8-2016

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WINTER GREENHOUSE PRODUCTION AND TISSUE CULTURE OF BASIL (*OCIMUM* SPP.)

By

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Horticulture

Under the Supervision of Professor Ellen T. Paparozzi

Lincoln, Nebraska

August, 2016

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University of Nebraska, 2016

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Basil (*Ocimum* spp.) is a popular annual culinary and medicinal herb contains essential oils. Numerous researchers have studied the effects of fertilizer level on the growth and oil yield of basil, but in most studies, basil was grown in the field during the summer. Our experiment was conducted in a controlled environment greenhouse using a capillary mat system during the winter with a goal of increasing dry weight for fresh produce and basil essential oil. The 7-month experiment was conducted to determine the optimal production timeline and fertilizer levels for eight basil cultivars grown in a soilless mix. We found that for most sweet basil cultivars (*Ocimum basilicum*), later harvest led to higher dry weight yield. In most cases, the additional supplement of slow release fertilizer (12N-3.1P-14.9K: 6g and 9g) increased the dry weight yield compared to the control (100 ppm N from 20N-4.4P-16.6K soluble fertilizer only), but there was no significant difference between the 2 levels. 'Mrs. Burns' Lemon' (*Ocimum basilicum*) had the largest flower dry weight. 'Holy' basil (*Ocimum sanctum*) didn't grow well in the capillary mat system, so tissue culture was thought to be a suitable production method. In this study, 3 media [Murashige and Skoog (MS) medium, Woody Plant medium (WPM), and DKW medium], and 4 treatments of plant growth regulators [the control, 0.1 mg indole-3-butyric acid (IBA)/L, 50 μM thidiazuron (TDZ), 0.1 mg IBA/L + 50 μM TDZ],

were compared to see whether they could be used to grow ‘Holy’ plants successfully. ‘Dolly’, a classic sweet basil cultivar was also tested to see if there was a different effect between basil species. We found that both ‘Holy’ and ‘Dolly’ explants cultured on DKW medium didn’t survive, and the explants cultured on MS medium grew better and had higher fresh and dry weight and heights than those grown on WPM. The explants supplied with TDZ didn’t grow well and had low survival rate while those supplied with nothing or IBA alone grew well.

Acknowledgements

I wish to express my sincere gratitude to Dr. Paparozzi, who provide me the chance to study abroad, and gave me the guidance and encouragement to carry out this project.

I sincerely thank my committee members Dr. Read and Dr. Ciftci for their kind suggestions and criticism.

I would also like to thank Elizabeth Conley and Benjamin Loseke, who trained me and taught me a lot in the past two years.

I'm also grateful to Alison Butterfield, Melinda Knuth and Clayton Osborn, we had a great time in greenhouse when working together.

I would like to thank all my teachers who had encouraged me and taught me knowledge and methods in the past two years.

Finally, I would like to express my deep appreciation to my parents and all my friends for their constant support and love.

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Literature Review

How basil is grown in the field

Ramezani et al. 2009, investigated the effects of different levels of phosphorus sprays on growth, yield and essential oil quality of basil (*Ocimum basilicum* L). The experiment was carried out in the southwest region of Iran, and the soil type was a clay-silt-loam with pH of 7.6. Basil seeds were sown by hand on April 24 and were provided with different levels of phosphorus spray. Zero, 2, 4, 6, 8, 10% spray treatments were applied at two stages: first, when 20% of the plants were in flower (54 d after sowing) and then later when 60% were in flower (12 d before harvesting). The plants were harvested 80 days after sowing. A sharp knife was used to cut the plant, leaving about 5cm above the ground surface. Harvested plants were dried at room temperature (less than 25℃) for 14 days. The dried leaves and flowers were put into a glass Clevenger-type apparatus for hydrodistillation and distilled for 3 hours.

The authors concluded that application of phosphorus improved the yield of essential oils. Two percent spray treatment was better than 0%, but the same as 4, 6, 8, 10% treatments.

Research by Daneshian et al. 2009, was carried out at the experimental area of Ankara University, Turkey. The aim of this research was to find out the effects of different nitrogen levels on components of essential oils from basil (*Ocimum basilicum* L). The soil of experimental plots consisted primarily of clay, silt and sand with pH of 8.06. Basil seeds were sown in the greenhouse on April 2-3 and the seedlings were transplanted to the experimental field on 24 May. Three levels of nitrogen (50, 100 and

150 kg ha^{-1}) were applied to the plants, and a control with 0 kg ha^{-1} was also set. Applications were carried out on 20 June and 7 August.Two cuttings were made on 23 July and 1 September, respectively when the plants started to flower. The cut plant parts were dried in the shade at room temperature. The dried leaves and stems were ground and then using a Clevenger-type apparatus distilled for 3 hours. Fifty g of sample needed 500mL water for hydrodistillation.

The authors concluded that the contents of essential oils in different nitrogen treatment were similar, but the proportions of major components (linalool and naphthalene) were different. The control and 150 kg N ha^{-1} had the highest average linalool content.

Research by Anwar et al. 2004 was carried out in India, and their objective was to study the effect of organic and inorganic fertilizers on growth of basil and quality of essential oils. The soil was sandy loam with pH of 8.38. The authors chose two organic manures: farm yard manures (FYM) and vermicompost. There were 6 treatments: control; FYM (10 t ha^{-1}); vermicompost (10 t ha^{-1}); inorganic fertilizer NPK (100:50:50 kg ha^{-1}); FYM (5 t ha^{-1}) + fertilizer NPK (50:25:25 kg ha^{-1}); vermicompost (5 t ha^{-1}) + fertilizer NPK (50:25:25 kg ha^{-1}). The seedlings were transplanted 45 d after sowing (mid-June). The plants were harvested at ground level at the end of October and essential oils were extracted by hydro-distillation.

The authors concluded that compared to the control, all the treatment with fertilizer had higher essential oil yield, and vermicompost +NPK had the highest oil

yield, followed by FYM+ NPK. The essential oils methyl chavicol and linalool were also higher with nutrient addition.

Majkowska-Gadomska et al. 2013 reported the effect of seedling planting time on concentrations of mineral compounds in basil (*Ocimum basilicum* L.) leaves. They grew 3 cultivars (sweet basil, purple basil and cinnamon basil). Seeds were sown in pots filled with highmoor peat (N-NO_3-100, P-80, K-215, Ca-1240, Mg-121 g dm^{-3}, pH5.9) on March 30 and April 14 each year (2010 and 2011). The seedlings were transplanted into the field (N-NO_3-11, N-NH_4-2, P-207, K-183, Ca-1070, Mg-77 mg dm^{-3}, pH 7.5) on May 19 and June 2. No fertilizer was applied during the season. Leaves were harvested while flowering at the end of July each year. The plants were cut 10 cm above the ground. Leaves were collected and then dried at 35℃ and then ground. The concentrations of macroelements (total nitrogen, phosphorus, potassium, magnesium, calcium, and sodium) and microelements (iron and copper) in basil leaves were determined.

The authors concluded that basil type determined the contents of macroelements (total nitrogen, phosphorus, potassium, magnesium, calcium, and sodium) in basil leaves, and sweet basil contained the highest concentrations of potassium and sodium. The planting time had an effect on the iron content as seedlings planted on May 19 had higher iron content.

Sifola and Barbieri 2006 investigated the effect of different levels of nitrogen fertilizer on fresh weight and essential oil content of three cultivars of basil (*Ocimum basilicum* L) in the Mediterranean area. Three cultivars of basil (‘Mostruoso mammouth’ (MM), ‘Genoveses profumatissimo’ (GP), ‘Napoletano a foglia di lattuga’ (NFL)) were grown in a clay-loam soil (42% sand, 27% silt, 31% clay, 7.3 pH). Two levels of nitrogen

were applied: 0 (control), 100 and 300 kg N ha^{-1} before transplanting. Seedlings were transplanted to the field on May 25, and then the plants were drip-irrigated every 3 days. The plants were harvested 49 days after transplanting (July 13) when the plants reached full bloom. The leaves and stems of plants were dried at 60℃. Dry weight and ratio of leaf and stem were measured. Essential oils were extracted from 300g fresh leaf samples by hydrodistillation for 135 min.

The authors concluded that nitrogen fertilization up to 300 kg N ha^{-1} improved yield of leaf fresh biomass, essential oil yield and oil concentration in leaf. However, it didn't influence the leaf-to-stem ratio, plant height and the number of branches per plant. The components of essential oil depended on the cultivar. MM and GP were rich in linalool and eugenol, while NFL was the richest in methyl chavicol.

Zheljazkov et al. 2008, studied the effect of sequential harvesting on essential oil content, composition and bioactivity of three basil genotypes (*O. basilicum* L. sweet basil: cvs. German and Mesten, *O. sanctum* L. holy basil: cv. Local). In March, the seedlings were produced in a greenhouse in 48-cell plastic trays with growth medium Metromix 300. The temperature in the greenhouse was 22-25℃ day and 18℃ night. The seedlings were irrigated once every day and fertilized every week with 1.8 g of 20-20-20 N-P_2O_5-K_2O dissolved in 300 ml of water.

The plants were transplanted to the field in May (40 days after sowing). The soil type of the field was Quitman sandy loam with 57% silt, 37% sand, 5% clay, 1.05% organic matter, pH of 6.5. The plants were irrigated and fertilized every week by subsurface drip tape. The totals of fertilizer for the whole growing season were 120, 80 and 100 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. The basil plants were harvested three

times and the dates were June 9, July 26 and October 22, each time when the plants reached full bloom. The plants were cut at 15 cm above the ground by hand and weighed for fresh weight. To get the dry weight, the plants were dried at 40℃.

Essential oil of basil was extracted by steam distillation, and the size of sample was 150g, including stems, leaves and flowers. The distillation time was 120 min. GC/MS was used to analyze the oil.

The authors found that the essential oil content of cv. German, cv. Mesten and cv. Local ranged from 0.40 to 0.75%, 0.50 to 0.72%, 0.17 to 0.50%, respectively. For sweet basil, the yields of herbage as well as essential oil increased in the second and improved again in the third harvest; for holy basil, the yield of herbage and essential oil increased in the third harvest. The major components of essential oil from sweet basil were linalool (30-40%) and eugenol (8-30%), but for the oil extracted from holy basil, the main constituent were eugenol (8-43%) and methylchavicol (15-27%).

Kandil et al. 2009 investigated the effect of source of nutrients (mineral or organic) and fertilization practices on 'Genovese' basil (*Ocimum basilicum* L.) growth and quality of essential oils. Two experiments were carried out during two successive seasons (2003 and 2004). 'Genovese' seeds were sown on March 9th, and after 45 d the seedlings were transplanted into the field. The soil type was a clay loam with pH of 7.25. The plants were cut twice during each growing season. The first cut was done during the first week of July and the second cut was done in the middle of October. The objective of the first experiment was to investigate the effect of fertilizer rate on growth, yield, oil content and quality of 'Genovese', and the treatments were control, 25%, 50%, 75%, and 100% of the recommended dose of fertilization: 238 kg ammonium nitrate/ha, 128 kg P

as super-phosphate/ha, and 114 kg K as potassium sulfate/ha. The second experiment investigated the effect of chemical and organic fertilizer on the same parameters. The treatments were 25% N as compost and 75% N as ammonium nitrate, 50% N as compost and 50% N as ammonium nitrate, 75% N as compost and 25% N as ammonium nitrate, 100% N as compost, and 100% N as ammonium nitrate**.** Fresh samples (100g) were distilled to obtain essential oils for 3 h using a cleavenger-type apparatus.

The authors concluded that increase of NPK rate improved the growth and yield of Genovese basil, but 50% of the recommended NPK gave 80% of the yield as compared to 100% recommended NPK. Organic fertilizer gave similar yields but higher quality (low proportion of estragole) than chemical NPK alone.

Research by Arabaci and Bayram 2004 investigated the effect of plant densities and nitrogen fertilization on agronomic and technologic characteristics of basil (*Ocimum basilicum* L.). The climate condition where the experiment was conducted was mild Mediterranean. Soil type was sandy-loam. The experiment was a split-plot design. Treatments were carried out with or without 50 kg/ha nitrogen in the main plot, and the subplots consisted of three different plant densities (20×20, 20×40, and 20×60 cm). Seeds were sown in January 2000, December 2001, and January 2002. After about 4 months, the seedlings were transplanted to the field. The plants were harvested at full flower stage for green herb yield, dry herb yields (dried at 35 ℃ for 72h), dry leaf yield, essential oil ratio (20g sample with 200 ml was distilled for 45 min using a neo-clevenger apparatus), essential oil yield, and composition of essential oil (GC was used).

The authors found that the 20×20 cm plant density with nitrogen fertilizer had the highest amount of green herb yield (4197.5 kg/ha), dry herb yields (1078.6 kg/ha), dry

leaf yield (671.1 kg/ha), and essential oil yield (5.164 L/ha). However, 20×20 cm plant density without nitrogen fertilizer had the highest essential oil rate.

Research by Zheljazkov et al. 2008 investigated yield and composition of 38 sweet basil (*Ocimum basilcum* L.) accessions grown in Mississippi. Seeds were sown in Metromix 300 growth medium in March, and placed in a greenhouse with a day temperature of 22-25℃ and 18℃ night. After germination, the seedlings were supplied with 20-20-20 NPK water soluble fertilizer weekly (1.8 g per 300 ml). In the experimental site, soil was sandy loam with 1.15% organic matter, 6% clay, 55% silt and 38% sand, pH 6.4. Raised beds were prepared and covered with black plastic mulch. After 45 d, the seedlings were transplanted into the holes in the black plastic. The plants were fertigated by drip irrigation tape. During the whole growing season, 120, 80, and 100 kg/ha N, P_2O_5 and K_2O from 20-20-20 was provided. The plants were harvested at full bloom and dried at 40 ℃ for dry weight. Dried samples (150 g) were distilled for 90 min using steam distillation to extract essential oils. GC-MS was used to identify the essential oil component.

The authors concluded that basil genotypes differed significantly in oil content (0.07-1.92%) and composition. The 38 accessions were divided into seven groups according to different major components such as linalool, linalool-eugenol, methyl chavicol, and methyl chavicol-linalool. The various chemotypes provide the chance to meet market requirements of specific basil oils or important components such as linalool, methyl chavicol, and eugenol.

Zheljazkov et al. (2008) investigated productivity, oil content, and composition of sweet basil (*Ocimum basilicum* L.) and holy basil (*Ocimum sanctum* L.) grown at four

locations in Mississippi. Seeds were sown in Metromix 300 growth medium in March, and placed in a greenhouse with a day temperature of 22-25℃ and night of 18℃.The seedlings were supplied with 20-20-20 NPK water-soluble fertilizer weekly (1.8 g per 300 ml). Six weeks after sowing, the seedlings were transplanted to black plastic-covered raised beds. The plants were fertigated by drip irrigation tape (20-20-20) during the whole growing season (120, 80, and 100kg/ha N, P_2O_5 and K_2O). The plants were harvested 7 weeks after transplanting at full bloom stage by cutting at 10 cm aboveground. Both fresh and dry weights (dried at 40 ℃) were recorded. Samples (150 g) of above-ground parts (leaves, flowers, and stems) were distilled for 120 min. GC-MS was used to identify the essential oil components.

The authors concluded that the major component of sweet basil (cv. German and Mesten) was linalool while the major components of holy basil (cv. Local) were methyl chavicol, eugenol, and eucalyptol. The 3 cultivars grew well at the 4 locations, and the herbage and oil yield were comparable to the results that were reported in previous literature.

How basil is grown in greenhouses

Succop and Newman 2004 planted sweet basil in three types of media: rockwool slabs (RW), perlite frames (PL) and commercial sphagnum peat/perlite/compost (PPC) and compared two hydroponic fertilizer formulations: conventional fertilizer reagents and an organic fertilizer liquid mixture (consisted of organic fermented poultry compost). The objective of this study was to investigate whether organic fertilizer can be used for basil grown in greenhouse, and whether discernable taste difference between plants grown

organically and conventionally could be detected. Seeds were sown on 11 June 1996 and the plants were weekly harvested from 7 August 1996 to 18 November 1996.

The study showed that basil grown in a greenhouse with organic fertilizer can be used for fresh market production. Sixty-nine percent of the panelists in taste test could tell the differences between basil grown using organic and conventional fertilizer, but there was no preference.

The study by Putwattana et al. 2010 was aimed at investigating whether the use of organic and inorganic fertilizer has effect on Cd immobilization in soil and if it could diminish accumulation of Cd in basil. They used uncontaminated potting soil (51% sand, 31% silt, 18% clay) to plant sweet basil and added organic (cow manure) and inorganic (silicate fertilizer: SiO_2 70%, Al_2O_3 13.66, Fe_2O_3 1.26, P_2O_5 0.017, CaO 1.12, MgO 0.2, Na_2O 1.33, and other minor elements) additives. The plants were grown in plastic pots in a greenhouse (25-28℃, 12/12h photoperiod, 60% relative humidity) and were harvested monthly. The harvested plants were dried at 85℃ in the oven for 48h, and then weighed for biomass.

The results showed that silicate fertilizer reduces the movement of Cd from roots to shoots, as a result Cd content decreased in the edible parts of basil.

Vieira and Simon 2000 investigated the chemical characteristics of market-available basil used for medicine, and 14 accessions of *Ocimum* were studied. The seeds were sown in a disinfected soil mix upon germination. Five seedlings were transplanted into 8 L pots and watered to pot capacity every day. The temperatures of the greenhouse were 26-30℃ day and 18-21℃ night. Cuttings of *O. basilicum* were also transplanted to

the field. The plants were harvested at full bloom and were stored in paper bags. Then the samples were dried at 38℃ for 15 days for oil analysis. The dried samples were put into a 2L flask, using a Clevenger apparatus to extract essential oils. The ratio of sample and water is 1:15 w/v.

The authors concluded that *O. basilicum* showed high 1,8-cineole (22%-relative percentage of total volatile oil), high linalool (49.7%), high methyl chavicol (47%) or high methyl (E)-cinnamate (65.5%) in essential oils.

Drying methods

Carvalho Filho et al. 2006, investigated the effects of harvesting time, drying temperature and drying period on chemical composition of basil (*Ocimum basilicum* L.) essential oils. The seeds were sown in cells with a mixture of washed coconut dust, bovine manure and carbonized rice husk (1:1:1). The seedlings were transplanted to a plant bed in a greenhouse after 30 days. A drip irrigation system was used to water the plants. Two harvestings were carried out, and the first harvest was carried out 40 days after transplant (March 6, 2003) during full bloom. The plants were cut "at 20 cm height from the soil." The second harvest was performed 53 days after the first harvest using the same procedures as the first. The combination of three harvesting periods (8:00 am, 12:00 pm, and 4:00 pm) and three drying temperatures (40, 50, 60℃) were the treatments. Leaves and inflorescences were dried and subjected to hydrodistillation for 3h using a Clevenger apparatus. A 3-liter round bottomed flask with 1.5 L water was used as the container of each sample.

The authors found that basil harvested during morning (8:00-12:00) produced higher essential oils yield. To obtain higher concentration of linalool (86.8%), the samples should be dried for 5 days. Lower temperature (40 ℃) could reduce the loss of essential oil while drying.

Di Cesare et al. 2003 investigated the effect of different drying methods on the content of volatile compounds and chlorophyll pigments in basil leaves. The authors purchased basil from the market and separated leaves and stems. Three drying methods were used: microwave-drying, air-drying and freeze-drying. In microwave-drying, samples (18 g) were dried at 270W for 1 min, 440 w for 2 min, 650 w for 1 min and 1100 w for 1 min. In air-drying, raw samples (18 g) were boiled in water for 20 seconds, and then were put into an air circulating drier. The samples were dried for 4.5 hours at 50℃.In freeze-drying, the authors used a freeze-dryer to dry raw leaves. It took 48 hours for blanched and 72 hours for raw leaves.

The author concluded that microwave-drying preserved more volatile compounds (eucalyptol, linalool, eugenol and methyl eugenol) as well as chlorophyll pigments than traditional methods. However, microwave-drying is not optimal for commercial production because only small samples can be dried in a microwave.

Tissue culture of Basil (Ocimum* spp.*)

Phippen and Simon (2000) reported shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.). They collected explant tissue including leaves at different development stages, cotyledons, petioles and stems. The plant tissue was surface-sterilized for 20 min in 20% bleach. The midrib section of the leaves was cut into

four 5*5 mm pieces and then placed abaxial down on the medium which was callus- and shoot-introduction. The medium contained Murashige and Skoog salts 4.33g/L, 100 mg myo-inositol/L, 0.4 mg thiamine/L, 30g sucrose/L, 7.5g bacteriological grade agar/L and different levels of thidiazuron (TDZ) (0-84 μM) (pH 5.8). The explants were maintained in darkness at 26℃ for two weeks. Then the explants were transferred to fresh new media and maintained in the same environment for another two weeks after callus formation. Isolated shoots (6-10mm) were cultured on MS medium without plant growth regulators for two weeks at 26℃ in darkness for shoot elongation and root initiation. After that the explants were transferred into fresh media and growing under 16h photoperiod of 25 μ mol/m^2*s at 27±1℃ for two weeks. When the plantlets formed a well-developed root system, they were transferred to soil.

The authors reported an efficient regeneration protocol for basil. MS basal medium with 16.8 μM TDZ was used to induce callus and shoots, and after 14 days the medium was refreshed. Two weeks later, the explants were transplanted to plant growth regulator-free medium for rooting. Within 20 days the plantlets were transplanted to potting soil and grew under greenhouse condition. Also, explants taken from the first true leaves have the highest morphogenesis response (largest number of explants and mean number of shoots per explant).

Begum et al. 2002 conducted an experiment to establish a suitable regeneration protocol for *O. basilicum* L. growing in Bangladesh using tissue culture. Nodal and shoot tip segments from mature plants were collected and surface-sterilized, and then cut into 1.0-1.5 cm long segments (each containing a single node). The explants were cultured on half-strength MS medium with different levels (0.1-2.0 mg/L) of 6-benzylaminopurine

(BAP) or kinetin (KN). For regeneration of complete plantlets, the shoots were cultured on half strength medium containing NAA, IBA or IAA either singly or in combination to promote rooting (pH 5.7±0.1).

The authors found that when the explants were cultured on half strength of MS medium supplemented with BAP and KN, the proliferation efficiency of nodal explants was higher than that of shoot tips. In addition, the highest frequency of healthy rooting was on MS medium with 0.1 mg/l NAA. If IBA was needed, 0.1 mg/l was best.

Bodhipadma et al. (2005) investigated whether or not tissue culture was a rapid and economic way to propagate holy basil (*Ocimum sanctum* L. cv. Dang) and produce essential oil, especially eugenol, from it. MS basal medium was used for the cultures. The medium was adjusted to pH 5.7, gelled with 0.8% (w/v) agar and autoclaved at 121℃ and 15 *psi* for 20 min. All the cultures were put under 16 h illumination from white fluorescent lamps (33,500 Lux), and the temperature of the room was 25℃. Mature dry seeds of holy basil were soaked in 1.5% (v/v) sodium hypochlorite to disinfect. After that, the seeds were rinsed 3 times by sterile distilled water. Then the seeds were placed on MS hormone-free medium and grown for 4 weeks.

The authors also tried callus induction and nodal culture. They used leaves from seedlings for callus induction and added different levels of 2, 4-D (0, 0.5, 1 and 2 mg/L) to the medium. As for nodal culture, the same procedure was carried out. The cultures were kept under the light for 8 weeks. The callus started to grow rapidly after 2 weeks, and shoots and roots in nodal culture also increased from week 2.

The plants were separated into roots, leaves, flowers and callus, and those plant materials were separately ground with pure methanol (2:1 mg/ml) using mortar and pestle. After that, the mixture was shaken at 250 rpm for 12h and then centrifuged at 14,000 rpm for 30 min. Syringe filters were used to filter the mixtures. For roots, leaves and flowers gas-chromatography was used to analyze the solvent extracts.

The authors found that, compared to the eugenol extracted from natural roots, leaves and flowers, callus from leaves of seed-germinated plantlets as well as leaves of nodal plantlets would also produce eugenol of good quality. The authors concluded that tissue culture could be an alternative method to propagate holy basil rapidly and economically. Also, callus from leaves of seed-germinated and nodal plantlets were appropriate for eugenol production. However, the authors didn't mention the quantity of eugenol produced from a tissue culture plant.

Hakkim et al. 2007 compared chemical composition and antioxidant property between plant parts (leaves, stems and inflorescences) and induced callus cultures of holy basil (*Ocimum sanctum* L.). Explants included leaves, stems and inflorescence. These parts were cut from 1-year-old holy basil plants and surface-sterilized for 3-5 min in Tween 80 and for 3 min in 0.1% mercuric chloride, and rinsed in 3 times in sterilized distilled water. The explants were cut to proper length (1 cm) and cultured on MS medium with 3.0% sucrose and 0.8% agar. Different levels of kinetin (0.1-0.5 mg/L) combined with 2, 4-D (1 mg/L) were also added separately to the medium. The temperature remained at 25±2 ℃ and the light was 1000-2000 lx for 12 hr per day. The explants were cultured for 8 weeks. Callus was also harvested and dried for extraction. The callus (1 g) was soaked in 80% methanol (10 ml) for 3 hr. Then the samples were

sonicated in an Ultrasonic Sonifier at 20 pulses for 20 min and the extract was centrifuged at 10000 rpm for 10 min.

The authors also collected field-grown basil samples to compare the chemical compositions of extracts to that from callus cultures. Leaves, stems and inflorescences were harvested and dried in shade for 7 days. Then the dried samples were powdered and 10 grams of sample were extracted twice using 500 ml methanol.

The authors found that the leaf explants showed maximum (99.1%) culture response (percentage of callus induction) when supplied with the combination of 2, 4-D (1 mg/L) and kinetin at 0.1 mg/L, while inflorescence and stem explants showed maximum (94.6% and 96.1%, respectively) culture response when supplied with the combination of 2, 4-D at 1 mg/L and kinetin at 0.2 mg/L. Rosmarinic acid extracted from in vitro callus cultures was more than those from field-grown plants.

Siddique and Anis 2007 studied the use of thidiazuron (TDZ) for *in vitro* propagation of basil (*Ocimum basilicum*). Shoot tips were excised from 2-year-old plants and then disinfected. The explants were cut into appropriate size (0.5-0.8cm) and cultured on Murashige and Skoog medium (pH 5.8) with various concentrations of TDZ (5, 25, 50, 75, 100 μ M). The explants were maintained at 24±2℃ under 16-h photoperiod with a photosynthetic photon flux density of 50 μ mol/m^2s. After 4, 8, 12, and 16 d, shoot tip explants were cultured in 50 cm^3 liquid MS media supplied with TDZ (5-100 μ M) and the control (no TDZ) for different treatment durations. All the explants were transplanted to fresh medium every 2 weeks. After 8 weeks' culture, the percentage of explants forming shoot, number of differentiated shoots and shoot length per plant were recorded.

For root induction, shoots with four or more leaves were excised and transferred to MS medium with different concentrations of IBA (0.5, 1.0, 1.5, 2.0, 5.0 μM). Root length, number and percentage of rooting were recorded after 4 weeks. Plantlets with well-developed shoot and roots were transferred to pots under 16-h photoperiod.

The authors found that the optimal level of TDZ supplementation to the medium for basil was 50 μM for 8-day induction period because it produced maximum regeneration frequency (78%), average shoot number (11.6 ±1.16) and length (4.8 ±0.43 cm). For root induction, the medium containing 1.0 μM IBA performed best.

Singh and Sehgal 1999 reported *in vitro* micropropagation of holy basil (*Ocimum sanctum* L.) from young inflorescences of mature plants. Young inflorescences (0.6-1.2 cm) were excised from a single mother plant and surface-sterilized in 0.2% aqueous mercuric chloride solution (with 5 drops of Tween-20 per 100ml solution) for 6-9 min. After repeatedly washing in sterile distilled water, the explants were cultured on Murashige and Skoog medium with pH 5.8 (added 3.0% sucrose and 0.8% agar). Different levels of plant growth regulators including 2, 4-D, IAA, BAP and TDZ were added to the medium. The cultures were maintained at 25±2 ℃ under continuous light (10000-2000 lux). Percentage of cultures responding, shoot and root numbers and length, and degree of response per culture were recorded every week. For rooting, shoots longer than 2.0 cm were transferred to MS alone. Percentage and number rooting were recorded after 3-4 weeks, and the plantlets were transplanted to autoclaved vermiculite mixed with disinfected garden soil (1:1).

The authors concluded that MS medium supplied with 2, 4-D and TDZ only produced non-morphogenetic callus. Shoots differentiated in 2-3 weeks when cultured on MS medium with BAP, and the rate of 1.0 mg/L produced the maximum number of shoots. However, when 1.0 mg/L BAP was combined with 0.05mg/L IAA, shoot number increased significantly.

Dode et al. 2003 reported in vitro propagation of basil (*Ocimum basilicum* L.) from cotyledons. Seeds were surfaced sterilized and placed on Murashige and Skoog medium containing 3% sucrose, 100 mg myo-inositol/L, 0.7% agar, at pH 5.8 for germination. After 12 days, cotyledonary leaves grew andwere used as explant tissue and cultured on the same medium with different levels of plant growth regulators. The explants were maintained at 28 ±1℃ under 16h photoperiod. Treatments were 0, 1, 2, 3, 4, and 5 mg BAP /L combined with 0.2 mg NAA /L. After 45d, regenerated shoots (1.5 cm) were removed to ½ plant growth regulator-free MS medium and cultured for 45 d, and finally transplanted to soil.

The authors found that the combination of 5 mg BAP /L and 0.2 mg NAA /L had the highest efficiency of shoot formation. They concluded that higher BAP concentrations induced an increase in shoot number and the percentage of explants with shoots. NAA inhibited root formation when combined with different levels of cytokinin (BAP 1-5mg/L), but the combinations improved callus formation.

Sahoo et al. 1997 reported a protocol for *in vitro* propagation of sweet basil (*Ocimum basilicum* L.) through nodal explants. Nodal explants were collected from 5-6 month-old *O. basilicum* plants. After surface sterilizing and rinse, the explants were cultured on Murashige and Skoog medium containing 3% sucrose, 0.8% agar, and 100

mg myo-inositol/L with different levels of plant growth regulators (pH 5.8). The treatments were: 0.1-2.0 mg BA /L, 0.1-0.8 mg GA_3/L either individually or in combinations. The cultures were maintained at 25 ±1℃ under 16h photoperiod of 35 μmol $m^{-2}s^{-1}$ irradiance. For rooting, shoots (3-4 cm) with 5-6 fully expanded leaves were transferred to 1/2 MS medium with IAA, IBA or NAA at 0.5-1.0 mg/L. When the plantlets were well-rooted and had 8-10 fully expanded leaves, they were moved into pots with autoclaved vermi-compost and maintained at 26 ±1℃ under 16h photoperiod for 3 weeks. The plants were then transplanted to pots with garden soil and kept under shade for 2 weeks before being moved to outdoors.

The authors found that a higher concentration of BA (1.0 mg/L) improved bud break while a lower concentration of BA (0.25mg/L) was suitable for further growth. When combined with GA_3 at 0.4 mg/L, medium with 1.0 mg BA /L enhanced the frequency of bud break. As for rooting induction, 1.0 mg IBA /L gave the best percentage rooting (93.0±1.27%), root number (9.7±0.15), and root length (5.6±0.15 cm).

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EFFECT OF FERTILIZER LEVEL AND HARVEST TIME ON WINTER GREENHOUSE PRODUCTION OF BASIL (*OCIMUM* SPP.) FOR ESSENTIAL OILS

Key words: production timeline, chemical fertilizer, soilless mix, capillary mat system

Abstract. Basil (*Ocimum* spp.) is a popular annual culinary and medicinal herb whose essential oils contain important compounds such as linalool, eugenol, and methyl chavicol. Numerous researchers have studied the effects of fertilizer level on the growth, yield and oil yield of basil, but in most studies, basil was grown in the field (soil) during the summer. Our research was conducted in a controlled environment greenhouse using a capillary mat system during the winter season with a goal of increasing dry weight for fresh produce and basil essential oil. The 7-month experiment was conducted to determine the optimal production timeline and optimal fertilizer levels for eight basil cultivars grown in a soilless mix. We found that for most sweet basil cultivars (*Ocimum basilicum* L.), later harvest time led to higher dry weight yield. The additional supplement of slow release fertilizer (12N-3.1P-14.9K: 6g and 9g) in the potting medium increased the dry weight yield (whole plant, leaf, and flower) compared to the control (100 ppm N from 20N-4.4P-16.6K soluble fertilizer only), but for most cultivars, the yield did not increase when the amount of slow release fertilizer was increased. 'Holy' basil (*Ocimum sanctum* L.) did not respond to high levels of fertilizer and didn't grow well in capillary mat system. 'Mrs. Burns' Lemon' (*Ocimum basilicum* L.) had the largest flower dry weight. Our results demonstrate that when certain cultivars of basil plants are grown during the winter, within a certain range, more fertilizer can increase the dry weight of whole plant, leaves and flowers.

INTRODUCTION

Medicinal herbs are a group of plants used for health purposes, so it is important to determine the specific constituents in these herbs (O'Hara *et al.*, 1998). Basil (*Ocimum* spp.) is a popular annual medicinal herb, and its essential oils were found to have antifungal and antimicrobial properties (Edris and Farrag, 2003; Kocić-Tanackov *et al.*, 2011; Saggiorato *et al.*, 2012). Numerous studies have been conducted to identify factors that influence basil leaves and whole plant yield (Arabaci & Bayram, 2004; Kandil et al., 2009; Ramezani et al., 2009) as well as essential oil characteristics, their chemical composition, and yield (Carvalho Filho *et al*., 2006; Di Cesare et al., 2003; Kandil et al., 2009; Lachowicz et al., 1996; Zheljazkov et al., 2008; Daneshian et al., 2009; Ramezani et al., 2009). Previous studies tried to determine a way to produce high biomass and essential oil yield, but most of these experiments were carried out in the field during summer, and only a few studies evaluated basil using soilless mix in the greenhouse (Succop & Newman 2004; Carvalho Filho *et al*., 2006). In addition, none of the studies that were carried out in the greenhouse used capillary mats or chemical fertilizers, which are inexpensive and convenient compared to organic fertilizers. In our experiment, eight basil cultivars were grown in a greenhouse during the winter. A capillary mat system (Paparozzi & Meyer, 2012) was used to fertigate the potted basil as it is convenient and saves labor. Our objective was to determine the production timeline necessary to produce basil with high foliage and flower mass and high essential oil yield for maximum profit during winter in Nebraska. To do this we trialed 3 fertilizer levels for each cultivar. We also wanted to determine which cultivars grew better in the greenhouse during the winter under a controlled environment because there are already growers producing fresh basil

for culinary use in Nebraska. In their system, unsalable plants will be discarded, wasted or given away for animal feed. If we create a standard growing system of basil for essential oils, the unsaleable plants could be used for oil production and more profit can be gained by local growers. At the same time, the standard growth system including fertilizer levels and timeline could help growers to arrange their production process more efficiently and gain a high plant and oil yield.

MATERIALS AND METHODS

Eight cultivars of basil (*Ocimum* spp.) were grown in a double-polyethylene greenhouse. Seeds were purchased from Johnny's Selected Seeds, Winslow, ME. The eight cultivars were: 'Italian Large Leaf' (*Ocimum basilicum*) (cultivar 1), 'Nufar' (*Ocimum basilicum*) (cultivar 2), 'Mrs. Burns' Lemon' (*Ocimum basilicum*) (cultivar 3), 'Aroma 2' (*Ocimum basilicum*) (cultivar 4), 'Dolly' (*Ocimum basilicum*) (cultivar 5), 'Holy' (*Ocimum sanctum*) (cultivar 6), 'Spicy Globe' (*Ocimum basilicum*) (cultivar 7), 'Genovese' (*Ocimum basilicum*) (cultivar 8). Most of them were popular cultivars. Among them, 'Italian Large Leaf', 'Nufar', 'Aroma 2', 'Dolly' and 'Genovese' are classic sweet basil type, 'Nufar' and 'Aroma 2' are Fusarium-resistant; 'Mrs. Burns' Lemon' is more like a lemon basil and has a citrus aroma; 'Spicy Globe' has a spicy aroma and is commonly used as an ornamental; Holy basil has unique aroma and it is different from all the other cultivars. Seeds of all the cultivars except Genovese (October 24th) were sown in October 3rd, 2014. Five weeks later, the seedlings were transplanted into 6-inch pots filled with a soilless mix consisting of sphagnum moss peat, vermiculite, and perlite (1:1:1). Plants in each pot were thinned to three plants after four weeks. The

pots were set on a capillary mat system for basic fertigation: a mat set between two layers of plastic, the top layer was white/black and the bottom layer was black. The top layer of plastic was cut so pots directly touched the mat. The water and fertilizer flowed through drip hoses to wet the mat, and in time roots grew through holes in the pots and into the mat.

The experiment was established as a randomized complete block design with six replications. The treatment design was a factorial with each pot at the experimental unit. Three nutrient treatments applied: 100 ppm N from 20N-4.4P-16.6K soluble fertilizer (treatment 0); 100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K osmocote (Everris NA Inc, Dublin, OH) which is a slow release fertilizer (treatment 1); 100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer (treatment 2). The greenhouse temperature was 70°F (21.1℃) day and 65°F (18.3℃) night.

In order to create a timeline, three harvests were carried out at different growth periods. The first harvest was carried out 15 weeks after sowing for all cultivars except ‘Holy’ plants, which were harvested 19 weeks after sowing. The largest plant in each pot was cut from the bottom and then dried at 60℃ for 3 days. The smaller of the two remaining plants in each pot was pinched to increase leaf numbers, which should contribute more to the essential oil yield. The second harvest was carried out 19 weeks after sowing for all cultivars except ‘Holy’ plants, which were harvested 22 weeks after sowing. The plant that had not been pinched in each pot was harvested. The third (final) harvest was carried out 25 weeks after sowing for all cultivars. Leaves, flowers, and stems

of plants from the second and third harvest were separated and put into white sugar bags to be dried at 40℃ for 1-6 days until determining dry weight.

All dry weight data were analyzed using analysis of variance (ANOVA) implemented in the GLIMMIX procedure of SAS (Littell, *et al*., 2006). The resulting LSMeans were evaluated using pairwise t-comparisons (alpha = 0.05).

RESULTS

Characteristics of Different Basil Cultivars:

'Holy' basil had a longer production timeline compare to the other 7 cultivars (Figure 1-1) because 'Holy' basil plants grew slow and flowered later. All the cultivars germinated in 3-4 days and set flower bud set after 12-13 weeks expect 'Holy', which germinated 5 days after seed sowing and set flower buds after 21 weeks.

'Italian Large Leaf', 'Nufar', 'Aroma 2', 'Dolly', and 'Genovese' are classical sweet basil cultivars (*Ocimum basilicum* L.) in our experiment (Figure 1-2). 'Aroma 2' was different in terms of a morphological pattern than other typical sweet basil cultivars in our experiment ('Italian Large Leaf', Nufar', 'Aroma 2', 'Dolly', and 'Genovese'): 'Aroma 2' appeared twisted and curved while other cultivars looked straight and had strong stems. 'Mrs. Burns' Lemon' (*Ocimum basilicum*), 'Holy' (*Ocimum sanctum*) and 'Spicy Globe' (*Ocimum basilicum*) plants looked and smelled quite different from all the other cultivars (classical sweet basil cultivars) (Figure 1-2). 'Holy' plants were short and small, and had small thin leaves and purple flowers and stems. 'Mrs. Burns' Lemon' had a lemony aroma and plenty of branches, leaves and large amount of flowers regardless of

pinching. ‘Spicy Globe’ smelled spicy and looked spherical, it had minimal pest damage.

Fertilizer Levels

From the experiment we found that different cultivars responded differently to the three fertilizer levels.

I Raw Data Plots

1 Harvest 1

1.1‘Italian Large Leaf’ plants (Figure 1-3)

The basic fertigation treatment (treatment 0) alone did not lead to a relatively high dry weight yield compared to the treatments with slow release fertilizer (treatment 1 and 2). In the six replications, 5 of them had higher dry weight when they were applied with a lower level of slow-release fertilizer (6g osmocote), with 3 of them showing an obvious difference. It seems that ‘Italian Large Leaf’ plants grow better at a relatively lower fertilizer level. This needs to be confirmed by statistics analysis. However, none of them had a whole plant dry weight larger than 18 g, which is relatively lower than the other sweet basil cultivars.

1.2 ‘Nufar’ plants (Figure 1-4)

The application of osmocote increased the dry weight. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 2 replications, plants with 6 g osmocote had higher dry weight in another 2 replications. The dry weight did not show obvious

difference between treatment 1 and 2 in the remaining 2 replications.

1.3 ‘Mrs. Burns’ Lemon’ plants (Figure 1-5)

Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 3 replications, plants with 6 g osmocote had higher dry weight in the other 3 replications.

1.4 ‘Aroma 2’ plants (Figure 1-6)

The application of osmocote increased the dry weight. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in all the 6 replications.

1.5 ‘Dolly’ plants (Figure 1-7)

The application of osmocote again increased the dry weight. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 5 replications, but 3 of them did not show obvious difference; plants with 6 g osmocote had higher dry weight in 1 replication.

1.6 ‘Holy’ plants (Figure 1-8)

In harvest 1, ‘Holy’ plants with treatment 0 were not big enough to harvest, they were treated as missing data. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 3 replications, and 1 of them did not show an obvious difference; plants with 6 g osmocote had higher dry weight in 3 replications. The dry weight of ‘Holy’ basil was lower than other cultivars (less than 9 g) except ‘Spicy Globe’.

1.7 ‘Spicy Globe’ plants (Figure 1-9)

The application of osmocote increased the dry weight, the dry weight with treatment 0 was very low and all of them were less than 1 g. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 3 replications, and 1 of them did not show obvious difference; plants with 6 g osmocote had higher dry weight in 3 replications, but the difference was not obvious. ‘Spicy Globe’ had the lowest dry weight yield in harvest 1, and none of the dry weight of plants reached 5g.

1.8 ‘Genovese’ plants (Figure 1-10)

The application of osmocote significantly increased the dry weight, but the difference was not as large as it of the other cultivars, the reason might be ‘Genovese’ seeds were sown 3 weeks later than the others. Plants with 9 g osmocote had higher dry weight than the plants with 6 g in 1 replication without obvious difference; plants with 6 g osmocote had higher dry weight in 4 replications, but the difference was not obvious in 3 replications. There was one replication had similar dry weight when the plants were applied with 2 levels of fertilizer.

2 Harvest 2

2.1 ‘Italian Large Leaf’ plants (Figure 1-11)

The dry weight of leaves, flowers and stems of ‘Italian Large Leaf’ varied among replications, they did not show an obvious effect due to fertilizer treatment level, either. The dry weight of leaves was about 20 g and for flowers was 1-3 g, which is lower than other sweet basil cultivars. However, the dry weight of stems looked close to other sweet basil cultivars.

2.2 ‘Nufar’ plants (Figure 1-12)

Slow release fertilizer increased the dry weight of leaves, flowers and stems compared to the control. Plants with 9 g osmocote had higher leaf dry weight than the plants with 6 g in 4 replications, but for flower dry weight, plants with 6 g osmocote had higher leaf dry weight than the plants with 9 g in 4 replications. It seems that high fertilizer level could increase leaf dry weight but not flower dry weight. As for stem dry weight, it was did not respond to osmocote amount.

2.3 ‘Mrs. Burns’ Lemon’ plants (Figure 1-13)

Slow release fertilizer increased the dry weight of leaves, flowers and stems compared to the control. Plants with 6 g osmocote had higher leaf dry weight than the plants with 9 g in 3 replications, so did flower and stem dry weight. Plants of ‘Mrs. Burns’ Lemon’ seemed responded to the lower fertilizer level. This cultivar also had a large quantity of flowers compared to all the other cultivars. Most of the plants’ flower dry weight was ranged from 10-40 g when the plants received osmocote, while other cultivars usually had less than 10 g flower dry weight. These plants also had a relatively high stem dry weight, but leaf dry weight was not obviously differ from other cultivars.

2.4 ‘Aroma 2’ plants (Figure 1-14)

Slow-release fertilizer increased the dry weight of leaves, flowers and stems compared to the control. Plants with 9 g osmocote had higher leaf dry weight than the plants with 6 g in 4 replications, and in 2 replications plants with 6 g osmocote had higher leaf dry weight. As for flower dry weight, in 4 replications plants with high fertilizer

level had higher dry weight than plants with low fertilizer level, though the difference was not obvious. However, in one replication, flower dry weight of the plant with 6 g osmocote was more than 4 times higher than the plant with 9 g. It might due to the high dry weight of the total plant. Since there were 3 plants in each pot, the plants in one pot completed with each other and some of them got more space, light or nutrition to grow larger than others.

2.5 ‘Dolly’ plants (Figure 1-15)

The dry weight of leaves, flowers and stems of ‘Dolly’ plants also increased when osmocote was supplied, but the difference between treatments with or without osmocote was not as obvious as for other cultivars. The difference between the 2 levels of slow release fertilizer treatments was not obvious, either. ‘Dolly’ is a high-yield cultivar, we found that its leaf dry weight was higher than the stem dry weight, while other cultivars usually had a higher stem dry weight.

2.6 ‘Holy’ plants (Figure 1-16)

In harvest 2, all the ‘Holy’ plants with basic fertilization (treatment 0) did not flower, and there were some plants with osmocote did not flower, either. The whole plant dry weight of ‘Holy’ basil was much lower than the other cultivars except ‘Spicy Globe’. None of the plants had leaf dry weight greater than 8 g, and flower dry weight was less than 0.14 g. Stem dry weight also were less than 4 g. Despite the fact that the dry weight yield was low, we could still see some difference between the control and treatments with osmocote, but the difference didn’t seem big.

2.7 ‘Spicy Globe’ plants (Figure 1-17)

‘Spicy Globe’ plants had very low dry weight in harvest 2. Their ‘globe’ shape and small size might contribute to the results. In most replications, treatments with osmocote had higher leaf, flower and stem dry weight than the control, but the 2 osmocote treatments seemed not differ a lot.

2.8 ‘Genovese’ plants (Figure 1-18)

‘Genovese’ plants performed similarly to other sweet basil cultivars. Dry weight values varied among treatments, and there was no obvious difference among replications for the osmocote treatments.

3 Harvest 3

3.1 ‘Italian Large Leaf’ plants (Figure 1-19)

In harvest 3, the application of osmocote increased the dry weight of leaves, flowers and stems of ‘Italian Large Leaf’ plants. The lower fertilizer level appeared better because plants with 6 g osmocote had higher leaf dry weights than plants with 9 g osmocote in 5 replications. Plants supplied with 6g osmocote also produced more flowers and stems.

3.2 ‘Nufar’ plants (Figure 1-20)

Slow-release fertilizer increased the dry weight of leaves, flowers and stems compared to the control in most replications except 3. The difference between treatments with or without osmocote was not obvious. Plants with 6 g osmocote tended to have

higher leaf dry weight than the plants supplied with 9 g in 3 replications. In 3 replications, plants with 9 g osmocote had higher leaf dry weight than the plants supplied with 6 g. Stem dry weight followed leaf dry weight. As for flower dry weight, plants with higher fertilizer level had higher dry weight in 3 out of 6 replications. The 2 treatments with osmocote had similar dry weights in 2 replications, and plants with 6 g osmocote produced higher flower dry weight in only one replication.

3.3 ‘Mrs. Burns’ Lemon’ plants (Figure 1-21)

‘Mrs. Burns’ Lemon’ produced a large amount of flowers when compared to all the other cultivars. Half of the plants had more than 80 g flower dry weight and one plant reached 170 g, while for other cultivars there were 0-2 plants that reached 80 g. Plants with 6 g osmocote had higher leaf and stem dry weight than the plants with 9 g in 3 replications, and in 3 replications plants with 9 g osmocote had higher leaf and stem dry weight than the plants with 6 g. In replication 5, the control had higher flowers and stems dry weight than the plant with 9 g osmocote.

3.4 ‘Aroma 2’ plants (Figure 1-22)

Slow release fertilizer increased the dry weight of leaves, flowers and stems compared to the control in all replications. However, the 2 levels of osmocote treatments had similar leaf and stem dry weight in most replications. In 4 replications, plants with 6 g osmocote had higher flower dry weights.

3.5 ‘Dolly’ plants (Figure 1-23)

The difference between the control and osmocote treatments was not obvious in 2 replications. Plants with 9 g osmocote had higher leaf, flower and stem dry weight than plants with 6 g osmocote in 3 replications. The dry weight of leaves and stems were similar, but for other cultivars, plants usually had higher stem dry weight.

3.6 ‘Holy’ plants (Figure 1-24)

In harvest 3, only 1 of 6 control plants flowered. ‘Holy’ plants had very low dry weight compared to all the other cultivars, and the difference among the 3 treatments was not as obvious as for other cultivars. In 3 replications, plants with 9 g osmocote had higher leaf, flower and stem dry weight than the control plants and the plants with 6 g osmocote.

3.7 ‘Spicy Globe’ plants (Figure 1-25)

‘Spicy Globe’ also had a relatively low leaf and stem dry weight, but it had similar flower dry weight with other cultivars. In 2 replications, plants with 9 g osmocote had higher leaf, flower and stem dry weight than the plants supplied with 6 g osmocote.

3.8 ‘Genovese’ plants (Figure 1-26)

In most replications, slow release fertilizer increased leaf, flower and stem dry weight. Plants with 9 g osmocote had higher leaf and stem dry weight than plants with 6 g osmocote in 4 replications, and in 1 replication plants with 6 g had higher leaf dry weight. As for flowers, in 3 replications, plants supplied with 6 g osmocote had higher dry weight, and in 2 replications plants with 9 osmocote had higher dry weight.

Raw Data Summary

For harvest 1-for all the cultivars slow-release fertilizer increased plant dry weight. ‘Genovese’ plants did not produce as much foliage plant growth as the other pesto cultivars, specially ‘Nufar’ and ‘Aroma 2’. ‘Dolly’ and ‘Italian Large Leaf’ produced the next greatest quantity of dry weight. Of the specially basil-‘Mrs. Burns’ Lemon’ grew better than ‘Spicy Globe’ or ‘Holy’.

For harvest 2-for all the cultivars slow-release fertilizer increased plant dry weight. ‘Italian Large Leaf’ produced less leaves and flowers compared to other pesto cultivars, while ‘Aroma 2) produced more flowers. Of the specially basil, ‘Mrs. Burns’ Lemon’ had large quantity of flowers and relatively high leaf and stem dry weight. ‘Holy’ and ‘Spicy Globe’, in contrast, produced low dry weight.

For harvest 3- for all the cultivars slow-release fertilizer increased plant dry weight. ‘Italian Large Leaf’ produced more stems compared to other pesto basil cultivars, while ‘Aroma 2’ had lower leaf dry weight. ‘Nufar’ and ‘Dolly’ had a relatively high leaf to stem ratio, which means these 2 cultivars may be good for fresh basil retail. The flower dry weight of ‘Mrs. Burns’ Lemon’ plants was obviously greater than all the other cultivars. ‘Holy’ still had low dry weight, but’ Spicy Globe’ had a normal level of flower dry weight.

II Statistics Analysis Results

1 Harvest 1

Except for dry weight of flowers in harvest 3, there were significant interactions between fertilizer treatments and cultivars (Table 1-1).

There were significant interactions among fertilizer treatments and cultivars (Table 1-1). In harvest 1, treatments with slow release fertilizer (treatment 1 and 2) showed more growth when compared to the basic fertilizer treatment for six cultivars (‘Italian Large Leaf’, ‘Nufar’, ‘Mrs. Burns’ Lemon’, ‘Aroma 2, ‘Dolly’, and ‘Genovese’) (Table 1-2a). Among them, only ‘Italian Large Leaf’ and ‘Aroma 2 showed significant difference between different amounts of slow release fertilizer: ‘Italian Large Leaf’ grew better with less fertilizer while ‘Aroma 2’ grew better with more fertilizer. Most of the plants of ‘Holy’ supplied with only basic fertilization were not big enough in harvest 1, and thus were treated as missing data. ‘Holy’ plants grew the same under both fertilizer levels that included osmocote. Cultivars of ‘Nufar’, ‘Mrs. Burns’ Lemon’, ‘Dolly’ and ‘Genovese’ grew well at either level of osmocote. Dry weights of ‘Spicy Globe’ at three fertilizer levels were not significantly different from each other.

When supplied with only basic fertilizer, all cultivars did not grow well (Table 1-2b). When fertilizer was increased, significant differences among cultivars were apparent. Compared to other cultivars, ‘Holy’ and ‘Spicy Globe’ plants grew less than ‘Italian Large Leaf’, ‘Nufar’, ‘Mrs. Burns’ Lemon’, ‘Aroma 2’, ‘Dolly’ and ‘Genovese’ at both levels of osmocote. ‘Italian Large Leaf’ and ‘Genovese’ at 9 g level of osmocote processed less plant mass than ‘Nufar’, ‘Aroma 2’ and ‘Dolly’.

2 Harvest 2

There were significant interactions between fertilizer treatments and dry weight of whole plant, leaf and flower in harvest 2 (Table 1-1). In harvest 2, ‘Nufar’, ‘Mrs. Burns’ Lemon’, ‘Aroma 2’, ‘Dolly’, and ‘Genovese’ in that plants receiving slow release fertilizer had higher whole plant and leaf dry weight than those with only basic fertilization (Table 1-3a & 1-4a). ‘Mrs. Burns’ Lemon’ had higher whole plant dry weight when supplied with less slow-release fertilizer (6g) than the treatment with more, but other cultivars respond similarly (Table 1-3a).

‘Mrs. Burns’ Lemon’ plants was significantly more whole plant than all others at 6g but not at 9g osmocote. ‘Holy’ and ‘spicy Globe’ had relatively lower dry weight compared to other cultivars when supplied with osmocote especially 9g (Table 1-3b).

As for leaf dry weight, all the cultivars except ‘Italian Large Leaf’, ‘Holy’ and ‘Spicy Globe’ grew better when supplied with osmocote, and the 2 fertilizer produced similar leaf dry weights (Table 1-4a).

When comparing the cultivars, ‘Dolly’ had higher leaf dry weight when supplied basic fertilization (treatment 0), and ‘Genovese’ and ‘Aroma 2’ had less leaves. However, when osmocote was added, the difference narrowed. ‘Holy’ and ‘Spicy Globe’ always had the lowest leaf dry weight in the 8 cultivars (Table 1-4b).

As for flower dry weight, only ‘Mrs. Burns’ Lemon’ and ‘Aroma 2’ had higher flower dry weight when supplied with slow release fertilizer (Table 1-5a). These two cultivars also had relatively high flower dry weight compared to other cultivars especially ‘Mrs. Burns’ Lemon’ (Table 1-5b).

Overall, 3 plant parts ‘Holy’ and ‘Spicy Globe’ still had lower dry weight and the supplement of slow-release fertilizer did not increase the dry weight (Table 1-3b, 1-4b & 1-5b).

3 Harvest 3

There were significant interactions among fertilizer treatments and whole plant and leaf dry weights (Table 1-1). In harvest 3, except for ‘Holy’ and ‘Spicy Globe’, slow-release fertilizer increased all the other cultivars’ whole plant dry weight (Table 1-6a). When comparing cultivars, ‘Holy’ had the lowest whole plant dry weight regardless of fertilizer levels (Table 1-6b).

Osmocote didn’t increase leaf dry weight of ‘Holy’ and ‘Spicy Globe’. ‘Italian Large Leaf’ supplied with less slow-release fertilizer (6 g) had higher leaf dry weight than the treatment with more (9 g), while other cultivars respond similarly to the 2 fertilizer levels (Table 1-7a). When plants were supplied with basic fertilization their difference was not obvious. When osmocote was added, the difference widened. ‘Italian Large Leaf’ and ‘Nufar’ produced more leaves when supplied with 6g osmocote while ‘Nufar’ and ‘Dolly’ produced more leaves when supplied with 9g (Table 1-7b).

Both treatment and cultivar influenced flower dry weight in harvest 3 (Table 1-8). Slow release fertilizer increased the flower dry weight compared to the treatment with basic fertilization only, but the amount (6g and 9g) of slow release fertilizer did not make any difference (Table 1-8a). ‘Mrs. Burns’ Lemon’ had the greatest flower dry weight and was significantly different from all the other cultivars, while ‘Holy’ had the lowest flower

dry weight and was significantly different from the other cultivars. All the other cultivars were not significantly different from each other (Table 1-8b).

Comparison of Three Harvest Periods

I Sweet basil types

'Italian Large Leaf' grew best at the lower fertilizer level (6 g osmocote) when supplied with the fertilizer in harvest 1 (Table 1-2a). In harvest 2, the difference between three fertilizer treatments was not that obvious (Table 1-3a), similar for the leaf and flower dry weight (Table 1-4a & 1-5a). When the harvest time was delayed until 25 weeks after sowing (harvest 3), the difference among three fertilizer treatments became obvious again and showed similar results in harvest 1 (Table 1-6a & 1-7a). 'Italian Large Leaf' showed a relatively low flower dry weight yield compared to other sweet basil cultivars in harvest 2 (Table 1-5b).

'Nufar', 'Aroma 2', 'Dolly', and 'Genovese' responded similarly to fertilizer application: treatments with slow release fertilizer had significantly higher dry weights, but no difference between the two fertilizer levels was observed (Table 1-2a, 1-3a, 1-4a, 1-5a, 1-6a & 1-7a). 'Aroma 2' plants grew better at the high fertilizer level (9 g slow release fertilizer) in early stage (harvest 1) (Table 1-2a), but this was not evident at the latter two harvest periods (Table 1-3a & 1-6a). These four cultivars were similar in dry weight of the whole plant in each harvest. 'Aroma 2' produced more flowers in harvest 2 compared to the other 3 cultivars (Table 1-5b), but the difference narrowed in harvest 3 (Table 1-8). 'Dolly' and 'Nufar' also had higher leaf to stem ratio than other sweet basil

cultivars (Figure 1-12, 1-15, 1-20 & 1-23).

II Specially basil cultivars

The dry weight of whole plants of ‘Mrs. Burns’ Lemon’ (*Ocimum basilicum*) was not significantly heavier than typical sweet basil cultivars in first stage (Table 1-2b). However, in the latter two harvest periods, the whole plant dry weight of ‘Mrs. Burns’ Lemon’ was larger than all the other seven cultivars (Table 1-3a & 1-6a). It was worth mentioning that ‘Mrs. Burns’ Lemon’ had the greatest amount of flower dry weight in both harvest 2 and 3 (Table 1-5b & 1-8b). In harvest 2, ‘Mrs. Burns’ Lemon’ grew better at the lower fertilizer level (Table 1-5a).

‘Holy’ plants need a longer production timeline compared to other cultivars, the plants with basic fertilization didn’t grow large enough to be harvested in harvest 1 (Table 1-2a). However, regardless of harvest period, ‘Holy’ plants always had the lowest dry weight yield regardless of the fertilizer level.

‘Spicy Globe’ also didn’t produce high biomass, but this cultivar produced relatively large amount of flowers in harvest 2 and 3 (Table 1-5b & 1-8b).

DISCUSSION

Fertilizer Treatment

From the data we found that for most basil cultivars, 100 ppm N from 20N-4.4P-16.6K soluble basic fertilizer did not lead to an adequate dry matter yield. Thus more fertilizer is needed when basil is grown in soilless mix in a capillary mat fertigation

system. When plants were applied with slow-release fertilizer (12N-3.1P-14.9K), most cultivars showed increases in dry weight, but when the amount of slow release fertilizer increase from 6g to 9g, dry weight did not always increase. Only one cultivar responded better to the lower fertilizer level (‘Italian Large Leaf’). Other research reported that increasing NPK rate increased the yield of ‘Genovese’, but the application of only 50% recommended NPK (238 kg/h as ammonium nitrate, 128 kg/ha P as super-phosphate and 114 kg/ha K as potassium sulfate) gave about 80% of the yield as compared to 100% recommended NPK (Kandil *et al.* 2009). However, in our growth system more experiments are suggested to figure out the best nutrient concentration.

As for optimal fertilizer levels for other sweet basils (*Ocimum basilicum*), there was field research reported that nitrogen fertilization up to 300 kg/ha (nitrogen rates in the experiment were 0, 100 or 300 kg/ha) increased above-ground and leaf fresh biomass (Sifola *et al*. 2006). While another research reported that phosphorus spray level did not affect fresh and dry weight of basil (Ramezani *et al*. 2009). When organic fertilizers were used, researchers reported that the combination of organic and inorganic fertilizer performed best with respect to dry weight yield (Anwar *et al.* 2005). However, those experiments were all carried out in field. Thus their results cannot be directly compared with ours since soil and soilless mixes work quite differently.

As for experiments carried out in a greenhouse, Carvalho Filho *et al*. 2006 also used a soilless mix (coconut dust, bovine manure, and carbonized rice husks 1:1:1) to grow sweet basil (*Ocimum basilicum*), but they used organic fertilizer (bovine manure $60m^3$/ha) which again cannot compare with ours (chemical fertilizer). Another

greenhouse experiment studied the effect of conventional (salt-based fertilizer) and organic fertilization on basil flavor. In that experiment, a hydroponic system with NPK concentration was 364 ppm, 78 ppm, and 308 ppm, respectively (Succop & Newman 2004). The study did not focus on fertilizer level effect, but the plants grew well with the above nutrient concentrations, this agreed with our results that 100 ppm N from 20N-4.4P-16.6K soluble fertilizer is not enough to reach good growth of basil.

In our experiment, when fertilizer level was increased, dry weight of 'Holy' did not significantly increase. 'Holy' also had the lowest whole plant, leaf and flower dry weight in each harvest (1-3) compared with all the other cultivars. The results showed that simply increasing the slow release fertilizer level was not the best way to improve the dry weight yield of 'Holy'. A study reported significant differences among the yield of holy basil (cv. Local) (*O. sanctum*) grown in four locations (Zheljazkov *et al.* 2008). The authors thought that the difference due to soil type and climate. The soil in the location that had the highest holy basil herbage yield had relatively lower concentrations of P and K compared to with the other three. This location also has the highest latitude. It seems that *O. sanctum* responds to a relatively lower fertilizer level, and thus the results have something in common with ours: high level of fertilizer doesn't increase the dry weight of 'Holy' basil.

Harvest Time

As expected, our experiment showed that the longer the production period, the higher the dry weight of whole plant, leaf and flower. However, considering the cost of running greenhouses, the loss due to pest, and convenient time arrangement for farmers,

the production period should not be as long as the third harvest. According to Sifola *et al.* (2006), basil plants at full bloom stage was the most appropriate time for commercial harvest, and this is about 7 weeks after transplanting. This stage is almost the same as harvest 1 in our experiment. However, we did not have very good performance of the cultivars in harvest 1. The reasons were multiple: first, we grew three plants in one pot with a certain level of fertilizer treatment. Thus, the plants might not have enough room and nutrients to grow well before one or two of them were harvested. Second, most of the research was done in the field during the summer when photoperiod was longer than in winter. Third, the increased amount of slow release fertilizer (from 6g to 9g) may be too small to see the difference in such a short time period. In future studies, we will try a higher level of slow release fertilizer, larger pot with fewer plants, and shorten the production period to fit commercial production.

'Holy' had a longer production timeline compared to the other cultivars (*Ocimum basilcum*). The result was similar to the research carried out by Zheljazkov *et al.* 2008. The authors found that cultivar Local (*O. sanctum*) had increased in herbage yield with the third cut (about 5 months after transplant) compared to both the first (about 1 month after transplant) and the second cut (about 3 months after transplant), and they thought that it was due to the establishment of root system. However, they harvested each plant three times, grew the plants in soil not pots and cut the plants at 15 cm above soil surface, which was different from our experiment.

Cultivar Choice

Since we are also interested in extracting essential oils from basil, we hoped to

improve basil dry weight yield through increasing chemical fertilizer level. ‘Italian Large Leaf’ may not be a good choice since it grew better at a lower fertilizer level. ‘Nufar’ which was fusarium-resistant, had relatively high plant dry weight yield, but was plagued by heavy thrips and aphid damage.

Previous research reported that the yield of essential oil of flowers and leaves from *Ocimum basilicum* was at least 10 times higher than the yield of stems (Chalchat *et al.*, 2008), and essential oils yield from *Ocimum basilicum* was higher in flowers than in leaves (Charles *et al.*, 1990). Mrs. Burns’ Lemon produced the most flower dry weight. If the results were pervasive among *Ocimum* spp., ‘Mrs. Burns’ Lemon’ might be an important cultivar for essential oil production. ‘Aroma 2’, ‘Dolly’, and ‘Genovese’ had typical sweet basil morphological characteristics and had relatively high dry weight yield during the experiment. They are good choices to grow in our system. ‘Holy’ and ‘Spicy Globe’ had low dry weight yield, and the increase of fertilizer seemed make no improvement, so these two cultivars are not good choices for dry mass. ‘Holy’ plants did not flourish under a constant water status system, this may due to where it originated geographically. ‘Spicy Globe’ was more for ornamentals not oil production (Simon *et al.*, 1999), and our results showed that the use of slow release fertilizer only influenced the dry weight of flower in ‘Spicy Globe’ in harvest period 3. Since the unique spicy smell of this cultivar was very appealing, more research should done to identify the compounds of the oils from ‘Spicy Globe.’

In future experiments, basil essential oils will be distilled, quantified and identified, and from these plants and the information will be analyzed to get a full answer for the recommendation for the setup of the standard growing system.

CONCLUSIONS

In this 7-month experiment, the later the harvest time, the more whole plant, leaf and flower dry weight the plants produced. However, other factors such as pest damage and cost of running a greenhouse during the winter should be considered when creating a production timeline. When basil is grown in soilless mix, 100 ppm N from 20N-4.4P-16.6K soluble basic fertilizer does not produce adequate dry matter yield; more fertilizer is needed. 'Mrs. Burns' Lemon' might be an important essential oil producer since it produced a very high flower quantity. Other typical sweet basil cultivars such as 'Aroma 2', 'Dolly', and 'Genovese' are also good choices to grow in greenhouse during winter, and 'Dolly' and 'Genovese' are particularly good for fresh harvest. However, neither 'Holy' nor 'Spicy Globe' plants grew well in our capillary mat system especially 'Holy' plants, which produced relatively lower dry weight yield. Also, 'Holy' plants had a longer production timeline than all the other cultivars.

For future experiment, we will distill essential oils from dry samples and do oil quantification and identification. We will rerun the experiment with adjusted fertilizer levels and appropriate cultivars to get more data.

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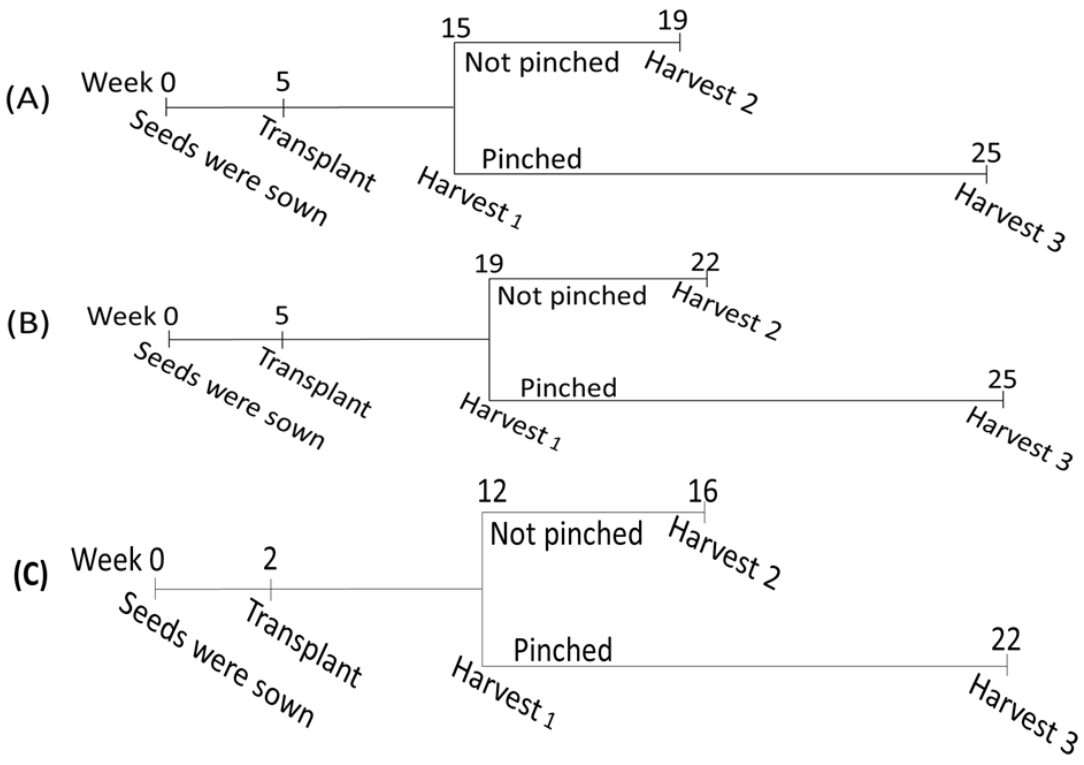

Figure 1-1. Production timelines during winter 2014-2015. (A) Production timeline for 'Italian Large Leaf', 'Nufar', 'Mrs. Burns' Lemon', 'Aroma 2', 'Dolly' and 'Spicy Globe' basil. (B) Production timeline for 'Holy' basil. (C) Production timeline for 'Genovese' basil.

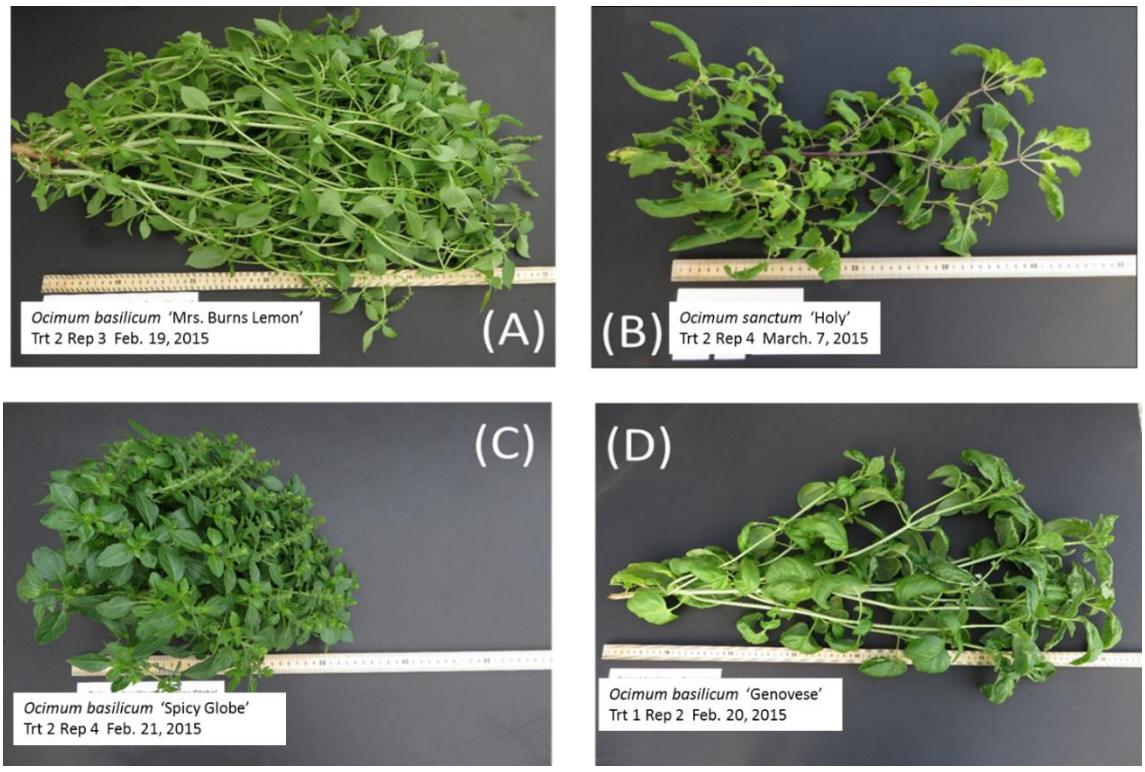

Figure 1-2. Different cultivars looked differently: (A) 'Mrs. Burns' Lemon' basil. (B) 'Holy' basil. (C) 'Spicy Globe' basil. (D) 'Genovese' basil, which is a classic sweet basil and looked similar with all the other sweet basil cultivars.

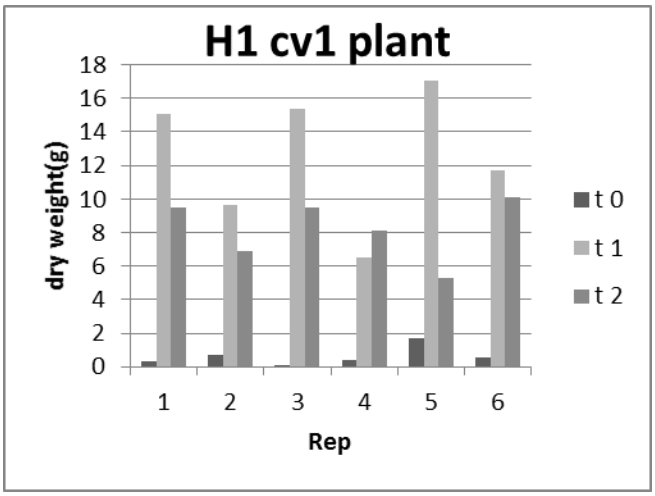

Figure 1-3. Total weight of whole plant of ‘Italian Large Leaf’ basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K osmocote; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

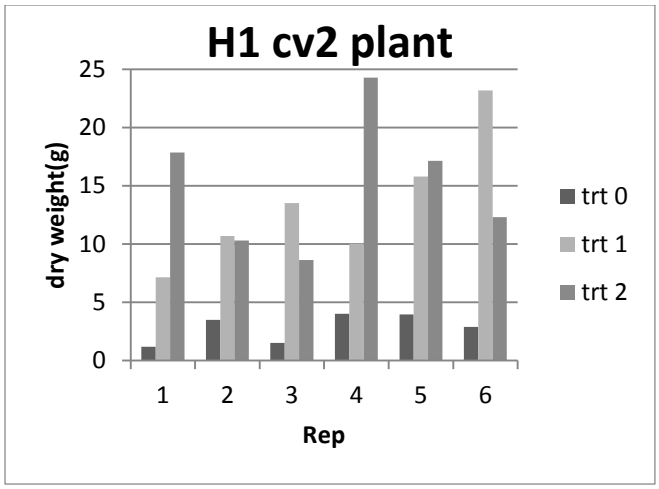

Figure 1-4. Total weight of whole plant of 'Nufar' basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K osmocote; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

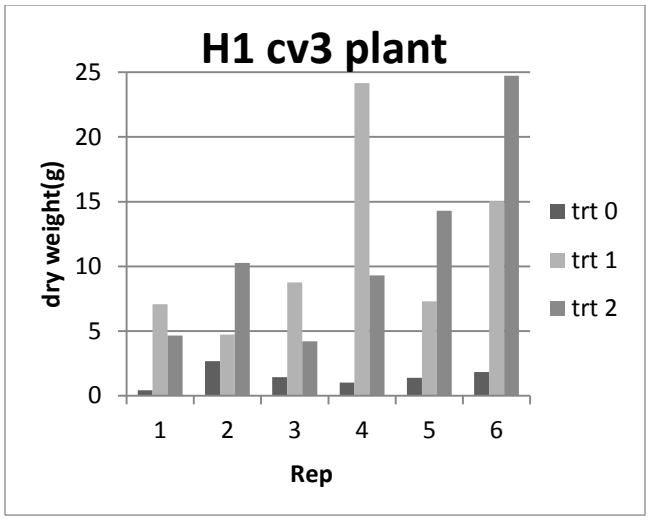

Figure 1-5. Total weight of whole plant of 'Mrs. Burns' Lemon' basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

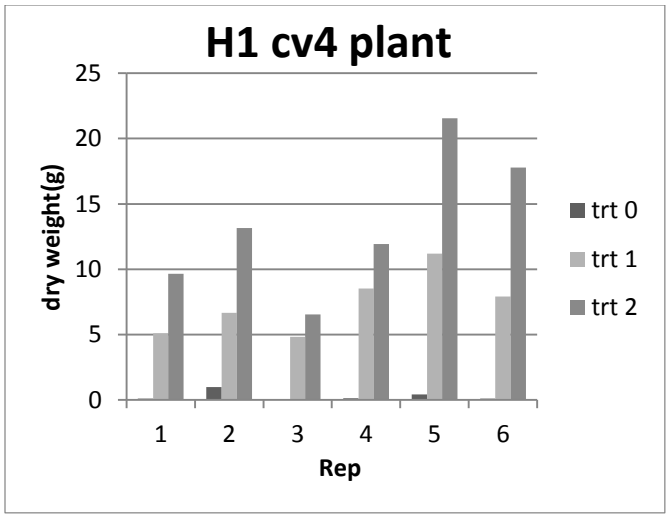

Figure 1-6. Total weight of whole plant of ‘Aroma 2’ basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

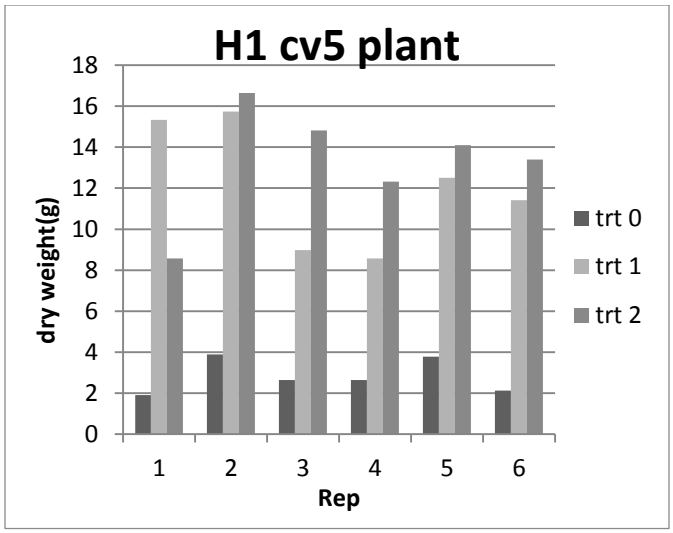

Figure 1-7. Total weight of whole plant of 'Dolly' basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

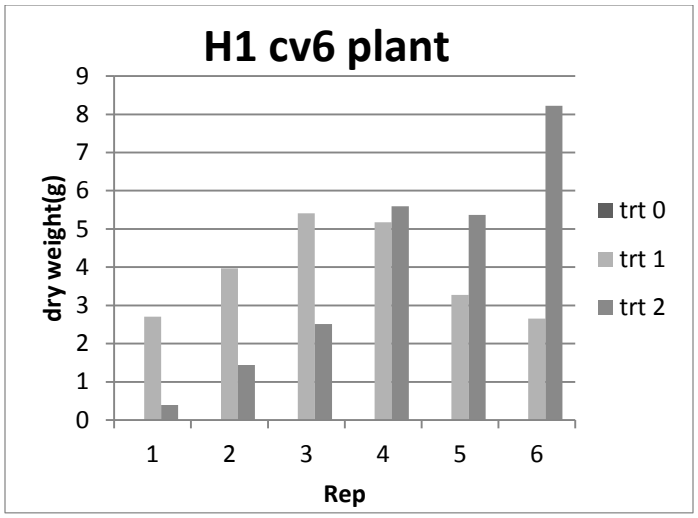

Figure 1-8. Total weight of whole plant of 'Holy' basil at harvest 1 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

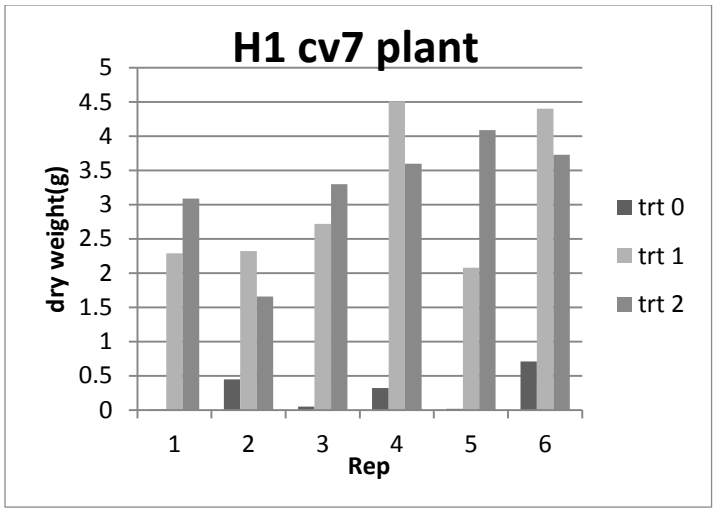

Figure 1-9. Total weight of whole plant of 'Spicy Globe' basil at harvest 1 (15 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

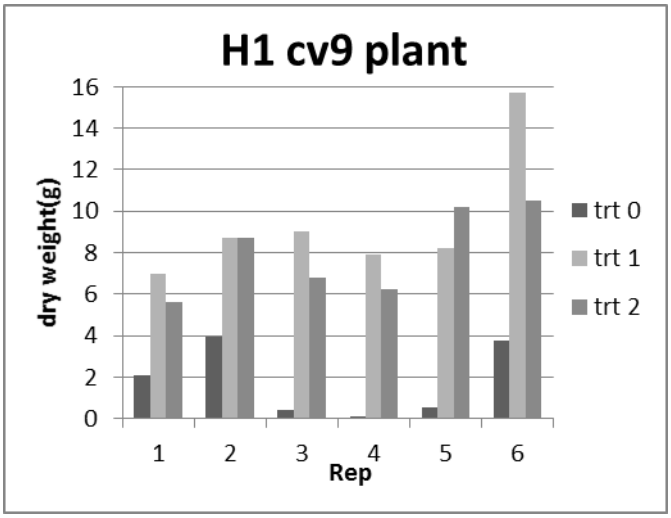

Figure 1-10. Total weight of whole plant of 'Genovese' basil at harvest 1 (12 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

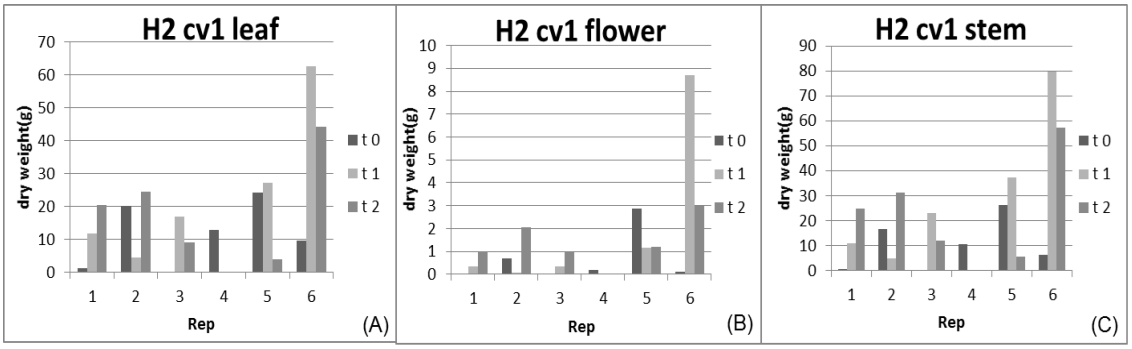

Figure 1-11. Dry weight of leaves (A), flowers (B) and stems (C) of 'Italian Large Leaf' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

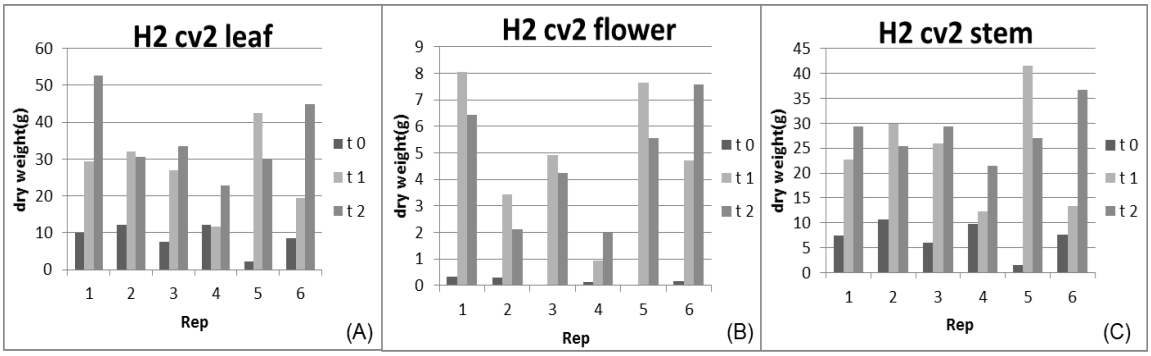

Figure 1-12. Dry weight of leaves (A), flowers (B) and stems (C) of 'Nufar' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

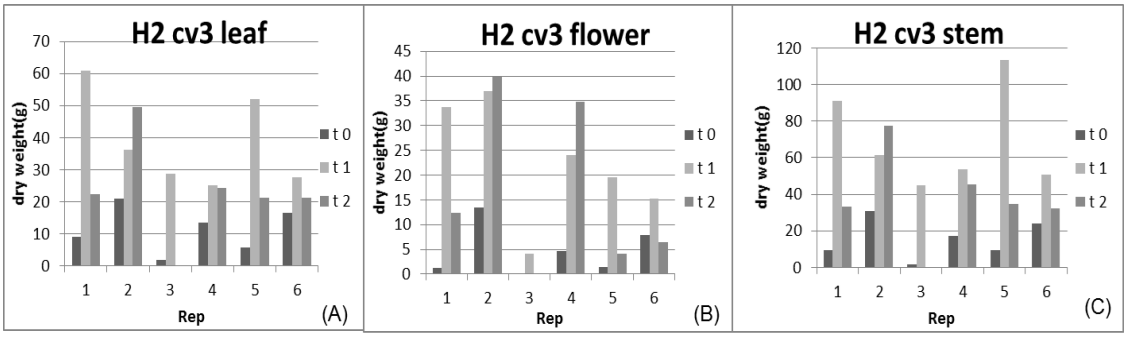

Figure 1-13. Dry weight of leaves (A), flowers (B) and stems (C) of 'Mrs. Burns' Lemon' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

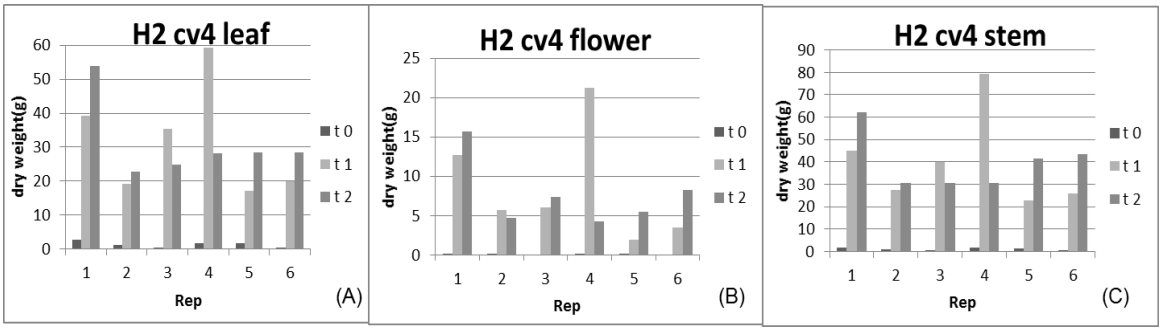

Figure 1-14. Dry weight of leaves (A), flowers (B) and stems (C) of 'Aroma 2' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

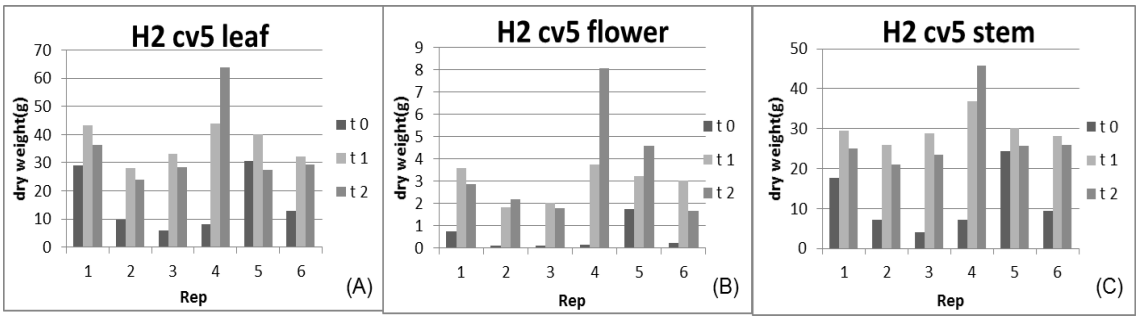

Figure 1-15. Dry weight of leaves (A), flowers (B) and stems (C) of 'Dolly' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

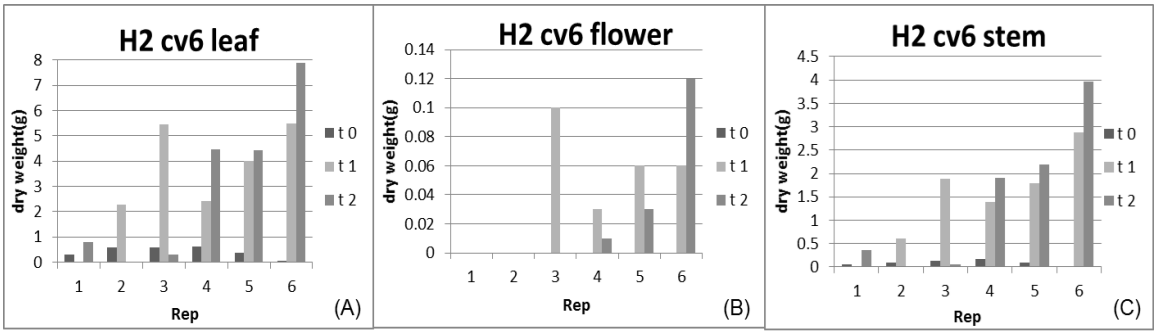

Figure 1-16. Dry weight of leaves (A), flowers (B) and stems (C) of 'Holy' basil at harvest 2 (22 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

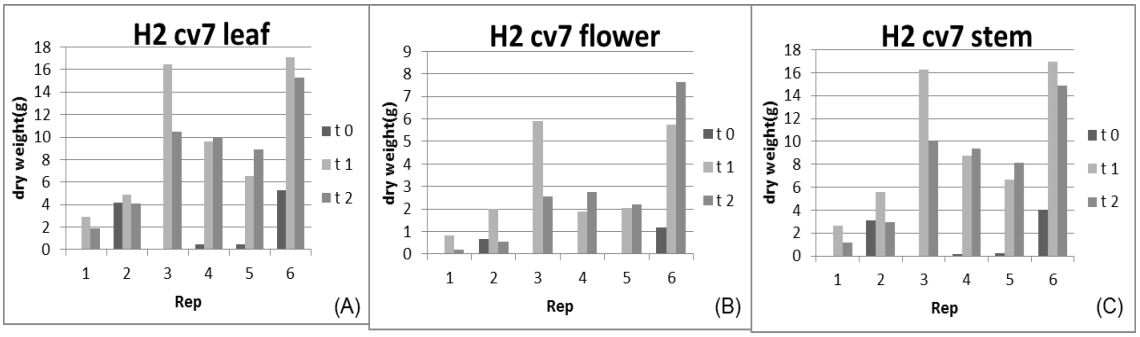

Figure 1-17. Dry weight of leaves (A), flowers (B) and stems (C) of 'Spicy Globe' basil at harvest 2 (19 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

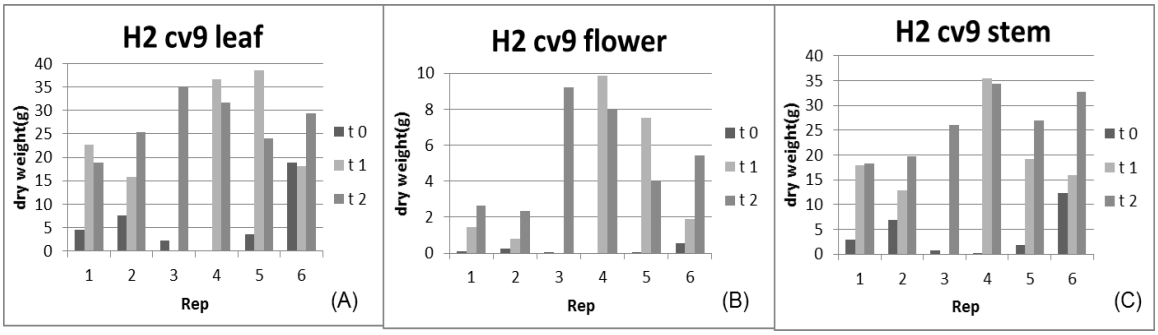

Figure 1-18. Dry weight of leaves (A), flowers (B) and stems (C) of 'Genovese' basil at harvest 2 (16 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

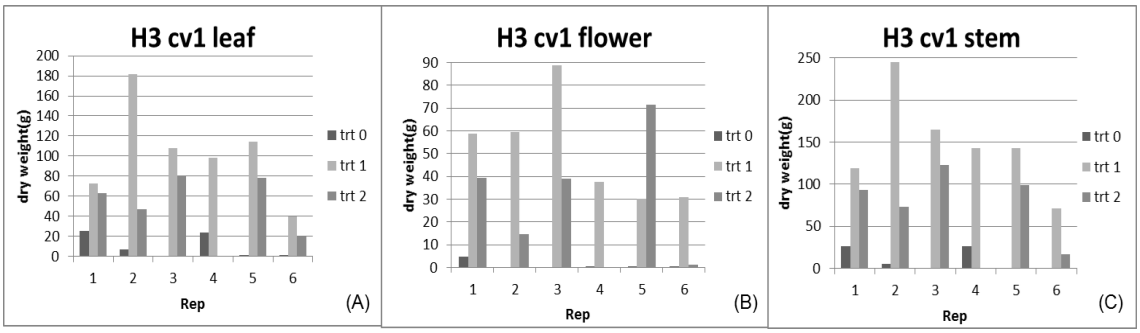

Figure 1-19. Dry weight of leaves (A), flowers (B) and stems (C) of 'Italian Large Leaf' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

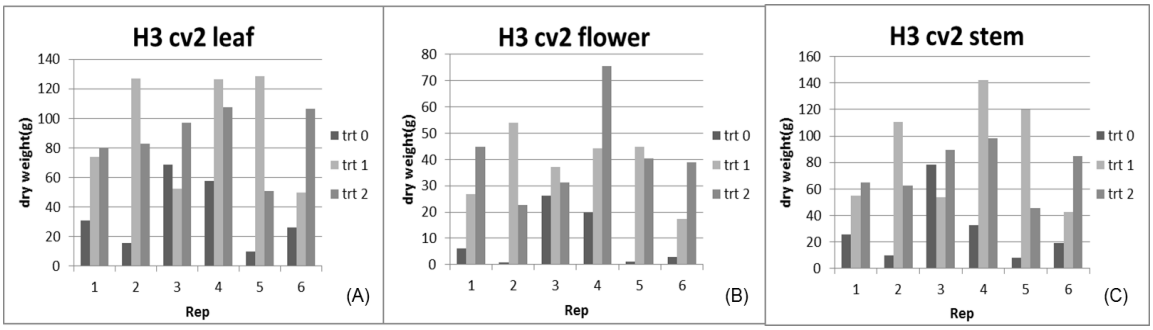

Figure 1-20. Dry weight of leaves (A), flowers (B) and stems (C) of 'Nufar' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

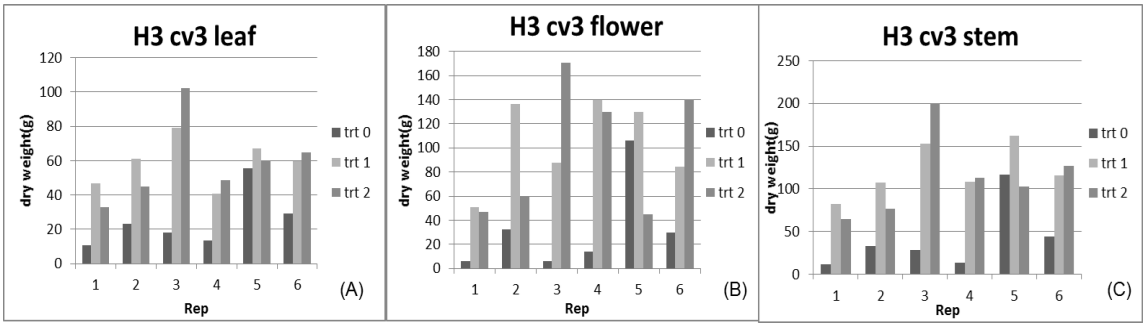

Figure 1-21. Dry weight of leaves (A), flowers (B) and stems (C) of 'Mrs. Burns' Lemon' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

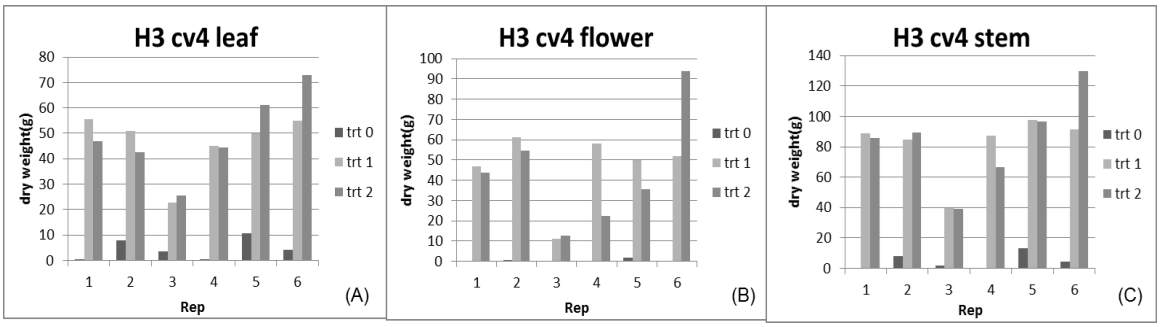

Figure 1-22. Dry weight of leaves (A), flowers (B) and stems (C) of 'Aroma 2' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

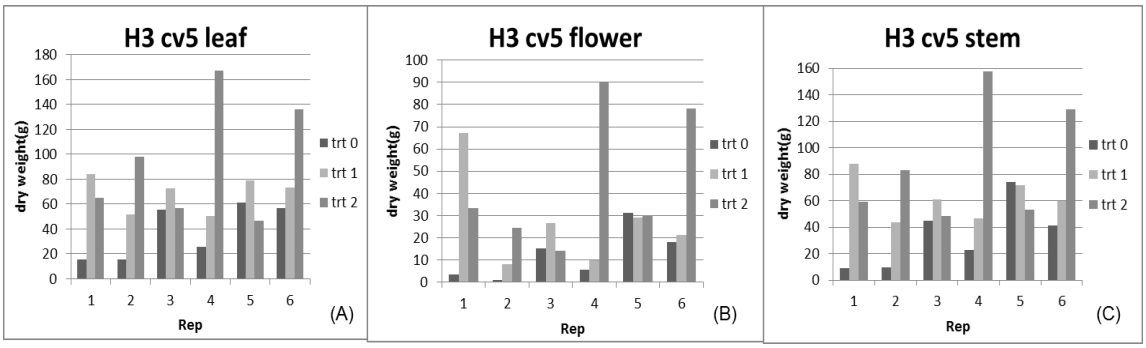

Figure 1-23. Dry weight of leaves (A), flowers (B) and stems (C) of 'Dolly' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

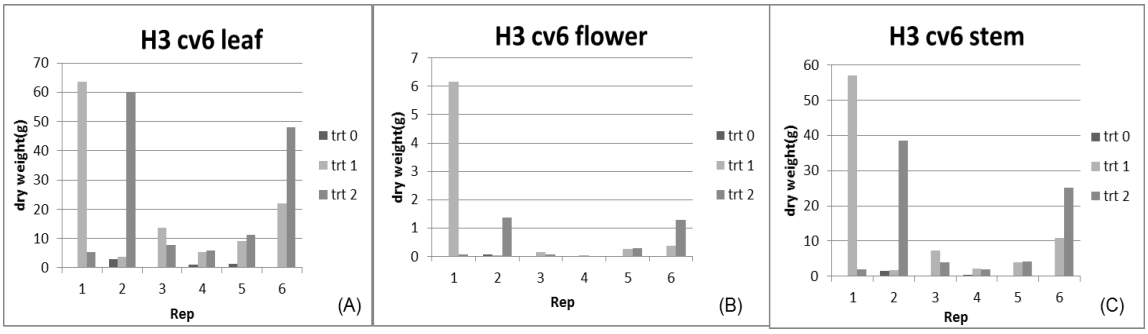

Figure 1-24. Dry weight of leaves (A), flowers (B) and stems (C) of 'Holy' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

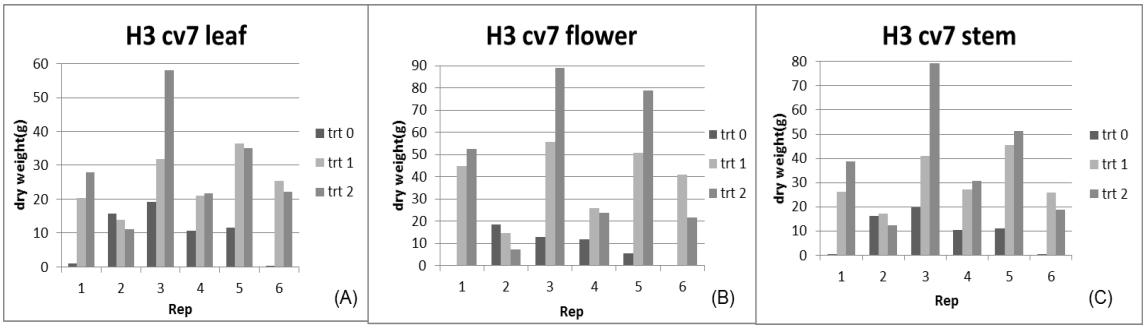

Figure 1-25. Dry weight of leaves (A), flowers (B) and stems (C) of 'Spicy Globe' basil at harvest 3 (25 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

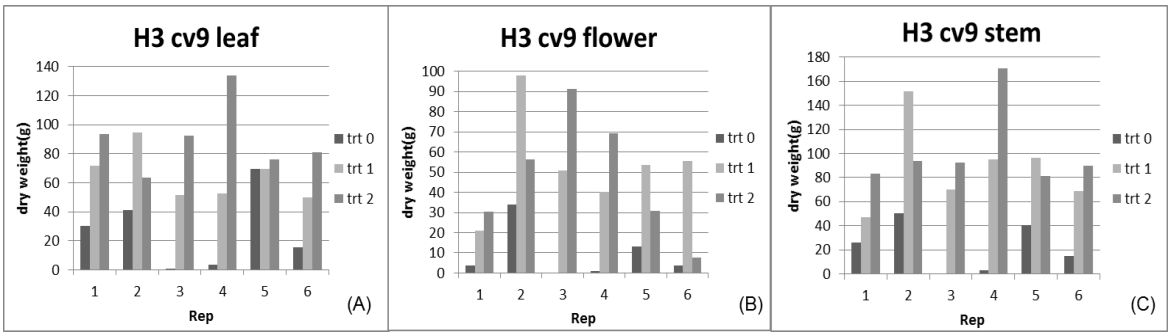

Figure 1-26. Dry weight of leaves (A), flowers (B) and stems (C) of 'Genovese' basil at harvest 3 (22 weeks after sowing) as identified by replication (rep) for the three fertilizer treatments. T0=100 ppm N from 20N-4.4P-16.6K soluble fertilizer; t1=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 6g of 12N-3.1P-14.9K slow release fertilizer; t2=100 ppm N from 20N-4.4P-16.6K soluble fertilizer plus 9g of 12N-3.1P-14.9K slow release fertilizer.

ANOVA	wp H1	wp H2	leaf H2	flower H2	wp H3	leaf H3	flower H3
treatment	**	**	**	**	**	**	**
cultivar	**	**	**	**	**	**	**
treatment*cultivar	**	**	*	**	**	**	NS
wp: whole plant				NS: not significant			
H: harvest				* Significant at p<0.05			
				** Significant at p<0.01			

Table 1-1. Analyses of variance for whole plant, leaf and flower dry weight for 8 cultivars treated with 3 levels of fertilizer levels. Harvest 1 was carried out 19 weeks after seeds sowing for ‘Holy’ basil, 12 weeks after seeds sowing for ‘Genovese’ basil, 15 weeks after seeds sowing for all the other cultivars; harvest 2 was carried out 22 weeks after seeds sowing for ‘Holy’ basil, 16 weeks after seeds sowing for ‘Genovese’ basil, 19 weeks after seeds sowing for all the other cultivars; harvest 3 was carried out 22 weeks after seeds sowing for ‘Genovese’ basil, 25 weeks after seeds sowing for all the other cultivars.

Cultivar	Whole Plant in Harvest 1*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	0.64±1.43a	12.57±1.43c	8.23±1.43b
'Nufar'	2.85±1.43a	13.40±1.43b	15.09±1.43b
'Mrs. Burns' Lemon'	1.47±1.43a	11.17±1.43b	11.25±1.43b
'Aroma 2'	0.29±1.43a	7.37±1.43b	13.44±1.43c
'Dolly'	2.83±1.43a	12.09±1.43b	13.31±1.43b
'Holy'	--	3.86±1.43a	3.92±1.43a
'Spicy Globe'	0.26±1.43a	3.05±1.43a	2.72±1.43a
'Genovese'	1.79±1.43a	9.43±1.43b	8.01±1.43b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-2a. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 1. In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Whole Plant in Harvest 1*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	0.64±1.43a	12.57±1.43ab	8.23±1.43c
'Nufar'	2.85±1.43a	13.40±1.43a	15.09±1.43a
'Mrs. Burns' Lemon'	1.47±1.43a	11.17±1.43abc	11.25±1.43bc
'Aroma 2'	0.29±1.43a	7.37±1.43cd	13.44±1.43ab
'Dolly'	2.83±1.43a	12.09±1.43ab	13.31±1.43ab
'Holy'	--	3.86±1.43de	3.92±1.43d
'Spicy Globe'	0.26±1.43a	3.05±1.43e	2.72±1.43d
'Genovese'	1.79±1.43a	9.43±1.43bc	8.01±1.43c

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-2b. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 1. In this table, 8 cultivars were compared within each fertilizer treatment.

Cultivar	Whole Plant in Harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	25.75±11.29a	57.90±11.29b	48.12±11.29ab
'Nufar'	16.10±10.31a	56.26±10.31b	68.54±10.31b
'Mrs. Burns' Lemon'	31.52±10.31a	129.92±10.31c	90.59±11.29b
'Aroma 2'	2.48±10.31a	80.29±10.31b	78.55±10.31b
'Dolly'	28.18±10.31a	69.62±10.31b	66.23±10.31b
'Holy'	0.52±10.31a	5.62±11.29a	5.25±11.29a
'Spicy Globe'	4.86±12.60a	22.11±10.31a	16.33±10.31a
'Genovese'	10.46±10.31a	51.02±11.29b	59.00±10.31b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-3a. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 2. In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Whole Plant in Harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	25.75±11.29ab	57.90±11.29b	48.12±11.29b
'Nufar'	16.10±10.31ab	56.26±10.31b	68.54±10.31ab
'Mrs. Burns' Lemon'	31.52±10.31a	129.92±10.31a	90.59±11.29a
'Aroma 2'	2.48±10.31ab	80.29±10.31b	78.55±10.31ab
'Dolly'	28.18±10.31ab	69.62±10.31b	66.23±10.31ab
'Holy'	0.52±10.31b	5.62±11.29d	5.25±11.29c
'Spicy Globe'	4.86±12.60ab	22.11±10.31cd	16.33±10.31c
'Genovese'	10.46±10.31ab	51.02±11.29bc	59.00±10.31b

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-3b. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 2. In this table, 8 cultivars were compared within each fertilizer treatment.

Cultivar	Leaf in Harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	13.33±4.58a	24.63±4.58a	20.43±4.58a
'Nufar'	8.79±4.19a	27.01±4.19b	35.71±4.19b
'Mrs. Burns' Lemon'	11.30±4.19a	38.40±4.19b	27.47±4.58b
'Aroma 2'	1.40±4.19a	31.69±4.19b	31.07±4.19b
'Dolly'	16.04±4.19a	36.80±4.19b	34.86±4.19b
'Holy'	0.43±4.19a	3.87±4.58a	3.54±4.58a
'Spicy Globe'	2.51±5.11a	9.58±4.19a	8.44±4.19a
'Genovese'	6.17±4.19a	26.42±4.58b	27.40±4.19b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-4a. LSMeans of leaf dry weight (g±SE) for 8 basil cultivars in Harvest 2. In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Leaf in Harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	13.33±4.58ab	24.63±4.58b	20.43±4.58bc
'Nufar'	8.79±4.19abc	27.01±4.19ab	35.71±4.19a
'Mrs. Burns' Lemon'	11.30±4.19abc	38.40±4.19a	27.47±4.58ab
'Aroma 2'	1.40±4.19bc	31.69±4.19ab	31.07±4.19ab
'Dolly'	16.04±4.19a	36.80±4.19ab	34.86±4.19a
'Holy'	0.43±4.19c	3.87±4.58c	3.54±4.58d
'Spicy Globe'	2.51±5.11c	9.58±4.19c	8.44±4.19cd
'Genovese'	6.17±4.19c	26.42±4.58ab	27.40±4.19ab

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-4b. LSMeans of leaf dry weight (g±SE) for 8 basil cultivars in Harvest 2. In this table, 8 cultivars were compared within each fertilizer treatment.

Cultivar	Flower in Harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	0.65±2.16a	2.09±2.17a	1.61±2.17a
'Nufar'	0.16±2.00a	4.95±2.00b	4.65±2.00b
'Mrs. Burns' Lemon'	4.76±2.00a	22.31±2.00b	18.31±2.16b
'Aroma 2'	0.04±2.00a	8.53±2.00b	7.62±2.00b
'Dolly'	0.52±2.00a	2.92±2.00a	3.53±2.00a
'Holy'	0.00±2.00a	0.05±2.16a	0.03±2.16a
'Spicy Globe'	0.35±2.37a	3.06±2.00a	2.65±2.00a
'Genovese'	0.16±2.00a	4.51±2.16ab	5.27±2.00b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-5a. LSMeans of flower dry weight (g±SE) for 8 basil cultivars in Harvest 2. In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Flower in harvest 2*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	0.65±2.16a	2.09±2.17cd	1.61±2.17d
'Nufar'	0.16±2.00a	4.95±2.00bcd	4.65±2.00bcd
'Mrs. Burns' Lemon'	4.76±2.00a	22.31±2.00a	18.31±2.16a
'Aroma 2'	0.04±2.00a	8.53±2.00b	7.62±2.00bc
'Dolly'	0.52±2.00a	2.92±2.00bcd	3.53±2.00bcd
'Holy'	0.00±2.00a	0.05±2.16d	0.03±2.16d
'Spicy Globe'	0.35±2.37a	3.06±2.00bcd	2.65±2.00cd
'Genovese'	0.16±2.00a	4.51±2.16bcd	5.27±2.00bcd

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-5b. LSMeans of leaf dry weight (g±SE) for 8 basil cultivars in Harvest 3. In this table, 8 cultivars were compared within each fertilizer treatment.

Cultivar	Whole Plant in Harvest 3*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	26.14±31.13a	301.32±28.45c	173.34±31.13b
'Nufar'	73.47±28.45a	217.79±28.45b	204.17±28.45b
'Mrs. Burns' Lemon'	98.47±28.45a	285.54±28.45b	271.51±28.45b
'Aroma 2'	9.91±28.45a	174.59±28.45b	177.20±28.45b
'Dolly'	84.37±28.45a	157.17±28.45ab	228.58±28.45b
'Holy'	1.38±28.45a	34.57±28.45a	36.15±28.45a
'Spicy Globe'	27.48±28.45a	93.99±28.45a	113.37±28.45a
'Genovese'	56.49±28.45a	206.28±28.45b	239.39±28.45b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-6a. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 3.

In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Whole Plant in Harvest 3*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	26.14±31.13abc	301.32±28.45a	173.34±31.13bc
'Nufar'	73.47±28.45abc	217.79±28.45bc	204.17±28.45ab
'Mrs. Burns' Lemon'	98.47±28.45a	285.54±28.45ab	271.51±28.45a
'Aroma 2'	9.91±28.45bc	174.59±28.45c	177.20±28.45bc
'Dolly'	84.37±28.45ab	157.17±28.45cd	228.58±28.45ab
'Holy'	1.38±28.45c	34.57±28.45e	36.15±28.45d
'Spicy Globe'	27.48±28.45abc	93.99±28.45de	113.37±28.45cd
'Genovese'	56.49±28.45abc	206.28±28.45bc	239.39±28.45ab

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-6b. LSMeans of whole plant dry weight (g±SE) for 8 basil cultivars in Harvest 3.

In this table, 8 cultivars were compared within each fertilizer treatment.

Cultivar	Leaf in Harvest 3*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	11.62±10.49a	102.49±9.57c	57.85±10.49b
'Nufar'	34.94±9.57a	93.08±9.57b	87.52±9.57b
'Mrs. Burns' Lemon'	24.99±9.57a	59.12±9.57b	58.94±9.57b
'Aroma 2'	4.58±9.57a	46.53±9.57b	48.88±9.57b
'Dolly'	38.26±9.57a	68.51±9.57b	95.02±9.57b
'Holy'	1.03±9.57a	19.56±9.57a	23.02±9.57a
'Spicy Globe'	9.69±9.57a	24.79±9.57a	29.33±9.57a
'Genovese'	24.73±9.57a	64.90±9.57b	90.09±9.57b

*Values within rows followed by the same letter are not significantly difference at p<0.05

Table 1-7a. LSMeans of leaf dry weight (g±SE) for 8 basil cultivars in Harvest 3. In this table, 3 fertilizer treatments were compared within each cultivar.

Cultivar	Leaf in Harvest 3*/g		
	100 ppm N from 20N-4.4P-16.6K (treatment 0)	100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)
'Italian Large Leaf'	11.62±10.49a	102.49±9.57a	57.85±10.49b
'Nufar'	34.94±9.57ab	93.08±9.57ab	87.52±9.57a
'Mrs. Burns' Lemon'	24.99±9.57abc	59.12±9.57d	58.94±9.57b
'Aroma 2'	4.58±9.57bc	46.53±9.57de	48.88±9.57bc
'Dolly'	38.26±9.57a	68.51±9.57bcd	95.02±9.57a
'Holy'	1.03±9.57c	19.56±9.57f	23.02±9.57c
'Spicy Globe'	9.69±9.57bc	24.79±9.57ef	29.33±9.57c
'Genovese'	24.73±9.57abc	64.90±9.57cd	90.09±9.57a

*Values within columns followed by the same letter are not significantly difference at p<0.05

Table 1-7b. LSMeans of leaf dry weight (g±SE) for 8 basil cultivars in Harvest 3. In this table, 8 cultivars were compared within each fertilizer treatment.

Fertilizer Treatment	Flower Dry Weight in Harvest 3/g
100 ppm N from 20N-4.4P-16.6K (treatment 0)	9.19±3.47b
100 ppm N from 20N-4.4P-16.6K +6g osmocote (treatment 1)	44.96±3.43a
100 ppm N from 20N-4.4P-16.6K +9g osmocote (treatment 2)	44.57±3.47a

Different letters with in each factor (fertilize treatment and cultivar) indicate least significant differences at p<0.05.

Table 1-8a. LSMeans of flower dry weight (g±SE) for 8 basil cultivars in Harvest 3. This table shows the main effect of treatments on flower dry weight.

Cultivar	Flower Dry Weight in Harvest 3/g
'Italian Large Leaf'	28.41±6.05b
'Nufar'	29.71±5.69b
'Mrs. Burns' Lemon'	78.62±5.69a
'Aroma 2'	30.29±5.69b
'Dolly'	28.19±5.69b
'Holy'	0.56±5.69c
'Spicy Globe'	30.78±5.69b
'Genovese'	36.67±5.69b

Different letters with in each factor (fertilize treatment and cultivar) indicate least significant differences at p<0.05.

Table 1-8b. LSMeans of flower dry weight (g±SE) for 8 basil cultivars in Harvest 3. This table shows the main effect of cultivars on flower dry weight.

EFFECT OF MEDIUM TYPE AND PLANT GROWTH REGULATORS ON MICROPROPAGATION OF ‘HOLY’ (*OCIMUM SANCTUM*) AND ‘DOLLY’ BASIL (*OCIMUM BASILICUM*)

Key words: tissue culture, production timeline, Indole-3-butyric acid, Thidiazuron

Abstract. ‘Holy’ basil (*Ocimum sanctum*) is an important medicinal herb which in our research had a longer production timeline and did not grow as well compared to other species in *Ocimum*. ‘Holy’ plant tissue culture was investigated in previous studies, so we thought that this may be a better production method. In this study, 3 types of media [Murashige and Skoog (MS) medium, Woody Plant medium (WPM), and DKW medium] and 4 treatments of plant growth regulators [the control, 0.1 mg indole-3-butyric acid (IBA) /L, 50 μM (11.0125mg/L) thidiazuron (TDZ), the combination of 0.1 mg IBA /L and 50 μM (11.0125mg/L) TDZ], were compared to see whether they could be used to grow ‘Holy’ plants successfully. ‘Dolly’, a classic sweet basil (*Ocimum basilicum*) cultivar was also tested to see if there was a different effect between basil species. We found that plants placed on DKW medium did not survive. The explants (nodes), which were supplied with TDZ didn’t grow well and had low survival rate after transplant to soilless mix. The explants supplied with nothing or IBA alone grew well. Both ‘Holy’ and ‘Dolly’ explants cultured on MS medium grew better and had higher fresh and dry weight and heights than those grown on WPM. Our results demonstrate that MS medium works well for basil tissue culture. After 9 weeks’ culture in medium, the explants can be transplanted to soilless mix.

INTRODUCTION

'Holy' basil (*Ocimum sanctum*) is known as Tulsi in India. It is an important medicinal herb in Southeast Asia (Baliga *et al*., 2012). The phytochemicals in 'Holy' basil show potential in cancer prevention and treatment (Baliga *et al*., 2012). From a preliminary experiment carried out during 2014-2015, we found that 'Holy' basil didn't grow well under our high nutrition capillary mat growth system. That is, it didn't increase dry weight or plant growth when supplied with high fertilizer levels. At the same time, our seed supplier—Johnny's Selected Seeds—had a crop failure of 'Holy' for two production seasons. So we hypothesized that tissue culture could be used for propagation and production of 'Holy' basil.

Numerous studies have investigated the micropropagation protocols of basil (*Ocimum* spp.) with Murashige and Skoog medium as the most commonly used for micropropagation of 'Holy' basil or other cultivars in *Ocimum sanctum* (Hakkim *et al*., 2007; Bodhipadma *et al*., 2005; Singh and Sehgal, 1999; Pattnaik and Chand, 1996).

So the objective of this research was to see if different media and hormone concentrations could be used to successfully grow 'Holy' basil until flowering. We also cultured 'Dolly' as a representative classic sweet basil (*Ocimum basilicum*) to compare the production timeline and growth condition with 'Holy'.

MATERIALS AND METHODS

Experiment 1

Seeds of 'Dolly' (*Ocimum basilicum*) and 'Holy' (*Ocimum sanctum*) basil were sown in flats of soilless mix (perlite, vermiculite, sphagnum moss peat 1:1:1) and grown

in the greenhouse. After five weeks, the explant material–nodes–were harvested and surface-sterilized in a 10% solution of sodium hypochlorite for 15min, and then rinsed in autoclaved distilled deionized water three times (5 min each). The segment with 2 axillary buds were cut into 5mm pieces and cultured on three types of medium in glass tubes (1 piece of segment in each tube). The media that used were: 1) Murashige and Skoog medium (MS) (Murashige & Skoog, 1962) (supplied with 20g sucrose/L and 6.5g agar/L); 2) Woody plant medium (WPM) (Lloyd & McCown, 1981) (supplied with 20g sucrose/L and 6.5g agar/L; 3) DKW salts medium (Driver & Kuniyuki, 1984; McGranahan, et al., 1987) (supplied with 20g/L sucrose and 6.5g/L agar. All the media were adjusted to pH 5.5-5.7 before autoclaving. The explants were maintained at 75 °F (23.89 °C), 14h photoperiod. The experiment was set up as a completely randomized design with 12 replications to see which medium promoted basil growth. The experimental unit was 1 tube. Data taken included the root and shoot numbers. The data were recorded daily for 3 weeks. As there were preliminary experiments and all results were obvious, no statistical analysis was performed.

Experiment 2

In experiment 1, none of the explants grew on DKW medium. MS medium and WPM were compared following the same process in experiment 1. The root and shoot number were recorded every day for 3 weeks. The experiment was designed as a completely randomized design with 32 replications, so there were 64 tubes with one node in each in total.

Experiment 3

In the third experiment, the object is to determine the optimal plant growth regulator treatment. Again five-week-old seedlings were harvested and the nodes with 2 axillary buds were cut into 5 mm pieces as explant material. The steps were the same as those in experiment 1. After 3 weeks' culture in plant growth regulator-free MS and WPM media, the explants were then transplanted to MS and WPM media each with one of 4 different plant growth regulator treatments: A) the control, no plant growth regulator was added; B) 0.1 mg IBA/L; C) 50 μM (11.0125mg/L) TDZ; D) 0.1 mg IBA/L and 50 μM (11.0125mg/L) TDZ. So there were 8 treatments in total and each of them had 8 replications. The experimental unit is one explant in a glass tube.

After 2 weeks, the explants were transferred to medium without plant growth regulators in a larger container—GA-7 (one explant in each GA-7) --and then grown for another 4 weeks. Plantlets with well-developed roots were cut near the medium surface and soaked in 0.815 g/L Hoagland basal salt mixture solution. Then the plantlets were transferred into pre-moistened soilless mix (sphagnum moss peat, perlite, vermiculite 1:1:1) in plastic pots (2.5" square×3.5" tall). The pots were set in flats with domes to keep moist. Humidity was gradually reduced over 2 weeks. Fifty ppm N 20-10-20 fertilizer was supplied with every watering. The plantlets were set in the same environment as the cultured explants. Pictures were taken every week to record the growth conditions of those plantlets. Three weeks after transplanting to soilless mix, the above-ground parts of plantlets were harvested and dried at 40 ℃ for 7 days. The fresh/dry weight, height, and node number were recorded. The experiment was designed as a completely randomized design with 8 replications, so there were 64 tubes with one node in each to start.

The fresh weight, height and dry weight data were analyzed using analysis of variance (ANOVA) implemented in the GLIMMIX procedure of SAS (Littell, *et al*., 2006). The resulting LSMeans were evaluated using pairwise t-comparisons (alpha = 0.05).

RESULTS

Medium Type

In the first experiment, the three medium types performed differently, and there were some common points for the two cultivars. The explants of both 'Holy' and 'Dolly' basil turned brown or stopped growing after culturing on DKW medium (Table 2-1 & 2-2). Most of the explants died the very next day after culture initiation. In experiment 1, 'Holy' basil plants grew well on both MS medium and Woody Plant medium because the number of explants with shoots and roots looked similar at different stages (Table 2-1). 'Dolly' showed similar results in this experiment (Table 2-2). When we compared the 2 cultivars, more explants of 'Dolly' rooted than 'Holy' at same stages, but 'Holy' grew more shoots than 'Dolly'. It seems that without any plant growth regulators, the nodes could also grow shoots and roots. 'Dolly' grew well on both MS medium and Woody Plant medium, while 'Holy' appeared visually to grow better on MS medium (Figure 2-1).

In experiment 2, when cultured on MS medium, more 'Holy' explants had roots (Table 2-3). As for 'Dolly', the difference between MS medium and WPM was not obvious (Table 2-4), but more explants cultured on WPM had roots.

Plant Growth Regulator and Medium Type

'Holy'

Culture Stage (week 0-9)

After 2 weeks' culture on medium with plant growth regulators, the 8 treatments (medium*plant growth regulators) showed obvious visual differences in morphological characteristics (Figure 2-3).

On MS medium, the explants in treatments with nothing and IBA (treatment A & B) grew normally. In contrast, when explants were supplied with TDZ (treatment C & D), their roots stopped growing and growth of shoots also slowed down, and some of leaves dropped during the culture (Figure 2-3).

On Woody Plant Medium, explants treated with TDZ grew slowly and even stopped, and many of them dropped leaves and turned brown or yellow (Figure 2-3). Most of the explants grown on WPM showed a problem: the part of explants that buried in the medium looked swollen at the bottom (Figure 2-4).

For both the media, explants treated with TDZ (treatment C & D) didn't grow well. For Woody Plant medium, only 5 of 8 replications survived to be transplanted before transplanting to soilless mix (Table 2-5).

After Transplanting (week 10-12)

Three weeks after transplanting to soilless mix, the difference found during the culture stage widened.

For MS medium, the 'Holy' explants in treatments with nothing and IBA (treatment A & B) grew normally and all of the replications survived after transplanting into soilless mix (Table 2-5). Three weeks after growing in soilless mix, roots had already

grown out of the pots and they were ready to be transplanted into larger containers (Figure 2-5). When we compared the 2 treatments, we also found that plantlets supplied with IBA seemed grow better and also had higher dry weight compared to the control (treatment A) (Figure 2-6). However, nearly half of the explants that were supplied with TDZ (treatment C & D) had no roots and thus died after transplanting to soilless mix (Table 2-5).

For Woody Plant Medium, the 'Holy' plantlets with same treatment also performed differently. After transplanting to soilless mix, most plants grew slowly. Plants supplied with nothing or IBA only grew better than those treated with TDZ (Figure 2-7) -- which was similar to the results on MS medium, and there was only 1 plantlet treated with TDZ that rooted. Plants treated with TDZ and IBA looked better than plants with TDZ only since they had more leaves (Figure 2-7).

In general, the 'Holy' plants cultured on WPM did not grow as well as those cultured on MS medium because they had fewer leaves (Figure 2-5 & 2-7), less biomass (Figure 2-7, Table 2-6), fewer node number (Table 2-6) and lower height (Figure 2-8, Table 2-6). However, more plants treated with only TDZ (treatment C) in WPM survived compared to those cultured on MS medium (Table 2-5). Those explants supplied with TDZ stopped growing or died in the production process, while the treatments without TDZ (treatment A and B) grew well and had more nodes and higher heights and biomass (Table 2-6). Plants with IBA appeared grow better than the control (Figure 2-5 & 2-7).

Up to the date that the harvest was carried out (week 12), none of the 'Holy' plants had set flower buds.

The fresh weight, dry weight and height of plants were statistically analyzed. For all the 3 response variables, ‘Holy’ plants cultured in MS medium had better results than those cultured in WPM (Table 2-7). When comparing fresh weight and plant height, plants supplied with treatments without TDZ (treatment A & B) grew better than those supplied with TDZ (Table 2-7). However, when comparing dry weight, the difference between treatments with or without TDZ only was not significant.

‘Dolly’

After 2 weeks of culture on medium with plant growth regulators, ‘Dolly’ plants also showed obvious differences among explants that were supplied with different treatments while being grown on the same medium.

Culture Stage (week 0-9)

After 2 week’s culture on medium with plant growth regulators, the difference between 2 media was not obvious, but the 4 plant growth regulator treatments showed obvious differences in morphological characteristics (Figure 2-9).

The explants in treatments with nothing and IBA (treatment A & B) grew and rooted normally. In contrast, when explants were supplied with TDZ (treatment C & D), their roots and shoots stopped growing, and their leaves kept emerging and enlarging and showed abnormal shape (Figure 2-10).

The segment buried in WPM also looked swollen which was the same with ‘Holy’ (Figure 10). Some of the explants grown on WPM and supplied with TDZ (treatment C & D) died at the apical growing point, while the explants grown on MS medium didn’t (Figure 2-11).

After Transplanting (week 10-12)

Three weeks after transplanting to soilless mix, the differences found in culture stage widened.

For MS medium, the explants in treatments with nothing and IBA (treatment A & B) survived more after transplanting into soilless mix (Table 2-8) and rooted well. Three weeks after growing in soilless mix, their roots had already grown out of the pots and they were ready to be transplanted into larger containers (Figure 2-12). However, nearly all the explants that were supplied with TDZ (treatment C & D) died after transplanting to soilless mix (Table 2-8) and none of them rooted.

For Woody Plant Medium, the shape of leaves looked narrower than normal ones (Figure 13). Among the explants supplied with TDZ (treatment C & D), only 1 of each treatment survived after transplanting (Table 2-8) and neither of them grew normally (Figure 2-13).

In general, the plants grown on WPM still did not grow as well as those grown on MS medium because they had abnormal appearance and lower survival rate (Table 2-8), smaller leaves (Figure 2-12 & 2-13), less biomass (Figure 2-14, Table 2-9), fewer nodes (Table 2-9) and lower height (Figure 2-15, Table 2-9). Most of the explants supplied with TDZ died in the first week after transplanting (Table 2-8) especially those cultured in MS medium, while the most of the plants that supplied with treatments without TDZ grew well. Plants supplied with nothing (treatment A) seemed to grow as well as plants supplied with IBA (Figure 2-12 & 2-13).

Similar to ‘Holy’ plants, none of the ‘Dolly’ plants had bud set at the harvest time.

The fresh weight, dry weight and height of plants were statistically analyzed. Since we didn’t get enough replications, we will look at the main effect. For all the 3 response variables, ‘Dolly’ plants cultured on MS medium had better results than those cultured on WPM (Table 2-10), which was similar with ‘Holy’. However, both fresh weight and dry weight didn’t show significant differences among all the 4 treatments (Table 2-10) though plants supplied without TDZ had more leaves (Figure 2-12 & 2-13). Plant height varied among treatments, and plants with IBA had relatively higher plant height, while plants with treatment C or D had the lowest.

DISCUSSION

From the results above, we can conclude that MS medium worked best in our experiment. However, we didn’t have enough replications to repeat, further studies may be needed. Although basil (*Ocimum* spp.) can grow to be a woody plant, the Woody Plant medium didn’t work as well as MS medium. Neither ‘Dolly’ nor ‘Holy’ plants nodes survived on DKW medium, which is also commonly used as a medium for woody plants.

As for plant growth regulator treatment, most of the previous studies supplied plant growth regulators at the very first stage of culture. In our experiment, we supplied plant growth regulators after 3 weeks’ culture on plant growth regulator-free medium. We found that for both cultivars, when supplied with TDZ, the explants didn’t grow normally like the other 2 treatments. Singh and Sehgal 1999 reported similar results in that, young inflorescences of ‘Holy’ basil cultured on MS medium supplemented with

TDZ produced only non-morphogenetic callus. However, callus formation wasn't recorded in these experiments, but it could be in future studies. The concentrations of TDZ they used ranged from 0.05-1 mg/L, while we used 11.0125mg/L (50 µM), which is much higher. We used this concentration based on a paper of Siddique and Anis 2007. They reported that to induce shoots of *Ocimum basilicum* from shoot tips, the optimal level of TDZ supplementation to medium was 50 µM for 8 days' duration. To induce roots, 1.0 µM IBA performed best.

Other hormones were also commonly used in other studies. Bodhipadma *et al*. 2005 investigated 'Dang' basil, which is also a cultivar of *Ocimum sanctum*, and found that callus from leaves developed best on MS medium supplemented with 0.5mg/L 2, 4-D. Hakkim et al. 2007 used 1 mg/L 2,4-D and 0.1-0.5 mg/L kinetin. Leaf explants with 0.1 mg/L kinetin showed maximum culture response. Dode *et al*. 2003 used cotyledons of *Ocimum basilicum* as explants to propagate, and medium containing 5 mg/L BAP and 0.2 mg/L NAA had the highest shoot formation efficiency. Begum *et al*. 2002 also used NAA to induce root formation and they found that it performed better than IBA or IAA. As for IBA, explants receiving 0.1 mg/L had formed the most roots.

Phippen and Simon (2000) used 16.8 µM TDZ to induced shoots from young leaf explants of several cultivars of *Ocimum basilicum* for 30 days, and then transplanted to plant growth regulator-free medium for 5 weeks before transplanting to soil. When comparing with our experiment, we had similar culture period (9 weeks), but in our experiment, the duration that plant growth regulators were supplied was shorter.

CONCLUSIONS

For tissue culturing ‘Holy’ plants, MS medium worked better when than Woody Plant medium and DKW medium. The nodes formed shoots and roots without plant growth regulators. When IBA was added, the plantlets appeared to grow better, but the height, fresh or dry weight was not different than the control. For both ‘Holy’ and ‘Dolly’, when TDZ was supplemented in the culture medium, the explants stopped growing or grew abnormally, and died quickly after transplanting to soilless mix. Nine weeks is supposed to be the timeline for ‘Holy’ plants to grow from explants (nodes) to plantlets that are ready to be transplanted into greenhouse condition. However, neither ‘Holy’ nor ‘Dolly’ plants flowered in the first 3 weeks after transplanting into soilless mix.

In future studies, short experiments using different media, plant growth regulators and concentrations of plant growth regulators (particularly lower levels of TDZ) should be conducted. The final experiments with more replications should also be conducted.

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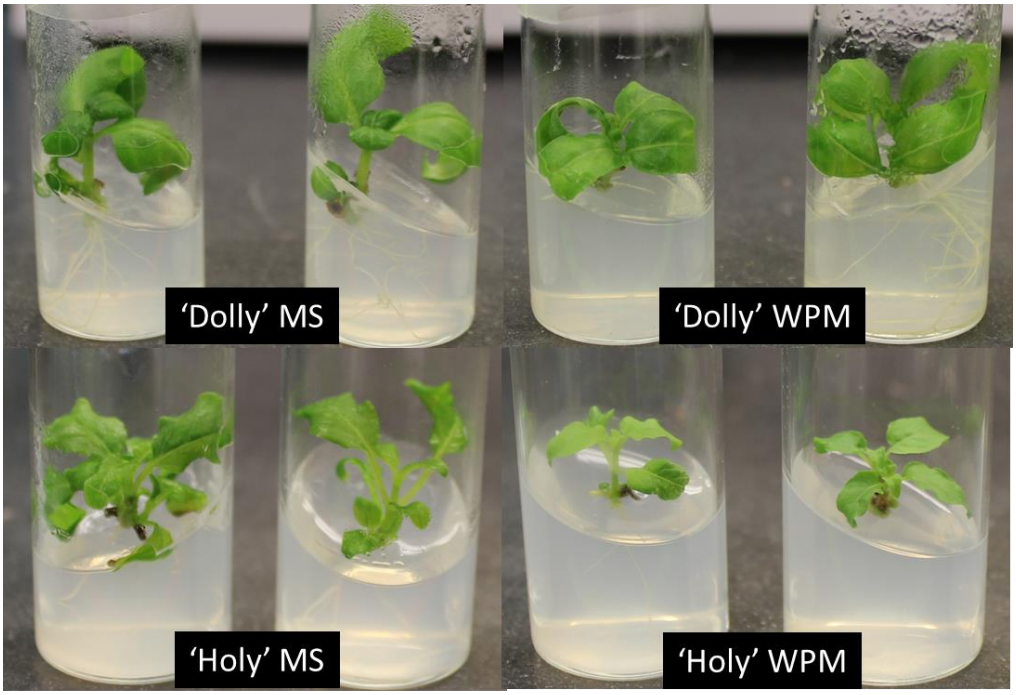

Figure 2-1. Experiment 2. Explants (nodes) of 'Dolly' basil and 'Holy' basil after 3 weeks of culture [plant growth regulator-free medium (Murashige and Skoog medium or Woody Plant medium)].

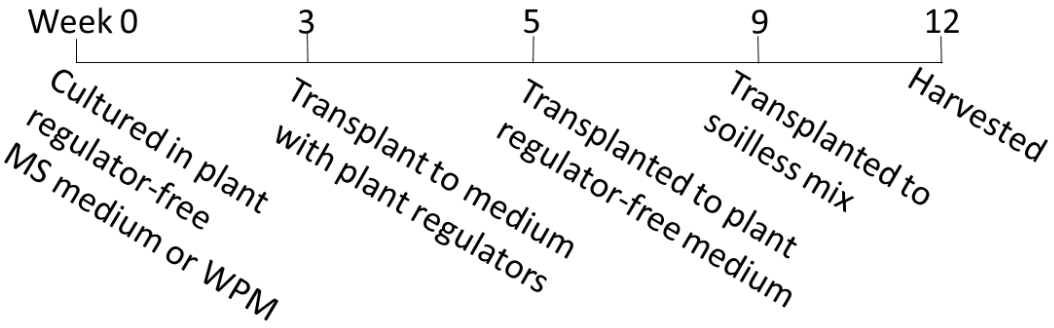

Figure 2-2. Production timeline for 'Holy' basil and 'Dolly' basil tissue culture in experiment 3.

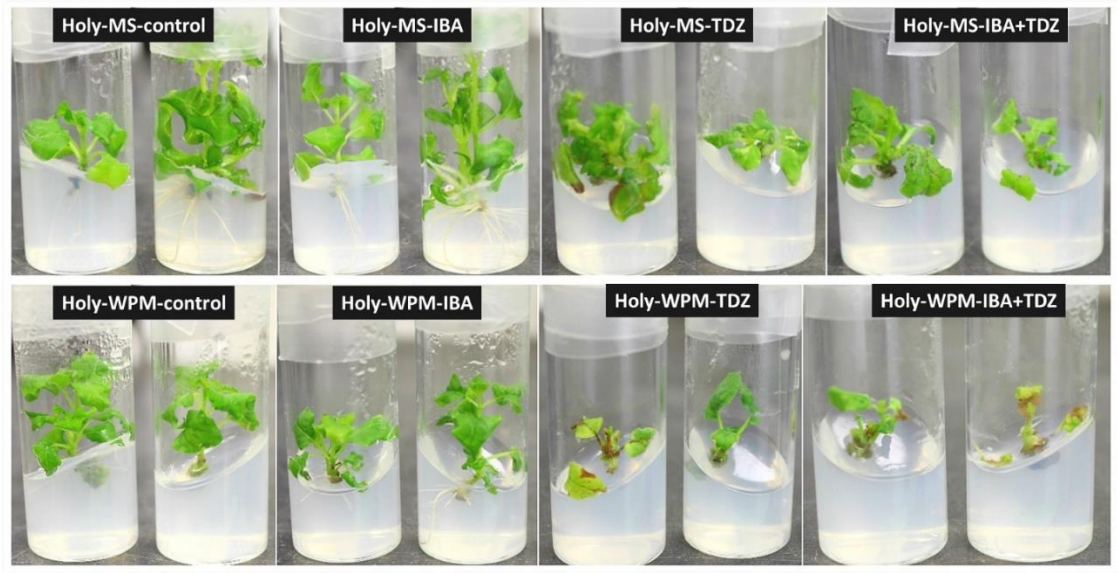

Figure 2-3. Experiment 3. ‘Holy’ basil plants cultured on Murashige and Skoog medium or Woody Plant medium-week 5. Two weeks after culturing in medium with plant growth regulators added.

Figure 2-4. ‘Holy’ basil explant treated with IBA and TDZ on Woody Plant medium in week 5 (after 2 weeks’ culture in medium with IBA and TDZ)—swollen nodal area.

Figure 2-5. Experiment 3. 'Holy' basil plants cultured on Murashige and Skoog medium-week 12. Three weeks after transplanting to soilless mix.

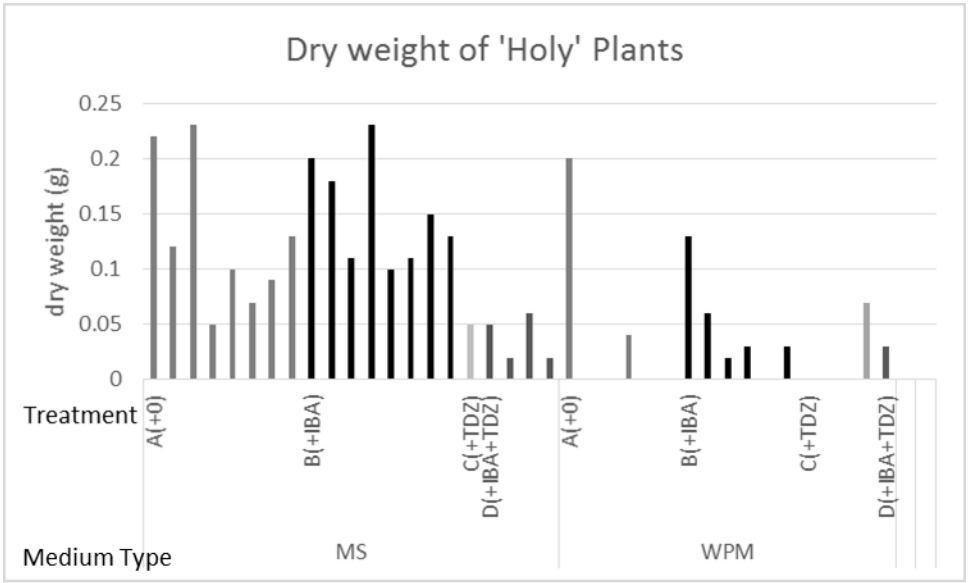

Figure 2-6. Total dry weight of 'Holy' basil plants after 3 weeks of growth in soilless mix.

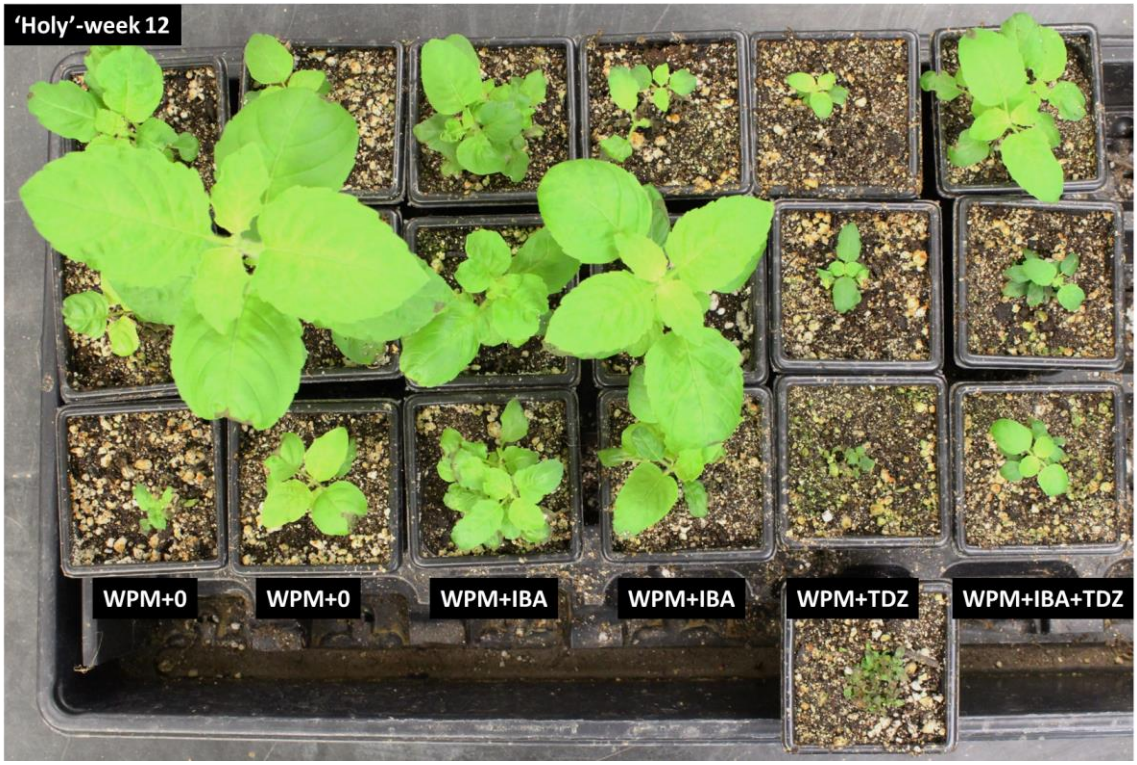

Figure 2-7. Experiment 3. 'Holy' basil plants cultured on Woody Plant medium-week 12. Three weeks after transplanting to soilless mix.

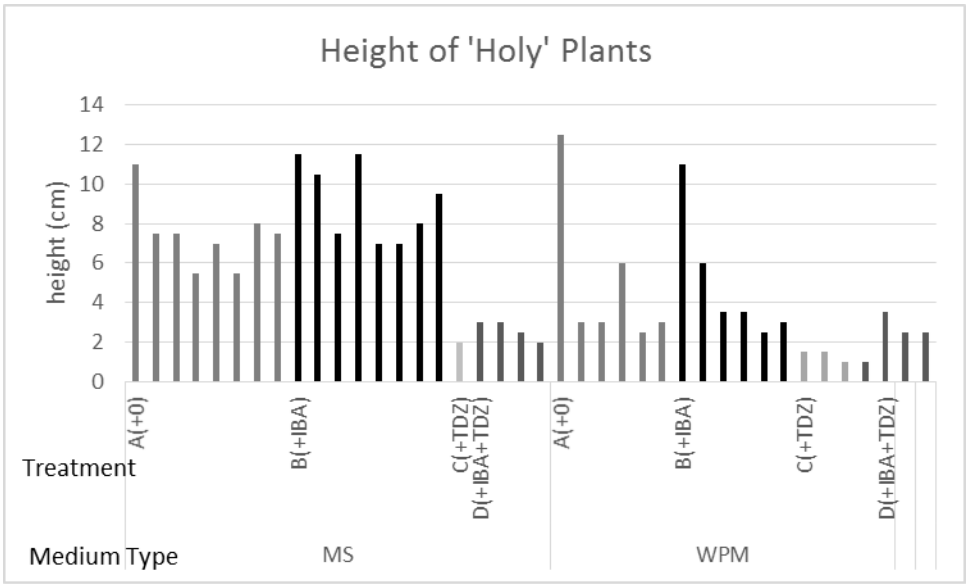

Figure 2-8. Height of 'Holy' basil plants at 12 weeks.

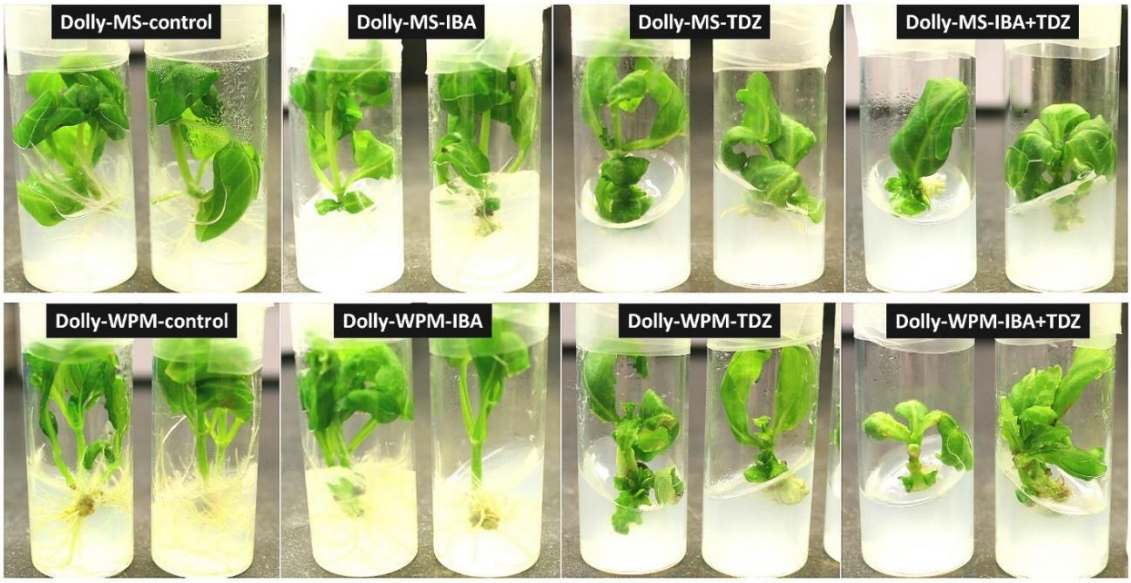

Figure 2-9. Experiment 3. ‘Dolly’ basil plants cultured on Murashige and Skoog medium or Woody Plant medium-week 5. Two weeks after culturing in media with plant growth regulators.

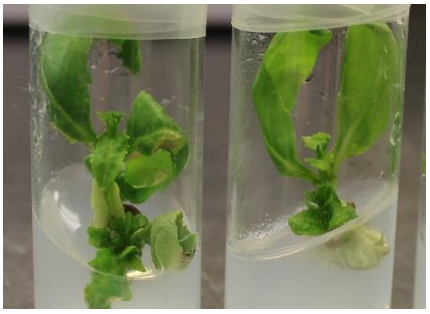

Figure 2-10. ‘Dolly’ basil explants treated with TDZ on Woody Plant medium in week 5 (after 2 weeks’ culture in medium with TDZ). The shoots and roots stopped growing and young leaves grew into abnormal shapes. The segment buried in medium also looked swollen which is same with ‘Holy’ basil.

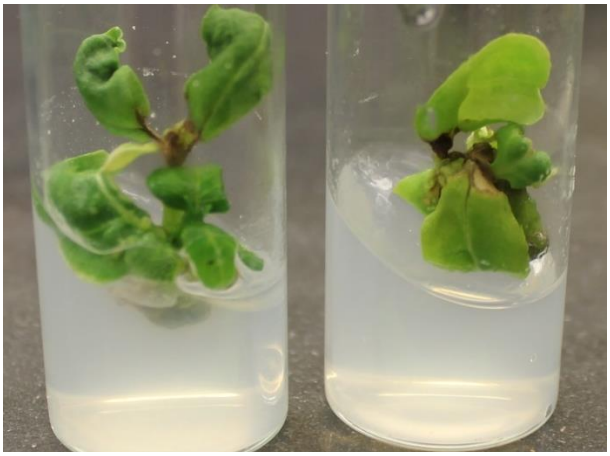

Figure 2-11. ‘Dolly’ basil explants treated with IBA and TDZ on Woody Plant medium in week 5 (after 2 weeks’ culture in medium with IBA and TDZ).

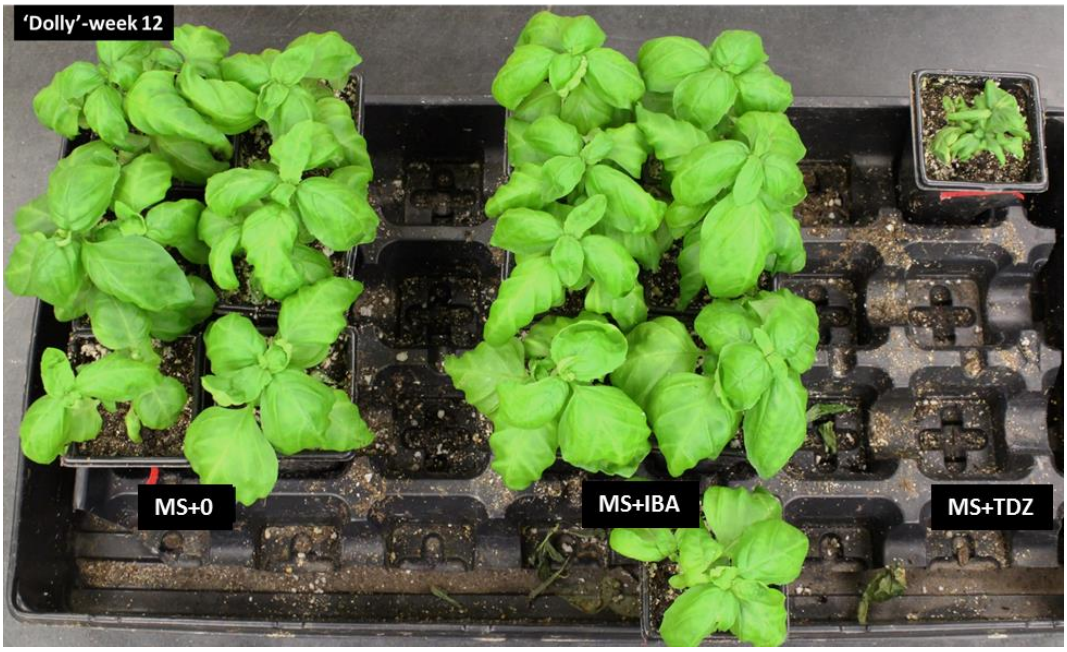

Figure 2-12. Experiment 2. 'Dolly' basil plants cultured on Murashige and Skoog medium-week 12. Three weeks after transplanting to soilless mix.

Figure 2-13. Experiment 3. 'Dolly' basil plants cultured on Woody Plant medium -week 12. Three weeks after transplanting to soilless mix.

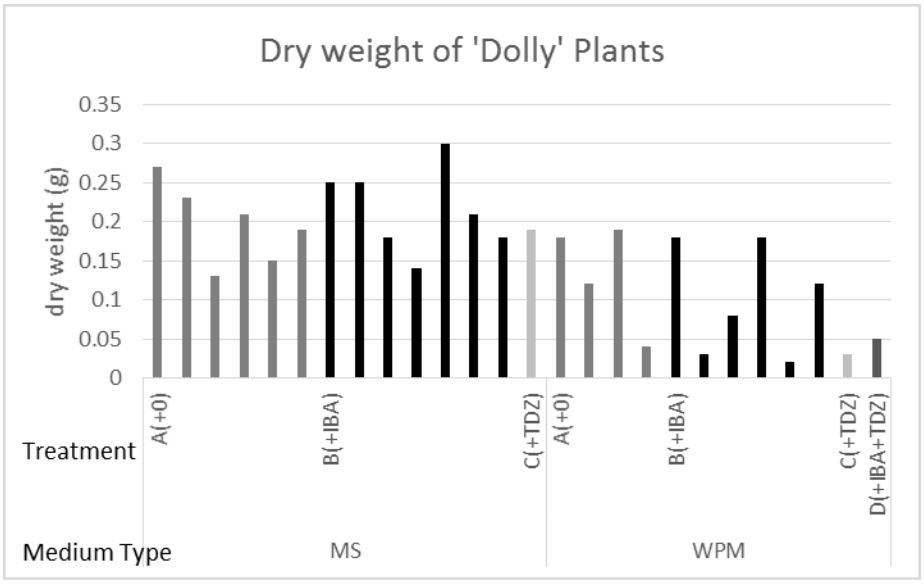

Figure 2-14. Total dry weight of 'Dolly' basil plants after 3 weeks of growth after transplant to soilless mix.

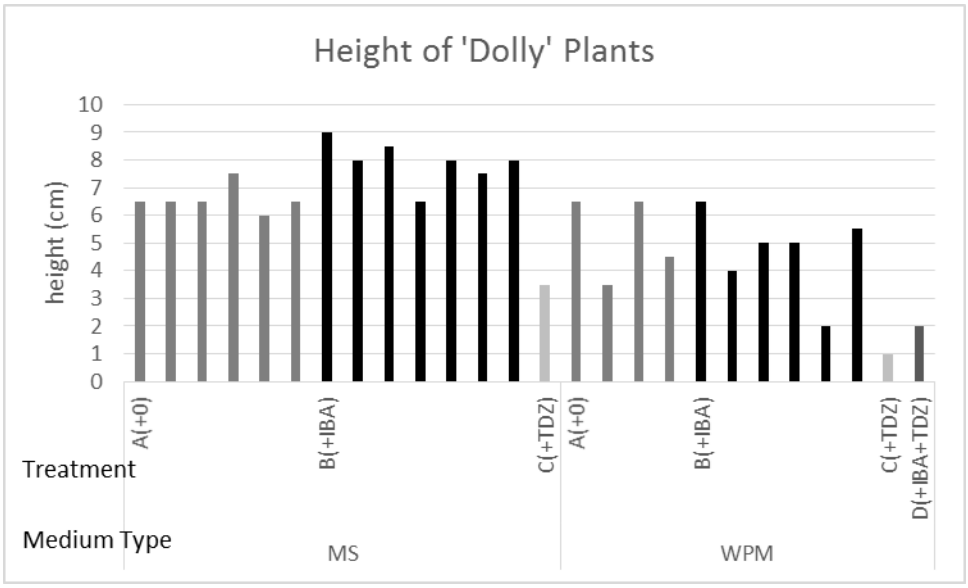

Figure 2-15. Height of 'Dolly' basil plants in week 12.

Media	Week	No. explant with shoots/total no. explant	No. explant with roots/total no. explant
Murashige and Skoog Medium	1	12/12	1/12
	2	12/12	3/12
	3	12/12	5/12
Woody Plant Medium	1	11/12	2/12
	2	11/12	2/12
	3	11/12	4/12
DKW Medium	1	0/12	0/12
	2	0/12	0/12
	3	0/12	0/12

Table 2-1. Experiment 1. Effect of medium type on regeneration of shoots and roots from nodes of ‘Holy’ basil (*Ocimum sanctum*) during the first three weeks in plant growth regulator-free media. The total number of shoots and roots were recorded in this table. Explants in MS medium rooted first after 6 days, and explants in WPM rooted first after 5 days.

Media	Week	No. explant with shoots/total no. explant	No. explant with roots/total no. explant
Murashige and Skoog	1	8/12	3/12
	2	10/12	5/12
	3	11/12	6/12
Woody Plant Medium	1	9/12	2/12
	2	11/12	5/12
	3	11/12	6/12
DKW Medium	1	0/12	0/12
	2	0/12	0/12
	3	0/12	0/12

Table 2-2. Experiment 1. Effect of medium type on regeneration of shoots and roots from nodes of ‘Dolly’ basil (*Ocimum basilicum*) during the first three weeks in plant growth regulator-free media. The total number of shoots and roots were recorded in this table. Explants in both MS medium and WPM rooted first after 6 days.

Media	Week	No. explant with shoots/total no. explant	No. explant with roots/total no. explant
Murashige and Skoog	1	31/32	2/32
	2	31/32	7/32
	3	31/32	16/32
Woody Plant Medium	1	29/32	0/32
	2	30/32	2/32
	3	31/32	4/32

Table 2-3. Experiment 2. Effect of medium type on regeneration of shoots and roots from nodes of 'Holy' basil (*Ocimum sanctum*) during the first three weeks in plant growth regulator-free media. The total number of shoots and roots were recorded in this table. Explants in MS medium rooted first after 6 days, and explants in WPM rooted first after 8 days.

Media	Week	No. explant with shoots/total no. explant	No. explant with roots/total no. explant
Murashige and Skoog	1	23/32	3/32
	2	30/32	22/32
	3	31/32	29/32
Woody Plant Medium	1	25/32	10/32
	2	30/32	30/32
	3	31/32	32/32

Table 2-4. Experiment 2. Effect of medium type on regeneration of shoots and roots from nodes of 'Dolly' basil (*Ocimum basilicum*) during the first three weeks in plant growth regulator-free media. The total number of shoots and roots were recorded in this table. Explants in MS medium rooted first after 6 days, and explants in WPM rooted first after 5 days.

Medium	Week	Survived number of 'Holy' plants/total no. after transplant to soilless mix			
		Treatment			
		A(+0)	B(+IBA)	C(+TDZ)	D(+IBA+TDZ)
MS	1	8/8	8/8	3/7	6/8
	2	8/8	8/8	1/7	4/8
	3	8/8	8/8	1/7	4/8
WPM	1	8/8	8/8	7/7	5/5
	2	6/8	6/8	5/7	3/5
	3	6/8	6/8	4/7	3/5

Table 2-5. Experiment 3. Number of plants surviving after transplanting to soilless mix-‘Holy’ basil.

Cultivar	Medium	Treatment	Replication	Height (cm)	Node no.	Fresh weight(g)	Dry weight (g)
Holy	MS	A(+0)	1	11	6	2.3	0.22
			2	7.5	5	1.31	0.12
			3	7.5	5	2.6	0.23
			4	5.5	5	0.58	0.05
			5	7	5	0.99	0.1
			6	5.5	6	0.87	0.07
			7	8	5	0.97	0.09
			8	7.5	5	1.21	0.13
		B(+IBA)	1	11.5	7	2.21	0.2
			2	10.5	6	1.97	0.18
			3	7.5	4	1.26	0.11
			4	11.5	8	2.6	0.23
			5	7	5	1.09	0.1
			6	7	5	1.03	0.11
			7	8	5	1.38	0.15
			8	9.5	6	1.45	0.13
		C(+TDZ)	1	2	2	0.42	0.05
		D(+IBA+TDZ)	1	3	2	0.68	0.05
			2	3	2	0.28	0.02
			3	2.5	2	0.42	0.06
			4	2	2	0.22	0.02
	WPM	A(+0)	1	12.5	6	2.15	0.2
			2	3	2	0.17	<0.01
			3	3	2	0.16	<0.01
			4	6	4	0.56	0.04
			5	2.5	2	0.1	<0.01
			6	3	2	0.06	<0.01
		B(+IBA)	1	11	6	1.25	0.13
			2	6	4	0.62	0.06
			3	3.5	3	0.36	0.02
			4	3.5	4	0.49	0.03
			5	2.5	2	0.11	<0.01
			6	3	3	0.49	0.03
		C(+TDZ)	1	1.5	2	0.14	<0.01
			2	1.5	2	0.04	<0.01
			3	1	1	0.01	<0.01
			4	1	1	0.8	0.07
		D(+IBA+TDZ)	1	3.5	2	0.47	0.03
			2	2.5	3	0.23	<0.01
			3	2.5	2	0.2	<0.01

Table 2-6. Experiment 3. Height, node number, fresh weight and dry weight of ‘Holy’ basil plants after 3 weeks transplant to soilless mix.

'Holy'	Whole Plant Fresh Weight (g)	Height (cm)	Whole Plant Dry Weight (g)
Medium Type			
Murashige and Skoog medium	1.080±0.142a	5.718±0.583a	0.102±0.013a
Woody Plant Medium	0.355±0.136b	3.283±0.560b	0.024±0.013b
Plant Growth Regulator Treatment			
A (+nothing)	0.950±0.156a	6.219±0.641a	0.084±0.015a
B (+IBA)	1.113±0.156a	7.112±0.641a	0.100±0.015a
C (+TDZ)	0.499±0.266ab	2.130±1.095b	0.047±0.025ab
D (+IBA+TDZ)	0.305±0.220b	2.540±0.905b	0.020±0.021b
Values within columns followed by the same letter are not significantly difference at p<0.05			

Table 2-7. LSMeans of fresh weight, height and dry weight (g±SE) for ‘Holy’ basil plants in week 12.

		Number of 'Dolly' plants/total no. after transplant to soilless mix			
Medium	Week	Treatment			
		A(+0)	B(+IBA)	C(+TDZ)	D(+IBA+TDZ)
MS	1	6/7	7/7	1/7	2/7
	2	6/7	7/7	1/7	0/7
	3	6/7	7/7	1/7	0/7
WPM	1	5/7	7/7	6/7	5/7
	2	4/7	6/7	1/7	1/7
	3	4/7	6/7	1/7	1/7

Table 2-8. Experiment 3. Number of surviving plants after transplant to soilless mix-‘Dolly’ basil.

Cultivar	Medium	Treatment	Replication	Height (cm)	Node no.	Fresh weight(g)	Dry weight (g)
Dolly	MS	A(+0)	1	6.5	5	4.75	0.27
			2	6.5	5	4.04	0.23
			3	6.5	4	1.91	0.13
			4	7.5	5	3.68	0.21
			5	6	4	2.54	0.15
			6	6.5	5	3.32	0.19
		B(+IBA)	1	9	7	4.1	0.25
			2	8	7	4.51	0.25
			3	8.5	6	2.91	0.18
			4	6.5	4	2.79	0.14
			5	8	5	5.21	0.3
			6	7.5	5	3.78	0.21
			7	8	6	2.83	0.18
		C(+TDZ)	1	3.5	2	2.96	0.19
	WPM	A(+0)	1	6.5	5	3.31	0.18
			2	3.5	3	2.04	0.12
			3	6.5	4	3.3	0.19
			4	4.5	3	0.98	0.04
		B(+IBA)	1	6.5	5	3.42	0.18
			2	4	2	0.78	0.03
			3	5	3	1.56	0.08
			4	5	4	2.95	0.18
			5	2	2	0.48	0.02
			6	5.5	5	2.3	0.12
		C(+TDZ)	1	1	1	0.6	0.03
		D(+IBA+TDZ)	1	2	1	0.93	0.05

Table 2-9. Experiment 3. Height, node number, fresh weight and dry weight of ‘Dolly’ basil plants after 3 weeks transplant to soilless mix.

'Dolly'	Whole Plant Fresh Weight (g)	Height (cm)	Whole Plant Dry Weight (g)
Medium Type			
Murashige and Skoog medium	3.050±0.418a	5.624±0.470a	0.182±0.024a
Woody Plant Medium	1.522±0.378b	3.179±0.425b	0.083±0.022b
Plant Growth Regulator Treatment			
A (+nothing)	2.834±0.331a	5.806±0.372ab	0.161±0.019a
B (+IBA)	2.835±0.289a	6.329±0.324a	0.159±0.017a
C (+TDZ)	1.780±0.735a	2.250±0.826c	0.110±0.042a
D (+IBA+TDZ)	1.694±1.061a	3.222±1.191bc	0.099±0.061a
Values within columns followed by the same letter are not significantly difference at $p<0.05$			

Table 2-10. LSMeans of fresh weight, height and dry weight (g±SE) for ‘Dolly’ basil plants in week 12.

Overall Conclusions

In our winter greenhouse growing system, the later the harvest time, the more whole plant, leaf and flower dry weight the basil plants produced. However, other factors such as pest damage and cost of running a greenhouse during the winter should be considered when creating a production timeline. When basil is grown in soilless mix, 100 ppm N from 20N-4.4P-16.6K soluble basic fertilizer does not produce adequate dry matter yield; more fertilizer is needed. 'Mrs. Burns' Lemon' plants could be an important essential oil producer since it produced a very high flower quantity. Other typical sweet basil cultivars such as 'Aroma 2', 'Dolly', and 'Genovese' are also good choices to grow in capillary mat system in greenhouse during winter. 'Dolly' and 'Genovese' are particularly good for fresh harvest. However, neither 'Holy' nor 'Spicy Globe' plants grew well in capillary mat system especially 'Holy' plants, which produced relatively lower dry weight yield. Also, 'Holy' plants had a longer production timeline than all the other cultivars.

For 'Holy' plant tissue culture, MS medium worked better when compared to Woody Plant medium and DKW medium. The nodes formed shoots and roots without plant growth regulators. When IBA was added, the plantlets appeared to grow better, but the height, fresh or dry weight was not different than the control. For both 'Holy' and 'Dolly', when TDZ was supplemented in the culture medium, the explants stopped growing or grew abnormally, and died quickly after transplanting to soilless mix. Nine weeks is supposed to be the timeline for 'Holy' plants to grow from explants (nodes) to plantlets that are ready to be transplanted into greenhouse condition. However, neither 'Holy' nor 'Dolly' plants flowered in the first 3 weeks after transplanting into soilless

mix. However, tissue culture may be a method to produce ‘Holy’ basil seedlings as there was a relatively high survival rate among plantlets.

Appendix

Composition of Murashige and Skoog medium, Woody Plant medium and DKW medium.

MURASHIGE AND SKOOG BASAL MEDIUM		
Component	Qty (mg/L)	Concentration (Molar)
Ammonium Nitrate	1650	20.61mM
Boric Acid	6.2	100µM
Calcium Chloride, Anhydrous	332.2	2.99mM
Cobalt Chloride·6H_2O	0.025	0.11µM
Cupric Sulfate·5H_2O	0.025	0.1µM
Na_2-EDTA	37.26	100µM
Ferrous Sulfate·7H_2O	27.8	100µM
Magnesium Sulfate	180.7	1.5mM
Manganese Sulfate·H_2O	16.9	100µM
Molybdic Acid, Sodium Salt, 2H_2O	0.25	1.03µM
Potassium Iodide	0.83	5.0µM
Potassium Nitrate	1900	18.79mM
Potassium Phosphate Monobasic	170	1.25mM
Zinc Sulfate·7H_2O	8.6	29.91µM
myo-Inositol	100	0.56µM
Glycine	2	26.64µM
Nicotinic Acid	0.5	4.06µM
Pyridoxine HCl	0.5	2.43µM
Thiamine	0.1	0.3µM
Grams of powder to prepare 1 liter	4.44g	
Grams of Agar to prepare 1 liter	6.5g	
Grams of sugar to prepare 1 liter	20g	

(Murashige & Skoog, 1962)

LLOYD AND McCOWN'S WOODY PLANT BASAL SALT MIXTURE		
Component	Qty (mg/L)	Concentration (Molar)
Ammonium Nitrate	400	5.0mM
Boric Acid	6.5	100µM
Calcium Chloride, Anhydrous	72.5	0.65mM
Calcium Nitrate	386	2.35mM
Cupric Sulfate $5H_2O$	0.25	1.0µM
Na2-EDTA	37.3	100µM
Ferrous Sulfate $7H_2O$	27.9	100µM
Magnesium Sulfate	180.7	1.5mM
Manganese Sulfate $2H_2O$	22.3	130mM
Molybdic Acid, Sodium Salt, $2H_2O$	0.25	1.03µM
Potassium Phosphate	170	1.25mM
Potassium Sulfate	999	5.68mM
Zinc Sulfate $7H_2O$	8.6	29.91µM
Grams of powder to prepare 1 liter	2.3g	
Grams of Agar to prepare 1 liter	6.5g	
Grams of sugar to prepare 1 liter	20g	

(Lloyd & McCown, 1981)

DKW BASAL SALT MIXTURE		
Component	Qty (mg/L)	Concentration (Molar)
Ammonium Nitrate	1416	17.7mM
Boric Acid	4.8	77.168µM
Calcium Chloride, Anhydrous	112.5	1.01µM
Calcium Nitrate·4H_2O	1367	8.3mM
Cupric Sulfate·5H_2O	0.25	1.0µM
Na2-EDTA	45.4	121.98µM
Ferrous Sulfate·7H2O	33.8	1.22µM
Magnesium Sulfate, Anhydrous	361.49	3.0mM
Manganese Sulfate·H_2O	33.5	0.2µM
Molybdic Acid, Sodium Salt, 2H_2O	0.39	1.61µM
Nickel Sulfate·6H_2O	0.005	8.95mM
Potassium Sulfate	1559	1.95mM
Potassium Phosphate Monobasic	265	72.19µM
Zinc Nitrate	17	
Grams of powder to prepare 1 liter	5.22	
Grams of Agar to prepare 1 liter	6.5	
Grams of sugar to prepare 1 liter	20	

(Driver & Kuniyuki, 1984; McGranahan, et al., 1987)