Effect of self and inter-cultivar grafting on growth and nutrient content in sweet basil (*Ocimum basilicum* L.)

Undergraduate Thesis

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Table of Contents

Chapter 1

A review of herbaceous fruiting vegetable grafting

Abstract

Grafting, combining the root system of one plant and the shoot system of another has been used to improve plant growth characteristics of tree fruit and nut crops for centuries. However, the application of this technique to herbaceous fruiting vegetable crops is relatively new and has been increasing over the last century. Initial modern grafts were made to confer resistance to soil-borne disease in cucurbits. However, grafting is now commonly performed on many fruiting cucurbit and solanaceous vegetables for reasons including resistance to soil borne pathogens, tolerance of adverse environmental conditions (such as temperatures, salinity and drought stress, flooding), enhanced nutrient use efficiency, increased yield and productivity, and greater fruit quality. Resistant rootstocks allow for production of susceptible scion cultivars either through major gene resistance or production of an extensive root system capable of handling pathogen loads. Higher performance of grafted plants under sub/supra optimal temperatures as well as salinity and drought in grafted plants is commonly observed and often attributed to increased production and activity of antioxidant enzymes. Grafted plants are also better able to withstand and recover from flooding with the use of rootstocks capable of quickly regenerating new roots. Rootstocks with large root systems and high rates of nutrient uptake have been shown to increase yield; similarly, vigorous rootstocks are commonly used in the production of vegetable crops as a means to improve yield, though the exact mechanisms controlling scion vigor promotion remain unclear. The effect of grafting on fruit quality is still debated, certain rootstocks have been shown to alter soluble sugar, lycopene, fruit shape, color, size and other

important marketable traits. This chapter serves as a review of the current applications of grafting in herbaceous vegetable production.

Introduction

While grafting of woody plants has long been integral to agriculture, grafting of herbaceous fruit and vegetable crops is a relatively new strategy for improving crop yields under adverse conditions. The first record of interspecific vegetable grafting comes from Japan in 1920 when watermelon (*Citrullus lanatus*) was grafted onto *Cucurbita* (squash or pumpkin) rootstocks in order to increase resistance to *Fusarium* wilt (Kubota, 2016). Since this initial graft, many other crops including members of Cucurbitaceae such as melons (*Cucumis melo*) and cucumbers (*Cucumis sativus*), as well as solanaceous crops such as tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), and eggplant (*Solanum melongena*), have been grafted using a variety of rootstocks and techniques. Like the initial application of grafting, vegetables are often grafted in order to imbue a susceptible cultivar with resistance to soilborne pathogens like bacteria, fungi and nematodes, reducing crop losses and the need for breeding resistance into commercial cultivars; however, grafting has also been shown to increase vigor and tolerance to unfavorable environmental conditions, improve fruit yield and quality, and reduce the need for agrochemical resource inputs (e.g., fertilizers). In this chapter, I will review current applications of grafting technology to address adverse environmental conditions and improve crop yield and quality.

Resistance to pathogens

Long the driving force behind grafting vegetables, resistance to pathogens remains an important advantage of grafting to this day. This resistance is especially important in regions of the world where there is limited land available for crop rotation, as continuous cropping of susceptible cultivars can lead to a rapid accumulation of inoculum for soil-borne pathogens like root knot nematode (*Meloidogyne* spp.), *Fusarium oxysporum*, *Verticillium*, *Monosporascus* *cannonballus*, *Phytophthora*, *Pseudomonas*, and *Didymella bryoniae* (Lee et al., 2010). Thus, it has been countries like Japan and Korea that have rapidly adopted grafted vegetables with reports of 92% of Japanese watermelon being grafted and 95% in Korea (Lee et al., 2010). Grafting as a means of conferring disease resistance is also becoming more important as countries around the world work to phase out fumigants like methyl bromide which are harmful to human and environmental health (Edelstein et al., 1999; Gaion et al., 2018; Lee et al., 2010).

In watermelon, many rootstocks have been utilized as a means of providing disease resistance including bottle gourd (*Lagenaria siceraria*), wild watermelon (*Citrullus lanatus* var. *citroides*) and *Cucurbita* species including interspecific hybrids (*C. maxima* X *C. moschata*) (King et al., 2010). *Cucurbita* hybrids especially have been known for conferring resistance to *Fusarium oxysporum* f. sp. *Niveum*, a devastating pathogen of watermelons that can persist in soil for many years (Álvarez-Hernández et al, 2015; Ling et al., 2013). In addition to simply reducing yield losses due to *Fusarium*, certain rootstocks, notably ‘Super Shintoza’, also confer resistance to *Verticillium* wilt and can reduce the formation of microsclerotia and therefore the inoculum for future growing seasons (Dabirian et al, 2017). Like watermelon, many of the rootstocks used for the production of melons (*Cucumis melo*) focus on resistance to *Fusarium*; this resistance is generally achieved through the use of melon rootstocks with major gene resistance to *Fusarium* isolates (King et al., 2010, Trionfetti Nisini et al., 2002). Other species can be used as rootstocks including *Benincasa hispida* and *Cucurbita* species, which can confer tolerance to all *Fusarium* isolates; however, issues can arise with fruit quality and graft compatibility (Calatayud et al., 2013; King et al., 2010; Trionfetti Nisini et al., 2002; Zhou et al., 2014). Grafting using Cucurbita and tolerant melon cultivars has also been shown to decrease crop losses associated with *Monosporascus* wilt; however other management strategies should be used in conjunction with grafting if *Cucurbita* rootstocks are used as they still become infected by the pathogen and can lead to build up of inoculum (Cohen et al., 2000; Edelstein et al., 1999). Grafting has also been used as a means to reduce crop losses from gummy stem blight caused by *Didymella bryoniae* (Crinó et al., 2007).

Tomato scions grafted onto resistant tomato rootstocks such as ‘Beaufort’ and ‘Maxifort’ have been shown to have excellent tolerance to many devastating pathogens such as *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium oxysporum* f. sp. *Radicis-lycopersici*, Root knot nematodes (*Meloidogyne* spp.), *Ralstonia solanacearum*, and *Verticillium* (Hamdi et al., 2009; Kunwar et al., 2015; Lee et al., 2010; Owusu et al., 2016; Papadaki et al., 2017; Polizzi et al., 2015; Yin et al., 2015). Resistance against these pathogens is the primary reason for grafting tomatoes in low input soil-based production systems such as high tunnels where conditions may be ideal for disease development (King et al., 2010). In eggplant production, numerous wild *Solanum* species such as *S. integrifolium*, *S. Torvum*, and *S. sisymbriifolium* can be used to improve tolerance to bacterial wilt, fusarium wilt, verticillium wilt and root knot nematodes (Gaion et al., 2018; King et al., 2010). Peppers, specifically *Capsicum annuum*, are less commonly grafted due to issues with incompatibility with other species, yet the worldwide trend towards reducing soil fumigant and nematicide makes finding suitable pathogen resistant rootstocks increasingly important (Gaion et al., 2018; Oliveira et al., 2009). *Capsicum annuum* and *Capsicum frutescens* rootstocks have been shown to confer resistance to two *Meloidogyne* species when grown in infested soils; however, scions grafted to species other than *C. annuum* tend to yield less and lower quality fruit (Oka et al., 2004; Oliveira et al., 2009). When used as rootstocks, three sweet pepper hybrids with known resistance to *Meloidogyne incognita* have also been shown to confer resistance to phytophthora wilt caused by *Phytophthora capsici* under controlled environment conditions (Santos and Goto, 2004).

Adverse temperatures

In addition to permitting cultivation in fields with high levels of pathogens, vegetable grafting has also become a valuable tool for increasing tolerance to adverse abiotic conditions.

For example, Rivero et al. (2002) concluded that grafted tomato plants produced fewer reactive oxygen species (ROS) under high temperatures and were better able to withstand heat stress than non-grafted plants. Cucumber plants grafted onto heat tolerant luffa (*Luffa cylindrica*) results in a similar reduction in damage to tissue by superoxide (O_2^-) when compared to non-grafted plants grown at high day/night temperatures of 36/31°C (Li et al., 2014). Conversely, Grafting can also be used as a means to overcome production limitations posed by suboptimal temperatures. A cold tolerant wild relative of cultivated tomato, *Solanum habrochaites*, has been shown to increase the growth rate of the commercial tomato cultivar 'Moneymaker' when used as a rootstock under low temperature conditions (Venema et. al., 2008). In cucurbits, rootstocks such as fig leaf gourd (*Cucurbita ficifolia*) and *Cucurbita maxima* X *Cucurbita moschata* interspecific hybrids are commonly used to increase cold tolerance of scions and can allow earlier planting dates for crops like watermelon (Davis et al., 2008). Grafting of watermelon onto cold-tolerant squash and calabash (*Lagenaria siceraria*) rootstock improves the storability of scion (Spalholz and Kubota, 2017). Additionally, cold tolerant fig leaf gourd (*Cucurbita ficifolia*), used as a rootstock for chilling sensitive cucumber, has been shown to increase plant biomass compared to non-grafted plants grown under suboptimal temperatures due to increased root production (Tachibana, 1982). This same combination has also been shown to reduce production of and damage from ROS and photoinhibition as well as increase the carbon assimilation rate and activity of antioxidant enzymes, resulting in better growth under cool conditions (Li et al., 2014; Zhou et al., 2007; Zhou et al., 2009). Similar increased activity of antioxidants like superoxide dismutase, has been shown to occur in the leaves of grafted watermelon under low temperature (Liu et al., 2003).

Drought and salinity

Salinity and drought stress represent another common limitation to vegetable production around the world, especially in soil-based protected culture settings where high levels of fertilizers are used (Huang et al., 2015). In both drought or high salinity conditions roots are unable to take up enough water to supply the plant, either due to a lack of water in the soil or high levels of ions in the soil water; this often induces a stress response resulting in the closure of stomata, which leads to production of harmful reactive oxygen species due to the lower concentrations of CO_2 (Cruz de Carvalho, 2008; Huang et al., 2015). In addition to osmotic stress, under saline conditions, plants can be subjected to ionic stress from the uptake of ions such as sodium and chloride. (Estañ et al., 2005; Niu et al., 2019). Under saline conditions, grafting has been shown to reduce uptake and translocation of toxic ions like sodium to the scion (Colla et al., 2012; Estañ et al., 2005; Fernández-García et al., 2002; Huang et al., 2015; Ruiz et al., 2006), improve mineral nutrition (Colla et al., 2012), increase water use efficiency (He et al., 2009) and increase activity of important antioxidant enzymes like superoxide dismutase and catalase as well as proline (He et al., 2009; Huang et al., 2015; Ruiz et al., 2006). Certain rootstocks have also been shown to improve tolerance to saline conditions by inducing earlier closure of stomata, preventing excess water loss in cucumber (Niu et al, 2019). Similar responses have also been observed under drought conditions. For example, grafted watermelons produce greater yields and display greater water use efficiency than their non-grafted counterparts under drought conditions (Rouphael et al., 2008). Liu et al. (2016) found that cucumbers grafted to luffa rootstocks were more tolerant of drought conditions due to increased production of and sensitivity to abscisic acid (ABA), resulting in reduced water loss, ROS production and increased activity of enzymatic and non-enzymatic antioxidants. Drought tolerant rootstocks have also induced similar reductions in ROS production and increases in antioxidant enzyme activity in tomato and tobacco (Liu et al., 2014; Zhang et al., 2019a).

Flooding

In addition to stress due to lack of water, grafting can also improve plant tolerance to flooding (Lee et al., 2010). For instance, grafting watermelon scions onto calabash rootstocks results in adventitious root formation and enhanced growth compared to non-grafted watermelon when grown under saturated field conditions (Yetisir et al., 2006). Additionally, certain eggplant rootstocks for use in tomato grafting have been reported to be tolerant of flooding (Black et al., 2003).

Nutrient use efficiency

Soils with nutrient imbalances can prevent optimal plant growth and yields; however, grafting, using vigorous rootstocks like ‘Beaufort’ for tomato, can increase the rootzone available for the uptake of water and nutrients, reducing recommended fertilizer rates by roughly half (Lee et al., 2010; Leonardi and Giuffrida, 2006; Oztekin et al., 2009). *Cucurbita moschata* and watermelon cultivar ‘Hongdun’, have been shown to improve the uptake efficiency of potassium in watermelon (Huang et al., 2013), while interspecific hybrid *Cucurbita* rootstock, along with a calabash interstock inserted between the rootstock and scion, can increase graft compatibility and uptake of important nutrients, including nitrate, phosphorus, potassium, calcium, manganese, and zinc, in melons (San Bautista et al., 2011). Additionally, Colla et al. (2011), found that grafting watermelon scions onto calabash and *Cucurbita maxima* X *Cucurbita moschata* rootstocks increased nitrogen use efficiency traits such as increased nitrogen uptake and nitrate reductase activity in the shoot and reduced the total necessary nitrogen. Similar results regarding increased uptake of nutrients like nitrate, phosphate and potassium, and nitrogen use efficiency are seen in Colla et al. (2010), and Salehi et al. (2010). This improved ability of grafted plants to scavenge nutrients makes grafting a powerful tool for minimizing fertilizer application, reducing costs to growers and the environment (Lee et al., 2010). In addition to increasing tolerance to low nutrient concentrations, grafting can also help to avoid issues associated with toxic levels of nutrients or heavy metals like copper, boron, cadmium (Edelstein et al., 2007; Kumar et al., 2015; Savvas et al., 2009). For example, in solutions with moderate levels of cadmium, tomatoes grafted to vigorous ‘Maxifort’ rootstocks exhibited improved nutrient status, reduced ROS production, increased activity of antioxidant enzymes and proline, and increased levels of metabolites involved in cadmium tolerance compared to non-grafted plants (Kumar et al., 2015).

Increased yield and productivity

The increased yields that are often associated with vegetable grafting, are generally observed as a result of grafted plants being tolerant or resistant to the biotic and abiotic constraints to production discussed earlier. For example, there are many studies showing that grafted plants increased yield under adverse environmental conditions such as salinity and drought (Dabirian et al., 2017; Flores et al., 2010; Sánchez-Rodríguez et al., 2012) as well as under high disease pressure (Edelstein et al, 1999; Gaion et al., 2018). However, other studies have indicated an increase in cucurbits, pepper, eggplant, and tomato yield under optimum growing conditions (Lee et al., 2010; Madeira et al., 2016; Sabatino et al., 2016; Zhang et al., 2019b) such as those seen in hydroponic greenhouse systems.

Fruit quality

There is still considerable debate on the effect grafting has on fruit quality, especially for melons and cucumbers. Among the quality characteristics that can be altered by grafting are vitamin and phenolic compounds with human health benefits. For example, increased levels of vitamin C and lycopene, both with important nutritional properties, have been recorded in grafted watermelon when compared to non-grafted controls (Proietti et al., 2008). Other compounds beneficial to human health such as phenolic antioxidants have also been shown to increase through grafting eggplant onto rootstocks of a wild relative, *S. torvum* (Sabatino et al., 2016). However, Moncada et al. (2013) reported higher levels of phenolic compounds in fruits from non-grafted plants and noted reduced marketability of fruits from scions grafted to *S.*

torvum due to darker and less vivid coloration. In addition to fruit nutritional quality, beneficial characteristics for post-harvest storage and handling such as reduced flesh discoloration, increased rind thickness and flesh firmness have been improved in watermelon by grafting on interspecific hybrid squash rootstocks (Kyriacou and Soteriou, 2015). Sweetness and flavor are other characteristics important to consumers; studies have also shown grafted tomatoes to produce fruits with greater soluble sugars and therefore greater marketability when grown under drought or salinity stress (Flores et al., 2010; Sánchez-Rodríguez et al., 2012). However other studies have reported decreases in sugars such as glucose and sucrose, total acidity and aromatic compounds (Milenković et al., 2020; Tripoidi et al, 2020). In addition to sweetness, certain melon cultivars can take on a squash like flavor when grafted to *Cucurbita* rootstocks (Lee et al., 2010; Traka-Mavrona et al., 2000). Furthermore, even characteristics like size (Lee et al., 2010) and shape of fruits can be altered by grafting; Tsabella et al. (2012) reported that grafting peppers could induce changes in fruit shape which could be inherited for several generations, potentially affecting salability.

Conclusion

Grafting of herbaceous fruiting vegetables has many useful applications including resistance to pathogens and abiotic stresses such as high salinity, drought, flooding, adverse temperatures and low/toxic nutrient levels, as well as increasing yield and fruit quality characteristics. As agricultural land continues to be lost to development, and growers face the challenges associated with a rapidly changing climate, it is likely that grafting will become even more widespread as a means to minimize inputs and maximize profits, even under non-ideal growing conditions.

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Chapter 2

Effect of self and inter-cultivar grafting on growth and nutrient content in sweet basil (*Ocimum basilicum* L.)

Abstract

Grafting of woody crops has been practiced by humans for thousands of years, but since the early 1900s, interest in grafting herbaceous vegetable crops has been increasing rapidly. This technique is known to improve plant nutrient uptake and use efficiency, tolerance to soil-borne disease and adverse environmental conditions, plant vigor and yield. Thus far, research on grafting has focused primarily on fruiting cucurbit and solanaceous vegetables; however, little is known about the response of leafy vegetables and herbs to grafting and whether these crops may also benefit from grafting. While typical compact architecture of these leafy greens makes grafting challenging, potential benefits include grafting vigor and rootstock disease resistance. In this study, the effects of grafting two cultivars of sweet basil on plant growth and inorganic nutrient content were examined. Cultivars ‘Nufar’ (N), a vigorous and Fusarium wilt resistant cultivar, and ‘Dolce Fresca’ (DF), a cultivar developed for compact, uniform growth, were selected. Four grafted treatments (scion/rootstock) were created by self-grafting (N/N and DF/DF) and reciprocal inter-cultivar grafting (DF/N and N/DF). Un-grafted plants (ug-N and ug-DF) served as controls. Following growth in a greenhouse, destructive root fresh and dry mass measurements were taken from half of the plants 21 days after transplanting (DAT) and all remaining plants were pinched at the second node. The second harvest for yield and tissue nutrient analysis was performed at 42 DAT during the first experiment and 35 DAT in the second experiment. After 21 DAT, leaf number, stem length, shoot mass, total mass, and

shoot:root fresh mass ratio were significantly lower in N/DF when compared to ug-N, suggesting that grafting vigorous N to DF rootstocks can limit yields of N. possibly due to preferential carbohydrate allocation to DF roots. The fresh shoot:root ratio from 42 DAT was significantly higher for DF/N than ug-D, in the first experiment, despite insignificantly different measures of root and shoot mass, suggesting that N rootstocks may have increased water and nutrient uptake efficiency. This was further supported by significantly higher levels of nitrogen content in DF/N than ug-DF or DF/DF. This study indicates inter-cultivar grafting can have significant effect on plant yield and nutrient content in basil. However, further research is needed to determine the specific mechanisms affecting graft union formation, and its effect on growth and nutrient transport in basil.

Introduction

While grafting has been used by humans for thousands of years to propagate tree fruit and nut crops, herbaceous vegetable grafting is a relatively new technique that has grown in popularity over the last century as a means to improve plant growth. Since the first modern record of vegetable grafting in the 1920's, when watermelon was grafted onto *Cucurbita* rootstocks in order to increase resistance to *Fusarium* wilt (Kubota, 2016), grafting has been widely adopted in many countries like Spain, Italy, Japan, Korea and Turkey (Bie et al., 2017). While disease resistance remains one of the most common reasons for grafting, numerous other beneficial applications for vegetable grafting have been identified including increased yields, reduced fertilizer requirements, improved quality and tolerance to environmental conditions such as adverse temperatures, salinity, flooding, and drought (Lee et al., 2010). To date, the majority of commercially grafted crops are solanaceous and cucurbit fruiting vegetables (Bie et al., 2017; Lee et al., 2010) However, green beans and artichokes have only recently been found to exhibit increased yields as a result of grafting (Koren et al., 2017). This indicates there is potential that many other untested crops such as leafy greens and herbs may also benefit from grafting.

Sweet basil is an annual herb in the family Lamiaceae and is a popular crop grown in many regions worldwide as a flavoring and for its essential oils (Makri and Kintzios, 2008; Walters et al., 2020). Various different production practices for basil exist including both open field and hydroponic production in greenhouses and indoor farms (Makri and Kintzios, 2008; Walters et al., 2020). While novelty basil grafted to a woody rootstock has been developed for home garden use (Histil Ltd., Ashkelon, Israel), commercially grown basil is not grafted. However, there is potential that grafted basil may provide some of the same benefits observed in other crops. While grafting has many benefits in fruiting vegetables, this study focused specifically on nutrient uptake, which has been shown to increase with use of vigorous rootstocks in tomato and watermelon (Colla et al., 2010; Colla et al., 2011; Leonardi and Giuffrida, 2006; San Bautista et al., 2011) and plant vigor and yield promotion which has been observed in many fruiting vegetables (Madeira et al., 2016; Masterson et al., 2016; Sabatino et al., 2016; Zhang et al., 2019). Two sweet basil cultivars, ‘Nufar’ (N), a vigorous and *Fusarium* resistant cultivar, and ‘Dolce Fresca’ (DF), a less vigorous cultivar developed for compact and uniform growth, were selected. In this study we compared un-grafted, self-grafted and inter-cultivar grafted basil to determine whether basil can be successfully grafted and whether these graft combinations result in a significant change in plant growth and nutrient content.

Materials and methods

Experimental timeframe and location

Two trials were conducted during the Autumn of 2020 and Winter of 2021. With the first trial running from 30 September 2020 to December 30 and the second from 15 January to 30 March. Both trials were conducted at the Howlett Hall Greenhouse in Columbus, OH.

Plant materials and growing conditions

For this experiment, two basil cultivars ‘Dolce Fresca’ (Territorial Seed Company, Cottage Grove, OR), and ‘Nufar’ (Johnny’s Selected Seeds, Waterville, ME) were used. Seeds were sown in 98 cell count plug trays (Hummert International, Earth City, MO) filled with BM1 all-purpose soilless potting mix (Berger Peat Moss Ltd., Saint-Modeste, Canada). Seeds were placed on the surface of the substrate and pressed down with a pencil eraser. Control plants were sown seven days after plants to be used for grafting. White plastic display trays (Hummert International, Earth City, MO) were placed under seeded plug trays and inverted on top, covering plug trays to exclude light and maintain high humidity. Seeds were germinated in a glass greenhouse set at 24/17℃ day/night with a dead band of ±1°C. Top cover trays were removed 108 hours after sowing, when approximately 85% of seedlings had visibly emerged. Following removal of top cover trays, plants were sub irrigated with water as needed by adding 2 litres of water to the undertrays and dumping excess liquid after 10 minutes. Twenty days after seeding, nutrient solution (Jack’s soluble 12-4-16 (JR Peters Inc., Allentown, PA) at 100 ppm N, 1.574 dS m^{-1}) was substituted for water.

After plants were removed from the graft healing chamber, 24 plants of each grafted treatment (N/N, DF/DF, N/DF, and DF/N) as well as 24 of each of the two ungrafted cultivars (N and DF) of a similar developmental stage were transplanted into 10 x 10 cm green square plastic pots filled with BM1 all-purpose soilless potting mix (Berger Peat Moss Ltd., Saint-Modeste, QC, Canada). Pots were arranged in a complete randomized block design consisting of six blocks each with 4 plants of each treatment. Pots were arranged in staggered rows, with 6 rows of 4 pots so that flat sides were 10-cm apart and corners of pots were touching, in order to achieve a plant density of 50 plants m^{-2}. Plants were fertigated with Jack’s soluble 12-4-16 (JR Peters Inc., Allentown, PA) at 100 ppm N and 1.5 dS m^{-2} using drip emitters. The irrigation system was controlled by a Sterling irrigation controller (Superior Controls Co Inc., Valencia, CA) and programmed to run for one minute, two four times daily. Seven days after transplanting, greenhouse temperature set points were raised to 25℃ during the day and 19℃ at night with a dead band of ±1℃. Throughout the growing period high pressure sodium lamps were used to maintain a daily light integral of 7.8 mol m^{-2} d^{-1}.

After 26 days, growing in the pots, plants were harvested. In each block, two plants of each treatment were cut just above the second node. Remaining plants were randomly respaced within the original block to achieve a planting density of 25 plants m^{-2} (2 plants of each treatment in each block). Growing conditions and irrigation were maintained at levels used prior to harvest.

Grafting treatments

In the first experiment plants were grafted 36 days after sowing. Four grafted treatments were created: 1: ‘Nufar’ self-grafted (N/N), 2: ‘Dolce Fresca’ self-grafted (DF/DF), 3: ‘Dolce Fresca’ scion grafted to ‘Nufar’ rootstock (DF/N), and 4: ‘Nufar’ scion grafted to ‘Dolce Fresca’ rootstock (N/DF) as depicted in Table 1. Five plants of each treatment were grafted at a time, this was repeated to achieve a total of 40 grafted plants of each treatment. For inter-cultivar grafts (N/N and DF/DF), rootstocks and scions with similar stem diameter were selected; the rootstock plug was removed from the cell tray and the hypocotyl cut at a 30° angle just below the cotyledons. A complementary cut was made on the scion as close to the media as possible. The cut edges of the scion and rootstock were then gently pressed together, ensuring the cambium was lined up, and secured by a 1.5-mm grafting clip. For self-grafted plants, a 30° cut was made below the cotyledons of a single plant, and the pieces rejoined with a 1.5-mm grafting clip. Throughout the grafting process, the plants were continuously misted with water using a hand spray bottle. As grafts were completed, plants were set in a new 98-cell tray on top of an upside down cell-tray inside a sealed clear polycarbonate healing chamber (47 L volume, 45.7 x 66 x 22.9 cm) (Rubbermaid, Atlanta, GA) with approximately 8 cm of water in the bottom.

Sealed boxes were placed in a growth chamber (Controlled Environments Ltd., Winnipeg, Canada) maintained at 28°C and under complete darkness. After 24 hours, the light intensity was increased to 60 μmol m^{-2} sec^{-1} (continuous light). After 5 days, the lid of the healing chamber was cracked open slightly for 48 hours before plants were removed.

Table 1. Grafted and un-grafted treatments

	'Dolce Fresca' Scion	**'Nufar' Scion**
'Dolce Fresca' Rootstock	DF/DF	N/DF
'Nufar' Rootstock	DF/N	N/N
Un-grafted controls	ug-DF	ug-N

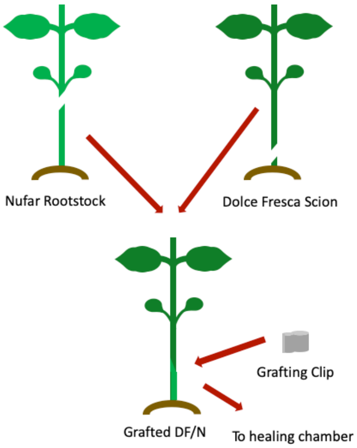

Figure 1. Grafting process for DF/N

Data collection

Plants were assessed for successful graft union formation following removal from the graft healing chamber. After grafting clip removal, plants with joined scions and rootstocks were subjected to a tug-test; if the scion and rootstock separated after pressure was applied by gently pulling rootstock and scion in opposite directions, graft union was considered unsuccessful.

Above ground plant biomass was harvested 21 DAT. Two plants of each treatment from each block were randomly selected and above ground biomass cut at the level of the soilless media. The remaining two plants of each treatment were cut just above the second nodes and the pots respaced as outlined previously. The number of leaves per plant were counted; only leaves with a blade length exceeding 1cm were considered for the leaf number count. Side shoots exceeding 1 cm of length were removed from the main stem by pulling and laid end to end along a metric ruler and the length recorded. Main stems were measured using a metric ruler as well. Values for side and main stem lengths were summed to give a total stem length value. To obtain destructive root mass measurements substrate was removed from the root system by gentle shaking followed by soaking and gently moving root systems in plastic tubs of water; roots were then removed and pressed lightly between two layers of paper towels to remove excess moisture. All shoot tissue and root tissue were weighed separately immediately following harvest. After drying for a minimum of six days at 55°C root and shoot dry mass was determined. The same harvest procedure was used for respaced plants during a second harvest 42 DAT in the first experiment and 35 DAT in the second experiment. Due to extensive thrips damage, ug-N, N/N and N/DF were excluded from analysis at 42 DAT in the first experiment.

Tissue nutrient analysis was conducted using plants harvest at 42 DAT in the first experiment. Following drying, leaf tissue was collected, ground and placed into sealed plastic 50 ml centrifuge tubes (Fisher Scientific, Hampton, NH). Tissue analysis was conducted by JR Peters Inc. (Allentown, PA) for essential plant macronutrients (N, P, K, Ca, Mg, and S) and six micronutrients (Fe, Mn, B, Cu, Zn, and Mo).

Statistical analysis

Statistical analysis was conducted using Excel (Microsoft Corporation, Redmond WA) and R (Rstudio, Boston, MA). An analysis of variance analysis was conducted to determine if treatment had a significant effect on plant growth and nutrient content. Tukey’s HSD (honestly significant difference) tests were conducted at $P \leq 0.05$ and used for separation of means.

Results and discussion

Graft union formation

No significant differences were found between the two experiments in terms of successful graft union formation and averaged survival rates can be observed in Table 2 . Graft union formation rates were not statistically different for N/DF, DF/DF, and DF/N but were significantly higher for N/N, which exhibited the highest rate (87.5%) of successful graft union formation (Table 2). Commonly identified causes of graft union failure in fruiting vegetables include genetic incompatibility, poorly matched rootstock and scion diameter, plants of an incorrect developmental stage, disease, poor technique, inadequate environmental conditions during healing, and insufficient duration of the healing period (Bumgarner and Kleinhenz, 2014). During testing for graft union formation many failed graft combinations were observed to have extensive adventitious root formation by scions; less extensive root formation was also observed in some successful grafts (Figure 2.). The formation of such adventitious roots can result from poor grafting technique or environmental conditions during healing and can increase the risk of graft failure in tomato (Meyer et al., 2017), and may partially explain the poor survival rate observed in this study. However, without additional experimentation, other common causes of graft union failure, mentioned previously, may also have contributed especially since adventitious root formation is known to be affected by environmental conditions during graft healing (Vega-Alfaro et al., 2020). Altering plant developmental stage at grafting, healing chamber conditions, and duration of healing used in grafting, and other techniques shown to increase grafted plant survival and reduce adventitious root formation such as application of ascorbic acid and leaf removal (Meyer et al., 2017; Vega-Alfaro et al., 2020) should be examined to identify conditions and techniques that maximize successful graft union formation in sweet basil.

Table 2. Rate of successful graft union formation (mean ± standard error). Means within a column that share a letter are not statistically different ($P \leq 0.05$)

Treatment	Survival (%) ± SE
N/N	87.50 ± 0.00 a
N/DF	63.96 ± 3.54 b
DF/DF	67.50 ± 5.00 b
DF/N	60.42 ± 2.08 b

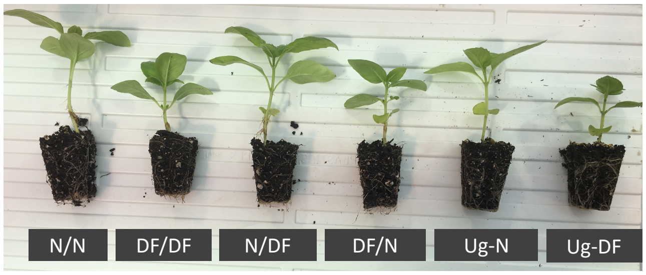

Figure 2. Image of successful graft combinations and un-grafted controls. Dried adventitious roots can be clearly observed for N/DF and N/N.

Growth parameters

Leaf number, stem length, and shoot mass were all significantly lower for ug-DF than for ug-N confirming the general classification of DF as less vigorous and N as vigorous (Table 3). Shoot fresh and dry mass were significantly lower for N/DF than ug-N 21 DAT in the first experiment. However, all shoot growth parameters (leaf number, side stem length, main stem length, fresh and dry shoot mass) as well as total mass and fresh shoot:root mass ratio were significantly lower for N/DF than either ug-N or N/N 21 DAT in the second experiment (Table 3). Root mass was not affected by treatment in the first experiment. However, at 21 DAT in the second experiment both fresh and dry root weight was significantly lower for DF/N than ug-DF (Table 3).

The reduction in yield (shoot mass) and other growth parameters observed when vigorous N scions were grafted to less vigorous DF rootstocks is similar to the common practice of using less vigorous scions to restrict shoot growth of vigorous cultivars of fruit trees in order to achieve higher planting densities. While the mechanisms of such growth reduction is still a matter of some debate, Foster et al. (2017) concluded that dwarfing rootstocks for apples effectively act as “supersinks” accumulating starch at the expense of shoot and root growth. While our results do show reduced scion (N) growth, ug-DF does not show a significantly reduced root mass when compared to ug-N but does exhibit a significantly lower yield. This indicates that instead of accumulating starch as reported in apples, assimilate devoted to DF roots may be used for additional root growth at the expense of shoot growth. It is also possible that DF rootstocks produce less of the shoot growth promoting cytokinins, leading to impaired shoot growth. The act of wounding itself may also affect assimilate allocation given that self-grafted DF plants in addition to inter-cultivar grafted DF exhibited lower root mass than ug-DF. This may be due to a physical restriction in the vascular system restricting flow of carbohydrates to the roots or a physiological response prioritizing shoot growth and reproduction.

Interestingly, while less vigorous DF rootstocks reduced growth the more vigorous N rootstocks did not significantly increase growth of DF scions. This finding contrasts with those of many other studies conducted on fruiting vegetables that report increased yields using vigorous rootstocks under ideal environmental conditions (Madeira et al., 2016; Masterson et al., 2016; Sabatino et al., 2016; Zhang et al., 2019). On the other hand, Traka-Mavrona et al. (2000) reported that vigorous *Cucurbita* rootstocks had no beneficial effect on melon yield, which was attributed to poor vascular connection between rootstocks and scions. Given the low graft success rate and significant reduction in total biomass for the interspecific grafts compared to un-grafted controls observed in our study, poor vascular connection may be one reason the expected increase in growth was not observed. While yield was not significantly increased for DF by grafting to N rootstocks, in the first experiment, DF/N exhibited a significantly higher ratio of fresh and dry shoot to root mass when compared to ug-DF 42 DAT (Table 3); in the second experiment harvests at 21 DAT produced root masses that were significantly smaller for DF/N than ug-DF (Table 3). This suggests that N rootstocks are more efficient at water and nutrient uptake and require less assimilate than DF and are capable of producing the same amount of shoot tissue with less root mass. The observed lack of yield promotion with vigorous rootstock (N) in this study is similar to a metanalysis that looked at 42 different tomato scions paired with the vigorous rootstock, ‘Maxifort’, and found yield increase to be highly variable depending on the scion cultivar used (Grieneisen et al., 2018). This indicates that there may be other scion mediated growth control factors, such as an insensitivity to increased nutrients from vigorous rootstocks influencing plant vigor and yield in grafted basil.

Table 3. Growth parameters (mean ± standard error) of treatments 21 and 42 DAT (experiment 1) and 21 and 35 DAT (experiment 2). Means within a column that share a letter are not statistically different (P ≤ 0.05)

	Harvest	Treatment	Leaf Number	Main Stem length (cm)	Side Shoot Length (cm)	Total Stem Length (cm)	Fresh Shoot Mass (g)	Shoot Dry Mass (g)	Root Fresh Mass (g)	Root Dry Mass (g)	Fresh S/R Ratio	Dry S/R Ratio	Total Fresh Mass (g)	Total Dry Mass (g)
Experiment 1	**21 DAT**	**ug-N**	28.67 ± 1.04 a	23.39 ± 0.59 a	10.60 ± 1.04 a	33.99 ± 1.27 ab	22.96 ± 0.41 a	1.57 ± 0.03 a	4.00 ± 0.24 ab	0.23 ± 0.01 a	5.97 ± 0.70 a	6.99 ± 1.06 a	26.96 ± 0.65 a	1.80 ± 0.04 a
		N/N	32.46 ± 1.72 a	22.60 ± 0.80 a	15.22 ± 2.18 a	37.83 ± 2.75 a	20.11 ± 0.95 a	1.39 ± 0.07 a	3.75 ± 0.19 a	0.23 ± 0.01 a	5.88 ± 0.90 a	6.88 ± 1.14 a	23.86 ± 1.14 a	1.62 ± 0.09 a
		N/DF	27.83 ± 1.33 a	21.06 ± 0.55 a	15.22 ± 2.19 a	32.83 ± 1.46 ab	17.96 ± 0.58 a	1.27 ± 0.05 a	3.66 ± 0.21 a	0.21 ± 0.01 a	5.20 ± 0.76 a	6.66 ± 1.37 a	21.62 ± 0.80 a	1.48 ± 0.06 a
		ug-DF	29.21 ± 1.09 a	16.47 ± 0.50 b	15.22 ± 2.20 a	30.54 ± 1.45 ab	12.45 ± 0.34 b	0.88 ± 0.03 b	3.66 ± 0.22 b	0.21 ± 0.01 a	3.72 ± 0.71 b	4.67 ± 1.15 b	16.04 ± 0.48 b	1.10 ± 0.04 b
		DF/DF	31.83 ± 1.51 a	15.48 ± 0.51 b	15.22 ± 2.21 a	29.64 ± 1.98 ab	11.38 ± 0.48 c	0.82 ± 0.04 c	3.66 ± 0.23 ab	0.18 ± 0.01 a	4.10 ± 0.38 b	4.80 ± 0.61 b	14.42 ± 0.62 b	1.00 ± 0.05 b
		DF/N	32.79 ± 1.67 a	15.32 ± 0.45 b	15.22 ± 2.22 a	31.40 ± 1.69 b	11.63 ± 0.49 c	0.81 ± 0.04 bc	3.66 ± 0.24 ab	0.18 ± 0.01 a	4.06 ± 0.63 b	4.73 ± 0.64 b	14.58 ± 0.71 b	0.99 ± 0.05 b
	42 DAT	**ug-DF**	34.08 ± 4.24 a	4.33 ± .090 a	15.22 ± 2.23 a	22.32 ± 3.91 a	13.41 ± 1.69 a	1.09 ± 0.16 a	3.66 ± 0.25 a	0.32 ± 0.03 a	2.30 ± 0.20 a	3.24 ± 0.24 a	19.09 ± 1.96 a	1.41 ± 0.19 a
		DF/DF	35.67 ± 4.09 a	2.79 ± 0.20 a	15.22 ± 2.24 a	23.22 ± 3.60 a	13.39 ± 1.54 a	1.09 ± 0.15 a	3.66 ± 0.26 a	0.28 ± 0.02 a	2.66 ± 0.22 ab	3.76 ± 0.32 a	18.29 ± 1.80 a	1.37 ± 0.16 a
		DF/N	46.75 ± 3.25 a	3.67 ± 0.09 a	15.22 ± 2.25 a	28.49 ± 2.03 a	17.35 ± 0.88 a	1.40 ± 0.08 a	3.66 ± 0.27 a	0.35 ± 0.01 a	3.42 ± 0.21 b	4.03 ± 0.16 a	22.52 ± 0.96 a	1.75 ± 0.09 a
Experiment 2	**21 DAT**	**ug-N**	34.38 ± 2.12 a	22.69 ± 0.62 a	18.77 ± 1.50 a	41.45 ± 1.73 a	24.79 ± 0.56 a	2.34 ± 0.06 a	6.09 ± 0.29 a	0.48 ± 0.02 a	4.69 ± 0.33 a	5.73 ± 0.41 ab	30.88 ± 0.63 a	2.82 ± 0.06 a
		N/N	37.46 ± 2.40 a	22.73 ± 0.42 a	22.52 ± 1.10 a	45.00 ± 1.41 a	24.61 ± 0.54 a	2.27 ± 0.04 a	5.84 ± 0.33 a	0.41 ± 0.03 ab	4.70 ± 0.27 a	6.21 ± 0.31 a	30.88 ± 0.64 a	2.68 ± 0.06 a
		N/DF	28.96 ± 1.91 b	19.33 ± 0.88 b	22.52 ± 1.11 b	30.18 ± 1.63 b	18.42 ± 0.88 b	1.61 ± 0.08 b	5.94 ± 0.28 a	0.35 ± 0.02 b	3.34 ± 0.14 b	5.06 ± 0.27 b	30.88 ± 0.65 b	1.95 ± 0.09 b
		ug-DF	25.58 ± 0.89 b	14.00 ± 0.56 c	22.52 ± 1.12 b	25.71 ± 1.06 bc	15.33 ± 0.71 c	1.21 ± 0.05 c	8.42 ± 0.31 b	0.45 ± 0.02 a	2.13 ± 0.10 c	3.18 ± 0.14 c	30.88 ± 0.66 b	1.95 ± 0.10 b
		DF/DF	24.08 ± 0.52 b	13.35 ± 0.38 c	22.52 ± 1.13 b	24.08 ± 1.65 bc	12.20 ± 0.40 c	0.96 ± 0.04 c	7.16 ± 0.28 ab	0.36 ± 0.02 b	2.12 ± 0.11 c	3.40 ± 0.15 c	30.88 ± 0.67 c	1.95 ± 0.11 bc
		DF/N	23.33 ± 0.90 b	13.36 ± 0.29 c	22.52 ± 1.14 b	22.48 ± 1.38 c	12.77 ± 0.61 c	0.99 ± 0.05 c	5.46 ± 0.28 a	0.31 ± 0.02 b	2.75 ± 0.20 bc	3.82 ± 0.20 c	30.88 ± 0.68 c	1.95 ± 0.12 c
	35 DAT	**ug-N**	40.00 ± 2.98 ab	4.53 ± 0.13 ac	32.98 ± 3.21 a	37.51 ± 3.20 a	31.00 ± 2.01 a	3.21 ± 0.31 a	8.73 ± 0.31 ab	0.74 ± 0.07 a	3.63 ± 0.21 a	4.56 ± 0.39 a	39.74 ± 2.12 a	3.95 ± 0.35 a
		N/N	35.67 ± 3.12 a	4.83 ± 0.09 a	28.38 ± 4.83 a	33.22 ± 4.89 a	25.22 ± 3.28 ab	2.79 ± 0.39 ab	7.90 ± 0.70 a	0.70 ± 0.06 a	3.09 ± 0.29 ab	3.77 ± 0.43 ab	33.12 ± 3.86 ab	3.49 ± 0.20 a
		N/DF	35.67 ± 3.13 a	4.54 ± 0.14 ac	28.38 ± 4.84 a	32.62 ± 2.83 a	29.35 ± 1.39 a	2.87 ± 0.17 a	8.16 ± 0.39 ab	0.60 ± 0.04 a	3.66 ± 0.18 a	4.89 ± 0.20 a	37.51 ± 1.60 ab	3.47 ± 0.20 a
		ug-DF	35.67 ± 3.14 b	3.59 ± 0.09 b	28.38 ± 4.85 a	36.88 ± 2.37 a	26 ± 1.33 ab	2.47 ± 0.14 ab	10.30 ± 0.75 b	0.75 ± 0.05 a	2.66 ± 0.18 bc	3.34 ± 0.07 b	36.88 ± 1.79 ab	3.22 ± 0.19 a
		DF/DF	35.67 ± 3.15 a	3.32 ± 0.11 b	28.38 ± 4.86 a	30.38 ± 1.33 a	20.79 ± 0.64 b	1.85 ± 0.07 b	9.78 ± 0.47 ab	0.57 ± 0.04 a	2.10 ± 0.06 c	3.21 ± 0.12 b	30.57 ± 1.05 b	2.42 ± 0.10 a
		DF/N	35.67 ± 3.16 ab	4.30 ± 0.10 c	28.38 ± 4.87 a	36.74 ± 3.62 a	25.91 ± 1.72 ab	2.30 ± 0.16 ab	8.55 ± 0.54 ab	0.60 ± 0.04 a	3.07 ± 0.19 ab	3.86 ± 0.13 ab	34.46 ± 2.08 ab	2.89 ± 0.20 a

Table 4. Nutrient concentration (mean ± standard error) 42 DAT in the first experiment. Means within a column that share a letter are not statistically different (P ≤ 0.05)

Treatment	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**S (ppm)**	**Fe (ppm)**	**Mn (ppm)**	**B (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**Mo (ppm)**
Ug-DF	5.2 ±.03 a	0.86 ±.02 a	5.2 ±.09 a	3.1 ±.3 a	0.8 ±.02 a	4137.5 ±61.50 a	105.9 ±.65 a	78.0 ±7.40 a	59.5 ±.57 a	16.1 ±1.075 a	121.6 ±5.57 a	0.2 ±.115 a
DF/DF	5.0 ±.02 a	0.86 ±.02 a	5.1 ±.04 a	3.0 ±.03 a	0.8 ±.01 a	3983.0 ±158.0 a	107.3 ±.15 a	47.4 ±6.34 a	53.2 ±.08 b	15.4 ±.45 a	115.3 ±2.23 a	0.3±.01 a
DF/N	5.4 ±.03 b	0.8 ±.01 a	5.2 ±.05 a	3.0 ±.06 a	0.7 ±0.0 b	3615.5 ±57.50 a	99.1 ±1.62 a	64.4 ±.35 a	46.8 ±.98 c	12.5 ±.11 a	95.5 ±1.69 b	0.3±.11 a

Nutrient Content

Only nitrogen, magnesium, boron and zinc were significantly affected by grafting. Content of magnesium and zinc were significantly lower in DF/N than ug-DF and DF/DF while boron was significantly reduced by both self- (DF/DF) and inter-cultivar (DF/N) grafting (Table 4). Suggesting that physical restriction at the graft union, possibly due to poor grafting technique, may have lower conductivity of water and nutrients transported by mass flow. Nitrogen content in the leaves of DF/N was significantly higher than ug-DF and DF/DF (Table 4). This suggests that 'Nufar' rootstocks may exhibit greater nitrogen uptake. This is similar to many studies which have found improved nutrient uptake in grafted plants with vigorous rootstocks such as 'Beaufort' in tomato (Leonardi and Giuffrida, 2006) and *Cucurbita* spp. for watermelon (Colla et al., 2010; Colla et al., 2011; San Bautista et al., 2011). Contrary to other studies that have cited increased yields in fruit yield and shoot growth as a result of enhanced nutrient uptake (Colla et al., 2010; Colla et al., 2011; Leonardi and Giuffrida, 2006) 'Dolce Fresca' yield was not increased by grafting, despite higher levels of nitrogen in scion tissue. This could be due to the relatively low fertility requirement of basil when compared to previously studied fruiting crops. Basil insensitivity to nutrient concentration was documented by Walters et al., 2018 who found increasing nutrient solution EC has a significant effect on nutrient content in basil tissue but no significant effect on basil growth. Similarly, Gillespie et al. (2020) observed that reduction in nutrient solution pH reduced uptake of phosphorus, calcium, magnesium, sulfur, boron, manganese, and zinc but had no significant effect on the growth of the two basil cultivars used in this study. This indicates that grafted basil is unlikely to exhibit the same level of yield increases observed in plants sensitive to nutrient levels as a result of increased nutrient uptake.

Conclusion

The results of this study indicate that inter-cultivar grafting can have significant effect on plant yield and nutrient content in sweet basil; principally, that low vigor DF rootstocks reduce the growth of N scions. However, additional research is required to determine the specific mechanisms affecting graft union formation, and the effects of grafting on vigor and nutrient transport in sweet basil.

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