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Effects of bottle gourd (*Lagenaria siceraria*) rootstocks on plant nutrient content of watermelon [*Citrullus lanatus* (Thunb.) Mats. & Nak.] leaf and nitrogen use efficiency

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Abstract
In this study, rootstock effects of bottle gourds on nutrient uptake and nitrogen use efficiency of watermelon were investigated. Watermelon seedlings were grafted onto Birecik, Skopje, Emphasis, 216 and FRGold. Non-grafted plants were used as control. The seedlings were transplanted and kept under plastic tunnel until climatic conditions were suitable for watermelon cultivation. Experimental area was amended with doses of 18 kg N da^{-1}, 20 kg P_2O_5 da^{-1} and 18 kg K_2O da^{-1} based on soil analysis. No micro-nutrient fertilizer was applied. While total P and 1/3 of N and K were applied as base fertilizer before transplanting, the second and third portions of the N and K were applied 20 and 40 days after transplanting, respectively. The experiment planned as a completely randomized block design and repeated four times, each replication has 15 plants. The seedlings were transplanted with 200 x 50 cm spacing. Macro and micro-nutrient contents were analyzed in fully grown seventh and eighth leaves from soot tips. While N, P, K and Ca contents of the leaves did not show significant differences between graft combinations and control plants, Mg concentration was significantly affected by rootstocks. All graft combinations had higher Fe concentration in the leaf than non-grafted plants. Cu and Zn concentrations showed significant variation based on rootstock. Higher nitrogen use efficiency (ton yield kg N^{-1}) was recorded in all graft combinations as compared to controls. Bottle gourd rootstocks used in this study enhanced the nutrient uptake, plant growth and total fruit yield.

Keywords: C*itrullus lanatus*; Grafting; Plant growth; Nutrient uptake; Yield

Su kabağı [*Lagenaria siceraria* (Mol) Standl] anaçlarının karpuz [C*itrullus lanatus* (Thunb.) Mats. & Nak.] yapraklarının bitki besin elementi içerikleri ve azot kullanım etkinliği üzerine etkileri

Öz
Bu çalışmada, su kabağı anaçlarının karpuzun bitki besin elementi alımına ve azot kullanım etkinliğine etkileri araştırılmıştır. Kalem olarak kullanılan Crimson Tide karpuz [C*itrullus lanatus* (Thunb.) Mats. & Nak.] çeşidi birisi köy çeşidi (Birecik) ve dördü su kabağı (*Lagenaria siceraria*) melezi (Skopje, Emphasis, 216 and FRGold) olan anaçlar üzerine aşılanmıştır. Aşısız Crimson Tide karpuz çeşidi kontrol olarak kullanılmıştır. Bitkiler dış koşullar uygun oluncaya kadar alçak plastik tünel altında yetiştirilmiştir. Bitkiler 18 kg N da^{-1}, 20 kg P_2O_5 da^{-1} ve 18 kg K_2O da^{-1} olacak şekilde gübrelenmiş, mikro element gübrelemesi yapılmamıştır. Fosforun tamamı taban gübresi olarak verilirken, N ve K üç eşit parçaya bölünmüştür. N ve K' un ilk kısmı taban gübresi olarak verilmiş, ikinci kısmı dikimden 20 sonra, üçüncü kısım ise dikimden 40 gün sonra verilmiştir. Deneme tesadüf blokları deneme desenine göre dört yinelemeli ve her yinelemede 15 bitki olacak şekilde kurulmuştur. Bitkiler 200 x 50 cm mesafelerle dikilmiş ve damla sulama yöntemiyle sulanmıştır. Bitki besin elementi analizleri sürgün ucundan sonra tam gelişmiş yedi ve sekizinci yapraklarda yapılmıştır. Aşılı ve aşısız bitkilerin yapraklarındaki N, P, K ve Ca açısından bir fark bulunmazken, Mg içerikleri önemli farklılıklar göstermiştir. Bütün aşılı bitkiler aşısız bitkilerden daha fazla Fe içeriğine sahip olurken, Cu ve Zn konsantrasyonları anaca bağlı olarak farklılık göstermiştir. Aşılı bitkiler daha yüksek azot kullanım etkinliği ve verim değerlerine sahip olmuşlardır. Bu çalışmada kullanılan anaçların bitki besin elementi alımını teşvik ettiği, bitkisel gelişimi ve verimi artırdığı tespit edilmiştir.

Anahtar Kelimeler: *Citrullus lanatus*; Aşılama; Bitkisel gelişim; Bitki besin elementi alımı; Verim

1. Introduction

Grafting in herbaceous plants is a widely used method for the sustainable cultivation of fruit bearing vegetables in Japan, Korea and some Asian and European countries, where those vegetable crops are produced intensively and continuously in the same fields without crop rotations (Lee, 1994; Oda, 1995). Sufficient evidence has been reported that the rootstocks influence the resistance to soil-borne diseases (Lee, 1994; Yetisir et al., 2003), tolerance to salinity (Yetisir and Uygur, 2010; Colla et al., 2010a), and resistance to chlorosis by nutrient deficiency in high soil pH conditions (Bavaresco et al., 1991; Sudahono and Rouse, 1994). Watermelon can be grafted onto interspecific or intraspecific hybrids of *Cucurbita* spp, *Lagenaria* spp, *Benincasa* spp, *Luffa* spp. and *Cirtullus* spp. for different purposes mentioned above. The most commercially used rootstocks for watermelon are *Cucurbita maxima* Duch. x *C. moshcata* Duch. interspecific hybrids and *Lagenaria siceraria* intraspecific hybrids in the grafted seedling market (Lee and Oda, 2003; Yetisir et al., 2007).

Bottle gourd, calabash [*Lagenaria siceraria* (Molina) Standl.] a member of the *Cucurbitaceae* family, is generally known as “the white-flowered gourd” because of its large flowers with white petals. *L. siceraria*, an annual, monoecious and vigorously climbing species, is one of the most ancient domesticated crop species for human utilization (Erickson et al., 2005; Clarke et al., 2006). Africa is believed as the center of genetic diversification of bottle gourds (Whitaker, 1971) and five wild species of bottle gourds were reported in Africa (Morimoto et al., 2005). Bottle gourd is extensively distributed and grown for different purposes in temperate, subtropical and tropical climate zones. Young tender fruits, leaves, shoot and tendrils of bottle gourds have been consumed as a vegetable. Immature young fruits are consumed similar way to zucchini by boiling, frying, stuffing or mixing with other vegetables. Seed, fresh tendril, shoot and leaf extracts of bottle gourds have been also used as medicine to treat different diseases such as diabetes, flatulence, hypertension, liver diseases, mellitus, and as a diuretic (Herklots, 1972; Moerman, 1998; Manandhar, 2002; Ghule et al., 2007). Besides, it has been utilized for several other purposes, such as containers, household tools, musical instruments, fishing net holders. Additionally, as mentioned above, bottle gourd hybrids are one of the most commercially used rootstocks for watermelon against different biotic and abiotic stress factors including fusarium wilt (Yetişir et al., 2003), salinity (Colla et al., 2005; Yetisir and Uygur, 2009, 2010) and water-logging of soil (Yetisir et al., 2006).

In previous studies, the rootstock effects on the leaf mineral content of the plants were explained by the root morphological characteristics (volume, hairiness, depth and branching). Castle and Krezdorn (1975) reported that the rootstocks capable of vigorous vertical and horizontal growth habit could promote the absorption of water and plant nutrients. The root volume and surface area interacting to soil solution are important plant characteristics affecting the amount of water and plant nutrients uptake (Taiz and Zeiger, 2008). Hence, vigorous growth of the aerial parts of the plants and higher yield was enhanced by these rootstocks; this fact being is related directly with the higher level of plant nutrients uptake such as N and K. However, Ruiz et al. (1997) reported that limited changes in the nutritional content of the scion were caused by rootstocks. Previously, it was reported that the foliar content of nutrients in fruit trees was more dependent on the genotype of the scion than the rootstocks (Chaplin and Westwood, 1980). Later, Tagliavini et al. (1992) reported that variation in uptake, translocation and utilization of plant nutrients was influenced by the vigor of the scion and the xylem sap nutrient status appeared to be more relevant to the vigor caused by the common effects of scion and rootstock parts in fruit trees. More recently, it was reported that the content of certain elements essential for the plant was changed by different rootstocks (Brown et al., 1994). Ruiz et al. (1997) reported that rootstock characteristics had significant effects on N, K, and Na content whereas P status was affected both by scion and the combination of rootstocks and scion in melon. Yield also was affected both by rootstocks and the interaction between rootstocks and scion. Nitrogen is the most crucial nutrient element in terms of plant growth, physiology and carbohydrate metabolism. It is a constituent of important metabolites such as chlorophyll, amino acids, nucleic acids, proteins, alkaloids, and protoplasm (Almodares et al., 2008). The N concentration of plant tissue is four and eight times more than K and P content, respectively (Taiz and Zeiger, 2008). The nitrogenous fertilization has been a significant tool for improving and ensuring crop yield and quality for last fifty years, especially for vegetables

(Greenwood, 1990). However, the nitrogenous fertilizers utilization has become one of the significant contributors to agriculture-related pollution through leaching, volatilization, and denitrification due to the high energy cost of nitrogenous fertilizers production (Smil, 2001), and its rapid mobility in the environmental (soil, water, atmosphere and living organisms) systems (Drinkwater et al., 1998; Limaux et al., 1999). Hence, the development of production and management systems to increase the ability of plants to uptake N is a growing concern to minimize the potential of N losses in the field of the agricultural research area. Furthermore, the breeding and use of new varieties with high nitrogen use efficiency (NUE) is able to lead to lower adverse effects on environmental systems through agricultural practices while ensuring sufficient production (Lynch, 1998). A simple technique for several horticultural crops to improve NUE in high-yielding cultivars is to graft them onto rootstocks capable of promoting NUE of the scion. Grafting in vegetables has been practiced for the purposes mentioned above for about a hundred years (Yetisir et al., 2007). However, published data about NUE in grafted vegetable crops is limited (Ruiz and Romero, 1999; Pulgar et al., 2000). In previous studies, improved NUE in melon and watermelon by grafting onto suitable rootstocks was reported (Pulgar et al., 2000; Colla et al., 2010b; Colla et al., 2011).

Information related to the relationship between scion and rootstock may be useful in the determination of the root systems tolerant to soils conditions that are deficient or toxic in some elements in the development of fertilization programs specific to particular rootstocks-scion combinations. The objective of this study was to determine the effect of different bottle gourd rootstocks on macro and micro-elements concentrations of leaves, nitrogen use efficiency and yield in watermelon grown in open field conditions.

2. Material and Method

2.1. Plant material and experimental establishment

The experiments were conducted out in the Horticultural Department of Agricultural Faculty in Çukurova University in spring seasons of 1999 and 2000. Watermelon [*Citrullus lanatus* (Thuanb.) Mat. & Nak.] cv. Crimson Tide (CT) was used as scion and bottle gourd [*Lagenaria Siceraria* (Molina) Standl.] genotypes Birecik (local bottle gourd landrace), 'Emphasis', 'Skopje', 'FRGold' and '216' (commercial bottle gourd hybrids) were used as rootstocks. Non-grafted Crimson Tide plants were used as the control. Plants were grafted by the hole insertion grafting technique described by Lee (1994) at the first true leaf stage. Seeds of the scion were sown to multipot filled with peat:perlite (v:v) mixture one week before (03.02.1999; 19.01.2000) in an unheated greenhouse to provide the equal diameter of scions and rootstocks. The seedlings were transplanted under a low plastic tunnel on 02.04.1999 and14.03.2000, and grown until outdoor climatic conditions (28.04.1999 and 30.04.2000) were suitable for watermelon cultivation. The experimental area was amended with doses of 18 kg N da^{-1}, 20 kg P_2O_5 da^{-1} and 18 K_2O kgda^{-1} based on soil analysis. No micro-nutrient fertilization was done. While total P and 1/3 of N and K were applied as base fertilizer before transplanting, the second and third portions of the N and K were applied 20 and 40 days after transplanting, respectively. Water was applied by drip irrigation system according to plant and soil observations. Plants were pollinated with native insect population. The experiments were designed as a completely randomized block design. The graft combinations were replicated four times with 15 plants for each replication. The average temperature during the growing season ranged from 8 °C to 25 °C, whereas relative humidity changed from 90% to 25%.The grafted and non-grafted control plants were grown in pots filled with soil taken from the watermelon production field (first part of the experiment was conducted) to determine effects of the rootstocks on plant vegetative growth. The experimental design was a randomized complete block design. The graft combinations were replicated three times with two plants. The plants were harvest 50 days after transplanting.

2.2. Plant sampling and analysis

The 7th or 8th fully expanded leaf from apex was collected (Bergmann, 1992) from 15 plants at the flowering stage from each replication.

Sampled leaf tissue was washed with tap water and rinsed three times with distilled water then the leaf samples were placed in an oven with forced air at 70 ^{o}C for 48 h and ground to a fine powder with a mill. The ground samples were stored in plastic bags until analysis. Macro and micro plant nutrient concentrations in fully developed leaves were determined. Total potassium (K) was analyzed by the flame photometer method (Lachica et al., 1973), total phosphorus (P) by the spectrophotometric method (Barton, 1948), calcium (Ca), magnesium (Mg), iron(Fe), copper (Cu), zinc (Zn) and manganese (Mn) were determined by Atomic-Absorption Spectrophotometry. Total N was analyzed by wet burning Kjeldahl method (Guzel et al., 1992). When the plants in pots harvested, they separated into root and shoot. The plant materials were dried in an air circulating oven at 70 ^{o}C for 48 h and then shoot and root dry weight (SDW and RDW) were determined (g plant^{-1}). Mature watermelon fruits were harvested on 14-25.06.1999 and 14.06.-03.07.2000, total fruit yield was recorded as ton da^{-1}. The nitrogen use efficiency was calculated as ton fruit yield kg for nitrogen applied.

2.3. Statistical analysis

Data were subjected to variance analysis by using SAS (2006). Means were compared by the LSD (Least Significant Difference) method at 5% significance level.

3. Results and Discussion

3.1. Nutrient uptake by leaves

Macro-nutrient contents of grafted and non-grafted watermelon plants were presented in Table 1. The concentrations of total N, P, K, and Ca in the leaf were not affected by rootstocks. The total Mg concentration of control and grafted watermelon was significantly affected by rootstocks. The highest Mg content was recorded in the control plants and it was followed by CT/Birecik graft combination, and other graft combinations had similar Mg concentration. The micro-nutrient contents of grafted and non-grafted watermelon leaves were significantly influenced by rootstocks except Mn (Table 2). All grafted plants had significantly higher Fe concentration than the control plants. Grafted plants absorbed about two folds more Fe from the soil than control plants. Rootstocks significantly influenced the Cu concentration of grafted and non-grafted watermelon leaf. The highest Cu concentration was recorded in CT/Skopje graft combination with 8.64 ppm while other combinations had similar results in terms of Cu concentration (6.18-7.09 ppm). Manganese concentration was not affected by rootstocks. Zinc concentration of grafted and the non-grafted plant was significantly affected and CT/Emphasis graft combination had higher Zn concentration than non-grafted control and other graft combinations. CT/FRGold graft combination had the lowest Zn concentration with 21.21 ppm. Rootstocks did not significantly increase macro-nutrient content (N, P, K, Ca and Mg) in unit DW of watermelon leaf. But total plant nutrients removed from the soil by plants was increased by rootstocks because the grafted plants produced significantly higher shoot and root dry weights. In contrast to current study, macro-nutrient concentrations of grafted watermelon leaf showed significant difference in our previous study (Yetisir et al., 2013). The difference between these two studies can be attributed to the variation in the rootstocks used. Because there were pumpkin and bottle gourd type rootstocks with different root characteristics and growth habit in the previous study. In agreement with our previous study, Fe, Cu and Zn concentration of the grafted watermelon leaf were significantly affected by rootstocks. As shown in macro-nutrients, total micro-nutrients removed from the soil were increased by grafting based on rootstock. However, in this study, the ranges of nutrient concentrations of the examined elements were the standard or acceptable values reported for watermelon (Bergmann, 1992), and there was no deficiency symptom.

3.2. Dry matter production and partitioning

Rootstocks effects on the shoot and root dry weights were significant and all graft combinations accumulated higher dry matters (shoot and root) than the control plants. Among the grafted plants the highest SDW was recorded in CT/FRGold graft combination while the lowest SDW was found in the plants grafted onto Emphasis. Increase in SDW as compared to the control plants ranged from 113% to 257%.

Table1. Macro nutrient uptake of Crimson Tide (CT) watermelon leaves grafted onto different rootstocks (g kg DW^{-1})

Graft combinations	N	P	K	Ca	Mg
CT/216	28.1	3.2	21.1	34.0	3.8 bc
CT/FRGold	27.4	3.4	19.8	39.1	3.4 c
CT/Emphasis	27.7	3.4	22.0	36.7	3.7 bc
CT/Skopje	27.2	4.0	28.8	33.7	3.8 bc
CT/Birecik	24.3	3.7	24.1	33.0	4.5 b
CT	28.3	3.4	21.0	33.5	5.8 a
Mean	27.2	3.5	22.8	35.0	4.13
$LSD_{0.05}$	ns	ns	ns	ns	0.88

Table 2. Micro element uptake of Crimson Tide (CT) watermelon leaves grafted onto different rootstocks (ppm)

Graft combinations	Fe	Cu	Mn	Zn
CT/216	298 a	7.04 b	73.40	24.3 bc
CT /FRGold	271 a	7.09 b	72.30	21.2 c
CT /Emphasis	267 a	7.06 b	64.50	27.7 a
CT /Skopje	275 a	8.64 a	62.60	26.1 ab
CT /Birecik	283 a	6.18 b	69.08	25.4 ab
CT	140 b	6.78 b	72.49	23.2 bc
Mean	255.9	24.66	69.01	7.13
$LSD_{0.05}$	50.84	1.2	ns	3.19

Root dry weight (RDW) changed significantly based on rootstocks and the grafted plants produced higher RDW than control plants. Among the graft combinations, CT/FRGold had lower RDW than other grafted plants (Figure 1).

It should be emphasized that nutrient concentrations in unit DW of plant tissue may not be found significantly different but higher biomass production due to vigorous root system results in higher uptake of the nutrients from the soil. In this study, grafted plants produced more biomass (Figure. 3) than the control plants. Hence, it can be concluded that grafted plants absorbed more plant nutrients and water than the control plants from the soil. This was explained by physical and physiological characteristics of rootstocks (Castle and Krezdorn, 1975). Uptake and translocation of water and inorganic nutrients depended on the vigor of the scion, and that the nutrient concentrations in the xylem appear to be more related to the vigor brought about by the combination of the root system and foliar parts (Tagliavini et al., 1992).

3.3. Yield

The grafted plant produced a higher yield than control plants and total fruit yield varied significantly based on rootstocks. The plants grafted onto Skopje and bottle gourd landraces Birecik rootstocks had the highest yield. Increase in yield compared to the control varied from 27% to 112% (Figure 2).

In agreement with previous study biomass accumulation and fruit yield were improved by bottle gourd rootstocks in this study. It has been reported that grafting onto vigorous rootstocks caused promoted vegetative growth at different ratios based on rootstock characteristics (Jeong, 1989; Kim and Lee, 1989; Itagi, 1992; Ito, 1992). Increased vegetative growth and fruit yield were attributed to the tolerance to soil-borne diseases (Ashworth, 1985; Lee, 1994), resistance to negative soil conditions (Behboudian et al., 1986; Walker et al., 1987; Picchioni et al., 1990; Bavaresco et al., 1991; Sudahono and Rouse, 1994; Yetisir and Uygur, 2010; Colla et al., 2010), improved water and plant nutrient absorption (Kota and Lou, 1989; Ruiz and Romero, 1999) and increased endogenous phytohormone production (Ruiz and Romero, 1999). In previous studies, the impact of rootstocks on water and plant nutrients uptake was explained by the physical characteristics of the root system such as root volume, length and dept. The effects of rootstock were principally attributed to morphological root characteristics, such as lateral and vertical growth habit that improved

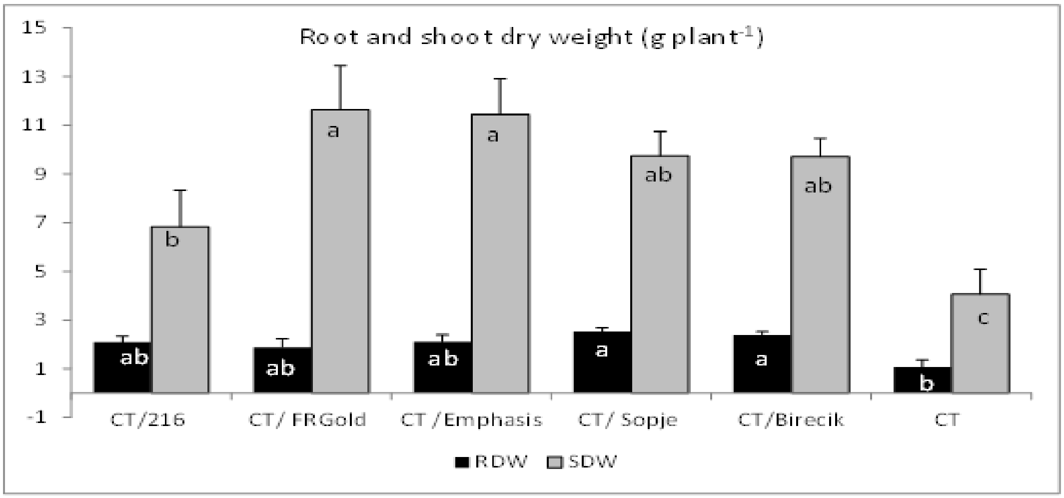

Figure 1. Shoot and root dry weight of grafted and non-grafted watermelon (g plant^{-1})
CT: Crimson Tide; SDW: Shoot Dry Weight; RDW: Root Dry Weight.

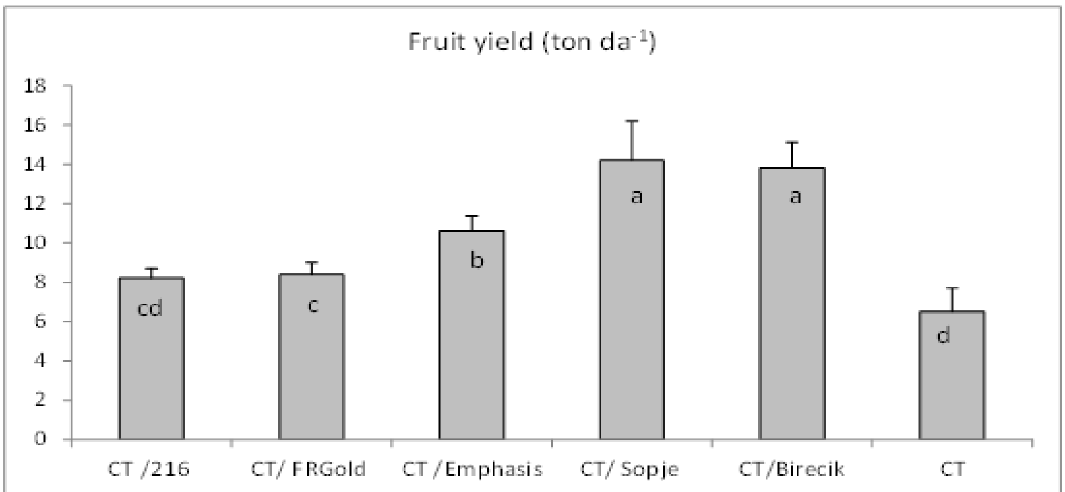

Figure 2. Total yield of grafted and non-grafted watermelon (ton da^{-1}). CT: Crimson Tide

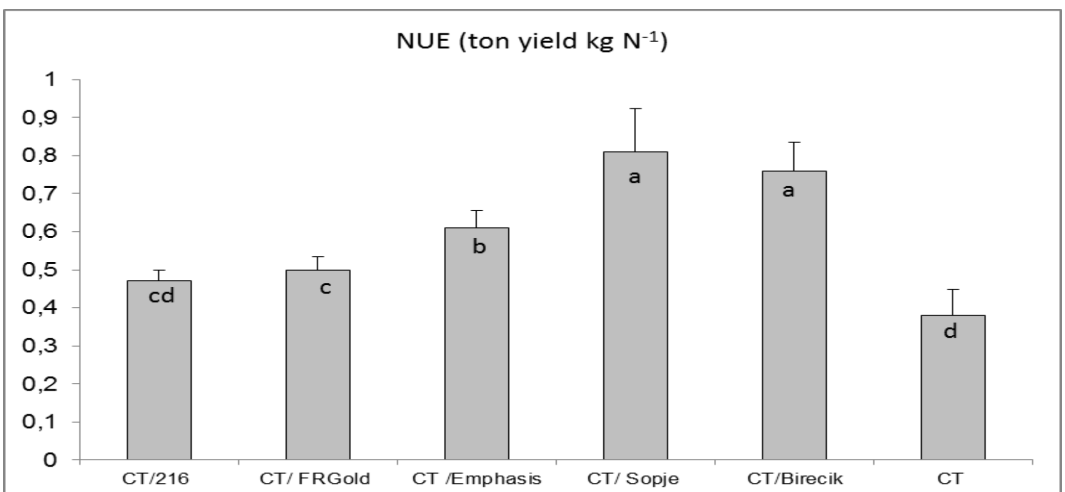

Figure 3. Nitrogen use efficiency (NUE) of grafted watermelon plants (ton yield kg N^{-1})
CT: Crimson Tide

the absorption of water and plant nutrients (Castle and Krezdorn, 1975). Vegetative growth was significantly affected by rootstocks and grafted plants on to local bottle gourds accessions produced higher fresh and dry weight (Yetisir et al., 2007). Vigorous root systems of rootstocks are often capable of absorbing water and plant nutrients more efficiently than scion roots and serves as a good supplier of endogenous hormones (Kota and Lou, 1989). The significant amount of increase in xylem sap with quite high concentrations of plant nutrients, organic substance and phytohormones such as gibberellins and cytokinin were reported in *Cucurbitaceous* crops (Masuda et al., 1981; Biles et al., 1989; Zijlstra et al., 1994). Ruiz and Romero (1999) noted that plant nutrients can be uptaken and utilized more effectively by grafted plants onto strong rootstocks than non-grafted plants in melon.

3.4. Nitrogen use efficiency

Nitrogen use efficiency was significantly higher in all graft combinations than non-grafted plants. Plants grafted onto Skopje and Birecik had about 2 folds higher NUE than non-grafted control plants. The highest NUE was determined in the plants grafted onto Skopje and Birecik with 0.79 and 0.75 tons fruit kg N^{-1}, respectively, while the lowest NUE was determined in the control plants with 0.38 ton fruit kg N^{-1} (Figure 3). The result showed that N was efficiently absorbed and utilized by the grafted plants as compared to non-grafted control plants. The higher dry matter and fruit yield in all grafted (Figure 1 -3) plants showed that total removed N from the soil by grafted plants was significantly higher than non-grafted control plants. Similarly, higher N uptake and utilization efficiency were reported in watermelon (Pulgar et al., 2000; Cola et al., 2010b), melon (Colla et al., 2011) in previous studies. Colla et al. (2010b) concluded that grafting melon onto certain rootstocks improved N uptake and utilization efficiency. Moreover, they suggest that increased nitrate reductase activity in grafted plants under low nitrate concentrations confirmed that certain rootstocks with higher N uptake and use ability could promote the NUE of grafted plants. The improved NUE of grafted plants can be attributed to physical (root volume, surface area, depth and length) and physiological (efficient uptake mechanism and nitrate reductase activity in roots) characteristics of the rootstocks.

4.Conclusions

In conclusion, grafting watermelon onto different bottle gourd rootstocks increased plant growth, total yield and NUE, but nutrient uptake of grafted watermelon leaf was not except Mg, Fe, Cu and Zn. Bottle gourd rootstocks did not cause a significant difference in plant nutrient content in unit dry weight in grafted watermelon leaves but grafted plants removed more plant nutrients because all graft combinations produced higher biomass than the control plants. Therefore, it may be concluded that bottle gourd rootstocks promote plant growth and yield without having a significant effect on quality regarding plant nutrients. Moreover, Birecik, local bottle gourd landrace, showed better performance than commercial rootstocks 216, FRGold and Emphasis in plant growth, yield and NUE after Skopje rootstocks. These results have shown once again that local plant genetic resources have a very valuable place in plant breeding to maintain sustainable agriculture and environment. Hence, studies on collection, characterization, conservation and utilization (agricultural and environmental aspects) of local plant germplasm should be supported and maintained.

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