6		
Grand IBA means		94.1	162.0	203.7	191.5					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 5b: Effects of rootstock cultivars, cutting position and NAA concentrations on total root length (cm)at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	133.9	223.0	172.1	176.3	138.8	Top	NAA concentration	37.39*
	Middle	139.1	193.7	150.3	161.0		145.2	Rootstocks	30.53*
	Bottom	45.0	91.5	101.1	79.2			Position	37.39*
	Means	106.0	169.4	141.1				NAA x rootstock	NS
							Middle	NAA x position	NS
'Natal briar'	Top	111.8	115.6	115.1	114.2	101.9	134.1	Rootstocks x position	60.76*
	Middle	79.6	124.8	117.2	107.2			Rootstock x position x NAA	NS
	Bottom	55.4	91.8	105.5	84.3		Bottom		
	Means	82.3	110.7	112.6			81.7		
Grand NAA means		94.1	140.1	126.9					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.4. Effects of rootstock cultivars, cutting position and auxin concentrations on root fresh weight (g) at 30 days after planting.

The root fresh weight was significantly ($p \leq 0.05$) influenced by the main factors of rootstock cultivars and cutting position in both NAA and IBA experiments (Tables 6a and 6b). Averaged across the cutting position and auxins, significantly ($p \leq 0.05$) higher root fresh weight was obtained from the rootstock 'Natal briar' than the rootstock 'Rosa progress' in both experiments (Tables 6a and 6b). Averaged across the IBA concentration and the rootstock cultivars, the RFW of the bottom position cuttings was significantly lower than those of the middle and top position cuttings but the RFW of the latter two positions were not significantly different from each other (Table 6a). Averaged across the NAA concentrations and the rootstock cultivars, root fresh weight significantly decreased from the top (0.97g) to the middle (0.80g) and bottom (0.60g) position cuttings (Table 6b). The root fresh weight was significantly ($p \leq 0.05$) influenced by the interaction between rootstock cultivars and the cutting position in the IBA experiment (Table 6a). The root fresh weight significantly increased from the bottom (0.65g) to the middle (1.03g) and to the top (1.27g) position cuttings in 'Natal briar'. In 'Rosa progress' the RFW of the top and middle position cuttings were significantly higher than the bottom position cuttings. However, the RFW of the former two positions were not significantly different from each other (Table 6a).

The main factor of IBA concentration and its interaction with the rootstock cultivar had significant ($p \leq 0.05$) effect on root fresh weight (RFW) (Table 6a). Irrespective of the cutting position and rootstock cultivar, the control had significantly lower (0.69g) root fresh weight than IBA treated cuttings. The RFW of IBA treated cuttings were however, not significantly ($p \leq 0.05$) different from each other. In 'Rosa progress', the control had significantly lower (0.62g)

root fresh weight than 0.4%IBA (0.90g) treated cuttings. In ‘Natal briar’, the control had significantly lower (0.75g) root fresh weight than IBA treated cuttings. The RFW of IBA treated cuttings were however, not significantly ($p \leq 0.05$) different from each other in both rootstock cultivars. NAA application had no significant ($p \leq 0.05$) effect on RFW of both rootstock cultivars (Table 6b).

Table 6a: Effects of rootstock cultivars, cutting position and IBA concentrations on root fresh weight (g) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.77	0.95	0.89	0.93	0.89	0.77	Top	IBA concentration	0.17*
	Middle	0.75	0.78	1.05	0.96	0.88		1.08	Rootstocks	0.12**
	Bottom	0.33	0.54	0.75	0.59	0.55			Position	0.15**
	Means	0.62	0.76	0.90	0.83				IBA x rootstock	0.22*
								Middle	IBA x position	NS
'Natal briar'	Top	0.98	1.35	1.30	1.46	1.27		0.96	Rootstocks x position	0.20*
	Middle	0.69	1.09	1.16	1.17	1.03	0.98		Rootstock x position x IBA	NS
	Bottom	0.59	0.56	0.78	0.66	0.65		Bottom		
	Means	0.75	1.00	1.08	1.10			0.60		
Grand IBA means		0.69	0.88	0.99	0.96					

*LSD= Least Significant Difference of means, * significant at p ≤ 0.05; ** significant at p ≤ 0.001, NS= Not significant*

Table 6b: Effects of rootstock cultivars, cutting position and NAA concentrations on root fresh weight (g) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.77	0.92	0.77	0.82	0.70	Top	NAA concentration	NS
	Middle	0.75	0.75	0.83	0.77		0.97	Rootstocks	0.11*
	Bottom	0.33	0.63	0.57	0.51			Position	0.13**
	Means	0.62	0.76	0.72			Middle	NAA x rootstock	NS
							0.80	NAA x position	NS
'Natal briar'	Top	0.98	1.14	1.22	1.11	0.83		Rootstocks x position	NS
	Middle	0.69	0.97	0.84	0.83		Bottom	Rootstock x position x NAA	NS
	Bottom	0.59	0.60	0.46	0.55		0.53		
	Means	0.75	0.90	0.84					
Grand NAA means		0.69	0.83	0.78					

*LSD= Least Significant Difference of means, *significant at p ≤ 0.05; **significant at p ≤ 0.001, NS= Not significant*

4.1.5. Effects of rootstock cultivars, cutting position and auxin concentrations on root dry weight (g) at 30 days after planting.

The main factors of rootstock cultivars and cutting position had significant ($p \leq 0.05$) effect on root dry weight (RDW) in both the NAA and IBA experiments (Tables 7a and 7b). Irrespective of cutting position and auxins, the rootstock 'Natal briar' had significantly higher RDW than the rootstock 'Rosa progress' in both experiments. Averaged across the rootstock cultivars and IBA and NAA treatments, significantly ($p \leq 0.05$) lower RDW was obtained from the bottom position cuttings than from the top and middle position cuttings in both experiments (Tables 7a and 7b). The root dry weight of the top and middle position cuttings were however, not significantly different from each other. The effect of cutting position was dependent on the rootstock cultivars in both experiments. In 'Natal briar', bottom position cuttings yielded significantly ($p \leq 0.05$) lower RDW than the top and middle position cuttings, however, the RDW of the latter two positions were not significantly different from each other in both experiments (Tables 7a and 7b). In 'Rosa progress', the cutting position had no significant effect on RDW (Tables 7a and 7b).

The main factor of IBA concentration significantly ($p \leq 0.05$) influenced the root dry weight (RDW) (Table 7a). Irrespective of the cutting position and rootstock cultivar, the control had significantly lower (0.09g) root dry weight than 0.4%IBA (0.12g) treated cuttings. The RDW of IBA treated cuttings were however, not significantly ($p \leq 0.05$) different from each other. NAA application had no significant ($p \leq 0.05$) effect on RDW of both rootstock cultivars (Table 7b).

Table 7a: Effects of rootstock cultivars, cutting position and IBA concentrations on root dry weight (g) at 30 daysafter planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.09	0.11	0.09	0.09	0.09	0.08	Top	IBA concentration	0.02**
	Middle	0.09	0.08	0.09	0.10	0.09		0.13	Rootstocks	0.02**
	Bottom	0.06	0.05	0.08	0.08	0.07			Position	0.02**
	Means	0.08	0.08	0.09	0.09				IBA x rootstock	NS
								Middle	IBA x position	NS
'Natal briar'	Top	0.14	0.15	0.17	0.18	0.16	0.13	0.11	Rootstocks x position	0.03*
	Middle	0.09	0.14	0.15	0.14	0.13			Rootstock x position x IBA	NS
	Bottom	0.05	0.09	0.13	0.09	0.09		Bottom		
	Means	0.09	0.13	0.15	0.14			0.08		
Grand IBA means		0.09	0.10	0.12	0.11					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 7b: Effects of rootstock cultivars, cutting position and IBA concentrations on root dry weight (g) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.09	0.11	0.09	0.09	0.08	Top	NAA concentration	NS
	Middle	0.09	0.09	0.08	0.09		0.12	Rootstocks	0.02**
	Bottom	0.06	0.06	0.06	0.06			Position	0.02*
	Means	0.08	0.09	0.08			Middle	NAA x rootstock	NS
'Natal briar'							0.10	NAA x position	NS
	Top	0.14	0.15	0.14	0.14			Rootstocks x position	0.03*
	Middle	0.09	0.13	0.11	0.11		Bottom	Rootstock x position x NAA	NS
	Bottom	0.05	0.08	0.09	0.07	0.11	0.07		
Grand NAA means	Means	0.09	0.12	0.11					
		0.09	0.10	0.09					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.6. Effects of rootstock cultivars, cutting position and auxin concentrations on shooting percentage (%) at 30 days after planting.

The main factors of cutting position and rootstock cultivars significantly ($p \leq 0.05$) influenced the shooting percentage in both experiments (Tables 8a and 8b). Irrespective of the rootstock and IBA concentration, the shooting percentage significantly increased from the bottom (67.7%) followed by the middle (79.0%) and top (89.9%) position cuttings (Table 8a). In the NAA experiment, bottom position cuttings yielded significantly lower shooting percentage than the middle and top position cuttings, however, the shooting percentage of the latter two positions were not significantly different from each other (Table 8b). Irrespective of the cutting position and IBA and NAA concentrations, the rootstock 'Rosa progress' had significantly higher shooting percentage than 'Natal briar'(Tables 8a and 8b).

The main factors of auxins and its interaction with the cutting position significantly ($p \leq 0.05$) influenced the shooting percentage in both NAA and IBA experiments (Table 8a and 8b). Irrespective of the rootstock cultivars and cutting position, the cuttings treated with 0.2%IBA (70.6%), 0.6%IBA (73.8%) and 0.4%IBA (80.7%) had significantly lower shooting percentage than the untreated cutting (90.5%). Cuttings treated with 0.4%IBA had significantly higher shooting percentage than those treated with 0.2%IBA. However, the shooting percentage of 0.6%IBA treated cuttings was not significantly different from the 0.2%IBA and 0.4%IBA treated cuttings (Table 8a). In 'Rosa progress', the control had significantly higher (94.8%) shooting percentage than 0.4%IBA (80.0%) treated cuttings however, the shooting percentage for the control was not significantly different from the 0.2%IBA and 0.6%IBA treated cuttings. In 'Natal briar', 0.2%IBA and 0.6%IBA treated cuttings had significantly lower shooting

percentage than the control and 0.4%IBA treated cuttings that were not significantly different from each other.

In the NAA experiment, averaged across the rootstock cultivars and the cutting position, the control (90.5%) had significantly higher shooting percentage than the 0.2%NAA (75.9%) and 1%NAA (79.6%) treated cuttings (Table 8b). In 'Rosa progress' the control had significantly higher shooting percentage than 1%NAA treated cuttings. However, the shooting percentage of the 0.2%NAA treated cuttings was not significantly different from the control and the 1%NAA treated cuttings (Table 8b). In 'Natal briar'the shooting percentage of 0.2%NAA (63.9%) treated cuttings was significantly lower than the control (86.1%) and 1%NAA (75.9%) treated cuttings, however, the shooting percentage of the latter two treatments were not significantly different from each other (Table 8b). The interaction between the cutting position and NAA concentration was also significant for shooting percentage (Table 8b). Bottom position cuttings treated with 0.2%NAA, had significantly ($p \leq 0.05$) lower (57.8%) percent shooted than the top (86.7%) and middle (83.3%) position cuttings. The shooting percentages of cuttings from the three positions treated with 1%NAA followed a similar trend to that observed in the 0.2%NAA treated cuttings. The top position cuttings untreated with NAA had significantly higher (98.6%) than the middle (84.7%) and bottom (88.1%) position cuttings.

Table 8a: Effects of rootstock cultivars, cutting position and IBA concentrations on shooting percentage (%) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	97.2	97.2	91.1	91.7	94.3	86.1	Top	IBA concentration	8.73**
	Middle	91.7	90.0	86.7	88.9	89.3		89.9	Rootstocks	6.17**
	Bottom	95.5	65.0	62.2	75.6	74.6			Position	7.56**
	Means	94.8	84.1	80.0	85.4			Middle	IBA x rootstock	12.34*
								79.0	IBA x position	NS
'Natal briar'	Top	100.0	73.3	95.6	73.3	85.6	71.7		Rootstocks x position	NS
	Middle	77.8	59.4	73.3	64.4	68.7		Bottom	Rootstock x position x IBA	NS
	Bottom	80.6	38.3	75.6	48.9	60.8		67.7		
	Means	86.1	57.0	81.5	62.2					
Grand IBA means		90.5	70.6	80.7	73.8					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; ** significant at $p \leq 0.001$, NS= Not significant*

Table 8b: Effects of rootstock cultivars, cutting position and NAA concentrations on shooting percentage (%) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	97.2	95.6	88.9	93.9	88.8	Top	NAA concentration	7.64**
	Middle	91.7	95.0	91.1	92.6		91.0	Rootstocks	6.24**
	Bottom	95.6	73.3	77.1	80.0			Position	7.64**
	Means	94.8	88.0	83.7			Middle	NAA x rootstock	10.81*
							85.3	NAA x position	13.24**
'Natal briar'	Top	100.0	77.8	86.7	88.1			Rootstocks x position	NS
	Middle	77.8	71.7	84.4	78.0		Bottom	Rootstock x position x NAA	NS
	Bottom	80.6	42.2	55.6	59.4	75.2	69.7		
	Means	86.1	63.9	75.6					
Grand NAA means		90.5	75.9	79.6					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.7. Effects of rootstock cultivars, cutting position and auxin concentrations on shoot height (cm) at 21 days after planting.

The shoot height was significantly ($p \leq 0.05$) influenced by the cutting position, rootstock cultivars and the interaction between the rootstock cultivars and cutting position at 21 days after planting in both IBA and NAA experiments (Tables 9a and 9b). Irrespective of the cutting position and the IBA and NAA concentrations, the rootstock 'Rosa Progress' produced significantly taller shoots than the rootstock 'Natal briar' (Tables 9a and 9b). Irrespective of the rootstock cultivars and the IBA and NAA concentrations, the bottom position cuttings exhibited significantly shorter shoots than the top and middle position cuttings, however, the latter two positions were not significantly different from each other (Tables 9a and 9b). In both experiments, the top position cuttings had significantly ($p \leq 0.05$) taller shoots than the bottom position cuttings in 'Rosa progress'. However, no significant difference was observed in shoot heights from both the top and middle positions cuttings. In 'Natal briar', the shoot heights among the cutting positions were not significantly different from each other in both experiments but the tallest shoots were obtained from the top position cuttings (Tables 9a and 9b).

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Table 9a: Effects of rootstock cultivars, cutting position and IBA concentrations on shoot height (cm) at 21 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	5.12	5.88	4.88	6.40	5.57	4.47	Top	IBA concentration	NS
	Middle	5.25	4.16	5.44	5.85	5.17		3.46	Rootstocks	0.62**
	Bottom	2.16	2.13	2.88	3.45	2.68			Position	0.76**
	Means	4.18	4.06	4.40	5.26				IBA x rootstock	NS
								Middle	IBA x position	NS
'Natal briar'	Top	1.41	0.98	1.56	1.40	1.34	0.81	2.87	Rootstocks x position	1.07**
	Middle	0.73	0.48	0.62	0.40	0.56			Rootstock x position x IBA	NS
	Bottom	0.65	0.47	0.76	0.30	0.55		Bottom		
	Means	0.93	0.64	0.98	0.70			1.61		
Grand IBA means		2.56	2.35	2.69	2.98					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 9b: Effects of rootstock cultivars, cutting position and NAA concentrations on shoot height (cm) at 21 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	5.61	4.99	4.42	5.01	4.04	Top	NAA concentration	NS
	Middle	5.61	4.03	5.09	4.91		3.13	Rootstocks	0.62**
	Bottom	2.14	2.43	2.01	2.19			Position	0.76**
	Means	4.46	3.82	3.84			Middle	NAA x rootstock	NS
							2.80	NAA x position	NS
'Natal briar'	Top	1.47	1.29	0.99	1.25			Rootstocks x position	1.09*
	Middle	0.94	0.65	0.48	0.69	0.83	Bottom	Rootstock x position x NAA	NS
	Bottom	0.54	0.54	0.55	0.54		1.37		
Grand NAA means	Means	0.98	0.83	0.67					
		2.72	2.32	2.26					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.8. Effects of rootstock cultivars, cutting position and auxin concentrations on leaf number at 30 days after planting.

The main factors of cutting position, rootstock cultivars and interaction between the two had significant ($p \leq 0.05$) effect on leaf number in both IBA and NAA experiments (Tables 10a and 10b). Averaged across the cutting position and the IBA and NAA concentrations, significantly higher leaf number was obtained from the rootstock 'Rosa progress' which had 1.9 times more leaves than the rootstock 'Natal briar' in both experiments. Irrespective of the rootstock cultivars and the IBA and NAA concentrations, the bottom position cuttings exhibited significantly lower leaf number than the top and middle position cuttings (Tables 10a and 10b), however, the latter two positions were not significantly different from each other. Significantly higher leaf number was obtained from the top and middle position cuttings than from the bottom position cuttings in 'Rosa progress' in both experiments (Tables 10a and 10b). The cutting position had no significant effect on leaf number of 'Natal briar' in both NAA and IBA experiments.

Table 10a: Effects of rootstock cultivars, cutting position and IBA concentrations on leaf number at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	14.50	15.28	15.47	15.73	15.25	14.10	Top	IBA concentration	NS
	Middle	15.28	16.17	15.92	15.43	15.70			Rootstocks	1.34**
	Bottom	7.18	13.15	12.68	12.35	11.34		11.90	Position	1.64**
	Means	12.32	14.87	14.69	14.51				IBA x rootstock	NS
								Middle	IBA x position	NS
'Natal briar'	Top	8.92	8.50	8.73	8.08	8.56	7.38	11.42	Rootstocks x position	2.32*
	Middle	7.25	7.10	6.80	7.38	7.13			Rootstock x position x IBA	NS
	Bottom	6.77	6.88	7.03	5.07	6.44		Bottom		
Grand IBA means	Means	7.64	7.49	7.52	6.84			8.89		
		9.98	11.18	11.11	10.67					

*LSD= Least Significant Difference of means, *significant at p ≤ 0.05; **significant at p ≤ 0.001, NS= Not significant*

Table 10b: Effects of rootstock cultivars, cutting position and NAA concentrations on leaf number at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	14.50	16.13	15.52	15.38	13.86	Top	NAA concentration	NS
	Middle	15.28	14.20	16.08	15.19		11.74	Rootstocks	1.42**
	Bottom	7.18	12.90	12.92	11.00			Position	1.74*
	Means	12.32	14.41	14.84				NAA x rootstock	NS
							Middle	NAA x position	NS
'Natal briar'	Top	8.92	7.17	8.18	8.09	7.13	11.10	Rootstocks x position	2.40*
	Middle	7.25	6.15	7.62	7.01			Rootstock x position x NAA	NS
	Bottom	6.77	6.13	6.02	6.31		Bottom		
	Means	7.64	6.48	7.27			8.65		
Grand NAA means		9.98	10.45	11.06					

*LSD= Least Significant Difference of means, *significant at p ≤ 0.05; **significant at p ≤ 0.001, NS= Not significant*

4.1.9. Effects of rootstock cultivars, cutting position and auxin concentrations on shoot fresh weight (g) at 30 days after planting.

The main factor of cutting position had significant ($p \leq 0.05$) effect on shoot fresh weight in both experiments (Tables 11a and 11b). Averaged across the rootstock cultivars and IBA treatments, significantly lower shoot fresh weight was obtained from the bottom position (1.86g) than from the middle position (2.63g) and top position (2.48g) cuttings. The SFW of the latter two cutting positions were however, not significantly different from each other (Table 11a). In the NAA experiment, the effect of cutting position on SFW was significant and followed a similar trend to that recorded in the IBA experiment (Table 11b). The rootstock effect was detected in the IBA experiment and the rootstock 'Rosa progress' produced significantly higher (2.46g) shoot fresh weight than the rootstock 'Natal briar' (2.19g) (Table 11a).

The interaction between rootstock cultivars and auxins had significant ($p \leq 0.05$) effect on shoot fresh weight in both experiments (Tables 11a and 11b). In 'Rosa progress', the control yielded significantly lower shoot fresh weight (1.90g) than IBA treated cuttings. The 0.2% IBA (2.40g) treated cuttings produced significantly ($p \leq 0.05$) lower shoot fresh weight than 0.4% IBA (2.87) treated cuttings (Table 11a). In 'Natal briar', the IBA treatment had no significant effect on shoot fresh weight (Tables 11a). In the NAA experiment, the control yielded significantly lower SFW than the 0.2%NAA treated cuttings (Table 11b) in 'Rosa progress' however, the SFW of 0.2%NAA treated cuttings was not significantly different from the control and 1%NAA treated cuttings. In 'Natal briar', the NAA treated cuttings recorded significantly lower SFW than the control (Table 11b).

Table 11a: Effects of rootstock cultivars, cutting position and IBA concentrations on shoot fresh weight (g) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	2.33	2.60	2.83	2.60	2.59	2.46	Top	IBA concentration	NS
	Middle	2.43	2.70	3.00	3.43	2.89		2.48	Rootstocks	0.23*
	Bottom	0.93	1.90	2.77	2.00	1.90			Position	0.29**
	Means	1.90	2.40	2.87	2.68				IBA x rootstock	0.47*
								Middle	IBA x position	NS
'Natal briar'	Top	2.53	2.47	2.20	2.27	2.37	2.19	2.63	Rootstocks x position	NS
	Middle	2.80	2.40	2.20	2.10	2.36			Rootstock x position x IBA	NS
	Bottom	1.87	2.13	2.13	1.60	1.82		Bottom		
	Means	2.40	2.18	2.18	1.99			1.86		
Grand IBA means		2.15	2.29	2.52	2.33					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 11b: Effects of rootstock cultivars, cutting position and NAA concentrations on shoot fresh weight (g) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	2.33	3.00	2.27	2.53	2.13	Top	NAA concentration	NS
	Middle	2.43	2.03	2.50	2.32		2.37	Rootstocks	NS
	Bottom	0.93	1.97	1.73	1.54			Position	0.30**
	Means	1.90	2.33	2.17			Middle	NAA x rootstock	0.40*
							2.37	NAA x position	NS
'Natal briar'	Top	2.53	1.87	2.20	2.20	2.10		Rootstocks x position	NS
	Middle	2.80	2.27	2.20	2.42		Bottom	Rootstock x position x NAA	NS
	Bottom	1.87	1.83	1.37	1.69		1.62		
	Means	2.40	1.99	1.92					
Grand NAA means		2.15	2.16	2.04					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.10. Effects of rootstock cultivars, cutting position and auxin concentrations on root fresh weight to shoot fresh weight ratio (RFW: SFW) at 30 days after planting.

The RFW: SFW was significantly ($p \leq 0.05$) affected by the main factor of rootstock cultivars in both IBA and NAA experiments (Tables 12a and 12b). Irrespective of the cutting position and IBA and NAA concentrations, the rootstock 'Natal briar' had 1.4 times higher RFW: SFW than 'Rosa progress'. The interaction of cutting position and rootstock cultivars was significant ($p \leq 0.05$) for RFW: SFW in the IBA experiment (Table 12a). In 'Natal briar', the RFW: SFW was significantly higher in the middle position (1.60) than the top (1.10) and bottom (1.16) position cuttings. However, there was no significant difference in RFW: SFW among the cutting positions of 'Rosa progress' (Table 12a).

IBA application had no significant ($p \leq 0.05$) effect on RFW: SFW of both rootstock cultivars (Table 12a). In the NAA experiment, the interaction between the rootstock cultivars and NAA concentration significantly ($p \leq 0.05$) influenced the root fresh weight to shoot fresh weight ratio (RFW: SFW) (Table 12b). The RFW: SFW of the 0.2%NAA (1.85) treated cuttings was significantly higher than the control (1.05) and 1%NAA (1.16) treated cuttings, however, the RFW: SFW of the latter two were not significantly different from each other in 'Natal briar'. NAA application had no significant effect on RFW: SFW of 'Rosa progress' (Table 12b).

Table 12a: Effects of rootstock cultivars, cutting position and IBA concentrations on root fresh weight to shoot fresh weight ratio at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	1.21	0.93	0.84	0.99	0.99	0.95	Top	IBA concentration	NS
	Middle	1.07	0.98	1.17	0.81	1.01		1.05	Rootstocks	0.28*
	Bottom	0.10	0.77	0.91	0.79	0.86			Position	NS
	Means	1.08	0.89	0.97	0.86				IBA x rootstock	NS
								Middle	IBAx position	NS
'Natal briar'	Top	1.23	1.13	1.02	1.03	1.10	1.29	1.30	Rootstocks x position	0.40*
	Middle	1.15	1.78	1.83	1.61	1.60			Rootstock x position x IBA	NS
	Bottom	0.76	1.04	1.36	1.47	1.16		Bottom		
	Means	1.05	1.31	1.41	1.37			1.01		
Grand IBA means		1.06	1.10	1.19	1.12					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 12b: Effects of rootstock cultivars, cutting position and NAA concentrations on root fresh weight to shoot fresh weight ratio at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	1.21	0.74	0.92	0.95	0.98	Top	NAA concentration	NS
	Middle	1.07	1.16	0.96	1.06		1.14	Rootstocks	0.30*
	Bottom	0.96	1.00	0.79	0.92			Position	NS
	Means	1.08	0.97	0.89			Middle	NAA x rootstock	0.60*
								NAA x position	NS
'Natal briar'	Top	1.23	1.66	1.11	1.33	1.35	1.22	Rootstocks x position	NS
	Middle	1.15	1.50	1.46	1.37			Rootstock x position x NAA	NS
	Bottom	0.76	2.39	0.90	1.35		Bottom		
	Means	1.05	1.85	1.16			1.33		
Grand NAA means		1.06	1.41	1.02					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.1.11. Effects of rootstock cultivars, cutting position and auxin concentrations on root dry weight to shoot dry weight ratio (RDW: SDW) at 30 days after planting.

The main factor of rootstock and the interaction between rootstock cultivars and cutting position had significant ($p \leq 0.05$) effect on RDW to SDW ratio in the IBA experiment (Table 13a). Irrespective of the cutting position and auxins, the rootstock 'Natal briar' had significantly higher (0.70) RDW to SDW ratio than 'Rosa progress' (0.53) (Table 13a). In 'Rosa progress' the RDW to SDW ratios of the three positions were statistically similar. In 'Natal briar', the middle position cuttings had significantly higher RDW to SDW ratio than the bottom and top position cuttings however, the RDW to SDW ratio of the latter two positions were not significantly different from each other (Table 13a). In the NAA experiment, RDW to SDW ratio was not significantly influenced by any treatment (Table 13b).

Table 13a: Effects of rootstock cultivars, cutting position and IBA concentrations on root dry weight to shoot dry weight ratio at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.69	0.49	0.52	0.54	0.56	0.53	Top	IBA concentration	NS
	Middle	0.75	0.44	0.21	0.51	0.48		0.57	Rootstocks	0.15*
	Bottom	0.69	0.40	0.42	0.68	0.55			Position	NS
	Means	0.71	0.44	0.38	0.58			Middle	IBA x rootstock	NS
								0.70	IBA x position	NS
'Natal briar'	Top	0.71	0.57	0.56	0.52	0.59	0.70		Rootstocks x position	0.20*
	Middle	0.76	1.03	1.05	0.87	0.93		Bottom	Rootstock x position x IBA	NS
	Bottom	0.29	0.52	0.64	0.94	0.60		0.57		
	Means	0.59	0.71	0.75	0.77					
Grand IBA means		0.65	0.57	0.56	0.68					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 13b: Effects of rootstock cultivars, cutting position and NAA concentrations on root dry weight to shoot dry weight ratio at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.69	0.52	0.46	0.56	0.58	Top	NAA concentration	NS
	Middle	0.75	0.55	0.49	0.59		0.64	Rootstocks	NS
	Bottom	0.76	0.52	0.44	0.58			Position	NS
	Means	0.73	0.53	0.47			Middle	NAA x rootstock	NS
							0.71	NAA x position	NS
'Natal briar'	Top	0.71	0.84	0.61	0.72	0.66		Rootstocks x position	NS
	Middle	0.76	0.83	0.89	0.82		Bottom	Rootstock x position x NAA	NS
	Bottom	0.29	0.53	0.53	0.45		0.51		
	Means	0.59	0.73	0.67					
Grand NAA means		0.66	0.63	0.57					

*LSD= Least Significant Difference of means, * =significant at $p \leq 0.05$; ** =significant at $p \leq 0.001$, NS= Not significant*

4.1.12. Effects of rootstock cultivars, cutting position and auxin concentrations on percentage survival (%) at 30 days after planting.

Percentage survival was significantly ($p \leq 0.05$) influenced by the rootstock cultivars and cutting position in both the IBA and NAA experiments (Tables 14a and 14b). Irrespective of the rootstock cultivars and IBA treatment, the percentage survival significantly decreased from the top (69.9%) to the bottom (45%) position cuttings. In the NAA experiment, bottom position cuttings yielded significantly lower percentage survival than the top and middle position cuttings, however, the percentage survival of the latter two positions were not significantly different from each other (Table 14b). Irrespective of the cutting position and NAA and IBA treatments, 'Rosa progress' exhibited significantly higher percentage survival than 'Natal briar' (Tables 14a and 14b).

In the IBA experiment, the main factors of IBA concentration and its interaction with the rootstock cultivars significantly ($p \leq 0.05$) influenced the percentage survival (Table 14a). Averaged across the rootstock cultivar and cutting position, the 0.2%IBA treated cuttings had significantly lower percentage survival (51.4%) than the control (63.1%). No significant difference in percentage survival was noted among the IBA treated cuttings. In 'Natal briar', the 0.2%IBA treated cuttings had significantly lower percentage survival (35.2%) than the control (54.0%) and 0.4%IBA (53.5%) treated cuttings (Table 14a) but not significantly different from 0.6%IBA treated cuttings. IBA treatment had no significant effect on percentage survival of 'Rosa progress'. NAA application had no significant ($p \leq 0.05$) effect on percentage survival of both rootstock cultivars (Table 14b).

Table 14a: Effects of rootstock cultivars, cutting position and IBA concentrations on percentage survival (%) at 30 days after planting.

Rootstock cultivars	Position	0% IBA	0.2% IBA	0.4% IBA	0.6% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	83.9	86.9	83.9	80.8	83.9	69.8	Top	IBA concentration	10.02*
	Middle	71.1	70.8	76.7	78.3	74.2		69.6	Rootstocks	8.19**
	Bottom	61.7	45.0	47.2	51.1	51.3			Position	9.03**
	Means	72.2	67.6	69.3	70.1				IBA x rootstock	15.09*
								Middle	IBA x position	NS
'Natal briar'	Top	66.1	43.3	63.3	48.3	55.3	45.7	58.6	Rootstocks x position	14.18*
	Middle	48.1	39.7	47.2	37.2	43.1			Rootstock x position x IBA	NS
	Bottom	47.8	22.5	50.0	34.4	38.7		Bottom		
	Means	54.0	35.2	53.5	40.0			45.0		
Grand IBA means		63.1	51.4	61.4	55.0					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 14b: Effects of rootstock cultivars, cutting position and NAA concentrations on percentage survival (%) at 30 days after planting.

Rootstock cultivars	Position	0% NAA	0.2% NAA	1% NAA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	83.9	83.3	82.8	83.3	70.8	Top	NAA concentration	NS
	Middle	71.1	73.3	72.2	72.2		70.6	Rootstocks	9.83**
	Bottom	61.7	60.0	48.9	56.9			Position	12.04**
	Means	72.2	72.2	68.0			Middle	NAA x rootstock	NS
							60.6	NAA x position	NS
'Natal briar'	Top	66.1	45.6	61.7	57.8	48.1		Rootstocks x position	NS
	Middle	48.1	46.7	52.2	49.0		Bottom	Rootstock x position x NAA	NS
	Bottom	47.8	29.4	35.0	37.4		47.1		
	Means	54.0	40.6	49.6					
Grand NAA means		63.1	56.4	58.8					

*LSD= Least Significant Difference of means, * =significant at $p \leq 0.05$; ** =significant at $p \leq 0.001$, NS= Not significant*

4.2 TOP GRAFTING STUDY

4.2.1. Effects of rootstock cultivars, cutting position and auxins on rooting percentage (%) of top grafted rose variety 'Inca' at 30 days after planting

The rooting percentage was significantly ($p \leq 0.05$) influenced by the main factor of rootstock cultivars and its interaction with the cutting position (Table 15). The grafts on the rootstock 'Natal briar' produced significantly higher (79.6 %) rooting percentage than on the rootstock 'Rosa progress' (70.4 %). In 'Rosa progress', the variety 'Inca' grafted on the top position cuttings had significantly lower (51.1%) rooting percentage than the the middle (72.2%) and bottom (87.8%) position cuttings. However, the rooting percentages of the latter two positions were not significantly different from each other. Though, the cutting position had no significant effect on rooting % in 'Natal briar', acropetal increase in rooting % was recorded (Table 15).

The main factor of auxin, and its interaction with the rootstock cultivars and the three way interaction of auxin x rootstock cultivars x position significantly ($p \leq 0.05$) affected the rooting percentage (Table 15). Averaged across the rootstock cultivars and cutting position, the control had significantly lower rooting percentage than the auxin treated cuttings. No significant difference was recorded between the auxin treated grafts. In 'Natal briar', the 0.4% IBA (91.1%) and 0.2% NAA (88.9%) treated grafts had significantly ($p \leq 0.05$) higher rooting % than the control (58.9%). 'Rosa progress' had a similar trend to that noted in 'Natal briar' though not significant.

Table 15: Effects of rootstock cultivars, cutting position and auxins on rooting percentage (%) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	33.3	43.3	76.7	51.1	70.4	Top	Auxins	11.12*
	Middle	73.3	86.7	56.7	72.2		67.8	Rootstocks	9.08*
	Bottom	93.3	86.7	83.3	87.8			Position	NS
	Means	66.7	72.2	72.2			Middle	Auxins x rootstock	15.72*
							77.2	Auxinx position	NS
'Natal briar'	Top	73.3	93.3	86.7	84.4			Rootstocks x position	15.72**
	Middle	56,7	90.0	100.0	82.2		Bottom	Rootstock x position x auxin	7.23*
	Bottom	46.7	83.3	86.7	72.2	79.6	80.0		
	Means	58.9	88.9	91.1					
Grand auxin means		62.8	80.6	81.7					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.2. Effects of rootstock cultivars, cutting position and auxins on root number of top grafted rose variety 'Inca' at 30 days after planting

The main factor of cutting position significantly ($p \leq 0.05$) influenced the root number (Table 16). Irrespective of auxin and rootstock cultivars, the variety 'Inca' grafted on the middle position cuttings had significantly higher root number than on the top (15.22) position cuttings but not significantly different from the bottom (18.63) position cuttings.

The main factor of auxins significantly ($p \leq 0.05$) influenced the root number (Table 16). Irrespective of cutting position and rootstock cultivars, the grafts of the control had significantly lower (11.67) root number than 0.2% NAA (20.38) and 0.4% IBA (21.92) treated grafts which, were however not significantly different from each other.

Table 16: Effects of rootstock cultivars, cutting position and auxins on root number of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	9.27	19.20	17.47	15.31	18.67	Top	Auxins	2.83*
	Middle	13.73	23.27	23.27	20.09		15.22	Rootstocks	NS
	Bottom	15.20	19.67	27.00	20.62			Position	2.83*
	Means	12.73	20.71	22.58				Auxins x rootstock	NS
							Middle	Auxins x position	NS
'Natal briar'	Top	11.73	17.93	15.73	15.13		20.11	Rootstocks x position	NS
	Middle	9.53	21.53	29.33	20.13	17.30		Rootstock x position x auxin	NS
	Bottom	10.53	20.67	18.73	16.64				
	Means	10.60	20.04	21.27			Bottom		
Grand auxin means		11.67	20.38	21.92			18.63		

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.3. Effects of rootstock cultivars, cutting position and auxins on the length of the longest root (cm) of top grafted rose variety 'Inca' at 30 days after planting.

The main factor of rootstock had significant ($p \leq 0.05$) effect on the length of the longest root (Table 17) and the variety 'Inca' grafted on 'Rosa progress' had significantly longer roots than on 'Natal briar'.

Table 17: Effects of rootstock cultivars, cutting position and auxins on the length of the longest root (cm) of top grafted rose cultivar 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	6.05	5.52	6.14	5.90	5.66	Top	Auxins	NS
	Middle	5.69	5.67	5.70	5.69		5.48	Rootstocks	0.44*
	Bottom	5.05	6.30	4.84	5.40			Position	NS
	Means	5.60	5.83	5.56			Middle	Auxins x rootstock	NS
							5.53	Auxins x position	NS
'Natal briar'	Top	5.21	4.81	5.17	5.07	5.17		Rootstocks x position	NS
	Middle	5.85	4.91	5.34	5.37		Bottom	Rootstock x position x auxin	NS
	Bottom	5.15	5.25	4.82	5.07		5.23		
	Means	5.40	4.99	4.11					
Grand auxin means		5.50	5.41	5.34					

*LSD= Least Significant Difference of means, * significant at $p \leq 0.05$; ** significant at $p \leq 0.001$, NS= Not significant*

4.2.4. Effects of rootstock cultivars, cutting position and auxins on total root length (cm) of topgrafted rose variety 'Inca' at 30 days after planting.

The main factors of rootstock cultivars and cutting position had significant effect on total root length (Table 18). Irrespective of cutting position and auxin treatment the variety 'Inca' grafted on 'Rosa progress' had significantly higher (105.7cm) total root length than on 'Natal briar' (88.5cm). Irrespective of auxin treatment and rootstock cultivars, the variety 'Inca' grafted on the middle position cuttings had significantly higher (110.0cm) total root length than on the top (84.0cm) position cuttings. The total root length of the grafts on the bottom position cuttings was however not significantly different from the grafts on the top and middle position cuttings (Table 18).

The main factor of auxins had significant effect on total root length (Table 18) and the grafts of the control exhibited significantly lower total root length (64.1cm) than 0.4% IBA (117.1cm) and 0.2% NAA (110.0cm) treated grafts which were not significantly different from each other.

Table 18: Effects of cutting position, auxin and rootstock cultivars on total root length (cm) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	57.3	107.0	107.2	90.5	105.7	Top	Auxins	18.68**
	Middle	79.2	131.1	134.9	115.1		84.0	Rootstocks	15.25*
	Bottom	75.9	126.3	132.6	111.6			Position	18.68*
	Means	70.8	121.5	124.9			Middle	Auxins x rootstock	NS
							110.0	Auxins x position	NS
'Natal briar'	Top	62.7	86.3	83.3	77.4	88.5		Rootstocks x position	NS
	Middle	55.7	103.4	155.5	104.9			Rootstock x position x auxin	NS
	Bottom	53.9	106.1	89.3	83.1		Bottom		
	Means	57.4	98.6	109.4			97.4		
Grand auxin means		64.1	110.0	117.1					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.5. Effects of rootstock cultivars, cutting position and auxins on root fresh weight (g) of top grafted rose variety 'Inca' at 30 days after planting

The main factors of auxin and position and two way interaction of auxin x position, rootstock cultivars x position and three way interaction of auxin x position x rootstock cultivars significantly ($p \leq 0.05$) influenced RFW (Table 19). Irrespective of auxin and rootstock cultivars, the variety 'Inca' grafted on the middle position cuttings had significantly higher RFW than the bottom and top position cuttings. The top position cuttings had significantly lower RFW than the bottom position cuttings. The variety 'Inca' grafted on the middle position cuttings of 'Rosa progress' had significantly higher RFW than on the top and bottom position cuttings (Table 19). In 'Natal briar', the variety 'Inca' grafted on the middle position cuttings had significantly ($p \leq 0.05$) higher (0.74g) RFW than on the top (0.63g) position cuttings. However, RFW of the variety 'Inca' grafted on the bottom position cuttings of 'Natal briar' was not significantly different from the top and middle position cuttings.

The main factor of auxin and its interaction with the cutting position significantly ($p \leq 0.05$) influenced RFW (Table 19). Averaged across the rootstock cultivars and the cutting position, the control had significantly lower (0.53g) RFW than the 0.4%IBA (0.69g) and 0.2%NAA (0.86g). With respect to the cutting position, the 0.2%NAA treated grafts from the top position cuttings had significantly ($p \leq 0.05$) lower (0.74g) RFW than middle (0.94g) and bottom (0.89g) position cuttings. The variety 'Inca' grafted on the middle position cuttings and treated with 0.4%IBA had significantly higher RFW than on the top (0.60g) and bottom (0.63g) position cuttings. Grafts from the three positions untreated with auxin were not significantly different from each other (Table 19).

Table 19: Effects of rootstock cultivars, cutting position and auxins on root fresh weight (g) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.46	0.66	0.66	0.60	0.69	Top	Auxins	0.06**
	Middle	0.58	1.17	0.77	0.84		0.61	Rootstocks	NS
	Bottom	0.54	0.76	0.60	0.63			Position	0.06**
	Means	0.53	0.86	0.63			Middle	Auxins x rootstock	NS
							0.79	Auxins x position	0.11*
'Natal briar'	Top	0.53	0.82	0.53	0.63	0.70		Rootstocks x position	0.09*
	Middle	0.56	0.70	0.97	0.74		Bottom	Rootstock x position x auxin	0.15**
	Bottom	0.50	1.01	0.64	0.72		0.68		
	Means	0.53	0.84	0.72					
Grand auxin means		0.53	0.86	0.69					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.6. Effects of cutting position, auxins and rootstock cultivars on root dry weight (RDW) (g) of top grafted rose variety 'Inca' at 30 days after planting.

The RDW was not significantly ($p \leq 0.05$) affected by the cutting position but by the main factor of rootstock cultivars and interaction between the auxin and cutting position (Table 20). Significantly higher RDW was obtained from the variety 'Inca' grafted on the rootstock 'Rosa progress' than on 'Natal briar' and the grafts on 'Rosa progress' had 1.2 times higher RDW than grafts on 'Natal briar' (0.81g) (Table 20).

The interaction between the auxin and cutting position significantly ($p \leq 0.05$) influenced the root dry weight (RDW) (Table 20). The variety 'Inca' grafted on the middle position cuttings and treated with 0.2%NAA had significantly lower (0.08g) RDW than on the top

(0.10g) position cuttings but not significantly different from bottom (0.10g) position cuttings. The variety 'Inca' grafted on the middle position cuttings and treated with 0.4%IBA produced significantly ($p \leq 0.05$) higher (0.10g) RDW than on the top (0.08g) and bottom (0.07g) position cuttings. The RDW of the non auxin treated grafts from the three positions were however, not significantly different from each other.

Table 20: Effects of rootstock cultivars, cutting position and auxins on root dry weight (g) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.10	0.11	0.08	0.10	0.09	Top	Auxins	NS
	Middle	0.10	0.07	0.12	0.10		0.09	Rootstocks	0.01*
	Bottom	0.09	0.11	0.07	0.09			Position	NS
	Means	0.10	0.10	0.09				Auxins x rootstock	NS
							Middle	Auxins x position	0.02*
'Natal briar'	Top	0.07	0.10	0.08	0.08		0.09	Rootstocks x position	NS
	Middle	0.08	0.09	0.09	0.08			Rootstock x position x auxin	NS
	Bottom	0.07	0.08	0.07	0.08	0.08			
	Means	0.07	0.09	0.08			Bottom		
Grand auxin means		0.08	0.09	0.08			0.08		

*LSD= Least Significant Difference of means, * significant at $p \leq 0.05$; ** significant at $p \leq 0.001$, NS= Not significant*

4.2.7. Effects of rootstock cultivars, cutting position and auxins on shoot height (cm) of top grafted rose variety 'Inca' at 21 and 28 days after planting.

The shoot height of top grafted rose variety 'Inca' was not influenced by the cutting position at 21 and 28 days after planting (Tables 21 and 22). The variety 'Inca' grafted on the rootstock 'Rosa progress' had significantly taller (3.00cm) shoots than on 'Natal briar'

(2.55cm) at 21 days after planting (Table 21) but the difference was lost by 28 days after planting (Table 22).

The main factor of auxin significantly ($p \leq 0.05$) influenced the shoot height at 21 and 28 days after planting (Tables 21 and 22). The grafts of the control were significantly shorter (1.85cm) than those of 0.2%NAA (3.00cm) and of 0.4%IBA (3.47cm) treated cuttings at 21 days after planting (Table 21). At 28 days after planting, the shoot height of the grafts treated with 0.4%IBA (9.73cm) was significantly higher than the control (6.63cm) and 0.2%NAA (8.59cm) treated grafts (Table 22).

Table 21: Effects of rootstock cultivars, cutting position and auxins on shoot height (cm) of top grafted rose variety 'Inca' at 21 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	1.98	3.57	3.81	3.12	3.00	Top	Auxin	0.51**
	Middle	1.95	3.57	3.95	3.16		3.08	Rootstocks	0.42*
	Bottom	1.83	2.79	3.59	2.74			Position	NS
	Means	1.92	3.31	3.78			Middle	Auxins x rootstock	NS
							2.55	Auxins x position	NS
'Natal briar'	Top	2.38	3.19	3.57	3.05	2.73		Rootstocks x position	NS
	Middle	1.48	2.69	2.71	2.29		Bottom	Rootstock x position x auxin	NS
	Bottom	1.50	2.21	3.19	2.30		2.52		
	Means	1.79	2.70	3.16					
Grand auxin means		1.85	3.00	3.47					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 22: Effects of rootstock cultivars, cutting position and auxins on shoot height (cm) of top grafted rose variety 'Inca' at 28 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	5.55	8.71	9.90	8.05	8.39	Top	Auxins	1.08*
	Middle	6.98	8.91	11.06	8.98		7.88	Rootstocks	NS
	Bottom	6.97	7.69	9.97	8.15			Position	NS
	Means	6.50	8.44	10.25			Middle	Auxins x rootstock	NS
							8.26	Auxins x position	NS
'Natal briar'	Top	5.53	8.88	8.69	7.70	7.57		Rootstocks x position	NS
	Middle	3.99	8.74	9.86	7.53		Bottom	Rootstock x position x auxin	NS
	Bottom	4.79	8.62	9.07	7.49		7.82		
	Means	4.77	8.75	9.20					
Grand auxin means		6.63	8.59	9.73					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.8. Effects of rootstock cultivars, cutting position and auxins on leaf number of top grafted rose variety 'Inca' at 30 days after planting

The leaf number of the grafts was not significantly ($p \leq 0.05$) influenced by the rootstock cultivar and cutting position (Table 23). However, the main factor of auxin significantly ($p \leq 0.05$) influenced the leaf number of the grafts (Table 23). The grafts treated with auxins had significantly higher leaf number than the control (8.88). The grafts treated with 0.4%IBA (11.29) produced significantly higher leaf number than the 0.2%NAA (10.31) treated grafts (Table 23).

Table 23: Effects of rootstock cultivars, cutting position and auxins on leaf number of top grafted rose variety 'Inca' at 30 days after planting.

RootstocksRootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	9.53	9.20	10.63	9.80	10.47	Top	Auxins	0.94*
	Middle	9.27	10.67	11.88	10.58		9.91	Rootstocks	NS
	Bottom	10.20	10.27	12.60	11.02			Position	NS
	Means	9.67	9.63	10.83			Middle	Auxins x rootstock	NS
							10.17	Auxins x position	NS
'Natal briar'	Top	9.00	10.07	11.00	10.02	9.85		Rootstocks x position	NS
	Middle	7.33	10.87	11.07	9.76		Bottom	Rootstock x position x auxin	NS
	Bottom	7.93	10.80	10.60	9.78		10.40		
	Means	8.09	10.59	10.89					
Grand auxin means		8.88	10.31	11.29					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.9. Effects of rootstock cultivars, cutting position and auxins on percent mortality (%) of top grafted rose variety 'Inca' at 14 days after planting

The percent mortality at two weeks after planting was significantly ($p \leq 0.05$) influenced by the main factors of rootstock cultivars, cutting position and the interaction of both (Table 24). Averaged across the rootstock cultivars and auxin treatment, the variety 'Inca' grafted on the top position (22.2%) cuttings had significantly higher percent mortality than on the bottom position (2.6%) cuttings. The variety 'Inca' grafted on the rootstock 'Rosa progress' (21%) had significantly higher percent mortality than on 'Natal briar' (2.2%). The variety 'Inca' grafted on the top position (40.0%) cuttings had significantly higher percent mortality than on the middle (17.8%) and bottom (5.2%) position cuttings in 'Rosa progress' (Table 24).

Percent mortality of the grafts was not significantly influenced by the auxin treatments, however, the highest % mortality was obtained from 0.2% NAA (25.9%) in 'Rosa progress' and 0.4% IBA (3.7%) treated grafts in 'Natal briar' (Table 24).

Table 24: Effects of rootstock cultivars, cutting position and auxins on percent mortality (%) of top grafted rose variety 'Inca' at 14 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	26.7	53.3	40.0	40.0	21	Top	Auxins	NS
	Middle	15.6	20.0	17.8	17.8		22.2	Rootstocks	4.73*
	Bottom	11.1	4.4	0.0	5.2			Position	5.79*
	Means	17.8	25.9	19.3			Middle	Auxins x rootstock	NS
							10.0	Auxins x position	10.14
'Natal briar'	Top	2.2	4.4	6.7	4.4	2.2		Rootstocks x position	8.19*
	Middle	0.0	2.2	4.4	2.2		Bottom	Rootstock x position x auxin	NS
	Bottom	0.0	0.0	0.0	0.0		2.6		
	Means	0.7	2.2	3.7					
Grand auxin means		9.3	14.1	11.5					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.10. Effects of rootstock cultivars, cutting position and auxins on shoot fresh weight (g) of topgrafted rose variety 'Inca' at 30 days after planting

The shoot fresh weight was not significantly ($p \leq 0.05$) influenced by the rootstock cultivar and cutting position (Table 25). However, the main factor of auxin and its interaction with the rootstock cultivars significantly ($p \leq 0.05$) influenced the shoot fresh weight (SFW) of 'Inca' (Table 25). Irrespective of the cutting position and rootstock cultivars, the control had significantly lower SFW than the auxin treated grafts (Table 25) which did not significantly differ from each other. In 'Rosa progress', 0.4%IBA treated grafts had

significantly higher (0.93g) SFW than the control (0.74g). However, the SFW of 0.2%NAA (0.85g) treated grafts was not significantly different from the control and 0.4%IBA treated grafts (Table 25). In 'Natal briar', significantly lower SFW was obtained from the control (0.44g) than the auxin treated grafts. The 0.2%NAA and 0.4%IBA treated grafts however, produced statistically similar SFW (Table 25).

Table 25: Effects of rootstock cultivars, cutting position and auxins on shoot fresh weight (g) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.66	0.69	0.95	0.77	0.84	Top	Auxins	0.11**
	Middle	0.67	1.00	0.96	0.88		0.79	Rootstocks	NS
	Bottom	0.88	0.87	0.90	0.88			Position	NS
	Means	0.74	0.85	0.93			Middle	Auxins x rootstock	0.17*
							0.77	Auxins x position	NS
'Natal briar'	Top	0.50	1.05	0.91	0.82	0.76		Rootstocks x position	NS
	Middle	0.35	0.72	0.90	0.66		Bottom	Rootstock x position x auxin	NS
	Bottom	0.47	1.030	0.90	0.80		0.84		
	Means	0.44	0.93	0.90					
Grand auxin means		0.59	0.89	0.92					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.11. Effects of rootstock cultivars, cutting position and auxins on shoot dry weight (g) of top grafted rose variety 'Inca' at 30 days after planting.

The main factor of cutting position and its interaction with rootstock cultivars significantly ($p \leq 0.05$) influenced the shoot dry weight (Table 26). Averaged across the rootstocks and auxin treatment, the middle position cuttings had significantly lower SDW than the top position cuttings and the SDW of the bottom position cuttings was not significantly different from the top and middle position cuttings (Table 26). The cutting position had no significant effect on SDW of 'Inca' grafted on the rootstock 'Rosa progress'. In 'Natal briar', the SDW of the variety 'Inca' grafted on the top position cuttings had significantly higher SDW than on the middle position cuttings. However, SDW of the former position was not significantly ($p \leq 0.05$) different from the bottom position cuttings.

The shoot dry weight (SDW) of the variety 'Inca' was significantly ($p \leq 0.05$) influenced by the main factor of auxin and the interaction between auxin x rootstock cultivars (Table 26). Irrespective of the cutting position and rootstock cultivars, the SDW of the grafts significantly increased with auxin treatment and the control (0.15g) had significantly lower SDW than the 0.2%NAA (0.19g) and 0.4%IBA (0.22g) treated grafts. The variety 'Inca' grafted on 'Rosa progress' cuttings treated with 0.4%IBA had significantly higher SDW than 0.2%NAA treated grafts. The SDW of the latter was however, not significantly different from the untreated grafts. Untreated grafts of 'Natal briar' had significantly lower (0.12g) SDW than 0.4%IBA (0.21g) and 0.2%NAA (0.20g) treated grafts.

Table 26: Effects of rootstock cultivars, cutting position and auxins on shoot dry weight (g) of topgrafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.17	0.17	0.22	0.19	0.19	Top	Auxins	0.02**
	Middle	0.15	0.18	0.24	0.19		0.20	Rootstocks	NS
	Bottom	0.22	0.16	0.22	0.20			Position	0.02*
	Means	0.18	0.17	0.23			Middle	Auxins x rootstock	0.03**
							0.17	Auxins x position	NS
'Natal briar'	Top	0.15	0.24	0.24	0.21	0.18		Rootstocks x position	0.03*
	Middle	0.08	0.16	0.18	0.14		Bottom	Rootstock x position x auxin	NS
	Bottom	0.13	0.21	0.02	0.18		0.19		
	Means	0.12	0.20	0.21					
Grand auxin means		0.15	0.19	0.22					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.12. Effects of rootstock cultivars, cutting position and auxins on root fresh weight to shoot fresh weight ratio (RFW: SFW) of top grafted rose variety 'Inca' at 30 days after planting.

The main factors of auxin, rootstock cultivars, cutting position and the interaction between rootstock cultivars and auxin significantly ($p \leq 0.05$) influenced the RFW: SFW (Table 27). Irrespective of cutting position and auxins, the variety 'Inca' grafted on the rootstock 'Rosa progress' had significantly higher RFW: SFW than on the rootstock 'Natal briar'. Irrespective of auxin and rootstock cultivars, the variety 'Inca' grafted on the middle position cuttings had significantly higher RFW: SFW than on the top and bottom position cuttings. However, the RFW: SFW of the latter two positions were not significantly different from each other (Table 27).

The main factors of auxin and its interaction with rootstock cultivars significantly ($p \leq 0.05$) influenced the RFW: SFW (Table 27). Averaged across the rootstock cultivars and cutting position, the control had significantly higher RFW/SFW ratio than the 0.4%IBA treated grafts, however, the RFW: SFW of 0.2% NAA treated grafts was not significantly different from those of the control but was significantly higher than those of 0.4% IBA treated grafts. The variety 'Inca' grafted on 'Natal briar' and treated with 0.2%NAA had significantly higher (1.03) RFW: SFW than the control (0.80) and 0.4%IBA (0.73) treated grafts. In 'Rosa progress', the variety 'Inca' grafted on the control had significantly ($p \leq 0.05$) higher (1.33) RFW: SFW than 0.2%NAA (0.93) and 0.4%IBA (0.81) treated grafts.

Table 27: Effects of rootstock cultivars, cutting position and auxins on root fresh weight to shoot fresh weight ratio (RFW: SFW) of topgrafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	1.07	0.78	0.59	0.81	1.02	Top	Auxins	0.18*
	Middle	1.71	1.00	1.09	1.26		0.81	Rootstocks	0.14*
	Bottom	1.21	1.00	0.77	0.99			Position	0.18**
	Means	1.33	0.93	0.81			Middle	Auxins x rootstock	0.25*
							1.14	Auxins x position	NS
'Natal briar'	Top	0.75	0.98	0.71	0.81	0.85		Rootstocks x position	NS
	Middle	1.01	1.23	0.80	1.01		Bottom	Rootstock x position x auxin	NS
	Bottom	0.62	0.88	0.67	0.72		0.86		
	Means	0.80	1.03	0.73					
Grand auxin means		1.06	0.98	0.77					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.13. Effects of rootstock cultivars, cutting position and auxins on root dry weight to shoot dry weight ratio (RDW: SDW) of top grafted rose variety 'Inca' at 30 days after planting.

The main factors of auxin, rootstock cultivars, cutting position and the interaction between the auxin and rootstock cultivars significantly ($p \leq 0.05$) influenced the RDW: SDW (Table 28). Irrespective of auxin and rootstock cultivars, the variety 'Inca' grafted on the middle position cuttings had significantly higher (0.64) ratio than on the bottom (0.47) and top (0.48) position cuttings. The variety 'Inca' grafted on the rootstock 'Natal briar' exhibited significantly higher RDW: SDW ratio than on 'Rosa progress'.

The main factor of auxin and its interaction with the rootstock cultivars significantly ($p \leq 0.05$) influenced the RDW: SDW (Table 28). Averaged across the rootstock cultivars and cutting position, the control had significantly higher RDW: SDW than the auxin treated grafts (Table 28). The variety 'Inca' grafted on'Rosa progress' cuttings and treated with 0.4% IBA (0.35) had significantly lower RDW: SDW than 0.2% NAA (0.55) but not significantly different from the control (0.45). The variety 'Inca' grafted on 'Natal briar' cuttings and not treated with auxin had significantly higher (0.89) RDW: SDW than 0.2% NAA (0.49) and 0.4% IBA (0.47) treated grafts.

Table 28: Effects of rootstock cultivars, cutting position and auxins on root dry weight: shoot dry weight ratio of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	0.42	0.62	0.36	0.46	0.45	Top	Auxins	0.13**
	Middle	0.64	0.48	0.36	0.49		0.48	Rootstocks	0.11*
	Bottom	0.30	0.54	0.33	0.39			Position	0.13*
	Means	0.45	0.55	0.35			Middle	Auxins x rootstock	0.18*
							0.64	Auxins x position	NS
'Natal briar'	Top	0.72	0.44	0.34	0.50	0.62		Rootstocks x position	NS
	Middle	1.22	0.49	0.68	0.80		Bottom	Rootstock x position x auxin	NS
	Bottom	0.73	0.54	0.39	0.55		0.47		
	Means	0.89	0.49	0.47					
Grand auxin means		0.67	0.52	0.41					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.14. Effects of rootstock cultivars, cutting position and auxins on grafting take (%) of top grafted rose variety 'Inca' at 30 days after planting.

The grafting take was significantly ($p \leq 0.05$) influenced by the interaction between rootstock cultivars and cutting position (Table 29). The grafts on the bottom (55.6%) position cuttings had significantly ($p \leq 0.05$) lower grafting take than on the top (76.7%) position cuttings in 'Natal briar', however, grafting take of the grafts on the middle position cuttings (66.7%) was not significantly different from those on the top and bottom position cuttings. The grafts on the bottom position cuttings of 'Rosa progress' had significantly higher (75.6%) grafting take than on the top (50.0%) position cuttings, however, the grafting take of the grafts on the middle (64.4%) position cuttings was not significantly different from the other two positions.

The grafting take was significantly ($p \leq 0.05$) influenced by the main factor of auxin (Table 29). Irrespective of the cutting position and rootstock cultivars, the 0.2% NAA (70.6%) and 0.4% IBA (75.6%) treated grafts had significantly higher grafting take than the control (48.3%), however, the grafting take of the grafts treated with auxins were not significantly different from each other.

Table 29: Effects of rootstock cultivars, cutting position and auxins on grafting take (%) of top grafted rose variety 'Inca' at 30 days after planting.

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	33.3	40.0	76.7	50.0	63.3	Top	Auxins	15.07*
	Middle	56.7	80.0	56.7	64.4		63.3	Rootstocks	NS
	Bottom	76.7	73.3	76.7	75.6			Position	NS
	Means	55.6	64.4	70.0			Middle	Auxins x rootstock	NS
							65.6	Auxins x position	NS
'Natal briar'	Top	66.7	76.7	86.7	76.7			Rootstocks x position	21.31*
	Middle	26.7	83.3	90.0	66.7	66.3	Bottom	Rootstock x position x auxin	NS
	Bottom	30.0	70.0	66.7	55.6		65.6		
	Means	41.1	76.7	81.1					
Grand auxin means		48.3	70.6	75.6					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.15. Effects of rootstock cultivars, cutting position and auxins on total number of harvestable stems of top grafted rose variety 'Inca'.

The treatments had no significant effect on total number of harvestable stems of grafted variety 'Inca' in the first and second flushes (data not presented). However in the third flush, the main factor of rootstock cultivars significantly ($p \leq 0.05$) influenced the total number of harvestable stems of top grafted rose variety 'Inca' (Table 30). Averaged across the auxins and cutting position, the variety 'Inca' grafted on the rootstock 'Rosa progress' had significantly higher number of stems than on 'Natal briar'.

The interaction between the auxin and cutting position significantly ($p \leq 0.05$) influenced the total number of harvestable stems of top grafted rose variety 'Inca' (Table 30). Top position cuttings untreated with auxin had significantly fewer number of harvestable stems of 'Inca' than the auxin treated cuttings. Bottom position cuttings treated with 0.2% NAA had significantly fewer (8.84) number of stems of 'Inca' than the control (14.83) and the number of stems of the control and 0.4%IBA treated cuttings (11.34) were not significantly different from each other . Auxins had no significant effect on grafts on middle position cuttings of both rootstock cultivars. (Table 30).

Table 30: Effects of rootstock cultivars, cutting position and auxins on total number of harvestable stems of top grafted rose variety 'Inca'. (3rd flush).

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	3.33	12.67	12.33	9.44	12.59	Top	Auxins	NS
	Middle	14.67	10.67	18.33	14.56		9.83	Rootstocks	2.65*
	Bottom	17.67	10.00	13.67	11.33			Position	NS
	Means	11.89	11.11	14.78			Middle	Auxins x position	5.62*
							12.22	Auxins x rootstock	NS
'Natal briar'	Top	8.00	12.00	10.67	10.22			Rootstocks x position	NS
	Middle	9.00	10.67	10.00	9.89	9.89		Rootstock x position auxin	NS
	Bottom	12.00	7.67	9.00	9.56		Bottom		
	Means	9.67	10.11	9.67			11.67		
Grand auxin means		10.78	10.61	12.33					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.16. Effects of rootstock cultivars, cutting position and auxins on flowering stem length (cm) of top grafted rose variety 'Inca'.

The treatments had no significant effect on stem length of grafted 'Inca' in the first and second flushes (data not presented). However, the main factors of rootstock cultivars and cutting position had significant ($p \leq 0.05$) effect on stem length of grafted rose variety 'Inca' in the third flush (Table 31). Irrespective of cutting position and auxins, stem length of 'Inca' grafted on the rootstock 'Natal briar' produced significantly ($p \leq 0.05$) shorter (72.34cm) stems than on the rootstock 'Rosa progress' (76.31cm). Irrespective of auxins and rootstock cultivars, taller stems of grafted 'Inca' were obtained from the top position cuttings than from the bottom position cuttings.

The interaction between auxin and cutting position and three way interactions of auxin x position x rootstock cultivars had significant ($p \leq 0.05$) effect on stem length of grafted rose variety 'Inca' in the third flush (Table 31). Bottom position cuttings of the rootstock cultivars untreated with auxin produced shorter stems than the auxin treated grafts. Stem length of the grafts treated with 0.4% IBA and 0.2%NAA were not significantly different from each other. The auxins had no significant effect on the top and middle position cuttings.

Table 31: Effects of rootstock cultivars, cutting position and auxins on flowering stem length (cm) of top grafted rose variety 'Inca' (3rd flush).

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	75.77	78.14	78.47	77.46	76.31	Top	Auxins	NS
	Middle	76.60	78.83	72.55	75.99		76.50	Rootstocks	2.35*
	Bottom	74.30	74.88	77.23	75.47			Position	2.88*
	Means	75.56	77.28	76.08			Middle	Auxins x position	4.99*
							74.41	Auxins x rootstock	NS
'Natal briar'	Top	82.22	73.37	71.03	77.46	72.34		Rootstocks x position	NS
	Middle	71.90	70.72	75.83	75.99		Bottom	Rootstock x position auxin	2.25*
	Bottom	61.49	72.42	72.07	75.47		72.07		
Grand auxin means	Means	71.87	72.17	72.98					
		76.50	74.41	72.02					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.17. Effects of rootstock cultivars, cutting position and auxins on stem diameter (cm) of top grafted rose variety 'Inca'.

The treatments had no significant effect on stem diameter in the first and third flushes (data not presented), however, in the second flush, the interaction between rootstock cultivars and cutting position significantly ($p \leq 0.05$) influenced the stem diameter of grafted

rose variety 'Inca' (Table 32). In 'Natal briar' the variety 'Inca' grafted on the top position cuttings had significantly higher stem diameter than on the bottom position cuttings. The variety 'Inca' grafted on bottom position cuttings of 'Rosa progress'had significantly higher stem diameter than on the top position cuttings. However, the stem diameter of the variety 'Inca' grafted on the middle position cuttings was not significantly different from the top and bottom position cuttings in both rootstock cultivars.

Table 32: Effects of rootstock cultivars, cutting position and auxins on stem diameter (cm) of top grafted rose variety 'Inca' (2nd flush).

Rootstock cultivars	Position	control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	1.29	1.30	1.28	1.29	1.45	Top	Auxins	NS
	Middle	1.37	1.45	1.56	1.46		1.44	Rootstocks	NS
	Bottom	1.87	1.19	1.76	1.61			Position	NS
	Means	1.51	1.31	1.53			Middle	Auxins x position	NS
							1.50	Auxins x rootstock	NS
'Natal briar'	Top	1.72	1.52	1.50	1.58	1.48		Rootstock x position	0.22*
	Middle	1.47	1.45	1.68	1.54		Bottom	Rootstock x position auxin	NS
	Bottom	1.18	1.28	1.57	1.33		1.48		
	Means	1.46	1.42	1.59					
Grand auxin means		1.48	1.37	1.56					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.2.18. Effects of rootstock cultivars, cutting position and auxins on stem weight (g) of top grafted rose variety 'Inca'.

The main factors of rootstock cultivars and cutting position had significant ($p \leq 0.05$) effect on stem weight of grafted rose variety 'Inca' in the second flush (Table 33a). Irrespective of the cutting position and auxins, stem weight of the variety 'Inca' grafted on the rootstock 'Natal briar' (42.76g) was significantly higher than on the rootstock 'Rosa progress' (38.47g). Irrespective of auxin and

rootstock cultivars, the variety ‘Inca’ grafted on the top position cuttings had significantly higher stem weight than on the middle position cuttings but not significantly different from those on the bottom position cuttings.

In the third flush, the main factor of cutting position and its interaction with the rootstock cultivars and auxins significantly ($p \leq 0.05$) affected stem weight of grafted rose variety ‘Inca’ (Table 33b). Irrespective of auxin and rootstock cultivars, the variety ‘Inca’ grafted on the bottom position cuttings had significantly lower stem weight than those grafted on the middle and top position cuttings. The stem weight of the latter two positions were however, not significantly different from each other. The variety ‘Inca’ grafted on the bottom position cuttings had significantly lower stem weight than those grafted on the top and middle position cuttings in ‘Rosa progress’. The cutting position had no significant effect on stem weight of grafts on the rootstock ‘Natal briar’ (Table 33b).

The variety ‘Inca’ grafted on the bottom position cuttings and untreated with auxins had significantly lower (33.99g) stem weight than those grafted on the middle (44.95g) and top (41.68g) position cuttings. The variety ‘Inca’ grafted on the bottom position cuttings and treated with 0.4%IBA had significantly lower (36.02g) stem weight than those grafted on the top (42.07g) position cuttings (Table 33b).

Table 33a: Effects of cutting position, auxins and rootstock cultivars on stem weight (g) of top grafted rose cultivar 'Inca' (2nd flush).

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand position means	LSD values	
'Rosa progress'	Top	47.27	39.89	40.87	42.67	38.47	Top	Auxins	NS
	Middle	33.78	39.07	34.26	35.70		43.29	Rootstocks	3.07*
	Bottom	35.73	40.26	35.10	37.03			Position	3.76*
	Means	38.92	39.74	36.74			Middle	Auxins x position	NS
							38.62	Auxins x rootstock	NS
'Natal briar'	Top	47.03	44.20	40.45	43.90	42.76		Rootstocks x position	NS
	Middle	45.02	42.94	36.62	41.43		Bottom	Rootstock x position auxin	NS
	Bottom	42.18	45.11	41.31	42.87		39.95		
	Means	44.75	44.09	39.46					
Grand auxin means		41.84	41.91	38.10					

*LSD= Least Significant Difference of means, *significant at p ≤ 0.05; **significant at p ≤ 0.001, NS= Not significant*

Table 33b: Effects of rootstock cultivars, cutting position and auxins on stem weight (g) of top grafted rose variety 'Inca' (3rd flush).

Rootstock cultivars	Position	Control	0.2% NAA	0.4% IBA	Position Means	Rootstock Means	Grand Position means	LSD values	
'Rosa progress'	Top	44.78	38.41	43.45	42.21	39.69	Top	Auxins	NS
	Middle	48.58	38.11	45.19	43.96		40.47	Rootstocks	NS
	Bottom	30.38	35.00	33.29	32.89			Position	3.75*
	Means	41.24	37.17	40.64			Middle	Auxins x position	5.30*
							41.50	Auxins x rootstock	NS
'Natal briar'	Top	38.58	36.96	40.68	38.74	38.87		Rootstocks x position	2.60*
	Middle	41.32	43.62	32.20	39.05		Bottom	Rootstock x position x auxin	NS
	Bottom	37.60	40.16	38.75	38.84		35.86		
	Means	39.17	40.25	37.21					
Grand auxin means		40.20	38.71	38.93					

*LSD= Least Significant Difference of means, *significant at p ≤ 0.05; **significant at p ≤ 0.001, NS= Not significant*

4.3. THE BIOCHEMICAL CONSTITUENTS OF THE CUTTING POSITIONS OF ROSE ROOTSTOCK CULTIVARS

4.3.1 Carbohydrate levels

4.3.1.1. Carbohydrate levels in the leaf and stem base of the stem cuttings at the time of planting as influenced by rootstock cultivars and cutting position in the self rooting experiment

Rootstock cultivars, cutting position and the interaction of both significantly ($p \leq 0.05$) influenced fructose, glucose and sucrose level in the leaf at the time of planting (Table 34). Irrespective of cutting position, leaves of 'Natal briar' had significantly higher fructose, glucose and sucrose level than those of 'Rosa progress'. Irrespective of the rootstock cultivars, the fructose and glucose contents significantly increased from the bottom to the top position cuttings (Table 34) and the sucrose content significantly increased from the middle position followed by the top and then bottom position cuttings (Table 34). In 'Natal briar', the fructose level significantly ($p \leq 0.05$) decreased from the top (1.47%) to the bottom (0.24) position cuttings. The effect of cutting position on the glucose level of the rootstock 'Natal briar' was significant and followed a similar trend to that detected in the fructose level. Middle position cuttings had significantly higher sucrose level than the top and bottom position cuttings in 'Natal briar' (Table 34).

In 'Rosa progress', the bottom position cuttings had significantly lower (0.38) glucose level than the middle (0.57) position cuttings. However, the glucose level of the top position cuttings was not significantly different from the middle and bottom position cuttings. The sucrose level significantly decreased from the bottom position to the top position cuttings in 'Rosa progress'. The fructose levels of the three cutting positions were however, not significantly different from each other in 'Rosa progress'.

Table 34: Effects of rootstock cultivars and cutting position on carbohydrate level (%) of the leaf on stem cutting at the time of planting

Parameter	Rootstock	Cutting position			Rootstock Means	LSD values	
		Top	Middle	Bottom			
Fructose	Rosa progress	0.46	0.59	0.47	0.51	Rootstock	0.12*
	Natal briar	1.47	0.56	0.24	0.76	Position	0.15**
	Means	0.97	0.59	0.36		Rootstock x position	0.21**
Glucose	Rosa progress	0.47	0.57	0.38	0.47	Rootstock	0.10*
	Natal briar	1.22	0.54	0.28	0.68	Position	0.12**
	Means	0.85	0.55	0.33		Rootstock x position	0.17**
Sucrose	Rosa progress	1.29	0.78	0.35	0.81	Rootstock	0.21**
	Natal briar	0.60	1.95	0.63	1.06	Position	0.17*
	Means	0.94	1.36	0.49		Rootstock x position	0.29**

*LSD= Least Significant Difference of means, * =significant at p ≤ 0.05; ** =significant at p ≤ 0.001, NS= Not significant*

In the stem base of the cuttings, the cutting position significantly ($p \leq 0.05$) influenced the fructose content (Table 35).The middle position cuttings exhibited significantly higher fructose content than the top position cuttings, however, the fructose content of the bottom position cuttings was not significantly differen from the other two positions (Table 35). The cutting position however, had no significant effect on the glucose and sucrose contents (Table 35).

Table 35: Effects of rootstock cultivars and cutting position on carbohydrate level (%) of the stem base of stem cutting at the time of planting

Parameter	Rootstocks	Cutting position			Rootstock Means	LSD values	
		Top	Middle	Bottom			
Fructose	'Rosa progress'	0.75	1.13	0.84	0.91	Rootstock	NS
	'Natal briar'	0.7	0.85	0.85	0.78	Position	0.16*
	Means	0.72	0.99	0.84		Rootstock x position	NS
Glucose	'Rosa progress'	0.72	0.83	0.76	0.76	Rootstock	NS
	'Natal briar'	0.8	0.78	0.76	0.78	Position	NS
	Means	0.76	0.8	0.76		Rootstock x position	NS
Sucrose	'Rosa progress'	0.04	0.01	0.04	0.03	Rootstock	NS
	'Natal briar'	0.06	0.04	0.04	0.05	Position	NS
	Means	0.05	0.03	0.04		Rootstock x position	NS

*LSD= Least Significant Difference of means, * =significant at p ≤ 0.05; ** =significant at p ≤ 0.001, NS= Not significant*

4.3.1.2. Carbohydrate levels in the stem base of cuttings during the first week after planting as influenced by rootstock cultivars and cutting position in the self rooting experiment

a. Effects of rootstock cultivars, cutting position and days after planting on endogenous fructose and glucose levels (%) of stem cuttings.

The fructose and glucose levels were significantly ($p \leq 0.05$) influenced by the day after planting and its interaction with cutting position and with rootstock cultivars (Tables 36 and 37). Significant decrease in fructose and glucose levels from day 0 to day 7 after planting were recorded irrespective of the cutting positions and rootstock cultivars (Tables 36 and 37). In 'Rosa progress' the fructose and glucose levels significantly decreased from day 0 to day 7 after planting. In 'Natal briar' the fructose and glucose levels significantly decreased from day 0 to day 3 after planting followed by a non significant decrease to day 7 after planting (Tables 36 and 37). Irrespective of the rootstock cultivars and the days after planting, top position cuttings exhibited significantly higher glucose level than the middle position cuttings, however the glucose level of the bottom position cuttings was not significantly different from the other two positions (Table 37). 'Rosa progress' had significantly lower glucose level than 'Natal briar' irrespective of the cutting position and days after planting. The fructose and glucose levels of the three cutting positions significantly decreased from day 0 to day 7 after planting (Tables 36 and 37).

Table 36: Effects of rootstock cultivars, cutting position and days after planting on endogenous fructose level (%) of stem cuttings.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.75	0.27	0.04	0.35	0.38	Top	Days	0.06**
	Middle	1.13	0.10	0.06	0.43		0.36	Rootstocks	NS
	Bottom	0.84	0.22	0.05	0.37			Position	NS
	Means	0.91	0.20	0.05				Day x position	0.09*
							Middle	Day x rootstock	0.09*
'Natal briar'	Top	0.70	0.21	0.21	0.37	0.39	0.40	Rootstocks x position	NS
	Middle	0.85	0.19	0.06	0.37			Rootstock x position x day	NS
	Bottom	0.85	0.22	0.19	0.42				
	Means	0.80	0.21	0.15			Bottom		
Grand day means		0.85	0.20	0.10			0.39		

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 37: Effects of rootstock cultivars, cutting position and days after planting on endogenous glucose level (%) of stem cuttings.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.72	0.32	0.06	0.37	0.36	Top	Days	0.04**
	Middle	0.82	0.13	0.06	0.34		0.40	Rootstocks	0.04*
	Bottom	0.76	0.25	0.09	0.36			Position	0.03*
	Means	0.77	0.23	0.07			Middle	Day x position	0.07*
							0.34	Day x rootstock	0.06*
'Natal briar'	Top	0.80	0.19	0.29	0.43	0.40		Rootstocks x position	NS
	Middle	0.78	0.16	0.70	0.34			Rootstock x position x day	0.10*
	Bottom	0.76	0.25	0.30	0.43		Bottom		
	Means	0.78	0.20	0.21			0.39		
Grand day means		0.77	0.22	0.14					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

b. Effects of rootstock cultivars, cutting position and days after planting on endogenous sucrose and total sugar levels (%) of stem cuttings.

The sucrose level was significantly ($p \leq 0.05$) affected by days after planting, rootstock cultivars, cutting position, interaction between rootstock cultivars and position and between days after planting and rootstock cultivars (Table 38). Irrespective of rootstock cultivars and cutting position the sucrose level significantly increased from day 0 to day 7 after planting. Averaged across the rootstock cultivars and days after planting, bottom position cuttings recorded significantly lower sucrose level than the middle and top position cuttings, however, the sucrose level of the latter two positions were not significantly different from each other. 'Rosa progress' had significantly lower sucrose level than 'Natal briar' irrespective of the cutting position and days after planting.

In 'Rosa progress', bottom position cuttings exhibited significantly lower sucrose level than the middle position cuttings however, the sucrose level in top position cuttings did not significantly differ from those in the other two cutting positions. In 'Natal briar' a significant decrease in sucrose level from the top to the middle position followed by a non significant decrease to the bottom was observed. In 'Rosa progress' the sucrose level significantly increased from day 0 to day 3 after planting followed by a non significant decrease to day 7 after planting (Table 38). In 'Natal briar' the sucrose level significantly increased from day 0 through day 3 to day 7 after planting.

The main factors of rootsock cultivars and day after planting and interaction of rootstock cultivars with cutting position and with days after planting and the three way interaction of auxin x position x rootstock cultivars significantly ($p \leq 0.05$) influenced the total sugar content (Table 39). Averaged across the cutting position and days after planting, the rootstock 'Natal

briar' exhibited higher total sugar level than 'Rosa progress'. The total sugar level significantly decreased from day 0 to day 7 after planting in 'Rosa progress'. In 'Natal briar' there was a significant decrease in total sugar level from day 0 to day 3 after planting followed by a non significant increase to day 7 after planting. Middle position cuttings exhibited significantly higher total sugar level than bottom position cuttings in 'Rosa progress' while in 'Natal briar' stem base of top position cuttings exhibited significantly higher total sugar level than the middle position cuttings.

Table 38: Effects of rootstock cultivars, cutting position and days after planting on endogenous sucrose level (%) of stem cuttings.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.04	0.63	0.34	0.34	0.33	Top	Days	0.10**
	Middle	0.01	0.47	0.83	0.44		0.51	Rootstocks	0.10*
	Bottom	0.04	0.39	0.25	0.23			Position	0.08**
	Means	0.03	0.50	0.48			Middle	Day x position	NS
							0.47	Day x rootstock	0.14**
'Natal briar'	Top	0.06	0.67	1.35	0.69	0.56		Rootstocks x position	0.14*
	Middle	0.04	0.85	0.63	0.51			Rootstock x position x day	0.24**
	Bottom	0.04	0.63	0.82	0.50		Bottom		
	Means	0.08	0.71	0.93			0.36		
Grand day means		0.04	0.60	0.70					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 39: Effects of rootstock cultivars, cutting position and days after planting on endogenous total sugar level (%) of stem cuttings.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	1.51	1.22	0.44	1.06	1.07	Top	Days	0.17**
	Middle	1.96	0.70	0.96	1.21		1.27	Rootstocks	0.14**
	Bottom	1.64	0.85	0.39	0.96			Position	NS
	Means	1.70	0.92	0.60			Middle	Day x position	NS
							1.21	Day x rootstock	0.24**
'Natal briar'	Top	1.55	1.06	1.85	1.49	1.34		Rootstocks x position	0.24*
	Middle	1.67	1.20	0.76	1.21			Rootstock x position x day	0.41*
	Bottom	1.65	1.10	1.27	1.34		Bottom		
	Means	1.62	1.12	1.29			1.15		
Grand day means		1.66	1.02	0.94					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.3.1.3. Carbohydrate levels in the stem base of cuttings during the first week after planting as influenced by rootstock cultivars and cutting position in the top grafting experiment.

a. Effects of rootstock cultivars, cutting position and days after planting on endogenous fructose and glucose levels (%) of stem cuttings.

Days after planting, rootstock cultivars, cutting position, interaction between days after planting and cutting position and three way interaction of days after planting x position x rootstock cultivars significantly ($p \leq 0.05$) influenced the carbohydrate level (Tables 40 and 41). The rootstock 'Natal briar' had significantly higher fructose and glucose levels than 'Rosa progress' regardless of the cutting position and days after planting (Tables 40 and 41). Significant decrease in fructose and glucose levels from day 0 to day 3 after planting followed by a significant increase to day 7 after planting was recorded regardless of the cutting position and rootstock cultivars (Tables 40 and 41). The bottom position cuttings recorded significantly lower fructose and glucose levels than the top and middle position cuttings regardless of the rootstock cultivars and days after planting (Tables 40 and 41), however the fructose and glucose levels of the latter two positions were not significantly different from each other. The fructose and glucose levels significantly decreased from day 0 to day 3 after planting in the middle position cuttings however, in the top and bottom position cuttings there was a non significant decrease to day 3 after planting. From day 3 after planting, the fructose and glucose levels significantly increased upto day 7 after planting (Tables 40 and 41).

Table 40: Effects of rootstock cultivars, cutting position and days after excision on endogenous fructose levels (%) in the stem base of cuttings in top grafting

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.27	0.18	0.34	0.26	0.23	Top	Days	0.08**
	Middle	0.24	0.03	0.58	0.28		0.31	Rootstocks	0.08*
	Bottom	0.15	0.15	0.12	0.14			Position	0.07**
	Means	0.22	0.12	0.35			Middle	Day x position	0.15*
							0.34	Day x rootstock	NS
'Natal briar'	Top	0.28	0.16	0.66	0.36	0.35		Rootstocks x position	NS
	Middle	0.59	0.11	0.48	0.39		Bottom	Rootstock x position x day	0.21**
	Bottom	0.15	0.17	0.53	0.30		0.22		
	Means	0.35	0.15	0.56					
Grand day means		0.29	0.13	0.45					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 41: Effects of rootstock cultivars, cutting position and days after excision on endogenous glucose level (%) of stem cuttings in top grafting.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.22	0.17	0.33	0.24	0.21	Top	Days	0.06*
	Middle	0.22	0.03	0.55	0.27		0.30	Rootstocks	0.06**
	Bottom	0.13	0.14	0.12	0.13			Position	0.05**
	Means	0.19	0.11	0.33			Middle	Day x position	0.11*
							0.32	Day x rootstock	NS
'Natal briar'	Top	0.28	0.21	0.59	0.36	0.34		Rootstocks x position	NS
	Middle	0.57	0.09	0.45	0.37		Bottom	Rootstock x position x day	0.15**
	Bottom	0.20	0.18	0.49	0.29		0.21		
	Means	0.35	0.16	0.51					
Grand day means		0.27	0.14	0.42					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

b. Effects of rootstock cultivars, cutting position and days after planting on endogenous sucrose and total sugar levels (%) of stem cuttings.

Days after planting, rootstock cultivars, cutting position, interaction between days after planting and cutting position and three way interaction of days after planting x position x rootstock cultivars significantly ($p \leq 0.05$) influenced the sucrose level (Table 42). The rootstock 'Natal briar' had significantly higher sucrose and total sugar levels than 'Rosa progress' regardless of the cutting position and days after planting (Tables 42 and 43). Regardless of the cutting position and the rootstock cultivars, the sucrose level significantly decreased from day 0 to day 3 after planting followed by a non significant decrease to day 7 after planting (Table 42). The middle position cuttings had significantly lower sucrose level than the top and bottom position cuttings whose sucrose levels did not differ significantly.

The interaction between rootstock cultivars and cutting position was significant for sucrose (Table 42). In 'Rosa progress', bottom position cuttings recorded significantly higher sucrose content than the middle and top position cuttings (Table 42), however, the sucose content of the latter two positions were not significantly different from each other. In 'Natal briar', top position cuttings exhibited significantly higher sucrose content than the middle and bottom position cuttings (Table 42), however, the sucose content of the latter two positions were not significantly different from each other. Irrespective of the rootstock cultivars, the sucrose level decreased from day 0 to day 3 after planting followed by a significant increase to day 7 after planting in the top position cuttings (Table 42). The sucrose level of the middle and bottom position cuttings were not significantly different from day 0 to day 7 after planting (Table 42).

The total sugar level was significantly affected by the rootstock cultivars, day after planting and interaction of cutting position with rootstock cultivars and also with days after planting (Table

43). Day 3 after planting recorded significantly lower total sugar level than days 0 and 7 after planting regardless of the cutting position and rootstock cultivars, however, the total sugar level of the latter two days were not significantly different from each other. In 'Natal briar', top position cuttings had significantly higher total sugar level than the middle and bottom position cuttings, however, the total sugar level of the latter two positions were not significantly different from each other (Table 43). The cutting position however, had no significant effect on the total sugar level of 'Rosa progress' (Table 43).

In 'Natal briar', the total sugar level significantly decreased from day 0 to day 3 after planting in the middle position cuttings however, the total sugar level in the top and bottom position cuttings recorded a non significant decrease from day 0 to day 3 after planting. From day 3 after planting, the total sugar levels significantly increased upto day 7 after planting in all the cutting positions (Table 43). In 'Rosa progress' the total sugar level significantly decreased from day 0 to day 7 after planting in the bottom position cuttings while in the middle position cuttings the total sugar level significantly decreased from day 0 to day 3 after planting followed by a significant increase to day 7 after planting (Table 43).

Table 42: Effects of rootstock cultivars, cutting position and days after planting on endogenous sucrose level (%) of stem cuttings in top grafting.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.37	0.31	0.26	0.31	0.46	Top	Days	0.19*
	Middle	0.45	0.11	0.55	0.37		0.73	Rootstocks	0.19*
	Bottom	1.10	0.96	0.03	0.70			Position	0.16*
	Means	0.64	0.46	0.28			Middle	Day x position	0.33
							0.43	Day x rootstock	NS
'Natal briar'	Top	1.14	0.80	1.49	1.14	0.77		Rootstocks x position	0.27*
	Middle	0.71	0.50	0.27	0.49		Bottom	Rootstock x position x day	0.47*
	Bottom	0.90	0.52	0.23	0.55		0.62		
	Means	0.92	0.61	0.66					
Grand day means		0.78	0.53	0.47					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

Table 43: Effects of rootstock cultivars, cutting position and days after planting on endogenous total sugar level (%) of stem cuttings in top grafting.

Rootstock cultivars	Position	Days post excision			Position Means	Rootstock Means	Grand position means	LSD values	
		0	3	7					
'Rosa progress'	Top	0.85	0.67	0.93	0.81	0.90	Top	Days	0.29**
	Middle	0.90	0.17	1.68	0.92		1.34	Rootstocks	0.24**
	Bottom	1.39	1.25	0.27	0.97			Position	NS
	Means	1.05	0.69	0.96			Middle	Day x position	0.50*
							1.09	Day x rootstock	NS
'Natal briar'	Top	1.70	1.16	2.74	1.87	1.42		Rootstocks x position	0.41*
	Middle	1.86	0.70	1.20	1.26		Bottom	Rootstock x position x day	0.71*
	Bottom	1.29	0.87	1.25	1.14		1.05		
	Means	1.62	0.91	1.73					
Grand day means		1.33	0.80	1.34					

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.3.2. Effects of rootstock cultivars, cutting position and days post excision on endogenous indole-3-acetic acid (IAA) and cytokinin concentrations in the stem base and bud region of cuttings in the self rooting experiment.

4.3.2.1. Effects of rootstock cultivars, cutting position and days post excision on endogenous IAA concentration in the stem base and bud region of cuttings.

The concentration of IAA in the stem base and bud region of cuttings was significantly ($p \leq 0.05$) influenced by the main factors of rootstock cultivars, the cutting position, the days post excision (Table 44) and the interaction between rootstock cultivars and day post excision (Figures 2 and 3). Irrespective of the rootstock cultivars and days post excision, top position cuttings of the the stem base and bud region of cuttings had significantly higher IAA concentration than the bottom position cuttings of the stem base and bud region of cuttings (Table 44). Irrespective of cutting position and days post excision, the rootstock 'Rosa progress' had significantly higher IAA concentration than 'Natal briar' in the stem base and bud region of cuttings (Table 44).

At the stem base, top position cuttings of 'Rosa progress'had significantly higher IAA concentration than bottom position cuttings on days 2 and 8 post excision while in 'Natal briar', top position cuttings had significantly higher IAA concentration than the bottom position cuttings on days 0 and 8 post excision (Figure 2a). The concentration of IAA decreased from day 0 to day 1 post excision in all the cutting positions of rootstock cultivars followed by an increase to the 8^{th} day except for the bottom position cuttings of 'Natal briar' that recorded a decreasing amount of IAA from day 4 to day 8 post excision (Figure 2a).

A significant ($p \leq 0.05$) interaction of cutting position with rootstock cultivars and also with day post excision was also detected in the bud region of cuttings. The IAA concentration tended to

increase from day 0 to day 8 post excision in all the cutting positions of the rootstock cultivars in the bud region of cuttings (Figure 2b). The concentration of IAA was not significantly different among the cutting positions of both rootstock cultivars for the first days after planting until days 4 and 8 post excision (Figure 2b). 'Rosa progress' had higher IAA concentration in the bud region of cuttings than 'Natal briar' irrespective of the cutting positions on day 4 after planting. The IAA concentration of the bottom position cuttings of 'Rosa progress' and top position cuttings of 'Natal briar' were not significantly different from each other on day 8 post excision, but were significantly lower than IAA in the top position cuttings of 'Rosa progress' and significantly higher than the bottom position cuttings of 'Natal briar' (Figure 2b).

Irrespective of the cutting position, the IAA level in the stem base of cuttings decreased from day 0 (27.8 pmol/g FM) to day 1 (19.3 pmol/g FM) post excision, but thereafter significantly increased to day 2 (55.0 pmol/g FM) post excision in 'Rosa progress'. From day 2 to day 8 (80.8 pmol/g FM) post excision the IAA concentration did not significantly change in 'Rosa progress' (Figure 3). Irrespective of the cutting position, the IAA level in the stem base of 'Natal briar' did not significantly change during the course of the experiment (Figure 3). In the bud region of cuttings, the IAA concentration significantly increased from day 0 to day 8 post excision in 'Rosa progress'. In 'Natal briar' the IAA concentration recorded a non significant increase from day 0 to day 8 post excision (Figure 3). At day 8 post excision, significantly higher IAA concentration was found in 'Rosa progress' than 'Natal briar' (Figure 3).

Table 44: Effects of main factors of rootstock cultivars, cutting position and days post excision on endogenous IAA concentration (pmol/g FM) in the stem base and bud region of cuttings

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	51.8	25.1	46.8	30.1	14	17.3	31.4	52.3	77.30
LSD	10.40**		10.40**		16.44**				
Stem base	49.1	34.5	49.8	33.7	36.6	22.4	41.3	49.4	59.7
LSD	10.04*		10.04**		15.87**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

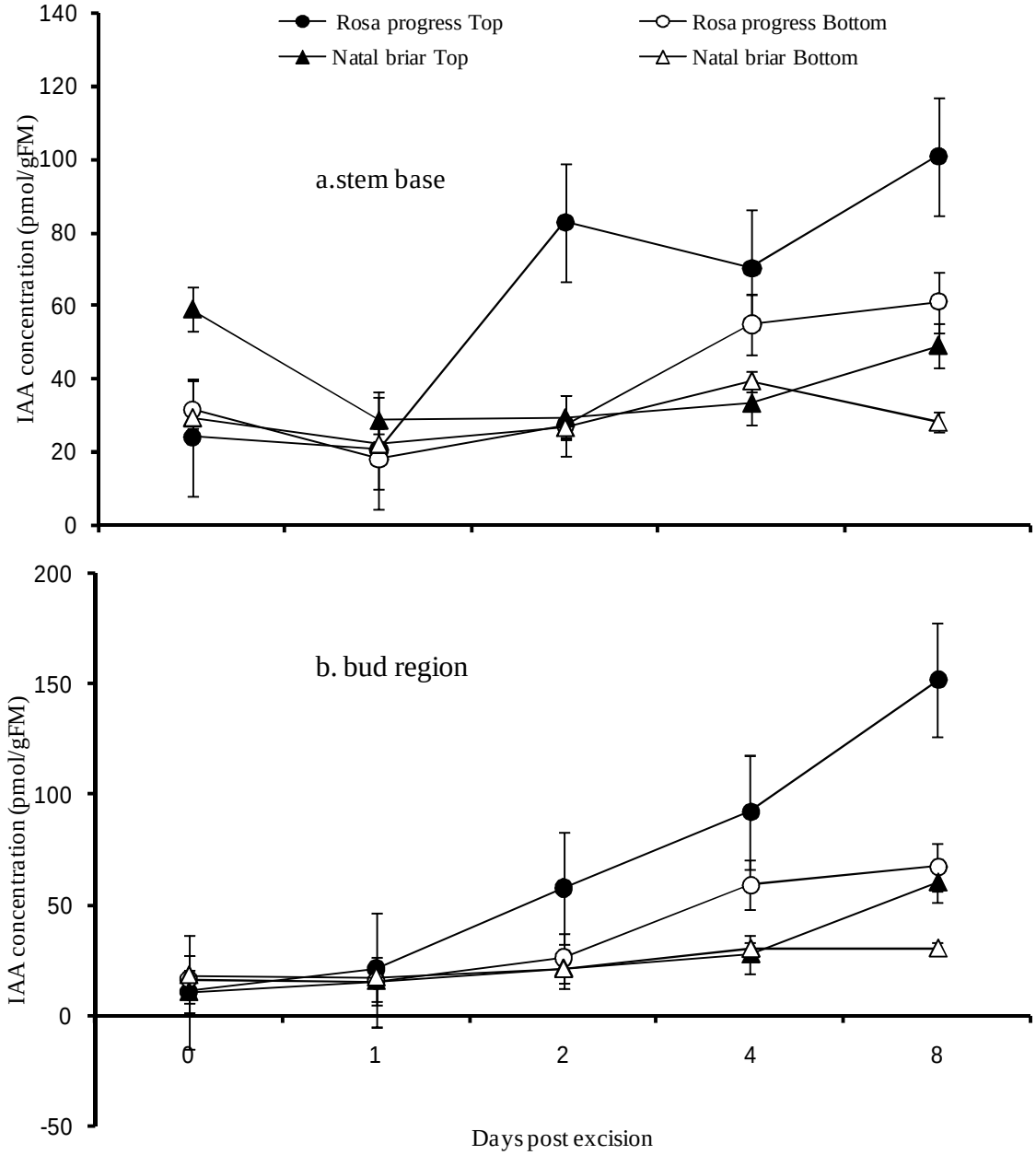

Figure 2: The time course of IAA in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

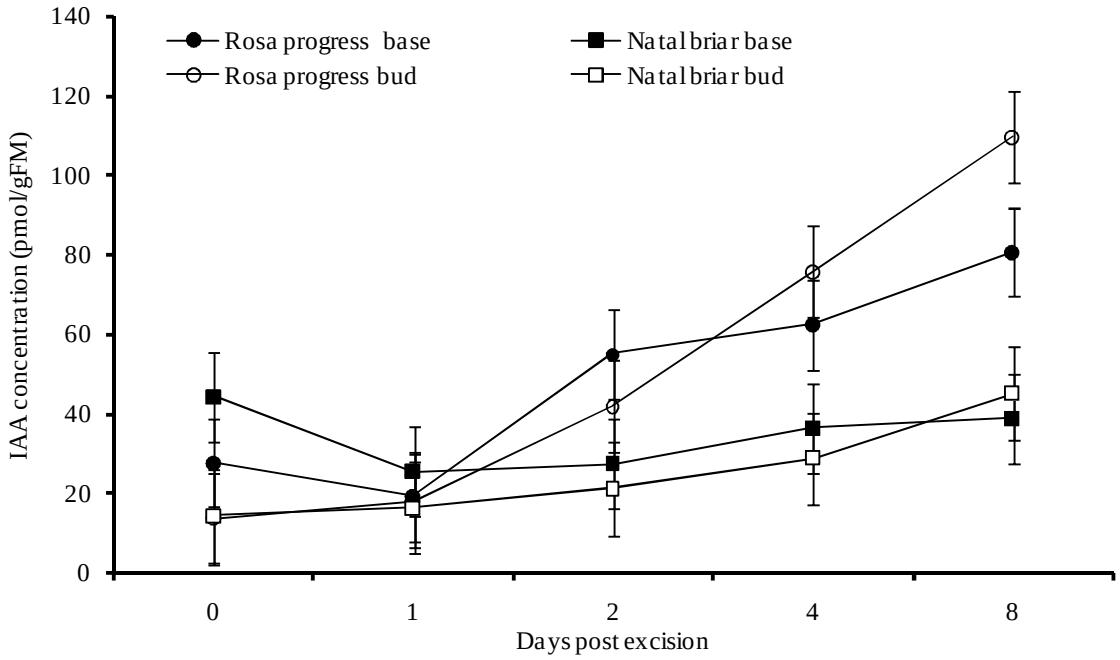

Figure 3: The time course of IAA in the stem base and bud region cuttings of rootstock cultivars

4.3.2.2. Effects of rootstock cultivars, cutting position and days post excision on endogenous IPR concentration in the stem base and bud region of cuttings.

The main factors of rootstock cultivars, cutting position and days post excision significantly ($p \leq 0.05$) influenced the IPR concentration in the stem base and bud region of cuttings (Table 45). Irrespective of the rootstock cultivars and cutting position, there was a significant increase in IPR concentration from day 0 (7.0 pmol/g FM) through day 1 (16.6 pmol/g FM) to day 2 (46.3 pmol/g FM) post excision followed by a significant ($p \leq 0.05$) decrease to day 4 post excision and a non significant increase to day 8 (23.6 pmol/g FM) post excision in the stem base region (Table 45). Irrespective of cutting position and days post excision, 'Natal briar' had significantly higher IPR concentration than 'Rosa progress' in the stem base and bud region of cuttings (Table 45). Irrespective of rootstock cultivars and days post excision, top position cuttings had

significantly higher IPR concentration than the bottom position cuttings in the stem base and bud region of cuttings.

Table 45: Effects of main factors of rootstock cultivars, cutting position and days post excision on endogenous IPR concentration (pmol/g FM) in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	46.6	119	106.3	59.2	12.2	58	160.6	82	101.2
LSD	18.46*		18.46**		29.19**				
Stem base	18	27.8	27.1	18.7	7	16.6	46.3	21.7	23.6
LSD	5.90**		5.90**		9.33**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

In the stem base of cuttings, the concentration of IPR remained statistically similar between days 0 and 1 post excision in bottom position cuttings of both rootstock cultivars, followed by a significant sharp rise to day 2 post excision thereafter a drop to day 4 post excision in all the cutting positions (Figure 4a). The concentration of IPR in the top position cuttings of 'Natal briar' significantly increased from day 0 to day 2 post excision while those of 'Rosa progress' were statistically similar from day 0 to day 1 post excision followed by a significant increase to day 2 post excision (Figure 4a). Between days 4 and 8 post excision, the concentration of IPR remained on a statistically similar level in the top position cuttings of 'Natal briar' and top and bottom position cuttings of 'Rosa progress' while those of the bottom position cuttings of 'Natal briar' increased to day 8 post excision. The IPR concentration in the top position cuttings of 'Rosa progress' decreased from day 2 to day 8 post excision (Figure 4a). On day 2 post excision,

‘Natal briar’ had significantly higher IPR concentration in the stem base of cuttings than ‘Rosa progress’ (Figure 4a).

In the bud region of cuttings, IPR concentration increased from day 0 to day 2 post excision followed by a decrease to day 4 post excision and thereafter the concentration of IPR remained on a similar level to day 8 post excision in all the cutting positions of both rootstock cultivars (Figure 4b). The top position cuttings of ‘Natal briar’ recorded significantly higher IPR concentration than the bottom position and both cutting positions of ‘Rosa progress’ on days 1, 2 and 8 post excision (Figure 4b).

Significant ($p \leq 0.05$) interaction between the rootstock cultivars and day post excision was noted in the bud region of cuttings (Figure 5). Averaged across the cutting positions, the IPR concentration significantly increased from day 0 to day 2 post excision followed by a significant decrease to day 4 post excision in both rootstock cultivars (Figure 5). Between days 4 and 8 post excision, the concentration of IPR remained on a similar level in both rootstock cultivars (Figure 5). ‘Natal briar’ had significantly higher IPR concentration than ‘Rosa progress’ on days 1, 4 and 8 post excision in the bud region of cuttings (Figure 5). In the stem base of cuttings, a similar but non significant trend to that recorded in the bud region of cuttings was noted except on day 2 post excision that ‘Natal briar’ had significantly higher IPR concentration than ‘Rosa progress’ (Figure 5).

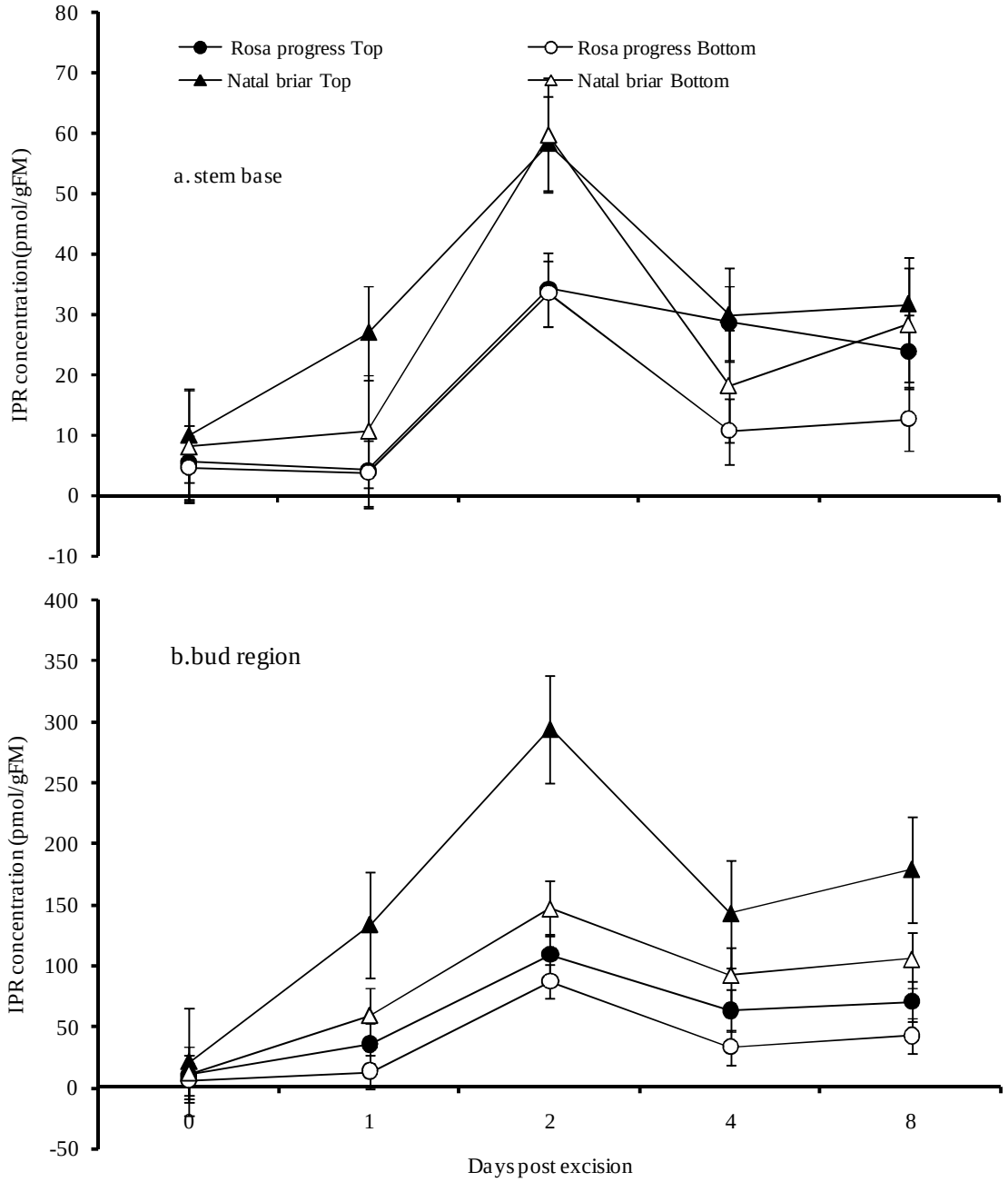

Figure 4: The time course of IPR in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

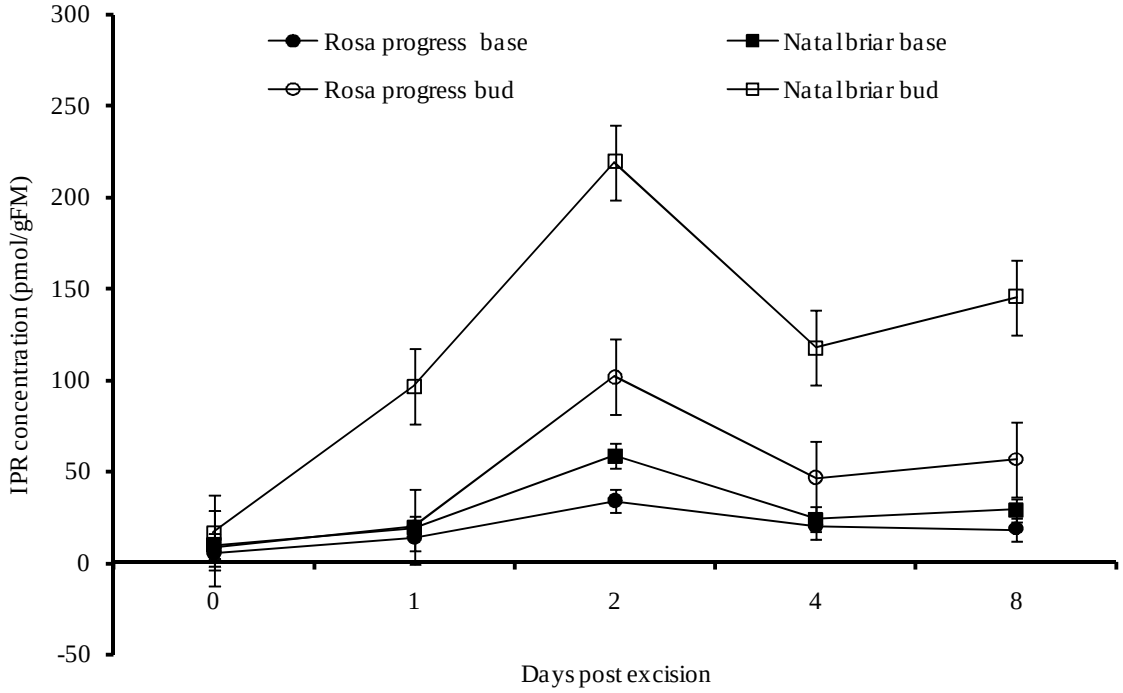

Figure 5: The time course of IPR in the stem base and bud region cuttings of rootstock cultivars

4.3.2.3. Effects of rootstock cultivars, position and days post excision on endogenous tr-Z concentration in the stem base and bud region of cuttings.

Day post excision significantly ($p \leq 0.05$) influenced tr-Z concentration in the stem base and bud region of cuttings (Table 46). Irrespective of rootstock cultivars and cutting position, a significant decrease in tr-Z concentration was observed from day 0 (526 pmol/g FM) through day 1 (484.7pmol/gFM) to day 2 (456.5pmol/g FM) post excision followed by a non significant increase to day 4 (463.8pmol/g FM) post excision and a non significant decrease to day 8 (454.8pmol/g FM) post excision in the stem base of cuttings. A similar trend was detected in the bud region except that there was a non significant increase in tr-Z concentration from day 2 to day 8 post excision. Irrespective of cutting position and day post excision, 'Rosa progress' had significantly ($p \leq 0.05$) lower concentration of tr-Z than 'Natal briar' in the bud region (Table

46). A similar trend but non significant, was detected in the stem base of cuttings. The concentration of tr-Z in the stem base and bud region of cuttings was not significantly ($p \leq 0.05$) affected by the interactions of the treatments.

Table 46: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous tr-Z concentration (pmol/g FM) in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	488.1	504.7	497.1	495.7	539.1	498.1	466.6	486.9	491.3
LSD	16.44*		NS		25.99**				
Stem base	471.2	483.1	473.9	480.4	526	484.7	456.5	463.8	454.8
LSD	NS		NS		25.89**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

4.3.2.4. Effects of rootstock cultivars, cutting position and days post excision on endogenous DHZR concentration in the stem base and bud region of cuttings.

The main factors of the rootstock cultivars, cutting position, days post excision (Table 47), interactions between rootstock cultivars and day post excision, rootstock cultivars and cutting position and day post excision and cutting position (Figures 6 and 7) significantly ($p \leq 0.05$) influenced the DHZR concentration of the stem base region of cuttings. Irrespective of the rootstock cultivar and days post excision, top position cuttings had significantly higher DHZR concentration than the bottom position cuttings (Table 47) in the stem base region of cuttings. The rootstock 'Rosa progress' had significantly higher DHZR concentration than 'Natal briar' in the stem base region of cuttings (Table 47). Irrespective of the rootstock cultivars and cutting position, the concentration of DHZR was statistically similar from day 0 to day 1 post excision

followed by a significant increase to day 2 post excision and thereafter a significant decrease to day 8 post excision in the stem base of cuttings (Table 47). In the bud region of cuttings, the concentration of DHZR remained on a statistically similar level from day 0 to day 1 post excision followed by a significant increase to day 2 post excision and thereafter a non significant increase to day 8 post excision (Table 47).

Table 47: Effects of the main factors of rootstock cultivars, cutting position and days post excision and on endogenous DHZR concentration (pmol/g FM) in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	786	786	804	769	471	518	915	941	1085
LSD	NS		NS		75.4**				
Stem base	839	608	787	661	494	595	1070	804	655
LSD	66.9**		66.9**		105.8**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

The concentration of DHZR remained at a statistically similar level from day 0 to day 8 post excision in all the cutting positions of both rootstock cultivars except the top position cuttings of 'Rosa progress' that recorded significantly higher DHZR concentration on day 2 post excision than the other positions in the stem base (Figure 6a). In the bud region of cuttings, the concentration of DHZR increased from day 0 to day 8 post excision in all positions of both rootstock cultivars but were not significantly different from each other except on day 2 post excision where the bottom position cuttings of both rootstock cultivars had significantly lower DHZR than the top position cuttings (Figure 6b).

In the stem base of cuttings, the concentration of DHZR significantly increased from day 0 post excision to day 4 post excision followed by a significant decrease to day 8 (674) post excision in 'Rosa progress' (Figure 7). In 'Natal briar', a significant increase was noted from day 0 post excision to day 2 post excision followed by non significant decrease to day 8 post excision (Figure 7). In the bud region of cuttings, there was a non significant ($p \leq 0.05$) increase in DHZR concentration from day 0 to day 1 post excision followed by a significant increase to day 2 post excision thereafter the concentrationof DHZR did not significantly change upto day 8 post excision in both rootstock cultivars (Figure 7).

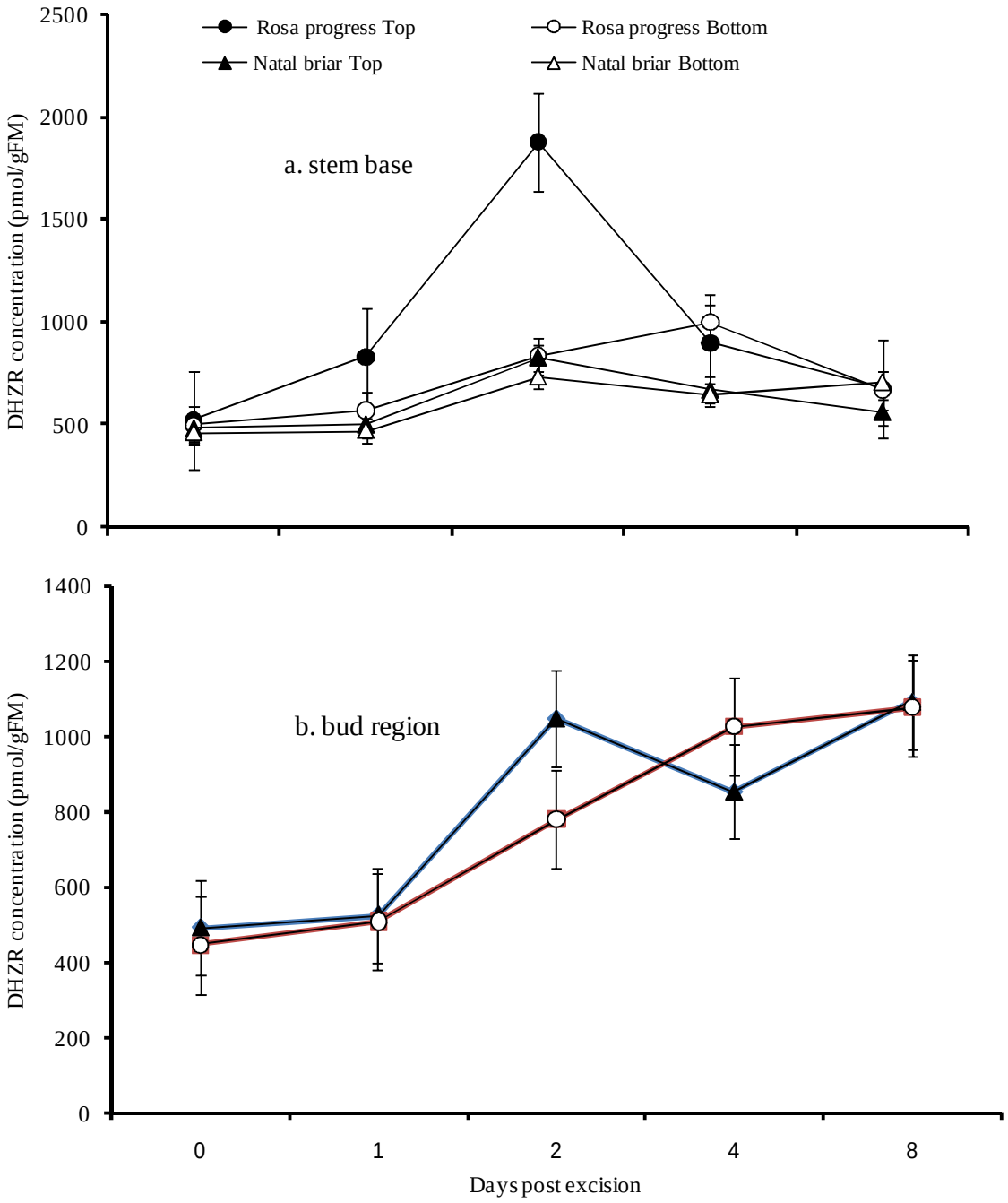

Figure 6: The time course of DHZR in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

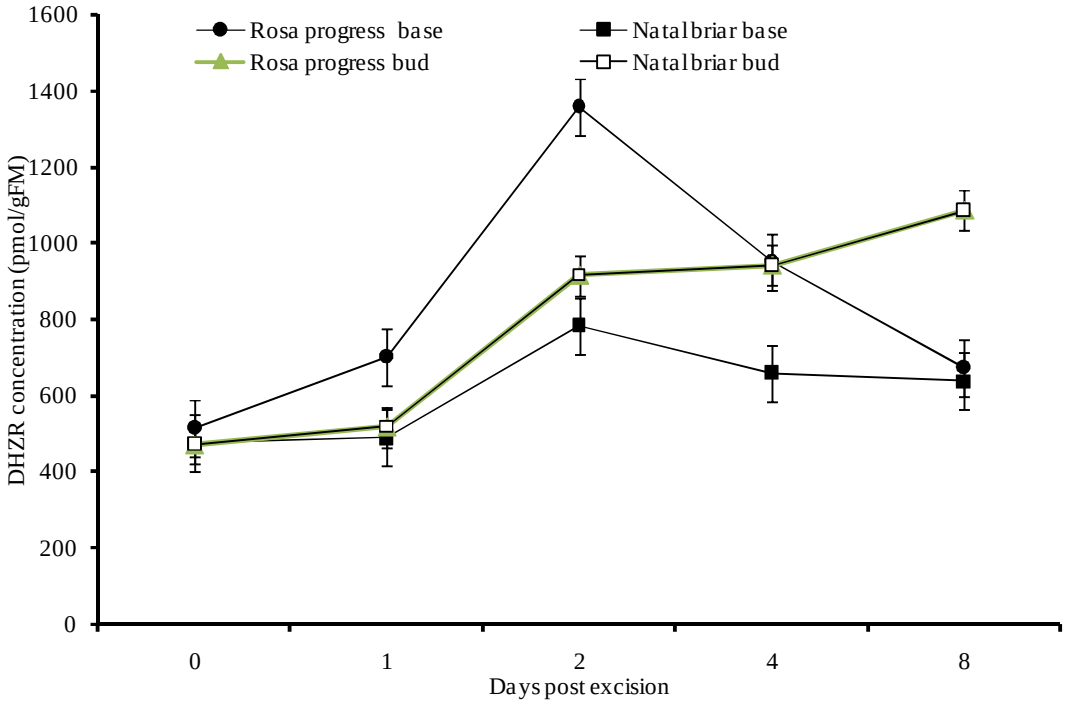

Figure 7: The time course of DHZR in the stem base and bud region cuttings of rootstock cultivars

4.3.2.5. Effects of rootstock cultivars, cutting position and days post excision on endogenous cis-ZR concentration in the stem base and bud region of cuttings.

The concentration of cis-ZR in the stem base and bud region of cuttings was significantly ($p \leq 0.05$) influenced by the rootstock cultivars and days post excision (Table 48). Irrespective of the cutting position and days post excision, 'Natal briar' had significantly higher cis-ZR concentration than 'Rosa progress' in the stem base and bud region of cuttings. Irrespective of cutting position and rootstock cultivars, cis-ZR concentration remained on a similar level from day 0 (3879 pmol/g FM) to day 1 (3292 pmol/gFM) post excision followed by significant increase to day 2 (4574 pmol/gFM) post excision in the bud region of cuttings. A significant decrease in the concentration was recorded from day 2 to day 4 post excision followed by a non significant decrease to day 8 (2560 pmol/g FM) post excision in the bud region of cuttings (Table 48). In the stem base of cuttings, the concentration of cis-ZR remained on a statistically similar

level from day 0 to day 1 post excision followed by a significant increase to day 2 post excision and thereafter a significant decrease to day 4 post excision and a non significant decrease to day 8 post excision (Table 48).

Table 48: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous cis-ZR concentration (pmol/gFM) in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	2179	4755	3340	3594	3879	3292	4574	3030	2560
LSD	773.8**		NS		1223.5*				
Stem base	1369	2289	1711	1947	2209	1737	2887	1337	975
LSD	274.1**		NS		433.3*				

*LSD= Least Significant Difference of means, * significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

In the stem base of cuttings, 'Natal briar' recorded significantly higher cis-ZR concentration than 'Rosa progress' on days 0 and 1 post excision. On day 2 post excision, bottom position cuttings of 'Natal briar' recorded significantly higher cis-ZR concentration than its top position and both cutting positions of 'Rosa progress' (Figure 8a). In 'Rosa progress', the cutting position had no significant effect on cis-ZR concentration on days 1, 2, 4 and 8 after planting except on day 0 after planting, where the bottom position cuttings had significantly higher cis-ZR concentration than the top position cuttings of the stem base region of cuttings. In 'Natal briar', the effect of cutting position on cis-ZR concentration was not significant on days 0,1,4 and 8 post excision except on day 2 post excision where the top position cuttings had significantly higher cis-ZR concentration than the bottom position cuttings of the stem base region of cuttings (Figure 8a).

Irrespective of the cutting position and days post excision ‘Natal briar’ exhibited significantly higher cis-ZR concentration in the bud region of cuttings than ‘Rosa progress’ on days 0, 1 and 2 post excision (Figure 8b). Bottom position cuttings of ‘Natal briar’ recorded significantly higher cis-ZR concentration than its top position and both positions of ‘Rosa progress’ on day 4 post excision however, on day 8 post excision there was no significant difference among the cutting positions of both rootstocks in the bud region (Figure 8b).

Significant ($p \leq 0.05$) interaction between rootstock cultivars and day post excision was recorded in the stem base and bud region of cuttings (Figure 9). The concentration of cis-ZR was not significantly different from day 0 to day 2 post excision in both rootstock cultivars and thereafter a significant decrease to day 8 post excision in ‘Rosa progress’ and a non significant decrease to day 8 post excision in ‘Natal briar’ was recorded in the bud region of cuttings (Figure 9). In the stem base of cuttings, the cis-ZR concentration did not significantly change from day 0 to day 1 post excision thereafter the concentration of cis-ZR significantly increased to day 2 post excision in both rootstock cultivars. From day 2 post excision, the concentration of cis-ZR significantly decreased to day 4 post excision and thereafter remained on a statistically similar level to day 8 post excision in both rootstock cultivars (Figure 9).

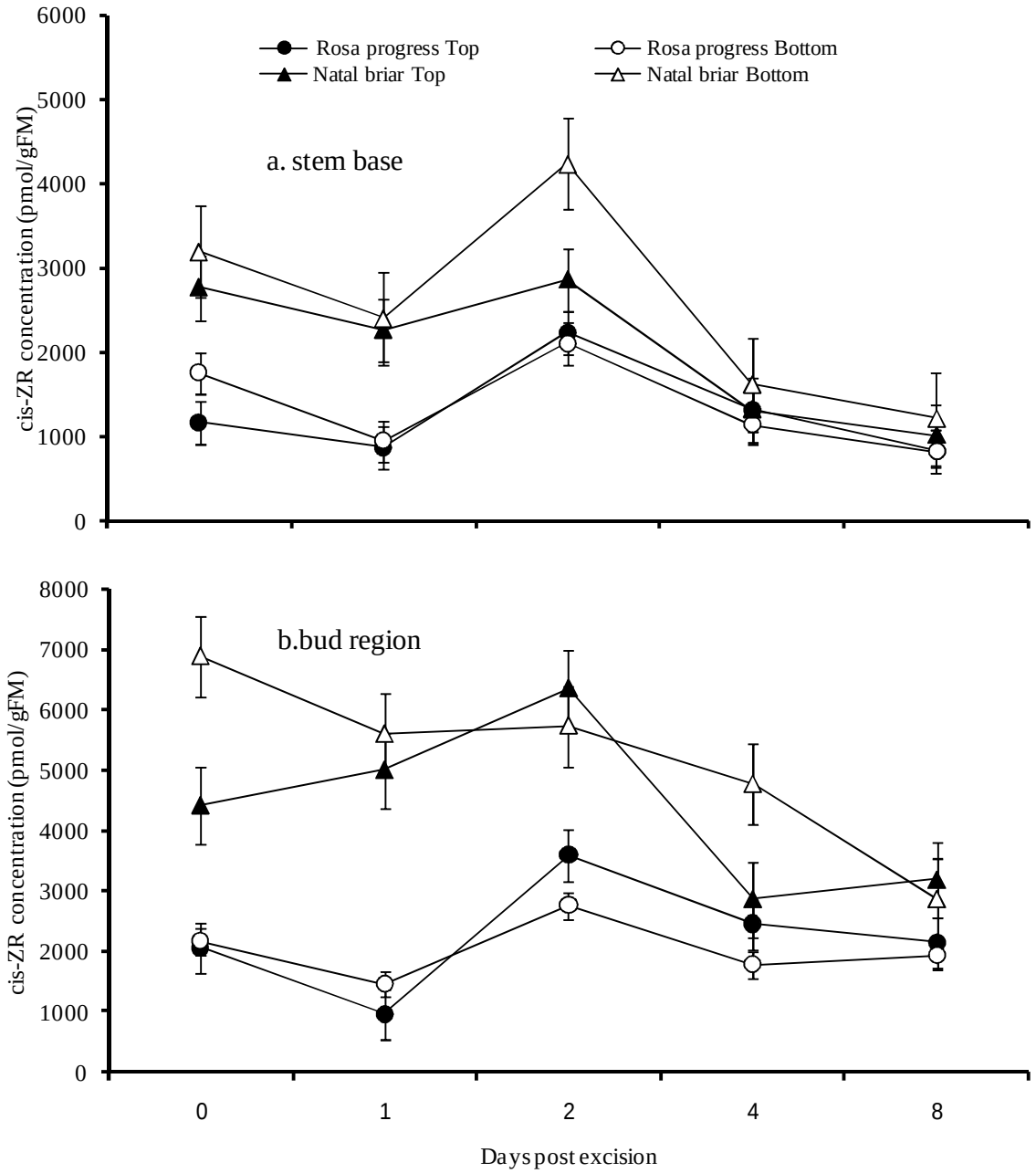

Figure 8: The time course of cis-ZR in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

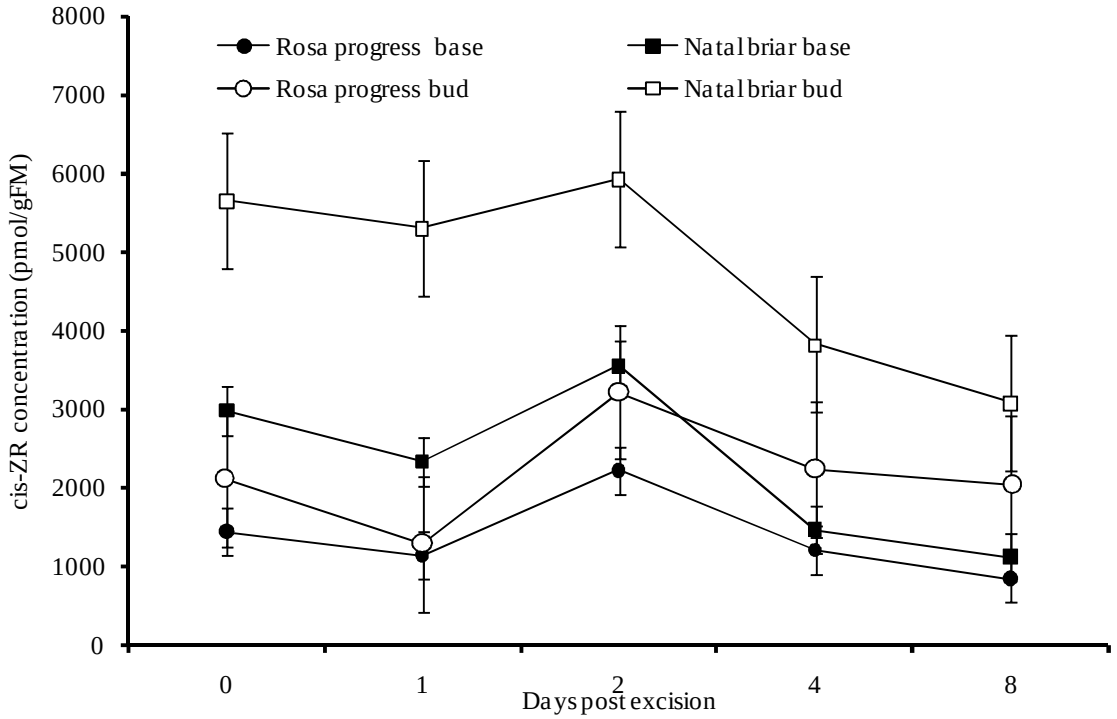

Figure 9: The time course of cis-ZR in the stem base and bud region cuttings of rootstock cultivars

4.3.2.6. Effects of rootstock cultivars, cutting position and days post excision on endogenous tr-ZR concentration in the stem base and bud region of cuttings.

Rootstock cultivars, cutting position, days post excision (Table 49) and interaction between cutting position and days post excision significantly ($p \leq 0.05$) influenced the tr-ZR concentration (Figures 10 and 11). Irrespective of cutting position and day post excision, 'Rosa progress' had significantly lower (464.7 pmol/g FM) tr-ZR concentration than 'Natal briar' (483 pmol/gFM) in the stem base of cuttings (Table 49). Irrespective of rootstock cultivars and days post excision, top position (489.2pmol/gFM) cuttings had significantly higher tr-ZR concentration than bottom position (458.5pmol/gFM) cuttings in the stem base of cuttings. Irrespective of rootstock cultivars and cutting position, the tr-ZR concentration in the stem base

of cuttings significantly increased from day 0 to day 2 post excision followed by a significant decrease to day 8 post excision (Table 49).

Table 49: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous tr-ZR concentration (pmol/g FM) in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	501	630	585	546	690	510	600	529	498
LSD	NS		NS		NS				
Stem base	464.7	483	489.2	458.5	449.6	481.8	514.2	472.1	451.5
LSD	12.0*		12.0**		18.98**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

In the stem base of cuttings, the concentration of tr-ZR did not significantly change during the course of the experiment except on days 1, 2 and 4 post excision (Figure 10a). On days 1 and 4 post excision, top position cuttings of 'Natal briar' recorded significantly higher tr-ZR concentration than the other positions (Figure 10a). On day 2, the bottom position cuttings of 'Rosa progress' exhibited significantly lower tr-ZR concentration than the top position and both cutting positions of 'Natal briar' (Figure 10a). In the bud region of cuttings, the concentration of tr-ZR did not significantly change during the course of the experiment except on day 0 post excision that the bottom position cuttings of 'Rosa progress' was significantly higher than that of the other positions (Figure 10b).

In the stem base of cuttings, the concentration of tr-ZR did not significantly change during the course of the experiment for both rootstock cultivars (Figure 11). In the bud region of cuttings, the concentration of tr-ZR did not significantly change during the course of the experiment

except on day 0 post excision that ‘Natal briar’ recorded significantly higher tr-ZR concentration than ‘Rosa progress’ (Figure 11).

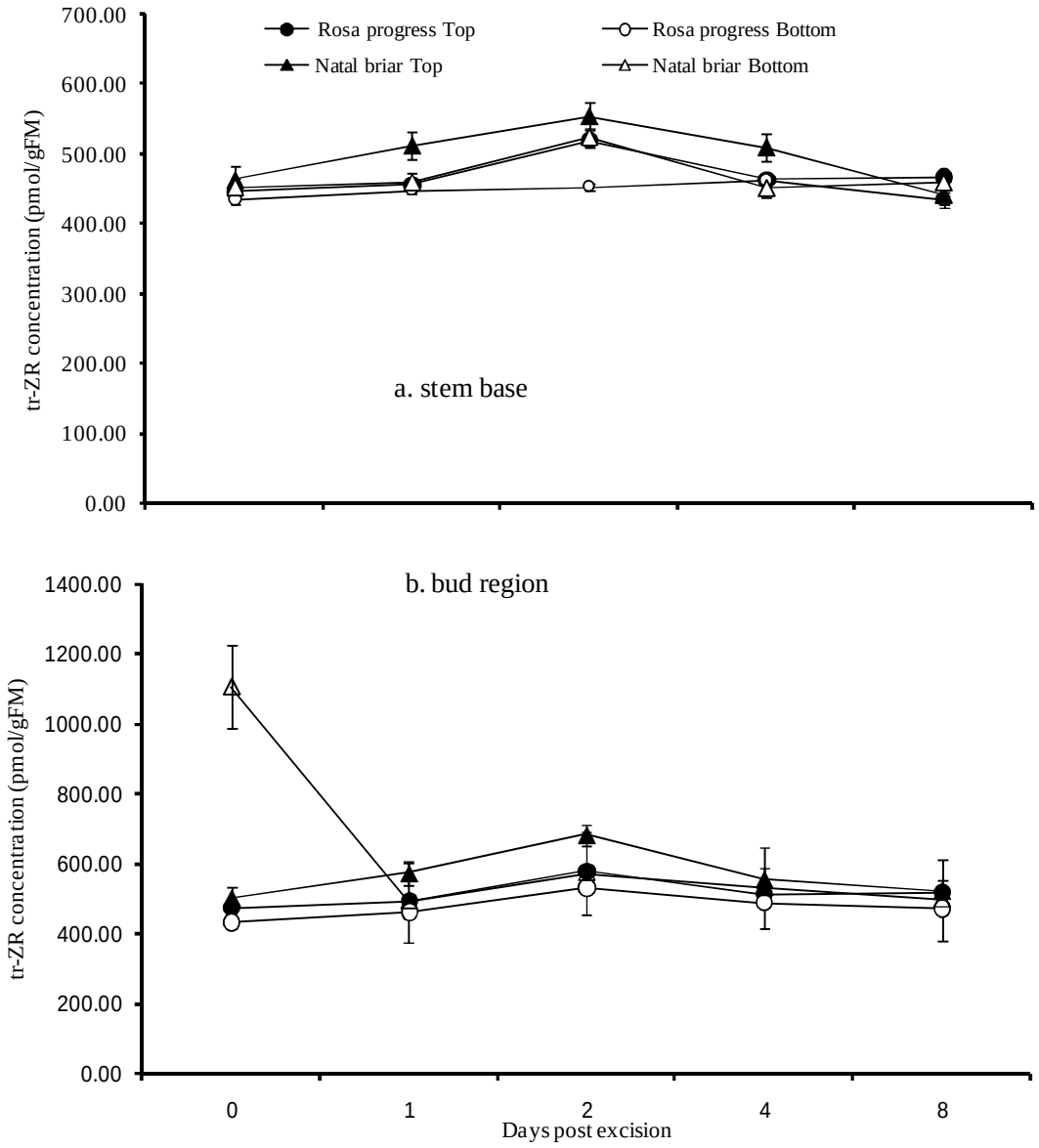

Figure 10: The time course of tr-ZR in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

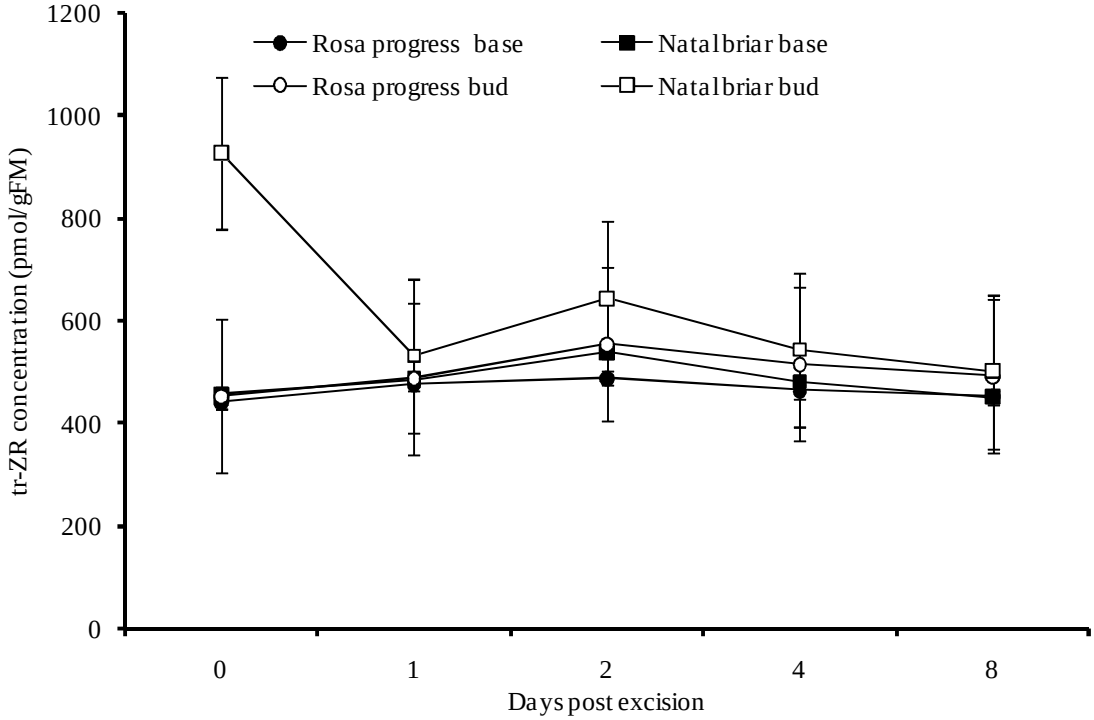

Figure 11: The time course of tr-ZR in the stem base and bud region cuttings of rootstock cultivars

4.3.2.7. Effects of rootstock cultivars, cutting position and days post excision on endogenous IAA: tr-Z in the stem base and bud region of cuttings.

The ratio of IAA:tr-Z in the stem base of cuttings was significantly ($p \leq 0.05$) influenced by the rootstock cultivars, cutting position, days post excision (Table 50), interaction between rootstock cultivars and day post excision and three way interaction (Figures 12 and 13). Irrespective of rootstock cultivars and day post excision, top position cuttings had significantly higher IAA: tr-Z than bottom position cuttings in the stem base of cuttings. Irrespective of cutting position and days post excision, 'Rosa progress' had significantly higher IAA: tr-Z than 'Natal briar' in the stem base of cuttings (Table 50). Averaged across the rootstock cultivars and cutting positions,

the IAA: tr-Z remained on a similar level from day 0 to day 1 post excision followed by a significant increase to day 2 post excision and thereafter a non significant increase to day 4 post excision and a significant increase to day 8 post excision in the bud region of cuttings. In the stem base of cuttings the IAA: tr-Z significantly decreased from day 0 to day 1 post excision followed by a non significant increase to day 2 post excision and thereafter a significant increase to day 4 post excision and a non significant increase to day 8 post excision (Table 50).

Table 50: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous IAA:tr-Z in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	0.05	0.05	0.05	0.05	0.03	0.03	0.05	0.06	0.09
LSD	NS		NS		0.02**				
Stem base	0.1	0.07	0.1	0.07	0.07	0.05	0.06	0.12	0.13
LSD	0.02*		0.02**		0.03**				

*LSD= Least Significant Difference of means, * significant at $p \leq 0.05$; ** significant at $p \leq 0.001$, NS= Not significant*

The interaction between cutting position and days after planting was significant for the IAA: tr-Z in the stem base of cuttings (Figure 12a). The IAA: tr-Z in the top position cuttings of 'Rosa progress' remained on a statistically similar level from day 0 to day 2 post excision followed by a significant increase to day 4 post excision and thereafter a non significant decrease to day 8 post excision (Figure 12a). In 'Natal briar' the IAA: tr-Z in the top position cuttings significantly decreased from day 0 to day 1 post excision followed by a non significant decrease to day 4 post excision and thereafter a significant increase to day 8 post excision (Figure 12a). The IAA: tr-Z in the bottom position cuttings did not significantly differ from day 0 to day 2 post excision however, the ratio significantly increased from day 2 to day 4 post excision in both

rootstock cultivars. From day 4 post excision, the IAA: tr-Z remained on a similar level to day 8 post excision in ‘Rosa progress’ and significantly decreased to day 8 post excision in ‘Natal briar’ (Table 12a) for the bottom position of the stem base region of cuttings.

In the bud region of cuttings, the IAA: tr-Z did not significantly change with days post excision for the cutting positions of both rootstock cultivars upto day 4 post excision. From day 4 post excision, the IAA: tr-Z of top position cuttings siginificantly increased to day 8 post excision while those of the bottom position cuttings remained on a statistically similar level to day 8 post excision in both rootstock cultivars (Figure 12b).

Averaged across cutting positions, the IAA: tr-Z in the stem base of cuttings of ‘Rosa progress’ remained on a similar level from days 0 to 2 post excision followed by a significant increase to day 4 post excision and thereafter remained on a similar level to day 8 post excision (Figure 13). In ‘Natal briar’, the IAA: tr-Z in the stem base of cuttings decreased from day 0 (0.09pmol/gFM) to day 1 (0.05pmol/g FM) post excision and remained on a similar level to day 2 post excision and thereafter slightly increased to day 8 (0.08 pmol/g FM) post excision (Figure 13). In the bud region of cuttings, the IAA: tr-Z slightly increased from day 0 to day 8 post excision in both rootstocks though the increases were not significant (Figure 13).

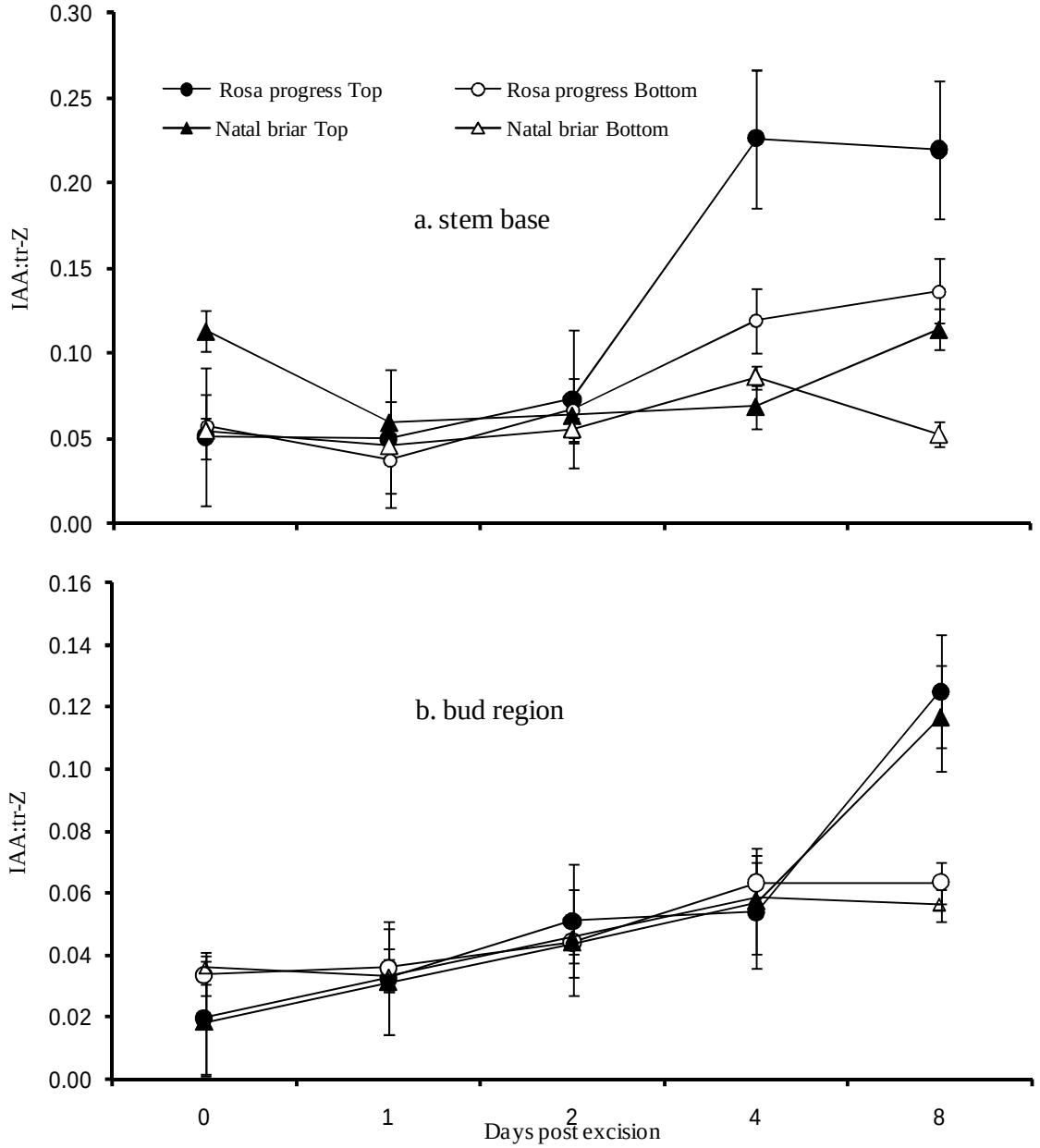

Figure 12: The time course of IAA: tr-Z in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

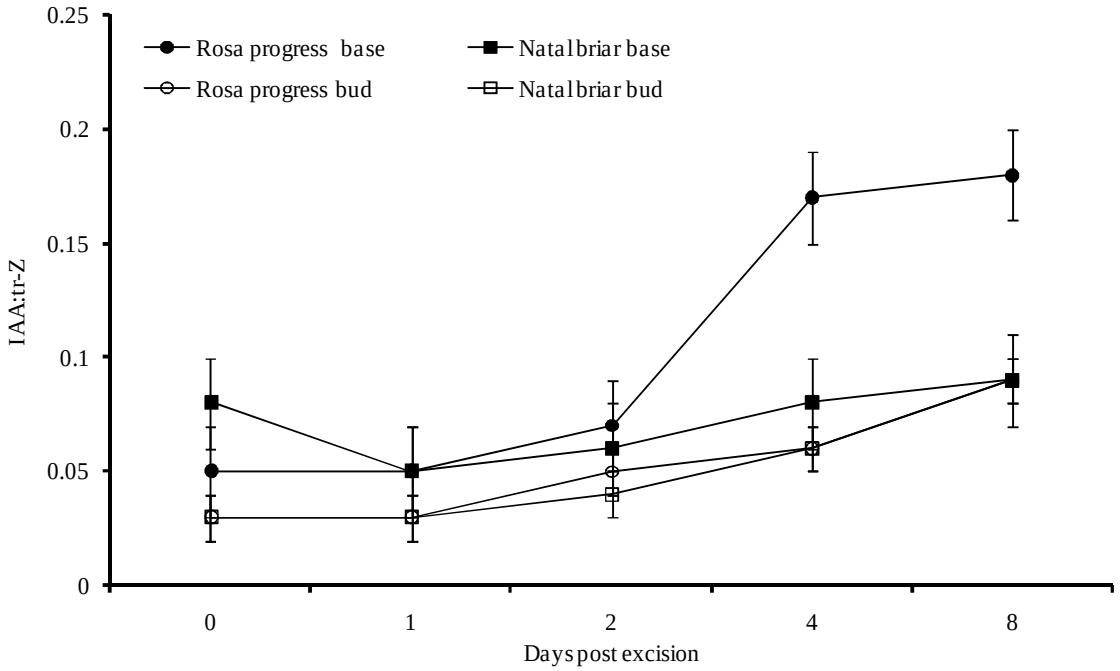

Figure 13: The time course of IAA: tr-Z in the stem base and bud region cuttings of rootstock cultivars

4.3.2.8 Effects of rootstock cultivars, cutting position and days post excision on endogenous IAA: tr-ZR in the stem base and bud region of cuttings.

The rootstock cultivars, the cutting position, the days post excision (Table 51), the interaction between rootstock cultivars and day post excision and three way interaction significantly ($p \leq 0.05$) influenced the IAA:tr-ZR ratio in the stem base of cuttings (Figures 14 and 15). Irrespective of rootstock cultivars and days post excision, top position cuttings had significantly higher IAA: tr-ZR than bottom (0.07pmol/gFM) position cuttings in the stem base of cuttings. Irrespective of cutting position and days post excision 'Rosa progress' had significantly higher IAA: tr-ZR than 'Natal briar' (0.07pmol/g FM) (Table 51).

Table 51: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous IAA: tr-ZR in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	0.05	0.05	0.05	0.05	0.03	0.03	0.04	0.05	0.09
LSD	NS		NS		0.02**				
Stem base	0.1	0.07	0.1	0.07	0.08	0.05	0.05	0.12	0.13
LSD	0.02*		0.02**		0.03**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

In the stem base of cuttings, the IAA: tr-ZR did not significantly change on days 1 and 2 post excision for the cutting positions of both rootstock cultivars except on days 0, 4 and 8 post excision (Figure 14a). On day 0 post excision, bottom position cuttings of 'Natal briar' exhibited significantly higher ratio than the top position cuttings and both cutting positions of 'Rosa progress' (Figure 14a). On the fourth day post excision top position cuttings of 'Rosa progress' exhibited significantly higher ratio than its bottom position cuttings which was significantly higher than the ratio of both cutting positions of 'Natal briar' (Figure 14a). On day 8 post excision, the top position cuttings of 'Rosa progress' recorded significantly higher ratio than the bottom position of 'Rosa progress' and top position cuttings of 'Natal briar' whose ratios were not significantly different from each other. The bottom position cuttings of 'Natal briar' recorded significantly lower ratio than the top position and both cutting positions of 'Rosa progress' on day 8 post excision (Figure 14a).

In the bud region of cuttings, the IAA: tr-ZR did not significantly change with days post excision for all the cutting positions of both rootstocks except on day 8 post excision where

the top position cuttings had significantly higher ratio than the bottom position cuttings of both rootstock cultivars (Figure 14b).

In the stem base of cuttings, the IAA to tr-ZR ratio for both rootstock cultivars was not significantly different from day 0 to day 2 post excision however, on days 4 and 8 post excision ‘Rosa progress’ had significantly higher ratio than ‘Natal briar’ (Figure 15). In the bud region of cuttings, the IAA: tr-ZR did not significantly change from day 0 to day 8 post excision in both rootstock cultivars (Figure 15).

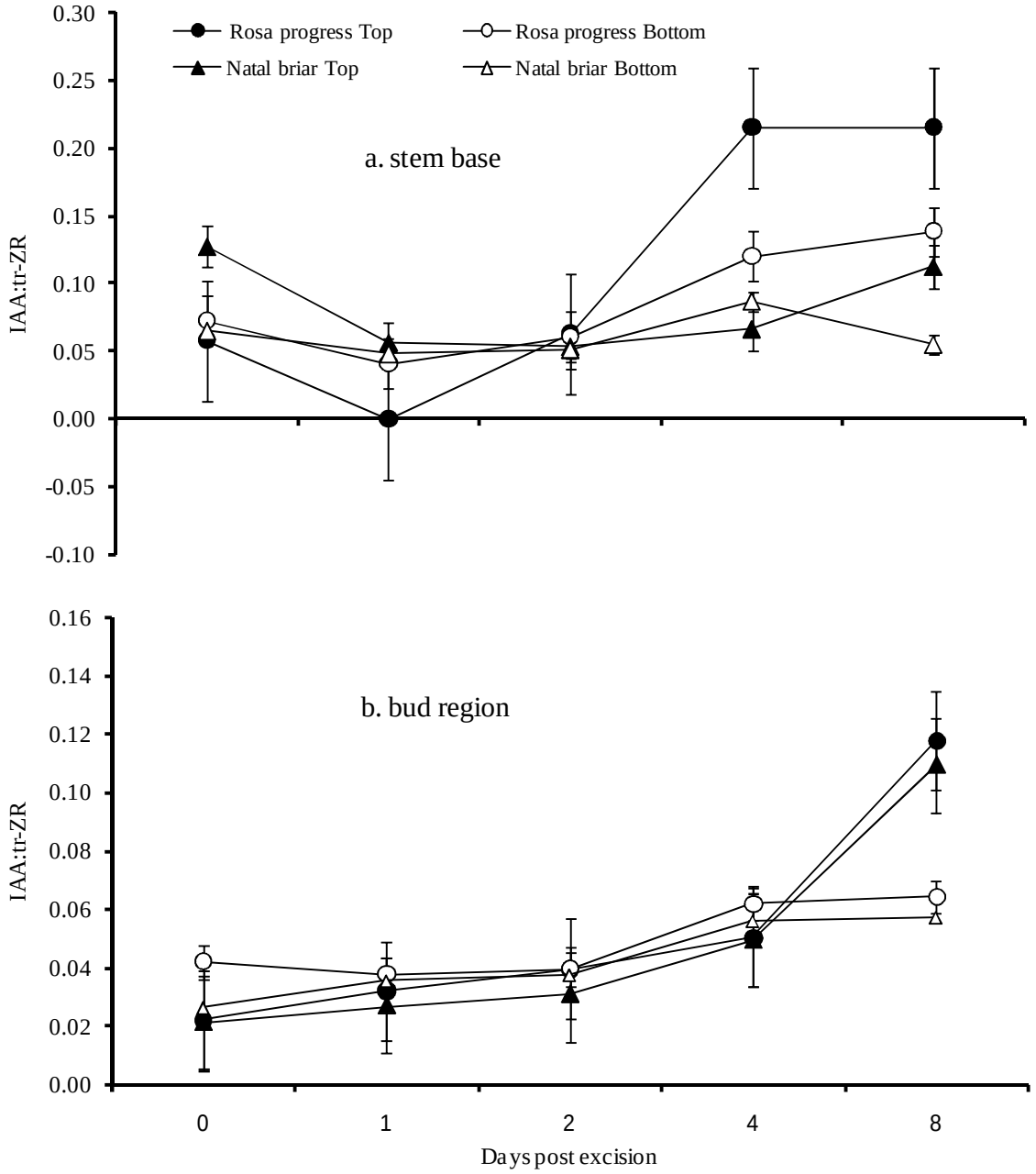

Figure 14: The time course of IAA: tr-ZR in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

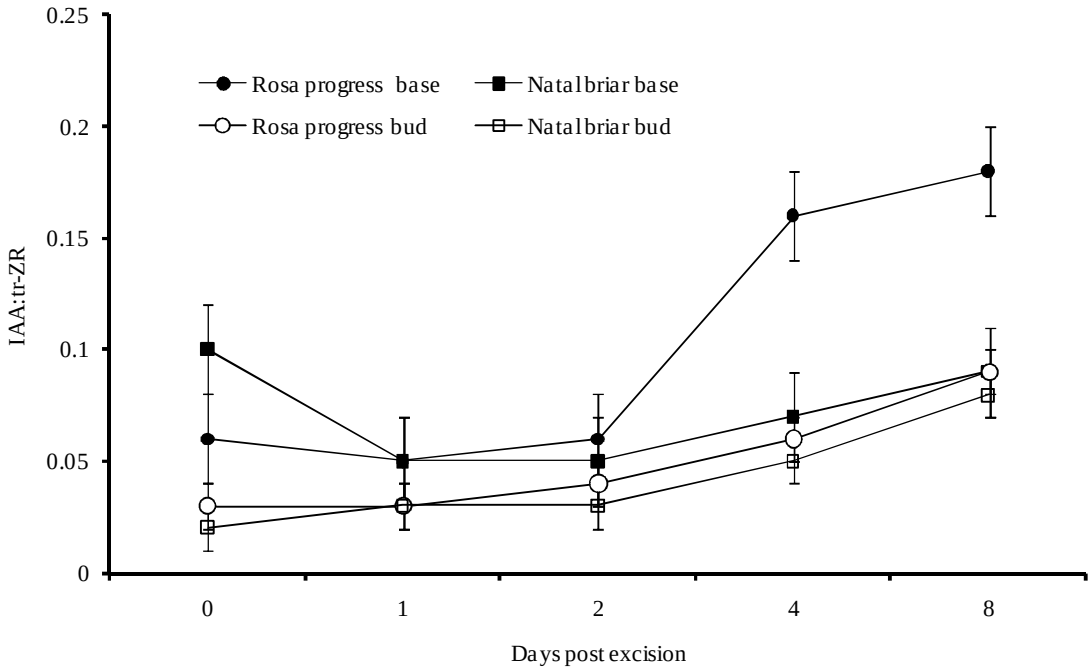

Figure 15: The time course of IAA: tr-ZR in the stem base and bud region cuttings of rootstock cultivars

4.3.2.9 Effects of rootstock cultivars, cutting position and days post excision on endogenous IAA: total cytokinin in the stem cuttings.

The rootstock cultivars, cutting position, days post excision and interaction between the rootstock cultivars and days post excision significantly ($p \leq 0.05$) influenced the IAA to total cytokinins ratio. 'Rosa progress' had higher IAA: total cytokinins than 'Natal briar' in the stem base and bud regions of the cuttings (Table 52). Irrespective of the cutting position and rootstock cultivars, the IAA: total cytokinins remained on a statistically similar level from day 0 to day 2 post excision followed by a significant increase to day 8 post excision in the bud region of cuttings (Table 52). In the stem base region of cuttings, the IAA: total cytokinins significantly increased from day 0 to day 1 post excision followed by a non significant decrease to day 2 post excision and thereafter a signicant increase to day 8 post excision (Table 52).

Table 52: Effects of the main factors of rootstock cultivars, cutting position and days post excision on endogenous IAA: total cytokinin in the stem base and bud region of cuttings.

Region	Rootstock cultivars		Position		Days post excision				
	'Rosa progress'	'Natal briar'	Top	Bottom	0	1	2	4	8
Bud	0.0132	0.0042	0.0108	0.0066	0.003	0.005	0.006	0.0121	0.0174
LSD	0.0039*		0.0039*		0.0062**				
Stem base	0.016	0.0096	0.016	0.0096	0.01	0.0088	0.0083	0.014	0.0228
LSD	0.003**		0.003**		0.0046**				

*LSD= Least Significant Difference of means, *significant at $p \leq 0.05$; **significant at $p \leq 0.001$, NS= Not significant*

In the stem base of cuttings, top position cuttings of 'Rosa progress' had significantly higher ratio than the bottom position and both cutting positions of 'Natal briar' on days 2 and 4 post excision (Figure 16a). On day 8 post excision, top position cuttings of 'Rosa progress' had significantly higher IAA:cytokinin followed by both the bottom position of 'Rosa progress' and top position of 'Natal briar' that were not significantly different from each other but were significantly higher than bottom position cuttings of 'Natal briar' (Figure 16a). In the bud region of cuttings, 'Rosa progress' had significantly higher ratio than 'Natal briar' on day 4 post excision while on day 8 post excision, top position cuttings of 'Rosa progress' had significantly higher IAA: cytokinin than both the bottom position of 'Rosa progress' and top position of 'Natal briar' that were not significantly different from each other but were higher than bottom position cuttings of 'Natal briar' (Figure 16b).

The IAA: total cytokinin remained on the same level from day 0 to day 4 post excision followed by a significant increase to day 8 post excision in the stem base region of cuttings in 'Rosa progress'(Figure 17). In 'Natal briar', a non significant decrease in the IAA: cytokinin was recorded from day 0 to day 2 post excision followed by a significant increase to day 8 post

excision in the stem base of cuttings (Figure 17). In the bud region of cuttings the ratio slightly increased from day 0 to day 8 post excision in ‘Natal briar’ while in ‘Rosa progress’ the ratio remained on the same level from day 0 to day 2 post excision followed by a significant increase to day 4 and day 8 post excision (Figure 17). ‘Rosa progress’ had significantly higher IAA to cytokinin ratio than ‘Natal briar’ on day 8 post excision (Figure 17).

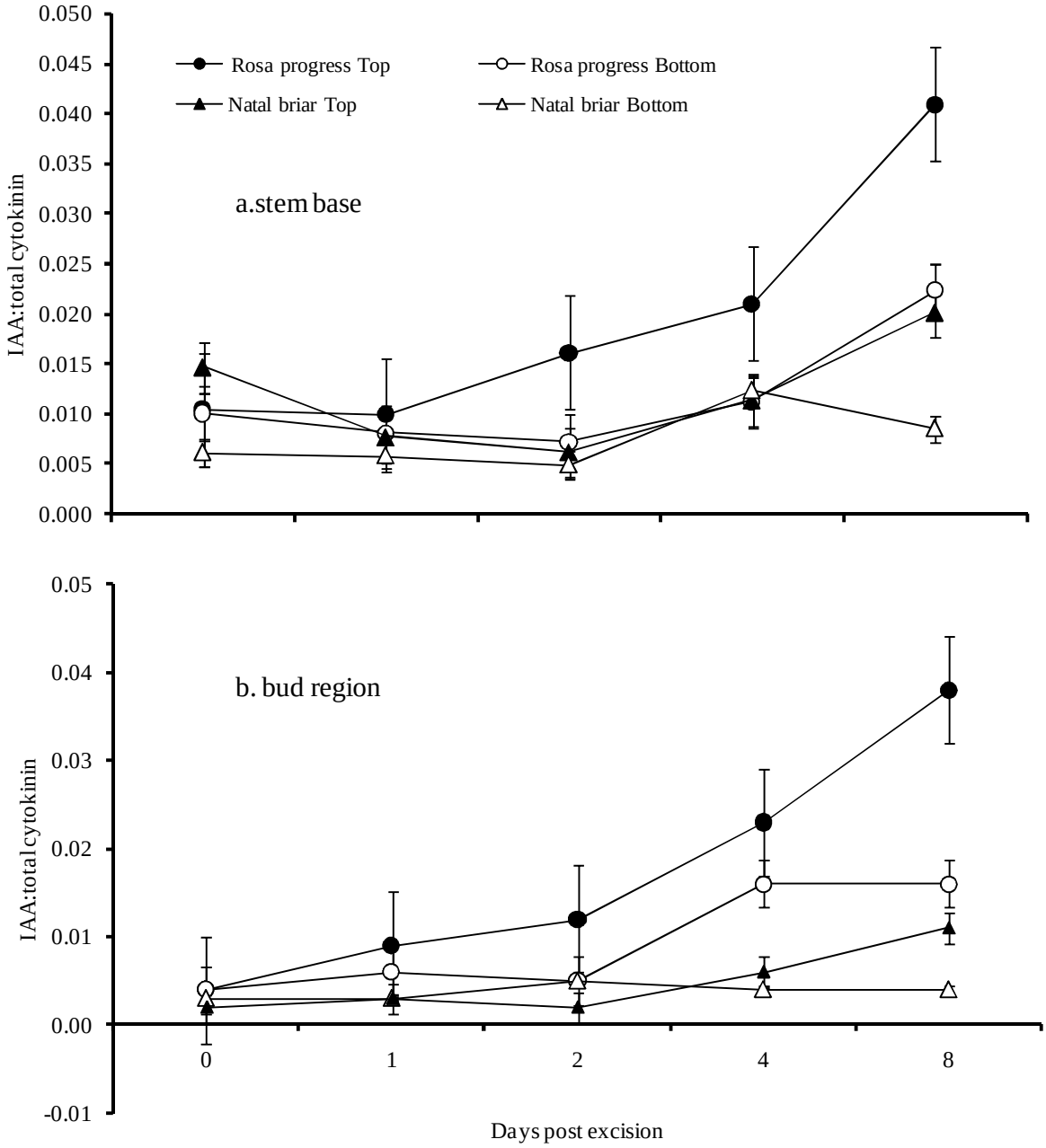

Figure 16: The time course of IAA: total cytokinin in a) the stem base and b) bud region of cutting positions of rootstock cultivars.

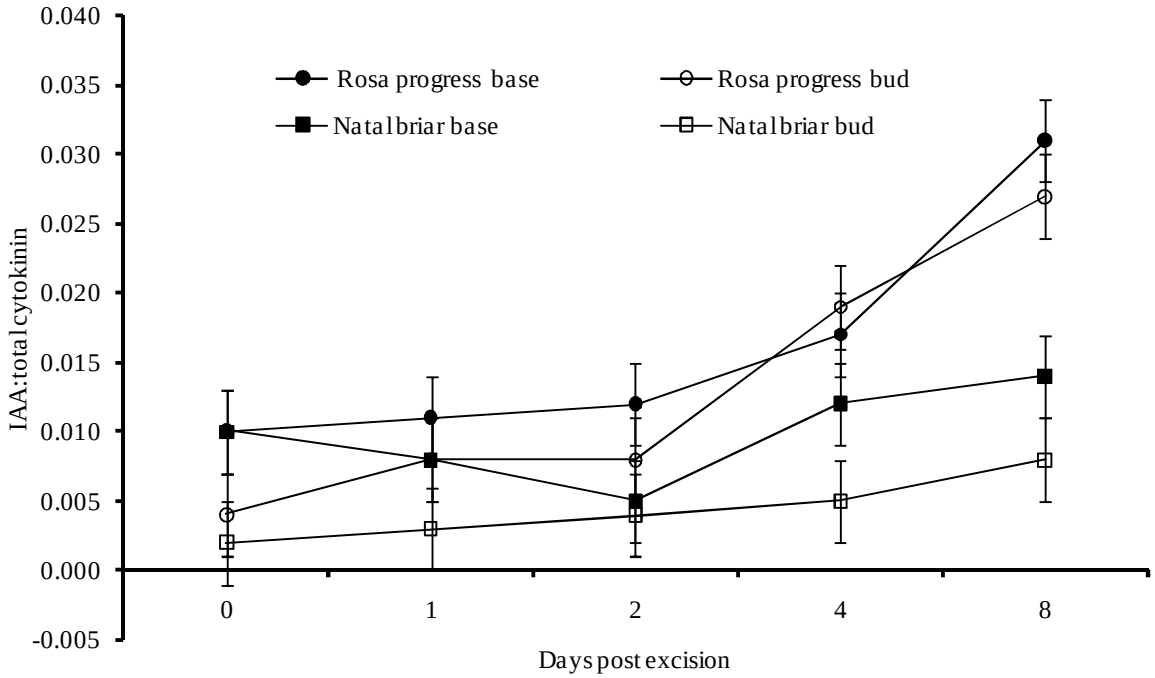

Figure 17: The time course of IAA: total cytokinin in the stem base and bud region cuttings of rootstock cultivars

4.3.4. Effects of rootstock cultivars, cutting position and days after planting on endogenous mineral nutrient level in the stem cuttings.

a. Zinc

In 'Rosa progress', at the time of severance, the middle position cuttings exhibited significantly higher zinc level followed by the top then bottom position cuttings (Figure 18). At day 3 after planting, the zinc level increased acropetally with top position cuttings exhibiting significantly higher zinc content followed by the middle and bottom position cuttings of 'Rosa progress'. On day 7, top position recorded significantly higher zinc level than the middle and bottom position cuttings. According to the days, the zinc level significantly decreased from day 0 to day 7 after planting in the middle position cuttings while in the bottom position cuttings the zinc level remained on a statistically similar level from day 0 to day 7 after planting in 'Rosa progress'. In

the top position, the zinc level significantly increased from day 0 to day 3 after planting followed by a slight decrease to day 7 after planting (Figure 18).

In 'Natal briar', at the time of severance, zinc level increased acropetally with bottom position exhibiting lower concentration in the stem base of cuttings (Figure 18). On day 3 after planting, top position cuttings had significantly higher zinc level than the middle position cuttings however, the zinc level of the top and bottom position cuttings were not significantly different from each other. On day 7 after planting, the top position cuttings recorded significantly higher zinc level than the other two positions that were however, not significantly different from each other. A slight increase in zinc level from day 0 to day 7 was noted in the bottom and top position cuttings. The zinc level in the middle position exhibited a sharp decline from day 0 to day 3 followed by a slight decrease to day 7 after planting.

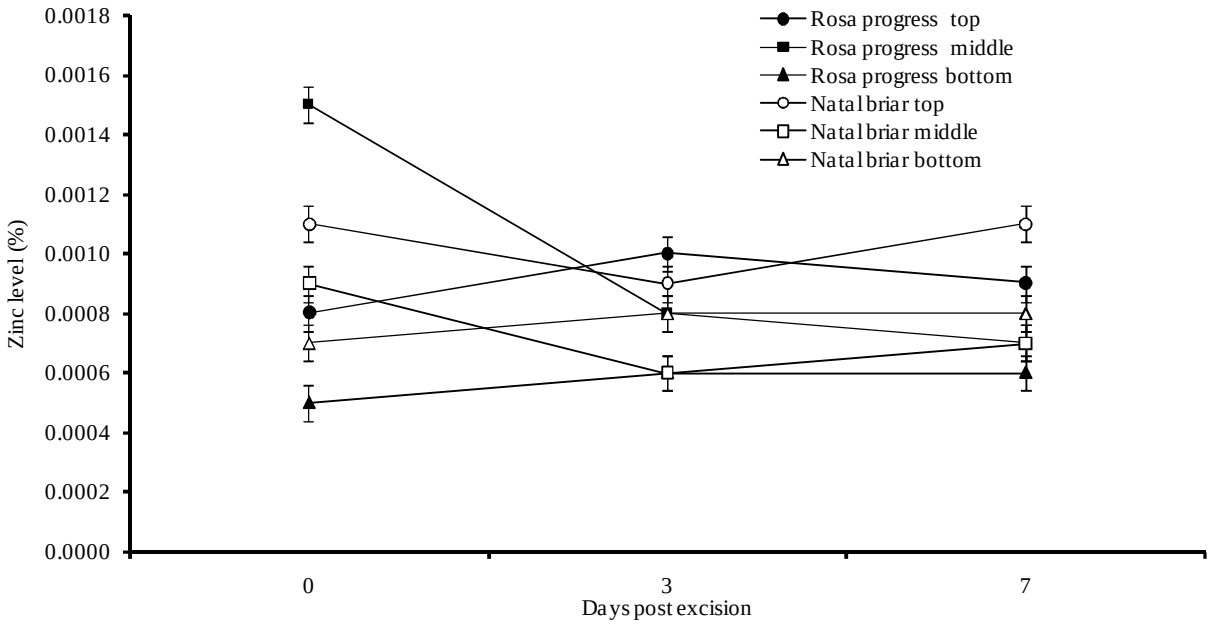

Figure 18: Effects of cutting position and days after planting on zinc level (%) of 'Rosa progress' and 'Natal briar'

b. Iron

In ‘Rosa progress’ the iron level decreased from day 0 to day 3 after planting in all the cutting positions followed by a significant increase to day 7 after planting in the middle and bottom position cuttings (Figure 19). However, the iron level in the top position cuttings significantly decreased from day 3 to day 7 after planting (Figure 19). On day 0 after planting, the bottom position cuttings had significantly lower iron level than the top and middle position cuttings however, the iron level for the latter two positions were not significantly different from each other. On day 7 after planting, the middle position cuttings had significantly higher iron level followed by the bottom and top position cuttings of ‘Rosa progress’.

In ‘Natal briar’, the effect of day after planting on iron level in the middle and bottom position cuttings followed a similar trend to that recorded in ‘Rosa progress’ (Figure 19). In the top position cuttings of ‘Natal briar’, the iron level significantly increased from day 0 to day 3 after planting followed by a significant decline to day 7 after planting. The iron level was significantly higher in top position of the ‘Natal briar’ than the other two positions at day 3 after planting. On day 7 after planting, the bottom position cuttings had significantly higher iron level than the middle and top position cuttings however, the iron level of the latter two positions were not significantly different from each other (Figure 19).

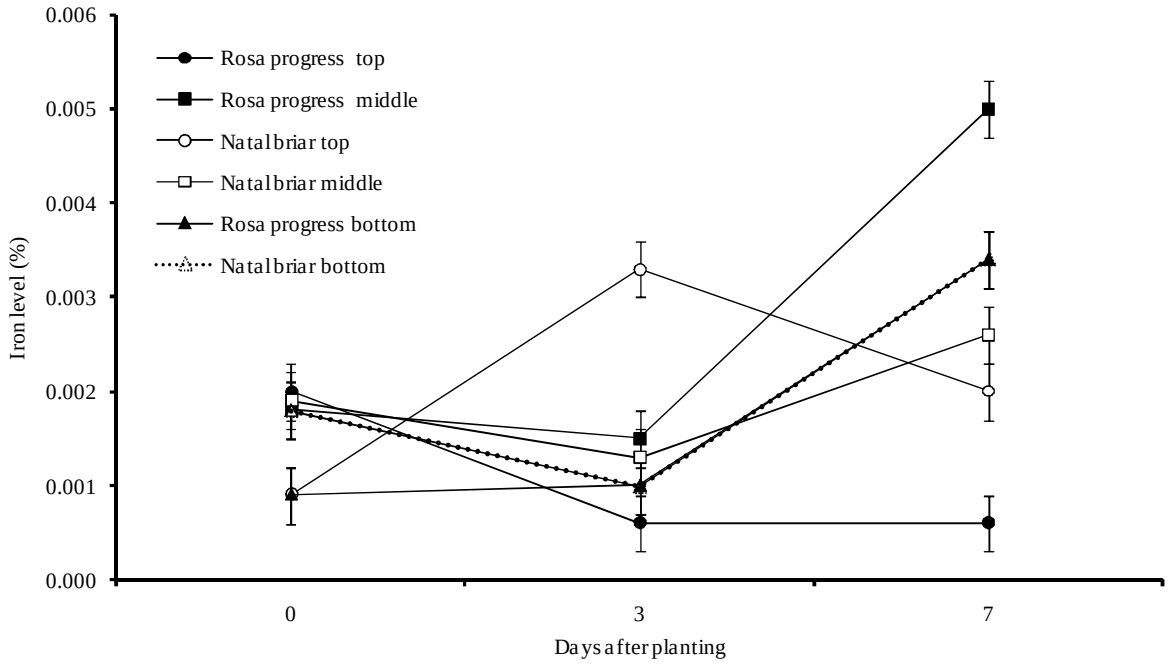

Figure 19: Effects of cutting position and days after planting on iron level (%) of 'Rosa progress' and 'Natal briar'

c. Manganese

The manganese content of the three cutting positions of both rootstock cultivars decreased from day 0 to day 7 after planting (Figure 20). On day 0 post excision, the middle position cuttings had significantly higher manganese level than the top and bottom position cuttings however, the manganese level of the latter two positions were not significantly different from each other in 'Rosa progress'. In 'Natal briar' the bottom position cuttings had significantly lower manganese level than the top and middle position cuttings however, the manganese level of the latter two positions were statistically similar on day 0 after planting. On day 3 after planting, top position cuttings had significantly higher manganese level than the middle and bottom position cuttings of 'Natal briar' and all the cutting positions of 'Rosa progress' , however the manganese level of

the latter two positions were not significantly different from each other. On day 7 after planting, top position cuttings of 'Rosa progress' had significantly lower manganese level than the middle and bottom position cuttings however, the manganese level of the latter two positions were statistically similar (Figure 20).

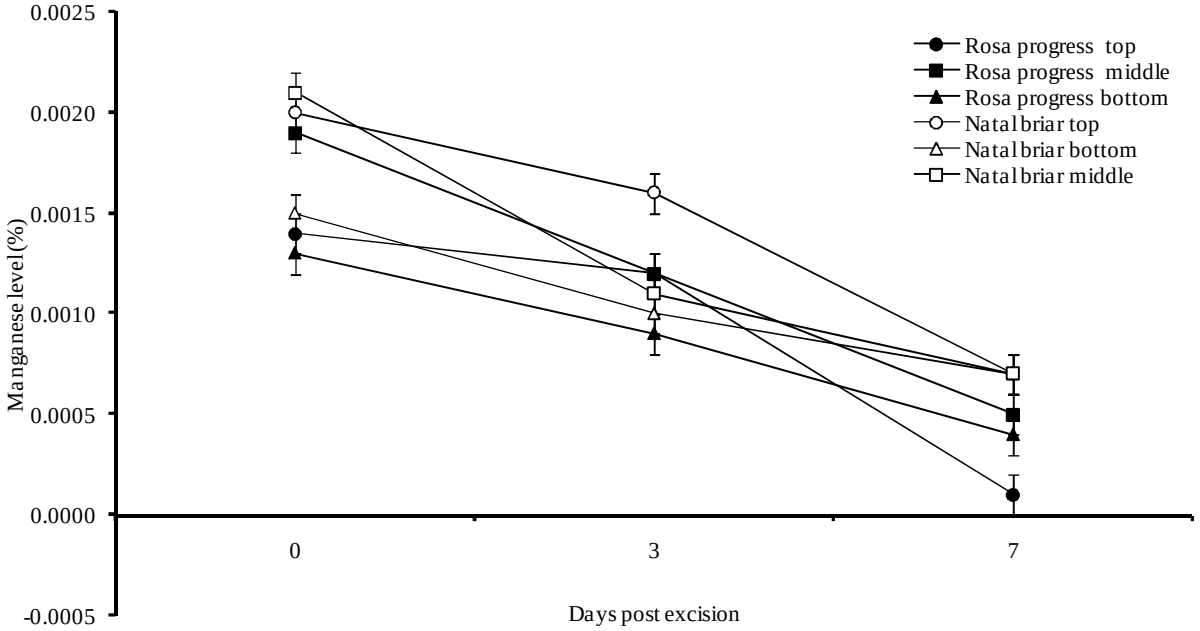

Figure 20: Effects of cutting position and days after planting on manganese content of (%) 'Rosa progress' and 'Natal briar'

d. Nitrogen

In 'Rosa progress', at the time of severance, top position cuttings had significantly lower nitrogen level than the other positions followed by a significant increase to day 3 after planting and thereafter a decrease to day 7 after planting (Figure 21). Middle position cuttings of 'Rosa progress' recorded a decreasing level of nitrogen from day 0 to day 7 after planting. Bottom position cuttings of 'Rosa progress' recorded a significant decrease in level of nitrogen from day 0 to day 3 after planting followed by an increase to day 7 after planting. In 'Rosa progress' middle position cuttings had significantly higher nitrogen level followed by the bottom and top

position cuttings on day 0 after planting while on day 3 after planting, bottom position cuttings had significantly lower nitrogen level than the other two positions that were however, statistically similar. Acropetal increase was noted for nitrogen level in 'Natal briar' with top position exhibiting significantly higher nitrogen level followed by the middle and bottom position cuttings throughout the days after planting (Figure 21). Across the days after planting, the nitrogen levels in each cutting position of 'Natal briar' statistically remained unchanged.

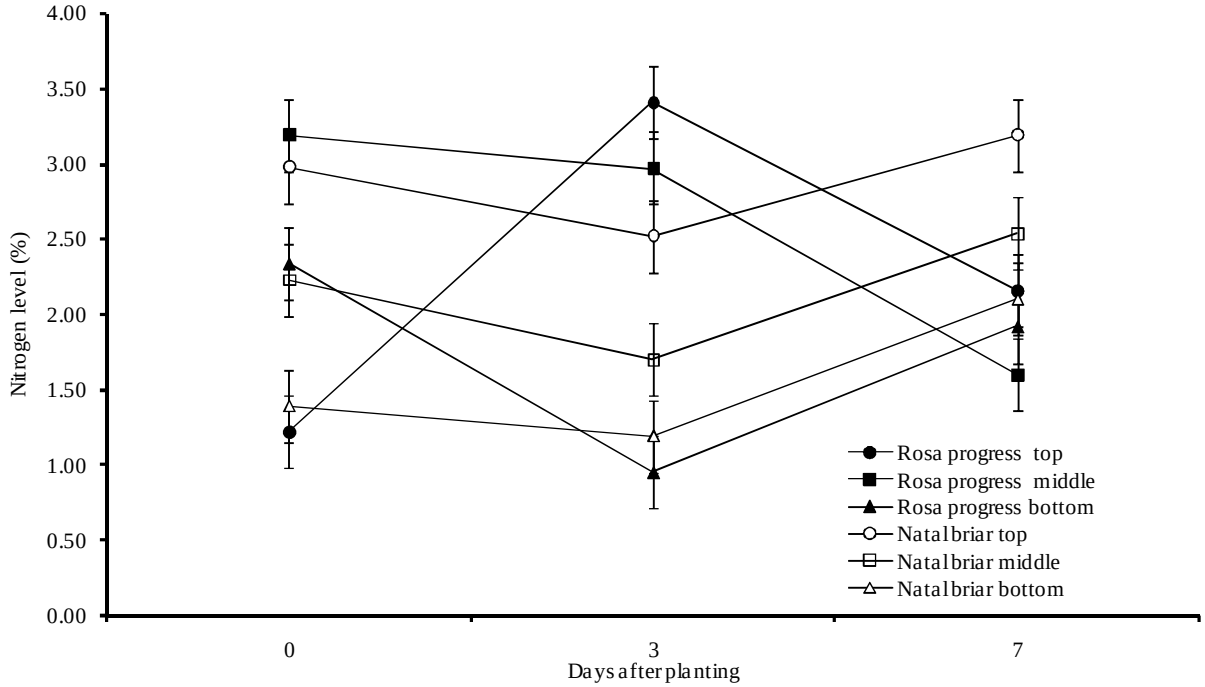

Figure 21: Effects of cutting position and days after planting on nitrogen level (%) of 'Rosa progress' and 'Natal briar'.

4.4 The relationship between mineral nutrient level and rooting percentage in the self rooting IBA experiment.

The zinc and manganese levels were positively correlated to rooting percentage from day 0 to day 7 after planting in 'Rosa progress' (Table 53). In 'Natal briar', a positive correlation between zinc and rooting was recorded on days 0 and 7 after planting and a negative correlation on day 3

after planting. The iron level was positively correlated to rooting on day 0 after planting followed by a negative correlation on days 3 and 7 after planting in 'Rosa progress'. In 'Natal briar', the iron level was negatively correlated to rooting on day 0 followed by a positive correlation on days 3 day 7 after planting (Table 53). The manganese level was positively correlated to rooting on day 0 followed by a negative correlation on days 3 and 7 after planting in 'Natal briar'. The nitrogen level was negatively correlated to rooting on day 0 followed by a positive correlation on day 3 after planting in 'Rosa progress'. In 'Natal briar', the nitrogen level was positively and strongly correlated to rooting from day 0 to day 7 after planting (Table 53).

Table 53: Correlation of rooting percentage and mineral nutrient level at the stem base of cuttings of rose rootstock cultivars

Mineral nutrient content	Rootstock cultivars	Rooting percentage		
		R		
		0	3	7
Zinc	'Rosa progress'	0.045	0.797	0.893*
	'Natal briar'	0.7	-0.045	0.297
Iron	'Rosa progress'	0.819*	-0.472	-0.297
	'Natal briar'	-0.49	0.561	0.427
Manganese	'Rosa progress'	0.257	0.559	0.651
	'Natal briar'	0.624	-0.65	-0.263
Nitrogen	'Rosa progress'	-0.753	0.836*	0.000
	'Natal briar'	0.868*	0.829*	0.856*

*NB: * Significant at probability level at 0.05.*

4. 5. The relationship between carbohydrate level and rooting percentage in the self rooting IBA experiment.

A negative correlation between rooting percentage and glucose level was recorded on days 0 and 7 after planting and a positive correlation on day 3 after planting in both rootstock cultivars (Table 54). The rooting percentage was negatively correlated to fructose level on all the days after planting in 'Rosa progress'. In 'Natal briar', a negative correlation of rooting percentage and fructose content was recorded on days 0 and 3 after planting however, on day 7, no relationship was recorded (Table 54). The sucrose level was positively correlated to rooting from day 0 to day 7 after planting in 'Rosa progress'. In 'Natal briar' a negative correlation was noted on day 0 followed by a positive correlation on days 3 and 7 after planting. A negative correlation between rooting and total sugar was observed on days 0 and 7 after planting however, a negative correlation was recorded on day 3 after planting in 'Rosa progress'. In 'Natal briar', day 0 recorded a negative correlation between rooting and total sugar followed by a positive correlation on days 3 and 7 after planting (Table 54).

Table 54: Correlation of rooting percentage and carbohydrate level at the stem base of cuttings of rose rootstock cultivars.

Carbohydrate level	Rootstock cultivars	Rooting percentage		
		R		
		0	3	7
Glucose	'Rosa progress'	-0.29	0.161	-0.657
	'Natal briar'	-0.272	0.55	-0.226
Fructose	'Rosa progress'	-0.207	-0.084	-0.247
	'Natal briar'	-0.509	-0.279	0
Sucrose	'Rosa progress'	0.032	0.527	0.063
	'Natal briar'	-0.339	0.251	0.105
Total sugar	'Rosa progress'	-0.243	0.274	-0.063
	'Natal briar'	-0.529	0.1	0.176

4.6. The relationship between rooting percentage and shooting percentage

There were positive correlations between rooting and shooting percentages in the self rooting experiments (Figures 22a and 22b).

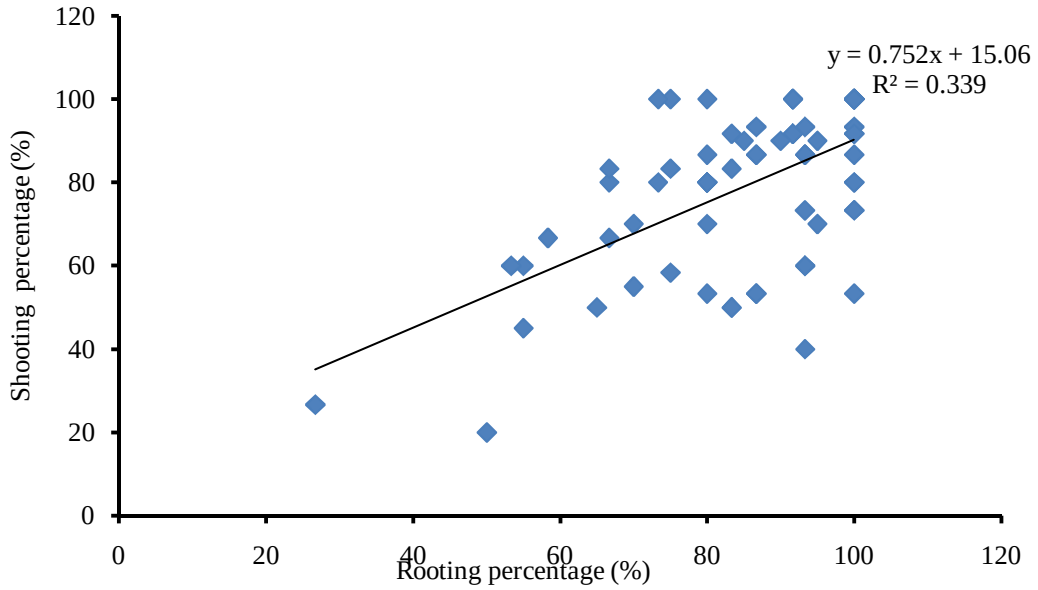

Figure 22a: Linear correlation between shooting and rooting percentages in the IBA experiment

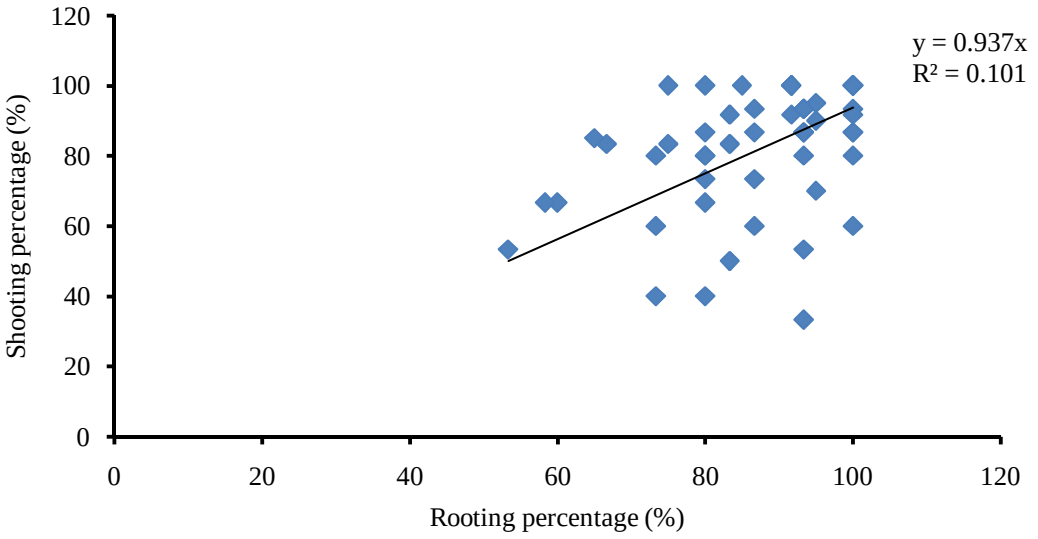

Figure 22b: Linear correlation between shooting and rooting percentages in the NAA experiment

4.7. The relationship between IAA concentration on the stem base and bud region of cuttings

There was a positive correlation between IAA concentration on the stem base and bud region of cuttings in 'Rosa progress' and 'Natal briar in the self rooting (Figures 23a and 23b)

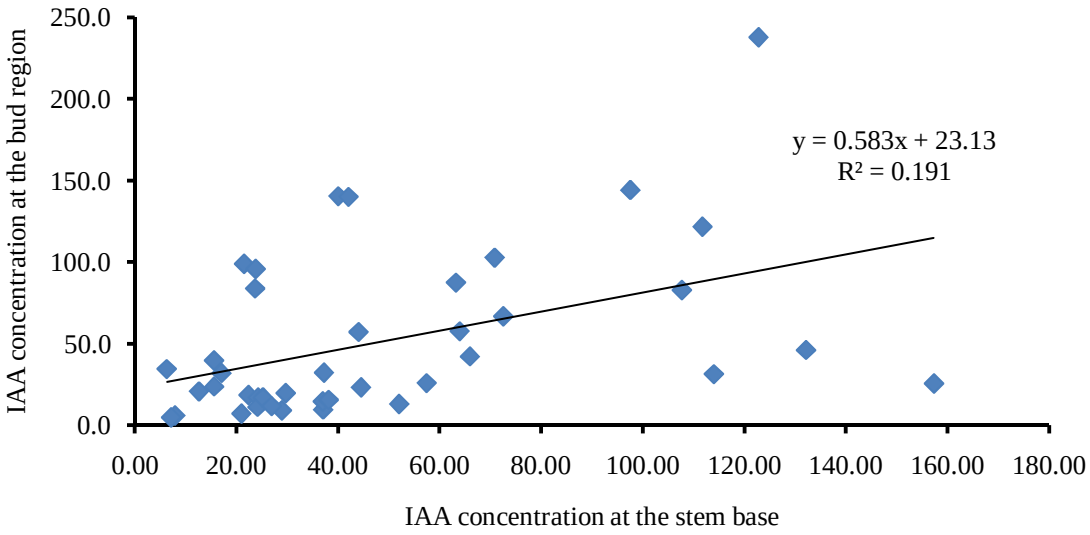

Figure 23a: Linear correlation between IAA concentration on the stem base and bud region of cuttings in 'Rosa progress'

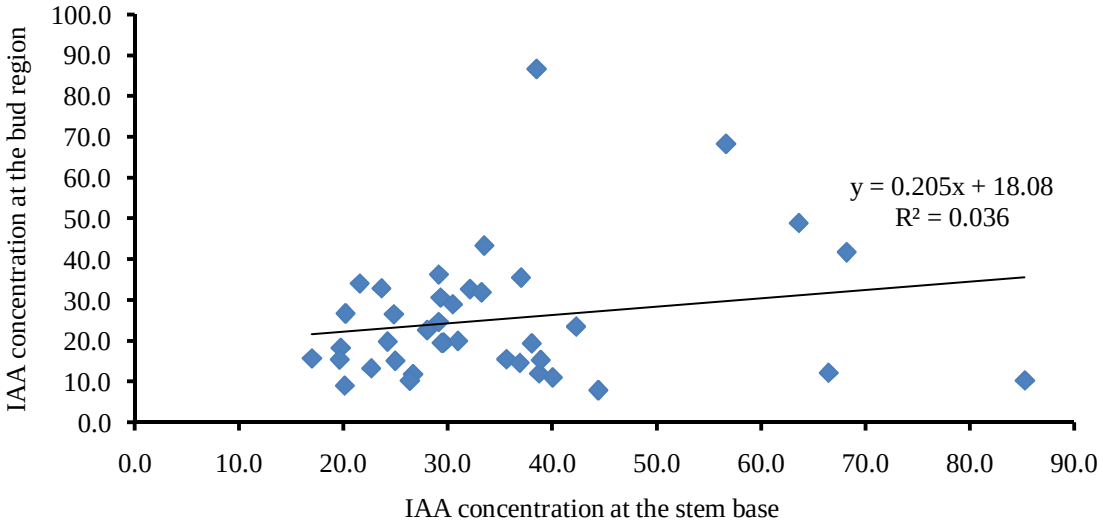

Figure 23b: Linear correlation between IAA concentration on the stem base and bud region of cuttings in 'Natal briar'

CHAPTER FIVE: DISCUSSION

5.1. Effects of cutting position of *Rosa hybrida* rootstock cultivars and its biochemical composition on growth parameters of self rooted cuttings.

5.1.1. Effects of cutting position of *Rosa hybrida* rootstock cultivars and endogenous carbohydrates on growth parameters of self rooted cuttings.

In the self rooting experiment, the results demonstrated that all the parameters related to the rooting and sprouting potential of stem cuttings of 'Rosa progress' and 'Natal briar' were significantly influenced by the cutting position on the stock plant shoot. The parameters related to rooting potential included rooting percentage (Tables 3a and 3b), root number (Tables 4a and 4b), root length (Tables 5a and 5b), root fresh and dry weights and (Tables 6a, 6b, 7a and 7b). The top and middle position cuttings exhibited higher rooting potential than the bottom position cuttings for most of the parameters measured in both rootstock cultivars.

The difference in rooting ability of the different positions could be due to differences in physiological age. Different reports have demonstrated that physiologically younger propagation material is more suitable for successful adventitious rooting (Hartmann *et al*., 2012). Physiological age increases with chronological age (cyclophysis). As such, the newest leaves at the top are chronologically the youngest, whereas the base of the stem is chronologically much older (Rasmussen *et al*., 2015). Physiologically mature plants may possess zones (the top of a plant, outer parts of a plant) which react more maturely than others (plant base, inner parts of a plant). This phenomenon is known as topophysis. Based on topophysis, chronologically adult plants can react physiologically as juvenile plant and this depends on specific plant part analysed (Spethman, 1997; Osterc, 2009). Rooting ability and axillary bud growth vary between cuttings originating from different positions on stock plant shoot. Such topophysic effects were also

observed in ‘Rosa progress’ and ‘Natal briar’. The topophysic effect was more pronounced in ‘Rosa progress’ than ‘Natal briar’ for most of the rooting potential parameters measured with bottom position cuttings exhibiting significantly lower rooting potential than top position cuttings (Tables 3a-7b). Rahbin *et al*. (2012); Suxia *et al*. (2009) and Taiz and Zeiger (2006) also demonstrated that upper cuttings had better rooting potential than lower cuttings.

The biochemical constituents such as the endogenous IAA, cytokinins, carbohydrates and mineral nutrients determined for the cutting positions of the two rootstock cultivars also varied and the top position cuttings exhibited higher biochemical constituents than the bottom position cuttings (Tables 34, 38, 44, 45, 47, 49-52). These variations in biochemical constituents with cutting position would explain topophysic effects on the rooting potential of the two rootstock cultivars. According to Araya *et al*. (2007), the difference in rooting ability of the cutting position can be related to the difference in the chemical composition of the shoots. Suxia *et al*. (2009) and Hartmann and Kester (1983) reported that the variation in rooting ability with cutting positions could be due to high concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds. Other researchers such as Suxia *et al*. (2009); Tchoundjeu and Leaky (2001); Chaummaravong (1998) and Ling (1993) attributed the higher rooting of top position cuttings to the presence of juvenile tissues which are actively differentiating and high in auxin concentration. It has been reported that there are cells in younger part of shoots (upper cuttings) which are metabolically more active than mature tissues and their cell wall is less ligneous and can absorb more auxin, water and nutrient for growth and development.

The carbohydrate analysis revealed that the top position leaves had significantly higher fructose and glucose levels than bottom position leaves at the time of severance (Table 34). The sucrose

level significantly increased from the middle position leaves to the top position and bottom position leaves. Carbohydrates contribute to the formation of adventitious roots by supplying energy and carbon necessary for cell divisions, establishment of the new root meristems and root formation itself (da Costa *et al*., 2013). The carbohydrate levels of the top and bottom position cuttings at the stem base where rooting occurs were not significantly different from each other (Table 35) and negatively correlated to rooting percentage at day 0 after planting (Table 54) and therefore the variation in rooting of the two positions at the time of severance may have been due to the activities of the original leaf on the stem cutting. The original leaf is the main source of carbohydrate due to photosynthesis. In addition, it may function as a source for the supply of auxin (indole-acetic acid), vitamins, organic nitrogen and rooting co-factors that are required for rooting. Sucrose is the most commonly found sugar in the phloem of angiosperms and immediate carbon substrate in plant tissues. It is usually hydrolyzed into its constituent monosaccharides (fructose and glucose) by the increased activities of cell wall invertase or decreased activities of vacuolar and cytosolic invertases before it can be utilized in metabolic processes (Kerner *et al*., 2000; Ahkami *et al.*, 2009). The hydrolysis of sucrose in the culture medium during root induction phase has been reported for *Rosa multiflora* (Rieka *et al*., 1997). Therefore within the stem cutting, there is a mixture of sucrose, glucose and fructose as observed among the cutting positions. The carbohydrate (glucose, fructose and sucrose) level increased acropetally in the rootstock 'Natal briar' with bottom position leaves (Table 34) recording lower levels than top position leaves and this was related to high root-shoot growth responces in top position cuttings (Tables 4a-14b). The original leaf of single-node soft wood cuttings has a strong effect on survival and rooting success (Thomas and Schiefelbin, 2004) and the growth of the axillary bud and primary shoot of cuttings depends on the supply of assimilates by the subtending leaf

(Thomas and Schiefelbin, 2004). In rose plant, the axillary bud outgrowth depends on the supply of carbohydrates by the leaf (Van Labeke *et al*., 2001). The high carbohydrate reserves at the time of severance observed in top and middle position leaves of both rootstock cultivars (Table 34) would ensure continuous supply of carbohydrates to the stem base during adventitious root formation, which recorded lower levels of sucrose (Table 38). Druege *et al*. (2004) and Rapaka *et al*. (2005) reported that the sugar, particularly sucrose level in the leaves at the time of planting has an important role for subsequent adventitious root formation in pelargonium cuttings by supplying the sugars to the stem bases where rooting occurs. The acropetal increase in rooting percentage recorded in both rootstock cultivars (Tables 3a and 3b) could be attributed to the high carbohydrate content of the top and middle position leaves at the time of severance. The low carbohydrate content of the bottom position leaf (Table 34) could be due to declined rate of photosynthesis with increasing leaf age.

The stem can be a source of energy or a storage buffer, through the reserves accumulated before severance, contributing to maintenance and growth of the roots or the primary shoot (Costa and Chall, 2002). The stem due to its respiratory activity (maintenance and growth), storage capacity and little photosynthetic activity acts as a sink for photoassimilates from the leaves. At the time of severance, the carbohydrate content of the stem base of the three positions was not significantly different for sucrose and glucose except for fructose that was significantly higher in the middle position than top position cuttings of both rootstock cultivars (Table 35).This was possibly due to the influx of fructose from the leaf of the stem cutting that recorded higher fructose content than the bottom position leaves (Table 34).

During the rooting process, the fructose and glucose content of the stem base cuttings decreased from day 0 to day 7 after planting in the three positions irrespective of the rootstock cultivars

(Table 36 and 37). The fructose, glucose and total sugars also decreased from day 0 to day 7 after planting in 'Rosa progress' (Tables 36, 37 and 39). In 'Natal briar', the fructose content decreased from day 0 to day 7 after planting while glucose and total sugars decreased from day 0 to day 3 after planting followed by a slight increase to day 7 after planting (Tables 36 and 37). The fructose and glucose being simple sugars, readily release energy for adventitious root formation than sucrose and this explains the decreased levels during the rooting process in the three cutting positions of both rootstock cultivars. Hussein *et al*. (2012) and Rolland *et al*. (2006) showed that direct uptake of glucose instead of sucrose induced higher rooting frequency in *Eurycoma longifolia*.

The amount of fructose and glucose in the stem base depend on the rate of sucrose hydrolysis and since the stem base recorded lower amounts of sucrose at the time of severance, the high carbohydrate content in the leaves and the current photosynthates would ensure the supply of carbohydrates to the stem base during the adventitious root formation. The slight increase in glucose and total sugars in the stem bases of 'Natal briar' from day 3 to day 7 after planting could be as a result of translocation of sugars from the original leaf as it recorded higher sugar content than 'Rosa progress' in the leaf of the stem cutting at the time of severance (Table 34) or enhanced accumulation of photosynthates to the rooting zone from the leaf during the rooting period (Druege *et al*., 2004).

At the time of severance the level of sucrose was low compared to glucose and fructose (Table 35). This could be due to increased activity of cell wall invertase activity in the first hours after excision (Ahkami *et al*., 2009). During the rooting process, there was increased accumulation of sucrose at the stem base of top position cuttings of 'Natal briar' and middle position cuttings of 'Rosa progress' (Table 38) which was positively correlated to high rooting percentage especially

on days 3 and 7 after planting (Table 54) and survival of the cuttings in both rootstock cultivars than bottom position cuttings and this would indicate high influx of assimilates from the leaves. Husen (2002) and Husen and Pal (2007) also reported positive correlations of sugars with rooting efficiency of teak cuttings. Our results were however, inconsistent to the findings of Druege *et al*. (2004) who indicated that low carbohydrate levels in cuttings at the beginning of rooting limit the speed or intensity of subsequent adventitious root formation.

The increased accumulation of sucrose to the stem bases in the top and middle position cuttings of 'Natal briar' and 'Rosa progress'(Table 38) could be due to high production of photosynthates in the top and middle position leaves than bottom position leaves (Table 34) or possibly due to decreased activity of the enzyme cell wall invertase. Cell division and cell enlargement during adventitious root formation require high input of energy and carbon skeletons and sucrose is a major carbon source formed in photosynthetically active tissues and translocated towards stem base for rooting. The basipetally translocated sucrose is, however, not only used to deliver energy for cell differentiation, a considerable portion of sucrose is converted to starch, which probably acts as the carbon source when the adventitious roots grow (Ahkami *et al*., 2009) and this would account for faster rooting. Moreover, increased basipetal transport of sugars in cuttings would also allow for accelerated co-transport of amino acids such as tryptophan, precursor to auxin synthesis, within the phloem along with sucrose (Winter *et al*., 1992). Sucrose therefore enhances the sensitivity to auxin (Anonymous, 2008; Calamar and de Klerk, 2002) whose involvement in the formation of adventitious root formation has been reported in many species (Ahkami *et al*., 2013; da Costa *et al*., 2013).

The bottom position cuttings recorded low sucrose content in the stem base of both rootstock cultivars and this corresponded to low rooting percentage (Tables 3a and 3b). Regardless of high

simple sugars (fructose and glucose) in the bottom position cuttings (Tables 36 and 37), without replenishment by sucrose due to lower level (Table 38), the rate of rooting process could be inhibited. This implies that low carbohydrates, for instance as a consequence of reduced leaf photosynthesis (Table 34), may decrease total sugar level (Table 39) that may hinder rooting, especially when root initiation occurs within the callus tissue. Increasing carbohydrate level in the stem bases of cuttings for instance, by dipping them in sucrose solution has been shown to improve rooting response of woody plants (Pallardy and Kozlowski, 2007).

Most of the bottom position cuttings lost their leaves and started to rot at the basal end before rooting and this contributed to low percentage survival and rooting. 'Rosa progress' had lower glucose, sucrose and total sugar content than 'Natal briar' and rotting was more pronounced in 'Rosa progress' than 'Natal briar'. Presumably low carbohydrate content of the bottom position cuttings contributed to the rotting since low carbohydrate content of tissues have been shown to enahnce their susceptibility to disease and sugars such as sucrose and glucose are known to induce disease resistance in plants (Druege *et al*., 2004; Howard and Harrison-Murray, 1995; Ypema *et al*., 1987).

The carbohydrate content of the stem base of cuttings was not the only factor responsible for the acropetal increase in rooting potential in both rootstock cultivars because the sucrose and total sugar contents for the top and bottom position cuttings were not significantly different from each other in 'Rosa progress' (Tables 38 and 39). In 'Natal briar', total sugars of the bottom position cuttings was not significantly different from the top and middle position cuttings (Table 39), and sucrose content in the middle and bottom position cuttings were not significantly different from each other (Table 38) and therefore, other factors such as auxins, apart from the carbohydrate

content of the leaves on the stem cuttings may have been responsible for acropetal increase in adventitious root formation.

Our results disagreed with the findings of Keeley *et al*. (2004) and Soonhuae and Limpiyaprapart (1996) who observed higher rooting percentage from the bottom position cuttings of 'Norton' grape vine rootstock and *Dipterocarpus alatus* respectively and attributed it to high storage carbohydrates in the thick size cuttings from the bottom position. Aini *et al*. (2010) also observed that basal stem base position cuttings of *Gonystylus bancanus* exhibited higher root number than the apical position cuttings and attributed it to large area of the bottom cuttings for storage of nutrients. Saifuddin *et al*. (2013) documented higher root length in cuttings taken from the basal positions in *P. pterocarpum* and attributed it to different carbohydrate storage and organogenic activity of different positions of stem cuttings. The contrasting results indicate that adventitious root formation is influenced by other internal factors such as auxins that may either act singly or interact with carbohydrates to effect rooting.

5.1.2. Effects of cutting position of *Rosa hybrida* rootstock cultivars and endogenous IAA and cytokinins on growth parameters of self rooted cuttings.

The results revealed that the stem base of the top position cuttings had significantly higher endogenous IAA, IPR, DHZR, tr-ZR, ratios of IAA to tr-Z, tr-ZR and total cytokinins than those of the bottom position cuttings (Tables 44, 45, 47, 49, 50, 51 52). These higher endogenous hormonal levels in top position cuttings may have contributed to the higher rooting and growth observed in the top position relative to the bottom position cuttings (Tables 3a-8b). Auxin is required for adventitious root formation on stems and the divisions of the first root initials are dependent on exogenous and endogenous levels of auxins (Ludwig-Müller, 2000; Kochhar *et al*., 2005). Cytokinins are involved in the regulation of polar auxin transport as well as induction of

auxin gradient and the subsequent coordination of cell division and differenciation (Pijut *et al*., 2011). The high concentration of IAA and ratios of IAA to cytokinins in the top position than the bottom position cuttings was associated with higher rooting ability as evidenced by high rooting percentage, root number, total root length, RFW and RDW (Tables 3a-7b) of top position cuttings in both rootstock cultivars. Ayoub and Qrunfleh (2006) and Geneve (1991) reported a positive correlation between IAA content and rooting ability of *Olea europaea L.* and *Hedera helix* cuttings respectively.

The lower root number associated with low auxin concentration in the bottom position cuttings was in agreement with the findings of Deen and Mohamoud (1996) in *Rosemarinus officinalis* L, Palanisamy and Kumar (1998) in *Azadirachta Indica*, Wassner and Ravetta (2000) in *Grindelia chiloensis* and Edgar (2012) in *Afzelia rhomboidea* Vidal. Auxins play a significant role in stimulating adventitious rooting from the stem cuttings of tree species (Poupard *et al*., 1994; Tchoundjeu *et al*., 2001). In addition to enhancing the rate of adventitious root development, auxin application has been found to increase the number of roots initiated per rooted cutting in a variety of species (Mesen *et al*., 1997; Palanisamy *et al*., 1998).

Significantly higher total root length was obtained from the middle position cuttings than bottom position cuttings in the IBA experiment (Table 5a) and since the total root length of the top and middle position cuttings were not significantly different from each other in both IBA and NAA experiments (Tables 5a and 5b), it is possible that the IAA concentration of the top position (IAA concentration analysed) and middle position (IAA concentration not analysed) cuttings were statistically similar and the high IAA presumably enhanced callus and root formation than the bottom position cuttings in both rootstock cultivars. Longer roots extract more water and mineral

nutrients required for plant growth. High total root length from the top position cuttings has been reported in *Gonystylus bancanus* (Aini *et al*., 2010).

Root weight, a function of root number, root length and root diameter, was also higher in the middle and top position cuttings (Tables 6a and 6b) and was associated with high IAA in the apical cuttings. Hartmann *et al*. (2011) also had similar results and in addition to the promotive effect of auxin, the apical cuttings are less differentiated and are therefore more prone to dedifferentiation. High root weight due to high root number and root length allows a plant to absorb more water and nutrients from the media to support shoot growth.

The bottom position cuttings had lower IAA concentration than the top position cuttings in both rootstock cultivars as was also reported in mature woody plants (Haffner *et al*., 1991). The IAA to cytokinin ratios were also low (Tables 50-52) in the stem bases of bottom position cuttings and these low levels of IAA and its ratio to cytokinins corresponded to the low rooting potential observed in bottom position cuttings (Tables 3a-7b). This could also be due to reduced polar auxin transport to the bottom position (Davies, 1995), increased peroxidase activity (enzyme that breaksdown IAA), less IAA biosynthesis or more conjugation of IAA to sugars and amino acids (Woodward and Bartel, 2005).

With respect to time course, there was increased accumulation of IAA in the bud region of cuttings from day 0 to day 8 post excisions (Table 44). Osterc *et al*. (2009) also recorded a similar trend in woody species though his measurements were up to the fourth day post excision. The ratio of IAA to cytokinins also increased during the rooting process (Tables 50-52). In the stem base of the cuttings there was increased accumulation of IAA from day 1 to day 8 post excision. The increased accumulation of IAA in the bud region of cuttings indicated that there was increased production of IAA from the bud and the leaf of the rooted cuttings as they act as

sites for synthesis of auxin (Hartmann *et al*., 2011) which is basipetally transported to the stem base for rooting. Basipetal transport and accumulation of auxin at the rooting zone has been reported in *Olea europaea L.* (Pio *et al*., 2005; Ayoub and Qrunfleh, 2006) *Petunia hybrida* (Ahkami *et al*., 2013) and *Pisum sativum* (Rasmussen *et al*., 2015) cuttings and has been repeatedly shown to precede adventitious root formation (Hausman *et al*., 1997; Guerrero *et al*., 1999). Elevated auxin concentration promotes production of new root primordial by activating the differentiation and elongation of phloem parenchyma cells adjacent to vascular bundles in the stem (Lund *et al*., 1996; De Klerk *et al*., 1999).

The results indicated that the IAA concentration in the stem base at the time of severance (day 0), is critical for rooting in 'Natal briar' since the high IAA in the top position cuttings at day 0 post excision also corresponded to the high rooting potential in top position cuttings. Furthemore, the IAA concentration in the bud region was lower than the stem base of the cuttings for the first four days post excision (Figure 3) and this may imply that the rate of IAA synthesis was low and hence little or no supply of IAA to the stem base of cuttings for rooting. Root induction and initiation in 'Natal briar' therefore depended on IAA that had accumulated at the time of severance. Smalley *et al*. (1991) also reported a positive correlation between the endogenous free auxin content and rooting percentage when auxin level was high at the time *Acerrubrum* cuttings were made. In 'Rosa progress', stem base of bottom position cuttings had higher IAA levels than those from the top position cuttings at the time of severance but, as the rooting process proceeded, there was enhanced accumulation of IAA in the top position cuttings than the bottom position cuttings (Figure 2a) indicating that there was increased production of IAA in the bud region of top position cuttings (Figure 2b). Rooting ability of 'Rosa progress'

was therefore related to the accumulation of IAA at the stem bases as has also been reported in *Olea europaea L.* cuttings (Ayoub and Qrunfleh, 2006).

Adventitious root development is a physiological process involving three phases; induction, initiation and expression (Kevers *et al*., 1997), each with a specific requirement that can even be antagonistic, but operate in a complementary fashion. These rooting phases are correlated with changes in endogenous auxin concentrations (Helor *et al*., 1996). During the rooting process, the concentration of IAA decreased from day 0 to day 1 post excision, remained on the same level to day 2 followed by an increase to day 8 post excision in all the cutting positions except top position cuttings of 'Rosa progress' (Figure 2a). Bellamine *et al*. (1998) found that the free IAA concentration has a transient increase during the induction phase, pass through a minimum at the initiation phase and may resume an increase in the expression phase. It is presumed that the first peak which corresponds to induction phase occurred on day 0 (24 hrs) post excision in both rootstock cultivars. The first peak (day 0) corresponded to high IAA (Table 44 and Figure 3), low concentrations of cytokinins (Figures 4a, 6a and 8a) and high ratios of IAA to tr-Z, tr-ZR and total cytokinins (Figures 12a, 14a and 16a). de Kerk *et al*. (1995) and Nag *et al*. (2001) reported that the first peak is as a result of lower peroxidase activity at the time of severance. Peroxidase enzymes are involved in IAA catabolism thereby reducing the amount of auxins for rooting.

Auxin has a rhizogenic action during the root induction phase (generally from cutting severance to 96hrs) and stimulates cells at the cutting phase to engage in the establishment of meristemoids (Garrido *et al*., 2002). The decrease observed in endogenous cytokinin during the day after the cutting excision would then allow the root forming processes to start. Moreover a decrease in cytokinin levels should increase the IAA: cytokinin quotient in the root forming tissue (Figures

12a, 14a and 16a), a change which is considered to be favourable for rooting (Mohammed, 1980). Bellamine *et al*. (1998) showed that anti-auxin present at the induction phase significantly inhibited adventitious root formation in poplar cuttings. Studies with carnation cuttings (Acosta *et al*., 2009) and mango cotyledons segments (Liu *et al*., 2012) showed the requirements of increased expression of auxin transporters and increase of polar auxin transport during the induction and formation phase of adventitious root formation.

The results were in agreement with the findings of Stefancic *et al*. (2006) who detected the auxin peak at 24hrs post excision in *Prunus* 'Gisela 5' leafy cuttings but contrary to the observations in *Prunus subhirtella* Autumnalis cuttings (Osterc *et al*., 2009) and *Vigna radiata* (Tartoura *et al*., 2004) where peak concentrations of IAA occurred before 24hrs post excision.

During the rooting process, the concentrations of IPR, DHZR and cis-ZR increased from day 1 to day 2 post excision followed by a decrease upto day 8 post excision for all the cutting positions of both rootstock cultivars except the top position cuttings of 'Natal briar' that recorded a significant increase in IPR concentration from day 4 to day 8 post excision (Figures 4a- 8a). Day 2 recorded lower IAA and ratios of IAA to tr-Z, tr-ZR and total cytokinins (Figures 2a, 12a, 14a and 16a) and it seemed to be the peak of cell division in the root initiation phase. The lower IAA levels in the root initiation phase which is assumed to have started from day 1 post excision with a peak on day 2 post excision may be as a result of activities of conjugating enzymes, higher peroxidase activity and increased cell division due to high cytokinin content (Tables 45-49).

Cytokinins stimulate cell differentiation into vascular tissues through spatially selective expression of cytokinin dehydrogenase gene in the transition zone (Dello Ioio *et al*., 2007). By maintaining high concentration of cytokinin on the second day, a larger number of cell divisions were possibly induced as well as meristemoids and development of root primordial. De Klerk *et*

al. (2001) noted that isopentenyladenide (IP) enhanced rooting in apple micro cuttings and that the promotion was higher when IP was applied during the second day.

Day 8 post excision recorded higher endogenous IAA concentration, ratios of IAA to cytokinins and lower concentrations of individual cytokinins and it seemed to be the expression phase of adventitious root formation. Tartoura *et al*. (2004) reported renewed increase in IAA concentration in the phase of growth and development of root primordial. The top position cuttings recorded higher IAA concentration than the bottom position cuttings in the stem base region of cuttings of both rootstock cultivars on day 8 post excision (Table 44) and this also corresponded to high rooting ability. The rapid increase in endogenous IAA concentration during the expression phase possibly enhanced growth of root primordial through the stem tissues and the establishment of vascular connections between the newly formed roots.

The induction and expression phases are characterized by high levels of IAA at the base of the cutting and high sensitivity to exogenous auxins (Gasper *et al.,* 2003) and relatively constant, low level of cytokinins (Taylor and Van Staden, 1997). Application of exogenous cytokinins at a higher concentration during these phases increases the concentration of endogenous zeatin and this inhibits rhizogenesis (Laplaze *et al.*, 2007) by disrupting primordial initiation and regular pattern of cell divisions. This leads to reduced root meristem size due to progressive decrease in the number of meristematic cells (Laplaze *et al*., 2007; Werner and Schmulling, 2009).

The high concentrations of individual cytokinins; IPR, DHZR and tr-ZR observed in the top position cuttings (Tables 45, 47 and 49) was associated with enhanced shoot growth as evidenced by high shooting percentage, shoot height and leaf number observed in top position cuttings of both rootstock cultivars (Tables 8a-10b). Many researchers have demonstrated acropetal increase in gowth of axillary buds of roses (Zieslin *et al*., 1976; Zamski *et al*., 1985;

Bredmose *et al*., 1999, 2004). The acropetal increase in shoot height was in agreement with the findings of Soundy *et al*. (2008) in *Lippia javanica* cuttings but contrary to the findings of Marcelis-Van-Acker (1994b) and Bredmose and Hansen (1996) who recorded increased shoot elongation in buds from basal positions in roses. Lower leaf numbers observed from the bottom position in both rootstock cultivars was in agreement with the findings of Kassahun *et al*. (2013) in *Stevia rebaudiana* but contrary to Soundy *et a*l. (2008) in *Lippia javanica* and Saifuddin *et al*. (2013) in *Peltophorum pterocarpum* who recorded more leaves in bottom position cuttings.

The acropetal increase in shoot growth was also attributed to high endogenous IAA concentration and IAA to cytokinin ratio (Tables 44, 50-52) that promoted adventitious root formation providing high number of root tips where cytokinins are synthesized. Cytokinins are involved in cell division, chloroplast biogenesis, bud and root differentiation and shoot meristem initiation and growth (Kuroha *et al*., 2009). Though cis-ZR has a lower biological activity in bioassays than tr-ZR (Sakakibara, 2006; Kaminek, 1982), it was the only cytokinin that was high in the stem base of bottom position cuttings (Table 48), although it was not significantly different from the top position cuttings, it possibly contributed partly to the low rooting percentage observed in bottom position cuttings.

The results showed that topophysically the younger the cuttings, the higher the percentage survival (Tables 14). Similar results were reported by Exadaktylou *et al*. (2009) in 'Gislea 5' cherry rootstock, Aini *et al*. (2010) in *Gonystylus bancanus*, Edgar (2012) in *Afzelia rhomboidea* Vidal, and Kassahum *et al*. (2013) in *Stevia rebaudiana*. A positive correlation between rooting percentage and shooting percentage was also recorded in both IBA (R^2=0.3392) and NAA (R^2=0.1013) experiments (Figures 22a and 22b) suggesting that the onset of axillary bud growth is accelerated as a result of accelerated root formation and implicating the possible involvement

of cytokinins from the root tips for shoot growth. Similar correlations were also recorded in Schefflera (Hansen and Kristensen, 2006). Bud outgrowth is essential in the supply of auxins and photoassimilates required for adventitious root formation. Roots tips provide sites for water and mineral nutrient uptake required for general plant growth. In addition, it is the site for cytokinin synthesis required for shoot growth. The slow bud growth in the bottom position cuttings of both rootstock cultivars was also related to low rooting ability and was possibly due to low IAA, cytokinins and the ratios of IAA to cytokinins (Figures 3b-17b). Zieslin *et al*. (1978) however, associated delayed bud growth with accumulation of inhibitory substances (abscisic acid) in the basal region of the parent plant.

The IAA concentration in the stem base of the cuttings was positively correlated to the levels in the bud region of cuttings of 'Rosa progress' (R^2=0.191) and 'Natal briar' (R^2=0.036) (Figures 23a and 23b). The high concentration of IAA in the bud region cuttings of 'Rosa progress' (Figure 3) ensured continous supply of auxins to the stem base for enhanced rooting. Auxins synthesized in shoot tips may be an important auxin source during adventitious root formation, since removal of the shoot apex decreased both the level of endogenous auxin in the basal portion of a cutting and the number of adventitious roots produced in *Pisum sativum* (Nordstrom *et al*., 1991). In the cuttings of 'Natal briar', the stem base contained higher IAA levels than the bud region. This possibly implied rapid transportation of endogenous IAA to the stem base through the phloem by polar auxin transport or through the xylem by mass flow.

The high concentration of IAA (Table 44) and ratios of IAA to tr-Z, tr-ZR and total cytokinins (Tables 50-52) in 'Rosa progress' enhanced adventitious root formation as evidenced by the high root number and total root length (Tables 4a, 4b, 5a and 5b) than 'Natal briar'. The formed roots provided sites for cytokinin synthesis consequently 'Rosa progress' had better shoot growth

and percentage survival as well, as was noted in shoot height (Tables 9a and 9b), leaf number (Tables 10a and 10b) and shooting percentage (Tables 8a and 8b) than 'Natal briar'. The partially initiated root primordial can synthesize cytokinins, thus becoming self sufficient for their hormones (Eriksen, 1974). The observed high shoot growth in 'Rosa progress' was also possibly contributed by the high concentration of DHZR recorded in this rootstock cultivar (Table 47 and Figure 7a). The high concentration of individual cytokinins observed in the rootstock 'Natal briar' was in contrast to the shoot growth parameters observed in this rootstock as it recorded lower shoot growth than 'Rosa progress' indicating that other factors other than cytokinins controlled the shoot growth of 'Natal briar'.

Though the concentrations of the individual cytokinins were higher in the top position cuttings, it did not lower the rooting potential of both rootstock cultivars indicating that the auxin: cytokinin rather than individual cytokinins is crucial in the process of adventitious root development in both rootstock cultivars. Numerous studies underline the role of cytokinins in root and shoot meristem development based on auxin-cytokinin interplay and due to antagonistic effects of auxin and cytokinin on shoot and root meristem development, the direction of plant morphogenesis depends on auxin: cytokinin in which high auxin:cytokinin favours adventitious root formation (Hartmann *et al.,* 2011). These two hormones are also responsible for maintenance of meristem activity, depending on the balance between cell division and cell differentiation (Dello Ioio *et al*., 2007).

5.1.3 Effects of cutting position of *Rosa hybrida* rootstock cultivars and endogenous mineral nutrient level on growth parameters of self rooted cuttings.

Mineral nutrition of the stock plant is an important factor in determining adventitious rooting capacity. At the time of severance, the zinc content in the rooting zone increased acropetally

with bottom position exhibiting lower concentration in ‘Natal briar’ (Figure 18). Middle position cuttings exhibited higher zinc content followed by the top then bottom position in ‘Rosa progress’ (Figure 18). High zinc content in top position cuttings on days 0 and 7 was positively correlated to higher rooting (Table 53) and survival than bottom position cuttings in ‘Natal briar’. The higher zinc content in the middle position than bottom position cuttings at the time of severance in ‘Rosa progress’ (Figure 18) was also positively correlated to rooting percentage (Table 53). Zinc stimulation of rooting has also been reported in *Eucalyptus globus* (Schwambach *et al*., 2005), ornamental shrubs (Pacholczak and Szydlo, 2008) and Dog Ridge rootstocks (Somkuwar *et al*., 2013).

Zinc plays an essential role in several plant metabolic processes, it activates enzymes and is involved in protein synthesis and carbohydrates, nucleic acid and lipid metabolism (Swietliek, 1999; Pahlsson, 1989; Marschner, 1995). Zinc ions are indispensible for the synthesis of tryptophan, the precursor for the biosynthesis of IAA. The high zinc content in the top position cuttings would imply high indole acetic acid as was observed in the IAA results (Table 44) and IAA is essential in promoting adventitious root formation.

The lower zinc content in the rooting zone of the bottom position cuttings of both rootstock cultivars at day 0 after planting (Figure 18) may impair auxin accumulation, which is considered an important event for induction of adventitious root formation (da Costa *et al*., 2013, Ahkami *et al*., 2013). Decreased rooting percentage and root number due to zinc deficiency has been reported in *Eucalyptus globus* microcuttings (Schwambach *et al*., 2005). A sharp drop of zinc level in the middle position cuttings of both rootstock cultivars and top position cuttings of ‘Natal briar’ on day 3 after planting possibly led to the low amounts of IAA recorded on day 2 post excision in the stem base cuttings (Figure 2a). Low amounts of IAA during the rooting

process would correspond to the root initiation phase characterized by high peroxidase activities and high cytokinin content (Figures 4a-11a) and this phase probably occurred on days 2 and 3 after planting. Days 0 after planting might represent the induction phase of adventitious root formation characterized by low peroxidase activity and high IAA content (Figure 2a).

There was increased accumulation of iron in the top position cuttings from day 0 to day 3 after planting followed by a decrease to day 7 after planting in 'Natal briar' (Figure 19). A negative correlation to rooting percentage with iron content at the time of severance (Table 53) would mean high IAA content as iron and manganese participates in the biosynthesis of peroxidases (Campa, 1991), so their low levels in the induction phase could cause a decrease in the activities of peroxidases involved in auxin catabolism (Fang and Kao, 2000). The positive effect of low iron content in the top position cuttings of 'Natal briar' on days 0 and 7 after planting is probably related to enhanced auxin action thereby increasing rooting percentage, root number and percentage survival (Tables 3a, 3b, 4a, 4b, 14a and 14b).

The high iron content in the top position cuttings of 'Natal briar' on day 3 after planting also corresponded to low zinc content (Figure 18) and would imply low IAA and high cytokinin content (Figures 2a-10a) as was recorded on day 2 post excision in the hormone results. Cytokinin is required for cell division during the root initiation phase. The decreased amounts of iron in the middle and bottom position cuttings in 'Natal briar' and all the positions in 'Rosa progress' from day 0 to day 3 after planting (Figure 19) was possibly due to utilization of the reserves in the stem tissues and the increase from day 3 to day 7 was possibly due to translocation of iron from the leaves since the roots had not developed for the uptake of the nutrients. It is also possible that the iron level was not sufficient enough to enhance the activities of peroxidise enzymes involved in IAA catabolism.

The decreased amount of manganese in all the cutting positions from day 0 to day 7 after planting (Figure 20) could have been due to utilization of the reserves in the stem base and it positively correlated to rooting in 'Rosa progress' (Table 53) and it is likely that the level was not sufficient enough to cause considerable IAA catabolism especially on day 3 after planting. Manganese is involved in IAA catabolism through the activities of peroxidise enzymes that reduces the amount of IAA required for rooting. In 'Natal briar', the rooting percentage was positively correlated to manganese content at the time of cutting severance (Table 53) indicating that the high amount of manganese at day 0 after planting was not sufficient enough to induce the activities of peroxidise enzymes involved in IAA catabolism. During the rooting process, the level of manganese decreased to day 7 after planting and was negatively correlated to rooting percentage (Table 53) possibly due to its negative effect on cell elongation causing a reduction on total root length and rooting percentage. Marschner (1995) reported that an increase in manganese in the expression phase would assure root growth, given that this nutrient is also involved in cell elongation.

The acropetal increase in nitrogen content from day 0 to day 7 after planting (Figure 21) was highly positively correlated to acropetal increase in rooting on day 3 after planting (Table 53) and percentage survival in 'Natal briar'. In 'Rosa progress', the rate of nitrogen accumulation in top position cuttings was higher than the other positions as noted with significantly lower nitrogen on day 0 followed by a sharp increase to day 3 after planting (Figure 21) that was highly correlated to rooting percentage (Table 53). Druege *et al*. (2000; 2004) and Zerche and Druege (2009) have also shown that high nitrogen supply to stock plants and the resulting elevated nitrogen content in herbaceous cuttings strongly promote adventitious root formation. The high amount of nitrogen in the top position cuttings could be due to high supply of nitrogen

from the top leaves to the stems as opposed to older leaves found at the bottom of the shoot. The bottom leaves on the shoot become chlorotic earlier probably due to low nitrogen for chlorophyll formation causing a reduction in production of photoassimilates. González-Real and Baille (2000), Bellert (1995) and Mor and Halevy (1979) also recorded that the mean values of leaf nitrogen decreased from the uppermost leaves to the bottom leaves of the plant. In contrast, the work on bitter almond (Kasim *et al*., 2009) and soft wood cuttings of white Forsythia (Yong Kweon and Kisun, 1996) showed a negative relationship between nitrogen concentration and rooting percentage. Nitrogen is required for synthesis of diverse nitrogenous compounds such as nucleic acid and protein which are necessary for root differentiation (Hambrick *et al*., 1985). Our contrasting results may be explained by the manner in which nitrogen relates to carbohydrate content and metabolism (Blazich, 1988). For instance, the findings in Tetraploid locust (Ling and Zhong, 2012) and *Thunbergia grandiflora* (Hussein, 2008) revealed a positive correlation of high carbon to nitrogen ratio to high rooting percentage.

5.2. Effects of cutting position of *Rosa hybrida* rootstocks and endogenous carbohydrates on growth and yield of top grafted 'Inca'

The bottom position cuttings exhibited higher rooting percentage, root number and grafting take (Tables 15,16 and 29) of top grafted 'Inca' than the top position cuttings in 'Rosa Progress'. The increased basipetal growth could have been attributed to high sucrose content recorded on bottom position cuttings (Table 42). Ezekiel (2010) and Saifuddin *et al*. (2013) also attributed high rooting to high availability of carbohydrate as a nutritional source and stem maturity of basal position cuttings. The decreased amount of sucrose to day 7 after planting (Table 42) would indicate that sucrose was being hydrolysed to fructose and glucose that readily releases energy for the rooting process and since sucrose is the most commonly found sugar in the

phloem of angiosperms and immediate carbon substrate in plant tissues, further supply of sucrose to the stem cutting depends on photosynthetic capacity of the leaf on the scion that only occurs once the graft union has formed successfully. In most cases, the formation of the graft union and adventitious roots occur simultaneously (Nazari *et al*., 2009) and since leafless stem cuttings of rootstocks were used, the amount of carbohydrate content especially sucrose at the time of severance (Table 42) as was recorded in the bottom position cuttings of 'Rosa progress' and top position cuttings in 'Natal briar' is important in adventitious root formation of grafts as evidenced by high rooting percentage (Table 15). Druege *et al*. (2004) also reported that low carbohydrate levels in cuttings at the beginning of rooting limit the intensity of subsequent adventitious root formation whereas application of sugars to the rooting medium increases subsequent root formation (Takahashi *et al*., 2003).

In 'Rosa progress', the high rooting percentage of the grafts was associated with high sucrose content of the bottom position cuttings (Table 42) and was in line with the findings of Hansen (1988); Al-Saqri and Alderson (1996) and Saifuddin *et al*. (2013). Similar results were also reported in root number of *Gonystylus bancanus* (Aini *et al*., 2010) but, contrary to the results of the self rooting study where low sucrose content was recorded in the stem base of bottom position cuttings and corresponded to low rooting percentage of both rootstock cultivars. The high amount of fructose and glucose (Tables 40 and 41) in the top and middle position cuttings and high sucrose content (Table 42) in the middle position cuttings recorded on day 7 after planting was inversely related to rooting percentage, root number and grafting take (Tables 15, 16 and 29) and it is possible that some other endogenous factors originating from the scion such as IAA played a role in rooting of grafts on apical stem cuttings rather than carbohydrates.

In the self rooting study the high IAA in the bud region of cuttings was basipetally transported to the stem base of the cuttings that recorded lower IAA levels during the rooting process. Therefore the supply of IAA to the stem base of the grafts depended on the activities of the scion and successful graft union healing which seemed to occur after day 7 of planting for the top and bottom position cuttings. The vascular connection of the grafts transports assimilates and endogenous growth regulators from the scion to the stem base for rooting (Hartmann *et al*., 2011).

In 'Natal Briar', there was acroptal increase in sucrose content (Table 42). The fructose and glucose content was also high for the top and middle position cuttings though, there was no significant difference among the three cutting positions. The high carbohydrate content in the top position cuttings was consistent to acropetal increase in rooting percentage and grafting take (Tables 15 and 29). During the rooting process, the sucrose content of the top position cuttings decreased from day 0 to day 3 after planting as it may have been utilized to provide the energy for cell differentiation and adventitious root growth (Ahkami *et al*., 2009). The increased accumulation of sucrose to day 7 after planting could have been due to supply of assimilates from the scion following healing of the graft union which seemed to occur early in the top position cuttings. The acropetal increase in root growth of grafts on the rootstock 'Natal briar' was consistent with acroptal increase in growth in the self rooting study indicating that the growth of the variety 'Inca' was influenced by the rootstock activity.

Rootstock effect on scion vigor has been reported in *Rosa canina* 'Inermis' (Fuchs, 1994) and has been attributed to increased rootstock activity through increased production of cytokinins and supply of water and nutrients required for shoot growth. The low rooting percentage and grafting take of bottom position cuttings was concomitant with low sucrose, glucose and fructose

content in the stem base cuttings of 'Natal briar'. This could be due to negative regulatory effect of low carbohydrates and hormones on mitotic activity of the cambium (Osterc *et al.*, 2009).

The higher total root length, FRW, RFW: SFW and RDW: SDW (Tables 18, 19, 27 and 28) of the grafts on the middle position cuttings than on the top position cuttings was inversely related to the sucrose content (Table 42) indicating that other factors such as the activities of the scion, low auxins in bottom position cuttings (Table 44), physiological maturity of the stem also regulated the root growth of both rootstock cultivars. Similar findings were reported in cuttings of *Rosa centifolia* (Al-Saqri and Alderson, 1996) and were attributed to different physiological and anatomical factors that influence the distribution of carbohydrates and other metabolites within the cuttings (Mujib, 1993). Our results for 'Natal briar' were contrary to the findings of Saifuddin *et al*. (2013) who recorded high root length with basal position cuttings in *P. pterocarpum* and attributed it to high carbohydrate storage and organogenic activity of different positions of stem cuttings.

The shoot growth parameters such as the shoot height, leaf number and SFW (Tables 21, 22, 23 and 25) were not significantly affected by the cutting position however, other researchers have reported increased shoot height and leaf number towards the basal position cuttings in *Rosa hybrida* cultivars (Bredmose *et al*., 2004) and *P. pterocarpum* cuttings (Saifuddin *et al*., 2013) respectively. The SDW of the grafts on the top position cuttings was higher than on the middle position cuttings in 'Natal briar' and was attributed to the high sucrose content of the top position cuttings (Table 42). Percentage mortality of the grafts significantly increased acropetally in 'Rosa progress' (Table 24) and coincided with low sucrose content of the top position cuttings (Table 42). Leakey (1983) attributed the poor rooting with apical cuttings to less favourable

water relations and relatively low mean total structural and water soluble carbohydrate content in *Triplochiton scleroxylon* cuttings (Leakey and Coutts, 1989).

Interaction between the rootstock cultivars and cutting position was noted for stem weight and stem diameter of the grafted variety 'Inca'. The grafts on the bottom position cuttings had lower stem weight than on the top and middle position cuttings in 'Rosa progress' (Table 33b). This was consistent with the self rooting study results where acropetal increase in SFW (Table 10a and 10b) was observed but inconsistent with the top grafting study results during propagation where cutting position had no significant effect on SFW (Table 35). The increased stem weight (Tables 33a and 33b) of the grafts could be due to increased activity of the rootstock that facilitated uptake of water and minerals required for shoot growth. In the propagation unit the root growth may be restricted by the propagation container thus hindering its activity resulting into reduced growth of the grafts.

Rootstocks influence on scion vigor has been reported in grafted vegetables and ornamental crops (Lee and Oda, 2003) and has been attributed to higher rootstock activity and increased synthesis of plant growth substances (Yetisir and Sari, 2003). Increase in stem weight of 'Inca' grafted on 'Rosa progress' and stem diameter on 'Natal briar' with apical cuttings may have been due to high IAA (Table 44) in both rootstocks and high sucrose content (Table 42) in 'Natal briar' that promoted root formation in grafts on the top position cuttings as evidenced by high root number and root length (Tables 16 and 18). The formed roots enhanced the uptake of water and mineral nutrients and production of cytokinins required for shoot growth and development. Hartmann *et al*. (1997) also attributed acropetal increase in growth to high IAA concentration.

Lack of significant effect of cutting position on stem weight of 'Inca' grafted on the rootstock 'Natal briar' indicates that the stem weight of the variety 'Inca' was probably dictated by the

activity of the scion 'Inca'. Scion effect on plant vigour has been reported especially where the scion has higher vigour than the rootstock (Cardinal *et al*., 2007).

The acropetal increase in stem diameter of 'Natal briar' and basipetal increase in stem diameter of the variety 'Inca' grafted on 'Rosa progress' (Table 32) was consistent to the high rooting % and grafting take of grafts on the top and bottom position cuttings respectively and was attributed to high sucrose content (Table 42) in these cuttings. The growth of the stem may be influenced by the nutritional status of the plant and the high rooting percentage and root number enhanced the supply of water and mineral nutrients required for sturdy growth and other physiological activities within the plant. Similar findings were reported in *Gonystylus bancanus* (Aini *et al*., 2010) and tree species (Saifuddin *et al*., 2013).

The rootstocks may affect either directly or indirectly scion characteristics such as vigor, nutrient status, flower yield and quality (Pertwee, 2000; Carbera 2002; Solis–Perez and Carbera, 2007). The variety 'Inca' grafted on the rootstock 'Rosa progress' exhibited significantly higher root length, total root length, shoot height, RFW: SFW, total number of harvestable stems and stem length (Tables 17, 18, 21, 27, 30 and 31) than 'Natal briar'. The rootstock vigorous root system (Tables 17 and 18) increases the efficiency of water and nutrient acquisition that possibly resulted in enhanced shoot growth of the variety 'Inca' grafted on 'Rosa progress (Tables 21, 30 and 31).

The faster growth rate trait of the rootstock 'Rosa progress' observed in the self rooting experiments (Tables 11a and 11b) was probably transmitted into the variety 'Inca' through the graft union contributing the high number of harvestable stems. Lee and Oda (2003) reported different responses of vegetative growth of the grafted combinations to be related to vigour of the rootstocks and compatibility of rootstocks and scion in vegetables and ornamental crops.

Other researchers attributed increased vigour of the shoot to other factors such as increased synthesis of plant growth substances such as cytokinin (Salehi *et al*., 2010; Yetisir and Sari, 2003), higher rootstock activity (De Varies and Dubois, 1990; Edelstein, 2004; Salehi *et al*., 2009) through enhanced uptake and translocation of water and minerals (Pheasant and Clarke, 1991).

In cut roses, the flower quality is determined interms of stem length and stem weight although, the two parameters do not always coincide while the yield is determined interms of number of stems/m^2 (Robert *et al*., 2001). The variety 'Inca' grafted on 'Rosa progress' had higher yield and stem quality interms of stem length (76. 31cm) than on 'Natal briar' (72.34 cm) and possibly due to high total root length and rootstock activity that enhanced the synthesis of cytokinins, uptake of water and nutrients required for shoot growth. Park and Jeong (2015) also reported that the yield and stem length of rose variety 'Pink Aurora' grafted onto the rootstock 'Multiflora' was lower than that in plants grafted on the rootstock 'Indica Major'. Our results were contrary to the findings Hu (2001) and Robert *et al*. (2001) who observed a negative relationship between flower quality and yield of grafted roses.

Rootstock differences in terms of yield and quality of grafted scions have also been reported in pomegranate (Karimi, 2011), Kiwi fruit orlova (2007) and apple (Sadowski and Gorski, 2003). Lack of rootstock effect on stem diameter of grafted 'Inca' (Table 32) was similar to the findings of Park and Jeong (2015) on rose variety 'Yellow Pink' grafted on 'Indica Major' and 'Multiflora'. This implied that the stem diameter was determined by the scion activities. Our results were however, inconsistent to the findings of Khosh-Khui and Zargarian (2010) where the rootstocks *Rosa chinensis* 'Masquerade' and 'Canina Inermis' increased the stem diameters of rose cultivars 'Golden Gate', 'Hocus Pocus' and 'Trixx'.

Higher RDW: SDW, rooting percentage and flowering stem weight (Tables 15, 28 and 33a) were obtained in variety 'Inca' grafted on the rootstock 'Natal briar'. Khosh-Khui and Zargarian (2010) also reported increase in fresh and dry weights of flowers stems of 'Golden Gate' and 'Hocus Pocus' budded on the rootstocks 'Canina inermis' and *Rosa chinensis* 'Masquerade'.

Shoot weight is a function of stem diameter, length and thickness of the leaves. Though stem diameter of 'Inca' was not influenced by the rootstock cultivars, 'Natal briar' had thicker stems than 'Rosa progress' (Table 32). In addition, morphologically 'Natal briar' has thicker stems and broader leaves than 'Rosa progress'. These traits were presumably transferred to the scion contributing partly to high shoot fresh weight of 'Inca' grafted on 'Natal briar'. The rootstock 'Natal briar' invested more energy in the development of roots as observed in high RDW: SDW (Table 28) and this ensured much supply of water, nutrients and cytokinins to the developing shoot. It is generally accepted that the root and shoot growth are interdependent with respect to supply of water, mineral nutrients, carbohydrates and phytohormones. Brouwer (1983) reported that the CO_2 uptake of leaves and water and mineral uptake of the roots must be balanced against one another and that biochemical signals, likely hormones, may be involved in the maintenance of optimum root-shoot ratios (Wit and Penning, 1983).

5.3. Effects of auxins, cutting position and rootstock cultivars on rooting, growth and yield of rose variety 'Inca'

In the self rooting study, the rooting percentage for 0.6%IBA (87.0%), 0.4%IBA (93.3%) and the control (82.4%) were not significantly different from each other in 'Natal briar' (Table 3a). 0.4%IBA seemed to be optimum for rooting as higher IBA concentration (0.6%IBA) decreased the rooting. da Costa *et al*. (2013) also reported inhibitory effect of high auxin concentration on root differentiation and growth. Al-Saqri and Alderson (1996) noted that application of auxin in

the range of 0.3-0.4% had a positive effect in increasing the rooting of cuttings of woody ornamental shrubs such as roses and Chinese hollyhock. Singh *et al*. (2011) also found that 4000 mg/L (0.4%) IBA under mist system had the greatest influence on rooting of *Bougainvilla spectabilis* cuttings. Though IBA treatment had no significant effect on rooting percentage in 'Rosa progress', the control had higher rooting percentage than 0.4% IBA treated cuttings (Table 3a).

Our results for IAA analysis showed that the rootstock 'Rosa progress' had higher level of endogenous auxins than 'Natal briar' (Table 44) and this possibly explains the high rooting percentage observed in the non auxin treated cuttings than the auxin treated cuttings. It is also possible that the concentration of exogenously applied IBA was slightly higher than the required amount thus slowing differentiation and the outgrowth of roots (da Costa *et al.*, 2013). Asl *et al*. (2012) and Kibbler *et al*. (2004) also reported comparable results for rooting percentage in both the auxin treated cuttings and the control in *Bougainvillea* species and *Backhousia citriodora* stem cuttings respectively and Davies (1984) postulated that a lack of any effect of auxins on promotion of rooting indicates other factors are of greater significance.

In the grafting experiment, the auxins promoted rooting irrespective of the type and concentration as 0.4%IBA and 0.2%NAA treated cuttings had higher rooting percentage than the control (Table 15). The effectiveness of auxin to raise rooting percentage of the cuttings could be through increasing cambial activity and differentiation of root primordia (Davies and Joiner, 1980) or by stimulating redistribution and mobilization of some auxin cofactors towards the base of cuttings. Low rooting percentage of cuttings untreated with auxin could be due to low endogenous auxin (Table 44) for root induction especially in 'Natal briar' though it had high carbohydrate content (Tables 40-43) than 'Rosa progress'.

The root number increased with NAA and IBA application in both rootstock cultivars in the self rooting (Table 4a and 4b) and top grafting (Table 16) studies. Galavi *et al*. (2013), Rahbin *et al*. (2012), Al-Saqri and Alderson (1996) and Ezekiel (2010) also obtained maximum number of roots with IBA in *Vitis vinifera, Cestrum nocturnum*, *Rosa hybrida* and tropical trees cuttings respectively. The exogenous auxins normally act by signaling the proteins to stimulate new cells and resulting in the initiation of numerous roots (Hamed *et al*., 2004; Durbak *et al*., 2012). Rahman *et al*. (2002) attributed the promotive effect of auxin on rooting to its role in stimulating cell division in the vascular cambium which leads to the formation of root primordial. The fine roots produced in the stem cuttings are capable of absorbing relatively more water and minerals due to the increment of root surface and root –soil interaction (Stokes *et al*., 2009). 'Rosa progress' recorded high IAA concentration (Table 44) and application of high concentration of NAA inhibited the number of roots formed because 0.2%NAA treated cuttings recorded significantly higher root number than 1%NAA treated cuttings (Table 4b).

Auxin treatment promoted total root length in both studies (Tables 5a, 5b and 18) irrespective of the concentrations. The increase in root length observed in the auxin treated cuttings could be attributed to accumulation of metabolites at the site of application of auxins, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins and cell division induced by the auxins (Shan *et al*., 2012). Polysaccharide hydrolysis provides energy for meristematic tissues of roots (Ezekiel, 2010). High concentration of auxins of 0.6%IBA and 1%NAA in both experiments inhibited root elongation as evidenced by reduced total root length after 0.4%IBA and 0.2%NAA treatments respectively (Tables 5a and 5b). da Costa *et al*. (2013) reported that high auxin levels are inhibitory to root differentiation and growth.

The control had lower root fresh weight than IBA treated cuttings in 'Natal briar' and 0.4%IBA treated cuttings in 'Rosa progress' in the self rooting study (Table 6a). A reduction in root fresh weight with 0.6%IBA (Table 6a) indicates inhibitory effect of very high concentration of auxin on root growth. In the top grafting study, generally the auxins promoted root growth as grafts on treated cuttings recorded significantly higher RFW than the control (Table 19). Top position cuttings treated with either 0.2%NAA or 0.4%IBA recorded lower RFW than the middle and bottom position cuttings though the top position cuttings had higher level of IAA and IAA:cytokinin (Tables 44, 51-52) than the bottom position cuttings. This could be due to inhibitory effect of high auxin concentration at the stem bases by inducing ethylene production and inhibiting root growth and bud break (da Costa *et al.*, 2013; De Klerk and Hanecakova, 2008).

Irrespective of the cutting position and rootstock cultivars, 0.4%IBA treated cuttings had significantly higher root dry weight than the control in the self rooting study (Table 7a) while in the top grafting study, the variety 'Inca' grafted on the middle position cuttings and treated with 0.4%IBA recorded higher RDW than the bottom and top position cuttings (Table 20). The low RDW in bottom position cuttings treated with 0.4% IBA than middle position cuttings could be due to anatomical barriers that reduces auxin uptake/ transport or juvenility factors such as sub-optimal levels of endogenous (Table 44) and applied auxins, low sensitivity to exogenously applied auxin (Stuepp *et al.*, 2014; Husen and Pal, 2006) or the healing of the graft union occured late and the supply of auxins from the scion was delayed. The top position cuttings recorded high concentration of IAA in the self rooting study (Table 44) and possibly high concentration of exogenously applied IBA retarded root growth (da Costa *et al*., 2013). Galavi *et al*. (2013) also reported maximum root fresh and dry weights from auxin treatment than the

control in grapes. Opuni-Frimpong *et al*. (2008) and Husen and Pal (2007) demonstrated that IBA has an important role in the development of adventitious roots, improving quality of roots and increasing root biomas.

In the self rooting experiment, the shoot height (Tables 9a and 9b), leaf number (Tables10a and 10b), and SDW (data not presented) were not influenced by the auxin treatment except the shooting percentage (Tables 8a and 8b) and SFW (Tables 11a and 11b). The auxins promoted SFW upto 0.4% IBA (Table 11a) and 0.2%NAA (Table 11b) in the IBA and NAA experiments respectively and above these concentrations, the SFW of 'Rosa progress' decreased and this may be due to the inhibitory effect of high auxin concentration on outgrowth of buds through the production of ethylene (De Klerk and Hanecakova, 2008). 'Natal briar' cuttings treated with 0.2%IBA had significantly lower shooting percentage than 0.4%IBA treated cuttings (Table 8a). This corresponded to high rooting percentage in 0.4%IBA treated cuttings of 'Natal briar' (Table 3a) indicating the involvement of root growth in shoot growth development through the production of cytokinins from the root tips. In 'Rosa progress' the control had significantly higher shooting percentage than 1%NAA treated cuttings possibly due to high endogenous IAA concentration (Table 44) in untreated cuttings and application of higher auxins inhibited shooting percentage (Table 8b).

The top position cuttings untreated with NAA recorded higher shooting percentage than the middle and bottom position cuttings (Table 8b). The difference was due to the difference in biochemical constituents such as high IAA and cytokinins in the top position cuttings (Tables 44-49) that promoted acropetal root and shoot growth in both rootstock cultivars. The bottom position cuttings had comparable carbohydrate content to the top position cuttings (Tables 38 and 39) but low IAA and cytokinin content that contributed to low shoot growth. The low

shooting percentage in bottom position cuttings treated with 0.2% NAA than middle and top position cuttings could be due to anatomical barriers that reduces auxin uptake or juvenility factors such as suboptimal levels of endogenous and applied auxins, high rooting inhibitors or low sensitivity to exogenously applied auxin (Stuepp *et al*., 2014; Husen and Pal, 2006).

In the grafting experiment, the shoot growth was promoted by auxin treatment as evidenced by high shoot height, leaf number and shoot fresh and dry weight. The grafts on the auxin treated cuttings had comparable shoot height (Tables 21 and 22), leaf number (Table 23) and SFW (Table 25) but higher than the untreated grafts. The higher SDW of 0.4%IBA than 0.2%NAA in 'Rosa progress' (Table 26) was possibly due to different concentrations of auxins or other factors such as higher stability and a slow rate of conjugation of IBA, so that the free IBA required to induce rooting will be available over a longer period of time than IAA or NAA (Krisantini *et al*., 2006). Increased shoot height with IBA application has been reported in miniature roses (Bredmose *et al*., 2004). Saiffudin *et al*. (2013) also obtained increased fresh shoot weight with IBA than the control and explained that the shoot growth is favoured by triggering enough growth of roots by IBA. The increase in leaf number with 0.4% IBA may be due to their significant effect on inducing vigorous rooting system by growth regulators thus enabling the cuttings to absorb more nutrients thereby producing more leaves as reported by Prati *et al*. (1999) and Stancato *et al*. (2003).

The promotive effect of auxin on the vegetative growth was due to enhancement of rooting ability by the auxins as recorded in rooting percentage, root number and total root length (Tables 15, 16 and 18) which could have led to more uptake of water and nutrients from the growing medium resulting in an increment in vegetative growth. Eartey (2007) explained that the concentration of endogenous auxin rises to the point that roots are initiated on the callus after

which, the roots will produce cytokinins that will be transported acropetally, as the concentration of cytokinin accumulates, it stimulates shoot formation. Cytokinins are involved in cell division, chloroplast biogenesis, bud and root differentiation and shoot meristem initiation and growth (Argueso *et al*., 2009; Kuroha *et al*., 2009). Hence the presence of endogenous cytokinin in the stem tissues (Wroblewska, 2013) with the addition of auxin into the basal ends might have eventually promoted the formation of shoot from the cuttings.

In the self rooting study, the RFW:SFW increased with NAA application in 'Natal briar' but significantly reduced when higher concentration of 1%NAA (Table 12b) was used indicating inhibitory effect of high auxin concentration on adventitious root formation (da Costa *et al*., 2013). The increased RFW: SFW with NAA application was concomitant to the high root and shoot growth with auxin treatment. In the top grafting study, the RFW: SFW of grafts on 'Rosa progress' (Table 27) was possibly determined by high endogenous IAA levels as recorded in the self rooting study (Table 44) and the activities of the scion since the control recorded higher RFW:SFW than the auxin treated cuttings (Table 27). High concentration of auxin reduced RFW: SFW of grafts on 'Natal briar' since higher RFW: SFW was obtained from 0.2%NAA treated cuttings than the control and 0.4%IBA.

The percentage survival as determined by the presence of roots and shoots was lower in 0.2%IBA treated cuttings than the control and 0.4%IBA treated cuttings in 'Natal briar' (Table 14). This was related to higher rooting (Tables 3a and 3b) and shooting percentages (Table 11a) indicating a positive correlation between shoot and root growth (Table 22). The root and shoot growth are interdependent with respect to supply of water, mineral nutrients and cytokinins from the roots and auxins and carbohydrates from the leaves. In the top grafting study, the grafting take increased with auxin treatment irrespective of the type and concentration (Table 29). Karimi

(2011) associated higher bud take percentage and IBA treatment with cell division stimulated by the auxin at the graft union.

Interaction between cutting position and auxin was noted for total number of harvestable stem and stem length (Tables 30 and 31). Bottom position cuttings treated with 0.2%NAA had lower number of harvestable stems than the control and 0.4%IBA treated cuttings possibly due to low conversion of NAA to IAA, a form that can easily be utilized for adventitious root development (Zolman *et al*., 2008). Top position cuttings untreated with auxin had significantly lower number of harvestable stems than the treated cuttings (Table 30). Since the top position cuttings recorded high IAA levels in the stem base and bud region of cuttings in the self rooting study (Table 44), the bud region supplied IAA to the stem bases and since leafless rootstock cuttings were used in grafting, the supply of IAA from the bud and leaf on scion to the stem base where rooting occurs depended on successful healing of the graft union which probably occurred late and auxin application promoted shoot growth of grafts on top position cuttings.

The bottom position cuttings untreated with auxin had significantly lower stem length than the treated cuttings. This could be due to low internal auxin as was recorded in the self rooting study (Table 44) and possibly the healing of the graft union occurred late and the supply of IAA from the the scion to the rooting zone was delayed. The stem length of the middle and top position cuttings were not significantly affected by auxin treatment. The promotive effect of IBA on the vegetative growth may be caused by the enhancement of rooting percentage and root growth on the treated cuttings which increases sites for cytokinin synthesis that is responsible for shoot growth. The enhanced root growth also ensured more uptake of water and nutrients from the growing medium resulting in an increment in vegetative growth.

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1: Summary

The objective of the study was to determine the effects of cutting position of rootstock cultivars and exogenously applied auxins on rooting ability, growth and yield of Rose (*Rosa hybrida*). The biochemical constituents including the mineral nutrient level, carbohydrate level, auxin and cytokinin levels in the cutting positions of the rootstock cultivars were also analysed and related to rooting and outgrowth of buds. The findings indicated that the top and middle position cuttings had higher rooting and sprouting potential than the bottom position cuttings for most of the parameters measured in both rootstock cultivars in the self rooting experiment. The rooting and sprouting potential were however, not significantly different for the former two positions. Significant interaction effect of rootstock cultivars and cutting position also influenced most of the parameters measured except rooting percentage, shooting percentage and leaf number. The variations were attributed to differences in carbohydrate, auxin, cytokinin concentrations and mineral nutrient content among the cutting positions.

Carbohydrates contribute to the formation of adventitious roots by supplying energy and carbon necessary for cell divisions, establishment of the new root meristems and root formation itself (da Costa *et al*., 2013). The high sucrose, fructose and glucose contents in the original leaf of top and middle position cuttings at the time of severance and in the stem bases during the rooting process was possibly responsible for the higher rooting and survival of cuttings from these positions relative to the bottom position cuttings for both cultivars (Tables 3a, 3b, 14a and 14b). The original leaf of single-node cuttings has a strong effect on survival and rooting success as it is the main source of carbohydrate due to photosynthesis. The carbohydrate content of the stem base of cuttings was not the only factor responsible for the acropetal increase in rooting potential

in both rootstock cultivars because during the rooting process the sucrose and total sugar contents for the stem bases of top and bottom position cuttings were not significantly different from each other in 'Rosa progress'. Similarly, in 'Natal briar', total sugars of the stem bases of bottom position cuttings was not significantly different from the top and middle position cuttings, and sucrose content in the stem bases of middle and bottom position cuttings were also not significantly different from each other yet the rooting potential increased acropetally. Therefore other endogenous factors such as auxin and mineral nutrient content were important in determining adventitious root formation of the cutting positions in both rootstock cultivars.

Auxin is required for adventitious root formation on stems and the divisions of the first root initials are dependent on exogenous and endogenous levels of auxins (Ludwig-Müller, 2000; Kochhar *et al*., 2005). The acropetal increase in rooting ability and survival along the rose shoots was consistent with acropetal increases in endogenous IAA and individual cytokinins; IPR, DHZR and tr-ZR in both rootstock cultivars (Tables 44, 45, 47 and 49). Positive correlation between IAA content in the bud and stem base region of cuttings was recorded and the high concentration of IAA in the bud region cuttings presumably ensured continuous supply of auxins to the stem base for rooting. The higher concentrations of individual cytokinins (Tables 45, 47 and 49) observed in the top position cuttings had a promotive effect on shoot growth parameters observed in the top position cuttings of both rootstock cultivars than bottom position cuttings.

During the rooting process, the concentrations of IAA, cytokinins and IAA:cytokinin changed from day 0 to day 8 post excision and were related to the physiological phases in the process of adventitious root development which included induction, initiation and expression phases. The bud region cuttings recorded a steady increase in IAA concentration from day 0 to day 8 post excision indicating that there was continuous production of IAA from the bud and the leaf on the

stem cutting during the rooting process. The first peak of IAA concentration and IAA to tr-Z, tr-ZR ratios in the stem bases of the cuttings occurred on day 0 post excision and was partly due to low concentration of cytokinins (Figures 4a-11a) or lower peroxidase activity and was presumed to be the root induction phase while the second peak on day 8 post excision represented the expression phase also characterized by rapid increase in endogenous IAA that enhanced growth of root primordial through the stem tissues and the establishment of vascular connections between the newly formed roots. Day 2 post excision was considered as the root initiation phase characterized by low IAA, IAA to tr-Z, tr-ZR ratios (Figure 2a, 15a-17a) and high cytokinins. Auxins and cytokinins are responsible for the maintenance of meristem activity, depending on the balance between cell divisions and cell differentiation (Dello Ioio *et al.*, 2007).

The high zinc and nitrogen in the top position cuttings was concomitant to high rooting ability in both rootstock cultivars. Nitrogen is required for synthesis of diverse nitrogenous compounds such nucleic acid and protein which are necessary for root differentiation. Zinc is indispensible for the synthesis of tryptophan, the precursor for the biosynthesis of IAA. The high zinc content in the top position cuttings would therefore lead to high indole acetic acid (Table 44) that is essential in promoting adventitious root formation. Low amounts of IAA would correspond to the root initiation phase characterized by high peroxidase activities and high cytokinin content (Figures 4a-11a) and this phase probably occurred on days 2 and 3 after planting.

Iron participates in the biosynthesis of peroxidases and the positive effect of low iron content in top position cuttings on days 0 and 7 after planting is probably related to enhanced auxin action thereby increased rooting and survival percentages (Tables 3a, 3b, 14a and 14b). The high iron content on day 3 after planting also corresponded to low zinc content and would indicate high cytokinin content as was recorded in the our cytokinin results (Figures 4a-11a).

Bottom position cuttings recorded lower IAA concentration, auxin to cytokinin ratio and carbohydrate than top position cuttings in both rootstock cultivars. This corresponded to low rooting observed in these cuttings. This could be due to reduced polar auxin transport to the bottom position or increased peroxidase activity. The low carbohydrate content of the bottom position leaf could be due to declined rate of photosynthesis with increasing leaf age. Most of the bottom position cuttings lost their leaves and started to rot at the basal end before rooting and this contributed to low percentage survival and rooting. The rotting was more pronounced in 'Rosa progress' and was possibly due to its lower carbohydrate content than 'Natal briar'. Stem rot, a non-pathogenic disorder has been reported to be due to carbohydrate starvation of tissues in *Syringa vulgaris* cuttings (Howard and Harrison-Murray, 1995).

The rootstock 'Rosa progress' recorded higher IAA concentration and lower concentrations of individual cytokinins except DHZR than the rootstock 'Natal briar'. The high bud sprouting potential noted in 'Rosa progress' was directly related to high concentration of DHZR–type cytokinin. The high root number recorded in 'Rosa progress' was probably due to the promotive effect of IAA. The rootstock 'Natal briar' recorded higher carbohydrate and cytokinin content than the rootstock 'Rosa progress'. It is possible that the root growth was favoured by the high carbohydrates and exogenously applied auxin. Though the cytokinin concentration was high in this rootstock, the shoot growth was slow compared to 'Rosa progress, and possibly other intrinsic factors influenced bud break.

In the top grafting experiment, the root-shoot growth parameters of the variety 'Inca' grafted on 'Rosa progress' were promoted basipetally while on 'Natal briar' acropetally and was consistent with high sucrose content in the stem bases of bottom and top position cuttings respectively. Several researchers have mentioned higher growth performance from bottom position cuttings

than the top position cuttings (Saifuddin *et al*., 2013; Ezekiel, 2010; Aini *et al*., 2010) and has been attributed to the availability of carbohydrate as a nutritional source and stem maturity of basal position cuttings. The rootstocks had significant effect on the growth and yield of the variety 'Inca'. The variety 'Inca' grafted on the rootstock cultivar 'Natal briar' had higher rooting percentage and stem weight than on the rootstock 'Rosa progress' which had high stem length and total number of harvestable stems. Both rootstock cultivars had statistically similar grafting take.

Auxin application had stimulatory effect on root growth of cutting positions of both rootstock cultivars. Among the auxins and their concentrations, 0.2% NAA and 0.4% IBA exhibited better rooting ability in the self rooting study while in the top grafting experiment 0.4% IBA had better rooting ability. Higher concentrations of 1%NAA and 0.6%IBA inhibited root and shoot growth for most of the parameters measured. Many studies have reported promotive effects of auxins on adventitious root formation.The promotive effect of auxin on the vegetative growth may be caused by the enhancement of rooting ability on the treated cuttings which leads to production of cytokinins from the root tips, more uptake of water and nutrients from the growing medium required for vegetative growth.

6.2: Conclusion

Rooting ability and auxillary bud growth varies among cuttings originating from different positions on stock plant. Such topophysic effects were observed in 'Natal briar' and 'Rosa progress'. The variation in rooting ability with cutting position has been reported by many researchers and the outcomes are contradictory. Auxins are known to promote adventitious root formation in many woody and non woody plants as reported by many researchers. This study provides indication that the investigated biochemical constituents may be involved in rooting and

bud outgrowth. Application of auxin further supported the role of auxin homeostasis in the observed rootsock cultivars and position-mediated adventitious root formation. The main findings and conclusions of the study are;

1. The biochemical constituents of the cutting positions such as endogenous carbohydrates, IAA, cytokinins and mineral nutrient contents had stimulatory effect on rooting ability and survival of 'Natal briar' and 'Rosa progress'. The acropetal increase in rooting ability and survival was due to higher biochemical constituents in the stem base of top position cuttings than the bottom position cuttings. The auxin to cytokinin ratio rather than individual cytokinins was critical in the process of adventitious root development since the high individual cytokinin in the top position cuttings was inversely related to rooting ability. The bud region of the top position cuttings recorded higher IAA and IAA to cytokinin ratios than the bud region of the bottom position cuttings.The high individual cytokinins; IPR, tr-Z, tr-ZR and DHZR in the stem base and bud region of top position cuttings was concomitant to high sprouting potential than those from the bottom position cuttings in both rootstock cultivars.
2. In the top grafting experiment, the root-shoot growth parameters of the variety 'Inca' grafted on 'Rosa progress' were promoted basipetally while on 'Natal briar' they were promoted acropetally and the trend was consistent with high sucrose content in the stem bases of bottom and top position cuttings respectively. The contrasting results with the self-rooting experiment, especially for 'Rosa progress', where the shoot-growth parameters were promoted acropetally, suggest that the scion had a promotive effect on rooting and growth of its grafts.

3. Auxin application had stimulatory effect on root growth of all cutting positions of both rootstock cultivars. Between the auxins and among their concentrations, 0.2% NAA and 0.4% IBA exhibited better rooting ability in the self rooting study while in the top grafting experiment 0.4% IBA had better rooting ability.
4. The rootstock cultivars also had varied rooting and sprouting potentials as well as biochemical constituents. The high endogenous concentration of DHZR–type cytokinin and IAA promoted sprouting and rooting potentials respectively in 'Rosa progress'. The rooting ability of the rootstock 'Natal briar' was favoured by the high carbohydrates and exogenously applied auxin.
5. In cut roses, the flower quality and yield are separate, but dependent parameters and usually these two may be expressed in an inversely proportional pattern when the total photosynthate is the same. In the grafting experiment, the rootstock 'Natal briar' could be a better rootstock for grafting the variety 'Inca' due to higher rooting percentage (79.89%) than 'Rosa progress' (70.4%). In terms of flower yield, the variety 'Inca' grafted on the rootstock 'Rosa progress' recorded higher number of harvestable stems than on the rootstock 'Natal briar'. Interms of stem quality, both rootstocks produced high quality stem lengths falling in the 2^{nd} grade (79-70 cm) though the variety 'Inca' graftd on 'Rosa progress'(76.31 cm) had taller shoots than 'Natal briar' (72.34 cm). The higher yield and stem length of 'Rosa progress' would possibly indicate higher economic returns than the rootstock 'Natal briar'. The longer stems can be cut to meet the desired market demand hence broader market scope than 'Natal briar'. The high stem weight of the variety 'Inca' grafted on 'Natal briar' than on the rootstock 'Rosa progress' may indicate high freight charges, though they can withstand breakages during post harvest handling.

6.3: Recommendations

1. In the self rooting experiment, the two rootstock cultivars can be successfully propagated using middle or top position cuttings to maintain genetic purity as they had higher root and shoot growth responses as well as biochemical constituents than bottom position cuttings.
2. In the grafting experiment, the rose variety can be successfully propagated using bottom position cuttings of the rootstock 'Rosa progress' and top position cuttings of 'Natal briar' which had more than 70% grafting take. In terms of production, the variety 'Inca' should be grafted on the rootstock 'Rosa progress' due to high stem length and total number of harveastable stems compared to 'Natal briar'.
3. Among the auxins, 0.4%IBA should be used due to high rooting ability.

6.4: Suggestions for further research

1. Since the cuttings for our experiment were stored at 2-4 ℃ for 1 day before sticking, the concentrations of IAA and cytokininins obtained probably does not depict the status of these two hormones at the time of cutting severence (0 day). It is therefore recommended that analysis for IAA at the time of severance and also changes in IAA concentration with storage period and their relationship to rooting of these rootstock cultivars be undertaken
2. To help understand the cause of low rooting in bottom position cuttings, auxin transport and peroxidase activity of the bottom position cuttings should be further explored.
3. Interaction between the exogenous and endogenous auxin may occur during the rooting process. The dynamics of endogenous IAA on auxin treated cuttings and comparison to rooting of the two rootstock cultivars should be investigated.

4. To partly understand the probable cause of high rooting in bottom position of ‘Inca’ grafted on the rootstock ‘Rosa progress’, the biochemical constituents of scion and stock should be established before, and during the rooting process.

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