

**ÇUKUROVA UNIVERSITY  
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

**PhD THESIS**

**Mohamed Dhamir KOMBO**

**ROOTSTOCK EFFECTS ON SEED YIELD AND QUALITY IN  
WATERMELON**

**DEPARTMENT OF HORTICULTURE**

**ADANA-2017**

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**DEPARTMENT OF HORTICULTURE**

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## ABSTRACT

### PhD THESIS

# ROOTSTOCK EFFECTS ON SEED YIELD AND QUALITY IN WATERMELON

Mohamed Dhamir KOMBO

ÇUKUROVA UNIVERSITY  
INSTITUTE OF NATURAL AND APPLIED SCIENCES  
DEPARTMENT OF HORTICULTURE

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Watermelon grafting has been conducted to meet various objectives such as soil-borne diseases control, tolerance against agronomic predicaments, improving yield and quality etc. This research was conducted to investigate rootstock effects on seed yield and quality in watermelon. The work conducted at field experimental areas and laboratories of Department of Horticulture of the University of Cukurova, one watermelon cv. Crimson sweet scion was used onto three different rootstocks (Cucurbita - NUN-9075, Lagenaria – Argentario and citron watermelon – PI296341). Observations and measurements started from seed to seed. That is from seed sowing, grafting, main stem length, diameter, number of nodes, biomass, pollen production and development, fruit yield and quality, and seed yield and quality. NUN-9075/CS and Argentario/CS graft combinations resulted in higher stem length, plant biomass, fruit yield and quality as well as higher seed yield, seed emergence and germination percentage. There was no significant difference observed between graft combinations in pollen viability, pollen germination and normal pollen production at 0.05 significant level. No significant difference between graft combinations observed in accelerated ageing (AA) and the seed germination was not very much decreased after AA of 192 h. NUN-9075 followed by Argentario rootstocks are recommended as the best rootstocks used in this study.

**Key words:** Fruit analysis, graft combinations, rootstocks, seed yield, seed quality.

ÖZ

DOKTORA TEZİ

**KARPUZLARDA TOHUM VERİM VE KALİTESİ ÜZERİNE FARKLI ANAÇLARIN ETKİLERİ**

**Mohamed Dhamir KOMBO**

**ÇUKUROVA ÜNİVERSİTESİ  
FEN BİLİMLERİ ENSTİTÜSÜ  
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Karpuzlarda aşılama, toprak kökenli hastalıkların kontrolü, tarımsal sorunlara karşı dayanıklılık, verim ve kaliteyi artırmak vb. amaçlarla yapılmaktadır. Bu araştırmanın amacı, anaçların karpuzda tohum verim ve kalitesine olan etkilerini araştırmaktır. Çukurova Üniversitesi, Bahçe Bitkileri Bölümü, deneme alanları ile laboratuvarlarında yürütülmüş olan bu tez çalışmasında, Crimson sweet karpuz çeşidi kalem olarak kullanılmış ve üç farklı anaç üzerine (Cucurbita - NUN-9075, Lagenaria-Argentario ve citron karpuz - PI-296341) aşılanmıştır. Gözlemler ve ölçümler tohumdan tohuma kadar yapılmıştır. Bunlar; tohum ekimi, aşılama, ana gövde uzunluğu, çapı, boğum sayısı, biyokütle, polen üretimi ve gelişimi, meyve verimi ve kalitesi, tohum verimi ve kalitesidir. NUN-9075/Crimson sweet ve Argentario/Crimson sweet aşı kombinasyonları, daha yüksek ana gövde uzunluğu, bitki biyokütlesi, meyve verimi ve kalitesinin yanısıra daha yüksek tohum verimi, tohum çıkış ve çimlenme yüzdesi göstermişlerdir. Polen canlılığı, polen çimlenmesi ve normal polen üretimi parametrelerinde aşı kombinasyonları arasında istatistiksel olarak 0,05 önem seviyesinde bir farklılık tespit edilmiştir. Hızlı yaşlandırma (HY) gözlenen aşı kombinasyonları arasında fark yoktur ve 192 saat hızlı yaşlandırma yapıldıktan sonra tohum çimlenmesi çok fazla azalmamıştır. Bu çalışmada bütün kullanılan anaçlarından, NUN-9075 ve Argentario en iyi anaçlar olarak önerilmektedir.

**Anahtar kelimeler:** Meyve analizi, aşı kombinasyonları, anaçlar, tohum verimi, tohum kalitesi.

## EXECUTIVE SUMMARY

This thesis work has been conducted at the experimental areas of Department of Horticulture for field work and laboratory work have been carried out at Biotechnology Center and cytology laboratories of the University of Cukurova in Adana, Turkey for two consecutive years, 2016 and 2017 spring seasons. Seed sowing and grafting of the seedlings was carried out at Antalya Seedlings Company in Antalya. Four genotypes were used, where watermelon (*Citrullus lanatus*) cv. Crimson sweet (CS) used as scion which was grafted onto other three different rootstocks namely; Cucurbita rootstock – NUN-9075, Lagenaria rootstock – Argentario and Citron watermelon – PI 296341, watermelon cv CS also used as control (non-grafted). Twenty plants were planted at a spacing of 3 m × 0.75 m that made 80 plants replicated in four repetitions (20 plants × 4 genotypes × 4 replications = 320 plants).

To ensure safety of the plants, low plastic tunnel was used once after transplanting and removed after two weeks. Plants were regularly irrigated and fertigated by using dripping system. Used fertilizers were Monoammonium Phosphate (MAP) and Potassium Nitrate (KNO<sub>3</sub>) for N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O supply were controlled by used handhoes and hand operated weeder was used between the lines. Pesticides were used to control anthracnose, red spider mites and aphids.

Days from transplanting to the time when main stem attained 10 cm long were counted and recorded. Other measurements such as main stem length, main stem diameter and number of nodes were measured once in every month for two months to determine the plant growth differences. Two plants at the end of each plot was uprooted for plant biomass assay, where number of leaves were counted, stem length, diameter, leaf fresh weight and stem fresh weight was weighed before oven dried to get dry weight. Also flowers occurrence was observed by counting days from transplanting to the occurrence of first male flower, first female flower, 50% male flowers and 50% female flowers. Pollen production capacity of the

plants was observed by using 10 flowers from each replication. Number of anthers per flower, pollen viability, pollen germination and normal pollen production from each graft combination and control were determined.

Fruits were left in the field for a bit longer for the seeds to get fully maturity, and harvesting was done once for all plants in both two years. Only cracked and rotten fruits were harvested before the actual intended harvesting time. Fruits were all weighed by using weighing balance to get total fruit yield ( $\text{kg m}^{-2}$ ), then 3 fruits from every replicate were analysed for single fruit weight (SFW), fruit height (FH), fruit diameter (FD), fruit rind thickness (FRT) and total soluble solids (TSS). Therefore, in each genotype 12 fruits categorically were analysed, in total 48 fruits were used for analysis in each year. Analysed fruits together with the rest of the other fruits were cut for seed extraction. Seeds were extracted, well washed and dried before seed analysis experiments. From every replicate, 50 seeds were used for seed germination test between paper, 50 seeds for seed emergence test by using inert sand, 10 g seed for seed moisture test and 100 seeds for accelerated aging test at 45 °C where by 25 seeds from each replicated were taken after every 48 h (48 h, 96 h, 144 h and 192 h consecutively).

NUN-9075 and Argentario genotypes resulted in higher seed germination percentage and higher survival rate in the initial start of the experiment before sowing in both two years. The seed germination percentage was between 92.59 – 98.33% and survival rate after grafting was between 81.33 – 93.33%.

There was a significant difference between graft combinations in days to when main stem attained 10 cm length. Earliest graft combination was NUN-9075/CS which attained 10 cm long after 8.25 days in 2017 growing season. Argentario/CS was the latest graft combination attained 10 cm stem length after 17.25 days in 2016 growing season.

Also significant difference between graft combinations observed in main stem length, main stem diameter and number of nodes. NUN-9075/CS graft combination resulted in higher values of main stem length, main stem diameter and

number of node in both years (90.60 cm, 9.19 mm and 17.45 in 2016 and 234.50 cm, 16.06 mm and 25.90 in 2017 growing season respectively).

Likewise in main stem length, stem diameter and number of nodes, also there was a significant difference between the graft combinations and control in plant biomass. NUN-9075/CS graft combinations resulted in highest average values in plant fresh weight and dry weight (3.84 and 0.55 kg.plant<sup>-1</sup> respectively), while lowest average values were obtained in non-grafted plants (control) (0.43 and 0.06 kg.plant<sup>-1</sup>).

No significant difference observed between graft combinations and control in number of anthers, total number of pollen, number of pollen per anther and number of pollen per flower at 5% level of significant. However, the highest number of pollen grains was observed in Argentario/CS (1891235.55) in 2016 growing season. Moreover, no significant differences observed between graft combinations in pollen viability, pollen germination percentage and normal pollen development.

NUN-9075/CS graft combination resulted in higher total fruit yield, seed yield and more number of seeds per fruit, while Argentario/CS graft combination resulted in higher values of main stem length, main stem diameter and more number of node.

NUN-9075/CS and Argentario/CS graft combinations resulted in higher seed germination percentage and germination rate, higher seed emergence percentage and seed emergence rate and higher accelerated aging.

According to the results of this study, NUN-9075 and Argentario are regarded as the best rootstocks for both fruit yield and quality, as well as seed yield and quality. Hence, they are recommended for commercial use in grafted watermelon seed production.



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## ABBREVIATIONS

AA	: Accelerated aging
cm	: Centimetre
CS	: Crimson sweet
DSL	: Days to 10 cm stem length
FAO	: Food and Agriculture Organization
FD	: Fruit diameter
FFF	: First female flower
FH	: Fruit height
FMF	: First male flower
FRT	: Fruit rind thickness
g	: Gram
GP	: Germination percentage
GS	: Germinated seed
GT	: Germination time
GTS	: Grafted seedlings
K <sub>2</sub> O	: Potassium
kg	: Kilogram
KNO <sub>3</sub>	: Potassium Nitrate
l	: Litre
LSD	: Least significant difference
MAP	: Monoammonium Phosphate
m <sup>2</sup>	: Square metre
m	: Metre
mg	: Milligram
ml/l	: Millilitre/litre
mm	: Millimetre
MSD	: Main stem diameter

MSL	: Main stem length
N	: Nitrogen
NaCl	: Sodium chloride
NL	: Number of Leaves
ns	: non-significant
NSS	: Number of seeds sown
Pg/f	: Polen grain per flower
P <sub>2</sub> O <sub>5</sub>	: Phosphorous
SD	: Sowing date
SE	: Seed emergence
SER	: Seed emergence rate
SFW	: Single fruit weight
SGD	: Seed germination rate
SGP	: Survived grafted plants
SGP	: Seed germination percentage
SMC	: Seed moisture content
SR	: Survival rate
TFY	: Total fruit yield
TSS	: Total soluble solid
TTC	: Triphenyltetrazolium chloride
50% MF	: 50% male flowers
50% FF	: 50% female flowers
°C	: Degree centigrade

## 1. INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) of the family *Cucurbitaceae* is one of the most important horticultural crop world wide nutritionally and economically and grown in both temperate and tropical regions (Pitrat et al., 1999; Bosgnin, 2002). Watermelon originated in South Africa, its cultivation has spreaded all over the world (Meeuse, 1962). It includes three subspecies; *C. lanatus* subsp. *lanatus* L., which represents the group of ancient cultigens such as citron watermelon, *C. lanatus* subsp. *mucosospermus* L., and *C. lanatus* subsp. *vulgaris* which represents the commonly modern cultivated sweet watermelon group (Ren et al., 2014). Besides watermelon, *Cucurbitaceae* family includes other crops such as melon, pumpkin, cucumber, squash and gourds. Watermelon is well known as a functional food that provides important nutritional and bioactive benefits (Perkins-Veazie et al., 2012). Other important healthy bioactive compounds contained in watermelon including phenolic compounds, carotenoids, citrulline, vitamins and flavonoids (Gunn et al., 2015). Watermelon is important for its nutritive value especially the red fleshed watermelon that contain high quantity of lycopene, a red pigmented carotenoid with antioxidant properties (Tomes et al., 1963; Di Mascio et al., 1989; Perkins-Veazie et al., 2006). Lycopene of red fleshed watermelon is believed to be almost 40% higher (4.81 mg/100 g) than tomato (3.03 mg/100g) (Naz et al., 2014). The flesh of watermelon contains about 91% water and other rich source compounds as antioxidant agents against oxygen free radicals particularly lycopene and  $\beta$ -carotene (Soteriou et al., 2014).

The World production of watermelon is led by China with 72 943 838 tonnes (66.7%), Iran 3 947 057 tonnes (3.61%), Turkey 3 887 324 tonnes (3.55%), Brazil 2 163 501 tonnes (1.97%) and Egypt 1 894 738 tonnes (1.7%) as the large world's producers (FAOSTAT, 2015). Turkey used to be the second world producer of watermelon before Iran took over in the recent years. Among the

regions in Turkey, Adana ranked the first with 18.7% for watermelon production followed by Antalya, İzmir and Diyarbakir.

Grafting is a process that involves the choice of rootstock and scion, grafting of the rootstock and scion, healing and acclimatization of the grafted plant. Grafting is an asexual plant propagation method that joins plant parts to live together, hence, they grow as one single plant (Besri, 2008). Grafting is an asexual propagation technique that have been used for fruit trees and vines over thousand years. For a better results of grafted plants, there are several points to consider in grafting work such as plant species, type of graft, incompatibility, environmental condition during and after grafting, growth activity of rootstock and the craftsmanship of grafting (Hartmann *et al.*, 2002). Vegetable grafting is a recent practice started in late 1920's and first began with the production of grafted watermelon onto a pumpkin (*Cucurbita moschata*) and later bottle gourd (*Lagenaria siceraria*) rootstocks, in Korea and Japan. Grafting was originally adopted in China, Japan and Korea, where land use was intensive and crop rotation was difficult. Subsequently, due to multiple derived benefits, grafting has been adopted in additional countries such as Australia (Tran-Nguyen *et al.*, 2012). It was introduced to western countries in early 1990's and currently has become a common practice in many parts of the world (Ashita, 1927; Lee, 2003; Davis *et al.*, 2008a; Lee *et al.*, 2010). However, as mentioned in many reports, the effective vegetable grafting have been started as early as 1940's. Vegetable grafting was introduced purposely to control soil-borne diseases such as Fusarium wilt (*Fusarium oxysporum*), root-knot nematodes (*Meloidogyne* spp.), increase water and nutrient uptake and promote plant growth, but now grafting have further been used to increase low temperature resistance, salt and wet soil tolerance, improving plant vigour and extending the duration of economic harvest time and minimize copper toxicity (Lee, 1994; Ruiz *et al.*, 1997; Yetisir and Sari, 2003; Davis *et al.*, 2008b; Rouphael *et al.*, 2008; Yetisir *et al.*, 2013; Liu *et al.*, 2015).

Type of grafting has a great influence on the compatibility of rootstock and scion, plant growth as well as the fruit yield and quality. There are many grafting methods available today in watermelon and vegetable grafting in general such as hole insertion grafting, tongue approach grafting, one cotyledon grafting, side insertion grafting (Lee, 1994; Oda, 1995; Lee and Oda, 2003; Hassell *et al.*, 2008). Root-removed grafting method resulted in high stem diameter, better plant growth and physiological index and seedlings survival rate of 88.6% compared to conventional cleft grafting (Qin *et al.*, 2016). Hole-insertion method with different insertion angles resulted in high grafting successful rate and high survival rate of 91% (Ma *et al.*, 2014). The multi-rootstock grafting method of bottle gourd and pumpkin had a highly significant interaction effect on stem diameter, number of leaves, single fruit yield, plot yield and fruit vitamin C content (Qin *et al.*, 2014).

Grafting faces many problems such as the commercial rootstocks are limited and due to the huge eruption of rootstocks that are unexamined, their effects on vegetable quality are not clear yet (Elazar and Zoran, 2014). The most common rootstocks for watermelon grafting are wild watermelon (*C. lanatus* var. *citroides*) (King *et al.*, 2010; Kong *et al.*, 2014), *Cucurbita* interspecific hybrids (*C. moschata* x *C. maxima*) and the bottle gourd accessions (*Lagenaria siceraria*) that are highly resistance to soil-borne fungi (Fredes *et al.*, 2017; Liu *et al.*, 2015). But the main problems are time and labour required, as well as high costs of grafted seedlings compared to non grafted ones (Djidonou *et al.*, 2013). Also graft incompatibility between rootstock and scion is also another problem that interferes with the grafting work. Andrews and Marquez (1993), reviewed graft incompatibility as more than graft failure which may be resulted by environmental conditions or lack of skills, it is about physiological factors such as wounding responses, lack of cellular recognition, presence of growth regulators or incompatibility toxins. However, some of these problems such as labour costs can be reduced by improving grafting methods that will make more use of grafted plants. Also

efficient grafting machines and robots that nowadays become more popular, increased grafting speed and improved survival rate (Taylor et al., 2006).

The use of suitable rootstocks offer many benefits but also rootstocks associate with negative effects of grafting, therefore, the choice of appropriate rootstock to be used in grafting is paramount important. Most of these benefits are same to those general importances of grafting but also including those directly associated with uptake, translocation and utilization of plant nutrients. The correct choice of rootstock can bring about a preferred success in the grafting work. The use of rootstock depends on place and purposes of grafting. In Japan *Lagenaria siceraria* is the most frequently used rootstock for watermelon grafting accounts for 64% followed by *Cucurbita* spp. (26%), *Benincasa hispida* (7%) while watermelon varieties only used to overcome *Fusarium* wilt problems (1%) (Kawaide, 1985). *Lagenaria* used mostly as a potential rootstock for watermelon and other cucurbits in some Asian and European countries to reduce incidences of soil-borne diseases, to promote vigour of the root system and because of its high compatibility rate (Lee and Oda, 2003; Lee, 1994; Oda, 1995; Yetisir and Sari, 2003; Karaca *et al.*, 2012). Edelstein *et al.* (2014), suggested the use exotic watermelon rootstocks since can prevent negative effects in fruit size, shape and quality but also tolerance to soil-borne pathogens.

Watermelon seed is very important in both as a source of plant material as well as human and animal food. Watermelon contains seed of different size, thickness, colour and texture of the seed coat depending on variety (Oyolu, 1977). Watermelon seeds are contained in the pulp and constituting about 1.9 – 4% of the fresh fruit (Kamel *et al.*, 1985). Seeds of Cucurbits are non-endospermic and have an epigeal germination (Singh *et al.*, 2001). Watermelon seeds obtained from fruits produced from either open pollinated plants, F<sub>1</sub> hybrid or triploid (seedless) watermelon. Understanding the watermelon seed germination is very important since germination and emergence of the seed give good indication of a good seed.

Watermelon is basically a cross pollinated crop, although some flowers of some plants within the population can bear andro-monoecious flowers. Therefore, in whichever method of watermelon seed production is used, great care and attention is very crucial such as isolation and roguing.

Seed yield of any crop varies considerably according to region, variety, soil, cultural practices and climate. In important *Cucurbits*, the average seed yield obtained from field grown is about 225 – 280 kg/ha for watermelon, 325 – 450 kg/ha for cucumber, 675 – 900 kg/ha for summer squash, 450 – 560 kg/ha for winter squash and 125 – 340 kg/ha for muskmelon (Ellis, 2006). Chopra *et al.*, (2006) found that seed yield of watermelon increased significantly when they applied glyphosate 1% along with one hoeing weed control.

Besides the agronomic practices and environmental conditions, the successful production of crop also depends on the quality of the seed used for sowing. Ambika *et al.* (2014), explained the importance of good quality seed as it increases yield by 15-20%. Seed yield and quality can be increased in different techniques. Many studies have been conducted to investigate the relationship between different techniques to seed yield and quality. Some of these studies that investigate the relationships to increased seed yield and quality are fruit-set order (Nerson, 2004); fruit shape and plant density (Nerson, 2005); plant spacing and pollen quality (Lima *et al.*, 2003); effect of fertilizers (Olaniyi and Tella, 2011); seed size (Ambika *et al.*, 2014). However, so far there is no study conducted to investigate the effect of rootstocks on seed yield and quality, therefore, the aim of this thesis is to study the best rootstock that increases seed yield and quality.



## 2. LITERATURE REVIEW

### 2.1. Grafting for Yield and Quality

Yetisir *et al.* (2003) studied the effect of different rootstocks on plant growth, yield and quality of watermelon. They grafted watermelon cultivar Crimson Tide on 10 different rootstocks (*Cucurbita moschata*, *Cucurbita maxima* and *Lagenaria siceraria* – open pollinated, Strong Tosa, Gold Tosa, P360, Skopje, Emphasis, 216 and FRG – hybrid cultivars) and found that *Lagenaria* rootstocks resulted in higher survival rate (95%). Grafted plants flowered about 10 days earlier and showed more vigorous vegetative growth than the control plants. Also grafted plants found to have higher fresh weight, more leaves and larger leaf area. Indeed *Lagenaria* rootstocks produced higher yield than *Cucurbita* type of rootstocks.

Alan *et al.* (2007) studied the effect of different rootstocks on watermelon plant growth, fruit yield and quality under low tunnels by comparing the grafted and ungrafted plants. They grafted watermelon (*Citrullus lanatus* (Thunb.) variety Crisby onto TZ-148 and RS-841, commercial hybrids of *C. maxima* x *C. moschata* and experimental rootstock (*Lagenaria siceraria*) cv. 64-18. They found that grafted plants improved plant growth and yield without any harmful effects on fruit quality.

Davis *et al.* (2008b) overviewed the effects of grafting on vegetable quality and came up with the same results as Roupheal *et al.* (2010) did, hence they recommended the careful choosing of rootstock/scion combinations for optimal fruit quality as well as the importance of conducting rootstock/scion combinations under multiple climatic and geographic conditions because many rootstocks have optimal temperature and moisture ranges.

Bruton *et al.* (2009), studied the influence of grafted watermelon on fruit firmness, lycopene content and total soluble solids by using five rootstocks. They used *Cucurbita ficifolia*, *Cucurbita maxima* x *C. moschata* hybrid and *Lagenaria*

*siceraria* rootstocks, and they found that *C. ficifolia* consistently increased fruit quality especially firmness values. They concluded that grafting could increase the fruit firmness by 25%.

Rouphael *et al.* (2010) in their review of the impact of grafting on product quality of fruit vegetables determined that fruit quality is affected by grafting through translocation of the metabolites via xylem, they further explained that the effects could be in fruit appearances (size, shape, colour and absence of decay and defects), firmness, texture, flavour (sugar, acids, and aroma volatiles) and health-related compounds such as desired compounds like vitamins, minerals, and carotenoids as well as undesirable compounds like heavy metals, pesticides and nitrates. However, they noted some conflicting reports on the changes in fruit quality due to grafting but clarified that the conflicts could be attributed by the differences in production methods, environment, type of rootstocks/scions used and harvesting date.

Alla *et al.* (2012) studied the effect of Nubian watermelon on plant growth, flowering, fruit and seed yield and fruit characteristics. They grafted Nubian watermelon onto bottle gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita moschata*), luffa (*Luffa cylindrica*) and fig leaf gourd (*Cucurbita ficifolia*). They found that bottle gourd rootstocks significantly increased number of hermaphrodite flowers, number of fruits/plant, number of seeds/fruit and fruit weight. Also grafting Nubian watermelon onto bottle gourd followed by pumpkin significantly increased fruit yield per m<sup>2</sup>, seed yield per m<sup>2</sup>, both weights of fruit yield (ton/feddan) and seed yield (ton/feddan) compared to Luffa and control.

Karaca *et al.* (2012) investigated the rootstock potential of 21 bottle gourds and 2 commercial rootstocks (*Lagenaria siceraria*) collected from Mediterranean region in Turkey on watermelon (Crimson Tide) for plant growth, yield and fruit quality. They found the survival rates of the grafted plants varied from 83% to 100%, higher plant vigour, higher plant dry weight 37% to 80% and higher total

yield. Finally they concluded that the Turkish bottle gourds have high rootstock potential for watermelon with regards to investigated parameters.

Mohamed *et al.* (2012) investigated the effect of watermelon grafting on plant growth and fruit quality by using Aswan F<sub>1</sub> as scion grafted onto Nun 6001 F<sub>1</sub>, Strongtosa F<sub>1</sub>, Tetsukabuto F<sub>1</sub>, Ferro F<sub>1</sub> and Shintoza F<sub>1</sub> rootstocks, found that grafting increased plant vegetative growth and fruit yield especially with Nun 6001 F<sub>1</sub> rootstock. Also they found lycopene content increased by 57% in fruit flesh. Hence, they concluded that watermelon grafting influences plant growth, productivity and fruit quality.

Oztekin *et al.* (2012) studied the effects of five different rootstocks, 3 interspecific squash rootstocks 'RS 841', 'Nunhems 9075' and 'Maximus - AG 1355' and 2 bottle gourd rootstocks 'Magis' and 'Argentario' on plant growth, fruit yield and quality. They grafted watermelon scion cultivar Crimson Tide onto these rootstocks. Results revealed higher plant fresh and dry weight, larger fruit size, more number of seeds, total soluble solids and highest total yield of 89.9% for the grafted plants that was obtained on Maximus rootstock compared to 66.3% that of non grafted plants.

Turhan *et al.* (2012) studied the influence of rootstocks on yield and fruit characteristics and quality of watermelon. They grafted watermelon cultivars 'Crimson Tide', 'Dumara' and 'Farao' scions onto hybrid squashes rootstocks 'Dynamo', 'RS-841' and 'Shintosa'. In this study, they observed that although grafting resulted in lower dry matter, total soluble solids, total sugar and titratable acid, but significantly influenced fruit shape index, rind thickness, fruit weight, total yield and marketable yield.

Candir *et al.* (2013) studied the phytochemical characteristics of grafted watermelon on different bottle gourds (*Lagenaria siceraria*) from Mediterranean region in Turkey. They conducted study to determine sugar, organic acid and carotenoid content of Crimson Tide (CT) watermelon grafted onto 21 local bottle gourd rootstocks. They found that grafting CT onto local bottle gourd rootstocks

improved total soluble solids (TSS), titratable acidity (TA), TSSTA ratio, sugar, organic acid and carotenoid ( $\beta$ -carotene and lycopene) contents. Also they observed that some graft combinations (CT/09-01 and CT/07-06) increased the fruit quality by revealing higher sucrose, lycopene and total carotenoid content.

Yetisir *et al.* (2013) used four gourd rootstocks Ferro, RS841 (*Cucurbita maxima*  $\times$  *C. moschata*), Argentario and Macis (*Lagenaria* hybrid) to investigate the plant nutrition partitioning on watermelon. They found that except Mg, Fe, Zn and Mn concentrations of seed all other plant nutrients content were significantly affected by rootstocks and scion, especially in leaf, rind, flesh and seed.

Besides the positive effects obtained by grafting with a single rootstock, the use of multiple rootstocks has also given outstanding results in fruit yield and quality as studied by Qin *et al.* (2014). They did a comparative analysis of the effects of single, dual and threefold grafting on the plant growth, fruit yield and quality in watermelon by using bottle gourd and pumpkin rootstocks. The analysis showed that the combined rootstock of bottle gourd and pumpkin has significant interaction effects on the stem diameter, number of leaves, single fruit weight, plot yield and fruit vitamin C content of the grafted watermelon plants.

Soteriou *et al.* (2014) evaluated physicochemical and phytochemical composition of watermelon fruit during ripening as affected by grafting. They assessed flesh reflectance colorimetry, texture, pH, titratable acidity and soluble solid, soluble carbohydrate, lycopene and citrulline during ripening from 30-50 days after anthesis in grafted and non grafted plants. Results showed that grafting increased firmness, titratable acids and lycopene content. Also they observed total sugars, soluble solids and ripening as a whole delayed in grafted plants that means fruit quality is elongated and this may benefit from belated harvest.

Petropoulos *et al.* (2014) examined the effect of grafting of watermelon hybrids 'Obla F1 and Vanessa F1' onto *Cucurbita maxima*  $\times$  *C. moschata* rootstock TZ 148 and *Lagenaria* spp. rootstock 'Dias F1' on the volatiles and yield of fruit

and plant growth. They found that grafted plants had higher growth rate, total yield and fruit number than ungrafted plants.

Kyriacou and Soteriou (2015) studied quality and postharvest performance of watermelon fruits by grafting diploid cultivars onto interspecific rootstock of *Cucurbita maxima* × *C. moschata* and found that the quality and stability were improved. Also they found that rootstock improved the flesh firmness and lycopene content and enhanced flesh color at postharvest.

Kyriacou *et al.* (2015), analysed the configuration of watermelon fruit quality for the effects of grafting, harvesting maturity and postharvest storage. The watermelon hybrid cv. Pegasus on interspecific hybrid squash rootstock TZ148 and stored the fruits after harvesting at 25 °C. They reported that the grafted watermelon rootstock TZ 148 resulted in increased pulp firmness by 46.5% before postharvest. Also grafted watermelon resulted in sucrose content increase in both preharvest and postharvest, while pulp acidity decreased steadily with ripening but was moderately increased by grafting. Indeed, citrulline content increased by 12.5% and hence, they concluded that grafting enhances pulp texture and bioactive composition.

O Huang *et al.* (2016), they did comprehensive mineral nutrition analysis of watermelon cultivar ‘Zaojia 8424’ grafted onto two rootstocks, bottle gourd (*Lagenaria siceraria*) ‘Jingxinzhen 1’ and pumpkin (*Cucurbita maxima* × *C. moschata*) ‘Qingyanzhen 1’ and non grafted (control). They observed that grafting significantly resulted in increased plant growth and single fruit weight of watermelon. Also they observed that watermelon grafted onto pumpkin rootstocks exhibited significantly higher root volume, root surface area and number of root tips compared to control. Moreover, they explained that grafting enhanced fruit flesh, rind firmness and rind thickness as well as total uptake (mg.plant<sup>-1</sup>) and concentration (mg.g<sup>-1</sup> DW) of N, K, Ca, Fe, Mg and Mn in root, stem, leaf, fruit rind and flesh. They revealed that the total uptake of nutrients of plants grafted onto bottle gourd and pumpkin was increase by 30.41% and 49.14% at fruit

development stage and by 21.33% and 47.46% at fruit maturation stage, respectively, compared to control.

zdemir *et al.* (2016) investigated the effects of rootstocks on storage and shelf life of grafted watermelons. They grafted 'Crimson tide' and 'Crisby' watermelon scions onto Ferro, RS841, Argentario and Macis rootstocks where they compared the postharvest quality during storage at 7 °C and 21 °C for 7 and 21 days. They observed that Ferro and RS841 rootstocks resulted in higher flesh firmness, thicker rind, lower ripening rating, more intense (higher C\*) brighter red (lower h° value) colour and higher lycopene content. Also they observed high scores (7.5/8.5) for taste from panelists and revealed that watermelon can be stored successfully at 7 °C for 21 days and additionally 7 days at 21 °C.

## 2.2. Grafting Against Soil-Borne Diseases

As previously explained the main goal of grafting vegetables was to control soil borne disease. Conducting research to find out the most tolerant rootstock against pathogens have become more important due to the vast emergence of the rootstocks used in grafting. Tolerant rootstocks not only ensure health plants but also assure quality yield without defects or disorders.

Yetisir *et al.* (2003) evaluated the potential of grafted watermelon for resistance to *Fusarium oxysporum* f.sp. *niveum* on some *Cucurbitaceae*, *Lagenaria*, *Luffa*, *Benincasa* and commercial rootstocks. Also they evaluated the effects of grafting on yield and quality of diseased plants. They found that all grafted plants and rootstocks were resistance to the three known races (0, 1 and 2) of *F. oxysporum* f.sp. *niveum* except watermelon cv. 'Crimson Tide' that was susceptible to race 2. Also fruit yield was positively (21-112%) affected by *Lagenaria* rootstocks but negatively affected (200-267%) by *Cucurbita* rootstock when compared with control.

Huitron-Ramirez *et al.* (2009), investigated the influence of grafted watermelon plant density on yield and quality in soil infected with melon necrotic

spot virus. They were looking for the alternatives of Methyl bromide (MB) because of the restriction due to its negative effects to the environment. Therefore, they used triploid watermelon cultivar Tri-X 313 grafted onto two rootstocks of *Cucurbita maxima* × *C. moschata* ('RS841' and 'Shintosa Camelforce') cultivated in open field soil infected with *Olpidium bornovanus* and melon necrotic spot virus that was fumigated with MB against one without fumigation. They found that grafted watermelon significantly increased average fruit weight, total yield and flesh firmness in soil without MB fumigation. Hence, they concluded that grafting may be considered as an alternative method to MB fumigation.

The effect of grafting on *Fusarium* also was studied by Mohamed *et al.* (2012) where they cultured the infected small pieces of roots and basal stems into potato dextrose agar and incubated at 28 °C for 3-7 day watermelon. They observed the survival rate of between 91.67% - 100% from grafted plants and 68.33 % for non grafted plants, the commonly isolated microorganism associated with root-rot and wilting of non-grafted watermelon was identified as *Fusarium oxysporum*. Finally, they concluded that grafting can be an alternative method to control *Fusarium* wilt in watermelon.

Buller *et al.* (2013) in their research on grafted, non-grafted and self-grafted triploid watermelon by using 'Emphasis' bottle guard (*Lagenaria sicerarea*) and 'Strong Tosa' interspecific squash hybrid (*Cucurbita maxima* x *C. moschata*) held in Oregon and Washington USA. They found that the grafted watermelon had significantly larger stem diameter than non-grafted and self-grafted plants. Also they observed that 'Crispn sweet' watermelon used as a scion had no foliar symptoms of *Verticillium*. However, they observed significantly lower *Verticillium* wilt severity in rootstocks.

Keinath and Hassell (2014), determined the frequency of infection of six rootstocks by *Fusarium oxysporum* f.sp. *niveum* races 1 and 2 and the field performance of watermelon grafted rootstocks of interspecific hybrid squash, bottle gourd and citron (citroides). Grafted and non-grafted watermelon and rootstock

plants were inoculated in the greenhouse with race 1, race 2 and water (control). They found that in both races, the frequency of recovery of *F. oxysporum* from scion and rootstock portions of inoculated watermelon plants grafted onto 'Ojakkyo' citron was greater than from watermelon plants grafted onto 'Shintosa Camel' and 'Strong Tosa' interspecific hybrid squash, and from plants grafted onto bottle gourd. For non-grafted plants inoculated with race 2, *F. oxysporum* was recovered from the base of more than 79% of all inoculated plants. Also they observed that grafted plants produced greater weight and number of fruits.

Liu *et al.* (2015) conducted research to find new rootstock that would be a solution against nematodes for cucumber, melon and watermelon due to the fact that nematode was controlled by soil fumigation with methyl bromide but it was banned because of high toxicity. Therefore, they tested a wild *Cucumis* species (*Cucumis pustulatus*) as a possible rootstock for cucumber, melon and watermelon. They measured the survival rate, plant growth, yield and fruit quality of grafted plants. From 16 species, 53 accessions were studied and 5 accessions exhibited high resistance to southern root-knot nematode, while 12 accessions exhibited resistance to *Fusarium* wilt. Ultimately, they found that *C. pustulatus* is suitable rootstock with simultaneous resistance to root-knot nematode and *Fusarium* wilt for cucumber, melon and watermelon.

### 2.3. Watermelon Seed Nutrient Content

Watermelon seed contains minerals and other nutrients that have very good functions in the body if consumed. The presence of amino acids especially high arginine content, indicates that watermelon seed has a potential of medicinal effect (El-Adaway and Taha, 2001).

Lakshmi and Kaul (2011), did a study on decorticated watermelon seed bought from a local market to assay the nutritional potential. They reported high macro and micro nutrients especially fat (46.8 g), protein (27.6 g), ash (2.8 g),

crude fibre (4.7 g), iron (7.3 mg), calcium (100 mg), zinc (5.2 mg) per 100 g from seed meal.

Rahman *et al.* (2013), observed that watermelon seed extracts possess antioxidant activity especially the total phenolic contents, flavanoids, nitric oxide and ascorbic acid with n-Hexane, chloroform and ethanol extracts. However, they revealed that the potency of antioxidant activities depends on the type of extract and that, n-Hexane extract of watermelon seeds possesses highest antioxidant activity *in vitro*.

Mehra *et al.* (2015) reported high concentration of nutrients from their study on estimation of nutritional, phytochemical and antioxidant activity of muskmelon and watermelon. They reported that watermelon seed contains high percentage of protein (34.22%) and carbohydrates (26.57%) compared to muskmelon. Other components found at high level in watermelon seed were 531.15 kcal energy, 31.99% fat, 3.64% ash, 0.1% fibre.

Tak and Jain (2016), in their study on nutrient potential of watermelon seed found high concentration of fat, carbohydrate, fibre, protein and energy values of 48.9 g, 8.9 g, 1.8 g, 32.6 g and 619 kcal respectively per 100 g of seed. They concluded that the watermelon seed flour due to high nutrient content can be successfully incorporated in vegetable gravy.

#### **2.4. Seed Priming**

Seed priming is used to enhance seed germination rate and seedling establishment. It has been reported in many reports that seed priming enhances seed germination performance of various crops (Shehzad *et al.*, 2012). There are different priming methods that can be used to improve germination. The priming methods include hydropriming (Huang *et al.*, 2002), osmopriming (Farooq *et al.*, 2007), halopriming (Siadat *et al.*, 2011), thermopriming (Yari *et al.*, 2012), biopriming (Moeinzadeh *et al.*, 2010) and solid matrix priming (Merreddy *et al.*, 2000).

Demir and Van de Venter (1999), investigated the effect of priming on performance of watermelon seeds under temperature and osmotic stress. Seeds subjected to either osmoconditioning (2%  $\text{KNO}_3$  at 20 °C for 6 days) or hydropriming (30 °C for 18 h) incubated at 15, 25 and 38 °C. They found that the seed germination mean time was decreased and germination percentage increased in osmoconditioning, and also seed emergence was enhanced at 15 °C.

Susila *et al.* (2013) studied the effect of priming on germination of watermelon seed under low temperature with  $\text{KNO}_3$  3% , Brassinosteroid 1, 2, and 3  $\mu\text{M}$  and Methyl Jasmonate 1, 2 and 3  $\mu\text{M}$  solutions for 24 h at 20 °C in darkness and distilled water as control. They reported the maximum radical length obtained in seeds primed with  $\text{KNO}_3$  3% + Me JA 1  $\mu\text{M}$ . Also under the same treatment they obtained early and higher emergence percentage.

Phat *et al.* (2015) studied the effect of priming on different concentrations of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), fusicoccin (FC) and gibberellic acid (GA) on germination and seedling uniformity of triploid watermelon. They pretreated Seedless Plus, Sinus and Sizero cultivars with water and  $\text{H}_2\text{O}_2$  (2 and 4%), FC (1, 5 and 10  $\mu\text{M}$ ) and GA (1, 5 and 10  $\mu\text{M}$ ). Their findings revealed that, treating seeds with 5  $\mu\text{M}$  GA and hydropriming helped to break seed dormancy, enhancing the final germination percentages in all cultivars and increasing the germination index in Sizero cultivar. Indeed, they recommended that seed priming treatments could be used in large scales also they concluded that hydropriming provides a simple, effective and costless method to improve seed germination and seedling vigour.

Mavi and Atak (2016) studied the effect of organic priming of watermelon seed emergence under low temperature. They primed Crimson sweet with leonardite (30 g moistened with 25 ml distilled water at 25 °C for 24 h), waste tea (12 g waste tea moistened with 40 ml distilled water at 25 °C for 24), potassium nitrate ( $\text{KNO}_3$  3%), polyethylen glycol (PEG 6000, -1.25 MPa), patula herbal tea (2 and 10 g dried petal/L) along with hydropriming and unsoaked seed (control). Their results showed that seed priming significantly increased seed germination

rate and germination percentage. Also the priming increased survival rate of the primed seeds compared to control.

### 2.5. Seed Germination

Hall *et al.* (1989) reported that water-imbibed 'Crimson sweet' watermelon seeds provided faster plant development and the differences were greater when soil temperatures ranged from less than the optimum (21.3 to 35.4°C) than dry seeds at temperatures below the optimum (15.7 °C).

Singh *et al.* (2001) studied the optimum temperature for testing watermelon seeds for cold germination ability, also screening of a set of cultivars for germination speed at 10, 14, 18 and 22 °C. They found a rapid and high germination percentage at 14 °C to about 90 to 95% for 'Blackstone and Starbrite' cultivars.

Bakhshandeh *et al.* (2015) evaluated the ability of hydrothermal time to describe the kinetics of watermelon (*Citrullus lanatus* cv. 'Crimson sweet') seed germination under different temperature and water potentials (10 °C, 28.34 °C and 40.8 °C). They observed that temperature and water potentials influenced germination rate and germination percentage.

### 2.6. Seed Yield and Quality in Watermelon

Many studies have been conducted to investigate seed yield and quality with different approaches. Lima *et al.* (2003) studied the effect of plant spacing and pollen quantity on squash seed yield and quality. They used three different spacing (0.8 × 0.3, 0.8 × 0.6 and 0.8 × 0.9 m) and two quantities of pollen (50% of anther and 100% anther). They observed that wider plant spacing increased average number of matured fruits and seed yield per plant. That means the higher the pollen quantity, the higher the seed yield per area.

The relationship between plant density and seed yield and quality also was studied by Nerson (2005) in muskmelon, he obtained the highest seed yield at 8 to

12 plants/m<sup>2</sup>. Moreover, he found the positive relationship between fruit size and seed size, that means the seed yield increased with the increase in fruit size.

Chopra *et al.* (2006) investigated the efficacy of glyphosate herbicide against obnoxious weeds by adopting a new spraying technique and its effect on seed yield and quality in watermelon (*Citrullus vulgaris* cv. Sugar baby) in Karnal, Hayrana India. They used two levels of glyphosate (1.0% and 0.5%) to spray on the foliage of the weeds 20 days after transplanting. They observed that application of 1.0% glyphosate increased fruit and seed yield. Also seed weight and seedling dry weight found to be at maximum at 1.0% glyphosate.

Walters and Taylor (2006), studied the impact of honey bees on seed set, fruit set and yield of *Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch. Ex Poir under field conditions. They used honey bees, bumble bees, carpenter bees and squash bees. In their results they observed that honey bees resulted in larger-sized fruit of pumpkin and yield of *Cucurbita pepo*, *C. moschata* and *C. maxima* increased by 26%, 70% and 78% respectively. They further explained that honey bees increased proportionally the seed number per pumpkin in *C. maxima* to about 61%.

Olaniyi and Tella (2011), investigated the effects of N and K fertilizers and their combinations on growth, seed yield and quality of Egusi watermelon (*Citrullus lanatus* (Thunb.) Mansf.). They applied two levels of Nitrogen (0 and 60 kg.Nha<sup>-1</sup>) and five levels of potassium (0, 10, 20, 30 and 40 kg.K<sub>2</sub>O ha<sup>-1</sup>) and their combinations. They obtained the best seed yield and quality at 60 kg Nha<sup>-1</sup> × 30 kg K<sub>2</sub>O ha<sup>-1</sup>. Indeed the plant growth traits such as plant height, number of leaves, nutrient uptake, seed yield and quality were significantly increased with increasing rates of fertilizers.

Xhou *et al.* (2012) conducted field experiment to investigate the relationship between leaf area and leaf length and width with seed yield in watermelon. They found that seed yield of watermelon was highly related with leaf area index. Seed yield increased with the increased leaf area.

### 3. MATERIALS AND METHODS

This study was conducted at Horticultural Experimental fields and Biotechnology Center of the Cukurova University Adana together with the Antalya Seed Company in Turkey in 2016 and 2017 spring seasons.

#### 3.1. Plant Materials

Four plant species of the family *Cucurbitaceae*, 2 hybrids, Cucurbita ‘NUN 9075’ (*Cucurbita maxima* x *Cucurbita moschata*) and Lagenaria ‘Argentario’ (*Lagenaria siceraria*); and 2 watermelon species, Citron watermelon (*Citrullus amarus* Schard) ‘PI296341’ and ‘Crimson sweet’ cultivar (*Citrullus lanatus*) were used in this research. ‘NUN 9075’, ‘Argentario’ and Citron watermelon were used as rootstocks while watermelon cv ‘Crimson sweet’ was used as control (non grafted) and also used as scion grafted onto all other rootstocks. Plants were allowed to self pollinate in the field. Seeds for rootstocks production and scions obtained from the Department of Horticulture seed stock and others were bought from Antalya Seedlings Company. Latin square design was used in assigning rootstocks to the blocks during experimentation.

##### 3.1.1. Characteristics of the Rootstocks

###### (a.) Rootstock 1: Cucurbita - NUN-9075

- It is a Cucurbita rootstock from Nunhems Company,
- It produces very strong plants,
- It has high grafting ability,
- It is resistant to *Fusarium oxysporum* f.sp *niveum* and *Verticillium*

###### (b.) Rootstock 2: Lagenaria – Argentario

- It is a type of rootstock produced from Syngenta Company

- It is a Lagenaria F<sub>1</sub> type hybrid which is especially suited for the early grown watermelons
- It is root cold tolerant
- It has strong lateral root system and very good quality

**(c.) Rootstock 3: Citron watermelon - *Citrullus amarus* Schard  
PI 296341**

- It is a very strong *Citrullus* type of rootstock
- Resistant to Fusarium wilt

**(d.) Scion: Watermelon *Citrullus lanatus* cv Crimson sweet.**

- It is an open pollinated watermelon variety
- High yielding
- Strong and vigorous plant

### 3.2. Methods

#### 3.2.1. Seed Sowing and Grafting

Seeds were sent to Antalya Seedlings Company for sowing and grafting. Seeds of Citron watermelon were sown on 29/01/2016 and that of C. Sweet (scion) sown on 26/01/2016, while those of NUN-9075 and Argentario were sown later (01/02/2016) for 2016 growing season because the previous delay in germination and the later germinate very fast. For 2017 year, sowing was done on 03/02/2017 for Citron watermelon, 13/02/2017 for Crimson sweet (Control/Scion) and 16/02/2017 for NUN-9075 and Argentario. Grafting was done on 23/02/2016 for the first year and 22/02/2017 for the second since sowing and soon after grafting seedlings were transferred to the raising room before being transferred to the nursery. Figure 3. 1 shows the grafting method. All seedlings were kept and managed in the same greenhouse in Antalya Seedling Company under the same

growing conditions. Two weeks after germination number of germinated seeds were recorded, and 4 weeks later Crimson sweet scion was grafted onto rootstocks by using splice/one cotyledon grafting technique.



Figure 3.1. Grafting; (a) joining Crimson sweet scion onto *Cucurbita* rootstock; (b) grafted seedlings

### 3.2.2. Transplanting

One month after grafting all plants were transported to Adana from Antalya and in the second day seedlings were transplanted to the main experimental field of Department of Horticulture of the Cukurova University in Adana, Turkey. Grafted plants and non grafted (control) were planted at a spacing of 3 m x 0.75 m apart on 30/03/2016 for the first year and 06/04/2017 for the second year. Each genotype replicated randomly 4 times and 20 plants were planted in each plot for each genotype. Soon after transplanting low plastic tunnels were established to protect young plants from cold temperature and heavy rains (Figure 3. 2) and 2 weeks later the tunnels were removed. Fertilizer applied 3 times during the growing period, as follows, after transplanting, during plant development and at flowering. Fertilizer was applied at a rate of 15:15:20 kg per 1000 m<sup>2</sup>, with pure nutrients as N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O. The mostly used fertilizers were Monoammonium Phosphate (MAP) and Potassium Nitrate (KNO<sub>3</sub>) with ratio of 18:18:18 and 20:20:20 respectively. During application, fertilizer was applied together with water during irrigation as fertigation. Frequently, plants were irrigated by dripping system whenever the need to do so observed. Weeds were controlled by using hand hoes to remove weeds between the plants and mechanically by weeder to remove weeds between the rows. Insect pests and diseases control was done by applying pesticides with the aid of knapsack sprayer, Antracol Combi chemical used to control anthracnose, Pascal used against aphids while Oberon was used against red spider mites.

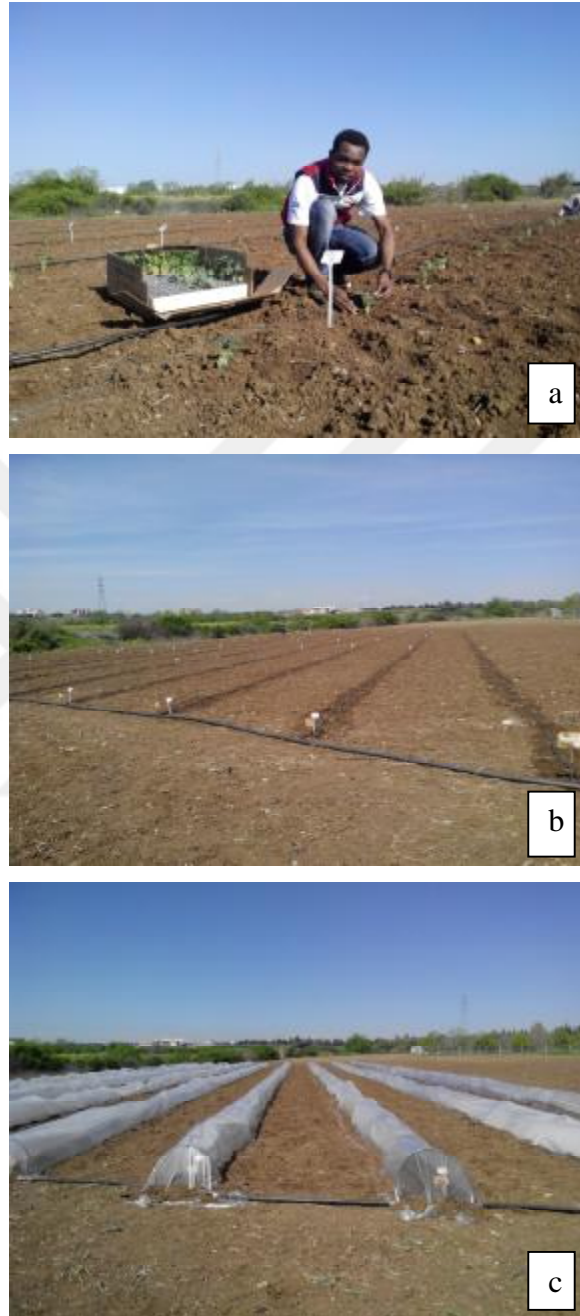


Figure 3.2. (a) Transplanting; (b) drip irrigation system watering the newly transplanted seedlings; and (c) low plastic tunnels soon after transplanting

### **3.2.3. Observation and Plant Measurements**

#### **3.2.3.1. Days Main Stem Formation to 10 cm Long**

Number of days were counted when at least 1-5 plants in every plot had the main stem reached 10 cm long since transplanting. At first measuring ruler was used on few plants to confirm the size of 10 cm long but then approximation of the plant size was done based on the first measurements.

#### **3.2.3.2. Flowering Time**

Flowering time is a number of days counted from transplanting to the first flowering of the flowers. The flowering of genotypes was observed in 4 areas, first flowering of male and female flower, 50% flowering of male and female flowers. The 50% flowering of the flowers was observed when at least half of the plants in a plot has initiated flowering.

#### **3.2.3.3. Main Stem Length, Stem Diameter and Number of Leaves**

First measurements of main stem length, main stem diameter and counting the number of nodes on main stem was carried out one month after transplanting and repeated one month later (06/05/2016 and 06/06/2016 for first growing season and 06/05/2017 and 06/06/2017 for the second growing season respectively). In every plot, 5 random plants were measured their main stem length from the ground level just after grafting union to the tip of the stem by using a measuring tape and then recorded. Main stem diameter was measured by using vanier caliper at the base of the stem and data recorded in millimeters (Figure 3.3 a & b). The leaves on the main stem were counted from the base to the tip and recorded. The measurements were repeated one month later, that is it was taken twice during the plant growth.



Figure 3.3. Plant growth measurements; (a) main stem length measurement; and (b) measuring the main stem diameter with digital vanier caliper

#### 3.2.3.4. Plant Biomass Content

At the initiation of fruit setting stage last 2 plants from every plot were cut at the soil surface level by using a sharp knife and the length of the main stem, diameter of the stem, fresh weight of leaves and stem were determined and number

of nodes and side shoots were counted and recorded. Leaves and stems were then oven dried at 70 °C for 3 days (Figure 3.4) to determine dry weight.



Figure 3.4. Plant biomass content measurements; (a) stem fresh weight; (b) leaf fresh weight

### 3.2.3.5. Pollen Production Experiment

To assess the capacity of pollen production among rootstocks, 10 flowers were used in every replication. Five mature flowers before opening when pale yellow colour started to develop (approximately one day before anthesis) were

selected and closed in the afternoon by using clips and picked in the next morning for pollen germination and viability. In the morning, after picking of 5 closed flowers from each replicate other 5 unopened flowers near to anthesis were picked and sent to the laboratory for anther counting and determination of pollen production.

**a). Anther counting:** Flowers were opened by using a pair of forceps, anthers were counted from each flower and then anthers were put in small film plastic boxes for drying (Figure 3.5). Every 1 box represented a replicate with total of 4 boxes per genotype and 5 flowers were put in every box (Gunver-Dalkilic and Dayi-Dogru, 2011). Soon after brought to the laboratory flowers were opened and anthers were removed and placed on a white paper at 27 - 28 °C over night for drying. After one day of drying, the dried anthers were put in small plastic film boxes and smashed by using a glass baguette to release pollen from anthers. Half of the pollen used in germination test and the other half used in pollen viability test.



Figure 3.5. Separation of anthers from flowers and counting the number of anthers

**b). Pollen germination:** Pollen germination was determined according to Eti (1991), medium was prepared by adding 12.5 g sucrose, 250 ppm boric acid and 1% agar into 100 ml of water. Then medium was poured into petri dishes and left to cool. Two folds of filter paper slightly wetted with distilled water was placed inside the cover of the petridish to provide moisture condition. By using fine sable brush, pollen was evenly spreaded on the medium, covered and left for 12 hours before counting the pollen numbers on microscope (Figure 3.6). Each replicate had 2 petri dishes and in every petri dish 3 different positions were selected randomly for pollen counting. Germinated pollen was determined by observing normal pollen with a protuded tail of at least the size of the pollen, but most pollen germinated with long tail more than 10 times to the size of the pollen (Figure 3.7). Germinated and non germinated pollens were counted and germination percentage was calculated.

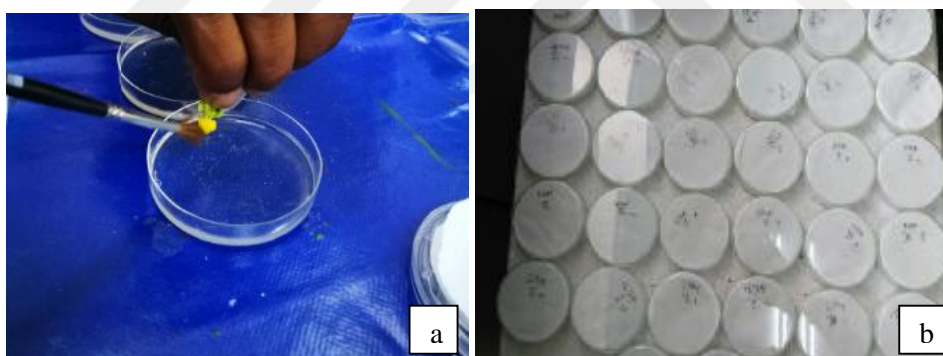


Figure 3.6. Pollen germination; (a) sowing pollen on a media in petri dish; (b) petri dishes with pollen soon after sowing waiting for microscopic observation

**c). Pollen viability:** After crushing the anthers to remove the pollen, 1% 2,3,5 Triphenyltetrazolium chloride (TTC) was prepared by adding 100 mg TTC and 5.4 g sucrose into 10 ml of water (Norton, 1966). Two separate drops of TTC was added on two different places on the slide and by help of a fine sable brush pollen was spreaded on the drop of TCC and covered by using slide cover. Slides

were left for 3 – 4 hours near the window with slight light. Then by using microscope viable, semi viable and non viable pollen were counted at 3 different places on each place with 2 slides per replicate and calculation was done to obtain the pollen viability percentage (Figure 3.8). Viability of the pollen was identified by its colour; viable (red colour), semi-viable (pink) and non viable (colourless/whitish) (Figure 3.9).



Figure 3.7. (a) Germinated and (b) non germinated pollen

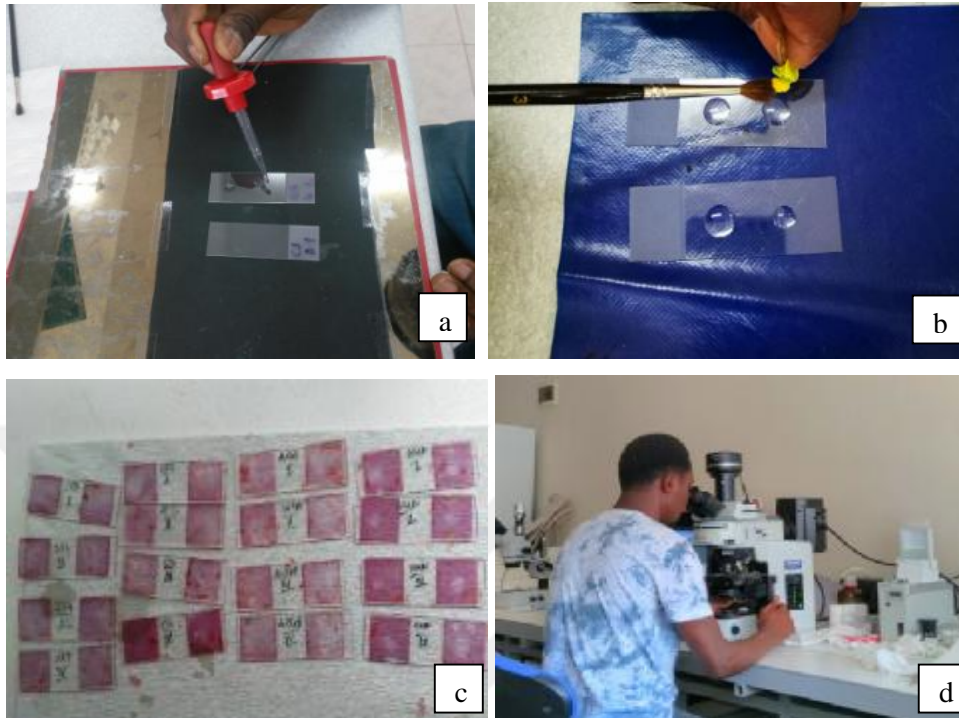


Figure 3.8. Pollen viability; (a) Putting drops of TTC on a microscopic slide; (b) sowing pollen on microscopic slide; (c) microscopic slides with pollen soon after sowing waiting for microscopic reading; (d) counting viable and non-viable pollen in a microscope

**d). Normal pollen development:** Boxes with anthers were left in the laboratory at a temperature of 27 - 28 °C for one week. One week later when anthers were well dried, 3 ml of water were added and by using glass baguette the anthers were crushed (Eti and Stosser, 1988). For proper spreading, not clubbing and to get homogenous solution of pollen one drop of liquid detergent was added. Then by using pasteur pipette one drop of solution was put on the two parts of hemocytometer, covered with slide cover and the results were read on microscope (Figure 3.10). The microscope with a special eye-objective lens with grids inside was used, only pollen within the grids area were counted (Figure 3.11). Well developed pollen (normal pollen) without any defects and abnormal pollen were determined. To obtain well developed normal pollen percentage, the number of

anthers per flower, number of pollen per flower and number of pollen per anther were calculated.

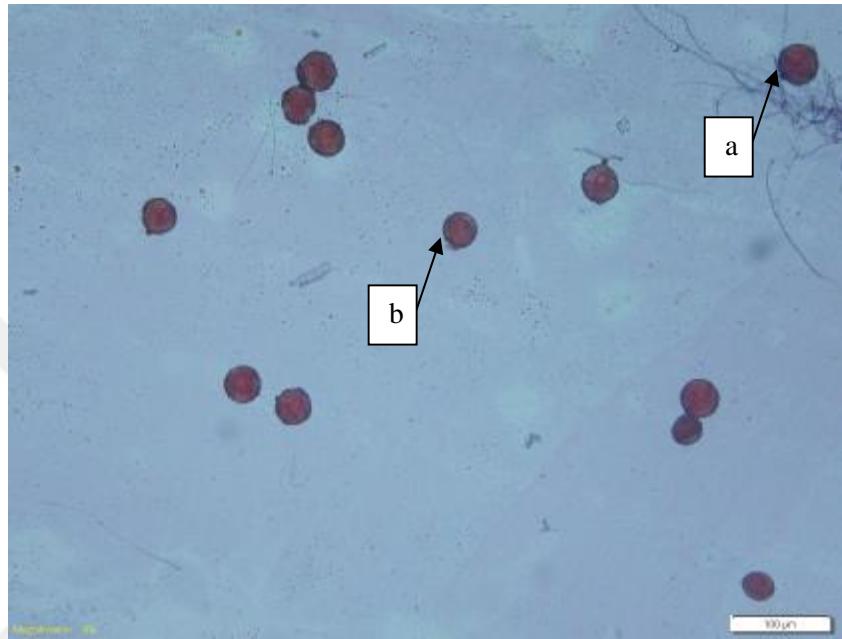


Figure 3.9. (a) Viable, (b) semi-viable and (c) non-viable pollen

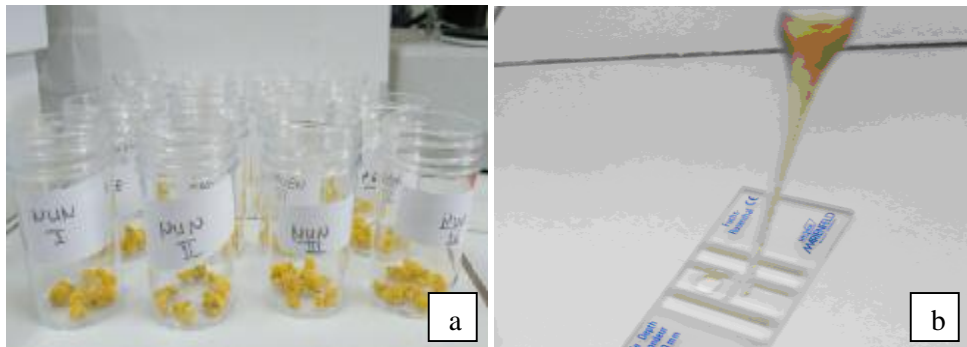


Figure 3.10. Normal pollen development; (a) anthers in the plastic boxes; (b) putting drops of pollen mixture on a hemocytometer by using pasteur pipette

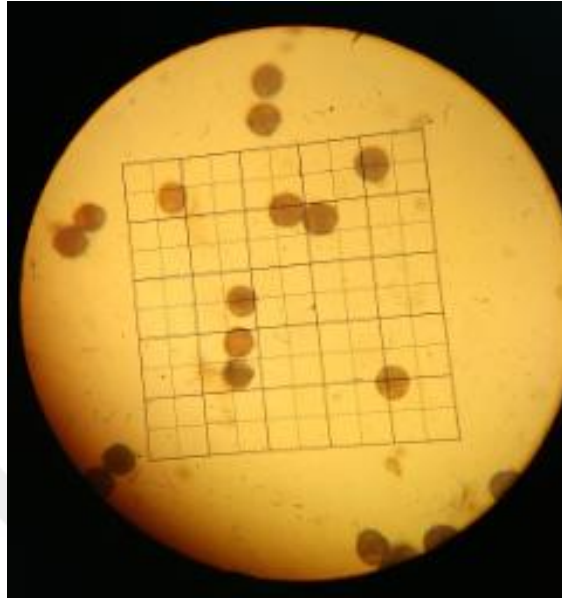


Figure 3.11. A snap taken to the microscope showing pollen within the grid area

#### **3.2.3.6. Fruits Harvesting**

Harvesting was done on 07/07/2016 for the first year and 28/06/2017 for the second year, that means in the first growing season it took 100 days from transplanting to harvesting and 83 days from transplanting to harvesting for the second growing season. Fruits were left for longer time in the field before harvest for better maturation of the seeds in the fruits. All fruits from all plots were harvested and weighed. Some cracked and decayed fruits were picked and weighed before the actual harvesting. During harvesting fruits were picked, put in the bags and grouped near the label of the respective replicate in every plot before they were carried to laboratory for analysis (Figure 3. 12 and 3.13).



Figure 3.12. Fruit harvesting, showing the biggest and heaviest fruit from NUN-9075/CS weighed 12.84 kg



Figure 3.13. Harvested fruits for seed extraction

**3.2.3.7. Total Fruit Weight/Yield**

All fruits were grouped according to source of rootstock and replication then they were carried to the laboratory. Fruits were weighed by using measuring balance to obtain total fruit weight. Total fruit weight in each plot was determined by dividing the weight of the particular fruit over plot area (18 plants x 0.75 m x 3 m = 40.5 m<sup>2</sup>) to get total fruit weight over area (yield in kg/m<sup>2</sup>). Three fruits were then taken from every replication for single fruit weight, fruit height, fruit diameter, fruit rind thickness, total soluble solid content, seed counting and seed weight determination.

**3.2.3.8. Fruit Analysis**

Three fruits were taken from every replication for analysis. Fruit weight (g) was measured by using measuring balance to get single fruit weight. For fruit analysis, each fruit was cut longitudinally into two halves then fruit height was measured by using a measuring ruler (cm) from the stem to the blossom end of the fruit. Fruit diameter (cm) was measured by measuring ruler while the fruit rind thickness (mm) was measured by using a digital vanier caliper (Mitutoyo CD-15D). Total soluble solid content (%) was measured by using a digital refractometer (ATAGO Pocket PAL-31) (Figure 3.14. a-f).

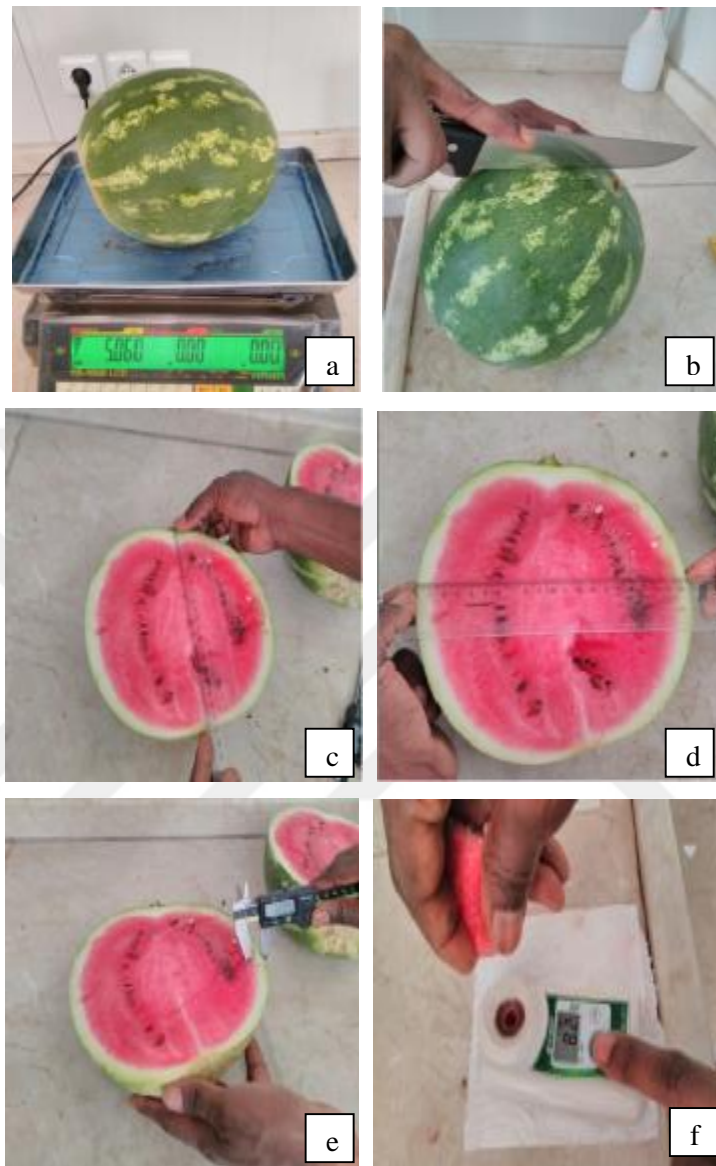


Figure 3.14. Fruit analysis; (a) single fruit weight; (b) longitudinal cutting of the fruit; (c) measuring the fruit height; (d) measuring the fruit diameter ; (e) measuring the fruit rind thickness by using a digital vanier caliper; and (f) measuring total soluble solids by using a digital hand refractometer.

**3.2.3.9. Seed Analysis Experiments**

After harvesting and weighing fruit were cut for analysis and seed extraction. Seeds were extracted by hands and then they were put in large containers well covered, labelled and left to ferment. Because the weather in Adana was very hot with the temperature range between 46 - 52 °C, it took 3 days for the flesh adhered on seed to ferment. Seeds were thoroughly stirred to further separate parts of fruit and pulp from the seed by using stick and washed with clean water until no pulp remained. Then they were put on sieves with very fine wiremesh, they left out side for few minutes to drain excess water before they were taken for indoor drying (Figure 3.15). Seeds left to dry for one weeks then they were taken to Biotechnology Laboratory of the Cukurova University for seed analysis tests. Seeds were tested for moisture content (SMC), germination rate and capacity, emergence rate and capacity as well as seed accelerated aging as a means to test seed vigour.



Figure 3.15. Seed extraction, washing and drying; (a) extracting seeds from fruits; (b) stirring and washing seeds in a large container; (c) draining extra water; and (d) drying by using sieves with small sized wiremesh



Figure 3.15. Continues...

**3.2.3.9.(1). Seed Sterilization**

Before being used for seed analysis tests, seeds were sterilized by soaking in vials with 3% sodium hypochloride for 10 min (Wang et al., 2013; Barbosa et al., 2016). Seeds were then rinsed 3 times with distilled water and then partially dried by using blotting paper before germination, emergence and seed accelerated aging tests (Fig. 3.16).



Figure 3.16. Seed sterilization with 3% sodium hypochloride for 5 min in the vials

**3.2.3.9.(2). Seed Moisture Content Test**

Seed moisture test was done to determine the moisture condition of the seed lot and to establish the optimal condition for seed storage. In every genotype 10 g of seed per repetition was previously weighed in order to get the initial weight, then oven dried at 130 °C for 1 h, then weighed again to get the final weight.

The seed moisture content in percentage (SMC) was calculated by the following formular.

$$\text{SMC (\%)} = [(\text{Initial seed weight} - \text{Final seed weight} / \text{Final seed weight}) \times 100]$$

### 3.2.3.9.(3). Seed Germination Test (Between Paper)

In each genotype seeds were divided according to replications as they were in the field, and in every replicate 50 seeds were counted. Filter paper was cut according to the size of the petri dishes, 50 seeds were placed between the filter paper and slightly moistured with 3 drops of water by using pasteur pipette. Petri dishes were labelled with the name of the genotype and number of replicate, then petri dishes were stored in the germination incubator at 25 °C. Number of germinated seeds were counted and recorded daily, once counted everyday, the germinated seeds were removed from the petri dish (Figure 3.17). At the end of the experiment, germination percentage (GP) and mean germination time (GT) were calculated according to Phat *et al.* (2015); Armin *et al.* (2010) and Souza *et al.* (2013) respectively.

$$\text{GP (\%)} = [(\text{Total number of seed germinated} / \text{Total number of seed sown}) \times 100]$$

$$\text{GT (days)} = [(\sum n_i \cdot t_i) / \sum n_i ]$$

Where,  $n_i$  = number of germinated seeds in that  $i^{\text{th}}$  day,

$t_i$  = number of days from sowing to the days of measurement.

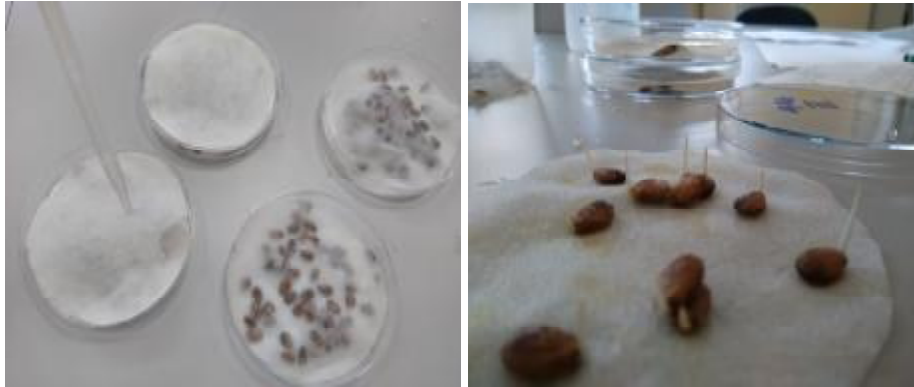


Figure 3.17. Sowing of seeds in the petri dishes for germination test (left) and germinated seeds (right)

#### 3.2.3.9.(4). Seed Emergence Test (Using Sand)

Pure clean sand collected from sand deposited by running water was used. Sand was well sieved, dried and sterilized in autoclave at 120 °C for 1 h. Then it was left to cool before being placed in a plastic trays of 45 cm × 30 cm × 8.5 cm size and seeds of both four genotypes were sown in one tray, replicated in four trays. Four slight shallow row of 2 cm deep were made and 50 seeds were smoothly sprinkled into the rows, covered with fine sand, firmed, slightly watered by a mist hand sprinkler and left on at 25 °C. Each row indicated one genotype and each plastic tray indicated a replicate. Rows were labelled with a name of the genotype, date of sowing and replication, watering also was applied every day with mist hand sprinkler. The germinated seeds were counted and recorded daily by observing the well emerged cotyledons above the sand surface, counting was done by cutting the emerged seedlings by using a pair of scissors (Figure 3.18). Emergence percentage and emergence time was calculated by using the same formular as used in germination.

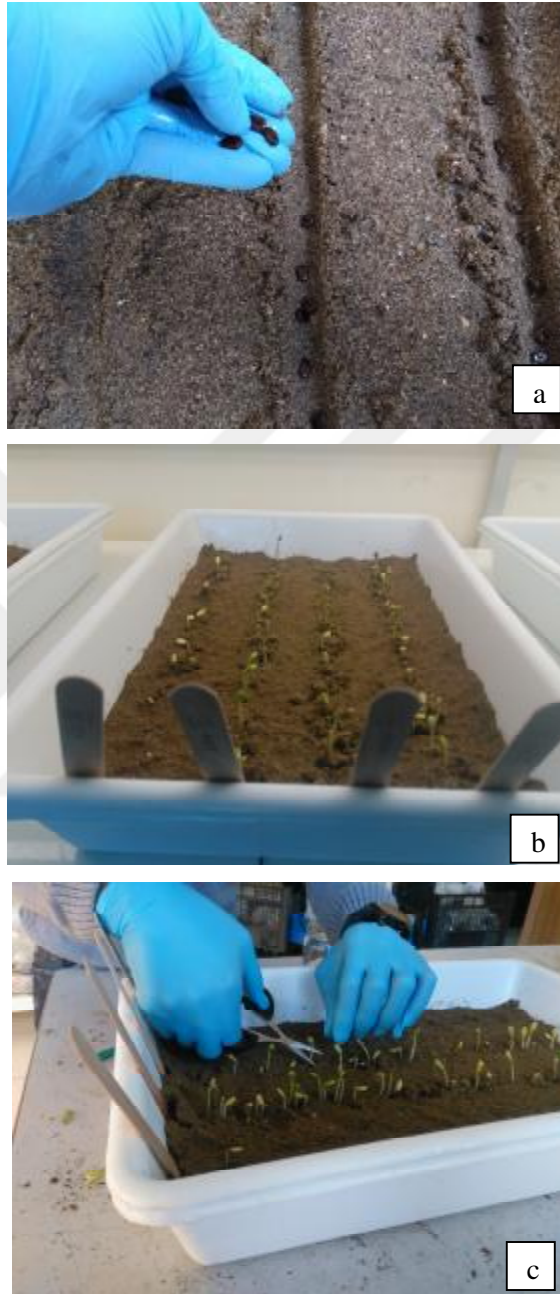


Figure 3.18. Seed emergence test; (a) seed sowing on inert sand in plastic tray; (b) emerged seeds; (c) cutting/counting emerged seedling

**3.2.3.9.(5). Accelerated Ageing Test**

Seed accelerated ageing test was conducted to determine the storage potential of the seed. The method used in this test was according to Mavi and Demir (2007). Each genotype had four replication and in each replication 100 seeds were counted. Seeds were stored in an oven at 45 °C and 100% relative humidity. A hard plastic container of was half filled with water and a petri dish with 100 seeds was floated on top of water then the container was covered with a lid and tied completely by using stretch to maintain 100% RH. From every replication 25 seeds were removed for germination test after 48 h, 96 h, 144 h and 192 h consecutively. Soon after removal of 25 seeds in a particular day, the container sealed and stored again (Figure 3.19).

The removed 25 seeds were put in a petri dish according to replication for germination test. Seeds were placed between two blotting papers, slightly moistured and stored in a germination incubator to determine the accelerated aging. Observation of the germinated seeds was carried out everyday and finally the germination percentage was calculated. Germination percentage (GP) was calculated by using the following formular as used by Phat *et al.* (2015).

$$GP (\%) = [(Total\ number\ of\ seed\ germinated / Total\ number\ of\ seed\ sown) \times 100]$$

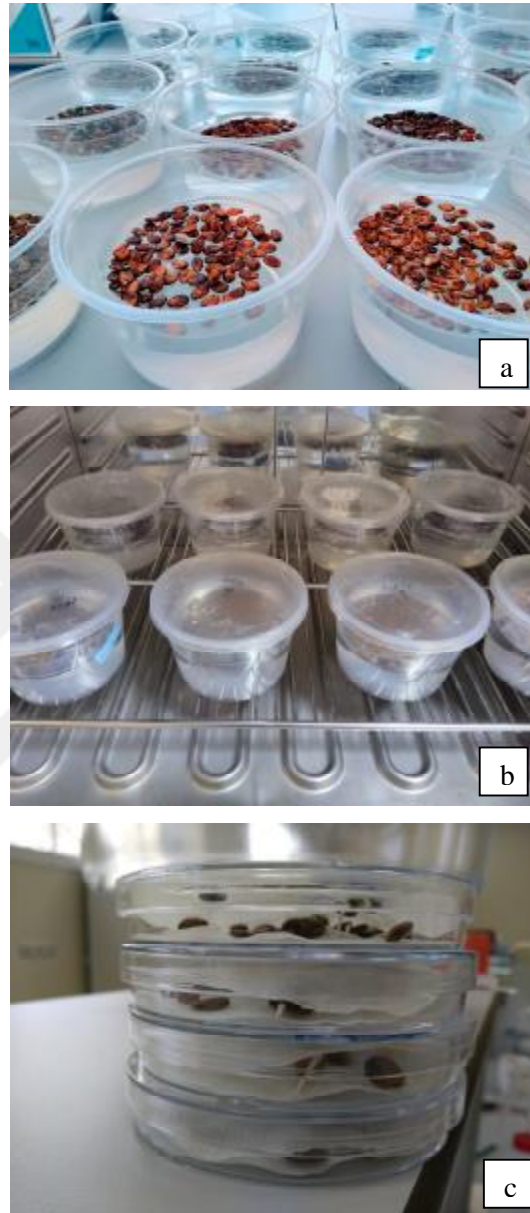


Figure 3.19. Seed accelerated aging test; (a) Seed in plastic container with water to create 100% humidity; (b) Seed in oven at 45 °C; (c) Seed germination for aging test after 45 °C temperature and 100% humidity treatment for 48 h, 96 h, 144 h and 192 h

**3.3. Statistical Analysis**

Measured and observed data was recorded in Microsoft Excel sheet and averages for each parameter was calculated. Significant difference between means was calculated by using Tukey Multiple Range Test at a significance level of  $P \leq 0.05$ . Statistical analysis was performed using JMP (v8.00, SAS Institute Inc., NC 27513-2414, USA) statistics software.





#### 4. RESULTS AND DISCUSSION

This chapter of the PhD thesis explains the results of both nursery, field and laboratory experiments conducted for two consecutive years (2016 and 2017).

##### 4.1. Seed Sowing and Grafting

Results of seed sowing and grafting carried out at the nursery and greenhouse before transplanting are presented in Table 4.1. for 2016 and for the year 2017 results are presented in Table 4.2. Seeds of NUN-9075 and Argentario sown earlier because the seeds germinated earlier compared to other species used in this research. NUN-9075 resulted in higher germination percentage of 98.33% than any other genotypes and citron while PI296341 resulted in lowest germination percentage (33.33%).

Table 4.1. Sowing date (SD), number of seeds sown (NSS), germinated seeds (GS), germination percentage (GP; %), grafted seedlings (GTS), survived grafted plants (SGP) and survival rate (SR) for 2016 growing season

Genotypes	SD	NSS	GS	GP (%)	GTS	SGP	SR (%)
Crimson sweet	26.01.2016	150	112	74.67	Scion	Scion	Scion
Citron (PI296341)	29.01.2016	150	50	33.33	49	44	89.80
Argentario	01.02.2016	120	112	93.33	111	96	86.49
NUN-9075	01.02.2016	120	118	98.33	111	95	85.59

However, it is vice versa for the survival rate, whereby PI296341 resulted into higher percentage of survival rate (89.80%) compared to NUN-9075 (85.59%) although with the very small difference.

In 2017 germination percentages and survival rates observed to be somehow lower compared to that of year 2016. NUN-9075 and Argentario resulted in the same germination percentage of 92.59% each, while Crimson sweet (control) resulted in lowest germination percentage (62.33%). Argentario resulted in highest percentage survival rate followed by NUN-9075, while PI296341 was the lowest (93.33%, 81.33% and 35.33% respectively).

Table 4.2. Sowing date (SD), number of seeds sown (NSS), germinated seeds (GS), germination percentage (GP), grafted seedlings (GTS), survived grafted plants (SGP) and survival rate (SR) for 2017 growing season

Genotypes	SD	NSS	GS	GP (%)	GTS	SGP	SR (%)
Crimson sweet	28.01.2017	300	187	62.33	scion	scion	scion
Citron (PI296341)	03.02.2017	216	159	73.61	150	53	35.33
Argentario	03.02.2017	216	200	92.59	150	140	93.33
NUN-9075	03.02.2017	216	200	92.59	150	122	81.33

The germination of *Cucurbita* 'NUN-9075' and *Lagenaria* 'Argentario' seeds were always higher than others, this is may be due to the nature of these materials in their genetic make up. It was also these genotypes observed to germinate and emerge earlier that's why their seeds were sown a bit later compared to Citron watermelon 'PI296341' and Crimson sweet (Control). The survival rates after grafting were always higher in Argentario followed by NUN-9075. Survival rate for these two rootstocks used in this study was similar to the results reported by Qin *et al.* (2014) who obtained maximum survival rate of 90% on bottle gourd and pumpkin rootstocks compared to watermelon rootstocks. Yetisir *et al.* (2003) found the same observations that *Lagenaria* rootstock resulted in higher survival rate (95%) followed by low survival rate in *Cucurbita* type of rootstocks (65%).

The higher survival rate in *Lagenaria* and *Cucurbita* type of rootstocks may be because of high compatibility between these rootstocks and watermelon scions.

## **4.2. Effect of Graft Combinations on Plant Growth**

### **4.2.1. Days to 10 cm Long Main Stem Formation**

Results pertaining to number of days observed when the main stem reached 10 cm long after transplanting and days from transplanting to flowering are presented in Table 4.3 for year 2016 growing season and Table 4.4 for year 2017 growing season. There was significant difference among rootstocks in number of days to 10 cm long main stem formation in both two growing seasons. However, in 2016 NUN-9075/CS graft combination and control formed 10 cm main stem earlier compared to Argentario/CS and 'PI296341/CS' graft combinations, they attained the length after an average of 14.25 and 13.25 days respectively. Argentario/CS was the late rootstock to have 10 cm long of the main stem (17.50 days). However, this was not the case for the second year (2017) growing season where all graft combinations and non-grafted (control) had their main stem reached 10 cm long earlier than in the previous year. In this year, the earliest rootstock was NUN-9075/CS which reached 10 cm after 8.25 days and 'PI296341/CS' reached its 10 cm long after 11.25 days. Although there was significant difference between genotypes in days to 10 cm length of the main stem but NUN-9075/CS and Argentario/CS were almost similar being the earlier graft combinations. Likewise PI296341/CS and Crimson sweet were more or less similar attained the length in almost the same day (11.25 and 10.25 respectively).

There was significant difference at 5% level of significance in days to flowering since transplanting among graft combinations and control for 2016 growing season in all observed flowering categories (Table 4.3).

NUN-9075/CS resulted in early flowering for male flower, female flower, 50% male flowers and 50% female flowers, the flowering was observed after 33.00, 44.75, 37.25 and 55.00 days respectively. In first male flower flowering,

PI296341/CS and control followed which resulted in male flower flowering after average days of 34.50 and 35.75 respectively, of which statistically they are the same. Argentario/CS, PI296341/CS and control resulted in statistically same average days of first female flower flowering at 5% level of significant although physically the number seemed to be different (53.50, 55.00 and 53.75 days respectively). NUN-9075/CS graft combination resulted in earlier occurrence of 50% male flowers, 50% of plants in a plot completed its flowering after 37.25 days, Argentario/CS took the longest time to complete flowering in its 50% plants. It was observed to have completed 50% flowering after 53.25 days since transplanting. PI296341/CS and control completed 50% flowering in the same day (43.00 and 43.75 days), 3 days more than NUN-9075/CS and 10 days less than Argentario/CS graft combinations. Argentario/CS and PI296341/CS resulted in late completion of 50% flowering, they completed after 59.00 and 59.50 days same with control.

Table 4.3. Days to 10 cm length main stem formation and days to flowering of first male flower (FMF), first female flower (FFF), 50% male flower (50% MF) and 50% female flower (50% FF) for 2016 growing season

Genotype	DSL 10 (cm)	FMF (days)	FFF (days)	50% MF (days)	50% FF (days)
Crimson Sweet (CS) (Control)	13.25 c	35.75 ab	53.75 a	43.75 b	59.75 a
PI 296341/CS	15.75 ab	34.50 ab	55.00 a	43.00 bc	59.50 a
Argentario/CS	17.50 a	40.00 a	53.50 a	53.25 a	59.00 a
NUN-9075/CS	14.25 bc	33.00 b	44.75 b	37.25 c	55.00 b
<i>Prob&gt;f</i>	0.0003	0.0181	0.0060	0.0002	0.0063
<i>LSD 5 %</i>	1.88	5.55	7.24	6.27	3.45

In the second year of crop cultivation statistically no significant differences observed among graft combinations and control in all flowering categories. However, the lowest average value in days to flowering of male flower observed in

control was 32.00 days and highest value was 34.00 days observed in PI 296341/CS. NUN-9075/CS graft combination as in first year resulted in earlier flowering in all other three flowering categories assessed in this research where first female flower, 50% male flower and 50% female flower flowered after average of 37.75, 36.00, and 41.50 days respectively. In general, 2017 growing season resulted in earlier flowering and completion of 50% flowering compared to 2016 growing season. In 2017, the first flower was observed after 32.00 days and 50% flowering completed after 45.00 days while in 2016 the first flower flowered after 33.00 days and 50% flowering was completed after 59.75 days since transplanting.

Table 4.4. Days to 10 cm length main stem formation and days to occurrence of first male flower (FMF), first female flower (FFF), 50% male flower (50% MF) and 50% female flower (50% FF) for 2017 growing season

Genotype	DSL 10 (cm)	FMF (days)	FFF (days)	50% MF (days)	50% FF (days)
Crimson Sweet (CS) (Control)	10.25 ab	32.00	39.75	36.50	44.75
PI296341/CS	11.25 a	34.00	40.75	37.00	45.00
Argentario/CS	9.00 b	33.75	39.25	37.75	42.50
NUN-9075/CS	8.25 b	33.50	37.75	36.00	41.50
<i>Prob&gt;f</i>	0.0056	0.2451	0.0644	0.3604	0.0819
LSD 5%	2.02	ns	ns	ns	ns

The differences in days to flowering for the first and second growing seasons may be explained as because of differences in plant growth was caused by the hail occurred in Adana in mid-May 2016 just one month and half since transplanting (Anonymous, 2016a; 2016b). The heavy snowy rain with heavy wind tore the plants' leaves and chopped off the growing twigs before covered with snow which resulted staggering growth of the plants. The hazzard caused the plants not only to delay in re-growth but also flowering and of course harvesting.

#### 4.2.2. Stem Length, Diameter and Number of Leaves

The measurements on main stem length, main stem diameter and number of leaves on the main stem were done one month after transplanting and repeated again one month later. Analyzed results of these plant growth parameters for year 2016 are shown in Table 4.5.

There were significant differences among graft combinations on main stem length and number of leaves in first measurement, and main stem length and main stem diameter in the second measurement. No significant difference observed between graft combinations on main stem diameter in first measurement and number of leaves during the second measurement (Prob > f = 0.052 and 0.505 respectively at 0.05 level of significant). Grafting watermelon cv 'Crimson sweet' scion onto Cucurbita rootstock 'NUN-9075/CS' graft combination resulted in higher average value in main stem length of 90.60 cm, main stem diameter 9.19 mm and number of leaves 17.45 for the first measurement, and also in the second measurement NUN-9075/CS resulted in 234.50 cm, 16.06 mm and 25.90 respectively compared to other graft combinations.

Table 4.5. Plant growth parameters. Main stem length (MSL), main stem diameter (MSD) and number of leaves (NL) measured in 2 times at one month interval for 2016 growing season

Genotype	MSL 1 (cm)	MSD 1 (mm)	NL 1	MSL 2 (cm)	MSD 2 (mm)	NL 2
Crimson Sweet (Control)	39.25 c	8.04	11.70 b	127.95 b	11.96 b	25.15
PI 296341/CS	45.90 bc	8.59	12.95 b	174.35 b	14.33 ab	26.20
Argentario/CS	55.15 b	7.91	13.05 b	178.45 ab	13.79 ab	22.02
NUN-9075/CS	90.60 a	9.19	17.45 a	234.50 a	16.06 a	25.90
<i>Prob&gt;f</i>	0.0001	0.052	0.0001	0.0022	0.0062	0.5046
<i>LSD 5 %</i>	10.14	ns	2.25	57.84	2.60	ns

However, in the average number of leaves, PI296341/CS graft combination also attained nearly similar value of number of leaves (26.20) with NUN-9075/CS. Non-grafted plants (Crimson sweet – control) resulted in lower values in main stem length, main stem diameter and number of leaves on the main stem for both measurements. It resulted in main stem length of 39.25 cm, main stem diameter 8.04 mm and 11.70 leaves for the first measurement and 127.95 cm, 11.96 mm and 25.15 for main stem length, main stem diameter and number of leaves in the second measurement respectively.

In investigating the plant growth by main stem length, main stem diameter and number of leaves during the 2017 growing season, from the statistical analysis, the graft combinations were found to be significant difference in all measured plant growth parameters at 0.05 level (Table 4.6). NUN-9075/CS resulted in higher average main stem length, main stem diameter and number of leaves in both measurements, the values were 76.31 cm, 10.16 mm and 12.94 for the first measurement and 376.06 cm, 17.70 mm and 37.06 respectively for the second measurement. Like in 2016 growing season, also in 2017 growing season, non-grafted (control) plants resulted in lowest values of plant growth parameters for the first and second measurements, only number of leaves parameter in the first measurement the lowest value was obtained in PI296341/CS (10.19). The measured values in Crimson sweet (control) were 52.19 cm main stem length and 7.72 mm main stem diameter at the first measurement and 211.06 cm main stem length, 13.30 mm main stem diameter and 22.19 number of leaves obtained during the second measurement.

Table 4.6. Plant growth parameters. Main stem length (MSL), main stem diameter (MSD) and number of leaves (NL) measured in 2 times at one month interval for 2017 growing season

Genotype	MSL 1 (cm)	MSD 1 (mm)	NL 1	MSL 2 (cm)	MSD 2 (mm)	NL 2
Crimson Sweet (Control)	52.19 b	7.72 b	11.81 ab	211.06 c	13.30 b	22.19 b
PI296341/CS	52.31 b	9.71 a	10.19 b	242.63 c	15.98 a	25.06 b
Argentario/CS	57.63 ab	9.26 a	11.13 ab	292.25 b	15.96 a	25.44 b
NUN-9075/CS	76.31 a	10.16 a	12.94 a	376.06 a	17.70 a	37.06 a
<i>Prob&gt;f</i>	0.0101	0.0040	0.0079	0.0001	0.001	0.0015
LSD 5%	19.11	1.54	1.86	49.62	2.15	8.25

Results of stem length and number of leaves in this study are higher than those obtained by Yetisir and Sari (2004) who obtained the highest stem diameter value of 26.27 cm in *Lagenaria* hybrid/CT and lowest value of 6.0 cm in *Luffa* (landrace)/CT, and most number of leaves in *Cucurbita maxima*/CT (21.67) and lowest in *Luffa cylindrica*/CT (10.17). Indeed, results obtained from this study in main stem length, stem diameter and number of leaves are higher compared to that obtained by Yetisir *et al.* (2006) and Gomez *et al.* (2017) who used Chitosan-PVA hydrogen with copper nanoparticles to improve growth of grafted watermelon.

#### 4.2.3. Plant Biomass Content

Plant biomass content further provides additional information on growth characteristic of a particular plant. In this study, the importance of rootstock has also been assessed in plant vigour through leaves and stem in both fresh and dry weight basis. There was a significant difference between rootstocks on plant fresh weight ( $\text{kg}\cdot\text{plant}^{-1}$ ) and plant dry weight ( $\text{kg}\cdot\text{plant}^{-1}$ ) at 5% level of significance with  $Prob > f = 0.0001$  for both measured parameters. Results of plant fresh weight and plant dry weight are presented in Figure 4.1. and 4.2.

NUN-9075/CS graft combination resulted in highest average value of total plant fresh weight and total plant dry weight (3.39 and 0.36  $\text{kg}\cdot\text{plant}^{-1}$  respectively). Lowest value for both total fresh weight and total plant dry weight was found in Crimson sweet (0.43 and 0.06  $\text{kg}\cdot\text{plant}^{-1}$ ). In 2017 growing season, same graft combination (NUN-9075/CS) resulted in higher average value in total plant fresh and total dry weight (3.84 and 0.55  $\text{kg}\cdot\text{plant}^{-1}$  respectively), compared to control and other graft combinations. Also non-grafted plants (control) resulted in lowest value in total plant fresh weight and total dry weight (0.90 and 0.17  $\text{kg}\cdot\text{plant}^{-1}$  respectively). Like in other parameters observed in this study, values of the second year were higher than that of first year.

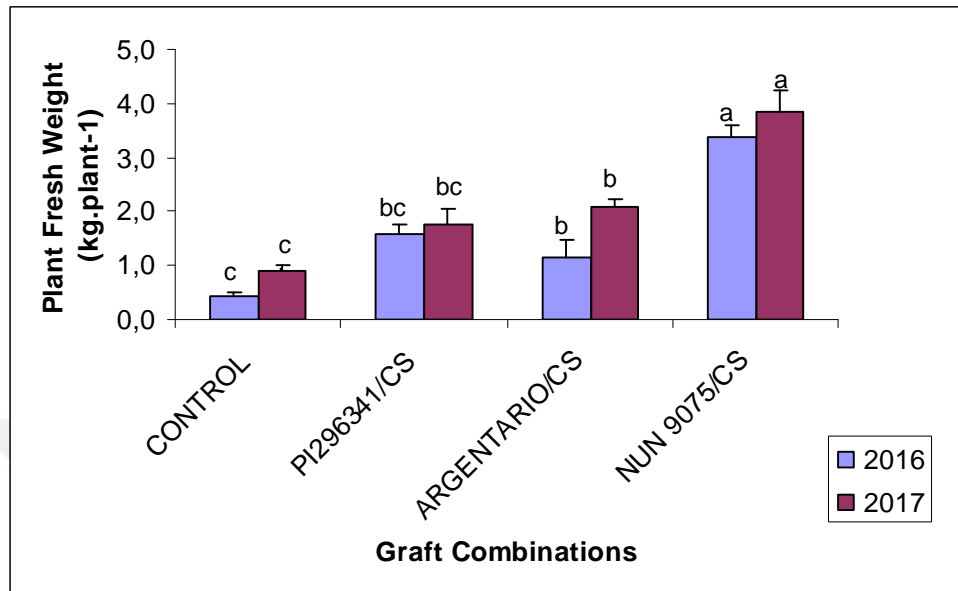


Figure 4.1. Effect of rootstocks on plant fresh weight. Data analyzed at 5% level of significance.

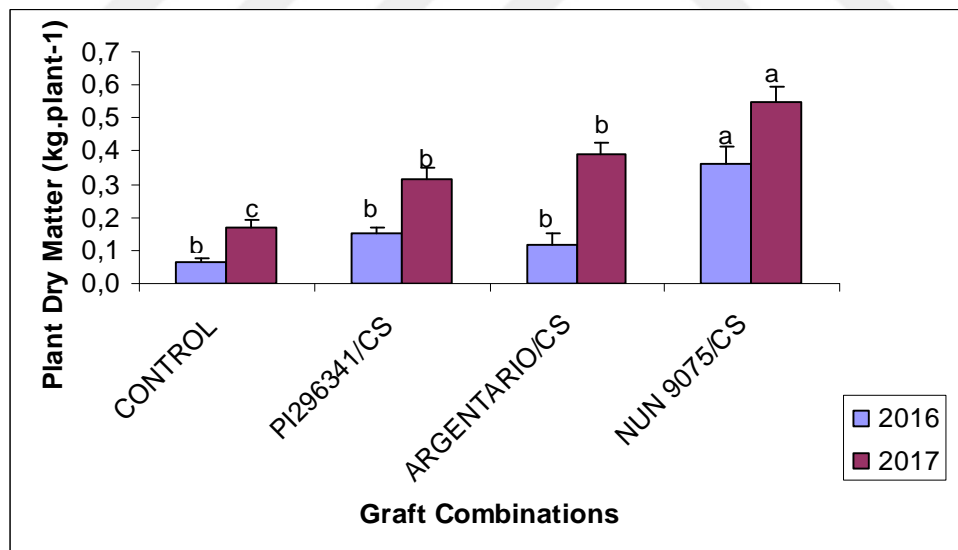


Figure 4.2. Effect of rootstocks on dry matter content. Data analyzed at 5% level of significance.

Results of this study showing that grafted plants result into higher biomass than non-grafted plants are in accordance with the results of Lee (1994), Ioannou (2001), Mohamed *et al.* (2014) and many more others who concluded that grafted plants produce much more foliage and vigorous plants compared to non-grafted plants. The great biomass in grafted plants is mostly an influence of and physical characteristics of the root system of the rootstocks which result in enhanced water and nutrients uptake (Salehi-Mohammadi *et al.*, 2009). The more the water and nutrients uptake especially nitrogen and transported to the sink, the more the growth of the leaves and stem that result in vigorous of the plant.

#### **4.3. Pollen Development**

Study on pollen development was made to assess the pollen capability in fertilizing female flowers so as to have a better prediction of the fruit and seed yield and quality production. To achieve this target, the study further estimated the number of anthers per flower, number of pollen per anther and number of pollen per flower, results are presented in Table 4.7.

Table 4.7. Anther analysis for pollen production

Graft Combinations	Total number of pollen in a box with 5 flowers		Number of pollen per anther		Number of pollen per flower	
	2016	2017	2016	2017	2016	2017
Control	1662728.43	913766.46	107083.57	60917.76	332545.69	182753.30
PI296341/CS	1525003.36	923844.40	101666.89	61589.63	305000.67	184768.90
Argentario/CS	1891235.55	1031386.10	124486.70	68759.07	378247.11	206277.20
NUN-9075/CS	1780408.49	1121775.10	113109.17	74785.00	356081.70	224355.00
<i>Prob&gt;f</i>	0.3	0.42	0.36	0.42	0.3	0.42
<i>LSD%5</i>	ns	ns	ns	ns	ns	ns

There was no significant difference among rootstocks on number of anthers per flower, number of pollen per anther and number of pollen per flower for both experimental years. All graft combinations and control resulted in the same average number of anthers per flower, all flowers observed to have average of 3.00 anthers. Also no significant difference among rootstocks at 5% level on number of pollen per anther and number of pollen per flower. Pollen grain production per flower (pg/f) ranged from 305000.67 (pg/f) in PI296341/CS to 378247.11 (pg/f) in Argentario/CS for 2016 growing season, and 182753.30 (pg/f) in control (Crimson sweet) to 224355.00 (pg/f) in NUN-9075/CS for 2017 growing season.

There was no significant difference between graft combinations and control for both growing years 2016 and 2017 observed in pollen viability, pollen germination and normal pollen production. The percentage pollen viability, percentage pollen germination and percentage normal pollen production ranged between 98.25% (PI296341/CS) to 99.69% (Control), 90.84% (control) to 96.23% (NU-9075/CS) and 96.98% (control) to 99.17% (Argentario/CS) respectively, for 2016 growing season. For 2017 growing season pollen viability percentage was between 98.40% (Control) to 100% in all graft combinations. Pollen germination percentage observed to be between 88.22% (control) and 97.22% (NUN-9075/CS) and normal pollen production ranged between 90.39% (Argentario/CS) and 99.38% (NUN-9075/CS) (Figure 4.3 and 4.4).

Generally, pollen production of the second year was lower compared that of first year. This may be explained as result of few flowers on the plant caused by heavy rain rained two days consecutively before the day of experiment. In the first year, flowers for pollen experiments were collected on 06/06/2016, in 2017 flowers were collected in the similar date to avoid difference between the growing seasons and graft combinations in pollen development experiments.

Good results obtained in pollen germination and viability in this study are also in line with those results reported by Sari *et al.* (1992) and Sensoy *et al.* (2003). Gok *et al.* (2007) who tested pollen viability and germination by using

2,3,5 Triphenyl Tetrazolium Chloride (TTC) and Flourescein di Acetate (FDA) in 45 watermelon genotypes, obtained nearly similar results to this study. They found highest pollen viability rates from 71.37% to 96.32% and pollen germination rates ranging from 19.62% to 89.43%. However, their results are somehow low compared to the results of this current study.

Results of this study showing no significant differences among graft combinations for all parameters in pollen development, are in line with the results reported by Stanghellini and Schultheis (2005) and Kwon *et al.* (2005). However, the results obtained in this study are much higher compared to them especially in pollen grain production per flower. Stanghellini and Schultheis (2005) obtained maximum number of pollen of 18125 pollen/flower and Kwon *et al.* (2005) obtained maximum number of pollen 52980 pollen/flower and minimum 16416 pollen/flower.

Also in pollen viability results of this study is equivalent to that of Sain and Joshi (2003), who reported the pollen viability range between 18.5% - 84.3% in *Citrullus lanatus* × *C. colocynthis* hybrids. Indeed, our results are similar to that of Freeman *et al.* (2008) who obtained pollen viability percentage values ranging between 97% - 99.2% in diploid watermelon pollenizier cultivars. Also McGregor and Waters (2013) obtained similar results to this study, they observed pollen viability ranged between 92.90 % - 96.90 % in crop wild relatives of watermelon.

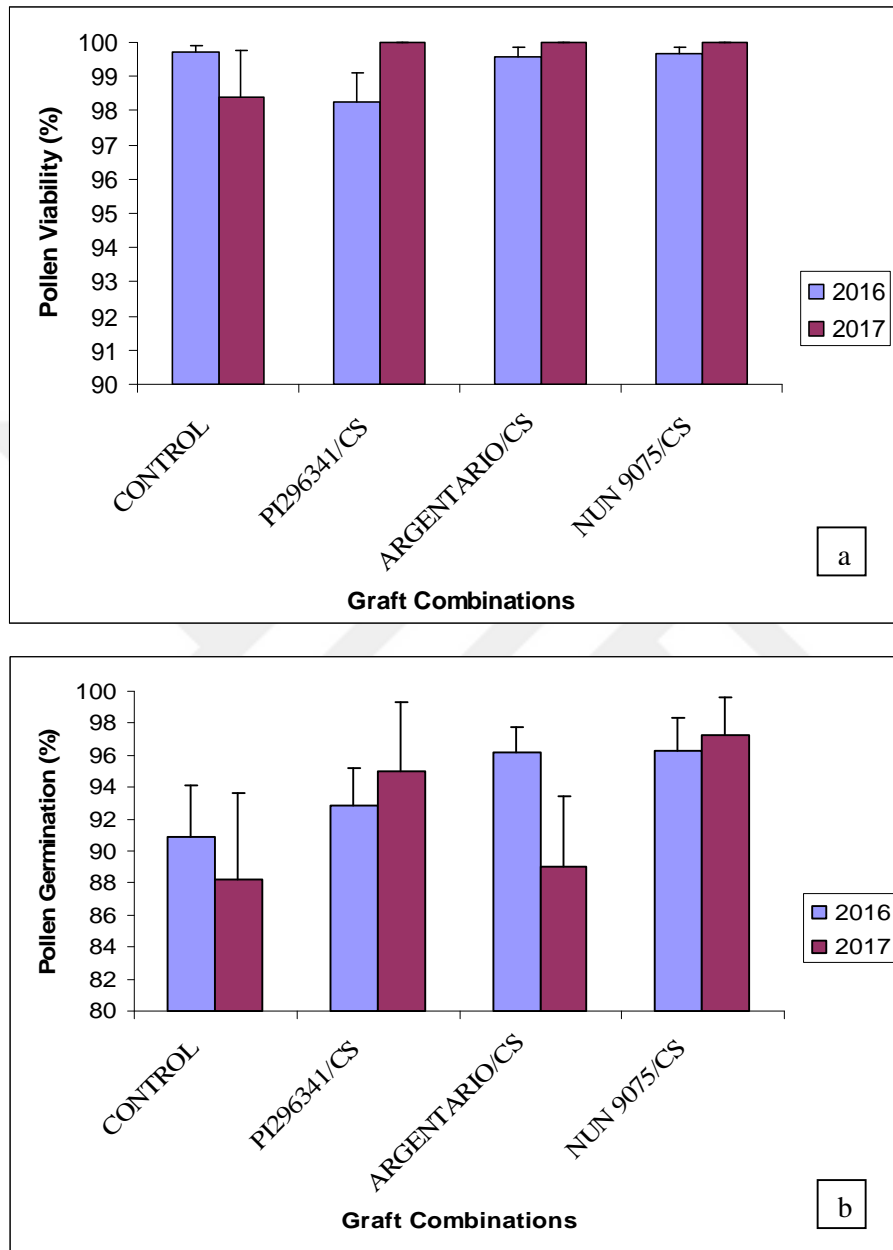


Figure 4.3. Effect of rootstocks on; (a) pollen viability; and (b) pollen germination in watermelon. Data analyzed at 5% level of significance.

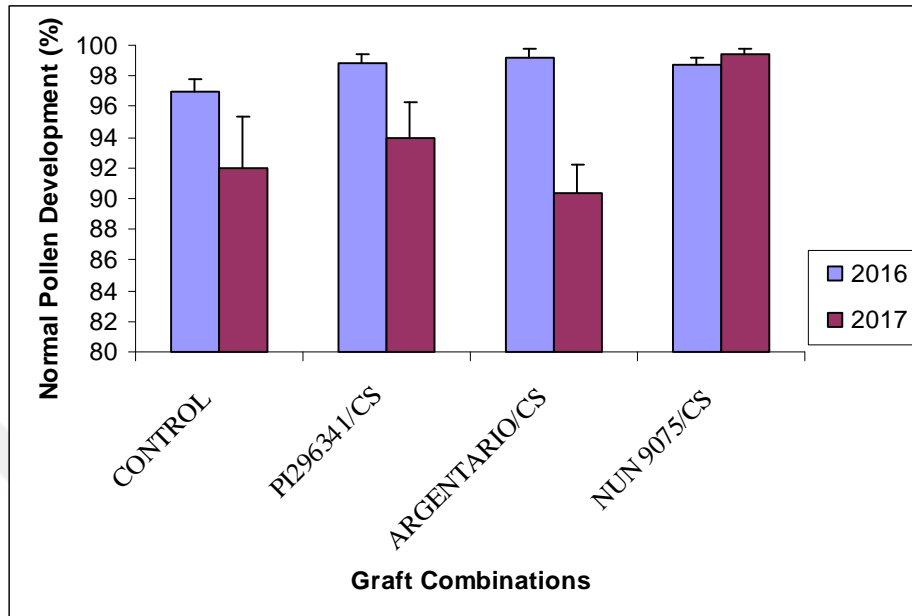


Figure 4.4. Effect of rootstocks on normal pollen production in watermelon. Data analyzed at 5% level of significance.

#### 4.4. Fruit Yield and Quality

Results of fruit yield and fruit quality for 2016 growing season are presented in Table 4.8. Photographs of the fruits according to their respective graft combination are shown in Figure 4.5. Total fruit yield ( $\text{kg.m}^{-2}$ ), single fruit weight (kg), fruit height (cm) and fruit diameter (cm) were significantly influenced by rootstocks, while no significant difference observed in fruit rind thickness (mm) and total soluble solids (TSS %). Grafted plants resulted in higher values in all assessed parameters under fruit yield and quality compared to control.

Table 4.8. Total fruit yield (TFY), single fruit weight (SFW), fruit height (FH), fruit diameter (FD), fruit rind thickness (FRT) and total soluble solids (TSS) of the fruits from graft combination and control for 2016 growing season

Graft combinations	TFY (kg/m <sup>2</sup> )	SFW (kg)	FH (cm)	FD (cm)	FRT (mm)	TSS (%)
Crimson Sweet (Control)	1.09 b	1.58 c	14.53 b	13.43 b	7.80	8.33
PI 296341/CS	2.51 b	4.73 bc	21.36 a	19.53 a	9.44	10.05
Argentario/CS	3.12 ab	6.52 ab	22.79 a	22.21 a	10.04	9.24
NUN-9075/CS	4.82 a	8.35 a	26.43 a	24.08 a	11.30	9.85
<i>Prob&gt;f</i>	0.0028	0.001	0.0007	0.0008	0.0911	0.101
LSD (0.05)	2.11	3.42	5.62	5.29	ns	ns

During the growing season of the year 2016, NUN-9075/CS graft combination resulted in higher fruit yield of 4.82 kg/m<sup>2</sup> followed by Argentario/CS which resulted in 3.12 kg/m<sup>2</sup>, while the lowest total fruit yield was obtained in control (1.09 kg/m<sup>2</sup>). Single fruit weight ranged from 1.58 kg (control) to 8.35 kg (NUN-9075/CS), the highest fruit height average value obtained in NUN-9075/CS (26.43 cm) and lowest value was obtained in control (14.53 cm). The average fruit diameter value was 24.08 cm obtained in NUN-9075/CS and lowest fruit diameter value was obtained in control (Crimson sweet).

In the second year (2017) higher yield was obtained compared to the first growing season. There was a significant difference at 5% level among graft combinations and control in total fruit yield, single fruit weight, fruit height, fruit diameter and TSS, no significant difference between graft combinations observed in fruit rind thickness ( $Prob > f = 0.76$ ). The highest yield was 6.39 kg/m<sup>2</sup> obtained in NUN-9075/CS and lowest yield was 1.49 kg/m<sup>2</sup> obtained in non-grafted plants (Crimson sweet). The highest single fruit weight value was 7.36 kg obtained in Argentario/CS while the lowest single fruit weight value was 4.77 kg obtained in non-grafted watermelon. Argentario/CS again resulted in higher fruit height, higher fruit diameter, higher fruit rind thickness and high percentage of TSS with values of 25.03 cm, 23.20 cm, 14.96 mm and 12.98% respectively. Lowest fruit height value was obtained in PI296341/CS graft combination (21.43 cm) and fruit rind thickness (13.75 mm), while lowest fruit diameter value was obtained in control (20.17 cm) and lowest TSS value was obtained in NUN-9075/CS (8.49%) (Table 4.9).

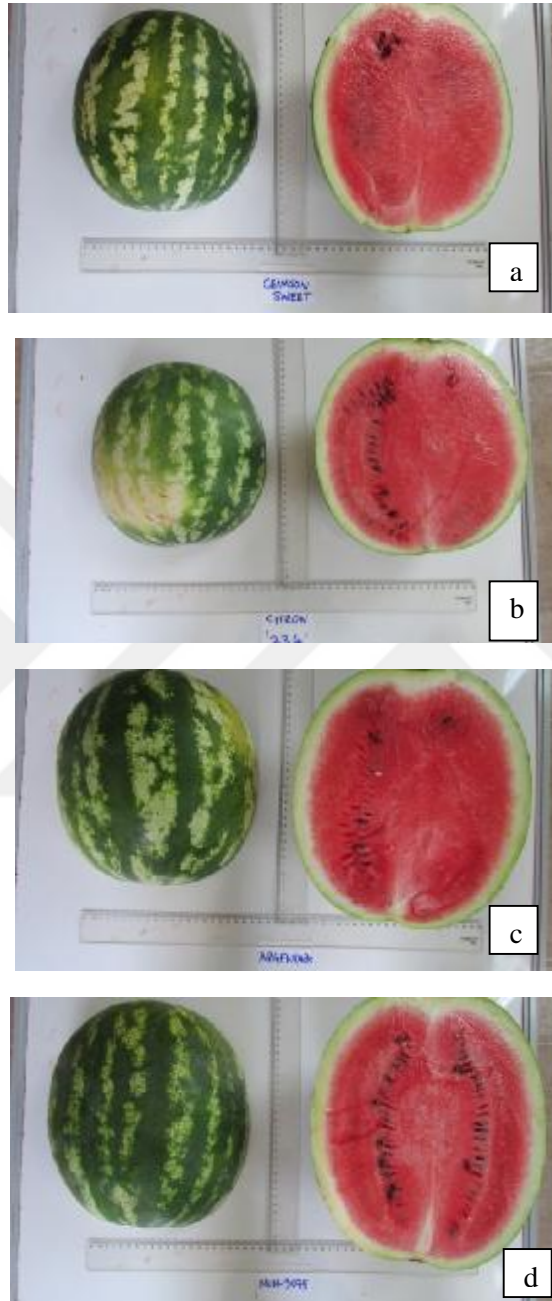


Figure 4.5. Fruit analysis; fruits of (a) Crimson sweet (CS) - control, (b) Citron - PI296341/CS, (c) Argentario/CS and (d) NUN-9075/CS

Table 4.9. Total fruit yield (TFY), single fruit weight (SFW), fruit height (FH), fruit diameter (FD), fruit rind thickness (FRT) and total soluble solids (TSS) of the fruits from graft combination and control for 2017 growing season

Graft combinations	TFY (kg/m <sup>2</sup> )	SFW (kg)	FH (cm)	FD (cm)	FRT (mm)	TSS (%)
Crimson Sweet (Control)	1.49 b	4.77 b	21.49 b	20.17 c	14.31	11.49 ab
PI 296341/CS	4.01 ab	4.98 ab	21.43 b	20.80 bc	13.75	12.50 a
Argentario/CS	4.92 a	7.36 a	25.03 a	23.20 a	14.96	12.98 a
NUN-9075/CS	6.39 a	7.12 ab	24.70 a	22.78 ab	14.88	8.49 b
<i>Prob&gt;f</i>	0.0019	0.0156	0.0201	0.0428	0.7554	0.0107
LSD 5%	2.68	2470.02	3.71	3.22	ns	3.39

Results obtained in this study that grafted watermelons resulted in higher yield and quality are similar to results obtained by Chaves *et al.* (2003), Yetisir *et al.* (2003), Yetisir and Sari (2003), Yetisir *et al.* (2007), Karaca *et al.* (2012), Turhan *et al.* (2012), Alan *et al.* (2007). Petropoulos *et al.* (2014) reported higher fruit yield, stem length, leaf area, fruit number and total dry matter on watermelon hybrid Obla F<sub>1</sub> × TZ 148 against non-grafted Obla F<sub>1</sub>. Indeed, results of this study is similar to that of Ozmen *et al.* (2015) who reported that grafting increased yield and the highest yield was 19.32 kg.plant<sup>-1</sup>. Marsic *et al.* (2016) report higher fruit yield and higher fruit rind thickness from grafted plants compared to non-grafted plants with the highest values obtained from Crimson sweet grafted on to DFCT and RS 841 *Cucurbita* rootstocks. Higher total yield is a result of fruit size and weight, but also the fruit numbers per plant or plot. Colla *et al.* (2006) reported that the causes of lowest yield in non-grafted watermelon plants (*Citrullus lanatus* L. Tex) associated with the reduction in fruit mean mass and number of fruits per plant. Indeed, Salam *et al.* (2002) reported that the higher yield in grafted watermelon plants are mainly due to the formation of more and larger fruits than the ungrafted ones.

The average TSS percentage for two years ranged between 8.33 – 12.98% in grafted watermelon in this study which is much higher than that reported by Turhan *et al.* (2012), they obtained the range of 7.32 – 9.53% TSS from Crimson Tide grafted on interspecific hybrid squash rootstock ‘Dynamo’. Yetisir *et al.* (2003) reported TSS content of 9.46% with Crimson sweet scion. Although, the TSS content in the second year of this study showed non-grafted Crimson sweet resulted in higher TSS value than *Cucurbita* rootstock (NUN-9075/CS) still it is higher compared to other rootstocks. Several reports have been reporting the negative effect of grafting in TSS especially the decrease in TSS content (Lopez-Galarza *et al.* 2004; Bie *et al.* 2010; Turhan *et al.* 2012; Alexopoulos *et al.* 2007) while others reported no difference in TSS between grafted and non-grafted plants (Miguel *et al.* 2004; Burton *et al.* 2009). In contrary, this current research in both

years revealed the positive effect of grafting on TSS content, where by the average TSS content of 13% was recorded in many fruits.

#### 4.5. Seed Yield and Quality

There was a significant difference between rootstocks in seed yield ( $\text{g.m}^{-2}$ ) while no significant difference between rootstocks was observed in number of seeds per fruit and weight of 1000 seeds at 5% significant level for the year 2016 (Table 4.10). Grafting resulted in higher seed yield compared to non-grafted plants. The highest average seed yield was  $16.68 \text{ g.m}^{-2}$  obtained in NUN-9075/CS graft combination and the lowest seed yield average value was  $4.08 \text{ g.m}^{-2}$  obtained in Crimson sweet.

The significant difference between graft combinations was also observed in number of seeds per fruit, NUN-9075/CS resulted in more number of seeds per fruit (558.11 seeds per fruit) and less number of seeds observed in Crimson sweet (241.25 seeds per fruit). Argentario/CS was followed by PI296341/CS (Citron watermelon) in number of seeds per fruit. Citron watermelon rootstock statistically resulted in the same number of seeds per fruit with NUN-9075/CS and Argentario/CS. Although no significant difference observed between graft combinations in weight of 1000 seeds, however, the weight ranged between 35.65 g - 40.74 g observed in PI296341/CS and Argentario/CS respectively.

Table 4.10. Seed yield, number of seeds per fruit and weight of 1000 seeds for 2016 growing season.

Graft Combinations	Seed yield ( $\text{g.m}^{-2}$ )	Number of seeds per fruit	Weight of 1000 seed (g)
Control	4.08 c	241.25 b	34.93
PI296341/CS	8.75 bc	477.83 a	36.14
Argentario/CS	11.27 ab	529.44 a	40.74
NUN-9075/CS	16.68 a	558.11 a	36.46
<i>Prob&gt;f</i>	0.0004	0.0021	0.2886
<i>LSD 5 %</i>	5.49	189.19	ns

As observed in fruit yield and other parameters recorded in this study, also in 2017 growing season observed to have more seed yield, number of seeds and weight of 1000 seeds. There was significant difference between grafted and non-grafted plants on seed yield ( $\text{g.m}^{-2}$ ), number of seeds per fruit and weight of 1000 seeds (g) at 5% level of significant (Table 4.11). The highest average value of seed yield was  $30.12 \text{ g.m}^{-2}$  observed in NUN-9075/CS and lowest seed yield was  $7.46 \text{ g/m}^2$  obtained in Crimson sweet (control). NUN-9075/CS again resulted in more number of seeds per fruit (805.00 seeds) and control resulted in fewer number of seeds (483.00 seeds). But the highest value of weight of 1000 seed was found in control which was statistically more or less the same with Argentario/CS and followed by NUN-9075/CS, PI296341 weight of 1000 seeds (32.52 g, 32.42 g, 31.10 g and 28.15 g respectively).

Table 4.11. Seed yield, number of seeds per fruit and weight of 1000 seeds for 2017 growing season. Data is the average of four replications.

Graft Combinations	Seed yield ( $\text{g.m}^{-2}$ )	Number of seeds per fruit	Weight of 1000 seed (g)
Control	7.46 b	483.00 b	32.42 a
PI296341/CS	14.48 b	607.00 b	28.15 b
Argentario/CS	27.26 a	607.50 b	32.52 a
NUN-9075/CS	30.12 a	805.00 a	31.10 a
<i>Prob&gt;f</i>	0.0008	0.005	0.004
<i>LSD 5 %</i>	12.20	197.32	2.95

The increased seed yield in grafted plants may be explained as the result of increased plant biomass, number and size of fruits and all these are caused by the effect of strong and deep root system of absorbing more water and nutrients. Edelstein and Nerson (2002), Nerson (2002, 2005), Nerson and Paris (2002) explained the increased seed yield is determined by many components but the main

one is the number of fruit per unit area. So far no study reported the effect of rootstock on seed yield and quality in watermelon, however, some researches reported the increase in seed yield in watermelon based on other parameters such as seed size Ambika *et al.* (2014), genotype and plant density (Edelstein and Nerson, 2002), fruit-set ratio (Nerson, 2004), fruit shape and quality (Nerson, 2005). Since seed is considered as the sink for receiving absorbed and manufactured metabolites, the higher the biomass and sources create more sink-source ratio which end up making more seeds.

#### **4.6. Determination of Seed Quality**

Results of seed moisture content, seed emergence percentage and rate, seed germination percentage and rate, and seed accelerated aging tests for 2016 growing season are presented in Table 4.12. There was no significant difference between graft combinations on seed moisture content (SMC, %) for both two growing seasons. In 2016 growing season the seed moisture content range was between 7.00 - 7.83% (Control and NUN-9075/CS) and 6.73 - 7.12% for 2017 growing season obtained in PI 296341/CS and Control, respectively (Table 4.13).

There was a significant difference between graft combinations in seed emergence percentage, seed emergence rate (days), seed germination percentage and seed germination rate (days). NUN-9075/CS resulted in high average value in seed emergence percentage (91.00%) and seed germination percentage (97.50%) and resulted in fewer average days in seed emergence rate and germination rate (7.14 days and 2.64 days respectively).

Table 4.12. Seed analysis results for seed moisture content (SMC), seed emergence percentage (SE), days of seed emergence (SED), seed germination percentage (SGP), days of seed germination (SGD) and accelerated ageing (AA) at different time for 2016 growing season.

Graft combinations	SMC (%)	SE (%)	SED (days)	SGP (%)	SGD (days)	AA 48 h (%)	AA 96 h (%)	AA 144 h (%)	AA 192 h (%)
Control	7.00	30.50 b	10.83 a	39.25 b	7.28 a	50.00	50.00	72.00 ab	40.00
PI296341/CS	7.76	61.00 ab	8.98 ab	68.50 ab	3.23 ab	63.00	53.00	57.25 b	48.00
Argentario/CS	7.62	86.50 a	7.86 b	97.00 a	2.56 b	96.00	87.00	80.00 ab	75.00
NUN-9075/CS	7.83	91.00 a	7.14 b	97.50 a	2.64 b	97.00	98.00	97.00 a	84.00
<i>Prob&gt;f</i>	0.1002	0.012	0.003	0.02	0.02	0.17	0.17	0.03	0.19
<i>LSD 5 %</i>	ns	52.14	2.185	53.03	4.4	ns	ns	33.65	ns

However, in seed germination rate NUN-9075/CS graft combination and Argentario/CS statistically attained the same rate (2.64 and 2.56 days respectively). Non-grafted plants resulted in lowest average seed emergence percentage, germination percentage, seed emergence and germination rate with averages of 30.50%, 39.25%, 10.83 days and 7.28 days, respectively. Generally, grafting resulted in more than 200% increase in seed emergence and germination, and 3 days earlier in seed emergence and 4 days earlier in seed germination compared to non-grafted plants. Significant difference between rootstocks in accelerated aging was found after 144 h (6 days) of seeds storage at 45 °C and 100% humidity. The average germination percentage ranging between 57.25 - 97.00%, with the highest germination percentage obtained in NUN-9075/CS and the lowest percentage was obtained in PI96341/CS. Although no significant difference between rootstocks observed after 48 h, 96 h and 192 h of seed storage at 45 °C and 100% humidity, yet the grafted plants attained higher germination percentage values compared to non-grafted. The percentages ranged between 50.00 – 97.00% after 48 h, 50.00 – 98.00% after 96 h and 40 - 84% after 192 h. In all days, the highest percentage value was observed in NUN-9075/CS and lowest percentage value was obtained in Crimson sweet (control). The ability of seeds to germinated after storage at 45 °C and 100% humidity was almost the same until at 192 h of seed storage, thereafter, a minimum decrease of germination percentage was observed. In every genotype at the 8<sup>th</sup> day there was an approximately 10% decrease in germination.

In the second year of cultivation, significant difference between rootstocks at 5% level was found in seed germination rate with NUN-9075/CS germinated after average of 7.06 days and Crimson sweet resulted in late germination (10.23 days). No significant difference between rootstocks and control observed in seed moisture content, seed emergence percentage, seed emergence rate, germination percentage and accelerated aging at all storage time.

Table 4.13. Seed analysis results for seed moisture content (SMC), seed emergence percentage (SE), days of seed emergence (SED), seed germination percentage (SGP), days of seed germination (SGD) and accelerated ageing (AA) at different time for 2017 growing season.

Graft combinations	SMC (%)	SE (%)	SED (days)	SGP (%)	SGD (days)	AA 48 h (%)	AA 96 h (%)	AA 144 h (%)	AA 192 h (%)
Control	7.12	89.00	7.09	86.00	10.23 a	95.00	86.00	95.00	86.00
PI296341/CS	6.73	90.00	7.41	92.50	7.94 ab	75.00	76.00	72.00	48.00
Argentario/CS	6.98	82.50	7.63	70.50	8.54 ab	89.00	92.00	86.00	93.00
NUN-9075/CS	7.03	75.50	7.80	71.50	7.06 b	92.00	90.00	83.00	73.00
<i>Prob&gt;f</i>	0.36	0.08	0.59	0.37	0.04	0.33	0.46	0.54	0.09
<i>LSD 5 %</i>	ns	ns	ns	ns	2.83	ns	ns	ns	ns

Seed germination and emergence tests has been conducted as a means to determine the seed quality that give insight of the seed performance during storage and or at the field (Gupta, 1993). This current study showed higher percentage of seed germination and seedling vigour that has been tested through seed emergence and accelerated aging. Seed emergence results of the grafted plants ranged between 61.00 - 91.00% and 75.50 - 90.00% for 2016 and 2017 growing seasons respectively, and seed germination of the grafted plants ranged between 68.5 - 97.5% and 70.5 - 92.50% for 2016 and 2017 growing seasons respectively. While the average accelerated aging of the grafted plants ranged between 53.00% as the lowest to 98.00% as the highest, with the overall average range between the rootstocks were between 80.00 and 90.00%. Decrease in germination after storage for the accelerated aging may be due to the fungal infection of the seeds caused by frequent opening of the incubator and the container. The incubator has to be shared with other people who were doing some other experiments that kept the experiment in a danger of contamination. Also the frequent opening of the container to remove seeds for germination might be the other reason of fungal infection and consequently the decrease in germination percentage especially after 8 days (192h) of seed storage at 45 °C and 100% humidity. However, the decrease in germination percentage was not that much lower as compared to that reported by Demir and Mavi (2008), who reported the decrease of germination of upto 48%. In other seed lots they obtained successful germination results in many of watermelon seeds between 90 and 100% that are nearly similar to the results of this study. Singh *et al.* (2001), reported results that are somehow lower compared to the results obtained in this research. They found highest germination of 84% and minimum days to germination and total germination of 6 and 10 days respectively in watermelon cv Charleston Gray at 22 °C. In contrary, the results obtained in this study revealed that the highest germination percentage was 97.50% which started after the 3<sup>rd</sup> day since sowing and germination completed on the 4<sup>th</sup> day in some graft combinations, especially NUN-9075/CS and Argentario/CS. This is proving that grafting

increases seed germination and in so doing it increases the quality of seeds. Moreover, result of this study is similar to that reported by Barbosa *et al.* (2016) who treated watermelon seeds with 1% sodium hypochloride before subjecting to 0.2% gibberellin, 0.2% cytokinin, 0.2% potassium nitrate and 0.2% calcium nitrate for 6 h at 25 °C and reported the highest germination percentage between 85 - 100%. Soares *et al.* (2016) who evaluated physiological and health quality of watermelon seeds through seed moisture content, seed germination and accelerated aging, found similar results to this study where they reported results of normal seeds in seed moisture content between 7.3 - 7.7%, seed germination 84 - 97% and accelerated aging 62 - 77%. Results of this thesis on seed germination and emergence are higher than that reported by Mavi and Atak (2016), but are similar to that reported by Jaskani *et al.* (2005), who reported germination percentage between 76.6 - 93.3% and maximum seed emergence of 93.3% after treating diploid and tetraploid watermelon seeds with different levels of BA, GA<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Baalbaki *et al.* (2009) concluded that seeds that have been exposed to high temperature between 40 - 45 °C and 100% relative humidity and be able to produce high germination after tolerating this aging treatment has a high possibility of producing high percentage of normal seedlings. Marcos-Filho (2015), explained high performance of seed in germination and vigour as an important initiative towards successful crop production and explained further that germination and vigour are the important factors influencing seed physiological potential which govern the capacity of seed under both favourable and unfavourable environmental conditions. Therefore, seeds of grafted plants tested in this study has a potential of high storage life, germinability and a capacity to express their vital functions under any environmental condition.

## **5. CONCLUSIONS AND RECOMMENDATIONS**

Importance of watermelon production is known all over the world and the techniques has been studied to increase its production and improve the quality in many ways. Grafting is one of the techniques that are used to increase yield and improve quality of watermelon via its potentials in soil-borne diseases control, water and nutrient absorption, improving plant vigour, tolerance to low temperature and salinity conditions and many more other advantages. In this current study conducted at the experimental areas and laboratories of the Department of Horticulture of Cukurova University grafting was used to investigated the rootstocks ability in seed yield and quality production. According to the results of this study as well discussed in the previous chapter, the following are conclusions.

NUN-9075 and Argentario graft have the highest germination percentage of 98.33 and 93.33% and highest survival rates of 85.59% and 93.33%, these were before grafting, but after grafting and harvesting the highest seed germination rate is 98.00% in the first year and even germination rate (days) reduced to an average of 3 days to complete germination. Therefore, grafting watermelon onto NUN-9075 and Argentario rootstocks increases both, germination percentage and rate.

According to results of this study as far as plant growth is concerned it is very clear that grafting increases plant growth of all parts from roots, stems, branches, leaves and fruits. The difference between grafted and non-grafted plants on the morphology of the plants can be easily compared morphologically. Indeed, NUN-9075 and Argentario rootstocks have high vigourousity effect to the plant, and they always result in robust plant growth and many fruits. However, in the beginning may delay to grow of which may be considered as a delay caused by the union effect. But once well established plants grow very fast.

Although some similarity in days to flowering were observed between grafted and non-grafted plants, however, some graft combinations especially NUN-9075/CS and Argentario have high potentials in early flowering, more flowers per

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plant and more viable pollen. This is very obvious since yield which is directly related to flowers that are receptive and easily conceive and more viable pollen, has been doubled on the rootstocks. Simply, it can be said that the effect of rootstocks on fruit, seed yield and quality started to be seen from the performance in flowering.

Moreover, the results of this study have been able to answer all research questions and lead to the accept of the hypotheses. Regarding these questions and hypotheses, the following is a list of answers.

- Due to great increase in seed yield and quality reported on grafted plants, it can be concluded that, there is a direct relationship between rootstocks and seed yield and quality.
- Rootstocks NUN-9075 and Argentario are the best rootstocks which improved seed yield. Although PI296341 rootstock showed highest quality value of seed in the second year on germination and emergence, yet NUN-9075 and Argentario remain to be the best because of their consistent performance in almost all parameters tested.
- Also as per the results of this research, it is concluded that seed yield and quality greatly relate to plant size and vigour. Because the best performed rootstocks (NUN-9075 and Argentario) showed highest yield and quality and highest values of stem length, stem diameter, number of leaves and plant biomass.
- Strong rootstocks ensure seed yield and quality, this may be because of the plant vigour caused by the ability of strong rootstock to absorb water and nutrients. In turn, the plant vigour (length of the stem, number of shoots and leaves) contributes more in manufacturing of photosynthates and net exporters of photoassimilates, hence channeling to fruit and seed as a storage sink due to higher source/sink ratio.

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PI 296341 rootstock has potentials in some traits such as high total soluble content, higher average number of seeds per fruit which is one of the factors that contribute to seed yield and strong rootstock. In the future this rootstock can be used to further investigate its ability in seed yield and quality production in combination of other high seed yielding rootstock such as NUN-9075 as dual grafting.

Also this research gives opportunity to further accentuate the good results of rootstocks especially NUN-9075 and Argentario on relationship between plant vigour and seed yield by assessing other physiological parameters such as leaf area, metabolites content and translocation, leaf size and position as well as number of flowers.

Furthermore, these rootstocks can be assessed for their ability to soil-borne diseases resistance.

Number of seeds depends on pollen quantity and viability, and in this research plants were left to self-pollinate of which the evenly efficiency of pollination may be questionable, and inconsidering the increasing number of grafted watermelon production in greenhouses, this research gives another opportunity to investigate the effects of rootstocks on seed yield and quality by using bees and or hand pollination.

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## **CURRICULUM VITAE**

Mohamed Dhamir KOMBO was born in Zanzibar, Tanzania in 1979, and finished his primary and secondary education in Zanzibar. He graduated his bachelor degree in Horticulture in the department of Crop Science of Sokoine University in Morogoro, Tanzania in 2009 and immediately in 2010 started his Master degree in Plant Sciences at Horticultural Production Chain Group of Wageningen University and Research Center in the Netherlands and graduated in 2012. He started his PhD studies in 2014 at the department of horticulture of the Çukurova University in Turkey. While persuing his PhD studies he had an opportunity to join to Erasmus Plus internship programs at Lancaster University in England and University of Palermo in Italy at different times.