



ADDIS ABABA UNIVERSITY
GRADUATE PROGRAMMES

This is to certify that the Dissertation prepared by Kebede Jobir, entitled Response of apple rootstocks (*MM.111*, *M.9*, and *MM.106*) and cultivars (*‘Anna’* and *‘Jonagored’*), to low soil pH of *Chencha*, Southern Ethiopia and submitted in partial fulfilment of the requirements for the Degree of Doctor of philosophy in (Biology: Botanical Sciences) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Acid soils are severe problems in many parts of Ethiopia, especially on the highland areas of the southern regions of the country. The coverage of acid soil of the country is estimated to be about 40% of the landmass of which 15.6% is strong acid soil. It is generally known that acid soils have adverse effects on growth and yield of apple trees. In view of the wide cultivation of apple in southern Ethiopia, a study was conducted to assess the effect of naturally occurring acidic soils and two low soil pH modified soils on three apple rootstocks (MM.111, MM.106, and M.9) and two apple cultivars ('Anna' and 'Jonagored'). The soil pH of the study site was 4.9; the pH was adjusted, down to pH 3.5 (in H₂SO₄) and higher to pH 6.3 (in lime). The apple rootstocks were grown under these three pH treatments and studied for one year. 'Anna' and 'Jonagored' cultivars grafted on these three rootstocks under similar pH treatments were also studied for three years. Data on vegetative growth and mineral content of the different plant parts as well as yield components was followed. There were significant growth differences between rootstocks. Under naturally occurring acidic soil (pH 4.9) the vegetative growth of rootstocks were severely affected compared to soil pH of 6.3. Similarly, strongly acidic soil of pH 3.5 severely decreased the growth of rootstocks in comparison with pH 4.9 and 6.3. Among the three rootstocks, maximum vegetative growth were recorded for MM.111 followed by M.9 whereas, least growth was registered for MM.106 in soil pH of 3.5. The increase in growth was pH related. The effect of low pH on rootstocks elemental status was significant. In soil pH of 3.5, MM.111 showed least concentration of Al in roots and Mn in leaves and maximum amount of Ca, Mg, P and N in soil pH of 3.5 which makes it the most the most tolerant of low pH followed by the rootstock M.9. On the other hand, least amount of Ca, Mg, P, N and highest concentration of Al in the roots and Mn leaves was recorded for MM.106 which was the least resistant of the rootstocks. Under naturally occurring acidic soil

(pH 4.9) both 'Anna' and 'Jonagored' cultivars grafted on MM.111, MM.106, and M.9 produced reduced vegetative growth, mean number of flowers and fruits compared to soil pH of 6.3. 'Anna' and 'Jonagored' grafted on MM.111 rootstock had the least growth with least number of flowers and fruits were recorded on MM.106 in soil pH of 3.5. On the contrary, both 'Anna' and 'Jonagored' grown on M.9 under soil pH of 6.3 produced more flower and fruits followed by MM.106 and least was found on MM.111. Among the two cultivars, more number of flowers and fruits were produced by 'Anna' than 'Jonagored'. Rootstock type had significant influence on scion leaf nutrient accumulation. The higher concentration of Al and Mn was found in leaf of both 'Anna' and 'Jonagored' grafted on all three rootstocks under naturally occurring acidic soil (pH 4.9) compared to soil pH of 6.3. In strongly acidic soil of pH 3.5, the accumulation of Al and Mn in leaves of 'Anna' and 'Jonagored' grafted on MM.106 was higher followed on M.9, and least was recorded on MM.111. Leaves of 'Anna' grown on MM.111 accumulated significantly higher concentration of macro-nutrients, Ca, Mg, P, and N, all within the sufficiency range for macronutrients, while, on M.9 and MM.106 the concentrations were all in the deficiency range. In contrast, nutrient absorption of 'Jonagored' was most efficient on MM.106 in uptake of Ca, Mg, P, and N, followed by M.9 and least on MM.111 under soil pH of 3.5. Similar findings were registered under pH of 4.9. The study showed that the rootstock MM.111 is the most tolerant of acid soils and could be used in the large acid soils of apple cultivating regions. The cultivars 'Anna' and 'Jonagored' grafted on MM.111 had the most tolerance of acid soils and least accumulation of the deleterious Al and Mn in their tissues. The implications of the findings are discussed and recommendations on future work suggested.

Key Words: apple, scion cultivars, rootstocks, growth, mineral content, pH levels.

ACKNOWLEDGEMENTS

I am grateful to express my heartfelt gratitude to my advisors, Prof. Masresha Fetene and Dr. Sheleme Beyene for their excellent guidance, advice, critical reading and comments, providing necessary materials, valuable suggestions and their friendly and positive approach they offered me during the course of this study.

I would like to acknowledge Plant Biology and Biodiversity Management of College of Natural Sciences of Addis Ababa University for facilitating financial matters and other administrative issues, especially Prof. Ensermu kelbesa, head of the department for his guidance, leadership and support.

My thanks are also extended to Arbaminch College of Teachers Education for providing me a study leave and Kalehiwot Protestant Church, Chench branch for allowing me the farm land, rootstocks and cultivars to do all field experiments by providing all the necessary facilities. My sincere appreciation also goes to Mr. Theoflos Tesfaye for his technical advices, management practices and all necessary support and facilitation while planning the research and also throughout the study. I also thanks to all workers of Chench Kalehiwot Protestant Church for their contribution to the success of this study.

I would like also to acknowledge with great pleasure all the members of Eco-physiology

research unit for their assistance and support in one way or another during the study.

Special thanks go to Awol Assefa for giving me his valuable time, providing equipment and showing me some operational procedures and also Mrs. Getahun Yemata, Yemane, Dr. Firew, Dr. Yigremachew, Biruk Abayneh and Dr. Yigardu for their excellent cooperation and assistance in various parts of my experiments..

My special thanks also go to Kedir Jemal and Kotebe University College department of Biology and also Bonn University Horticultural Institute Germany especially to Prof. George Noga and Dr. Lankes for their cooperation and support during my study. I am sincerely grateful to friends at Chenchu for their assistance and support during my fieldwork especially; I would like to thank Ayalew Abera, Habtamu Abera and Aynalem Fiseha whose friendship and support made my fieldwork more enjoyable.

Finally, my heartfelt thanks and love go to my wife Yishamu Tesema for her moral support, encouragement, patience and understanding throughout the period of my study. I am very grateful to my elder son Bayew Kebede (Abishkind) for his unreserved support and encouragement in my study and my thanks also goes to my children Melaku and Biruk.

Above all, I thank the Almighty God, who led me through all the difficult times and gave me endurance and strength to complete my study.

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LIST OF ABBREVIATIONS

CEC	Cation exchange capacity
GDP	Gross domestic production
LSD	Least significance difference
LDW	Leaf dry weight
MM	Malling Merton
RDW	Root dry weight
RL	Root length
TCSA	Trunk cross sectional area
SDW	Shoot dry weight

CHAPTER ONE

1. INTRODUCTION

Agriculture in Ethiopia is the main source of income in which about 81 % of the population is engaged and generate revenue in the sector. Agriculture, accounting for about 45% of GDP and 85% of total employment, is the dominant sector in the national economy of Ethiopia (CSA, 2010). The main role of agriculture is to produce sufficient amount of food and ensure food security. In the last four decades, Ethiopia has been facing tragic drought and food shortage. Several studies have been conducted in the areas of cereal crops, improving soil degradation, use of high yielding crop varieties and land management practices to enhance productivity, all of which are involved in the long-term sustainable solution to the country's food security needs. Among these studies more attention was placed on the cereal-based system of the northern, central, and eastern highlands of Ethiopia to address food security problems. Conversely, one of the most economically important fruits, apple which can be cultivated with similar geographical conditions was unnoticed for years. Studies conducted to enhance the productivity of this high income source for the growers were rare and unknown. Recently, there are different apple fruit cultivars that have been extensively grown separately or as mixed farming with other crops in some areas of the country. However, information on interactive effect of scion-rootstocks, lower soil pH, and mineral nutrients, which play vital roles for sustainable increase in the yield of the cultivars were insufficient.

Temperate fruits including apples, pears, peaches, almonds and others were introduced to Ethiopia before 40 to 50 years. Systematic research approaches have been started 30 years back in Agricultural Research Centers such as Holetta Agricultural Research Center (HARC) and the State Farms (Tsedey). However, the progress of studies that promote temperate fruits' production was not as needed. Presently, propagation of temperate fruits becomes an important component of development programs throughout the highlands of Ethiopia. Production and sales of apple fruits can have great contribution to the economy of farmers. Nowadays, the cultivation of apple trees by farmers is increased because of its importance as a source of income, since apple fruits as well as apple rootstocks used for grafting of scions are propagated and sold for apple growers within the country in relatively high price. The farmers have benefited from the high price, which increased their earnings and improved the livelihood of their family. Nevertheless, apple growing was not developed and expanded as it should be for a number of reasons among which knowledge on the impact of naturally occurring acidic soil in the area, and lack of information / on the degree of tolerance of rootstocks and cultivars to low soil pH are considered to be the major ones.

Soil occurring in the warm and humid zones of the west and southern part of Ethiopia, particularly those lying on well-drained landscape, are usually highly leached and very acidic due to the high rain fall intensity and continuous crop cultivation for many years (Taye and Hofner, 1993). The fertility status of these soils is very low due to the removal of nutrients in the harvest products, losses through erosion and leaching of basic cations (Murphy, 1959; Taye and Hofner, 1993) and lack of proper cultural practices, which attribute to the deficiency of nutrient and low soil pH. Under low soil pH condition, the major factors limiting the

growth and yield of apple fruit trees are aluminium and manganese toxicities, low availability of Ca, Mg, and some of the micronutrients, and low P availability due to high fixation by exchangeable aluminium and/ or oxides of iron and aluminium (Anandan *et al.*, 1984). Excessive Mn concentrations in plant tissues can alter various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Ducic and Polle, 2005; Lei *et al*, 2007). Both aluminium and Mn under lower soil pH condition, affect the growth of apple rootstocks and cultivars mainly by disrupting the uptake of essential nutrients such as, calcium and magnesium. Although, the influence of these nutrients in acidic soil conditions affect the growth and yield of apple rootstocks and cultivars, much attention was not given in studying their tolerance to low soil pH. There exists a wide variety of findings on Al toxicity and tolerance mechanisms in agricultural plants under acidic soil conditions (Raese, 1992). But comprehensive data relating to mechanisms of tolerance to low pH and aluminium ions by fruit-bearing plants are lacking.

According to Mesfin (2007) the coverage of acid soil in Ethiopia is close to 40% of the landmass and from this 15.6% is strongly acidic. In addition to the previously mentioned causes of soil acidity, the type of parent materials from which the soils are formed and continuous use of acid forming fertilizers such as urea and DAP (Cooke, 1982) could also decrease pH of the soil. Traditional practices, continuous removal of basic cations through crop harvest could further enhance the depletion of essential nutrients and contribute to increase in soil acidity. In southern Ethiopia, particularly in Chenchu high lands, farmers practice this traditional farming, which includes removal of crops by totally pulling out of the soil without leaving any residues, for years. Basic cations responsible for counteracting the

acidity are lost with the residues, and hence traditional farming system results in soil degradation, reduced fertility and soil acidification.

Soil acidity is therefore an increasing concern of crop production in most high lands of Ethiopia such as Chench. The increase in income and profits from apple were strongly correlated with fruit size, together with shape and colour, as these are the most important fruit quality characteristics to consumers (Flavio and Marco, 2006; Spohn, 2011). However, fruits produced in this area lack these standard qualities due to several reasons including, insufficiency of chilling, poor management, pests and diseases, and low soil pH. Among these, soil acidity was considered to be the major one (Tromp *et al.*, 2005). Soil acidity plays a vital role in apple production and integrated corrective measures should be taken to minimize its impact on apple fruits. Thus, research undertakings on the growth and productivity of apple rootstocks and cultivars under such acidic soil need more attention. Apple production was practiced for many years under acidic soil condition (pH 4.9) of Chench. However, there was no study on the impact of this acidic soil on growth and yield of apple rootstocks and cultivars in the area. Besides, data were not available on growth and yield responses of the rootstocks and cultivars to different soil acidity levels. And the degree of tolerance of apple rootstocks and cultivars to toxic minerals, uptake and utilization of essential elements were also not documented. Thus, the present study was initiated to identify the effect of low soil pH on growth and yield of apple rootstocks and cultivars. Specifically the study was aimed to address the effect of naturally occurring acidic soil of the area, and two other pH treatments below and above the soil pH of study site, on growth and yield of apple rootstocks and cultivars.

1.1. Justification of Study

In the highly degraded study area of Gamo Gofa Zone Chench, Southern Ethiopia, nowadays, apple is regarded as a food security fruit. Farmers practice to grow apple rootstocks of different kinds for sale, and cultivars that bear fruits in their garden significantly contributes for improving the stability of the food supply. This is because of the fact that, the high cost of apple rootstocks and apple fruits enhances their income and able farmers' to fulfil the basic necessary needs of their family. Apple rootstocks and apple fruits primary strategic importance can be considered as its role in income generating, and helps to prevent food shortage. Farmers can buy all the necessary needs from elsewhere and can survive famine when other food crops fail. Because of this, apple rootstocks and cultivars have great economic importance in contributing the food security of the farmers.

On the other hand, in the areas where there is shortage of farmlands due to soil degradation or over population, apple growing can be an alternative. Apple is one of the few fruits that can tolerate long-term storage without significant loss of quality, adaptability to different climates and can be grown in any types of soil conditions (Ryugo, 1988). The apple tree within a small area of home garden if well managed; produces higher yield and income relative to other cereals for households facing a shortage of land. It is also possible for the farmers to cultivate cereal crops and vegetables as mixed farming with apple cultivars. The cultivars drop off their leaves and enter their dormancy period while the underneath crops and vegetables were grown

during July to September under Ethiopian condition. Thus, it can help farmers as a safe guard for ensuring its food security.

Despite its desirable attributes, apple growing received very little research attention as compared to that given to other cereal based system in Ethiopia. Although, apple growing began in the early 1950's, in Ethiopia by Chenchä *Kalehiwot* protestant church, its spread to wider area of the country is limited. Due to this, research undertaking on apple productivity was not much known. Kebede (unpublished data) made a study on "Comparison of growth and yield of apple (*Malus domestica* Borkh.) tree cultivars at three altitudes in Gamo Gofa Zone, South Ethiopia". In this study on seven apple tree varieties he identified cultivars that have higher yield, in relation to altitude. Apart from this, there is no information with regard to impact of naturally occurring acidic soil of Chenchä (pH 4.9) on widely used apple rootstocks and cultivars growth, and yield in the area where farmers used to grow them for many years.

Although vigour's rootstock, a key source for high apple fruit yield in the high lands of Ethiopia where the soil pH is mostly acidic is crucial for the sustainable productivity of apple fruits, this also remained far from scientific investigation. There are no research data available as a base to use for advising farmers on the degree of tolerance of apple rootstocks that should be used for grafting in the area where the soil pH is a serious issue. Furthermore, information on the effect of scion/ rootstock and low soil pH interaction on growth, flowering, fruit set, and nutrient accumulation in the leaves is scarce despite the effect of strongly acidic soil on

the growth and yield of apple cultivation. The study therefore, stems from this understanding and was conducted to address the following hypothesis and objectives.

1.2. Research hypothesis

The problem of this study is to evaluate the effect of naturally occurring acidic soil of Chenchu on growth and yield of apple rootstocks and cultivars, and determines the difference in tolerance level of selected apple rootstocks and cultivars to soil pH of 3.5, 4.9 and 6.3, in the study site.

The hypothesis of this study is that, difference in soil acidity does not affect growth and yield of apple rootstocks and cultivars.

1.3. Objectives of the study

1.3.1. General objective: The main aim of this study is to evaluate the effect of different levels of soil acidity on growth and yield of selected apple rootstocks and cultivars, in Chenchu.

1.3.2. Specific objectives:

Four specific objectives have been identified that lead to a logical progression through the thesis:

- 1) To evaluate the effect of low pH on soil elemental status,

- 2) To investigate the vegetative growth of apple rootstocks as influenced by soil pH,
- 3) To evaluate effect of rootstock type and low soil pH on growth and yield of 'Anna' and 'Jonagored' cultivars, and
- 4) To examine effect of rootstock type and low soil pH on concentration of minerals in leaves of 'Jonagored' and 'Anna' cultivars.

CHAPTER TWO

2. LITERATURE REVIEW

Apple rootstocks and cultivar fruits are the most popular commercially productive trees, providing small farmers with a valuable source of income, and at the same time offering an economical solution to the Chenchu degraded environment. The soil of this study area was clay loam with pH of 4.9. Apple trees prefer an acidic soil with a pH range of 5.5 to 6.8 (Tromp *et al.*, 2005). It is well known that apple trees do not grow well at low soil pH levels which may cause some nutrients to be unavailable, and others become soluble and released in excess to apple trees.

2.1. Origin and spread of the domesticated apple

The cultivated apple is likely the result of interspecific hybridization and at present the binomial *Malus ×domestica* has been generally accepted as the appropriate scientific name (Korban and Skirvin, 1994). The main ancestor of apple is now considered to be *Malus sieversii*, which is wild from the Heavenly Mountains (Tien Shan) to the edge of the Caspian Sea (Morgan and Richards, 1993). This species is diverse and wild trees bearing the full range of forms, colors, and tastes are found in Kazakhstan and other independent countries of Central Asia formed from the breakup of the Soviet Union and especially around Alma Ata (Father of Apples). This is the area of greatest diversity and the centre of origin.

Morgan and Richards (2002) reported that, cultivation of apple has been practiced by Greeks and Romans, their travels and invasions spread cultivars throughout Europe and Asia. Later cultivation was concentrated around the medieval monasteries. Cultivars were selected and propagated in very early times, for grafting was known at least 2000 years ago. During the late 19th and 20th centuries, *Malus domestica* cultivars were grown or bred in Europe, Russia, and North America, and were introduced throughout the world and forming the basis for most current commercial apple production (Way *et al.*, 1990; Janick *et al.*, 1996).

On the other hand, until the latter half of the twentieth century most of the world's apple cultivars were chance seedlings selected by fruit growers (Khanizadeh and Cousineau, 1998). Thus it seems that, apples were probably improved through selection over period of thousands of years. As mentioned in Dick *et al.* (1986) apples were brought to North America with Columbus in the 1490's and missionaries during the 1800's. Furthermore, in the mid 1900's, irrigation projects in Washington State began and allowed the development of the multibillion-dollar fruit industry of which apple was the leading fruit. Dick *et al.* (1986) indicated that, through selection and research, low chilling requirement cultivars were developed. The development of these new tropical cultivars of low chilling requirement was opening the possibilities of growing apple across African countries.

Apples were first brought to South Africa by the Dutchman Jan Van Riebeeck in 1654 from St. Helena (Morgan and Richards, 2002). As Westwood (1993) described, some of these apple cultivars with low chilling requirements are thought to have derived from North America. Known cultivars such as Anna, Jonagold, Golden Delicious, Red Delicious and Granny Smith

were all chance seedlings (Bultitude, 1989; Westwood, 1993). Gala and Crispin (Mutsu) are the result of breeding experiment (Bultitude, 1989). In countries with lower latitudes the cultivars grow at relatively higher elevation. The favorable altitudinal range are areas with 1300 m and above (Rice *et al.*, 1990) or between 1000 and 3000 masl (Westwood, 1993). Godfrey and Berke Tsehai Tuku (1986) earlier reported that cultivars of deciduous fruit crops imported and planted were believed to have low chilling requirements, of these cultivars were only partially satisfied.

Ethiopia is a country found in the lower latitude (about 5⁰) that possess higher elevations above 3000 m, diversity of physical factors such as, humidity, rainfall, and cloud cover or fog which are important in choosing the site for growing of different cultivars. The cultivars introduced were those with low chilling requirements. In Ethiopia the cold and heavy rainy months are few comparative to more months of sunshine. Cultivars grown in the country were adapted to this climatic condition and become productive. Among different cultivars ‘Anna’ and ‘Jonagored’ are also those apple fruits that were adapted to low duration of cold months, mid of June to mid of September and bear fruits in the longer seasons of sunshine.

2.2. Ecology of apple trees

Apple tree grows at elevation of 1300 m and above altitude and with the temperature range of 5-10°C (Rice *et al.*, 1990). In agreement with this Westwood (1993) stated that, temperate fruits that are grown in lower latitudes based on the area and cultivar require a minimum elevation at which adequate chilling occurs, usually between 1000 and 3000 m. Ethiopia is

located in eastern Africa between 30 and 150 North latitude and 330 and 480 East longitude with a land area of 1.223 million km² and occupies a huge land mass of Africa. It is a country of great altitudinal diversity ranging from lowlands as low as 116 meters below sea level to highlands more than 4620 meters above sea levels. Although the country is found in the tropics, owing to the moderating influence of high altitude, the central, south and south western highlands generally enjoy a temperate and pleasant climate with average daily temperatures rarely exceeding 20⁰C (Admasu Tsegaye, 2002). Most of the highlands have rainfall in the range of 600 to 2700 mm (Balesh Tulema, 2005).

Apple trees grow and bear good fruits in loamy and sandy loam soils, as well as in sandy soils with the addition of organic fertilizations (Doronina and Terekhina, 2009). In addition to this the same author explained that, depression of growth and development is observed in saline, swampy and peaty soils. Others (Tromp *et al.*, 2005) reported that, very arid areas of the world are also largely unsuitable for temperate fruit production, unless abundant supplies of good, non-saline water can be acquired for irrigation purposes. The same authors further stated that, areas with very heavy rainfall are also unsuited to tree fruit production on account of the increased incidence of many damaging disease and the effects of the rainfall on the soil structure and aeration.

In Ethiopia, the domestic apple (*Malus domestica* Borkh.) tree cultivars were first grown in the South Western plateaus of Gamo Gofa zone, Chenchha district with clay soil type and altitude of 2700 masl. Its local temperature zones are *dega* with mean annual temperature of 9⁰ to 15.5⁰C (GGZAO, 1990). These fruit producing cultivars are grown on the highlands of

the study area that are prone to degradation and infertility with an altitudinal range of 2000 to 3200 masl and above where the temperature is cold at winter and sunny and hot at summer. Apple cultivars dispose several ecological significances, while possessing huge economic potentials to buffer and improve the economy of the communities living there. Thus, apple tree cultivars expansion improves soil conservation by reducing soil erosion there by stabilizing soils. On the other hand, the non-destructive harvest of apple tree fruits is also used for conservation of the cultivars and protection of the vast marginal degraded fragmented of highland ecosystems.

2.3. Significant of apple growing in Ethiopian economy

Ethiopia is predominantly an agrarian country and agricultural production plays an important role in Ethiopian economy. About 80-85% of the population is dependent on agriculture for its livelihood. The sector contributes for about 54 % of the gross domestic product (GDP) and about 90 % of foreign currency earnings (CSA, 2000; Eyasu Elias, 2003). The agricultural sector therefore, forms the basis for the overall socio-economic development of the country. However, agricultural production in Ethiopia still remains to be predominantly traditional. Agricultural practice had been and still struggled with adverse soil and climatic conditions. Similar to other farming systems apple growers also employ an age old poorly developed farming practices. Even though the cultivation of apple rootstocks and cultivars were not long, due to apple rootstocks and fruits high source of income , studies conducted in this sector that promote the growth and yield of cultivars should need more attention. As such, the country's agriculture is still far from playing its strategic role in the overall economic development of the country; mainly due to some reasons, among which the poor

performance of Ethiopian agriculture and poor soil fertility were considered to be the major factors in addition to, low variable rainfall, and soil acidity. However, recent agricultural practices exercised to enhance agricultural yield should also be focused in the cultivation of apple cultivars that produce more yield and quality fruits.

Apple fruits are cultivated widely in temperate latitudes or at high elevation in the tropics on all continents except Antarctica (Way *et al.*, 1990). The fruits are popular and have high economic values in most developed countries because of the many ways that they can be consumed, their medicinal values, convenience and durability (Hempson and Kemp, 2003). The tree fruit industry in British Columbia's (BC) interior represents more than 800 growers operating orchards that generate \$130 million in wholesale revenue, contributing \$900 million in economic activity, and directly employing 1,500 people at the grower, packer, and processor levels. In addition, the industry provides economic spin-off to partner industries such as the transportation sector, food processing and retail environments. Apples comprise approximately 80% of the total BC tree fruit production, with 98% of this grown in the Okanagan Valley (BC Fruit Growers Association, 2010). Thus, apple cultivation provides employment to workers in the farming, transportation, marketing, delivery industries as well as wholesale and retail sales outlets. Due to this and its other economic supremacy over other fruits, apples are widely grown in many countries such as China, USA, Japan, Australia, and others. According to Westwood (1993), a lot of research in most of the developed countries focuses on producing improved rootstocks and cultivars that have better performance of yield, resistance to climatic and soil conditions, frost or diseases. On the other hand, commercial apple production is increasingly concentrated in countries and in growing districts that have a

strong comparative advantage in apple production and marketing such as China and USA as a leading production countries (O'rourke, 1994). Recently, China dominating global apple production, was producing 52.3 % of the world's apple fruit and USA, the third apple producing accounted only for 7.2 % of worldwide produced apple fruit (Ballard *et al.*, 2011). Thus, apple trees are adapted to different climates. They are cultivated from Northern Europe down to the tropics. Most of the old cultivars require a long rest period, but new selections with fewer chilling requirements allow them to be cultivated in subtropical areas.

Apple had been introduced to Chench, a district (*wereda*) in the Gamo Gofa administrative zone of the southern region of Ethiopia, has nearly 50 years of experience in growing highland fruits including apple, pears and plums. The area is conducive to producing more than 100 varieties of apples among which some are Anna, 'Jonagored' , Granny Smith, Gala, Crispin and Princessa serving as a resource base for the rest of the country. Current apple fruit production in Chench is about 15 metric tonnes per year while the overall country production is estimated to be about 50 metric tonnes. Since the total production does not meet the demand, the country imports about 350 metric tonnes of apple fruits mainly from South Africa, Iran, China and Israel (HARC, 2010). There is therefore, an unmet market demand for the Chench apple. Apples of accepted size (medium and large) are sold to the Ethiopian Fruit and Vegetable Marketing Share Company (Etfruit) at 5.5 and 6.5 dollar/kg in 2012, according to size. In 2012, about 30% of the 15,000 kg total apple production in the Chench area met the standard market size classifications and could be sold to the company. The remaining 70% were sold to local traders and individual consumers at 2-3 dollar/kg. Etfruit sells the fruit through its retail channels with profit margins of about 42 and 43% respectively (HARC,

2010). The market price of imported apples ranges between 3 and 4 dollar/kg. If production is increased and marketability improved through on-farm technical assistance focusing on fruit tree expansion of selected rootstocks, and on the production of selected grafted seedling cultivars that produce quality fruit, apples could generate a substantial income for the farmers of our country and contribute significant role for development of Ethiopian economy.

Farmers in Chenchu currently, have practiced to grow these cultivars widely and benefit from its yield due to the high cost of apple rootstocks, and fruits. Apple rootstocks and fruits income is growing and accounts for the majority of Chenchu apple growers' income. At present, mixing mostly with small quantities of a wide range of crops such as cereals, cabbage, tubers, spices on holding usually less than one hectare per household also are produced apple fruits. Some Ethiopian farmers are separately producing apple fruits at their home garden. Nowadays, apple production is expanding in different areas of the country other than southern Ethiopia including North Shewa (Degem, Hedabu, Abote, and Alidoro and Dedre Birhan), Gurage, Dabat, etc. (HARC, 2010). Thus, these promising fruits become the source of income and help to overcome difficulties of some farmers that practiced apple cultivar growing in feeding their households and improve their livelihoods.

2.4. Rootstocks for Apple Cultivars

Rootstocks are the interface between the scion and the soil, providing anchorage, water, nutrients, and disease protection that ultimately affect the productivity and sustainability of the orchard (Gennaro *et al.*, 2012). A variety of rootstocks were used for the same scion species because they impart different properties (Castle, 1987). On the other hand, rootstocks can be

selected for traits such as dwarfism, early bearing, disease resistance, and winter hardiness. It has been reported that, more than twenty horticultural characteristics are affected by the rootstocks including leaf nutrient status, vigor and size, depth of rooting, low temperature tolerance, adoption to adverse soil conditions including resistance to soil acidity and drought, disease resistance and fruit quality (Castle, 1987; Tromp *et al.*, 2005). The scions of apple cultivars are propagated by grafting buds or shoots onto the rootstocks. Therefore, the root system is genetically different from the above-ground portion of the apple tree (Lorraine *et al.*, 2012). Apple trees grown from seed will not grow into the same variety from which the seed originated, but will be a new and unique type of apple that may or may not be good (Lorraine *et al.*, 2012).

2.4.1. Rootstock and its influence on vigor

The rootstock has far more effect on the ultimate size of the tree than the scion cultivar (Tromp, 2005). Apple rootstocks vigor can be determined destructively by measuring the dry weight of whole tree over certain period. However, a simple and reliable alternative is to measure trunk cross sectional area (TCSA) which has good relationship with tree size and weight when the trees are dormant (Tromp, 2005). According to Smith (1975), rootstocks greatly affect the vigor, productivity of plants and quality of the fruit by their differences in absorption and translocation of mineral elements from soil which ultimately affect the overall performance of the plants. In agreement with this, Roy and George (1987) revealed that, rootstocks profoundly affect water and nutrients uptake from the soil especially nitrogen which is of prime importance as it affects the photosynthetic rate, production of carbohydrates

and proteins, which in turn control plant growth and development. Antonio and Carlos (2009) showed the influence of the rootstocks on N content in the petioles was significant. In addition to this, it affects phosphorous and potassium absorption that has a key role in maintaining fruit quality.

A number of studies have reported that, different rootstocks induce clear differences in growth vigour and other features of scion cultivars (Wertheim, 1998), influences aspects of fruit quality such as ripening and storability (Lord *et al.*, 1985), mineral composition (Drake *et al.*, 1988) and size (Marini *et al.*, 2002). However, the effects of most of the rootstocks studied were not consistent from site to site and varied overtime (Yahya *et al.*, 2004). Thus, apple cultivars obtain a right balance of nitrogen to phosphorous based on the types of rootstocks which are directly related to nutrient and water use efficacy of the cultivars (Roy and George, 1987; Taiz and Eduardo, 2002). Rootstocks mainly determine the size of the tree, the training system to be applied, the quality and quantity of the produced fruit as well as its adaptability in the interaction with the scion (Webster, 1995). Thus, rootstock selection is one of the most important decisions in apple management.

Besides, researchers stated that, rootstock is one of the most important biotic factors influencing fruit quality (Skrzynski and Gastol, 2007). Similarly, rootstocks also may have important effects on the performance of scion in apple, including precocity, photosynthetic rate and nutrient uptake (Fallahi *et al.*, 2001). The interaction of the rootstock with the soil has also been expressed through the ability of its root system to develop and to extract adequate nutrients and water from the soil. This can be considered the source of the vigour of a

rootstock and vigour differences conferred to the scion, are often more apparent in poor soils. Soils low in soil moisture or soil available nutrients are often better exploited by rootstocks known to impart vigorous growth to scions. The vigour of rootstock/scion combinations can also be influenced by other factors, particularly rootstock compatibility to the acidic soil environment, seasonal soil moisture levels, soil fertility, irrigation and compost / manure application. A rootstock that confers high vigour to a scion may prolong the growth period of the shoots into the maturation period of fruit potentially delaying sugar accumulation due to the additional competition for assimilates (Craig, 2004). In addition to this, recent advances in the science of genetics/genomics of apple rootstocks show that at the molecular level different rootstocks have the ability to turn on/off or increase/decrease the production of proteins in the scion (trunk, leaves and even fruit) (Gennaro *et al.*, 2012).

2.4.2. The clonal rootstocks for apple cultivars

Grafting of fruit cultivars upon clonal rootstocks has a long tradition, but until well into the 12th century there was little sound knowledge on this issue (Wertheim, 1998). According to (Westwood, 1993 and Esmaeil *et al.*, 2002), in 1912 researchers at the East Malling research station at Kent, England began to gather dwarf and semi-dwarfing rootstocks named 'Paradise' and 'Doucin' respectively from around the world. They tested and characterized the Paradise stocks of Europe, some of which had been used for centuries and introduced these dwarfing stocks as Malling (M) series in 1917, each clone with Roman numerical such as, MI, MIV, and MIX. Recently the numbers were preceded by the letter M from Malling. Later, the

Roman figures were replaced by Arabic ones and thus become M.9, M.27, M.26, and so on (Wertheim, 1998).

2. 4.3. Apple rootstocks breeding and selection

The next logical step was ‘to make’ improvements through breeding and again England took the lead (Wertheim, 1998). A first aim was to find rootstocks resistant to woolly apple aphid (*Eriosoma lanigerum*), a noxious insect on apple roots in many countries. Collaborated efforts between East Malling and the John Innes Institute at Merton, started in 1922 and intensified in 1928, leading to the ‘Merton Immune’ or MI-series (MI 778 to 793) in the 1930s and the ‘Malling-Merton’ (MM.101 to 115) in 1950s (Wertheim, 1998). The author stated all were resistant to the root pest mentioned; a resistance based upon one of the crossing parents ‘Northern Spy’, itself a precocious, semi dwarfing rootstock. Thus, the size controlling Malling Merton (MM) rootstock series with their desired characteristics of both parents are incorporated into the MM series which are used to control size, resistance to woolly apple aphids, improved anchorage, scion compatibility, and precocity. The cultivar of stocks tested which are important for their character of resistance, vigor then designated as MM.106, MM.111, MM.104, and so on (Wertheim, 1998; Esmacil *et al.*, 2002).

It has been reported that, conventional breeding is still the main method used to obtain new rootstocks. However, from making the initial cross between chosen parents and releasing anew rootstock, usually takes on average 30 years and it is, therefore an extremely expensive procedure (Tromp *et al.*, 2005). They further revealed that, following the breeding and initial

selection of a new rootstock, its field evaluation is a time consuming process, taking between 8 and 15 years for dwarfing and invigorating rootstocks, respectively. An added complication is that, there are often significant interactions between location, cultivar and rootstock performance. A reason for location effect on rootstock performance might be that, some rootstocks are more sensitive than others to a biotic stress factors associated with the prevailing climatic or soil conditions. Thus, for reliable information on rootstock performance in a certain area, evaluation should be carried out regionally and separately for relevant soil types and cultivars (Wertheim, 1998; Tromp *et al.*, 2005).

2.4.4. Types of apple rootstocks

Rootstocks are of different types depending on their multiplication methods or raising methods. Seedling rootstocks are those developed from germinated seeds and are not genetically uniform (UWECE, 2001). The advantages of production of seedlings are their relatively simple and economical and well adapted to mass propagation methods. The others are clonal rootstocks, which are propagated vegetatively. Each clonal individual plant is genetically the same as all the other plants in the clone and can be expected to have identical growth characteristics in a given environment (Hartmann *et al.*, 2002). Unlike seedling rootstocks clonal rootstocks propagation by vegetative means avoids genetic variations resulting in true to type progenies (Wertheim, 1998). Rootstocks that are used for grafting are classified according to tree size as dwarf, semi-dwarf, and semi-vigorous and how they are propagated. Among other type's apple rootstocks M.9, MM.106, and MM.111 have

been well known and used in various types of soils and plantation systems (Tromp *et al.*, 2005).

Each type of fruits has its own particular rootstocks. In general it has been known that the rootstock must be of the same genus as the scion in order to produce successful graft (UWECE, 2001). However, among the grafted rootstocks, there could be performance differences due to scion/ rootstock interaction, soil and climatic conditions or vigour differences induced by rootstocks which will be manifested, irrespective of the environmental conditions. The types of apple rootstocks used for our study are MM.111, MM.106, and. M9. These rootstocks are widely used by apple growers of study area for many years.

2.4.4.1. MM.111

This rootstock was produced by crossing Northern Spy with Merton 793 in the 1920s. MM.111 rootstock is a semi- vigorous, and is quite capable of supporting itself without staking. It produces large numbers of very fine roots with good anchoring capability. The large numbers of fine roots give a large surface area and able this rootstock for mopping up water and nutrients in the soil. It has been reported that, MM.111 is greatly valued for its ability to grow in poor soils, drought tolerant, bears fruits early and also shows good resistance to woolly apple aphids as compared to other vigorous stocks (Wertheim, 1998; John, 2011).

2.4.4. 2. MM.106

According to Wertheim (1998) the most popular of the MM series is MM.106 (Northern Spy x M.1). It was developed in collaboration by the East Malling and Merton research stations in the early 20th century. It is a semi-vigorous rootstock resistant to wooly apple aphid, absence of root suckers, and good anchorage which needs no staking. However, fruit size and quality is generally less than M.9 (Wertheim, 1998). The author further stated that, MM.106 is a good rootstock, but rather vigorous and unsuitable for poorly drained soils. Although semi-dwarfing MM.106 causes high yield efficiency and fruit size can be smaller than M.9 and is more productive with early cropping (Ferree & Warrington, 2003).

2.4.4.3. M.9

This is the most commonly used dwarf rootstocks throughout the world both for one-union trees, producing a tree 25-35% the size of seedling, and for inter stem trees (Wertheim, 1998). It was selected as a chance seedling in 1879 in France. Under normal culture the shoots of M.9 are thick and stout, slightly curving to one side at the base in stool beds, with reddish bark, slightly pubescent, and also forming small nodules at each side of bud nodes. M.9 roots fair well under favourable stool bed culture, but the roots are brittle and break easily, so trees on M.9 need to be supported. M.9 tends to sucker from the roots, which is a special problem due to susceptibility of this stock to fire blight (Wertheim, 1998). M.9 was one of the first standardized rootstocks released by East Malling Research Station in the 1920s, with a lineage going back to the 'Paradise' rootstocks used for many centuries in Europe. M.9 is probably the

most widely-planted of all rootstocks, and the core of commercial apple production. There are a very large number of clones of M.9, with varying qualities and vigors.

According to Mohammad *et al.* (2012), M.9 was introduced as a dwarf that induces excellent yield, precocity, efficiency and large fruit size. Because of its lack of vigor, it should only be used where the soil is fertile and never where there is any competition from grass or weeds. Cultivars on M.9 are very productive and do not produce strong roots and whatever shape is grown, it must be strongly supported by a stake. Such trees are seldom over nine feet tall when mature and usually start bearing within the first or second year of planting (Pretson, 1955).

2.5. Description of Apple Cultivars

2. 5.1. Biology and morphology

According to Doronina and Terekhina (2009), the height of apple cultivars ranges from 3-6 to 10-14 m tall. Diameter of the trunk reaches 90 cm. Crown wide branching, sometimes spherical or ovoid. Shoots remain pubescent for a long time. Buds are ovoid-conical. Leaves are 5-10 cm long, ovoid, with pointed peak and rounded or slightly heart-shaped base, crenate-serrate, pubescent. Leafstalk is no more than 1/3 of the blade length. Chromosome number: $2n=34, 51, 68$ (Doronina and Terekhina, 2009).

2. 5.1.1. Floral Biology

Jules *et al.* (1996), described that, the flowers of apple cultivars and seedlings vary considerably in size and petal shape, and in color from white to deep pink. The petals are white when open, but have red-pink undersides when opening, hence the "pink" bloom stage. The ovary is inferior, embedded in the floral cup or hypanthium, containing 5 locules, usually 2 ovules per locule. The inflorescence is a cyme of 4-6 flowers, with the center flower opening first; the central flower is often called the "King bloom", and has the potential to produce a larger fruit than other flowers. Flowers are produced terminally from mixed buds (containing both leaves and flowers) on spurs, or to a lesser extent on long shoots (Jules *et al.*, 1996). Spurs form on 1- 2-year-old and older wood, and generally grow only a fraction of an inch each year. In Ethiopia, where the rainy season is June to August flowering is mostly expected from October to December. The uniformity of fruit set and maturity and harvest may differ from area to area depending on favorable climatic condition in Ethiopia. However, most cultivars fruit set and maturity is from end of December to March, some let fruit yield may extend to June.

2. 5.1.2. Fruit Biology

Apple is named pome fruit with thick hypanthium which is tissue connecting sepals, petals and stamens; enlarges to become the part we eat. The seed-bearing ovary (core) is surrounded by a thick, fleshy hypanthium that is not part of the pericarp. It derived from the fusion of the bases of the perianth segments (petals and sepals). Some references state that the hypanthium may also contain tissue from the receptacle (Hickey and King, 2001). In the pome, the

thickened, fleshy hypanthium is fused with the ovary wall or core. The outer skin of an apple is the multi serrate epidermis of the hypanthium surrounded by a waxy cuticle. Depending on the variety, the skin contains red anthocyanin pigments. The skin color of apples ranges from red to yellow to green. Fruits have sour, sour-sweet or sweet taste. The seeds are contained in chamber in the center of fruit and are relatively small and black, and mildly poisonous. Apple is crispy and juicy fruit. Its size can vary from 2 to 4 inches (Esau, 1977). Since the fruit contains tissue not derived from the pericarp, it is called an accessory fruit. This is the typical fruit of certain members of the rose family (Rosaceae), including apple, pear, quince and loquat.

Apple fruit is reported to be composed of active substances which are approximately 300 bio substances such as organic acids, tannins, pectin, essential oils, vitamin C, vitamin B, potassium, calcium, phosphorous, iron, and sodium. Researchers reported effects in such a way that, "An apple a day keeps the doctor away" - the old saying still holds true. The apple is reported to lower cholesterol, be good for digestion, and protect against colon cancer. It can also lower blood pressure, benefit nerves and muscles, and lower the risk of cardiovascular disease (Hessayon, 2000).

2. 5. 2. Description of certain introduced Apple cultivars in Chench

Terence (2011) study indicated that, since the mid 1980's a series of new cultivars have been introduced to the world. Among the new cultivars produced, some cultivars were quickly discarded but others such as Anna, Granny Smith, Gala, Jonagold, 'Jonagored' , Empire, Fuji and Pink Lady have become important world varieties. Most of these cultivars were introduced to Chench apple growing region about 40 to 50 years ago. It has been reported that, there are more than 7,500 known cultivars of apples in the world (Elzebroek & Wind, 2008). These cultivars vary in their yield and the ultimate size of the tree, even when grown on the same rootstock. Commercially popular apple cultivars are soft but crisp. Other desired qualities in modern commercial apple breeding are a colourful skin, absence of russeting, ease of shipping, lengthy storage ability, high yields, disease resistance, typical 'Red Delicious' apple shape, and popular flavour. Modern apples are generally sweeter than older cultivars, as popular tastes in apples have varied over time. In our study, among the others we used apple cultivars 'Anna' and 'Jonagored' that have attractive fruit shape and colour with good sweet taste and early fruiting.

2. 5. 2.1. 'Anna' (Apple Chinago)

'Anna' apple cultivar is an Israeli selection with a remarkably low chill requirement (only 200-300 chill hours) by Mr. Abba Stein and was commercially planted in 1850 (Baker, 2000). It is large and has light greenish-yellow skin with a slight red blush. The 'Anna' apple fruit is sweet, slightly tart, crisp with a creamy white flesh. 'Anna' apples are good for fresh eating, apple sauce, or homemade pies. The cultivar produce at an early age and the fruit stores very

well. Pollinator required, and it also has 60% self-fertility (Spohn, 2011). Among the introduced cultivars ‘Anna’ was the most adapted which perform best in the Wet, and Moist ‘*Dega*’ and ‘*Woyna Dega*’, 1900 to 2800 masl. Kebede (unpublished) observed that, the productivity of ‘Anna’ was more in areas below 2700masl with more in 2200masl.

2. 5. 2. 2. ‘Jonagored’

The apple cultivar ‘Jonagored’ naming was related with Jonagold. Jonagold was bred in 1943 in Geneva, New York, and released in 1968. It got its name because it was a cross between Jonathan and Golden Delicious, and so the first four letters of each word were combined to give the new name. Jonathan was once a very popular apple with really good flavour, but unfortunately it could get very soft, and over the years, possibly as people’s teeth improved, the Jonathan became less popular. In an attempt to make a firmer Jonathan, it was crossed with Golden Delicious (an apple which is harder), and the result was Jonagold. Everyone agreed that this was a good daughter, having inherited the flavour from its mother (Jonathan) and firm texture from its father (Golden Delicious). Jonagold became very popular in Ireland, because it does well in cold climates, and it was widely planted in the early 1980’s. However, it has now been replaced with a better colored version called ‘Jonagored’ (Morren, 1987).

‘Jonagored’ was discovered in 1980 by Mr. Morren in Belgium, and he began propagating them in 1981. The original ‘Jonagored’ arose by accident, with one branch on a Jonagold tree giving really red fruits, and this branch was then propagated to give more of the same. The original ‘Jonagored’ tree, which is still alive and well, and it is amazing to see the

faintly colored Jonagold apples on part of the tree, and the bright red ‘Jonagored’ apples on the other part (Morren, 1987). ‘Jonagored’ apples are available from the Celtic Orchards growers from November onwards.

‘Jonagored’ is a red Jonagold variety which was discovered in Belgium towards the beginning of the 1980s. It is very widespread in Northern Germany, as colours are more optimal in this climate than those of the standard Jonagold. Winter chilling requirements for most varieties are 500 to 1000 hr. below 45° F (7° C). There are relatively few varieties among which ‘Jonagored’ are adapted to mild winter climates (chill hours below 300) (Morren, 1987).

2.6. Cultivation and Management of Apple Cultivars

The growth and productivity of the apple tree cultivars is primarily based on the knowledge of the grower to decide on geographical area (climate) and soil condition that best suits with the cultivars. Moreover, during nursery propagation the grower has to know those rootstocks (suckers) that are compatible with the scion. Incompatibility resulted in yield failure and susceptibility to disease. According to Rob and Peter (2009) there are potentially a range of factors affecting the level of compatibility between rootstock and scion, the most obvious being the anatomy and morphology of the graft union itself. A successful graft union is one where xylem and phloem integrity is maintained between rootstock and scion with no adverse impacts on upward flow in the xylem of water, mineral elements and root to-shoot signaling compounds, or downward flow in phloem of carbohydrates or other essential factors for root

function (Rob and Peter, 2009). In addition to this, proper orchard management is required regardless of rootstocks selected. Some of these were, site selection, mulching and manuring, pruning and thinning, and method of preventing or controlling apple diseases and pests.

Flower and fruit thinning of apple is an important practice used to regulate crop load and improve fruit quality at harvest (Milić *et al.*, 2011). Reducing the number of fruits per tree increases the relative amount of leaf areas per fruit, and hence the availability of photo-assimilates for the remaining fruit. According to Meland (2009) most apple cultivars have an over-abundant flower density and percentage fruit set. Production practices to regulate the numbers of flowers and fruits are therefore necessary to overcome alternate bearing and to enhance fruit quality. Removing of weak flowers by using machine as in developed fruit production system or mechanical shaking to drop off weak flowers or hand picking is essential to optimize fruit size. A low crop load decreased fruit yield, but improve fruit weight and fruit quality. Therefore, most producers attempt to increase fruit size by reducing the number of flowers and fruits on a tree. Large size fruits with good shape and colour are preferred by buyers. The management activities on thinning of flowers and fruits are the most essential practice that improves fruit quality (Milić *et al.*, 2011).

Apple trees grown under favourable conditions will set more fruit than they are capable of successfully carrying to maturity. According to Meland (2009), as fruit begins to grow, the tree produces a hormone that affects flower bud differentiation for fruit production the following year. The same author stated that, leaving too many fruits on the tree increases embryo production which triggers the release of a hormone that decreases the amount of

flower buds for next year. This makes apple trees alternate bearing (the first year they have lots of fruit and the next year, very little). Reducing the number of fruits per tree increases the relative amount of leaf areas per fruit, and hence the availability of photo-assimilates for the remaining fruit (Meland, 2009). Failure to remove excess fruit decreases formation of flower buds for the following year and causes trees to produce a crop only every other year (Lorraine *et al.* , 2012). Other previous study also reported that, return bloom was greater when trees were thinned early to a low crop load, because low crop loads gave larger fruit, higher soluble solid content (SSC) values, and more highly colored fruit (Meland, 2009).

This removal of flowers or fruit increase the ratio of leaf area to fruit, resulting in an increased amount of available assimilates supporting fruit growth (Palmer *et al.*, 1991). Early removal of fruit lets results in larger fruit size at harvest (Jones *et al.*, 1992). Thus, fruit thinning is considered as one of the most important cultural practice affecting fruit quality and cropping consistency (Basak, 1999).

2.7. Effect of Soil acidity on the Availability of Nutrients

The availability of plant nutrients is considerably affected by soil pH. Calcium, potassium, and magnesium are positively charged alkaline elements adsorbed on negatively charged particles of clay or humus fractions (Webster, 2005) which are lost with increasing acidity while, phosphorous is more available. Acidity can also enhance the solubility of minor elements which may be deficient due to low solubility in high pH includes aluminium, manganese and iron. Acid soils comprise up to 50% of the world's potentially arable land and thus are a significant limitation to fruit production worldwide (Von Uexküll and Mutert,

1995). On most acid soils, there are several limiting factors for plant growth, other than aluminum (Al), which includes toxic levels of manganese (Mn), iron (Fe), and deficiencies of some essential elements, such as phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), and some micronutrients (Hong *et al.*, 2006). The mineral nutrient availability to apple rootstocks and cultivars are dependent on the quantity of each nutrient in the soil, and its availability. Among the others, pH is an important chemical property of the soil due to its effect on the availability of nutrients and also to the activity of soil microorganisms. It has been reported that in the area with very low soil pH (< 5.0), cations of Al^{3+} , Fe^{2+} , and Mn^{2+} , normally bound to negatively charged soil colloids, become solubilized and are available for plant uptake (Tisdale *et al.*, 1993; Marschner, 1997; Tromp *et al.*, 2005; Alan *et al.*, 2009). Even though these micronutrient ions are only needed in trace amounts, they can reach phytotoxic levels if too highly concentrated (Tisdale *et al.*, 1993; Marschner, 1997; Tromp *et al.*, 2005). In contrast, the macronutrients (N, P, K, Ca, and Mg) are less available below pH 5.0, such that they can become deficient in the plant (Tisdale *et al.*, 1993; Marschner, 1997; Tromp *et al.*, 2005; Alan *et al.*, 2009).

On other study, low soil pH made K less available in such a way that it is fixed or exists in a form of non-exchangeable K. Since plants eventually obtain exchangeable K from this source, soil pH becomes an important factor in this regard (Grewal and Kanwar, 1976). Soil acidity can be a preexisting problem in highly weathered soils (due to leaching of exchangeable basic cations such as Ca^{+2} and Mg^{+2}), soils with low cation exchange capacity (CEC), and/or the presence of organic acids released by plant roots, microorganisms, and decomposing humus (Tisdale *et al.*, 1993; Marschner, 1997). For the healthy growth and sufficient yield of plants

the presence of proper balance of macro and micronutrients in the soil plays a significant role. However, these conditions were a severe problem for the plants that had been grown in the area where the soil pH is low. Other study reported that, in the soil with low pH value, nitrogen, phosphorus and potassium were tied up in the soil and not available for uptake of the plants (Tromp *et al.*, 2005).

Alan *et al.* (2009) reported that, the concentration of available N are less sensitive to pH than concentration of available phosphorous. They indicated that, under low soil pH < 6, P starts forming insoluble compounds with iron (Fe) and aluminum (Al), and at soil pH higher than 7.5, P starts forming insoluble compounds with calcium (Ca). The same researchers concluded that, in order for P to be available for plants, soil pH needs to be in the range of 6.0 and 7.5 (Alan *et al.*, 2009). Although soil potassium and nitrogen did not exhibit significant trends with changing pH, for a given soil, the availability of both potassium and nitrogen generally decreases with decreasing soil pH. Thus, soils with low pH cannot hold potassium and nitrogen very well, resulting in more leaching loss and poor response to potassium and nitrogen fertilizers.

2.7.1. Effect of Al and Mn toxicity on the Availability of Nutrients

In low soil pH, aluminum (Al) toxicity has been reported as a major agricultural problem, and has been intensively studied in several species of plants. The studies revealed that, due to solubility of aluminum in low soil pH, plant growth exhibited undeveloped root system, a variety of nutrient-deficiency symptoms, and consequently decrease in yield (Imran *et al.*, 2007). The main reason reported was that, Al interferes with uptake, transport and utilization

of essential nutrients including Ca, Mg, K, P, Fe, and Mn (Imran *et al.*, 2007). Researchers (Ryan *et al.*, 1993; Bolan *et al.*, 2003) revealed that the primary target of Al toxicity is the root apex which causes stunting of the primary root and inhibition of lateral root formation. Other study showed that, long-term exposure of plants to Al, in addition to inhibition of root growth further lead to P, K, Ca and Mg deficiencies (Haug and Vitorello, 1996). Thus, the ultimate consequence is reduced plant biomass.

The relationship between pH and dissolved manganese in the soil is similar to that just described for aluminium, except that manganese only increases 100 fold when the pH drops from 5.0 to 4.0 (Filiz and Meral , 2007). Toxic levels of manganese interfere with the normal growth processes of the above ground plant parts. This usually results in stunted, discoloured growth and poor yields. Similarly in more reduced pH values below 5.0, iron is also soluble in sufficient quantities to be toxic to the growth of some plants. Filiz and Meral (2007) stated that, under low soil pH, complex aluminum and iron hydroxy ions are tightly adsorbed on clays and displace the Ca and Mg. These ions block some of the negative charge sites of the colloid, reducing its cation ex- change capacity. As exchangeable calcium and magnesium are lost by leaching, the acidity of the soil gradually increases. These low levels of calcium and magnesium were known to cause stock vigor problems on apple cultivars. In humid regions, there is a direct correlation between the pH and the amounts of the two elements. When the pH of a mineral soil is low, large amounts of aluminum, iron, and manganese are soluble. In contrast to this, with the increase of pH level, precipitation takes place and the amounts of these ions in soil solution becomes less and less. At neutrality plants may suffer from lack of available manganese and iron (Fey *et al.*, 1978; Richard, 1998).

The interaction effect of the three apple rootstocks, cultivars and low soil pH treatments has been expressed through the ability of the rootstocks to develop and extract adequate nutrients from the low soil pH. The amount of nutrients accumulated in the vegetative organs of the three different apple rootstocks, and leaves of cultivars under study can be considered as the source of the vigour of a rootstock, and vigour differences conferred to the rootstocks, are often more apparent in different soil acidity. Thus, among the three different apple rootstocks studied, vigour's rootstocks may demonstrate vigorous growth and accumulate more nutrients in their tissues, and also in leaves of grafted cultivars. This ability to extract nutrients from the low soil pH may depend on differences of rootstocks to tolerate the influence of soil acidity.

The nutrient status in response to soil acidity was not much known in the literature for various apple rootstocks. In addition to this, there is insufficient information on nutrient deficiency ranges for apple rootstocks MM.106, MM.111, and M.9 and cultivars 'Anna' and 'Jonagored' that had been grown under different soil acidity treatments. Most of the literatures do not adequately explain the effects of soil acidity on the nutrient status of rootstocks and fruit trees organs. This also holds true for the three apple rootstocks and cultivars under study. Thus, in this study we investigated the accumulation of eight nutrients (Ca, Mg, Mn, P, N, Fe, Al, and K) in the root, leaf, and shoot, of the three apple rootstocks, and in leaves of two different cultivars grafted on these rootstocks.

2.7.2. Effect of Soil Cation Exchange Capacity (CEC) on availability of nutrients

The soil nutrient availability for apple rootstocks and cultivars grown under low soil pH treatments in a micro plot was also influenced by soil CEC. It has been known that, the CEC of a soil represents the total amount of exchangeable cations that the soil can adsorb (Michael, 2007). Soils having a high CEC were essential in apple tree plantation, as the soil would be able to hold more nutrients. In contrast, soils with low CEC affect the growth and yield of apple fruits not only by missing some important nutrients, but would also not be able to hold onto nutrients (Brady and Weil, 2002). So, even adding nutrients to a soil with a low CEC would not be very effective in improving fertility unless soil conditioning was applied that would help improve the CEC as well (Michael, 2007). Plants that grow in soils with a high CEC value do not have to spend as much energy looking for minerals and water and therefore are able to devote it towards growth.

However, a soil with a high CEC can also hold more of the acidic hydrogen cations (H^+). Soil particles and organic matter have negative charges on their surfaces. Mineral cations can adsorb to the negative surface charges or the inorganic and organic soil particles. Once adsorbed, these minerals are not easily lost when the soil is leached by water and they also provide a nutrient reserve available to plant roots. These minerals can then be replaced or exchanged by other cations (i.e., cation exchange) (Gruhn *et al.*, 2000).

Plant roots take up nutrients as either cations, which are positively charged (potassium, calcium, magnesium) or anions, which are negatively charged (nitrate, phosphate). Plants

absorb more cations than anions and each time a positively charged cation is absorbed by the plant it has been known that, they must excrete appositively charged hydrogen ion into the soil to maintain electrical balance. Accumulation of these hydrogen ions in the soil results in acidification (Gruhn *et al.*, 2000).

2.8. Effect of Rootstocks on Vegetative growth, Flowering, and Fruit set, of ‘Anna’ and ‘Jonagored’ Cultivars

Rootstocks can markedly affect the vegetative growth of the scions. It has been reported that, rootstocks have significant effect on trunk diameter, plant height, leaf area, number of branches and yield of apple scions (Tahereh *et al.*, 2011). The trunk cross-sectional area was found to bear a linear relationship to the total above-ground weight of apple trees (Westwood and Roberts, 1970; Barden and Marini, 2001).

In another study, Neeme *et al.* (2006) showed that, the rootstock had a major influence not only on the growth vigour but also on number of flower clusters, fruit set and development per apple cultivars. The apple cultivar usually initiate flowers just after shoot growth ceases and when leaves are dropped (Frank and Dennis, 1996).The cultivars flower initiation and bud break depends on the vigour of rootstocks types and adequacy of cold temperature in which the cultivar grows (Westwood, 1993). Peter and David (1995) stated that, the effect of rootstock on flowering appears to be due to internal control mechanisms which affect the proportion of spurs that become floral. In agreement with this, several studies have showed that, apple rootstocks do influence the concentrations and movements of plant hormones

(auxins, gibberellins, cytokinins and abscisic acid) within the tree (Soumelidou *et al.*, 1994; Kamboj *et al.*, 1997). It is likely that these differences are in some way involved in their influence on floral bud initiation in the scion. Other study reported that, rootstock effects on flowering may be due to differences among rootstocks in phosphorus uptake from the soil or differences in distribution within the tree which is positively related to rootstock vigour (Peter and David, 1995).

Apple rootstock thus, shows differences on the growth vigour and blooming of cultivars grown on them. Previously, Ferree (1976) revealed that, the increased precocity induced by dwarfing rootstocks showed that, trees on M.9 and M.26 developed significantly more flower clusters than on the semi-standard MM.106 and MM.111. Other study by Webster (1985) showed that, the rootstock had influence on number of flower clusters per tree, and during the first year the most dwarfing stocks were more precocious, but later his data indicated that, the number of flower cluster per tree decreased as the vigour of the rootstock decreased. On the other hand, for apple cultivars to be fruitful it must not only produce flowers, but these flowers must set and develop into fruits. Blasco *et al.* (1976) found significant differences between rootstocks on initial and final fruit set. It has been known that apple cultivars flowering establish the potential for crop load. Fruit set and development determine how well that potential is realized, with pollination complete (Tromp *et al.*, 2005). It has been reported that, certain rootstocks may induce smaller fruits than another one, but also a higher yield per unit growth (Tromp *et al.*, 2005). In addition to these rootstocks has an effect on fruit ripening, colour, shape, firmness, and soluble solid content composition.

2.9. Effect of Rootstocks on Leaf Mineral Accumulation of Apple

Cultivars

Rootstock has been shown to influence leaf mineral nutrition, in a number of studies, but results have been inconsistent (Peter and David, 1995). The same authors worked on the uptake of phosphorous by different rootstocks reported that, trees of 'Delicious' and 'Golden Delicious' grown on MM.106 rootstock had higher phosphorous levels than similar trees on MM.111 rootstock (Peter and David, 1995). In contrast, West and Young (1988) cited in Peter and David (1995) found similar phosphorous concentrations in 'Delicious' trees grown on these rootstocks but lower concentrations in 'Golden Delicious' trees on MM.106 compared with those on MM.111.

Previous study (Esmael *et al.*, 2002) on the differences in uptake of nutrients by same rootstocks grafted on different scions revealed that, leaves of 'Jonathan' cultivar grafted on four different rootstocks (M1, M2, M7, M13) showed significant differences in N, P, and Mg but not in K or Ca, while leaves of 'Rome Beauty' cultivar grown on similar type of rootstocks showed significant differences in all nutrients tested including K and Ca. They also reported significantly higher K in the leaves of 'Rome Beauty' cultivar on M.1 rootstocks than on Western Seedling. It has been indicated that, rootstocks affect scion leaf mineral concentration in apples (Kucukyumuk *et al.*, 2011). Abdalla *et al.* (1982) reported that, 'Delicious' apple trees on dwarfing rootstocks had more yield efficiency and higher leaf Mn but lower leaf K than trees on vigorous rootstocks. Fallahi *et al.* (1984) observed that 'Starkspur Golden Delicious' apple tree on M.26 had higher leaf Mg than those on the other tested rootstocks.

The nutrient uptake and translocation ability of the different plant cultivars and their rootstocks must also be taken into account for plant growth because these differences can affect yield and quality (Giordano and Mortvedt, 1974). By determining which genotype is sensitive or tolerant to which nutrient, it would be possible to choose which cultivar should be grown for higher yield and quality. To get a better quality of fruits, apple growers should take into consideration mineral nutrient content of apple (Campeanu *et al.*, 2009). Also determining plant nutrient uptake capacity is important to know how much nutrient is required for a cultivar (Jimenez *et al.*, 2004).

Esmael *et al.* (2002) described that the leaf mineral concentration of apple cultivars depend on the effect of rootstocks on nutrient requirements which is directly related to the influence of rootstocks to the tree size of cultivars. Further the same author showed that, xylem sap collected from various apple rootstocks contained different concentrations of minerals suggesting that major genetic variation may exist in rootstocks with respect to their ability for uptake and translocation of minerals from the soil. Thus, scion–rootstock combination is fundamental for optimal growth, water and nutrient uptake and transport (Carmen *et al.*, 2010). Rootstock effects occur because each rootstock has a different root growth pattern, which influences nutrient uptake. Different scion species regulate uptake, transport and metabolism of nutrients to varying degrees (Pitman, 1975). Therefore, every scion/ rootstock combination must be sampled individually. Among the other organs, leaf nutrient status is considered to be the most reliable indicator of apple cultivars response to effects of different rootstocks and cultivars grown under different low soil pH treatments. It has been known that, the leaf is metabolically very active, functioning as the site of photosynthesis, which determines the primary processes occurring within the plant. Leaves are also a major site of

carbohydrate and mineral storage. Consequently, the mineral status of the leaves not only influences the efficacy of photosynthesis but also reflects the nutrient status of the plant and therefore its fruitfulness. Although other organs within the plant may act in a similar manner, the leaf is the most readily available source of tissue for nutrient analysis used to determine the differences in the vigour of apple rootstocks/scion combination to the effect of soil acidity (Kucukyumuk *et al.*, 2011).

According to Joseph (2004), the basis behind leaf analysis which is widely used to evaluate the nutritional status of apple trees is that, optimum ranges exist for the level of each nutrient in the leaf and maximum quality/ yields occur when nutrients are maintained within these ranges. If the level of a nutrient falls outside of its optimum range, corrective measures should be taken. The table below provides nutrient concentration levels indicative of deficient, or optimum for seven essential elements in apple cultivar leaves (Table1).

Table 1: Nutrient concentration levels in leaves of apple cultivars

Interpretations for nutrient levels		
Nutrient	Deficient (below)	Optimum (within)
Nitrogen (%)	1.80	2.0 - 2.25
Phosphorus (%)	0.2	0.20 - 0.30
Potassium (%)	1.00	1.25 - 1.75
Calcium (%)	0.70	1.20 - 1.60
Magnesium (%)	0.20	0.25 - 0.40
Manganese (ppm)	40	40 – 100
Iron (ppm)	100	100 – 300

Source: Joseph Heckman (2004)

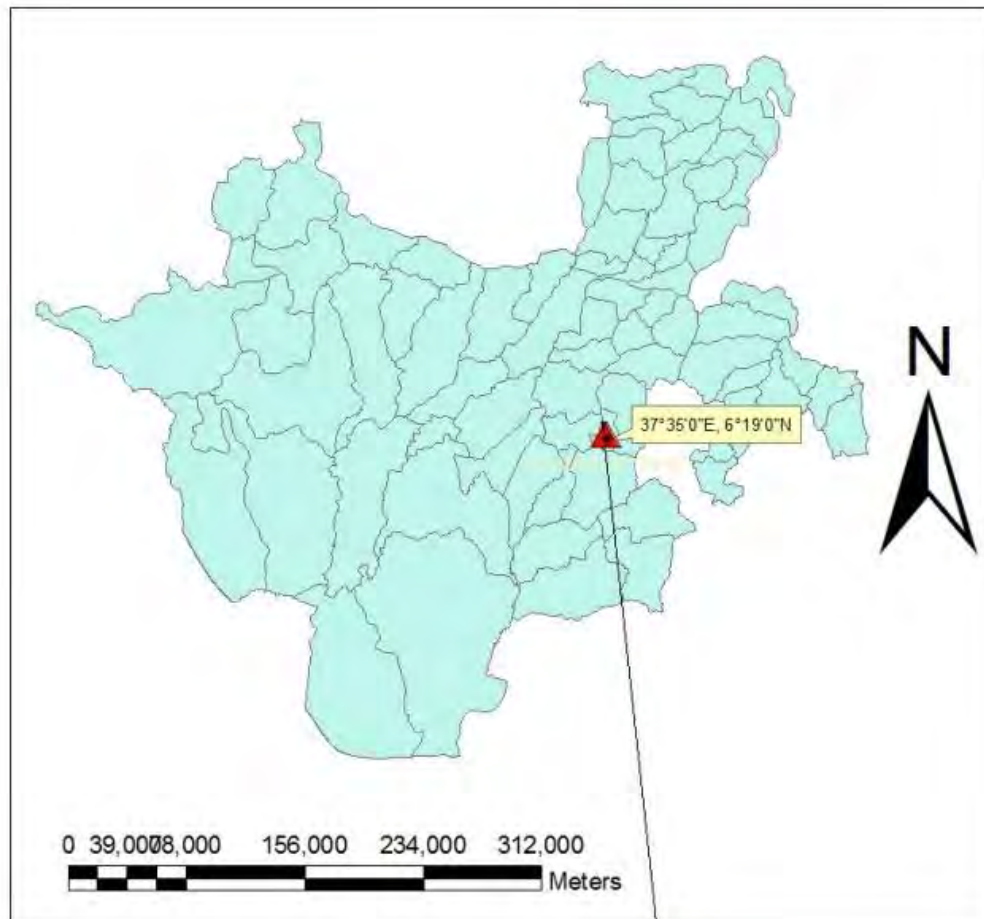
CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was carried out in Chenchu district Gamo Gofa administrative zone, Southern Nations, Nationalities and Peoples Region (SNNPR). The study site is situated at 6°19' 0" N latitude and 37°35'0"E longitude with altitude of 2700masl and about 545 km south of Addis Ababa. The district is categorized under Gamo highlands, Enset and barely livelihood zone and currently known for its high apple fruit production.

The topography of the area is hilly, with slope ranging from 5-60%. The area is characterized by high population density (about 350 people/km²) and small farm sizes (average 0.5 ha). Mixed farming of crop livestock systems is common in the area. Cattle and sheep are the major livestock kept by households, whereas enset, barely, wheat, and peas, are the main plant products. Potato, loose cabbage (locally called *yabesha gomen*) and apple fruit are the major cash crops of the area. Local agricultural labour is not common in this livelihood zone, but poor households seeking cash migrate to nearby town areas in search of employment as daily labour during the months of January to March. The soil of Chenchu is clay loam in texture with 77 percent Al saturation (Mugwira and Haque, 1993; Haile and Boke, 2011). The soil has a weak medium sub angular blocky structure with consistence that is friable when moist and sticky / slightly plastic under wet condition.



Legend

- ▲ Study Site (CHENCHA)
- SNNPR_Wereda
- Ethio_Regions



3.2. Climate

The mean annual precipitation exceeds 1086.04 mm with slight fluctuation over the years. The rainfall pattern is bi-modal, allowing two cropping seasons in a year (i.e., the long and the short rains). The long rainy season extends from July to November whereas; the short rainy season is between March and May. The month with the minimum rain fall is December (Fig 2).

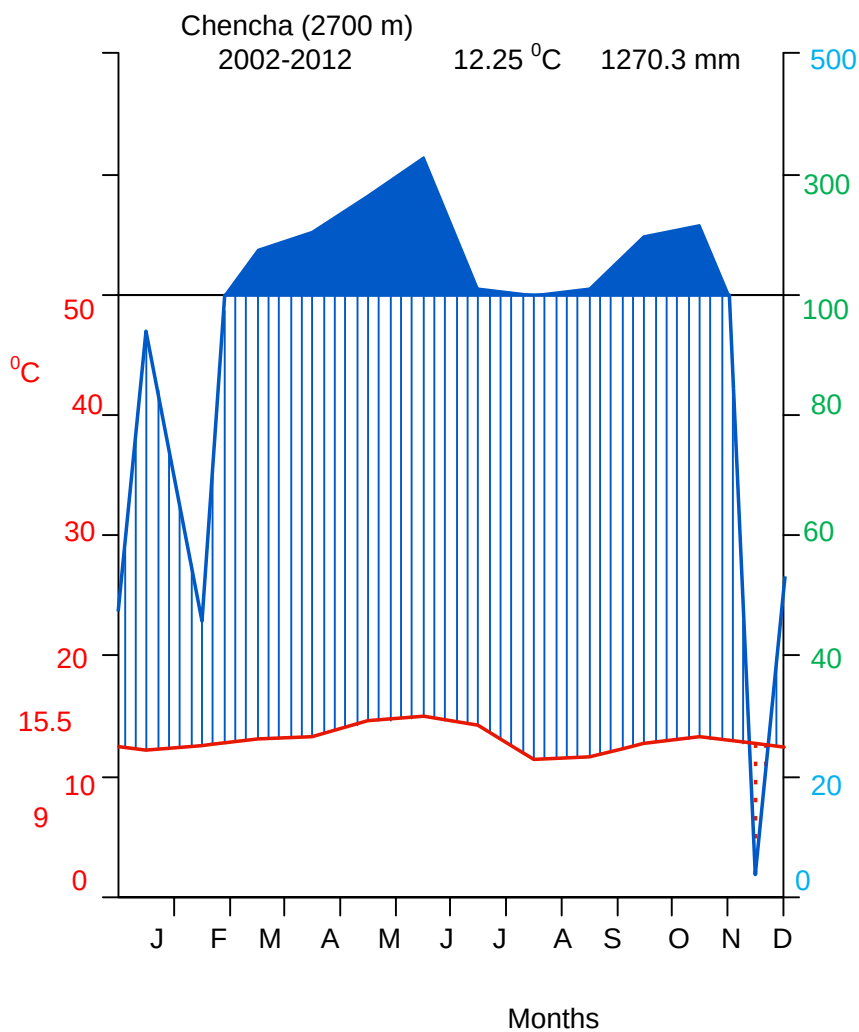


Figure 2: Klimadiagram of the study area. Wet periods are hatched black (for precipitation above 100 mm) Source: Chench meteorological substation.

3. 3. Description of Laboratory and Field Experiments

The thesis consists of four separate trials undertaken both in the study area and in the Ecology and Eco-physiology laboratory at Addis Ababa University between December 2010 and December 2013. The first study dealt with investigation of the three apple rootstocks leaf, shoot and root nutrient accumulation under the three soil pH treatments. The second study dealt with evaluation of vegetative growth parameters of apple rootstocks MM.111, MM.106, and M.9 under naturally occurring acidic soil (pH 4.9), and two different low soil pH treatments (pH 3.5 and 6.3) both in the field and laboratory. The third experiment was on assessment of the growth and yield of apple cultivars, ‘Anna’ and ‘Jonagored’ grafted on three different rootstocks at three soil pH levels. The fourth examined the leaf mineral composition of the two apple cultivars, ‘Anna’ and ‘Jonagored’ grafted on three different rootstocks at three soil pH treatments. The fieldwork was carried out on experimental micro plot established at Chenchawereda Tolola district on apple growers’ field. Weeds were cleared when needed, apple aphids were removed by hand when appearing, no fertilizers were added during the experimental period and the watering was by gardener and natural rainfall.

3.3.1. Soil acidification in micro plot

The research was set in polythene bags considered as micro-plots (Sas and Mercik, 2004). A total of 225 polythene bags having 85cm height and 40cm in diameter were filled with 40 kg of soil. The polythene bags contained soil with sufficient amounts of nutrients and one of three different pHs: 3.5, 4.9 and 6.3. Each polythene bag had only one pH level. The pH of the

experimental soil (4.9) was used as a control, whereas, adjusted pH levels (4.9 +/- 1.4) were used as the other two treatments. The differences in growth and yield of the three rootstocks and cultivars to the three pH levels were evaluated. The different acidity levels were maintained by using 0.1 M sulfuric acid and lime (CaCO_3) for pH 3.5 and 6.3, respectively. The amounts of sulfuric acid needed to reduce soil pH, and lime to increase soil pH was based on the initial pH (4.9) and the desired pH for the treatments. The pH was monitored at the end of the experiments and remained stable for all pH values throughout the experimental period.

To adjust the soil to low soil pH of 3.5, 98% stock solution of sulfuric acid with specific gravity or density of a solute of 1.84 was used. The amount of 0.1M sulfuric acid used to acidify 40 kg of soil to pH of 3.5 was 7200ml (V_2). Thus, 7160ml of water was mixed with 40 ml of concentrated sulfuric acid from stock solution which adds up to 7200ml were used for one micro plot. To obtain pH of 6.3 40 kg soil mixed with 8L of 0.1M CaCO_3 (V). Therefore, 80 gram of solid lime was dissolved in 8000ml of water for each micro plot. For both pH 3.5 and 6.3 treatments, the prepared solution was mixed with 40 kg of soil in a polythene bag to a depth of 50 cm prior to planting the rootstocks. In addition, one kg of organic material in the form of compost was added and uniformly mixed with the soil before the rootstocks were planted.

Vegetatively propagated apple rootstocks, MM.106, MM.111 and M.9, were then planted, one per micro-plot. The experiment consisted of 9 treatment combinations, 2 examined factors: three pH levels 3.5, 4.9 and 6.3, three apple rootstocks MM.106, MM.111, and M9 in 5

replications ($5 \times 3 \times 3 = 45$) following Sas and Mercik (2004). The rootstocks were allowed to grow from December 2010 to December 2011.

3.3.2. Soil sample and analysis

The soil used for micro plot was analyzed to assess the suitability of the root environment and identifying nutrient deficiencies and imbalances. The soil samples were collected from the test field at the end of November 2010 for physicochemical analysis. The soil was sampled from surface layer (0 to 15 cm) using an auger from six random spots across the field. Soil samples were air dried and ground to pass a 2mm size sieve. The pH of the soils was measured in water and potassium chloride (1M KCl) suspension in a 1:2.5 (soil: liquid ratio) potentiometrically using a glass-calomel combination electrode (Van Reeuwijk, 1992). Cation exchange capacity (CEC) and exchangeable bases (Ca, Mg and K) were analysed using ammonium acetate extraction procedure (Chapman, 1965). Ca and Mg were measured with an atomic absorption spectrophotometer whereas, flame photometer was used to measure K contents. The total N was analyzed using the Kjeldahl digestion, distillation and titration method as described by Schuman *et al.* (1973). Available soil P was analysed following the procedure of Olsen *et al.* (1954) extraction method as recommended by Tekalign Mamo *et al.* (2002) for Ethiopian soils. Exchangeable aluminium was determined by 1N KCl extraction (McLean, 1965) and measured with an atomic absorption spectrophotometer. Available Fe and Mn were extracted by DTPA as described by Sahlemedhin and Taye (2000) and measured with an Atomic Absorption Spectrophotometer. The particle size analysis was carried out by the modified sedimentation hydrometer procedure (Bouyoucos, 1962). The soil analysis was conducted in

Ecology and Ecophysiological laboratory at Addis Ababa University, and in JiJe pvt.ltd, laboratory.

The compost sample, which was prepared for three months, was collected from the *Kalehiwot* protestant church. The sample was air-dried and sieved through (2-mm mesh) to eliminate coarse rock and plant material and thoroughly mixed to ensure uniformity. The sample was then stored in a dry place at room temperature to minimize disturbance of the microbial population. From this compost sample, a sub-sample was taken for the determination of total nitrogen and phosphorus. The total nitrogen and available phosphorous contents were analyzed following Kjeldahl (Schuman, *et al.*, 1973) and (Olsen *et al.*, 1954) methods respectively.

3.4. Effect of Low soil pH on Tissue Mineral content of Apple rootstocks

3.4. 1. Plant sample collection and preparation

The apple rootstocks organs (root, shoot, and leaf) were analyzed to assess their differences in nutrient accumulation in response to soil acidity. Samples of ten young leaves that were fully expanded near base of current year's growth were collected. Leaves were air dried under the shade and then dried in an oven at 70°C to constant weight. Completely dried leaves were pooled in to one sample to be analysed in three technical replicates, ground to pass 1 mm sieve and stored in airtight plastic bottles for nutrient analysis. The available youngest branches of shoots without buds from the middle portion of the stem were randomly removed

by sharp stainless steel cutter after removing the leaves one from each replicate a total of five all replicas. The shoot samples were air dried under the shade and then dried in an oven at 70°C to constant weight. The root branches of all five replicas under three pH treatments were also removed from the wet soil without damaging the main roots and gently dipped in water for removing adhered soil and dried under the shade and finally in an oven at 70°C to constant weight.

3.4. 2. Plant sample analysis

For the determinations of P, K, Ca, Mg, Fe, and Mn concentrations, 0.5 g of samples were dry ashed in muffle furnace at 550°C for 8 h, and the ash was dissolved in 4 ml 3N HCl and filled up with distilled water as described in Kucukyumuk and Erdal (2011). Phosphorus contents of samples were determined by vanadate-molybdate colorimetric method of Wantabe and Oleson (1963) as described in Antonio *et al.* (2005). Ca, Mg, Fe, and Mn concentrations were determined using atomic absorption spectrophotometer and K by flame emission spectrophotometers according to procedure described by Kacar and Inal (2008). Aluminium was determined using Wet Ash Extract – Direct Nitrous Oxide Acetylene Flame atomic absorption spectrophotometer method. Nitrogen concentration in samples was determined according to modified Kjeldahl method in which 0.5 g sample digested in concentrated H₂SO₄ and distilled with NaOH (40%). The ammonium N was fixed in H₃BO₃ (2%) and titrated with 0.1N H₂SO₄ following the procedure described by Kucukyumuk and Erdal (2011).

3.5. Effect of Soil acidity on Vegetative growth of Apple rootstocks

The growth parameters of apple rootstocks under naturally acidic soil and two different low soil pH treatments for one year were evaluated. Leaf area, TCSA, number of branches, and total rootstocks length were measured three times, starting at three months after planting and with three months from February 2011 to November 2011. The full dormancy period where the rootstocks drop their leaves in August, was considered as time of rest and data were not collected during the dormancy period.

3.5.1. Vegetative growth measurements

A total of 15 leaves/rootstocks were measured by using leaf area meter LA- AM 100, and the average leaf area was recorded. Trunk circumference (cm) of all five replicates was measured at 5 cm above the ground and converted to Trunk Cross Sectional Area (TCSA) following Yahya and Teryl (2004). The circumference was changed to diameter ($D = C / \pi$), and TCSA was calculated using the formula: $TCSA (cm^2) = \pi (D / 2)^2$, where, $\pi = 3.14$. To determine height of the rootstocks, total length above the ground was measured and number of branches was counted following (Esmaeil *et al.*, 2001; Said *et al.*, 2008) method. The average of one year three round vegetative growth parameters of the rootstocks under pH treatments was analyzed.

3.5. 2. Dry weight measurements

Three apple rootstocks were randomly pulled out of the soil without damaging the roots and their root lengths were separately measured and average was recorded. A total of 30 leaves (10 fully developed leaves/ rootstocks) were dried at 70°C to constant weight and their weights were taken based on Soheil *et al.* (2009) method. The roots were separated from the above ground part (by cutting at soil surface line); both roots and shoots were dried in an oven at 70°C to constant weight. The leaves, roots and shoots were separately weighed and the average was recorded for each rootstock.

3.6. Data Collection on Vegetative growth, Flowering and Fruit set of Apple cultivars

On the second experiment, the same rootstocks grown for three months in a polythene bag under similar pH levels, were grafted with ‘Anna’ and ‘Jonagored’ and then allowed to grow for three years. The study site was divided in to three blocks (block one pH 3.5; block two pH4.9; block three, pH 6.3). The experiment consisted of 18 combinations with 10 replications, including 3 examined factors: 3 pH levels 3.5, 4.9 and 6.3, three apple rootstocks M.106, M.111 and M.9, and 2 apple cultivars ‘Anna’ and ‘Jonagored’ ($10 \times 3 \times 3 \times 2 = 180$) following Sas and Mercik (2004). Tree spacing between rows and blocks were 1.2 and 1.8m, respectively. Growth measurements were taken for both cultivars, ‘Anna’ and ‘Jonagored’ during their

vigorous growth after winter dormancy during three seasons from December 2011 to December 2013. Flowers and fruits count were conducted for three successive seasons from October 2011 to November 2013 following (Blasco, 1976; Crasweller and Ferree, 1983; Neilsen, *et al.*, 1990; Elfving and Schechter, 1993; Sas and Merick, 2004; Przemyslaw and Maciej, 2006; Meland, 2009) methods.

3.6.1. Vegetative growth

A total of 30 fully developed leaves from ten replications were measured and the average leaf area (cm²) was recorded. The scion shoot length of the replicates was also measured 20 cm above the soil surface every year and the average was taken. Similarly, the number of branches was counted following Esmaeil *et al.* (2001) and Said *et al.* (2008) methods. Trunk circumference was measured on each tree every year after leaf drop (in August) at a premarked location 20 cm above the soil surface and converted to trunk cross sectional area (TCSA cm²) following Yahya and Teryl (2004) and Mohamed *et al.* (2012) method. TCSA has been used to relate tree size (Westwood and Roberts, 1970; Barden and Marini, 2001), and also to determine the percent of standard size against known combinations (Lombard *et al.*, 1985; Westwood *et al.*, 1986). At the end of the study year, leaf dry weight was determined destructively, gram of the sample of four fully developed leaves of ‘Anna’ and ‘Jonagored’ cultivars per replica. A total of forty leaves as a representative for ten replicates were dried in oven at 70°C to constant weight.

3.6. 2. Flowering

Whole tree counts of clusters were made on each rootstock in ten replications to determine the rootstock's influence on flower cluster numbers. The number of flower clusters on each tree was counted within 2 to 3 days of full bloom, three times from October, 2011 to October, 2013. The spur of flower buds, which contain on average five flowers, were counted as one. Flower spurs that was damaged by frost was discarded. Counting was conducted from the bottom of the branch to the top. Each branch counted for their flower spur bud was marked to avoid repetition. The mean total number of flowers counted per cultivar was recorded per season.

3.6. 3. Fruiting

The whole tree of both 'Anna' and 'Jonagored' cultivars grafted on each rootstock were studied to determine the scion-rootstock and low soil pH effect on fruit set. After pollination apple flower petals wilted within one to two weeks. The total number of fruit sets were counted following the wilting of bloom, and drop off of immature fruits within three weeks in every season from October 2011 to November 2013. Yield was based on the mean total number of fruits counted per cultivar.

3.7. Plant sampling and Analysis for Leaf Mineral content of ‘Anna’ and ‘Jonagored’ Cultivars

3.7.1. Plant sampling and sample preparation

The main purpose of leaf analysis was to evaluate the efficacy of MM.111, MM.106, and M.9 rootstocks nutrient absorption and transport to both ‘Anna’ and ‘Jonagored’ cultivars, and also to observe the response of scion-rootstocks combination to the effect of low soil pH. Leaf analysis was conducted for major and minor elements, Ca, Mg, N, P, Fe, Mn, K and Al during the year 2013.

Leaf samples from bearing apple cultivars, ‘Anna’ and ‘Jonagored’ were collected from the mid-region of current season shoots after the terminal bud formed in mid-October at the end of the third study year, 2013. Leaves showing insect, disease, or mechanical damage were not selected for sampling. Four leaves per cultivar a total of 40 fully expanded leaves for all 10 replicates were removed and used for LDW and grinded for nutrient and aluminum analysis.

3.7.2. Plant analysis

Preparation of the leaf samples consisted of washing and rinsing with distilled water, drying at 70 °C to constant weight. Leaf dry weight for each scion /rootstocks were separately measured and average was recorded. In order to determine P, K, Ca, Mg,

Fe, and Mn concentrations, 0.5 g of samples were dry ashed at 500⁰C for 8 h, and the ash was dissolved in 4 ml 3N HCl and filled up with pure water according to the procedure described by Kucukyumuk and Erdal (2011). Phosphorus contents of samples were determined by vanadate-molybdate colorimetric method of Wantabe and Oleson (1963) as described in Antonio *et al.* (2005). K, Ca, Mg, Fe, and Mn concentrations were determined using atomic absorption spectrophotometer and flame emission spectrophotometers according to procedure described by Kacar and Inal (2008). Aluminium was determined using Wet Ash Extract – Direct Nitrous Oxide Acetylene Flame atomic absorption spectrophotometer method. Nitrogen concentration in samples was determined according to modified Kjeldahl method in which 0.5 g sample digested in concentrated H₂SO₄ and distilled with NaOH (40%). The ammonium N was fixed in H₃BO₃ (2%) and titrated with 0.1N H₂SO₄ following the procedure described by Kucukyumuk and Erdal (2011) in Ecology and Ecophysiological laboratory at Addis Ababa University and Jije pvt.ltd, Laboratory.

3.8. Statistical Analysis

The data were subjected to analysis of variance to find out treatment effects and/or interactions between factors. Analysis of variance was carried out using MINITAB statistical package version 14. The mean separation was performed by Duncan's multiple range tests at the level of the significance (LSD at $P < 0.05$) (Duncan, 1955). Sigma plot 10 was used to present the analyzed data in different graphs. The results were elaborated separately for each measurements value. Throughout the text, differences between treatment means is considered significant when $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1. Characterization of Experimental Soil

The experimental soil had a pH of 4.9 before the apple rootstocks were planted. It was silty clay in texture having 14% Sand, 43 %Silt, and Clay 43%. The total nitrogen (0.23 %) content of the soil declined (0.13%) after a year in the treated soil (Table 2). The exchangeable bases were in the order of Ca (9.97) $\geq$ Mg (3.47) $\geq$ K (0.78) Cmol(+). And their respective values decreased to 3.70, 1.11 and 0.62 Cmol(+)/kg soil in the treated soil (Table 2). Contrasting results were found for Al and Mn. The exchangeable Al was high in soil pH of 3.5 whereas the total Mn was high in pH 4.9 (Table 2). On the other hand, the one kg of compost mixed with the soil contains total nitrogen (1.7%) and available phosphorous (16.5 mg/kg). The three pH levels showed insignificance differences at the end of both experiments.

Table 2: Soil chemical properties of the experimental site.

pH	Total %	mg/kg (Total)			Exchangeable Bases and CEC(Cmol+)/kg soil				Exchangeable Cmol(+)/kg soil
	N	Fe	Mn	P	Ca	Mg	K	CEC	Al
4.9	0.23	7950.0	760.21	23.61	9.97	3.47	0.78	23.59	4.2
3.5	0.13	834.53	108.60	22.27	3.70	1.11	0.62	17.05	11.4

4.2. Effect of Low pH on Soil Elemental Status

The nutrient levels in the tissues of apple rootstocks under study were used to evaluate the degree of uptake differences in response to the effect of low soil pH treatments. The accumulations of seven nutrients and Al in three different apple rootstocks organs (leaves, shoots, and roots) were analyzed and the results were organized separately. We observed that, the amount of Al, Mn, Fe, Ca, Mg, N, K, and P were significantly different in root, shoot, and leaf of MM.111, MM.106, and M9 rootstocks (appendix Table 3).

4.2.1 Macronutrients (Ca, Mg, N, K and P)

Significant effect ($P \leq 0.05$) of low soil pH was registered for rootstocks leaf calcium concentration. Three rootstocks had decreasing Ca content with decreasing pH (Table 3). The differences between rootstocks in leaf calcium content were also significant ($P < 0.001$) (Table 7) with MM.111 having the highest level, and MM 106 the lowest at all pH values. In roots, the Ca content varied between rootstocks. There was no clear tendency for decreasing Ca with decreasing pH, but the content at pH 3.5 was the lowest in all rootstocks (appendix Table 3).

Magnesium accumulation in leaves of rootstocks according to the result of two way analysis of variance was not significantly influenced by low soil pH treatments. The differences between rootstocks in leaf Mg content were also statistically not significant (Table 7). Also in the roots, the Mg content was variable for each of the rootstocks, with no clear tendency for decrease with decreasing pH (Table appendix 3).

Nitrogen content in leaves of apple rootstocks were not influenced by soil pH and did not differ significantly between rootstocks. The contents of N in roots were however showing a trend for lower values at lower pH in all three rootstocks (appendix Table 3), Comparing the rootstocks organs, more N content was found in leaves, compared to roots and shoots, indicating that N is efficiently transported from roots to leaves of all rootstocks and constitutes a lesser part of the plant structure in roots and shoots than in leaves (appendix Table 3).

The effect of low soil pH on rootstocks leaf K accumulation was not significant. However, the differences between rootstocks in leaf K content were statistically significant ($P < 0.001$). M9 had comparatively high levels of K at all three pH values, whereas those of MM106 were low at pH 6.3, and in MM.111 low at both pH 4.9 and pH 6.3. In the roots, K values for M9 were the highest at all pH values, followed by MM.106 and the lowest in MM. 111 at all pH values.

Table 3: Effect of low soil pH on concentration of macro nutrients (dw⁻¹) in leaves of rootstocks. Values are the means of 3 repetitions $\pm$ SE.

Rootstocks	Macro nutrients(mg kg ⁻¹)	pH		
		3.5	4.9	6.3
MM.111	Ca	10955 $\pm$ 2.9c	11880 $\pm$ 5.8b	13150 $\pm$ 2.8a
	Mg	2550 $\pm$ 5.9a	2340 $\pm$ 11.8b	1310 $\pm$ 5.9c
	N%	2.78 $\pm$ 0.05a	2.77 $\pm$.04a	2.36 $\pm$.04b
	K	12250 $\pm$ 16.7a	5000 $\pm$ 16.7c	5500 $\pm$ 33.3b
MM.106	Ca	2030 $\pm$ 17.6c	3385 $\pm$ 2.9b	5390 $\pm$ 2.9a
	Mg	1180 $\pm$ 5.9b	2330 $\pm$ 17.6a	1090 $\pm$ 2.9c
	N%	2.27 $\pm$ 0.04a	2.09 $\pm$.05a	1.39 $\pm$.05b
	K	12500 $\pm$ 33.3b	16500 $\pm$ 33.3a	7000 $\pm$ 16.7c
M.9	Ca	4015 $\pm$ 2.9c	6510 $\pm$ 5.8b	8735 $\pm$ 2.9a
	Mg	2060 $\pm$ 2.9c	2270 $\pm$ 5.9b	2320 $\pm$ 11.8a
	N%	2.32 $\pm$.04b	2.36 $\pm$.04b	2.57 $\pm$.04a
	K	16500 $\pm$ 16.7a	16000 $\pm$ 16.7b	15500 $\pm$ 16.7c

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. *dw⁻¹ = dry weight

Phosphorous content in rootstocks tissue was not affected by low soil pH treatments. However, the differences between rootstocks in tissue P content were statistically significant ($P < 0.001$) (Table 8). In the leaves, the P values were the highest for MM.106, except at pH 3.5, followed by MM.111 and were the lowest in M.9 (Fig 3). In roots MM.106 had the highest values, followed by M9 and the lowest in MM.111 (appendix Table 3). The interaction effect of rootstocks and low soil pH treatments in terms of leaf P content was significant ($P \leq 0.05$).

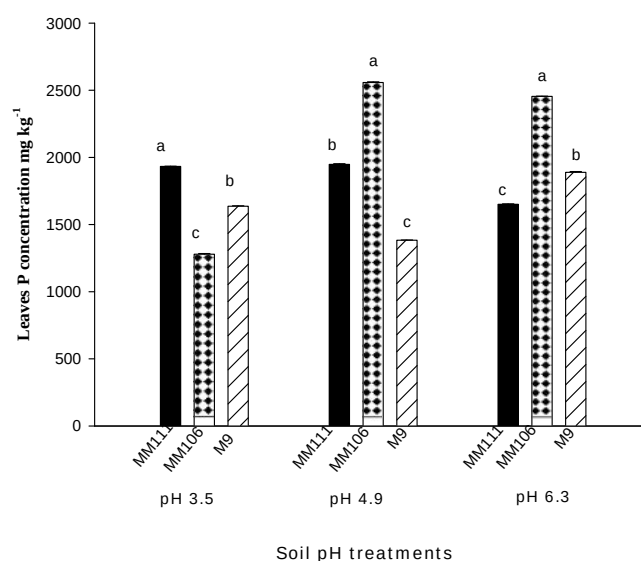


Figure 3: Effect of low soil pH on rootstocks leaf phosphorous accumulation. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Among the rootstocks absorption of phosphorous was significantly affected by excess accumulation of aluminium in roots of MM.106. Significant negative correlation was recorded between leaf phosphorous content and roots aluminium accumulation (Table 4). On the other hand, for MM.111 and M.9 under low soil pH treatments positive significant correlation was recorded for the effect of Al on leaves P content (appendix Table 1 and 2).

Table 4: Correlation coefficient of Al concentration in roots and P accumulation in leaves of MM.106 rootstock.

Source		AL	P
AL	Pearson Correlation	1	-.844**
	Sig. (2-tailed)		.004
P	Pearson Correlation	-.844**	1
	Sig. (2-tailed)	.004	

** . Correlation is significant at the 0.01 level (2-tailed).

4.2.2. Al, Mn and Fe

The effect of low soil pH on rootstocks Al content was significant ($P \leq 0.05$) as was the differences between rootstocks in leaf Al content ($P < 0.001$) (Table 7). Roots and leaves from all samples showed a consistent increase of Al content with decreasing pH (appendix Table 3). Comparing the rootstocks, MM106 had the highest Al content in both roots (appendix Table 3) and leaves, followed by M9, whereas MM.111 had the lowest Al contents at all pH values (Table 7). The root Al accumulation was significantly negatively correlated with Ca, Mg, N, and K nutrients in the leaves (Table 5).

Table 5: Correlation coefficient of Al concentration in roots and Ca, Mg, N and K accumulation in leaves of apple rootstocks.

	Source	Al	Ca	Mg	N	K
Al	Pearson Correlation	1	-.537**	-.224*	-.346**	-.285**
	Sig. (2-tailed)		.000	.044	.002	.010
Ca	Pearson Correlation	-.537**	1	.483**	.444**	.158
	Sig. (2-tailed)	.000		.000	.000	.159
Mg	Pearson Correlation	-.224*	.483**	1	.525**	.534**
	Sig. (2-tailed)	.044	.000		.000	.000
N	Pearson Correlation	-.346**	.444**	.525**	1	.684**
	Sig. (2-tailed)	.002	.000	.000		.000
K	Pearson Correlation	-.285**	.158	.534**	.684**	1
	Sig. (2-tailed)	.010	.159	.000	.000	

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

The effect of low soil pH on rootstocks Mn content was significant ($P \leq 0.05$) as was the differences between rootstocks in leaf Mn content ($P \leq 0.005$) (Table 8). Leaves from all samples showed a consistent increase of Mn content with decreasing pH

(appendix Table 3). Comparing the rootstocks, MM106 had the highest Mn content in leaves, followed by M9, whereas MM.111 had the lowest Mn contents at all pH values (Table 7). In shoots of M.9, Mn accumulation was high in pH of 3.5 low in pH 4.9 and least was recorded in pH 6.3 indicated less translocation to the leaves compared to other rootstocks (appendix Table 3). In roots of MM.106, less Mn was found followed by M.9 while highest concentration was recorded in roots of MM. under soil pH of 3.5. Unlike others, shoot Mn content of MM.111 was found less in soil pH of 3.5 and 4.9. High Mn content was found in leaves of M.9 followed by MM.106 and least was recorded for MM.111 in soil pH of 6.3 (appendix Table 3). The interaction effect of rootstocks and low soil pH treatments in terms of Mn content was not significant. Similar to Al, highest Mn accumulation was observed with decreased in soil pH to 3.5 while least was recorded under soil pH of 6.3 (Table 7).

Table 6: Correlation coefficient between Mn and Ca concentration in leaves of apple rootstocks.

	Source	Mn	Ca
Mn	Pearson Correlation	1	-.237*
	Sig. (2-tailed)		.033
Ca	Pearson Correlation	-.237*	1
	Sig. (2-tailed)	.033	

*. Correlation is significant at the 0.05 level (2-tailed).

Rootstocks tissue iron content was also not influenced by low soil pH treatments. However, the differences between rootstocks in tissue Fe content were significant ($P \leq 0.05$) (Table 8). Comparing the three rootstocks differences on the basis of Fe accumulation, roots of M.9 obtained the highest followed by MM.106, and least was found in MM.111 under acidic soil of pH 3.5. The amount of iron in leaves of M.9

was higher low in MM.111, and least was found in MM.106 under soil pH of 3.5. Among the three rootstocks, the highest concentration of iron was found in root, shoot and leaves of M.9 compared to other two rootstocks (appendix Table 3).

In all three rootstocks, the roots iron content was found high under naturally occurring acidic soil of pH 4.9. On the other hand, except in leaves of M.9, least iron was recorded under soil pH of 6.3. The highest concentration of Fe was found in roots than shoots and leaves (appendix Table 3). On the other hand, the interaction effect of rootstocks and low soil pH treatments on tissue Fe accumulation was not significant. The increase in Fe concentration with increase in soil pH was not uniformly observed for rootstocks under study. However, the Fe concentration in leaves of M.9 increased with increase in level of soil pH, while the decrease in amount of Fe was found for MM.111. On the other hand, MM.106 rootstock recorded more concentration of Fe in soil pH of 4.9 compared to pH 3.5 and 6.3 (Table 7).

Table 7: Effect of low soil pH on micronutrients and element (dw^{-1}) accumulation in leaves of apple rootstocks. Values are the means of 3 repetitions $\pm$ SE.

Rootstocks	Nutrients (mgkg^{-1})	pH		
		3.5	4.9	6.3
MM.111	Al	135 $\pm$ 5.9a	65 $\pm$ 13.3b	48 $\pm$ 1.8b
	Mn	195.2 $\pm$ 1.2a	48.4 $\pm$ 4.7b	9 $\pm$ 2.4c
	Fe	401 $\pm$ 6.2a	219 $\pm$ 2.4b	159.0 $\pm$ 1.5c
MM.106	Al	756 $\pm$ 3.5a	178 $\pm$ 4.7b	130 $\pm$ 2.9c
	Mn	318.2 $\pm$ 2.9a	137.2 $\pm$ 4.1b	30.1 $\pm$ 2.9c
	Fe	274 $\pm$ 2.4c	453 $\pm$ 5.9a	334 $\pm$ 5.9b
M.9	Al	235 $\pm$ 5.9a	107 $\pm$ 4.1b	96 $\pm$ 6.7b
	Mn	238.2 $\pm$ 4.7a	89.1 $\pm$ 5.2b	56.1 $\pm$ 2.9c
	Fe	705 $\pm$ 8.8c	880 $\pm$ 5.9b	960 $\pm$ 17.6a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by

Duncan test. * dw^{-1} = dry weight

Table 8: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on MM.111, MM.106 and M9 rootstocks leaves nutrients and element concentration.

Source	nutrients and element	P-value	nutrients	P-value	nutrients	P-value
Differences between rootstocks	Ca, K, P and Al (element)	0.001 (s)	Mg, N	> 0.05 (ns)	Mn, Fe (s)	≤ 0.05 (s)
Effect of low soil pH	Ca, and Al (element)	0.05(s)	Mn (s)	0.001 (s)	Mg, N, P,K, Fe (ns)	> 0.05 (ns)
Interaction effect of rootstocks and low soil pH	Ca, K, P, Mg, Fe, Mn and Al (element)	> 0.05 (ns)	P	0.05 (s)		

* ns = not significant, s = significant

4. 3. Vegetative Growth of apple rootstocks as influenced by soil pH

4.3.1 Root length

At the end of the study, the root length under all three pH levels varied. The effect of pH on the root length was statistically significant ($P < 0.001$) as was differences between rootstocks in terms of root length were also recorded (Table 11). The rootstock MM.111 had the longest followed by M.9 whereas the stunted root growth was observed for MM.106 in soil pH of 3.5(Fig 4c). The increase in root length was found first rank for MM.111second for M.9 and least for MM.106 in soil pH of 4.9. In soil pH of 6.3, MM.111 had the largest root length followed by MM.106 and least was observed for M.9 (Table 9).



4.3.2 Above ground parts (Leaf area, number of branches, trunk diameter, height and shoot length)

4.3.2.1. Leaf area and total number of branches

The result of analysis of variance showed that, there were significant differences between rootstocks ($P < 0.001$) and treatments ($P < 0.001$) in leaf area. The highest leaf area was recorded for MM.111 followed by M.9 while MM.106 was the least under strongly acidic soil of pH 3.5 (Fig 5). The observed differences in leaf area could be related to differences in rootstocks vigor to strongly acidic soil. However, significant increment in leaf area for MM.106 was observed under pH 6.3 compared to M.9 rootstock. On the other hand, the interaction effect of rootstocks and low soil pH treatments on leaf area was statistically not significant. However, rootstocks leaf area increased with increase in pH level. Maximum growth in leaf area was observed under pH 6.3. The result of Duncan LSD showed that, across low soil pH the growth in leaf area of the same types of rootstocks significantly differs (Table 9).

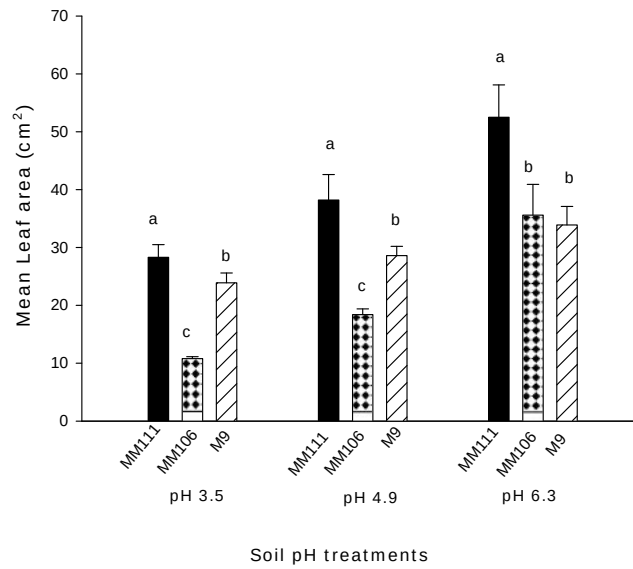


Figure 5: Effect of low soil pH on rootstocks mean leaf area. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the average LA (cm²) $\pm$ SE, 3 leaves/rootstocks a total of 15 for replication.

On the other hand, the total number of branches was significantly ($P < 0.001$) affected by low soil pH treatments as was differences between the rootstocks were also statistically significant ($P < 0.001$) (Table 11). The highest number of branches was recorded for MM. 111 followed by M.9 whereas the least number was observed for MM.106 under all soil pH (Table 9). The interaction effect of rootstocks and low soil pH treatments on number of branches was statistically not significant. However, the amount of branches increased for all three rootstocks with increase in level of soil pH (Table 9).

Table 9: Effect of low soil pH on vegetative growth of apple rootstocks.

Rootstocks	Growth	pH		
		3.5	4.9	6.3
MM.111	Root length(cm)	60.3 ± 2.9a	63.7 ± 4.7a	68 ± 1.8a
	Leaf area (cm ²)	28.3± 2.2b	38.22± 4.4b	52.5 ± 5.6a
	Total branches	7 ± 1.5b	9.6 ± .72a	10.6 ± .72a
MM.106	Root length(cm)	21.7± 2.4c	37.3± 1.2b	44.7± 1.2a
	Leaf area (cm ²)	10.8± .33b	18.4± 1.0b	35.6 ± 5.3a
	Total branches	2.2 ± .30b	4 ± .72b	6.6 ± .72a
M.9	Root length(cm)	28.7± 1.2b	38.8± 1.8a	41.3± 2.4a
	Leaf area (cm ²)	23.8 ± 1.7b	28.6 ± 1.6a	33.9 ± 3.2a
	Total branches	5.3 ± .72b	7.6 ± .72a	9 ± .72a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Root length is the average length of three rootstocks $\pm$ SE, Leaf area is the average of the sum of 15 leaves $\pm$ SE, 3/replica from 5 rootstocks.

4.3.2.2. Trunk Cross Sectional Area

The analysis of variance showed that, pH treatments significantly ($P < 0.001$) affected the trunk growth of rootstocks as was differences in trunk growth between the rootstocks were also statistically significant ($P < 0.001$) (Table 11). Under soil pH of 3.5, the thickest trunk was obtained for MM.111 followed by M.9 and least was for MM.106. Similarly, under soil pH of 4.9, MM.111 recorded the highest trunk growth followed by M.9 while, MM.106 was the least. With the increase in soil pH to 6.3, M.9 recorded thickest trunk growth followed by MM.111 and the least was observed for MM.106 (Fig 6). The interaction effect of rootstocks and low soil pH treatments in terms of trunk diameter was also significant ($P < 0.001$). Duncan LSD showed that,

across soil pH the trunk growth among the rootstocks differs. The trunk growth increased with increasing levels of soil pH. The highest trunk growth for all three rootstocks was obtained under pH 6.3, while the lowest was recorded in pH 3.5 (Table 10).

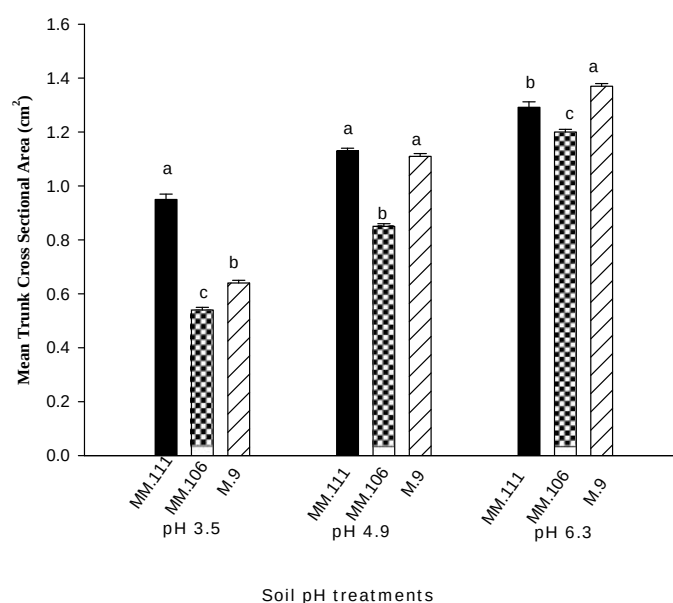


Figure 6: Effect of low soil pH on rootstocks trunk growth. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the average TCSA (cm²) $\pm$ SE, 5 cm above the ground.

4.3.2.3. Height of Rootstocks across low soil pH

The analysis of variance showed that, height of rootstocks was significantly ($P < 0.001$) affected by levels of low soil pH as was difference between rootstocks in total height ($P < 0.001$) (Table 11). Of the three rootstocks studied, MM.111 had highest, M.9 recorded intermediate while MM.106 was the shortest. MM.111, M.9, and MM.106 under soil pH of 3.5, had heights of 80.8 (± 37.5 cm), 42.6 (± 10.2 cm) and

38.7($\pm$ 9.0cm) respectively (Fig 9). The interaction effect of rootstocks and low soil pH treatments in terms of total height was also significant ($P \leq 0.05$). According to Duncan LSD differences in height of same rootstocks across low soil pH was recorded (Table 10). The lowest and highest heights of the rootstocks were recorded under soil pH of 3.5 and 6.3, respectively. MM.111 recorded maximum height followed by both other two rootstocks that showed no differences under all soil pH levels (Fig 9).

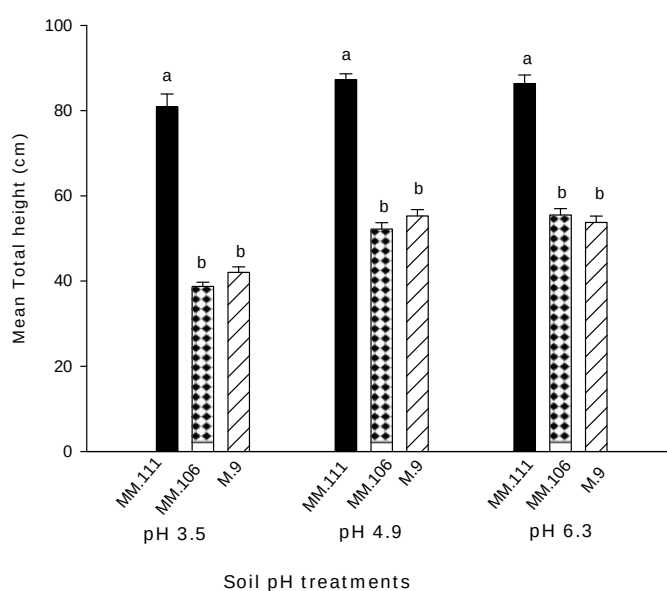


Figure 7: Effect of low soil pH on rootstocks total height. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the total height (cm) $\pm$ SE, above the ground.

Table 10: Effect of rootstocks, low soil pH and its interaction on vegetative growth.

Rootstocks	Growth Parameters	pH		
		3.5	4.9	6.3
MM.111	Total height(cm)	80.8±37.5a	87.2±31.9a	86.3±29.1a
	TCSA (cm ²)	0.95± .02c	1.13 ± .01b	1.29 ± .02a
	LDW(g)	30.6± .72c	52.4 ± 1.6b	74 ± 2.0a
	SDW(g)	111±6.5c	167.2± 4.1b	377.5± 4.1a
	RDW(g)	71.1±3.5c	114.5± 2.4b	168.7± 4.7a
MM.106	Total height(cm)	38.7± 9.0b	52.6± 9.1a	55.4±10.3a
	TCSA (cm ²)	0.54 ± .01c	0.85±.01b	1.2± .01a
	LDW(g)	10 ± .94c	27.3 ± .94b	31.8 ±1.6a
	SDW(g)	66.7± 3.5c	169.4± 5.3b	293.6± 1.8a
	RDW(g)	50.5± 2.9c	63.9± 1.8b	108± 4.7a
M.9	Total height(cm)	42.6±10.2b	55.2±12.0a	53.7±10.3a
	TCSA (cm ²)	0.64±.01c	1.11±.01b	1.37± .01a
	LDW(g)	14 ± 1.6b	25.9 ±1.6a	29.2 ± .96a
	SDW(g)	62.6± 1.2b	76.5± 3.5a	78.5± 1.8a
	RDW(g)	29.20 ± 2.4b	41.7 ± 3.5 a	45.3 ± 2.9 a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH.

4.4. Effect of low soil pH on dry weight production of apple root stocks

4.4.1. Leaf dry weight

Significant effect of low soil pH treatments were found in leaf dry weight among the rootstocks as was difference between rootstocks was also significant ($P < 0.001$) in terms of leaf dry weight (Table 11). Under soil pH of 3.5, MM.111 rootstock obtained highest value while least was for MM.106 and M.9 recorded intermediate. The

maximum leaf dry weight under soil pH of 4.9 was found for MM.111 followed by MM.106 and least was observed for M.9. In soil pH of 6.3, MM.111 showed more gram of leaf dry weight compared to MM.106 and least was found for M.9 indicated MM.111 as more resistant to effect of low soil pH than other two rootstocks (Fig.8). The interaction effect of rootstocks and low soil pH treatments on leaf dry weight was also significant ($P < 0.001$). Duncan LSD showed that all three rootstocks recorded differences in leaf dry weight across soil pH. The leaf dry weight increased with increasing levels of low soil pH while reduced leaf dry weight was recorded under pH 3.5. The highest was found for MM.111 followed by MM.106 while M.9 recorded least (Table 10).

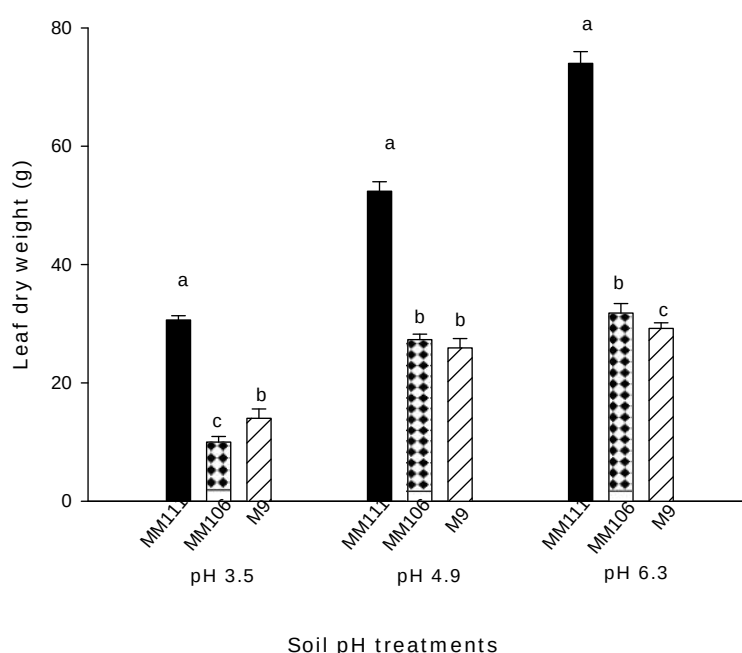


Figure 8: Effect of low soil pH on rootstocks leaf dry weight. Different letters above bars indicate difference between rootstocks at same pH. Bars showed gram of LDW $\pm$ SE.

4.4.2. Shoot dry weight

Rootstocks shoot dry weight was significantly ($P < 0.001$) influenced under low soil pH treatments. The differences between rootstocks in shoot dry weight were also significant ($P < 0.001$) (Table 11). The maximum shoot dry weight was recorded for MM.111 followed by MM.106 while M.9 was least. Among the rootstocks in strongly acidic soil of pH 3.5, a highest value was recorded for MM.111 whereas least was for M.9 and MM.106 was intermediate. The positive counteract of MM.106 to soil pH of 4.9 registered more growth followed by MM.111, and least was recorded for M.9. In soil pH of 6.3 the highest shoot dry weight was found for MM.111 followed by MM.106, while least was recorded for M.9 (Fig. 9).

The interaction effect of rootstocks and low soil pH treatments on shoot dry weight was also significant ($P < 0.001$). The result of Duncan LSD computed showed all three rootstocks recorded differences in shoot dry weight across low soil pH. The shoot dry weight increased with increasing levels of low soil pH. The highest leaf dry weight under pH 6.3 was obtained for MM.111 while least was for M.9 and intermediate for MM.106 (Table 10).

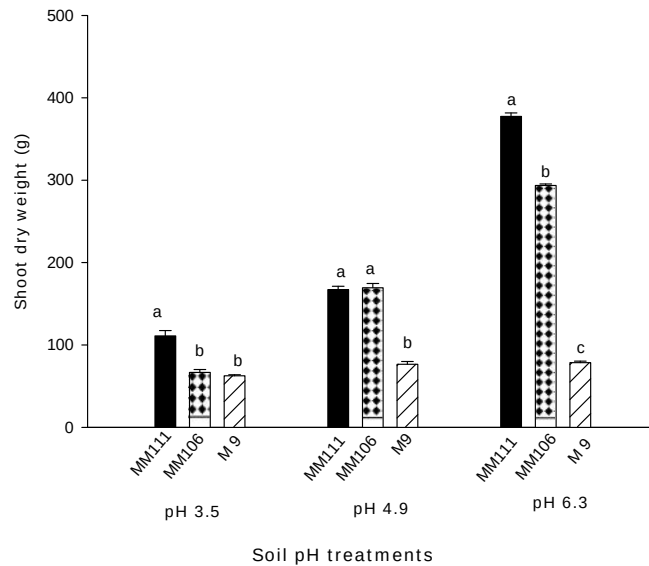


Figure 9: Effect of low soil pH on rootstocks shoots dry weight. Different letters above bars indicate difference between rootstocks at same pH. Bars showed gram of SDW $\pm$ SE.

4.4.3. Root dry weight

Significant ($P < 0.001$) differences between apple rootstocks in root dry weight as was differences between rootstocks in root dry weight were also significant ($P < 0.001$) (Table 11). The highest value of root dry weight was found for MM.111, low for MM.106 whereas M.9 recorded least value under strongly acidic soil of pH 3.5. On the other hand, under naturally occurring acidic soil of pH 4.9, the growth in root dry weight was more for MM.111, less for MM.106 and least was found for M.9. In soil pH of 6.3, similar to other pH levels highest value was found for MM.111 followed by MM.106 and least was recorded for M.9 (Fig. 10). The interaction effect of rootstocks and low soil pH treatments in terms of root dry weight was also significant

($P < 0.001$). The result of Duncan LSD showed that, across low soil pH rootstocks showed differences in root dry weight. The highest root dry weight was found with increase in pH level to 4.9 and 6.3 while least was recorded under pH 3.5 (Table 10).

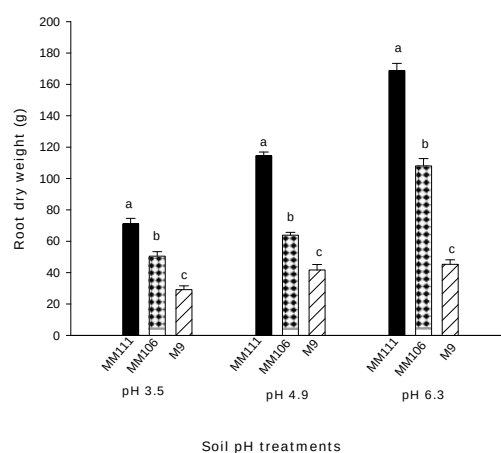


Figure 10: Effect of low soil pH on rootstocks root dry weight. Different letters above bars indicate difference between rootstocks at same pH. Bars showed gram of RDW $\pm$ SE.

Table 11: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on vegetative growth and dry weigh of MM.111, MM.106 and M9 rootstocks under three levels of soil pH treatments.

Source	Vegetative growth	P-value	Vegetative growth	P-value	Vegetative growth	P-value
Differences between rootstocks	RL,RDW,SDW,LDW,TCSA, Total height, total number of branches	0.001 (s)	--	--	--	--
Effect of low soil pH	RL,RDW,SDW,LDW,TCSA, Total height, total number of branches	0.001 (s)	-----	-----	-----	-----
Interaction effect of rootstocks and low soil pH	RDW,SDW,LDW,TCSA	0.001 (s)	RL, total height	$P \leq 0.05$ (s)	total number of branches	$P > 0.05$ (ns)

* ns = not significant, s = significant, RL=root length, RDW= root dry weight, LDW= leaf dry weight, SDW= shoot dry weight, TCSA = trunk cross sectional area.

4.5. Effect of Rootstock Type and Low Soil pH on Growth and Yield of ‘Anna’ and ‘Jonagored’ cultivars

4.5.1. ‘Jonagored’

In this experiment ‘Jonagored’ cultivar was grown on three types of rootstocks planted under low soil pH treatments. In the first growing season, 2011 due to early age of growing much difference were not observed. However, increased differences were particularly evident from 2012, with more difference in third year, 2013.

4.5.1. 2. Total height

According to the result of analysis of variance, there were statistically no significant differences in growth of total height between ‘Jonagored’ cultivars. However, the effect of low soil pH on total height of ‘Jonagored’ grafted on all three rootstocks was significant ($P < 0.001$) (Table 12). The interaction effect of ‘Jonagored’ and low soil pH treatments on total height was statistically not significant. However, the difference in total height was observed in soil pH of 6.3. ‘Jonagored’ grafted on MM.111 recorded highest growth followed by MM.106 and lowest was found on M.9 (Table 12).

Table 12: Effect of rootstock type, low soil pH and its interaction on vegetative growth of ‘Jonagored’ cultivar .

Cultivar/ Rootstocks	Vegetative growth	pH		
		3.5	4.9	6.3
‘Jonagored’ /MM.111	Total height(m)	0.93 ±5.9.c	1.17± 9.2b	1.34 ±7.5a
	Total branches	2.5 ± .08b	2.7 ± .08b	7 ± .08a
	LDW(g)	18.4 ± .50c	20.1 ± .53b	23.0 ± .56a
‘Jonagored’ /MM.106	Total height(m)	0.85± 6.9b	1.02± 8.4b	1.15±5.3a
	Total branches	1.4 ± .08b	3.0 ± .4a	4.5 ± .08b
	LDW(g)	14.2 ± .50b	18.2 ± .40a	19.6 ± .51a
‘Jonagored’ /M.9	Total height(m)	0.87±11a	.98±10.5a	1.15±10.4a
	Total branches	2.4 ± .08a	2.8 ±.08a	3.10 ± .4a
	LDW(g)	16.7 ±.67b	19.0 ± .51b	20.7 ± .47a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH.

Table 13: Two way Analysis of Variance which tested the effects of cultivar /rootstocks, low soil pH and interaction of these factors on vegetative growth and dry weight of Jonagored under three levels of soil pH treatments.

Source	Vegetative growth	P-value	Vegetative growth	P-value
Differences between Jonagored	Total height, total number of branches	>0.05 (ns)	LDW --	0.001 (s) --
Effect of low soil pH	Total height, total number of branches and LDW	0.001 (s)	-----	-----
Interaction effect of rootstocks and low soil pH	Total height, LDW	>0.05 (ns)	total number of branches	$P \leq 0.05$ (s)

* ns = not significant, s = significant,

4.5.1.3. Trunk Cross Sectional Area

The effect of low soil pH on trunk growth of ‘Jonagored’ grafted on all three rootstocks was significant ($P < 0.001$) as was differences between ‘Jonagored’ in terms of trunk growth (Table 16). The trunk growth increased with an increase in pH levels and year of growth. Least growth for all Jonagored was recorded under soil pH of 3.5 across all growing season while maximum was in pH of 6.3. The increase in trunk thickness was also age dependant. Maximum and least trunk growth was recorded in 2013 and 2011 respectively (Table 14). Throughout the three years of study ‘Jonagored’ grafted on MM.111 recorded more trunk diameter followed on M.9 and least was on MM.106 in soil pH of 3.5. In soil pH of 6.3 ‘Jonagored’ grown on M.9 registered maximum trunk growth while least on MM.106 in all three successive seasons (Table 14). On the other hand, the interaction effect of ‘Jonagored’ and low soil pH treatments in terms of trunk diameter was significant ($P < 0.001$) (Table 16).

Table 14: Effect of rootstock type and low soil pH on TCSA (cm^2) of ‘Jonagored’ throughout three successive seasons

Jonagored trunk cross sectional area(cm^2) per cultivar/year of growth									
pH	2011			2012			2013		
	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9
3.5	2.5±.08b	2.1±.28b	3.4±.08b	4.5±.08b	3.7±.28b	3.4±.08b	5.7±.08b	3.2±.01b	4.8±.08b
4.9	3.5±.08a	3.9±.08a	4.1±.55b	4.5±.08a	4.6±.08a	4.1±.55b	5.7±.43b	3.15±.03b	5.1±.01b
6.3	3.7±.33a	4.1±.55a	6.1±.55a	7.7±.33a	6.1±.55a	9.1±.55a	10.9±.16a	8.6±.01a	12.2±.504a

Means with same letters in columns per year indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH (columns) indicated differences between same cultivar/rootstock combinations under different level of soil pH.

4.5.1.4. Total number of branches

The total number of 'Jonagored' branches grafted on three rootstocks was significantly ($P < 0.001$) affected by low soil pH treatments as was differences between 'Jonagored' in terms of total number of branches (Table 13). In soil pH of 3.5, 'Jonagored' grown on MM.111 produced more branches followed on M.9 and least was observed on MM.106. However, under naturally occurring acidic (pH 4.9) the highest number of branches was observed for 'Jonagored' grown on MM.106 less on M.9 and least number were recorded on MM.111 (Fig 11). The interaction effect of 'Jonagored' and low soil pH treatments on total number of branches was also significant ($P \leq 0.05$).

Duncan LSD showed that, differences in number of branches across low soil pH was recorded for Jonagored grown on all three rootstocks. The numbers of branches increased with an increase in level of soil pH. More number of branches was recorded in pH 4.9 with highest in soil pH of 6.3. Under soil pH of 6.3, the maximum number of branches was registered for 'Jonagored' grafted on MM.111 followed on M.9 and least numbers was found on MM.106 (Table 12).

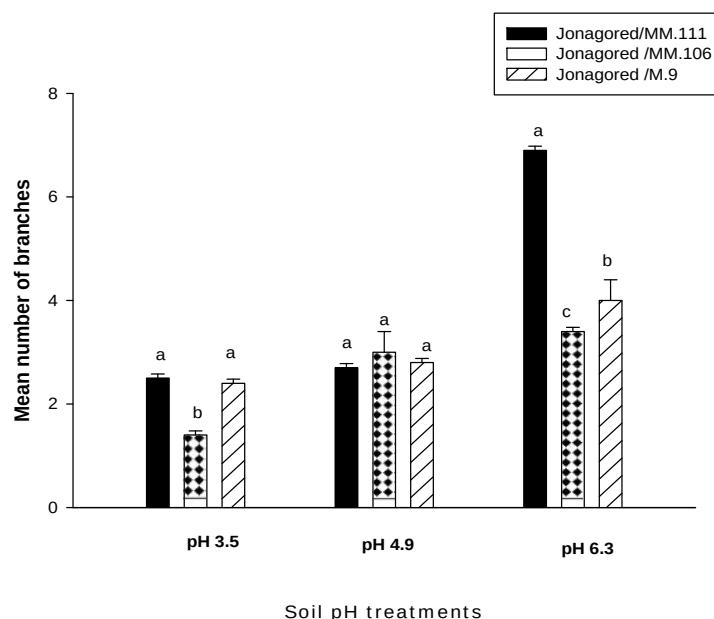


Figure 11: Effect of rootstock type and low soil pH on mean number of ‘Jonagored’ branches. Different letters above bars indicate difference between ‘Jonagored’ grafted on rootstocks at same pH. Bars showed the mean number of branches $\pm$ SE.

4.5.1.1. Leaf Area and Leaf dry weight

The result of analysis of variance showed that, the leaf area of ‘Jonagored’ grafted on all three rootstocks were significantly affected ($P < 0.001$) by low soil pH treatments as was differences between ‘Jonagored’ grafted on different rootstocks in terms of leaf area were also statistically significant ($P < 0.001$) (Table 16). The growth in leaf area increased with an increase in pH levels and year of growth. Least growth for all Jonagored was recorded under soil pH of 3.5 across all growing season while maximum was in pH of 6.3. The increase in leaf area was also age dependant.

Least and maximum growth in leaf area was recorded in 2011 and 2013 respectively (Table 15). Throughout the three years of study ‘Jonagored’ grafted on MM.111 recorded more leaf area followed on M.9 and least was on MM.106 in soil pH of 3.5. In soil pH of 6.3 ‘Jonagored’ grown on M.9 registered maximum growth in leaf area while least on MM.111 in all three successive seasons (Table 15). In soil pH of 4.9, maximum leaf area was observed for ‘Jonagored’ grown on MM.111 followed on MM.106 whereas least was recorded on M.9 (Table 15). The interaction effect of ‘Jonagored’ and low soil pH treatments in terms of leaf area was also significant ($P < 0.001$). Duncan LSD revealed that, differences in growth of Jonagored grown on same types of rootstocks showed differences in growth of leaf area across low soil pH. The increase in growth was high in 2013 while least in 2011. Similarly increase in leaf area was recorded with increase in soil pH to 4.9 and 6.3. Maximum leaf area was registered for ‘Jonagored’ grafted on M.9 followed on MM.106, and least was found on MM.111 (Table 15).

Table 15: Effect of rootstock type and low soil pH on Leaf area (cm^2) of ‘Jonagored’ throughout three successive seasons.

Jonagored leaf area per cultivar/year of growth									
	2011			2012			2013		
pH	Jonna gored/ MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9
3.5	15.2± .3b	9.7± 3.2b	13.9 ± .4b	18.2± .1b	13.7± 2.2b	17.9 ± .61b	25.3± .84b	18.7± 1.2b	20.9 ± .61b
4.9	17.1± .1a	11.5± .54b	16.8 ± .9b	22.1± .1a	18.5± .84b	20.8 ± .62b	29.1± 1.1a	22.5± .84b	21.8 ± .62b
6.3	20.8 ± .1b	20.9 ± .9a	22.7 ± .7a	23.8 ± .1b	25.2 ± .9a	30.7 ± 2.7a	25.8 ± 1.1b	28.2 ± .3a	39.7 ± 2.7a

Means with same letters in columns per year indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH (columns) indicated differences between same cultivar/rootstock combinations under different level of soil pH.

Table 16: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on TCSA and leaf area of ‘Jonagored’ from 2011 -2013.

Source	Trunk cross sectional area(cm ²) and leaf area (cm ²)of Jonagored per years					
	2011	p-value	2012	p-value	2013	p-value
Differences between ‘Jonagored’ cultivar	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)
Effect of low soil pH	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)
Interaction effect of rootstocks and low soil pH	TCSA and Leaf area	0.05(s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)

ns = not significant, s = significant

The leaf dry weight of ‘Jonagored’ was significantly influenced by soil pH treatments ($P < 0.001$) as was difference in leaf dry weight of ‘Jonagored’ cultivar was also significant in terms of leaf dry weight (Table 13). Under soil pH of 3.5, leaf dry weight of ‘Jonagored’ grown on MM.111 was highest lowest on M.9 and least was found on MM.106 (Table 11). Under naturally occurring acidic soil (pH 4.9) similar to pH 3.5, the leaf dry weight of ‘Jonagored’ grafted on MM.111 was high followed on M.9 and least was found on MM.106 compared to pH of 6.3. The data showed that, ‘Jonagored’ grown on MM.106 was found least in leaf dry weight under all three pH treatments indicated weak scion-rootstock combination compared to MM.111 and M.9. On the other hand, the interaction effect of ‘Jonagored’ and low soil pH treatments on leaf dry weight was statistically not significant. Significant increase in leaf dry weight in relation to increased low pH level was not observed (Table 12).

4.5.1.5. Mean number of flowers

The effect of low soil pH on total number of ‘Jonagored’ flowers was statistically significant ($P < 0.001$) throughout three years. However, the differences in number of flowers between ‘Jonagored’ cultivars were statistically not significant in first growing season but significant in second and third growing season. Similar result was recorded for interaction effect (Table18). The flower production increased with an increase in growing season highest in 2013, least in 2011 and also in level of pH, highest in soil pH of 6.3 least in soil pH of 3.5 (Table17).

Table 17: Flowering of Jonagored as influenced by different rootstocks and low soil pH treatments.

Number of flowers per cultivar									
pH	2011				2012			2013	
	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9	Jonna gored / MM111	Jonna gored / MM106	Jonna gored / M9
3.5	8	4	7	10	12	11	11	16	20
4.9	10	8	11	11	14	16	13	19	24
6.3	12	11	13	14	17	19	17	22	28

Table 18: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on mean number of ‘Jonagored’ flowers from 2011 -2013.

Source	Number of flowers per years					
	2011	p-value	2012	p-value	2013	p-value
Differences between Jonagored cultivar	Number of flowers	>0.05 (ns)	Number of flowers	0.001 (s)	Number of flowers	0.001 (s)
Effect of low soil pH	Number of flowers	0.001 (s)	Number of flowers	0.001 (s)	Number of flowers	0.001 (s)
Interaction effect of rootstocks and low soil pH	Number of flowers	>0.05 (ns)	Number of flowers	0.001 (s)	Number of flowers	0.001 (s)

ns = not significant, s =significant

4.5.1.6. Mean number of fruits

Fruit set of ‘Jonagored’ cultivar grafted on MM.111, MM.106, and M.9 rootstocks were studied for three successive seasons. The first bearing season, 2011 the fruits counted were less for ‘Jonagored’ compared to 2012 and 2013(Fig.12). The effect of low soil pH on total number of ‘Jonagored’ fruits and differences in number of fruits between ‘Jonagored’ cultivars were also significant ($P < 0.001$) during three years of fruit bearing years. In significance interaction effect was recorded only in first fruit bearing season (Table 20). The number of fruits increased with an increase in growing season with highest fruit number in 2013 under soil pH of 4.9 and highest in soil pH of 6.3. The highest number of fruits was counted for ‘Jonagored’ cultivar grafted on M.9 and MM.106 while least fruits were counted for Jonagored grafted on MM.111 (Fig.12). In low soil pH of 3.5, the total number of ‘Jonagored’ fruits counted on MM.111 was high while least in pH 4.9 and 6.3 throughout growing season.

The analysis of correlation coefficient showed positive significant ($r = .448$, $p < 0.001$) relationship between ‘Jonagored’ mean trunk diameter and mean number fruits. Similarly, positive significant ($r = .202$, $p < 0.001$) correlation was observed between leaf area and number of fruits for ‘Jonagored’ grafted on all three rootstocks under low soil pH treatments (Table 19).

Table 19: Correlation coefficient of ‘Jonagored’ TCSA, LA, and number of fruits.

Source		TCSA	LA	Fruits
TCSA	Pearson Correlation	1	.461**	.448**
	Sig. (2-tailed)		.000	.000
LA	Pearson Correlation	.461**	1	.202
	Sig. (2-tailed)	.000		.056
Fruits	Pearson Correlation	.448**	.202	1
	Sig. (2-tailed)	.000	.056	

** Correlation is significant at the 0.01 level (2-tailed).

Table 20: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on mean number of ‘Jonagored’ fruits from 2011 -2013.

Source	Number of fruits per years					
	2011	p-value	2012	p-value	2013	p-value
Differences between Jonagored cultivar	Number of fruits	< 0.05 (s)	Number of fruits	0 .001 (s)	Number of fruits	0 .001 (s)
Effect of low soil pH	Number of fruits	0 .001 (s)	Number of flowers	0 .001 (s)	Number of fruits	0 .001 (s)
Interaction effect of rootstocks and low soil pH	Number of fruits	>0.05 (ns)	Number of fruits	0 .001 (s)	Number of fruits	0 .001 (s)

ns = not significant, s =significant

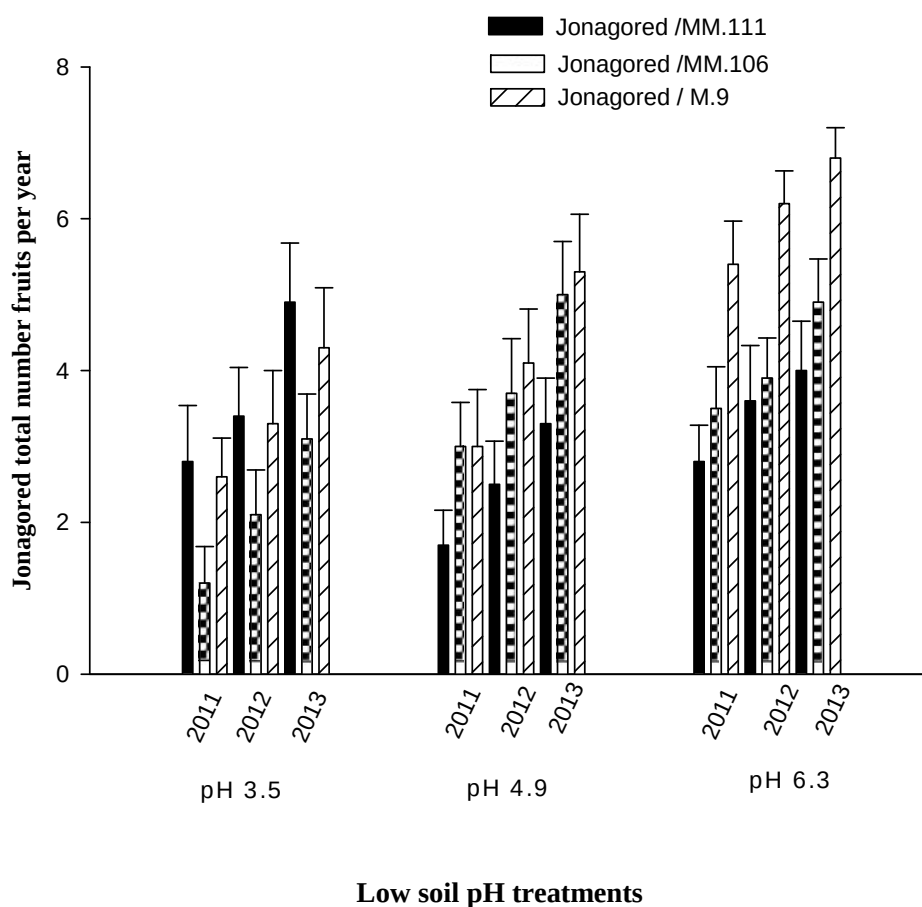


Figure 12: Effect of rootstock type and low soil pH on number of ‘Jonagored’ fruits per three successive seasons.

4.5.2. Anna

‘Anna’ was the second apple cultivar grafted on three different rootstocks that had been grown under soil pH of 3.5, 4.9, and 6.3. Similar to ‘Jonagored’, in the first growing season, 2011 due to early age of growing much difference were not

observed. However, increasing differences were particularly noticeable from 2012, with more differences in third year, 2013.

4.5.2.1. Total height

The effect of low soil pH on total height of ‘Anna’ was significant ($P \leq 0.05$) as was difference between ‘Anna’ cultivars in terms of total height (Table 22). ‘Anna’ showed highest growth on MM.111 followed on MM.106 and least height was recorded on M.9 under pH of 3.5. Under naturally occurring soil pH of 4.9, ‘Anna’ on MM.111 showed highest followed on MM.106 and least was recorded on M.9. The maximum height found for ‘Anna’ grown on MM.111, next on MM.106 and least growth was found on M.9 in soil pH of 6.3 (Fig 13).

The interaction effect of ‘Anna’ and low soil pH treatments on total height was statistically not significant. The increase in height with increase in pH level was not significant. The result of Duncan LSD showed that, there was differences in growth of total height for grafted on same rootstocks across low soil pH (Table 21).

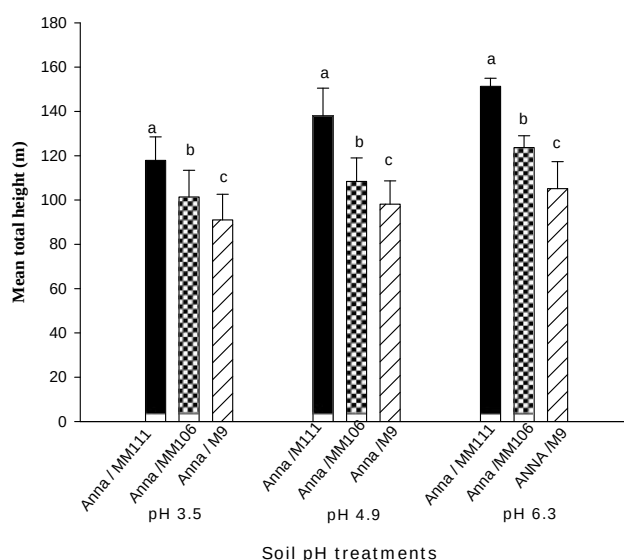


Figure 13: Effect of rootstock type and low soil pH on mean total height of ‘Anna’ cultivar. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the mean number of total height above the grafted union $\pm$ SE.

Table 21: Effect of rootstock type, low soil pH and its interaction on vegetative growth of ‘Anna’.

Cultivar/ Rootstocks	Growth Parameters	pH		
		3.5	4.9	6.3
Anna/ MM.111	Total height(m)	1.18 $\pm$ 10.6b	1.38 $\pm$ 12.5ab	1.51 $\pm$ 3.7a
	Total branches	3.8 $\pm$.08b	6.1 $\pm$.08a	5.4 $\pm$.08a
	LDW(g)	19.3 $\pm$.56b	21.1 $\pm$.56a	22.3 $\pm$ 1.6a
Anna/ MM.106	Total height(m)	1.01 $\pm$ 12.03b	1.08 $\pm$ 10.6b	1.24 $\pm$ 5.3a
	Total branches	2.3 $\pm$.05b	2.4 $\pm$.05b	5.6 $\pm$.08a
	LDW(g)	15.1 $\pm$.75b	21.1 $\pm$.63a	21.9 $\pm$.56a
Anna/ M.9	Total height(m)	.91 $\pm$ 11.6b	.98 $\pm$ 10.5b	1.05 $\pm$ 12.2a
	Total branches	3.2 $\pm$.11c	4.4 $\pm$.08b	5.7 $\pm$.08a
	LDW(g)	17.1 $\pm$.59a	18.2 $\pm$.75a	18.05 $\pm$ 1.9a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH.

Table 22: Two way Analysis of Variance which tested the effects of cultivar /rootstocks, low soil pH and interaction of these factors on Anna vegetative growth and dry weight.

Source	Vegetative growth	P-value	Vegetative growth	P-value
Differences between Anna	Total height, total number of branches and LDW	0.001 (s)	---	--
Effect of low soil pH	Total height, total number of branches and LDW	0.001 (s)	-----	-----
Interaction effect of rootstocks and low soil pH	Total height	>0.05 (ns)	total number of branches, LDW	$P \leq 0.05$ (s)

ns = not significant, s =significant

4.5.2.2. Trunk Cross Sectional Area

The result of analysis of variance showed that, low soil pH significantly ($P < 0.001$) affected the trunk thickness of ‘Anna’ as was differences in trunk diameter between ‘Anna’ cultivars (Table 25). The interaction effect of ‘Anna’ and low soil pH treatments in terms of trunk diameter was also significant ($P < 0.001$). The trunk growth increased with an increase in pH levels and year of growth. Least growth for all Anna was recorded under soil pH of 3.5 across all growing season while maximum was in pH of 6.3. The increase in trunk thickness was also age dependant. Maximum and least trunk growth was recorded in 2013 and 2011 respectively (Table 23). Throughout the three years of study ‘Anna’ grafted on MM.111 recorded more trunk diameter followed on M.9 and least was on MM.106 in soil pH of 3.5. In soil pH of 6.3 ‘Anna’ grown on M.9 registered maximum trunk growth while least on MM.106 in all three successive seasons (Table 23). The increase in trunk growth was recorded with increase in pH levels. Under soil pH of 4.9 the trunk growth of ‘Anna’ on MM.111 was high low on M.9 and least value was recorded on MM.106. Thickest and

thinnest trunk was recorded for ‘Anna’ grafted on M.9 and MM.106 respectively under soil pH of 6.3 (Table 23). Similar to ‘Jonagored’ , no change was observed with pH differences for ‘Anna’ grafted on MM.106.

Table 23: Effect of rootstock type and low soil pH on TCSA (cm²) of ‘Anna’ per year of growth.

pH	Anna trunk cross sectional area(cm ²) per cultivar/year								
	2011			2012			2013		
	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9
3.5	3.5±.51a	1.96±.18c	3.1±.50b	4.5±.51a	3.96±.18c	4.1±.50b	6.2±.09b	4.6±.08c	5.5±.08c
4.9	3.7±.35a	2.6±.31b	3.6±.17b	5.7±.35a	4.6±.31b	4.6±.17b	6.9±.08a	5.7±.08b	6.0±.08b
6.3	4.1±.55a	4.06±.33a	5.6±.17a	6.1±.55a	5.06±.33a	7.6±.17a	6.9±.08a	6.1±.08a	8.5±.08a

Means with same letters in columns per year indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH (columns) indicated differences between same cultivar/rootstock combinations under different level of soil pH.

4.5.2.3. Total number of branches

The effect of low soil pH on total number of ‘Anna’ branches grafted on all three rootstocks was significant ($P < 0.001$) as was differences in total number of branches between ‘Anna’ cultivars (Table 22). The interaction effect of ‘Anna’ and low soil pH treatments on total number of branches was also significant ($P \leq 0.05$). The formation of ‘Anna’ branch increased under soil pH of 4.9 and with highest branch number under pH of 6.3. ‘Anna’ grafted on M.9 recorded highest number of branches followed on MM.106 and least was found on MM.111 under pH of 6.3(Fig.14). In contrast, in soil pH of 3.5 ‘Anna’ grafted on MM.111 formed more branches followed on M.9 and least was found on MM.106. The increase in number of branches of

‘Anna’ grown on MM.106 was not pH related. More number of branches was recorded for ‘Anna’ grown on MM.111 followed on M.9 whereas least number was recorded for ‘Anna’ grafted on MM.106 in soil pH of 4.9 (Fig. 14). According to the result of Duncan LSD differences in number of branches was recorded for Anna grown on same types of rootstocks across low soil pH (Table 21).

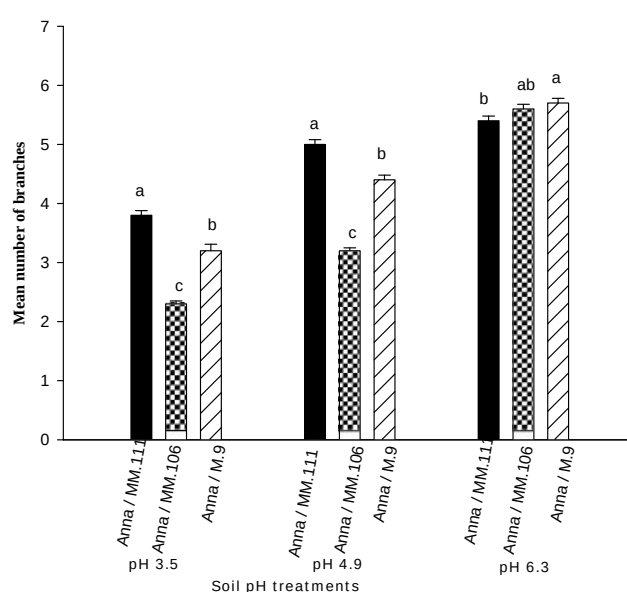


Figure 14: Effect of rootstock type and low soil pH on mean number of ‘Anna’ branches. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the mean number of branches $\pm$ SE.

4.5.2.4. Leaf area and Leaf dry weight

The result of analysis of variance showed, soil pH significantly ($P < 0.001$) influenced the leaf area of ‘Anna’ as was differences between ‘Anna’ grafted on different rootstocks in terms of leaf area (Table 25). The growth in leaf area increased with an

increase in pH levels and year of growth. Least growth in leaf area was recorded under soil pH of 3.5 across all growing season while maximum was in pH of 6.3. The increase in leaf area was also age dependant. Least and maximum growth in leaf area was recorded in 2011 and 2013 respectively (Table 24). Throughout the three years of study ‘Anna’ grafted on MM.111 recorded more leaf area followed on M.9 and least was on MM.106 in soil pH of 3.5. In soil pH of 6.3 ‘Anna’ grown on MM.111 registered maximum growth in leaf area while least on M.9 in all three successive seasons (Table 24). The interaction effect of ‘Anna’ and low soil pH treatments in terms of leaf area was significant ($P \leq 0.05$). The growth in leaf area increased with increase in pH levels. Under pH 6.3 we found highest leaf area for ‘Anna’ grown on MM.111 next on MM.106 whereas smallest leaf area was recorded on M.9 (Table 24).

Table 24: Effect of rootstock type, low soil pH on Leaf area (cm²) of ‘Anna’ per year of growth.

pH	Anna leaf area((cm ²) per cultivar/year of growth								
	2011			2012			2013		
	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9
3.5	14.1 ± .3b	11.2 ± 1.2b	12.7 ± .3b	22.2 ± 1.3b	16.2 ± 1.4b	20.7 ± 1.3b	30.2 ± .1b	21.03 ± .6b	23.9 ± 1.1b
4.9	20.5 ± .3b	16.2 ± 1.0b	17..5 ± .7b	25.9 ± 1.5b	20.02 ± 1b	22.5 ± .7b	32.9 ± .5b	25.06 ± .3b	24.8 ± .78b
6.3	27.5 ± 2a	24.1 ± .8a	22.3 ± .9a	32.5 ± 2.6a	27.1 ± 1.8a	24.3 ± .9a	49.6 ± 3a	37.0 ± 2.8a	28.8 ± 1.9a

Means with same letters in columns per year indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH (columns) indicated differences between same cultivar/rootstock combinations under different level of soil pH.

Table 25: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on TCSA and Leaf area of from 2 Anna from 2011 -2013.

Source	Trunk cross sectional area(cm ²) and leaf area (cm ²)of Anna/ year					
	2011	p-value	2012	p-value	2013	p-value
Differences between 'Anna' cultivar	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)
Effect of low soil pH	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)
Interaction effect of rootstocks and low soil pH	TCSA and Leaf area	0.05 (s)	TCSA and Leaf area	0 .001 (s)	TCSA and Leaf area	0 .001 (s)

ns = not significant, s =significant

The effect of low soil pH on leaf dry weight of 'Anna' was significant ($P < 0.001$) as was differences in terms of leaf dry weight (Table 22). In soil pH of 3.5, 'Anna' grafted on MM.111 recorded highest leaf dry weight followed on M.9 and least was recorded for 'Anna' grafted on MM.106. However, compared to M.9, the leaf dry weight of 'Anna' grafted on MM.106 increased with an increase in soil pH to 4.9 and to 6.3 (Fig. 15). The interaction effect of 'Anna' and low soil pH treatments on leaf dry weight was significant ($P \leq 0.05$). According to Duncan LSD differences were recorded for same Anna / rootstocks combination under different level of soil pH. The highest leaf dry weight was recorded with an increase in pH levels. Under soil pH of 6.3, the highest leaf dry weight was recorded for 'Anna' grown on MM.111 followed on MM.106 while least was found on M.9 (Table 21).

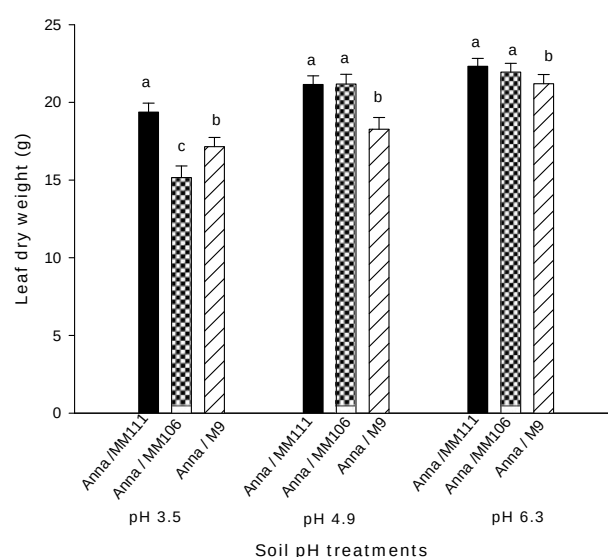


Figure 15: Effect of rootstock type and low soil pH on leaf dry weight of ‘Anna’ cultivar. Different letters above bars indicate difference between rootstocks at same pH. Bars showed the mean number of LDW $\pm$ SE.

4.5.2.5. Mean number of flowers

The productivity of ‘Anna’ was determined by counting all flowers after a year, for three successive seasons. Despite the fact that a few cultivars flowered and set a few fruits in 2011, all trees begun flowering and fruiting in 2012 and with more crop load in 2013. The flowers counted per season were used to evaluate the effect growing season in number of flowers among the cultivar rootstocks combinations.

The effect of low soil pH on total number of ‘Anna’ flowers grown on three rootstocks was significant ($P < 0.001$) as was the differences in number of flowers between ‘Anna’ in all three successive seasons. Interaction effect was not significant in first season of flower production (Table 27). The number of flower produced

increase with pH levels and years of growth with highest in pH 6.3 and in growing season of 2013 respectively while least was recorded in pH 3.5 and 2011. In soil pH of 4.9 and 6.3 Anna grafted on MM.111 and M.9 respectively produced maximum number of flowers while least was on MM.106 (Table 26).

Table 26: Flowering of Anna as influenced by different rootstocks and low soil pH treatment

pH	Number of flowers per cultivar/year								
	2011			2012			2013		
	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9	Anna/ MM111	Anna/ MM106	Anna/ M9
3.5	13	4	6	15	8	11	24	13	18
4.9	14	10	8	19	13	17	20	18	27
6.3	16	12	17	23	16	20	28	19	36

Table 27: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on mean number of ‘Anna’ flowers from 2011 -2013.

Source	Number of flowers per t years					
	2011	p-value	2012	p-value	2013	p-value
Differences between ‘Anna’ cultivar	Number of flowers	0 .001 (s)	Number of flowers	0 .001 (s)	Number of flowers	0 .001 (s)
Effect of low soil pH	Number of flowers	0 .001 (s)	Number of flowers	0 .001 (s)	Number of flowers	0 .001 (s)
Interaction effect of rootstocks and low soil pH	Number of flowers	>0.05(ns)	Number of flowers	0 .001 (s)	Number of flowers	0 .001 (s)

ns = not significant, s =significant

4.5.2.6. Mean number of fruits

The growth and fruit production of cultivars depend on type of apple rootstocks used. The differences were related on rootstocks types and their resistant to low soil pH. The amount of fruits produced by ‘Anna’ grafted on MM.111, MM.106, and M.9 was studied for three consecutive years. Since 2011 was the first bearing season, less mean fruits were counted compared to 2012 and 2013(Fig. 16).

The effect of low soil pH on total number of ‘Anna’ fruits; differences in number of fruits between ‘Anna’ cultivars were significant ($P < 0.001$) in all three successive seasons while interaction effect was not significant in first growing season (Table 29). The number of fruits increases with an increase in level of soil pH and year of growth. The highest number of fruits was counted in soil pH of 6.3 and end of growing season, 2013(Fig.16). Anna was more productive on MM.111 under all three pH levels during the three years of fruit production least on MM.106 in pH 3.5 and 4.9. However in 2013 Anna on MM.111 was in least rank. The number of fruits counted for Anna on M.9 increased with an increase in level of soil pH and year of growth with maximum fruit count in soil pH of 6.3 and 2013 respectively (Fig.16).

The analysis of correlation coefficient showed positive significant ($r = 0.352$, $p < 0.001$) relationship between ‘Anna’ trunk diameter and number of fruits. Conversely, negative significant($r = -0.095$, $p < 0.001$) correlation was observed between leaf area and number of fruits of ‘Anna’ cultivar grafted on three rootstocks under low soil pH treatments (Table 28).

Table 28: Correlation coefficient of ‘Anna’ TCSA, LA, and number of fruits.

Source		TCSA	LA	Fruits
TCSA	Pearson Correlation	1	.430**	.352**
	Sig. (2-tailed)		.000	.001
LA	Pearson Correlation	.430**	1	-.095
	Sig. (2-tailed)	.000		.375
Fruits	Pearson Correlation	.352**	-.095	1
	Sig. (2-tailed)	.001	.375	

** Correlation is significant at the 0.01 level (2-tailed).

Table 29: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on mean number of ‘Anna’ fruits from 2011 -2013.

Source	Number of fruits per years					
	2011	p-value	2012	p-value	2013	p-value
Differences between ‘Anna’ cultivar	Number of fruits	0 .001 (s)	Number of fruits	0 .001 (s)	Number of fruits	0 .001 (s)
Effect of low soil pH	Number of fruits	0 .001 (s)	Number of flowers	0 .001 (s)	Number of fruits	0 .001 (s)
Interaction effect of rootstocks and low soil pH	Number of fruits	>0.05(ns)	Number of fruits	< 0 .05 (s)	Number of fruits	0 .001 (s)

ns = not significant, s =significant

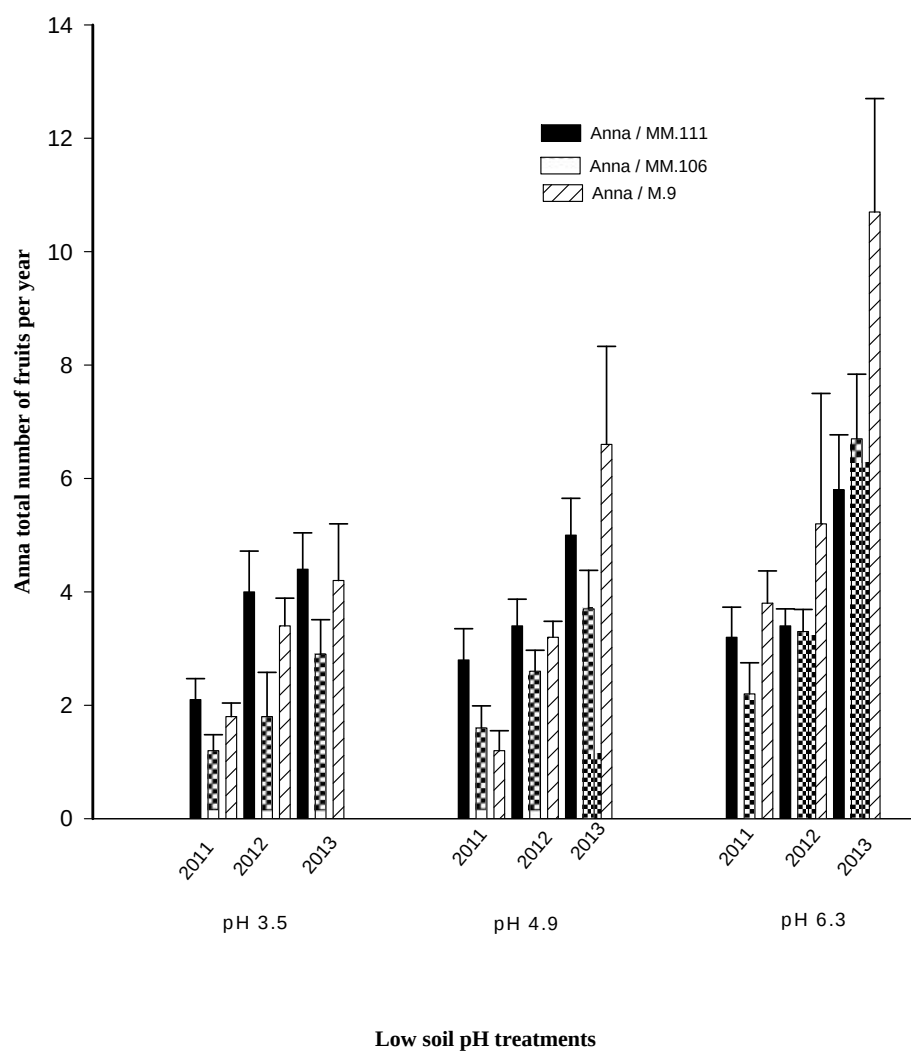


Figure 16: Effect of rootstock type and low soil pH on number of ‘Anna’ fruits per three successive seasons.

4.6. Effect of Rootstock Type and Low Soil pH on concentration of minerals in leaves of ‘Jonagored’ and ‘Anna’ cultivars

The apple rootstocks used in this study may differ in their absorption and translocation of the necessary minerals to the scion. Therefore, the tolerance differences among the rootstocks to low soil pH were determined by analyzing the accumulation of nutrients in the leaves of both ‘Anna’ and ‘Jonagored’ cultivars.

4.6.1 ‘Jonagored’

4.6.1.1 Macronutrients (Ca, Mg, N, K and P)

Calcium concentration in leaves of ‘Jonagored’ grown on three rootstocks had significantly ($P < 0.001$) affected by low soil pH treatments as was differences between ‘Jonagored’ in amount of leaves Ca concentration (Table 35). In soil pH of 3.5, ‘Jonagored’ grafted on MM.106 contain highest calcium (7815 mg kg^{-1}) followed on MM.111 (7690 mg kg^{-1}) and least was found on M.9 ($6757.50 \text{ mg kg}^{-1}$). Similar ranks for rootstocks as pH 3.5 but with increase in Ca content was recorded under pH 4.9 (Fig. 17). The interaction effect of ‘Jonagored’ and low soil pH treatments on leaves Ca content was also significant ($P < 0.001$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in Ca concentration under different levels of soil pH (Table 30). In leaves of ‘Jonagored’, the amount of Ca increased with increase in level of soil pH. The maximum uptake of Ca was recorded in soil pH of 6.3 whereas least was found in pH 3.5 (Table 30). Under soil pH of 6.3, the maximum concentration of Ca was found in leaves of ‘Jonagored’ grafted on

MM.106 followed on MM.111 while least amount was recorded on M.9. The maximum uptake and transport of calcium was found in leaves of ‘Jonagored’ grafted on MM.106, contrary to ‘Anna’ which recorded least on similar rootstock.

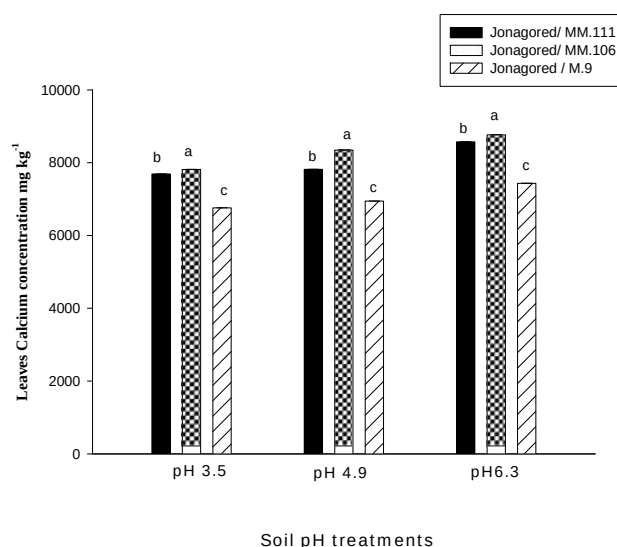


Figure 17: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves Ca content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Magnesium content in leaves of ‘Jonagored’ was significantly ($P < 0.001$) affected by low soil pH treatments as was differences in leaves Mg concentration between ‘Jonagored’ cultivars (Table 35). In pH of 3.5 ‘Jonagored’ grafted on MM.106 contain highest Mg (2110 mg kg⁻¹) followed on MM.111 (1820 mg kg⁻¹) and least was found on M.9 (1650.52 mg kg⁻¹). Under naturally occurring acidic soil (pH 4.9) similar to pH 3.5 the concentration of Mg in leaves of ‘Jonagored’ grafted on MM.106 (2213 mg kg⁻¹) contain maximum followed on MM.111 (1895 mg kg⁻¹) and

minimum content was found on M.9(1683.3 mg kg⁻¹) (Fig. 18). The interaction effect of ‘Jonagored’ and low soil pH treatments on leaves Mg content was significant ($P < 0.001$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in Mg concentration under different levels of soil pH (Table 30). The amount of Mg in leaves of ‘Jonagored’ increased with an increase in soil pH. Under soil pH of 6.3 the amount of Mg in leaves of ‘Jonagored’ grafted on MM.106 was highest (2397 mg kg⁻¹) followed on MM.111 (1943 mg kg⁻¹) and least was found on M.9 (1683.3 mg kg⁻¹) (Table 30).

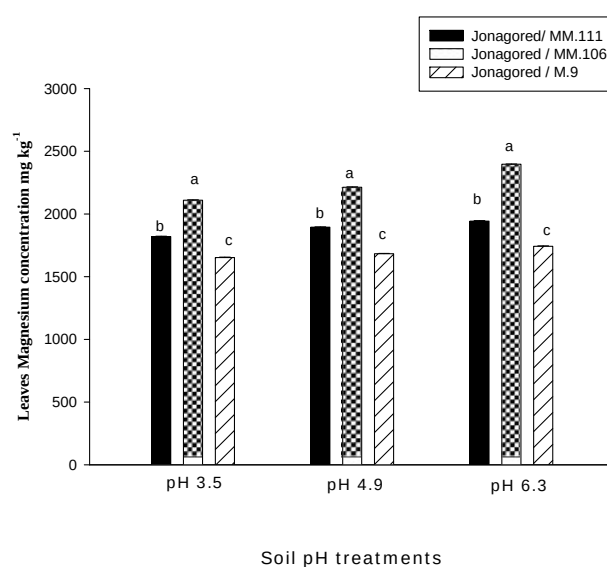


Figure 18: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves Mg content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Table 30: Effect of rootstock type, low soil pH and its interaction on vegetative growth of on leaf macro nutrients (dw⁻¹) of ‘Jonagored’ . Values are the means of 3 repetitions ± SE.

Cultivar/ Rootstocks	Macro nutrients(mg kg ⁻¹)	pH		
		3.5	4.9	6.3
‘Jonagored’/ MM.111	Ca	7690.00 ± 2.94c	7816 ± 3.5b	8573 ± 3.8a
	Mg	1820.00 ± 2.9c	1895 ± 1.76b	1943 ± 4.11a
	N	1.81 ± .029c	2.02 ± .0117b	2.16 ± .047a
	K	20000.0 ± 2.9c	21134 ± 4.1b	23642 ± 3.5a
	P	1867.0 ± 4.1c	1886.0 ± 3.5b	1907.5 ± 4.1a
‘Jonagored’/ MM.106	Ca	7815.00 ± 2.9c	8346.00 ± 3.5b	8765 ± 3.8a
	Mg	2110.00 ± 2.94c	2213 ± 3.5b	2397 ± 1.17a
	N	2.35 ± .029b	2.39 ± .023ab	2.46 ± .035a
	K	16500.0 ± 15.6c	17100 ± 29.4b	17833 ± 3.5a
	P	2041.5 ± 2.9c	2106.3 ± 3.5b	2263 ± 1.76a
‘Jonagored’ / M.9	Ca	6757.50 ± 4.1c	6945 ± 2.94b	7432 ± 3.5a
	Mg	1652.50 ± 3.5c	1683.3 ± 1.76b	1742 ± 3.5a
	N	2.06 ± .035a	2.09 ± .052a	2.1 ± .029a
	K	26812.5 ± 3.5c	26940 ± 2.35b	27110 ± 2.9a
	P	1896.0 ± 1.76c	1913 ± 3.5b	1954 ± 2.35a

Means with same letters in rows indicate non-significant differences at P< 0.05 by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH. *dw⁻¹ = dry weight

Potassium concentration in leaves of ‘Jonagored’ was significantly (P< 0.001) affected by low soil pH as was differences in amount of leaves K content between ‘Jonagored’ grafted on three rootstocks were significant (Table 35). The maximum uptake of potassium was obtained in leaves of ‘Jonagored’ grafted on M.9 (26812.5 mg kg⁻¹) followed on MM.111 (20000 mg kg⁻¹) and minimum concentration was found on MM.106 (16500 mg kg⁻¹) in soil pH of 3.5 (Fig. 19). On the other hand, the interaction effect of ‘Jonagored’ and low soil pH treatments on leaves K content was also significant (P< 0.001). Duncan LSD showed that Jonagored grafted on same

types of rootstocks differs in K concentration under different levels of soil pH (Table 30). The highest amount of potassium in ‘Jonagored’ leaves was observed in the treatment with soil pH of 6.3 whereas least was found in pH 3.5. In soil pH of 4.9 and 6.3 the highest amount of potassium was found in leaves of ‘Jonagored’ grown on M.9 followed on MM.111 and least was found on MM.106 (Table 30).

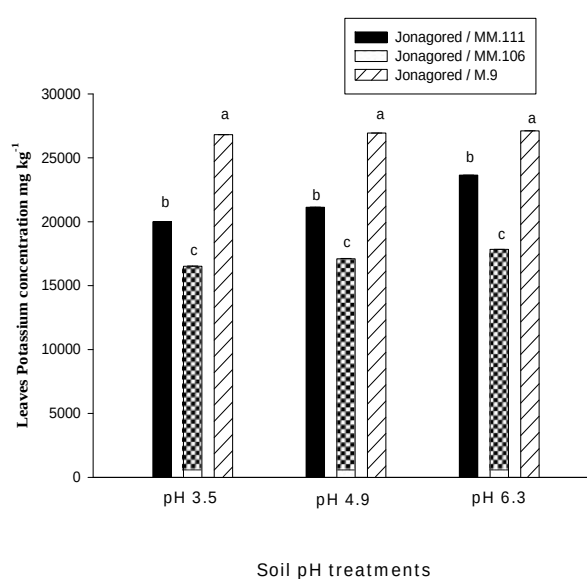


Figure 19: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves K content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

The concentration of phosphorous in leaves of ‘Jonagored’ grown on three rootstocks was significantly ($P < 0.001$) affected by low soil pH treatments. The differences between ‘Jonagored’ in amount of leaves P concentration was significant ($P < 0.001$) (Table 35). Under soil pH of 3.5, the highest accumulation of phosphorous was found in leaves of ‘Jonagored’ grown on MM.106 ($2041.5 \text{ mg kg}^{-1}$) followed on M.9 (1896 mg kg^{-1}) whereas least amount was recorded on MM.111

(1867 mg kg⁻¹) (Fig 20). The interaction effect of ‘Jonagored’ and low soil pH treatments in terms of leaves P content was also significant ($P < 0.001$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in P concentration under different levels of soil pH (Table 30). In leaves of ‘Jonagored’, the amount of phosphorous accumulation increased with increase in pH level. The maximum and minimum concentration was recorded under pH 6.3 and 3.5 respectively. In soil pH of 4.9 and 6.3 the phosphorous content of ‘Jonagored’ grown on MM.106 was highest followed on M.9 and least was found in leaves of ‘Jonagored’ grown on MM.111 (Table 30).

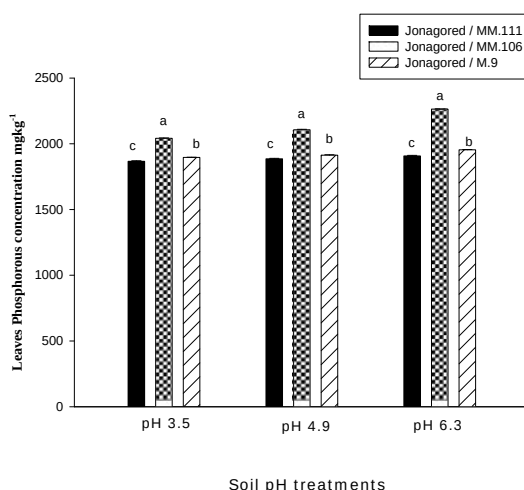


Figure 20: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves P content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Nitrogen concentration in leaves of ‘Jonagored’, was significantly ($P < 0.001$) affected by low soil pH treatments as was differences between ‘Jonagored’ in leaves N accumulation (Table 35). Maximum amount of nitrogen was found in leaves of ‘Jonagored’ grafted on MM.106 (2.35%) followed on M.9 (2.06%) and minimum

value was obtained on MM.111 (1.81%) in soil pH of 3.5 (Fig.32). In pH 4.9 the maximum content of N was registered in leaves of ‘Jonagored’ grown on MM.106 (2.9%) followed on M.9 (2.09%) and least was recorded on MM.111 (2.02%). The interaction effect of ‘Jonagored’ and low soil pH treatments on leaves N content was also significant ($P \leq 0.05$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in P concentration under different levels of soil pH (Table 30). The leaf nitrogen content increased in pH 4.9 and highest value was recorded under soil pH of 6.3. In soil pH of 6.3, ‘Jonagored’ grown on MM.106 obtained highest (2.46%) N content followed on MM.111 (2.16%) whereas least was found in leaves of ‘Jonagored’ grafted on M.9 (2.10%) (Table 30).

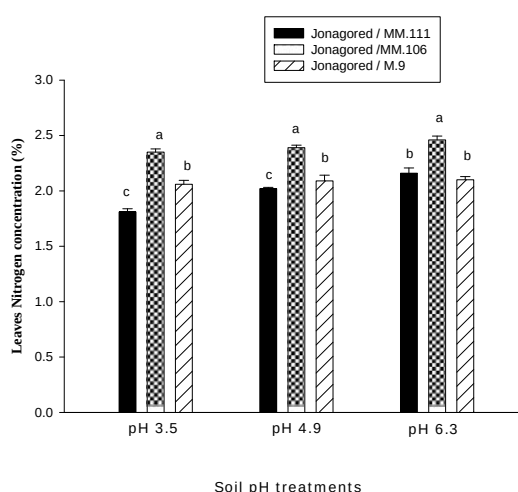


Figure 21: Effect of rootstock type and low soil pH interaction on ‘Jonagored’ leaves N content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

4.6.1. 2 Al, Mn and Fe

Aluminium concentration in leaves of ‘Jonagored’ was significantly ($P < 0.001$) affected by low soil pH treatments as was differences in leaves Al concentration

between ‘Jonagored’ cultivars (Table 35). Leaves of ‘Jonagored’ on MM.106 rootstock had significantly higher (158.5 mg kg⁻¹) concentration of Al followed on M.9 (127.5 mg kg⁻¹) and minimum content was found on MM.111 (96.5 mg kg⁻¹) in soil pH of 3.5. Under naturally occurring acidic soil (pH 4.9), ‘Jonagored’ grafted on MM.106 contain high Al (134 mg kg⁻¹) low on M.9 (101.3 mg kg⁻¹) and least on MM.111 (43 mg kg⁻¹) (Fig 22). The Al content in leaves of ‘Jonagored’ on M.9 was found high (64.5 mg kg⁻¹) followed on MM.106 (55.4 mg kg⁻¹) whereas least amount was found on MM.111 (21.5 mg kg⁻¹) under soil pH of 6.3. On the other hand, the interaction effect of ‘Jonagored’ and low soil pH treatments on leaves Al content was also significant ($P < 0.001$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in Al concentration under different levels of soil pH (Table 31). The increase in leaves Al accumulation was recorded with decrease in low soil pH.

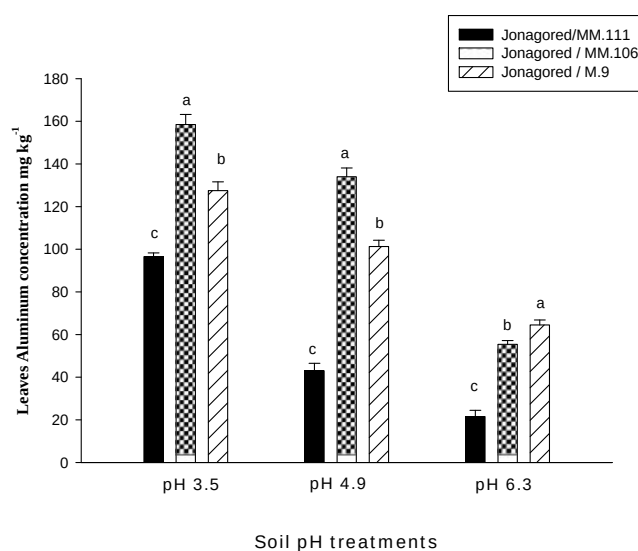


Figure 22: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves Al content.

Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Manganese concentration in leaves of 'Jonagored' was significantly ($P < 0.001$) affected by low soil pH treatments as was differences in leaves Mn concentration between 'Jonagored' cultivars (Table 35). Leaves of 'Jonagored' grown on MM.106 recorded the highest concentration of Mn (201 mg kg^{-1}), lowest on M.9 ($171.35 \text{ mg kg}^{-1}$), and least on MM.111 ($101.95 \text{ mg kg}^{-1}$) under soil of pH 3.5. The uptake and transport of M.9 and MM.111 for Mn was high in 'Jonagored' compared to 'Anna' grafted on similar rootstocks. Under soil pH of 4.9, maximum Mn content was found in leaves of 'Jonagored' grown on MM.106 (174.5 mg kg^{-1}), least was on MM.111 (85.3 mg kg^{-1}) whereas M.9 recorded intermediate (133 mg kg^{-1}) (Fig 23). Under soil pH of 6.3, Mn was high in leaves of 'Jonagored' grafted on MM.106 (98 mg kg^{-1}), followed by M.9 (85 mg kg^{-1}) whereas least concentration was found in leaves of 'Jonagored' grown on MM.111 (53 mg kg^{-1}) (Fig 23).

The interaction effect of 'Jonagored' and low soil pH treatments on leaves Mn content was also significant ($P < 0.001$). Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in Mn concentration under different levels of soil pH (Table 30). The solubility of manganese decreased with increase in soil pH to 6.3. In soil pH of 6.3 the accumulation of Mn in leaves of 'Jonagored' grown on all three rootstocks was less compared to other two soil pH (Table 31).

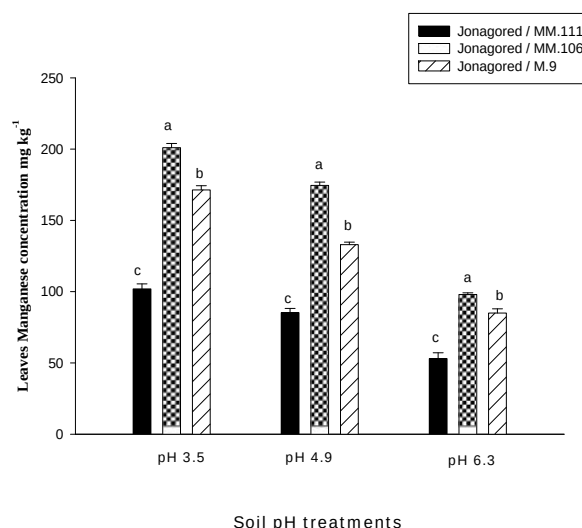


Figure 23: Effect of rootstock type and low soil pH on ‘Jonagored’ leaves Mn content. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

According to the result of analysis of variance, the amount of iron in leaves of ‘Jonagored’ was significantly ($P < 0.001$) influenced by low soil pH. The differences in leaves Fe concentration between ‘Jonagored’ cultivars were also significant ($P < 0.001$) (Table 35). Under soil pH of 3.5, the amount of iron found in leaves of ‘Jonagored’ grafted on M.9 was highest ($166.38 \text{ mg kg}^{-1}$), lowest on MM.111 ($136.30 \text{ mg kg}^{-1}$) and least was found on MM.106 ($117.40 \text{ mg kg}^{-1}$) (Fig 32). Under naturally occurring acidic soil (pH 4.9), the amount of iron found in leaves of ‘Jonagored’ grown on M.9 was high ($146.30 \text{ mg kg}^{-1}$) followed on MM.111 (112.5 mg kg^{-1}) and least content was recorded on MM.106 (103 mg kg^{-1}). In soil pH of 6.3 next to M.9 more iron content was found in leaves of ‘Jonagored’ grafted on MM.106 followed on MM.111 (Fig 24). Highest concentration of iron in all three pH levels were registered in leaves of both ‘Anna’ and ‘Jonagored’ grafted on M.9. On

the other hand, the interaction effect of ‘Jonagored’ and low soil pH treatments on leaves Fe content was also significant ($P < 0.001$).

Duncan LSD showed that Jonagored grafted on same types of rootstocks differs in Fe concentration under different levels of soil pH (Table 31). The solubility of iron increased with decrease in soil pH to 3.5. In soil pH of 3.5 the accumulation of Fe in leaves of ‘Jonagored’ grown on all three rootstocks was high whereas least concentration was found in pH 6.3 (Table 31).

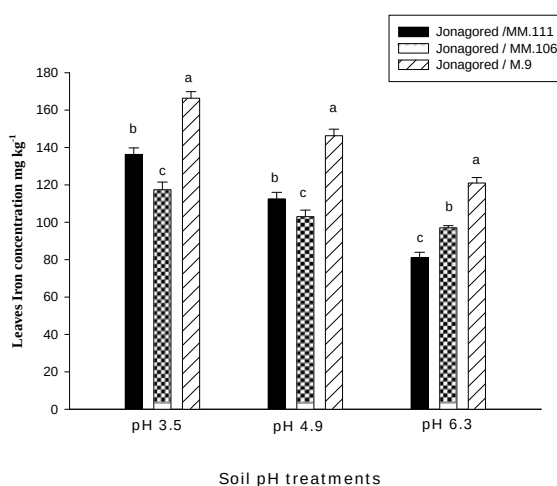


Figure 24: Effect of rootstock type and low soil pH on leaves Fe content of ‘Jonagored. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

The correlation coefficient between toxic nutrients and element on growth and yield of Jonagored cultivar was analysed. The result showed that, Al and Mn were negatively significantly correlated with yield of Jonagored cultivar (Table 32).

Table 31: Effect of rootstock type, low soil pH and its interaction on vegetative growth of on leaf Al, Mn, and Fe concentration (dw^{-1}) of ‘Jonagored’. Values are the means of 3 repetitions $\pm$ SE.

Cultivar/ Rootstocks	Nutrients (mg kg^{-1})	pH		
		3.5	4.9	6.3
Jonagored /MM.111	Al	96.5 $\pm$ 1.76a	43 $\pm$ 3.5b	21.5 $\pm$ 2.9c
	Mn	101.95 $\pm$ 3.5a	85.3 $\pm$ 2.9b	53 $\pm$ 4.1c
	Fe	136.30 $\pm$ 3.5a	112.5 $\pm$ 3.5b	81 $\pm$ 2.9c
Jonagored /MM.106	Al	158.5 $\pm$ 4.7a	134 $\pm$ 4.1b	55.4 $\pm$ 1.76c
	Mn	201 $\pm$ 2.94a	174.5 $\pm$ 2.35b	98 $\pm$ 1.17c
	Fe	117.40 $\pm$ 4.1a	103 $\pm$ 3.5b	97 $\pm$ 1.17c
Jonagored /M.9	Al	127.5 $\pm$ 4.1a	101.3 $\pm$ 2.9b	64.5 $\pm$ 2.35c
	Mn	171.35 $\pm$ 2.9a	133 $\pm$ 1.76b	85 $\pm$ 2.94c
	Fe	166.38 $\pm$ 3.5a	146.3 $\pm$ 3.5b	121 $\pm$ 2.9c

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH. * dw^{-1} = dry weight

Table 32: Correlation coefficient between toxic nutrients and element with growth and yield of Jonagored cultivar

Source		Al	Mn	Fe	Branch es	Total height	Mean flower	Mean fruit	TCSA (cm^2)	Leaf area (cm^2)
Al	Pearson Correlation	1	.925*	.642**	.130	.286	-.279	-.306	-.106	-.313
	Sig. (2-tailed)		.000	.000	.517	.149	.158	.120	.599	.112
Mn	Pearson Correlation	.925**	1	.728**	-.008	.097	-.111	-.121	-.053	-.287
	Sig. (2-tailed)	.000		.000	.969	.631	.581	.548	.792	.147
Fe	Pearson Correlation	.642**	.728*	1	.095	.080	.094	.072	.083	.079
	Sig. (2-tailed)	.000	.000		.637	.692	.639	.721	.679	.696

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

4.6.2 Anna

4.6.2.1. Macronutrients (Ca, Mg, N, K and P)

The analysis of variance showed that low soil pH significantly ($P < 0.001$) affected the leaves calcium content of 'Anna' grafted on three rootstocks as was differences in leaves Ca concentration between 'Anna' (Table 35). 'Anna' grafted on MM.111 accumulate highest Ca (13200 mg kg^{-1}) followed by M.9 (9660 mg kg^{-1}) whereas least was recorded for 'Anna' grafted on MM.106 (8295 mg kg^{-1}) under soil pH of 3.5. Under soil pH of 4.9 'Anna' grown on MM.111 recorded maximum concentration of Ca followed on M.9 and least was found on MM.106. In soil pH of 6.3, 'Anna' on MM.106 recorded more calcium next to MM.111 whereas least concentration was recorded for M.9 (Fig 25).

The interaction effect of 'Anna' and low soil pH treatments on leaves Ca content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in Ca concentration under different levels of soil pH (Table 33). The concentration of calcium in leaves of 'Anna' increased with an increase in soil pH to 4.9 and highest accumulation was found in soil pH of 6.3 (Table 33). In all three levels of soil pH, 'Anna' grown on MM.111 recorded vigorous uptake and transport of calcium compared to MM.06 and M.9 rootstocks. Calcium content of 'Anna' grown on MM.111 was significantly higher than all other rootstocks across low soil pH.

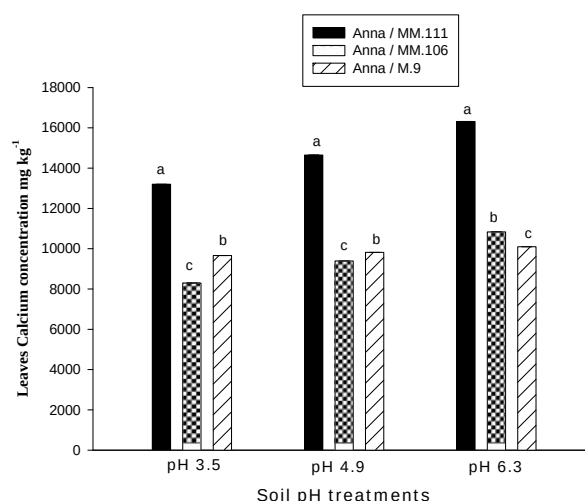


Figure 25: Effect of rootstock type and low soil pH on leaves Ca content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Table 33: Effect of rootstock type, low soil pH and its interaction on vegetative growth of on leaf macro nutrients (dw^{-1}) of Anna. Values are the means of 3 repetitions $\pm$ SE.

Cultivars/ Rootstocks	Macro nutrients(mg kg^{-1})	pH		
		3.5	4.9	6.3
Anna/ MM.111	Ca	13200 $\pm$ 2.9c	14650 $\pm$ 2.9b	16315 $\pm$ 2.9a
	Mg	2240 $\pm$ 2.9c	2370 $\pm$ 2.9b	2457 $\pm$ 4.1a
	K	16500.0 $\pm$ 17.6c	16635.2 $\pm$ 2.9b	16750 $\pm$ 2.9a
	P	2493.5 $\pm$ 1.76c	2571.3 $\pm$ 4.1b	2646.4 $\pm$ 3.5a
Anna/ MM.106	Ca	8295 $\pm$ 2.9c	9390 $\pm$ 2.9b	10835 $\pm$ 2.9a
	Mg	1875 $\pm$ 2.9c	2313 $\pm$ 1.76b	2435 $\pm$ 2.9a
	K	16250.0 $\pm$ 2.9c	16310 $\pm$ 2.9b	16445.3 $\pm$ 2.9a
	P	1779.5 $\pm$ 5.3c	1869.4 $\pm$ 5.3b	1945.5 $\pm$ 2.9a
Anna/ M.9	Ca	9660 $\pm$ 2.9c	9816 $\pm$ 3.5b	10095 $\pm$ 2.9a
	Mg	2110 $\pm$ 2.9c	2200.3 $\pm$ 2.9b	2340 $\pm$ 2.9a
	K	17500.0 $\pm$ 5.8c	18900 $\pm$ 5.8b	19100 $\pm$ 2.9a
	P	1787.0 $\pm$ 4.1c	1814 $\pm$ 2.4b	1871 $\pm$.58a

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Letters across soil pH indicated differences between same rootstock under different level of soil pH. dw^{-1} = dry weight

Magnesium accumulation in leaves of 'Anna' was significantly ($P < 0.001$) affected by low soil pH. According to analysis of variance the differences in leaves magnesium concentration between 'Anna' cultivars were also significant ($P < 0.001$) (Table 35). In soil pH of 3.5 'Anna' grafted on MM.111 accumulate highest Mg (2240 mg kg^{-1}) followed on M.9 (2110 mg kg^{-1}) whereas least was recorded for 'Anna' grafted on MM.106 (1875 mg kg^{-1}). Under naturally occurring acidic soil (pH 4.9) 'Anna' grafted on MM.111 accumulate highest magnesium (2370 mg kg^{-1}) followed on MM.106 (2313 mg kg^{-1}) whereas least was recorded for 'Anna' grafted on M.9 ($2200.3 \text{ mg kg}^{-1}$) (Fig 26).

The interaction effect of 'Anna' and low soil pH treatments in terms of leaves Mg content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in Mg concentration under different levels of soil pH (Table 33). The accumulation of Mg in leaves of 'Anna' increased with an increase in level of soil pH to 4.9 and maximum in pH 6.3. Highest Mg content was recorded in leaves of 'Anna' grafted on MM.111 (2457 mg kg^{-1}) followed on MM.106 (2435 mg kg^{-1}) whereas least was recorded for 'Anna' grafted on M.9 (2340 mg kg^{-1}) in soil pH of 6.3 (Table 33).

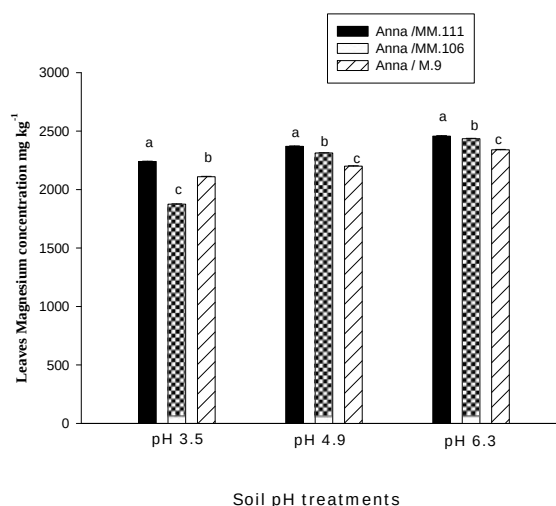


Figure 26: Effect of rootstock type and low soil pH on leaves Mg content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Significant ($P < 0.001$) effect of low soil pH on potassium content of ‘Anna’ leaves was recorded as was differences in leaves potassium concentration between ‘Anna’ cultivars (Table 35). Potassium content of ‘Anna’ leaves grown on M.9 (17500 mg kg^{-1}) was high followed on MM.111 (16500 mg kg^{-1}) and least content was found on MM.106 (16250 mg kg^{-1}) in soil pH of 3.5 (Fig.27). The interaction effect of ‘Anna’ and low soil pH treatments on leaves K content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in K concentration under different levels of soil pH (Table 33).The increased in ‘Anna’ leaves K content was recorded with an increase in level of soil pH to 4.9 and maximum in pH 6.3. The amount of potassium was high in leaves of ‘Anna’ grafted on M.9 under all three pH levels with highest concentration under soil pH of 6.3 followed on MM.111 and least was on MM.106 (Table 33).

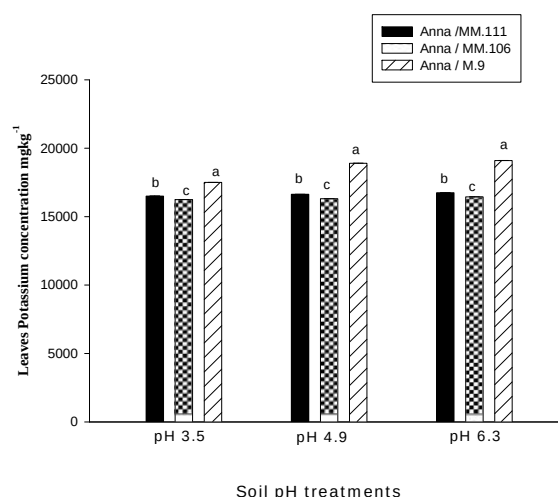


Figure 27: Effect of rootstock type and low soil pH on leaves K content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Phosphorus had a marked influence on fruit quality although it has little effect on yield. Significant ($P < 0.001$) effect of low soil pH treatments was found on phosphorous content of ‘Anna’ leaves grown on three different rootstocks (Table 35). The differences in leaves P concentration between ‘Anna’ cultivars were also significant ($P < 0.001$) (Fig 36). Highest phosphorous contents were found in leaves of ‘Anna’ grown on MM.111 ($2493.5 \text{ mg kg}^{-1}$) followed on both M.9 (1787 mg kg^{-1}) and MM.106 ($1779.5 \text{ mg kg}^{-1}$) under soil pH of 3.5 (Table 33). We observed that, the absorption and transport of phosphorous by MM.106 was more than M.9 with an increase in low soil pH. Among the rootstocks maximum uptake and transport was found in leaves of ‘Anna’ grafted on MM.111 whereas least was found for M.9 in all three different pH levels (Fig 28). The interaction effect of ‘Anna’ and low soil pH treatments on leaves P content was also significant ($P < 0.001$).

Duncan LSD showed that Anna grafted on same types of rootstocks differs in P concentration under different levels of soil pH (Table 33). The increase in ‘Anna’ leaves P content was recorded with an increase in level of soil pH with maximum under pH 6.3. In soil pH of 4.9 and 6.3 next to MM.111 high content of phosphorous was found in leaves of ‘Anna’ grafted on MM.106 than M.9 (Table 33).

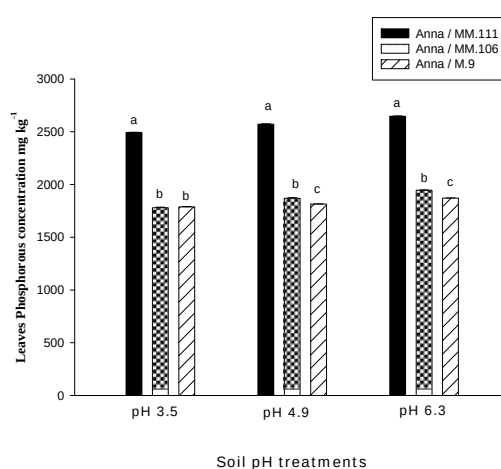


Figure 28: Effect of rootstock type and low soil pH on leaves P content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Nitrogen is important element required for plant growth and development. The result of analysis of variance showed that, the effect of low soil pH on leaves nitrogen content of ‘Anna’ was not significant. Similarly, no significant differences were found in leaves N content of ‘Anna’ grown on all three rootstocks. Similarly, the interaction effect of ‘Anna’ and low soil pH treatments on leaves N content was also statistically not significant (Table 35). However, among the rootstocks the data showed the nitrogen levels for ‘Anna’ were similar on all three rootstocks at soil pH of 3.5. In

leaves of Anna, the nitrogen content increased with an increase in soil pH to 4.9 and 6.3 (Table 33). Under naturally occurring acidic soil of pH 4.9, there were no significant differences in nitrogen content in ‘Anna’ grown on the three rootstocks. (Fig. 29).

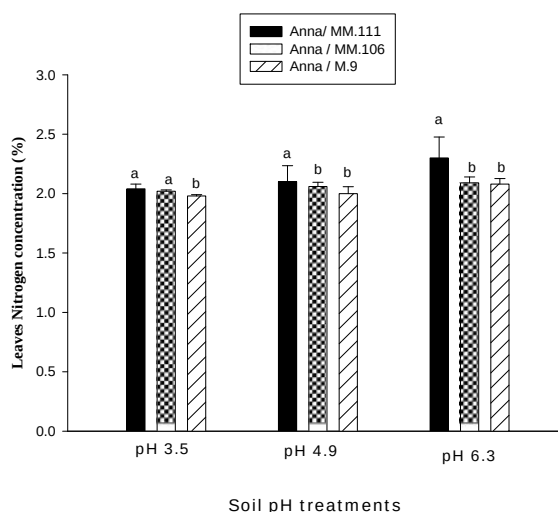


Figure 29: Effect of rootstock type and low soil pH on leaf N concentration of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

4.6.2.2. Al, Mn and Fe

The effect of low soil pH on Al content of ‘Anna’ leaves grown on three rootstocks was significant ($P < 0.001$) as was differences in leaves Al accumulation between ‘Anna’ cultivars (Table 35). The uptake and concentration of aluminium in leaves of ‘Anna’ grafted on MM.106 (196.7 mg kg^{-1}) was highest followed on M.9 (134.3 mg kg^{-1}) whereas least was on MM.111 (87.4 mg kg^{-1}) in pH 3.5. Under soil pH of 4.9 the amount of aluminium found in leaves of ‘Anna’ grafted on MM.106 were high (121

mg kg⁻¹), low on M.9 (98 mg kg⁻¹) and least on MM.111 (56 mg kg⁻¹) (Fig 34). The interaction effect of ‘Anna’ and low soil pH treatments on leaves Al content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in Al concentration under different levels of soil pH (Table 34). The amount of aluminium decreased with an increase in soil pH. In soil pH of 6.3 more aluminium was recorded for ‘Anna’ grafted on MM.106 (85.4 mg kg⁻¹) followed on M.9 (74.5 mg kg⁻¹) and least was found in leaves of ‘Anna’ grown on MM.111 (31.5 mg kg⁻¹) (Table 34).

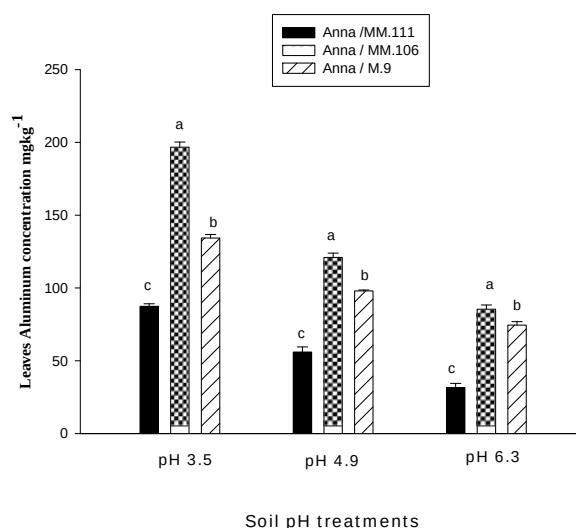


Figure 30: Effect of rootstock type and low soil pH on leaves Al content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

The concentration of manganese in leaves of ‘Anna’ was significantly ($P < 0.001$) affected by low soil pH treatments as was differences in leaves Mn concentration between ‘Anna’ cultivars (Table 35). Among the three rootstocks the highest amount

of manganese was found in leaves of ‘Anna’ grafted on MM.106 (220.38 mg kg⁻¹), followed on M.9 (114.5 mg kg⁻¹) and least was found on MM.111 (113 mg kg⁻¹) in soil pH of 3.5. Under naturally occurring acidic soil (pH 4.9), highest value was recorded for ‘Anna’ grown on MM.106 (114 mg kg⁻¹) followed on M.9 (85 mg kg⁻¹) and least was recorded on MM.111 (48 mg kg⁻¹) (Fig 31). The interaction effect of ‘Anna’ and low soil pH treatments in terms of leaves Mn content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in Mn concentration under different levels of soil pH (Table 34). The amount of Mn in leaves of ‘Anna’ decreased with an increase in soil pH. Under soil pH of 6.3, the highest Mn content was found in leaves of ‘Anna’ grown on M.9 (43 mg kg⁻¹) followed on MM.106 (33 mg kg⁻¹) while, least was recorded on MM.111 (26 mg kg⁻¹) (Table 34).

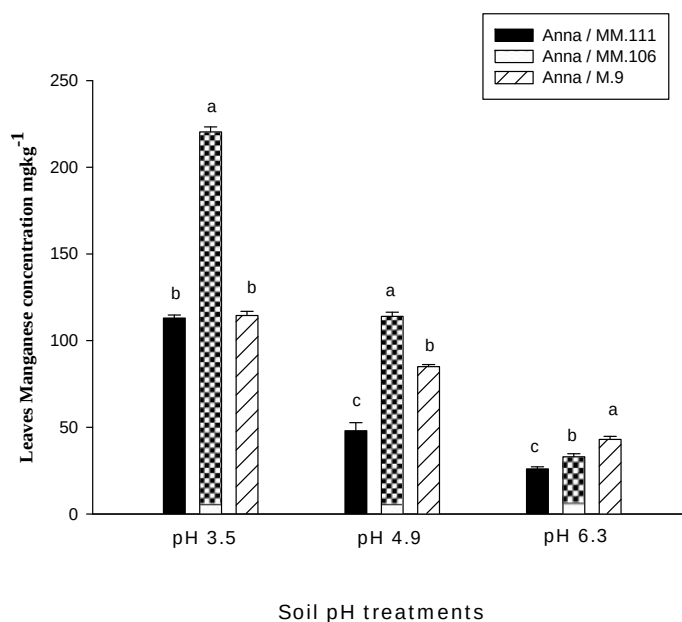


Figure 31: Effect of rootstock type and low soil pH on leaves Mn content of Anna. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

The result of analysis of variance showed significant ($P < 0.001$) effect of low soil pH on leaves iron content of ‘Anna’ as was differences in leaves Fe concentration between ‘Anna’ cultivars (Table 35). Contrary to Al and Mn, maximum iron was found in leaves of ‘Anna’ grafted on MM.111 ($170.10 \text{ mg kg}^{-1}$) followed on M.9 ($169.10 \text{ mg kg}^{-1}$) whereas least was recorded for ‘Anna’ grown on MM.106 ($149.05 \text{ mg kg}^{-1}$) in soil pH of 3.5 (Fig 34). Under naturally occurring acidic soil of pH 4.9, ‘Anna’ grown on M.9 contain more Fe (118 mg kg^{-1}) followed on MM.106 (103 mg kg^{-1}) and least was on MM.111 (93 mg kg^{-1}). Similar ranks for rootstocks but with less value were recorded in soil pH of 6.3 (Table 34).

The interaction effect of ‘Anna’ and low soil pH treatments on leaves Fe content was also significant ($P < 0.001$). Duncan LSD showed that Anna grafted on same types of rootstocks differs in Fe concentration under different levels of soil pH (Table 31). Leaves of ‘Anna’ Fe content decreased with an increase in soil pH. The highest was recorded under strongly acidic soil of pH 3.5, low in soil pH of 4.9 whereas least amount was found in soil pH of 6.3 (Table 34).

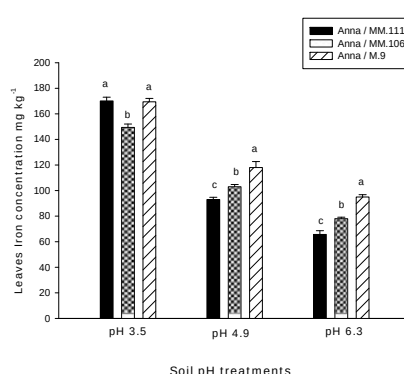


Figure 32: Effect of rootstock type and low soil pH on Fe content of ‘Anna’ leaves. Different letters above bars indicate difference between rootstocks at same pH. Values are the means of 3 repetitions $\pm$ SE.

Table 34: Effect of rootstock type, low soil pH and its interaction on vegetative growth of on leaf Al, Mn, and Fe concentration (dw^{-1}) of Anna. Values are the means of 3 repetitions $\pm$ SE.

Cultivar/ Rootstocks	Nutrients (mg kg^{-1})	pH		
		3.5	4.9	6.3
Anna/ MM.111	Al	$87.4 \pm 1.76a$	$56 \pm 3.5b$	$31.5 \pm 2.9c$
	Mn	$113.00 \pm 1.76a$	$48 \pm 4.7b$	$26 \pm 1.2c$
	Fe	$170.10 \pm 2.9a$	$93 \pm 1.76b$	$65.7 \pm 2.9c$
Anna/ MM.106	Al	$196.7 \pm 3.5a$	$121 \pm 2.9b$	$85.4 \pm 2.9c$
	Mn	$220.38 \pm 2.9a$	$114 \pm 2.4b$	$33 \pm 1.76c$
	Fe	$149.05 \pm 2.65a$	$103 \pm 1.76b$	$78 \pm 1.17c$
Anna/ M.9	Al	$134.3 \pm 2.4a$	$98 \pm .58b$	$74.5 \pm 2.4c$
	Mn	$114.50 \pm 2.4a$	$85 \pm 1.17b$	$43 \pm 1.76c$
	Fe	$169.10 \pm 2.65a$	$118 \pm 4.7b$	$95 \pm 1.76c$

Means with same letters in rows indicate non-significant differences at $P < 0.05$ by Duncan test. Data are means of three replicates. Letters across soil pH indicated differences between same rootstock under different level of soil pH. dw^{-1} = dry weight

Table 35: Two way Analysis of Variance which tested the effects of rootstocks, low soil pH and interaction of these factors on leaves nutrients and element concentration of Jonagored and Anna.

Source	nutrients and element	P-value	nutrient	P-value
Differences between Jonagored and Anna	Ca, K, P, Mg, Fe, Mn, N and Al (element)	0.001 (s)	N	> 0.05 (ns) for Anna
Effect of low soil pH	Ca, K, P, Mg, Fe, Mn, N and Al (element)	0.001 (s)	N	> 0.05 (ns) for Anna
Interaction effect of rootstocks and low soil pH	Ca, K, P, Mg, Fe, Mn, N and Al (element)	> 0.05 (ns)	N	> 0.05 (ns) for Anna

ns = not significant, s = significant

The correlation coefficient analysis showed that the accumulation of excess toxic nutrient and element negatively correlated with most vegetative growth and yield of Anna cultivar. Al and Mn was significantly negatively correlated with yield of Anna cultivar (Table 36).

Table 36:Correlation coefficient between toxic nutrients and element with growth and yield of Anna cultivar

Source		Al	Mn	Fe	branch es	Total heigh t	Mean flower	Mean fruit	TCSA (cm ²)	Leaf area(c m ²)
Al	Pearson	1	.925**	.642**	-.019	.192	-.451*	-.253	-.360	-.639
	Correlation									
	Sig. (2-tailed)		.000	.000	.923	.337	.018	.202	.065	.000
Mn	Pearson	.925**	1	.728**	-.184	.103	-.185	-.180	-.117	-.575
	Correlation									
	Sig. (2-tailed)	.000		.000	.357	.608	.355	.368	.563	.002
Fe	Pearson	.642**	.728**	1	.067	.216	.005	-.020	.094	.236
	Correlation									
	Sig. (2-tailed)	.000	.000		.740	.279	.979	.923	.640	.236

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

CHAPTER FIVE

DISCUSSION

5.1. Characterization of Experimental Soil

The CEC estimates the number of exchange sites in a given soil sample that would be capable of holding positively charged nutrients (Brady and Weil, 2002). The positively charged nutrients that were considered in this study include calcium, magnesium, and potassium. These basic cations are adsorbed onto either clay particles or organic matter (SOM) of the soil of the micro plot at initial pH levels. Previous study showed that, a soil suitable for fruits should have a CEC of 25-35 cmol(+) kg⁻¹; whereas values lower than 20 indicated a soil with low buffering and potentially poor fertility (Webster, 2005). The results of the present study revealed that, the soil pH of 4.9 had (23.59), (9.97), (3.7) and (0.78) of CEC, Ca, Mg and K contents, respectively. According to Pam and Brain (2007) all the values were high, except Ca, which was in the moderate category. The values of Ca, Mg, K and CEC decreased to 3.7, 1.11, 0.62 and 17.05 cmol(+) kg⁻¹, respectively, with decreasing the pH to 3.5 and all could be considered low, except K that was under moderate category (Webster, 2005; Pam and Brain, 2007).

According to Kamprath (1970) the available exchangeable Al in soil at pH of 4.9 and 4.5 was 0.60 and 0.91 meq/100g respectively. In our experiment high exchangeable Al (4.2 meq/100g soil) was recorded under soil pH of 4.9 (Table 2). The exchangeable Al was further increased to 11.4 meq/100 g soil when the soil pH was

reduced to 3.5. This could be considered as very high in accordance with Kamprath (1970) (Table 2). The content of Al in soils increases under soil pH < 5.5 whereas Ca, Mg, and K decrease (Pam and Brain, 2007). The decrease in amount of exchangeable bases could be due to the presence of high soluble Al in soil pH of 4.9 and highest in pH 3.5 that displaced the basic cations from the active exchange sites of the soil.

According to Sims and Johnson (1991) the critical levels of available Fe and Mn for crop production are 2.5-5 mg kg⁻¹ and 3.8 mg kg⁻¹ respectively. Therefore, the average mean values of available Fe and Mn found in both soil of pH 4.9 (7950 mg kg⁻¹ and 760.21 mg kg⁻¹ respectively) and 3.5 (834.5 mg kg⁻¹ and 108.60 mg kg⁻¹ respectively) qualified above the adequate range for the rootstocks and cultivars grown in the micro plot (Table 2). The accumulation of Fe and Mn might be due the high content of hydrogen ions (H⁺) which increase the solubility of Mn and Fe in acid soils (Rhodes, 1978). Reports on the total Mn content in soils are variable showing values from 15 to 17 mg kg⁻¹ (Hue and Mai, 2002), 20 to 10,000 mg kg⁻¹ (Sparks, 1995) and 450 to 4,000 mg kg⁻¹ soil (Adriano, 2001).

According to Landon (1991), available soil P level of < 5 mg kg⁻¹ is rated as low; 5-15 mg kg⁻¹ as medium and > 15 mg kg⁻¹ is rated as high. The available P of the soils of the study site qualified for the high range (Table 2). The high contents of P found in both 4.9 and 3.5 pH level could possibly be due to the farmers' continuous usage of animal manure and compost to promote the soil fertility of the farm land used for our experiment. Low soil pH < 5 was reported to reduce the availability of P in the soil (Pam and Brain, 2007). Even though P decreased in strongly acidic soil of pH 3.5, the amount of available P found was adequate for the uptake of the rootstocks. Following

the rating of total N of > 1% as very high, 0.5 to 1% high, 0.2 to 0.5% medium, 0.1 to 0.2% low and < 0.1% as very low N status as indicated by Landon (1991), soil pH of 4.9(0.23%) qualified for the medium range while soil pH of 3.5(0.13%) qualified for the low range indicating that the availability of N decreased with the decrease in soil pH < 5 (Pam and Brain, 2007).

5.2. Effect of Low Soil pH on Rootstocks Soil elemental status

A plant's ability to endure various pH levels depends largely on its ability to take up and utilize nutrients at varying concentrations in the soil solution. The maximum availability of the primary nutrients required for plant growth is greatest at a pH value between 6.5 and 7.5(IPNI, 2010). Most temperate fruits thrive best in soils with pH of 5.5 to 6.5 (Webster, 2005). The effect of low soil pH on rootstocks tissue nutrient concentration was significant ($P < 0.001$) in the present study for some nutrients. In apple rootstocks leaves, MM.111, MM.106, M.9 grown under soil pH less than 6.3, we found significantly increasing contents of Al, Mn, and Fe with decreasing in soil pH (appendix Table 3).

The standard values of nutrient levels in apple tree leaves established by Joseph (2004) indicates the ranges for N, P, K, Ca, and Mg, 2.00-2.25, 0.20-0.30, 1.25-1.75, 1.20-1.60, 0.25-0.40, and that of Fe and Mn as 100-300 and 40-100 mg kg⁻¹, respectively. Data on nutrients concentrations in leaves indicated significant differences among the rootstocks (appendix Table 3). In strongly acidic soil of pH 3.5, 2.78, 0.19, 1.22, 1.09, and 0.25% N, P, K, Ca and Mg; as well 401 and 195.2 mg kg⁻¹

Fe and Mn, respectively were found in leaves of MM.111. The result showed that, the amounts of P, K, and Ca in leaves of MM.111 treated under pH 3.5 were below the range. The effect of low soil pH on P and K absorption was statistically not significant. However, concentration of these nutrients in leaf of MM.111 and MM.106 was inadequate. In leaves of MM.106, concentrations of N, P, K, Ca and Mg were 2.27, 0.12, 1.25, 0.20 and 0.11%; while that of Fe and Mn were 274 and 318.2 mg kg⁻¹; respectively, indicating the amount of P, Ca, and Mg below the optimum range. The Mg values were even lower at pH 6.3, indicating no clear effect of low soil pH on Mg absorption by MM.106 rootstock. In leaves of M.9, 2.32, 0.16, 1.65, 0.40 and 0.20% N, P, K, Ca, and Mg; as well as 705 and 238.2 mg kg⁻¹ Fe and Mn respectively, were measured. P, Ca, and Mg concentrations in this rootstock were below the range. Uptake and transport of K by M.9 were higher than both MM.111 and MM.106 rootstocks. This could be due to genetic makeup of this rootstock (appendix Table 3). The availability of Ca, Mg, K, and P were limited at low pH; this is because under low soil pH, the solubility of Al, Mn, and Fe increases. The presence of excess Al block the uptake of these largely required nutrients by the roots. However, the increase in leaves Ca concentration was observed with an increase in level of soil pH (Table 3). This is in accordance with Sas and Mercik (2004) who reported that, the concentration of Ca in rootstocks was high under high soil pH.

The amount of soluble Al and Mn accumulation was high in roots and leaves of apple rootstocks respectively (appendix Table 3). It seems that, Al was more accumulated in roots while Mn was transported to the leaves. Our result agrees with finding of Marschner (1995) who reported more Al in roots and Mn in leaves under acidic soil. Mn was rapidly released from the roots into the xylem, reaching photo synthetically

active leaves via the transpiration stream (Millaleo *et al.*, 2010). In this study we observed that excess Mn in leaves was negatively correlated with Ca. This showed that Mn interfere the utilization of Ca in leaves of the rootstocks. In accordance with this it has been reported that, excessive Mn concentrations in leaves can alter utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Ducic and Polle, 2005; Lei *et al.*, 2007). Tolerance differences were observed in Al and Mn toxicity. Among the three rootstocks, under the soil pH of 3.5 the concentration of Al in roots (549 mg kg^{-1}) and Mn in leaves (195.2 mg kg^{-1}) of MM.111 were lowest, whereas Ca and Mg, were highest in the leaves indicating highest resistance of MM.111 to the effect of Al on uptake of nutrients which could be by secreting aluminium binding compounds followed by M.9 roots, which had (879 mg kg^{-1}) Al, and 238.2 mg kg^{-1} Mn in the leaves. In contrast, the highest concentrations of Al (1560 mg kg^{-1}) in roots and Mn (318.2 mg kg^{-1}) in leaves were obtained for MM.106 with low amounts of Ca and Mg in leaves under 3.5 pH.

The correlation coefficient revealed that Al was negatively correlated with Ca, Mg, N and K (Table 5). Thus, the decrease in amount of essential nutrients was caused due to the negative effect of Al on roots to take up these nutrients (appendix Table 3). The accumulation of high Al in roots of MM.106 under pH 3.5 suppress the uptake of essential nutrients, and also inhibited root elongation associated with the observed (Fig.4) severe changes in root morphology, and resulted in curved, stubby and stiff root apices as stated by Vardar *et al.* (2006). Due to its least tolerance to the effect of Al under soil pH of 3.5, the root growth of MM.106 was severely reduced (Table 9). This resulted in minimum uptake of nutrients compared to M.9 rootstock whereas MM.111 with highest root length absorbed more minerals than other two rootstocks.

Under the original acidic soil (pH 4.9) the nutrients concentrations in the leaves of MM.111 were 2.77% N, 0.19% P, 0.50% K, 1.18% Ca, 0.23% Mg, and 219 mg kg⁻¹ Fe and 48.4 mg kg⁻¹ Mn. The nutrient levels were within the range, except for P, K, and Ca contents of the leaves, which were below the range. In leaves of MM.106 2.09% N, 0.25% P, 1.65% K, 0.33% Ca, 0.23% Mg, 453 mg kg⁻¹ Fe and 137.2 mg kg⁻¹ Mn, were recorded showing the amounts of Ca and Mg below the range. Similarly, in leaves of M.9, 2.36, 0.13, 1.60, 0.65, and 0.22% N, P, K, Ca, and Mg; and 880 mg kg⁻¹ Fe and 89.1 mg kg⁻¹ Mn, respectively, was recorded. The contents of Ca, P, and Mg were also below the range in leaves of M.9.

The highest and lowest amounts of nutrients in leaves of the three rootstocks under pH 4.9 treatment were mainly due to differences in response to the high soluble Al, associated with fewer amounts of exchangeable base cations (Table 2). The accumulation of toxic minerals Al, Fe and Mn increased with decrease in pH (Table 7) in the tissues of apple rootstocks. In accordance with this early report indicated that, the decrease in soil pH increases the available Fe, Mn, and also Al contents (Lindsay, 1991). In soil with pH below 5.5 both aluminium and manganese concentrations reach toxic levels (Kamprath, 1970; Vardar *et al.*, 2006). The presence of these excess toxic elements caused adverse effect on essential nutrient absorption by competing with root binding surfaces and caused some nutrient deficiency range under different pH levels. In the leaves of the rootstocks, the excess toxic nutrients also obstruct the utilization by interacting with major nutrients causing reduced growth and weak vigour of the rootstocks (Ducic and Polle, 2005). This study showed that, next to pH 3.5 the naturally acidic soil (pH 4.9) of study area caused the release

of toxic mineral elements by increasing their solubility (Table 7). Thus, intensive addition of lime is required to reduce the concentration of both Al^{3+} and Mn^{2+} in the soil solution and thereby improve apple rootstocks and cultivars growth and yields.

Under the soil pH of 6.3 the nutrients concentrations in the leaves of MM.111 were 2.36 N%, 0.16 P%, 0.55K%, 1.31Ca%, 0.13 Mg%, 159.0 Fe mg kg⁻¹ and 9 Mn mg kg⁻¹. The nutrient levels P, Mg, K, and Mn were below the range while, Ca and N were within the range. In leaves of MM.106, 0.39 N%, 0.24 P%, 0.70K%, 0.53 Ca%, 0.10Mg%, 334 Fe mg kg⁻¹ and 30.1 Mn mg kg⁻¹ was recorded showing the amounts of Ca, Mg, K, and N were below the range. Similarly in leaves of M.9, 2.57 N%, 0.18 P%, 1.55 K%, 0.87 Ca%, 0.23 Mg%, 960 mg kg⁻¹ Fe and 56.1 mg kg⁻¹ Mn, (Table 3 and 7 respectively). The contents of P, N, Mg, K, Mn, and Fe were within the range while Ca content was below the range. This showed that, in soil pH of 6.3 the nutrient uptake and transport of M.9 were more vigorous followed by MM.111 whereas MM.106 was found least. The decrease in uptake below the range was not related with availability of soil nutrients of experimental site (Table 2). Thus, the possible reason might be the genetic variation in uptake of nutrients between the rootstocks under different level of low soil pH. Potassium which is important for fruit colour, winter hardiness, tree growth and disease resistance (Lorraine *et al.*, 2012) was highly absorbed by M.9 and qualified for the established range (Table 3). The dwarf M.9 was the most widely used rootstocks in developed countries like Germany, where apple fruits were intensively cultivated under soil pH of 6.7. Our finding agrees with Amir and Fallah (2009) who reported M.9 as the most vigorous rootstock in uptake of nutrients than MM.111 and MM.106 under soil pH of 7.3 in Iran. Contrary to this, Kucukyumuk and Ibrahim (2011) reported that MM.106 and MM.111 are cultivars

with the maximum nutrient uptake than M.9 under soil pH of 6.5 in Turkey indicating that performances of rootstocks differ across locality or from site to site (Wertheim, 1998; Tromp *et al.*, 2005).

In our study, apple rootstocks showed differences not only in uptake but also in translocation of phosphorous to the leaves. Under strongly acidic soil of pH 3.5, phosphorous concentrations were relatively low in leaves of MM.106 (1279 mg kg⁻¹) as compared to the concentrations in roots, 1904 mg kg⁻¹. Significant negative correlation between leaf P content and root Al accumulation indicated that the transport of phosphorous to the leaves was inhibited by excess Al in the roots of MM.106 rootstocks (Table 4). In accordance with this, previous study revealed that the presence of Al often resulted in precipitation of P in the root cell walls and/or intracellular space (Weiqiangyang and Barbara, 1997; Terence *et al.*, 2002). Other study on inhibition of P also showed that the P transport was limited because of non-metabolic AlPO₄ accumulation in the root cell (Weiqiangyang and Barbara, 1997). In contrast to MM.106, positive significant correlation (appendix Table 1 and 2) between leaves P content and roots Al accumulation for MM.111 and M.9 indicated insignificance effect of Al on leaves P content of both rootstocks. In our micro plot experiment, the result of tissue analysis revealed that among the three apple rootstocks examined, MM.111 accumulated the highest amounts of Ca, Mg, N, and P in leaves and the least concentrations of Al in roots and Mn in leaves, showing MM.111 as the most tolerant to strongly acidic soil of pH 3.5. In contrast, apple rootstocks MM.106 had the lowest contents of Ca, Mg, N, and P in the leaves (Table 3), but highest concentration of Mn in leaves and Al in roots (appendix Table 3) indicating the cultivar to be the least tolerant, whereas M.9 rootstock was found to be intermediate. However, leaf Fe and K concentrations of M.9 was high in soil pH of

3.5 (appendix Table 3) showing the markedly absorptive capacity of this rootstock for both nutrients compared to MM.111 and MM.106 rootstocks.

5.3. Vegetative Growth of apple rootstocks as influenced by soil pH

Highly significant ($P < 0.001$) differences were recorded between rootstocks in growth of root length. This showed that, the accumulation of soluble Al in root surface severely affected the continuous, normal sustainable growth of roots which consequently reduced the uptake of nutrients from the soil, caused reduced vegetative growth. The amount of Al from soil test indicated that, soil exchangeable aluminum increased from 4.2 meq /100g at a soil pH of 4.9 to 11.4 meq /100g at a soil pH of 3.5 (Table 2). This increased in Al solubility due decrease of soil pH to 3.5 significantly affected the growth by interfering the absorption of Ca, Mg, K, and N that are largely required by rootstocks (Table 5). Our study support previous work of Neilsen *et al.* (1994) who reported that, the increased availability and toxicity of Al and Mn elements in acidic soils significantly reduced uptake of essential elements and consequently plant growth and yield. Significant ($P < 0.001$) interaction effect of rootstocks and low soil pH on vegetative growth was recorded. In our experiment, we found that, the three apple rootstocks vegetative growth increased with incremental increases in soil pH, from 3.5, to 4.9 and to 6.3 as did final root length and dry weights in nearly all rootstocks (Table 10). The result of our findings in the current study support previous investigations by Sas and Mercik (2004) who reported that, strongly acidic soil limited the growth of apple rootstocks P.22, M26, and M9 in comparison with soils having higher soil pH in Poland.

In soil pH of 3.5, MM.111 registered maximum growth of leaf area, total height, number of branches, root length, and dry weight (root, shoot, leaf) followed by M.9 except for root and shoot dry weight which recorded least whereas reduced growth was recorded for MM.106 (Table 9 and 10). This showed that, MM.111 strongly resisted the negative effect of excess Al and Mn on uptake under soil pH of 3.5 and thus absorbed and transport adequate nutrients. Conversely, MM.106 which failed to counteract the effect of Al registered reduced growth while M.9 recorded intermediate considered as vigorous rootstocks next to MM.111. Due to the presence of excess Al and Mn under soil pH of 3.5, rootstocks root growth; nutrient uptake and transport were affected. The presence of high Al not only suppresses the absorption and usage of largely required soil nutrients, but also reduced rootstocks growth by inhibiting the development of their roots (Ryan *et al.*, 1993). It has been reported that trunk diameter was used to determine the vigor of apple rootstocks (Webster, 2005). Trunk cross sectional area is often used as a convenient non-destructive estimate of overall rootstock size (Wertheim, 1998; Barden and Marini, 2001).

Significant differences in growth of TCSA were found between rootstocks (Table 11). Highest value of TCSA was recorded for MM.111 followed by M.9 while MM.106 showed least trunk growth under acidic soil of pH 3.5 (Fig. 6). The thickest and thinnest trunk growth indicated the highest and least tolerance differences between MM.111 and MM.106 respectively to the effect of low soil pH. Although MM.111 showed more growth than others, reduced data of TCSA were recorded for all three rootstocks under soil pH of 3.5. The interaction effect of rootstocks and low soil pH on trunk diameter was also significant (Table 11). According to the result of Duncan LSD same type of rootstocks under different levels of soil pH showed differences in

trunk growth. The trunk diameter for all rootstocks increased with increase in soil pH (Table 10). This indicated that, with an increase in soil pH the availability of largely required nutrients was more while the amount of toxic minerals was less.

Under naturally occurring acidic soil (pH 4.9) MM.111 recorded highest growth in leaf area, TCSA, total height, number of branches, root length, and dry weight (root, leaf) except for shoot dry weight followed by M.9, except for root, shoot, and leaf dry weight and least was recorded for MM.106 (Table 9 and 10). The root and leaf dry weight of MM.106 was more than M.9 while the shoot dry weight recorded was higher than both rootstocks. This showed that, progress increased in growth was observed for MM.106 with an increase in low soil pH indicated, the uptake and transport of nutrients by MM.106 was improved due to increase in root growth with the decrease in amount of Al and Mn that severely reduced root growth (Fig 4) compared to M.9 rootstock.

In soil pH of 6.3, contrary to soil pH of 4.9, Al and Mn solubility decreased, and effect of toxicity was found insignificant. The increased in growth of all three rootstocks was observed in pH 4.9 and highest in soil pH of 6.3. This could be due to reduced or absence of Al from root surfaces that block the entry of basic cations and consequence availability of more essential nutrients. Comparing the three rootstocks under soil pH of 6.3, the maximum growth in leaf area, total height, TCSA, number of branches, root length, and dry weight (shoot, root, leaf) was recorded for MM.111 followed by MM.106 except TCSA and number of branches, and least was registered for M.9 (Table 10 and 11). MM.111 does not show difference in total height grown in different level of soil pH. This showed that, the weak performance of MM.106 under

soil pH of 3.5 and 4.9 improved due to less or no effect of Al and Mn under soil pH of 6.3. It can be inferred that, the potential uptake and transport of nutrients by MM.106 was high under least amount of Al and Mn in pH 6.3, resulted in increased growth of vegetative parameters. Among the three rootstocks progress increase in growth of TCSA was recorded for M.9 with highest value in soil pH of 6.3 than other rootstocks (Fig 6). This indicated that, the uptake and translocation of nutrients by M.9 was higher under soil pH of 6.3.

The vegetative growths of apple rootstocks under study were affected by level of all soil pH. However, effect of severity was more in pH 3.5, low in pH 4.9, and least was found in pH 6.3. According to result of Duncan LSD, same types of rootstocks showed differences in vegetative growth across low soil pH (Table 9 and 10). The strongest performance of MM.106 in soil pH of 6.3 for most parameters except TCSA was contrary to soil pH of 3.5 showed higher growths in leaf area, total height, and root length than M.9 (Table 9 and 10). As low soil pH affected nutrient availability, it is not unpredicted to see changes in vegetative vigor with varying soil pH. Many studies have concluded that, strong soil acidification was found to be a limiting factor for growth and yield of apple trees (Nielsen *et al.*, 1994; Vardar *et al.*, 2006).

5.4. Effect of Rootstock Type and Low Soil pH on Growth and Yield of ‘Anna’ and ‘Jonagored’ cultivars

Rootstock as an integrated part of fruit tree strongly influences its vigour, productivity and quality of fruits. However, the performance of rootstocks differs from site to site and/or region to region, and thus, there is no universal rootstock for all climatic and soil conditions (Wertheim, 1998; Tromp *et al.*, 2005; Przemyslaw and Maciej, 2006). The growth and yield of both cultivars had significantly affected by the interaction of rootstocks and low soil pH treatments. The increase in trunk growth and leaf area was depending on level of soil pH and year of growth. Thickest trunk and maximum leaf area was recorded in pH 6.3 under growing season of 2013 whereas thinnest trunk and minimum leaf area was under soil pH 3.5 in 2011 year of growth (Table 14 and 15 respectively). In soil pH of 3.5, throughout the three successive growing season thickest trunk and large leaf area was noted for ‘Anna’ grafted on MM.111 followed on M.9 and the thinnest trunk and smallest leaf area was observed on MM.106. Under similar soil pH ‘Jonagored’ grafted on MM.111 recorded thicker trunk with larger leaf area but less than Anna, and the thinnest trunk and smallest leaf area of ‘Jonagored’ were found on MM.106, while M.9 was intermediate (Table 14 and 15 respectively). Duncan LSD showed that both cultivars grafted on same types of rootstocks differ in growth of total height across soil pH. In soil pH of 3.5 ‘Anna’ grafted on MM.111 recorded highest total height followed on MM.106 and least was recorded on M.9 whereas ‘Jonagored’ recorded more height on MM.111 followed on M.9 and least was observed on MM.106 (Table 21 and 12 respectively).

Despite this, next to MM.111 both ‘Jonagored’ and ‘Anna’ grown on M.9 recorded more number of branches and leaf dry weight whereas reduced growth was found on MM.106 in soil pH of 3.5 (Table 21 and 12 respectively). This proved MM.111 as the most vigorous rootstocks under strongly acidic soil in transport of nutrients to the scion whereas MM.106 was least while M.9 was intermediate. Therefore, under low soil pH (3.5) of study site MM.106 was not recommended for any of the cultivars.

Under naturally occurring acidic soil (pH 4.9) highest vegetative growth was recorded for ‘Anna’ and ‘Jonagored’ grafted on MM.111 compared to other two rootstocks indicated maximum absorption and transport of MM.111 rootstock. On the other hand, both cultivars grown on MM.106 and M.9 registered differences in total number of branches. Highest was recorded for ‘Anna’ on M.9 whereas ‘Jonagored’ recorded highest on MM.106 in total height. ‘Anna’ on MM.111 recorded high growth in leaf dry weight, and total height followed on M.9 while least was found on MM.106. Conversely, ‘Jonagored’ grown on MM.111 recorded high growth in total height, followed on MM.106 and least was found on M.9. However, more growth of ‘Jonagored’ leaf dry weight was found on M.9 rootstock (Table 12). This showed that, the difference in scion rootstocks combination resulted in performance differences among the rootstocks under similar soil pH. Thus, before planting selection of compatible rootstocks seems to be crucial.

The vegetative growth of both ‘Jonagored’ and ‘Anna’ were significantly reduced under naturally occurring acidic soil (pH 4.9) compared to soil pH of 6.3 treatments. This was caused due to excess accumulation of Al and Mn that influenced the

absorption of other essential nutrients (Table 5). Under this acidic soil, the rootstocks nutrient uptake was constrained by insufficient root growth caused mainly by excess Al toxicity. In soil with pH below 5.5, aluminum, manganese and iron become soluble and can reach levels toxic to plants which affect the uptake and utilization of calcium and magnesium nutrients (Foy, 1992; Millaleo *et al.*, 2010). Thus, toxic minerals reduced absorption of nutrients and also excess Al decreased size and branching of root growth. This additive effect on rootstocks cumulatively reduced the growth of both cultivars grafted on these rootstocks. Thus, we observed that, the naturally occurring acidic soil (pH 4.9) of study site as a major limiting factor for improved apple fruit production of study site which needs instant attention by all apple growers and stakeholders.

In soil pH of 6.3, both ‘Jonagored’ and ‘Anna’ grown on M.9 had thickest TCSA, followed on MM.111 and thinnest on MM.106 throughout the three successive growing season (Table 14 and 23 respectively). Contrary results were recorded for both ‘Anna’ and ‘Jonagored’ grown on MM.111 and M.9 in number of branches and leaf area. ‘Anna’ grown on MM.111 recorded highest leaf area followed on M.9 and least was found on MM.106 in all three years of growth (Table 23). Conversely, highest leaf area was found for ‘Jonagored’ grown on M.9 followed on MM.106 while least was found for ‘Jonagored’ grafted on MM.111 under soil pH of 6.3 (Table 15). In soil pH of 6.3, ‘Anna’ grown on M.9 produced more number of branches, but low leaf area whereas ‘Jonagored’ on MM.111 produced more number of branches but least leaf area (Table 12 and 21 respectively). This was caused due to differences in interaction effect of rootstocks and grafted scions on growth in branches and leaf area under low soil pH treatments. Our results support the findings of Jones (1986)

who reported that, rootstock have a major control in shoot growth. In general, both ‘Jonagored’ and ‘Anna’ cultivars on same rootstocks under similar low soil pH treatments showed considerable differences in all data of growth parameters. This could be due to the compatibility and tolerance differences among the scion-rootstocks combinations.

5.4.1. Mean number of flowers

The number of flowers of ‘Anna’ and ‘Jonagored’ was significantly affected by low soil pH and year of growth. Despite the differential growth associated with pH treatments, number of flowers showed the respond of rootstocks to soil acidification. Previous study reported that, apple rootstocks control not only total growth, but also the proportion of buds that become floral (Pierre-éric *et al.*, 2006). The flower production was increased with an increase in level of soil pH and year of growth (Table 17). The highest was counted in soil pH of 6.3 and end of growing season, 2013 while least was in pH 3.5 and first fruit bearing season 2011. This indicated that under strongly acidic soil due to inadequate of largely required nutrients few flower productions were recorded. The number of flowers in 2011 was few due to early growing season. Anna grafted on MM.111 produced more number of flowers while least on MM.106 and intermediate on M.9 throughout three years whereas Jonagored on MM.106 and M.9 produced more number of flowers while least on MM.111. In soil pH of 6.3 both cultivars grown on M.9 produced maximum number of flowers compared to other rootstocks (Table17). This indicated that the absorption of nutrients used for flower production by rootstocks differs. Thus both cultivars flower

production was depend on types of rootstocks used. Peter and David (1995) reported that, rootstock effects on flowering may be due to differences among rootstocks in phosphorus uptake from the soil or differences in distribution within the tree.

MM.111 rootstock that tolerate the influence of excess Al and Mn on nutrient uptake under strongly acidic soil condition of pH 3.5 supply adequate nutrients and water to the scion causing higher production of flowers. In contrast, both cultivars grown on MM.106 produced least flowers indicated inadequate supply of nutrients to the scion which showed the least tolerant of this rootstock to the effect of Al and Mn toxicity.

It has been known that, in absence of soil acidity stress the cultivars on their branches produced spurs which bear five flowers that developed in to five fruits. Under soil pH of 4.9 we observed dispersed spurs that produced less than five numbers of flowers. The appearances of floral structures were smaller in size and easily dropped off when touched. This showed that, under naturally occurring acidic soil (pH 4.9) the decreased in size of rootstocks roots due to the effect of excess Al severely affected the uptake and transport of largely required nutrients resulted in production of less abundance and weaker flowers which contributes for reduced yield of the cultivars.

The highest numbers of flowers were counted in soil pH of 6.3 for ‘Anna’ grown on M.9 followed on MM.111 and least was recorded on MM.106 whereas ‘Jonagored’ on M.9 produced more flowers followed on MM.106 and least flower numbers was counted on MM.111 (Table 17 and 26 respectively). In absence or reduced toxicity of Al and Mn under soil pH of 6.3, cultivars grown on M.9 was more productive than other two rootstocks. Our finding disagrees with El-Shammaa, *et al.* (2011) who

stated MM.106 as the most suitable rootstocks for 'Anna' apple trees grown in clay soils in Egypt. In pH 6.3, both cultivars most spurs bear five flowers and they were in abundance at the third growing season, 2013. This could be due to the negligible effect of Al on root growth; rootstocks under this pH treatment absorb and transport more nutrients to the scion. The flower formation of 'Jonagored' grafted on MM.106 increased under soil pH of 6.3 while least on MM.111. In contrast, 'Anna' grown on MM.106 showed least flower formation while highest was recorded on MM.111 in soil pH of 6.3 (Table 17 and 26 respectively). This showed that, unlike Jonagored Anna is not productive on MM.106. Thus, the types of rootstocks determine the flower production of cultivars under similar pH treatments.

5.4.2. Mean number of fruits

The abundant flowers produced by apple cultivars set fruits following bloom shading. However, all flowers were not developed into fruits. Flowers produced by the cultivars was reduced due to natural abortion by the cultivars to minimize the number, damage mainly by spring frost observed in temperate areas, insufficiency of nutrients, low temperature or soil condition among which soil acidity was the major one. Temperature also affects pollen germination. The average mean annual temperature of 12.25⁰C within the study area qualified the adequate temperature for pollen germination. Webster (2005) reported that, most scion cultivars of tree fruit require temperature of 10⁰C or more for effective pollen germination and growth. The effect of low soil pH on fruit set of both 'Jonagored' and 'Anna' was statistically significant (Table 20 and 29 respectively). The differences in fruit set were determined by degree of rootstocks tolerance to the effect of low soil pH. Minimum number of

fruits was registered for 'Anna' and 'Jonagored' in soil pH of 3.5 with progressive increase in pH 4.9 and with maximum fruit number under soil pH of 6.3 under all three successive seasons (Fig 16 and 12 respectively). This indicated that, soil pH below 6.3 significantly decreased the yield of both 'Anna' and 'Jonagored' cultivar under study site.

According to data of 2011, 2012, and 2013, the number of fruits registered for both 'Anna' and 'Jonagored' differs on type of rootstocks, level of soil pH and year of growth. However, the fruit production of both cultivars increased with increase in level of pH to 6.3 and year of growth 2013. Conversely least fruit count was obtained under pH 3.5 in growing season of 2011(Fig 16 and 12 respectively).

In all three successive seasons both cultivars on MM.106 were the least productive under strongly acidic soil of pH 3.5. In this soil pH, we observed poorly developed few number of spur leaves surrounding early fruit set. A healthy and abundant supply of spur leaves is crucial for the set and early development of the fruit of apple trees (Webster, 2005). The number of fruits counted for both 'Jonagored' and 'Anna' cultivars showed statistically significant differences (Table 20 and 29 respectively). Throughout three successive years, 'Anna' grafted on MM.111 produced highest fruits, lowest on M.9 and least was recorded on MM.106 under acidic soil of pH 3.5 (Fig 16). Similar results with rootstocks types but with more number of fruits were recorded for 'Jonagored' on MM.111 followed on M.9 and least was on MM.106 under low soil pH of 3.5 (Fig 12). The results showed that, 'Anna' with more flowers than 'Jonagored' on three rootstocks produced less fruits indicated flower shading and fruit drop of 'Anna' in low soil pH of 3.5 was more compared to 'Jonagored'

grown under similar conditions in all growing seasons. Both cultivars grown on acid-tolerant rootstocks, such as MM.111 and M.9 were highly recommended. The only rootstock under study which appears to affect fruit set negatively was MM.106 in soil pH of 3.5.

Under naturally acidic soil (pH 4.9), ‘Anna’ on MM.111 produced more fruits followed on M.9 and least was found on MM.106 (Fig 16). In contrast, highest number of fruits was recorded for ‘Jonagored’ grown on MM.106 and M.9 in ranking order and least was found on MM.111 in all three successive seasons (Fig 12). This contrasting differences between the two cultivars indicated differences in the effect of rootstocks and low soil pH interaction on fruit production of the scions. Contrasting results were found in flower formation and fruit set for ‘Anna’ grafted on MM.111 and M.9 rootstocks in soil pH of 4.9. We observed that, ‘Anna’ on MM.111 produced more flowers than ‘Anna’ on M.9 in first growing season. However, comparing the amount of flowers produced with the number of fruit set, we found more for ‘Anna’ grown on M.9 compared to MM.111 indicated flower shading and failure of fruit set of ‘Anna’ on MM.111 was sever than M.9. Under soil pH of 3.5 and 4.9 the fruits formed were less in size, with deformed shape showing deficiency symptoms of bitter pit, scar and less skin firmness compared with pH 6.3 which showed more improved fruits. In soil pH of 4.9 more fruit drop was observed compared to pH 6.3, indicated failure of maturation which is a symptom of nutrient deficiencies. The main possible explanations are the shortage of essential nutrient uptake and translocation by rootstocks due to accumulation of soluble toxic elements. This caused leaves to send insufficient photosynthetic products to the fruit which could result in an imbalance in the hormones within the fruit and which leads

to fruit drops as reported by Webster (2005). This study therefore showed that, the fruit yield of the cultivars grown under naturally occurring acidic soil of study area was severely affected.

The number of fruits counted increased for both Jonagored and Anna with increase in growing season and level of soil pH. Maximum number of fruits was counted in soil pH of 6.3 and third year of growing season (Fig12 and16 respectively). This indicated that, with the increase in growing season the size and absorptive capacity of roots increased and also in pH 6.3 the effect of Al and Mn on rootstocks nutrient uptake and transport decreased while availability of largely required nutrients were increased. Under soil pH of 6.3, 'Anna' on all rootstocks registered highest number of fruits than 'Jonagored' in all seasons. The highest number of fruits was registered for 'Anna' grown on M.9 followed on MM.111 and least was found on MM.106 (Fig 16). Conversely, 'Jonagored' grafted on M.9 produced more fruits followed on MM.106 whereas least fruit number was recorded for 'Jonagored' grown on MM.111 (Fig 16). This indicated that, the productivity of both cultivars grafted on M.9 increased with an increase in soil pH. M.9 was one of the most widely used rootstocks in developed countries in the area where the soil pH is > 6.5. High fruit set of both 'Anna' and 'Jonagored' grown on dwarf M.9 rootstock confirms its increase in potential uptake and transport of minerals with an increase in soil pH to 6.3. MM.111 with vigorous performance than other two rootstocks under decreased soil pH of 3.5 and 4.9 failed adequate fruit set in both cultivars under pH of 6.3. Thus, in this study site MM.111 was not recommended under higher soil pH condition.

The fruit set of both ‘Jonagored’ and ‘Anna’ increased with an increase in thickness of trunk diameter across low soil pH. Positive correlation coefficient was recorded between trunk diameter and number of fruits (Table 19 and 28 respectively) under all growing season. The number of fruits increased with an increase in trunk size throughout the seasons. This agrees with previous results of Wesley and James (2001) who reported that, apple cultivars cumulative yield had close relationship with trunk cross sectional area. MM.106 with thinnest TCSA for both cultivars produced minimum fruits indicated, cultivars on weaker stocks produced less wood with few number of fruits whereas MM.111 with thickest produced maximum fruits under soil pH of 3.5. This showed that, vigorous MM.111 rootstock produced the most wood which produced it faster in the first season of growth, form more fruits. However, both cultivars grown on MM.111 showed thinnest TCSA in soil pH of 6.3 produced least fruits compared to M.9 and MM.106. Our result agrees with Esmaeli *et al.* (2002) who reported that, Fuji apple cultivars on MM.111 was least productive and produced the lowest amount of yield than MM.106 and M. 9 in high soil pH of Parma.

M.9 with thickest TCSA for both cultivars recorded highest fruit count under soil pH of 6.3. Compared with MM.106, ‘Anna’ and ‘Jonagored’ grown on M.9 produced highest fruit number. In another study, Sotiropoulos (2008) reported that, Imperial cultivar grafted on MM.106 produced high yield than on M.9 under soil pH of 6.8 in Greece. This confirmed differences in rootstocks performance from area to area. Researchers revealed that, apple rootstocks performance differed due to scion-rootstock interaction or due to inconsistent of rootstocks from site to site as well as from region to region (Yahya *et al.*, 2004). Wertheim (1998) also previously stated that, it is almost impossible to get a good worldwide impression of the rootstock

situation from literature data only. It has been repeatedly shown that, there are interactions between sites, cultivars and rootstocks, it is best to use the results from rootstocks trials carried out regionally with relevant cultivars. Results from a trial obtained in one area may provide incorrect information for a different area (Wertheim, 1998).

Thus, in our study site under strongly acidic soil of pH 3.5 both ‘Jonagored’ and ‘Anna’ had more number of fruits on MM.111 followed on M.9, and least was found on MM.106 rootstock (Fig. 12 and 16 respectively). Our study agrees with Sas and Mercik (2004) for M.9 who reported, Gala and Jonagold fruits were highest on rootstock P.22, less on M.9 and fewest on M.26 under acidic soil condition in Poland. Przemysla and Maciej (2006) also found similar results for ‘Jonica’ cultivar grown on M.9 rootstock, after P.22 and P.29 rootstocks in Krakowa, Poland.

5.5. Effect of Rootstock Type and Low Soil pH on concentration of minerals in leaves of ‘Jonagored’ and ‘Anna’ cultivars

The rootstocks had a significant effect on scion leaf mineral concentration in apples (Kucukyumuk and Erdal, 2011). Leaf analysis was widely used to evaluate the effect of rootstocks on mineral nutritional status of apple cultivars (Antonio *et al.*, 2005; Kucukyumuk and Erdal, 2011). Rootstock brings about its effect on the scion by influencing the amount of minerals taken up and translocated through the tree (Kennedy *et al.*, 1982; Olien and Lakso, 1984; Higgs and Jones, 1991; Fallahi *et al.*, 2002). We suppose leaves nutrient levels in both ‘Anna’ and ‘Jonagored’ most accurately reflect the cultivars uptake of nutrients. The statistical analysis showed

significant differences in nutrient content between ‘Anna’ and ‘Jonagored’ grafted on three rootstocks ($P < 0.001$), effect of low soil pH ($P < 0.001$), and their interaction ($P < 0.001$) (Table 35). The accumulation of Al and Mn increased with the decrease in soil pH whereas largely required essential nutrients increased with increase in pH level. In soil pH of 3.5, the highest concentration of Al and Mn was found in leaves of both ‘Anna’ (Fig 30 and 31 respectively) and ‘Jonagored’ (Fig 22 and 23 respectively) grafted on MM.106 followed on M.9 and least was registered on MM.111. This indicated, MM.111 as the most tolerant to the effect of soluble forms of Al and Mn whereas MM.106 was found least tolerant while M.9 exhibit intermediate. According to Duncan LSD differences in leaves Al, Mn, and Fe were recorded for Jonagored and Anna grafted on same types of rootstocks across low soil pH (Table 31 and 34 respectively).

The maximum uptake of Fe was found in leaves of ‘Jonagored’ grown on M.9 followed on MM.111 and least was found on MM.106 (Fig 24). In contrast, highest amount of Fe was found in leaves of ‘Anna’ grown on MM.111 followed on M.9, and least was found on MM.106 (Fig 32). Our results disagree with Erdal *et al.* (2008) who stated high Fe uptake was found in leaves of apple cultivar ‘Redchief’ grown on MM.106 in Turkey. The Fe leaves content of both ‘Anna’ and ‘Jonagored’ grafted on same rootstocks showed differences due to difference in rootstocks uptake and transport from root to leaves under low soil pH. Differences in scion –rootstocks combination affect the uptake and transport of soil mineral nutrients (Wertheim, 1998).

The standard values of nutrient levels in apple tree leaves established by Joseph (2004) indicates the ranges for N, P, K, Ca, and Mg, 2.00-2.25, 0.20-0.30, 1.25-1.75, 1.20-1.60, 0.25-0.40 %, and that of Fe and Mn 100-300 and 40-100 mg kg⁻¹ respectively. The leaf nutrient contents of the same cultivar grown on three different rootstocks, lack consistency across low soil pH. Under soil pH of 3.5, leaves of ‘Anna’ grown on MM.111 significantly accumulate high concentration of macro nutrients, Ca, Mg, P, and N, 1.3, 0.22, 0.24, and 2.04% respectively, while it is intermediate on M.9, Ca, Mg, P, and N, 0.96, 0.21, 0.17, and 1.98% respectively. In contrast, the amount of Ca, Mg, P, and N, 0.82, 0.18, 0.17, and 2.0% respectively were least in leaves of ‘Anna’ grown on MM.106. According to Duncan LSD differences in leaves macronutrients were recorded for ‘Anna’ grafted on same types of rootstocks across soil pH (Table 33). The nutrient content of ‘Anna’ on MM.111 qualified the sufficiency range whereas ‘Anna’ on M.9 and MM.106 showed nutrient deficiency range for all nutrients except N obtained for ‘Anna’ grafted on MM.106. This showed that, under similar soil pH, the nutrient uptake and transport of rootstocks to the scion differed. This indicated both MM.106 and M.9 failed to absorb adequate nutrients to the scion of ‘Anna’ compared to MM.111.

In contrast, ‘Jonagored’ nutrient absorption under low soil pH of 3.5 was most efficient on MM.106 in uptake of Ca, Mg, P, and N, 0.78%, 0.21, 0.20, and 2.35% respectively, followed by M.9, Ca, Mg, P, and N, 0.67, 0.21, 0.18, and 2.06 respectively. Whereas, least content of Ca, Mg, P, and N, 0.76, 0.18, 0.18, and 1.81% respectively were recorded in leaves of ‘Jonagored’ grown on MM.111 (Table 29). This showed that, the uptake and translocation of nutrients to the scion was based on the types of rootstocks used. According to the result of Duncan LSD, differences in

leaves macronutrients were recorded for Jonagored grafted on same types of rootstocks across soil pH (Table 30). However, relative to the sufficiency ranges, all macronutrients registered were below the optimum, except for ‘Jonagored’ P and N grafted on MM.106 and N on M.9. The highest P content (0.23%) above the range in leaves of ‘Jonagored’ grafted on MM.106 indicated the potential uptake and translocation of MM.106 for P under soil pH of 3.5. The maximum P uptake and transport of ‘Jonagored’ grown on MM.106 was registered in soil pH of 6.3(Fig 20). However, under similar soil pH, ‘Anna’ grafted on MM.106 registered low concentration of P below the range (0.17%) indicated weak absorption and slow transport to the leaves of ‘Anna’ (Fig 28). This showed that, the effect of rootstocks on uptake of P depends on types of scion used. Our result agrees with Amiri *et al.* (2009) for ‘Jonagored’ grafted on MM.106 who reported that, cultivars grafted on MM.106 accumulated highest P in their leaves under pH 7.3 in Iran. Previous report also indicated that, leaves of Imperial cultivar grafted on MM.106 contain more P in soil pH of 6.8 in Greece (Sotiropoulos, 2008). Our result confirms this observation too. Similar to Anna, in leaves of ‘Jonagored’ across low soil pH no micronutrient, Fe and Mn was found below standards.

Highest K concentration was recorded in leaves of ‘Jonagored’ cultivar grafted on MM.111 and M.9 above optimum indicated high absorptive capacity of both rootstocks for K. Whereas, on MM.106 the amount obtained was within the range across low soil pH (Fig 19). Conversely, K uptake and translocation registered in leaves of ‘Anna’ grafted on all three rootstocks qualified the sufficiency range (Fig 27). This indicated that, the uptake and transport of nutrients to the scion was mainly determined by the rootstocks on which the cultivars were grown. These findings agree

with the earlier report by Abdolhossein (2012) that, rootstocks directly influence the ability of grafted cultivars for absorption of nutrients from soil. The effect of naturally occurring acidic soil (pH 4.9) in leaf nutrient content of ‘Anna’ and ‘Jonagored’ cultivars grafted on MM111, MM106, and M9 was examined. The result showed that, ‘Jonagored’ grafted on MM.106 registered highest Ca, Mg, K, P, and N, 0.83, 0.22, 1.71, 0.21, and 2.39 % respectively followed on MM.111 Ca, Mg, K, P, and N, 0.78, 0.18, 2.1, 0.18%, and 2.02 % and least Ca, Mg, K, P, and N, 0.69, 0.16, 2.6, 0.19, and 2.09 % respectively were in leaves of ‘Jonagored’ grown on M.9 (Table 29). In reference with sufficiency range (Joseph, 2004) except for Ca, the nutrient level of ‘Jonagored’ on MM.106 was found within sufficiency range whereas ‘Jonagored’ on MM.111 and M.9 recorded below the range except for K which registered above the range and N within the range. This showed that, rootstock types had significant influence on leaf mineral elements of ‘Jonagored’ scion. The data showed that, ‘Jonagored’ was more compatible on MM.106 in uptake of nutrients under soil pH of 4.9 than other two rootstocks.

Under original low soil pH (4.9), ‘Anna’ grafted on MM.111 recorded highest content of Ca, Mg, K, P, and N, 1.46, 0.23, 1.66, 0.25, and 2.1 % respectively whereas on MM.106 high value of Mg, P, and N, 0.23, 0.19, and 2.06 respectively while low value for that of Ca and K, .93, and 1.63 respectively was recorded. In contrast, least value of Mg, P, and N, 0.22, 0.18, and 2.00% while high value of Ca and K, 0.98, 1.89% respectively were registered in leaves of ‘Anna’ grown on M9 rootstock (Table 33). In reference with sufficiency range (Joseph, 2004) the nutrient levels registered for ‘Anna’ grafted on MM.111 qualified the sufficiency range whereas ‘Anna’ on MM.106 and M.9 recorded below the range except for MM.106 K, and N

which is above and within the range respectively. This indicated that, the uptake and transport of nutrients to the leaves of 'Anna' by MM.111 was markedly greater than 'Anna' on weaker rootstocks MM.106 and M.9 under pH 4.9 treatments. On the other hand, N uptake was more in pH 4.9 with highest in pH 6.3 (2.09 and 2.08%) for scion of 'Anna' grafted on MM.106 and M.9 respectively (Fig 29). Our result agrees for M.9 with Aguirrel *et al.* (2001) who reported cultivars grown on M.9 rootstock under pH 7, were more efficient in N uptake in peninsular Malaysia. Highest concentration of Al, Mn, and Fe was recorded in leaves of both 'Anna' (Fig 30, 31 and 32 respectively) and 'Jonagored' (Fig 22, 23 and 24 respectively) under pH of 4.9 while least amount was found in pH 6.3.

It has been known that, the solubility of these toxic minerals was increasing with the decrease in soil pH mostly below 5.5 (Kamprath, 1970). In acidic soil of study site (pH 4.9) the accumulation of these toxic elements was found to be excess in soil where both cultivars were grown. The presence of Al, Mn, and Fe in there soluble form inhibits the uptake and usage of other essential nutrients. Due to the shortage of these largely needed nutrients, the growth and yield of both 'Anna' and 'Jonagored' was adversely affected. The result of correlation coefficient between toxic Al and Mn was negatively significantly correlated with growth and yield of Jonagored and Anna cultivar (Table 33 and 35 respectively). This showed that the soluble toxic minerals reduced the growth in trunk diameter and leaf area and caused cumulative effect on flowering and fruit set of both cultivars under study. In accordance with this, Von Bennewitz *et al.* (2011) mentioned, the macro and micro elements as a key factors that affect plant growth, yield and fruit quality to be considered in apple orchards. None of the micronutrients, Fe and Mn was found below standards.

Under soil pH above 6.0, soluble Al that interfere the uptake of these essential nutrients is almost certainly not a problem (Kamprath, 1970). More nutrient uptake and translocation for both cultivars grafted on three rootstocks was recorded in soil pH of 6.3. In reference with nutrient range, ‘Anna’ grafted on MM.111 recorded maximum content of Ca, Mg, K, P, and N, 1.63, 0.24, 1.67, 0.26, and 2.3 % respectively whereas that of Fe and Mn, 65.7 and 26 mg/kg respectively was minimum indicated the effect of low soil pH on MM.111 nutrient uptake and transport to the scion was negligible. Under similar soil pH, ‘Anna’ grafted on MM.106 contain Ca, P, and Mg, 1.08, 0.19, and 0.24 % respectively were below the sufficiency range and amount of Fe and Mn, 78 and 33 mg kg⁻¹ respectively was lowest whereas the amount of K (1.64%) and N (2.09 %) qualified the range (Table 33 and 34). This showed that, the uptake and transport of MM.106 for most of the nutrients were less vigorous than ‘Anna’ grown on MM.111. On the other hand, ‘Anna’ grown on M.9 registered low Mg, P, and Ca, 0.23, 0.18, and 1.00 respectively below the range whereas the N (2.08%) and K (1.91%) were within the range, and that of Fe and Mn, 95 and 43 mg kg⁻¹ were found closely within the adequate level. This indicated that, the uptake and transport of minerals by M.9 to the scion was more with increase in pH level compared to MM.106.

On the other hand, in soil pH of 6.3 in reference with apple leaves nutrient sufficiency range established by Joseph (2004), ‘Jonagored’ grafted on MM.111 recorded highest content of K(2.36%), N(2.16%), Mn (53 mg kg⁻¹) qualified the range whereas P(0.19%, Ca (0.85 %), Mg (0.19%) and Fe (81 mg kg⁻¹) was recorded below the range. Unlike Anna, reduced nutrient uptake and transport of MM.111 to ‘Jonagored’

was registered. This showed that, ‘Anna’ was more compatible with MM.111 than ‘Jonagored’ under pH 6.3. In leaves of ‘Jonagored’ grafted on MM.106 the amount of P, Mg, K, and N, 0.22, 0.23, 1.78, and 2.46% respectively and that of Fe and Mn, 97 and 98 mg kg⁻¹ respectively were found within the range while Ca (0.87%) was below the sufficiency range (Table 30 and 31). This indicated that, ‘Jonagored’ was more productive on MM.106 than ‘Anna’ under soil pH of 6.3. Similar to Anna, the amount of Mg, P, and Ca, 0.17, 0.19, and 0.74% respectively found in leaves of ‘Jonagored’ grown on M.9 were below the range whereas N and K, 2.1 and 2.71% respectively qualified the range and that of Fe and Mn, 97 and 98 mg kg⁻¹ respectively was within adequate level.

Research results showed that, the differences among rootstocks uptake and translocation was mainly caused by different nutrient absorption capacities through the roots (Kucukyumuk and Erdal, 2011). Thus, under low soil pH treatments, both ‘Anna’ and ‘Jonagored’ cultivars prefer different rootstocks. According to our finding, in soil pH of 3.5 leaves of ‘Anna’ on MM.111 recorded highest nutrient content in contrast to ‘Jonagored’ that registered maximum nutrient on MM.106. It can be inferred that rootstocks MM.111 and MM.106 differs greatly in uptake and transport of nutrients depending on type of grafted scion. In this study, mineral absorptive and transport of M.9 for ‘Anna’ was next to MM.111, while for ‘Jonagored’ was after MM.106. Our results disagree with previous work of Amir and Fallah (2009) who reported that, leaves of ‘Golden Delicious’ and ‘Gala’ grafted on M.9 contain high mineral than MM.111 and MM.106 under pH 7.3 in Iran. These showed lack of uniformity on the performance of rootstocks from site to site. On the other hand, the accumulation of toxic Al, Mn, and Fe elements in leaves of both

‘Anna’ and Jonagored grafted on MM.106 was high, followed on M.9 and least was found on MM.111. Under soil pH of 6.3 among the three rootstocks both ‘Anna’ and ‘Jonagored’ cultivars grafted on M.9 accumulate highest concentration of Fe in their leaves (Table 34 and 31 respectively). It can be inferred that M.9 rootstock differs greatly in uptake and ability to transport Fe than both MM.111 and MM.106 rootstocks. Similar result was previously reported by Amiri *et al.* (2009) under soil pH of 7.3 in Iran.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The present study provides information on the respond of the three different apple rootstocks and two cultivars to naturally occurring acidic soil (pH 4.9) and other two (3.5 and 6.3) modified soil pH treatments. It provides information on the effect of low soil pH, rootstocks, and the interaction of these factors on vegetative growth (leaf area, TCSA, total height, number of branches), dry weight (root, leaf, and shoot), and yield of cultivars. It also revealed the solubility of toxic minerals and its influences on the availability of essential nutrients. In relation with toxic nutrients differences in uptake of soil minerals by the three apple rootstocks and two cultivars were explained. The major findings of the study were summarized as follows.

The results showed that low soil pH significantly ($P < 0.001$) reduced the availability of essential nutrients and increase the amount of soluble Al and Mn and thus, affected the rootstocks mineral absorption and transport. Significant ($P < 0.001$) differences between rootstocks were also recorded in uptake of nutrients. Among the rootstocks under strongly acidic soil of pH 3.5, the maximum amount of largely required and least toxic nutrients were recorded for MM.111 whereas the highest amount of toxic and least essential nutrients were observed for MM.106 while M.9 was found intermediate. Thus, under acidic soil condition MM.111 was the most tolerant rootstocks while MM.106 was least. Strongly acidic soil significantly reduced the growth of rootstocks under study. The increase in growth was observed with increase in soil pH to 4.9 and highest in pH 6.3. Among the three rootstocks, maximum

vegetative growth and dry weight was recorded for MM.111 whereas least growth was registered for MM.106 while M.9 recorded intermediate in soil pH of 3.5. Therefore, under acidic soil of experimental site (4.9), MM.111 was the most tolerant rootstocks while MM.106 was least tolerant & M.9 was intermediate. Thus, MM.111 and M.9 was recommended for grafting in ranking order.

Low soil pH significantly influences the growth and yield of both cultivars under study. 'Anna' and 'Jonagored' grafted on MM.111 rootstock had maximum trunk growth, highest number of flowers and fruits in soil pH of 3.5. On the contrary, both 'Anna' and 'Jonagored' grown on M.9 under soil pH of 6.3 recorded highest trunk growths more flower and fruits. Among the two cultivars, more number of flowers and fruits were produced by 'Anna' than 'Jonagored'. This indicated that the three rootstocks under study were more compatible with 'Anna' cultivar under experimental site.

Rootstock type and low soil pH had significant influence on scion leaves nutrient accumulation. The highest concentration of Al and Mn was found in leaves of both 'Anna' and 'Jonagored' grafted on all three rootstocks under naturally occurring acidic soil (pH 4.9) compared to soil pH of 6.3. In strongly acidic soil of pH 3.5, the accumulation of toxic nutrients in leaves of 'Anna' and 'Jonagored' grafted on MM.106 was highest while essential nutrients were least. In contrast, leaves of 'Anna' grown on MM.111 significantly accumulate maximum concentration of macro nutrients qualified the sufficiency range while, least amount of toxic nutrients was recorded. In general, based on the results of the present study it can be concluded that MM.111 was the most tolerant rootstock to strongly acidic soil (pH 3.5) followed by

M.9 and the least tolerant was MM.106. 'Anna' and 'Jonagored' grafted on MM.111 produced the highest number of flowers and fruits followed by those grafted on M.9 and the least were recorded on MM.106 under acidic soil of pH 3.5. Maximum number of fruits was recorded for both cultivars grown on M.9 in soil pH of 6.3. Therefore, for experimental site of Chenchu under pH 4.9 Anna was productive on MM.111 & least on MM.106. Thus, MM.106 was not recommended for grafting with Anna. Conversely, Jonagored was most productive on M.9 and MM.106, but least on MM.111. Thus, MM.111 was not recommended for grafting with Jonagored. In pH 6.3 both cultivars were recommended to grow on M.9.

6.2. Recommendation

The soil acidity of the experimental site causes adverse effect on growth and yield of apple rootstocks and cultivars. The apple fruit standard quality of the study site was mainly affected by effect of low soil pH that affects the availability of essential exchangeable bases. Thus, deficiency symptoms of bitter pit, cork spot, internal breakdown were observed on matured apple fruits of experimental site. Thus emerged research ideas in this study and recommended for further action are as follows.

- The naturally occurring acidic soil (pH 4.9) was the major causes which affect the growth and yield of apple rootstocks and cultivars of the study site, thus before planting the cultivars corrective measures should be taken by adding lime and thus maintain a pH > 6.3.
- The growth and yield of cultivars under acidic soil condition of experimental site was determined by rootstocks types used. Thus, rootstocks that are compatible with the cultivars should be identified before planting.

- The apple fruit quality under acidic soil was mainly affected by nutrient deficiency especially of calcium. Thus soil nutrient tests should be conducted during apple blooming and fruit set and corrective measures should be taken.

Aspects that need future investigation:-

1. Further study on effect of other rootstock types, low soil pH and their interactions on growth and yield of other apple cultivars.
2. Further study on growth and yield of other cultivars grown on M.9 under soil pH greater than 4.9 under different localities or regions.
3. Study on effect of compost (manure) supply and low soil pH interaction on growth and yield of apple cultivars.

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8. APPENDIX

Table 1: Correlations coefficient between AI and P for MM.111

		AL	P
AL	Pearson Correlation	1	.861**
	Sig. (2-tailed)		.003
P	Pearson Correlation	.861**	1
	Sig. (2-tailed)	.003	

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2: Correlations coefficient between AI and P for M.9

		AL	P
AL	Pearson Correlation	1	.887**
	Sig. (2-tailed)		.001
P	Pearson Correlation	.887**	1
	Sig. (2-tailed)	.001	

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3: Concentration of nutrients and mineral elements in rootstocks organs from three apple rootstocks grown under different levels of low soil pH. Values are the means of 3 repetitions $\pm$ SE.

Nutrients mg/Kg	Soil pH treatments	Apple Rootstocks								
		MM111			M9			MM106		
		leaf	shoot	Root	leaf	shoot	Root	leaf	shoot	Root
Ca	3.5	10955 $\pm$ 2.9a	10180 $\pm$ 5.9a	201 $\pm$ 9.6a	4015 $\pm$ 2.9b	2880 $\pm$ 5.9a	1995 $\pm$ 2.9a	2030 $\pm$ 17.6c	2090 $\pm$ 2.9b	333.9 $\pm$ 5.9b
	4.9	11880 $\pm$ 5.8a	13465 $\pm$ 2.9a	1950 $\pm$ 29.4a	6510 $\pm$ 5.8b	8560 $\pm$ 5.9a	8595 $\pm$ 1.2a	3385 $\pm$ 2.9c	2930 $\pm$ 5.9b	1850 $\pm$ 5.9b
	6.3	13150 $\pm$ 2.8a	4040 $\pm$ 5.9a	1320 $\pm$ 11.8a	8735 $\pm$ 2.9b	3630 $\pm$ 11.8a	2110 $\pm$ 5.9a	5390 $\pm$ 2.9c	4045 $\pm$ 5.9b	1995 $\pm$ 2.9b
Mg	3.5	2550 $\pm$ 5.9a	1460 $\pm$ 5.9a	70 $\pm$ 2.9a	2060 $\pm$ 2.9a	1430 $\pm$ 17.6a	1280 $\pm$ 5.9a	1180 $\pm$ 5.9a	630 $\pm$ 2.9a	1020 $\pm$ 2.9a
	4.9	2340 $\pm$.11.8a	1680 $\pm$ 5.9a	410 $\pm$ 5.9a	2270 $\pm$ 5.9a	1080 $\pm$ 5.9a	2070 $\pm$ 5.9a	2330 $\pm$ 17.6a	1310 $\pm$ 5.9a	1200 $\pm$ 24.5a
	6.3	1310 $\pm$ 5.9a	1340 $\pm$ 11.8a	490 $\pm$ 2.9a	2320 $\pm$ 11.8a	960 $\pm$ 5.9a	920 $\pm$ 11.8a	1090 $\pm$ 2.9a	3400 $\pm$ 2.9a	1190 $\pm$ 2.9a
Fe	3.5	401 $\pm$ 6.2b	193 $\pm$ 1.8b	775 $\pm$ 2.9b	705 $\pm$ 8.8a	1013 $\pm$ 7.2a	7380 $\pm$ 5.9a	274 $\pm$ 2.4ab	254 $\pm$ 5.9ab	3335 $\pm$ 5.9ab
	4.9	219 $\pm$ 2.4b	206 $\pm$ 3.5b	3635 $\pm$ 2.9b	880 $\pm$ 5.9a	354 $\pm$ 5.9a	7800 $\pm$ 5.9a	453 $\pm$ 5.9ab	222 $\pm$ 5.9ab	7660 $\pm$ 5.9ab
	6.3	15.9 $\pm$ 1.5b	276 $\pm$ 3.5b	2550 $\pm$ 29b	960 $\pm$ 17.6a	375 $\pm$ 5.9a	3170 $\pm$ 5.9a	334 $\pm$ 5.9ab	762 $\pm$ 5.9ab	1333 $\pm$ 1.3ab
K	3.5	12250 $\pm$ 16.7b	2350 $\pm$ 16.7b	690 $\pm$ 5.9b	16500 $\pm$ 16.7a	7000 $\pm$ 16.7a	4500 $\pm$ 6.7a	12500 $\pm$ 33.3b	2710 $\pm$ 5.9b	2000 $\pm$ 16.7b
	4.9	5000 $\pm$ 16.7b	2100 $\pm$ 16.7b	990 $\pm$ 5.9b	16000 $\pm$ 16.7a	4250 $\pm$ 16.7a	5000 $\pm$ 16.7a	16500 $\pm$ 33.3b	3990 $\pm$ 5.9b	3420 $\pm$ 16.7b
	6.3	5500 $\pm$ 33.3b	2800 $\pm$ 16.7b	1140 $\pm$ 1.7b	15500 $\pm$ 16.7a	11500 $\pm$ 5.9a	2390 $\pm$ 5.9a	7000 $\pm$ 16.7b	4000 $\pm$ 16.7b	2820 $\pm$ 6.7b
P	3.5	1933 $\pm$ 1.8b	1145 $\pm$ 2.9b	1162 $\pm$ 1.2b	1636 $\pm$ 3.5a	1636 $\pm$ 3.5a	1784 $\pm$ 2.3a	1279 $\pm$ 5.3a	1220 $\pm$ 5.9a	1904 $\pm$ 2.3a
	4.9	1948 $\pm$ 4.7b	1338 $\pm$ 4.7b	1072 $\pm$ 3.5b	1383 $\pm$ 1.8a	1443 $\pm$ 2.9a	1726 $\pm$ 3.5a	2558 $\pm$ 4.7a	1160 $\pm$ 5.9a	2172 $\pm$ 3.5a
	6.3	1651 $\pm$ 3.5b	1115 $\pm$ 2.9b	624 $\pm$ 2.3b	1889 $\pm$ 5.3a	1591 $\pm$ 2.3a	2498 $\pm$ 5.3a	2454 $\pm$ 2.3a	1487 $\pm$ 4.1a	2618 $\pm$ 4.7a
Mn	3.5	195.2 $\pm$ 1.2b	5.9 $\pm$.59b	142.1 $\pm$ 5.9b	238.2 $\pm$ 4.7a	153.3 $\pm$ 1.8a	136.9 $\pm$ 3.5a	318.2 $\pm$ 2.9a	74.5 $\pm$ 2.4a	108.3 $\pm$ 4.7a
	4.9	48.4 $\pm$ 4.7b	3.4 $\pm$.59b	4 $\pm$.59b	89.1 $\pm$ 5.2a	52.9 $\pm$ 1.2a	71.9 $\pm$ 3.2a	137.2 $\pm$ 4.1a	21.1 $\pm$ 2.9a	36.1 $\pm$ 3.5a
	6.3	9 $\pm$ 2.4b	38.6 $\pm$ 4.7b	7.7 $\pm$ 1.2b	56.1 $\pm$ 2.9a	44.2 $\pm$ 2.4a	24.1 $\pm$ 2.4a	30.1 $\pm$ 2.9a	36.2 $\pm$ 3.5a	59.4 $\pm$ 2.4a
Al	3.5	135 $\pm$ 5.9b	87 $\pm$ 2.9b	549 $\pm$ 2.4b	235 $\pm$ 5.9b	175 $\pm$ 2.9b	879 $\pm$ 5.2b	756 $\pm$ 3.5a	235 $\pm$ 2.9a	1560 $\pm$ 11.8a
	4.9	65 $\pm$ 13.3b	127 $\pm$ 1.8b	342 $\pm$ 5.9b	107 $\pm$ 4.1b	155 $\pm$ 2.9b	657 $\pm$ 5.9b	178 $\pm$ 4.7a	564 $\pm$ 2.4a	1145 $\pm$ 2.9a
	6.3	48 $\pm$ 1.8b	97 $\pm$ 4.1b	108 $\pm$ 4.7b	96 $\pm$ 6.7b	118 $\pm$ 4.7b	435 $\pm$ 5.9b	130 $\pm$ 2.9a	390 $\pm$ 5.9a	775 $\pm$ 2.9a
N%	3.5	2.78 $\pm$.05a	1.65 $\pm$.02a	0.67 $\pm$.01a	2.32 $\pm$.04b	1.16 $\pm$.04b	0.59 $\pm$.05b	2.27 $\pm$ 0.04c	0.77 $\pm$.04c	0.51 $\pm$.04c
	4.9	2.77 $\pm$.04a	1.69 $\pm$.05a	0.700 $\pm$.01a	2.36 $\pm$.04b	0.89 $\pm$.05b	1.3 $\pm$.02b	2.09 $\pm$.05c	0.78 $\pm$.05c	1.38 $\pm$.05c
	6.3	2.36 $\pm$.04a	1.59 $\pm$.05a	1.3 $\pm$.2a	2.57 $\pm$.04b	0.93 $\pm$.20b	1.48 $\pm$.05b	1.39 $\pm$.05c	1.01 $\pm$.001c	1.2 $\pm$.02c

Means $\pm$ SE (n=3) with different letter are significantly different at p<0.05 by LSD test within apple rootstocks