

**Effect of Pot Size on Flowering and Fruiting Dates in Pepper**

**Summer Internship Report**

**(4 July to 31 August 2022)**

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## **Introduction**

Pepper (*Capsicum* spp.) is an annual herbaceous plant belonging to the *Solanaceae* family (Awuku & Egyir, 2018). There are numerous varieties and subspecies of pepper, with the most common in the market being chili and bell pepper (*C. annuum*). Unlike other vegetables, pepper can produce spicy sensation when it is consumed, due to the secondary metabolite capsaicin. The amount of capsaicin among pepper varieties greatly varies, making different peppers have different spiciness level and therefore diverse utilization.

Peppers are native to Central and South America (Awuku & Egyir, 2018), and now pepper has become one of the most popular crops in East, South, and Southeast Asia, and Latin America. According to the data released by Food and Agriculture Organization, the biggest pepper (green) producer was China, with over 16.6 million tons in 2020. Mexico set at the second place, with 2.8 million tons productions in the same year. In Indonesia, pepper has high economic value and the demand continues to increase every year, as the population is increasing and industries that require pepper are developing and expanding (Subagyo et al., 2010).

Breeding via conventional approaches requires selection of complementary parental genotypes with desired traits, followed by crosses and a series of selection and advancement of superior progenies to release candidate cultivars that are suitable to consumer demands (Wanga, Shimelis, Mashilo, & Laing, 2021). These processes take years to be done, but vary by crop and climate. Modern technologies, such as double haploid breeding and speed breeding, are applicable to reduce the breeding duration (Wanga et al. 2021). In speed breeding, the environment of production is adjusted to the best condition which plant growth can be optimum, aiming to shorten vegetative and reproductive stage. With speed breeding protocol, 3-9 generations can be completed within 1 year (Wanga et al. 2021) depending on plant species. Speed breeding protocol can be implemented for faster breeding program and shorter time advancing to the next generation.

Pepper is a photoperiod insensitive plant, thus extended photoperiod does not facilitate the growth of pepper plants (Dorais, Yelle, & Gosselin, 1996). Morphology and growth of pepper also show no change under the temperature range from 23/20 to 27/20 °C (Hwang, An, Pham, Cui, & Chun, 2020), meaning pepper growth is less affected by temperature, although extremes in temperature certainly have an effect. Therefore, our goal is to manipulate additional environmental factors, besides photoperiod and temperature to breed pepper faster. With this experiment, we would like to determine the effect of pot size on pepper growth. The result of this experiment can be the reference for selecting the correct pot size to shorten breeding time in the future.

## **Materials and methods**

In this study we evaluated the effect of pot size on three WorldVeg-developed chili lines (AVPP9813 [early maturing], AVPP0303 [mid-season maturing] and AVPP0512 [late maturing]) and two WorldVeg-developed sweet pepper lines (AVPP0412 [early maturing] and AVPP0504 [mid-season maturing]) (Table 1). Lines were selected for evaluation based on their respective flowering and maturing dates across multiple seasons under open field production. Our experiment was conducted in a controlled environment greenhouse at World Vegetable Center (Shanhua, Tainan, Taiwan). Seeds were sowed on April 29<sup>th</sup> and transplanted into three different pot sizes (3-inch, 5-inch, and 7-inch) on May 29<sup>th</sup>. During this study, environmental data were collected using a Hobo Pro V2 temperature/relative humidity sensors (U23-001) installed under a plastic

cloche to protect from direct sun exposure in the greenhouse. The soil used in this study had been sterilized before usage.

For this experiment we used different pot size and pepper entries with different maturing time to compare the effect of each treatment on flowering and fruiting time. As the comparison, this report also includes the data of the same experiment conducted in previous season by pepper breeding team of World Vegetable Center.

Table 1. Plants and treatments used in the experiment. AVPP number stands for Asian Vegetable Research and Development Center Pepper Breeding number. Each entry was replicated three times each with five plants.

Rep I	Rep II	Rep III	AVPP number	Remark	Pot Size
1	10	12	AVPP9813	Early	3"
2	6	15	AVPP0303	Medium	3"
3	7	11	AVPP0512	Late	3"
4	9	13	AVPP0402	Early	3"
5	8	14	AVPP0504	Medium	3"
16	24	27	AVPP9813	Early	5"
17	21	29	AVPP0303	Medium	5"
18	22	28	AVPP0512	Late	5"
19	23	30	AVPP0402	Early	5"
20	25	26	AVPP0504	Medium	5"
31	33	35	AVPP0402	Early	7"
32	34	36	AVPP0504	Medium	7"

After transplanting, we checked every plant for its flowering, fruit setting and maturing every day. For flowering, we recorded the date when the first flower started to open (anthesis). We used ruler to measure the length of fruits and recorded the date at the time one of the fruits in a plant had reached 3 cm in length.

For maturity date, we recorded the date when 50% of the first mature fruit in a plant had changed the color into red or yellow. Plant, height and width measurement were done once a plant had reached maturity. Using tape measure, we measured the plant from its base (or soil surface) to the highest growing tip for height and from one growing tip to the farthest growing tip for width. After these measurements, the fruits were harvested after they were completely mature and seeds were extracted for further experiments.

The same experiment was conducted in springtime and we did additional experiment along with this experiment to check the seeds from previous season. Collected seeds underwent length and weight measurement and germination rate test, which are described below. In the future, pepper breeding team of World Vegetable Center will do the same test to the seeds produced from this experiment.

### Seed Length and Weight

To evaluate seed size, we measured the length and weight of seeds. Seed length and weight measurement was done for 20 and 50 seeds, respectively. For seed length, 20 seeds were lined up and measured using electric caliper. For seed weight, 50 seeds were weighted using Mettler Toledo

scale. For every entry, in total 72 plates, both measurements were done with 2 repetitions. Seeds from these tests were further proceed to seed germination rate test.

### **Seed Germination Rate**

Seed germination rate test was intended to evaluate the viability of seeds after speed breeding program. To evaluate germination, seeds produced during previous season were evaluated. Seed germination rate test began on July 21<sup>st</sup> and ended on August 18<sup>th</sup>. For every entry, we used two replications each consisting of 25 seeds. For germination testing, filter paper was placed inside petri dish and 25 seeds were arranged in 5x5 pattern. The filter paper was moistened using 3 ml of distilled water and afterwards only gave appropriate amount of water based on water content inside petri dish. Seeds were grown in growth chamber maintained at 25°C. Observations were done every Thursday from July 28<sup>th</sup> to August 18<sup>th</sup>. In every observation, took pictures of every petri dish to see the germination progress. Some seed might be infected by fungi and it could affect seed germination. Therefore, we removed seeds that were severely infected and change filter paper as needed.

### **Drought Stress to Speed Breeding Protocol**

Before doing drought stress protocol, we measured the amount of water required for soil to be saturated in 3" pot. We took 6 chili pepper and bell pepper plants to conduct this pre-test. For this, water was gradually added until water dropped from the bottom of pot and we checked the amount of water consumed to water one pot. Soil was considered as saturated when water started to drop from the bottom of pot. After calculating the average of water requirement of one pot, water control plant based on the average and only half amount of control plant for drought plants twice a day. According to the average, we watered every control and drought plant with 100 mL and 50 mL of water, respectively. Afterward, the standard of watering changed to 70 and 35 mL and the dryness of soil as the benchmark of watering. The reason will be stated on result and discussion.

In order to establish speed breeding on pepper, we did an additional test of drought stress using two accessions (AVPP9813 and AVPP0504) of pepper. In this experiment, there were 3 replications with total of 12 plants in each. Plants were subjected to two treatments, normal watering (control) and drought stress. We took picture of plants in every 5 days. In every replication, we selected one chili and bell pepper plant from normal and drought treatment as representative. Photos were taken from front and above side of plant. Due to the lack of time, the data of this experiment will not be included in this report and might be released by pepper breeding team of World Vegetable Center in the future.

### **Statistical Analysis**

In this study, we will compare the data from previous experiment and the data collected in this experiment. The comparison is aimed to see the difference in number of days for a plant to reach flowering, fruit setting, and maturity stage. Moreover, in order to check the influence of weather, this report will include the weather data from April 29<sup>th</sup> to August 19<sup>th</sup> and previous growing season. Weather data were collected in every 1 hour and this report will process the data in mean (average) per day. All data collected were further processed in R studio and by performing analysis of variance (ANOVA) and Tukey's HSD test.

## Result and Discussion

### Weather and Relative Humidity

The temperature and relative humidity of the greenhouse were an average of 29.5°C and 81.1%, respectively (Fig. 1). Nevertheless, the results are lower than previous growing season, which were an average of 23.7°C and 72.0% (Fig. 1). The overall temperature of first growing seasons was lower than the second growing season. Meanwhile, the overall RH in second growing season was higher than first growing season overall (Fig. 2).

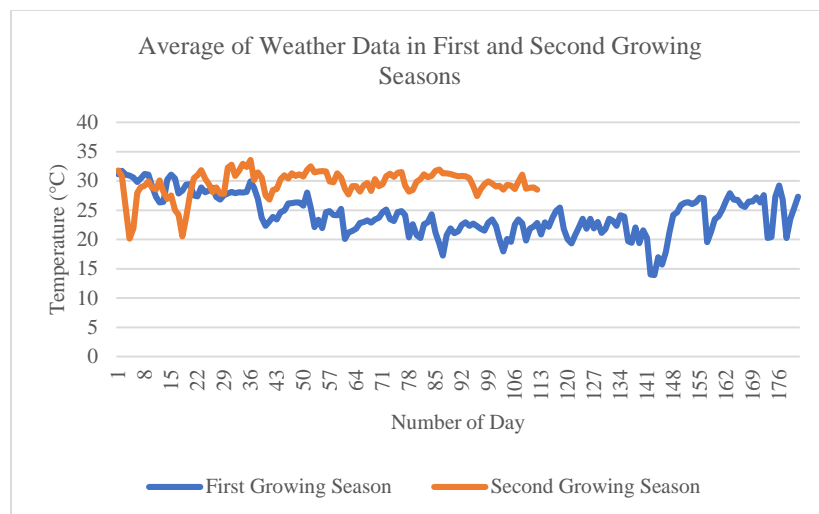


Figure 1. The trend of temperature in first growing season and second growing season. Weather data of first and second growing season was collected daily from October 8<sup>th</sup> to February 28<sup>th</sup> and April 29<sup>th</sup> to August 19<sup>th</sup>, respectively.

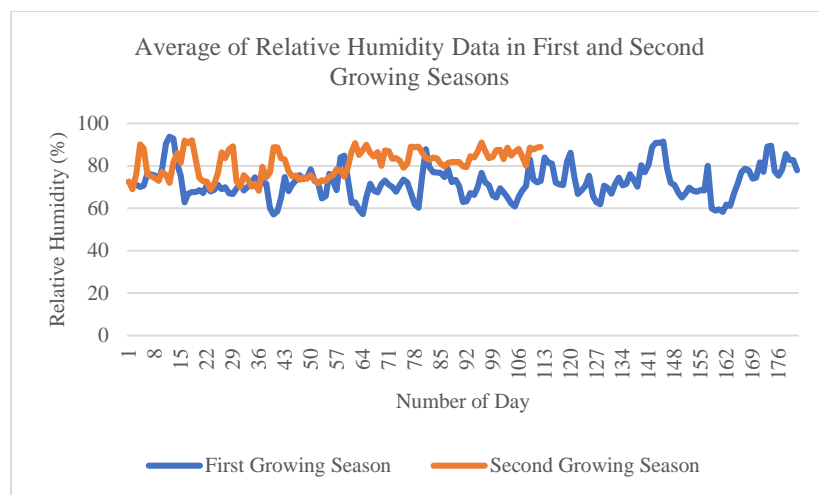


Figure 2. The trend of Relative Humidity (RH) in first growing season and second growing season. Relative humidity data of first and second growing season was collected daily from October 8<sup>th</sup> to February 28<sup>th</sup> and April 29<sup>th</sup> to August 19<sup>th</sup>, respectively.

## Pot Size Experiment

Some of the plants were damaged during this study by mechanical and biological factor. Mechanical damage happened when workers were watering plants and accidentally broke the branch with flower or fruit on it. Meanwhile, most of the biological damage could happened because of thrips and mites and other abiotic and biotic factors (Fig. 3). There were 2 plants with severe damage, and they could not bear flower and fruit. These damages were affecting the result of plant growth rate.



Figure 3. Damages caused by biological factor. (A). One of the sweet pepper fruits was damaged. (B). Chili plant with severe damage. All the leaves were curling and the plant loss its ability to produce flower.

Several sweet pepper plants reached maturity before the fruit had reached 3 cm in length. Fruit would reach its biggest size before becoming mature. Therefore, we assumed that the fruit would not grow bigger and calculate the fruit setting date by deducting 1 week from maturity date because the variety that underwent this condition required 1-2 weeks to change its fruit color.

- **Plant Height and Width**

In chili and sweet pepper, we applied two and three pot size treatments, respectively. Due to the lack of space for roots to grow, plant grown in 3" pot was stunted compared to 7" pot (Figs. 4 and 5). For fruit number, plant with 3" pot treatment produced less fruit than 7" pot treatment. However, this result might not directly contribute to the speed breeding because only a single fruit is required for seeds extraction. Our findings contradict those of Coon et al. (2017) who found no difference in plant size resulting from different pot sizes in dwarf ornamental pepper.



Figure 4. Chili pepper plants, with WorldVeg accession number AVPP0303, grown in two different pot sizes. (A). Using 3" pot and (B). 7" pot to grow chili pepper.



Figure 5. Sweet pepper plants, with WorldVeg accession number AVPP0402, grown in three different pot sizes. (A). Using 3” pot, (B). 5” pot, and (C). 7” pot to grow sweet pepper.

Chili and sweet pepper accessions used in this experiment had different plant height and width in 2 growing seasons. In first and second growing seasons, there are significant differences in plant height and width of each accession. Moreover, chili pepper plant height and width increased compared to the first growing season (Fig. 6). Sweet pepper plants also show the same trend as chili pepper (Fig. 7). We assume this condition was caused by the weather and humidity in second growing season that suit vegetative growth.

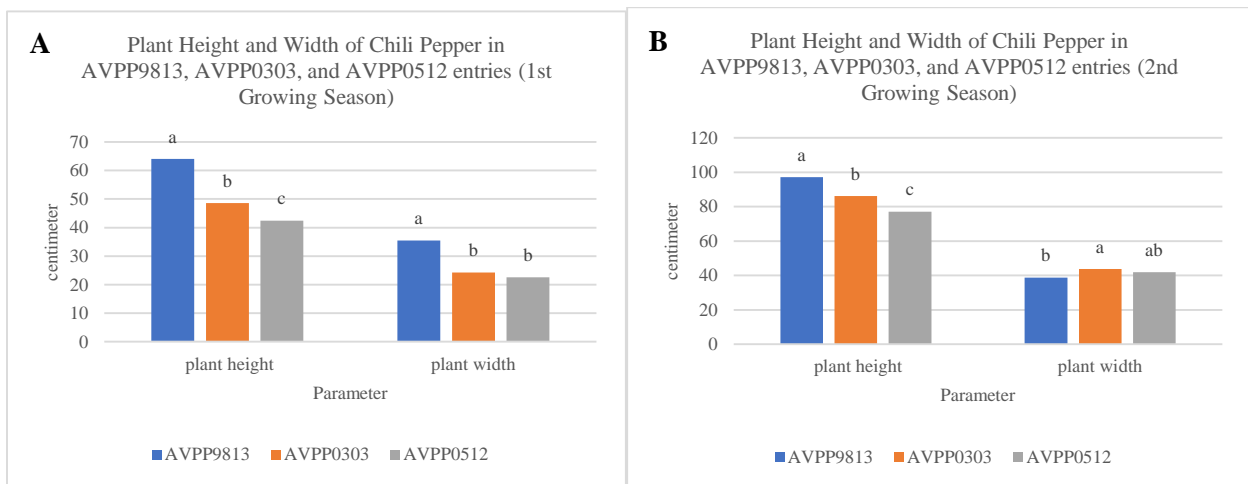


Figure 6. The chart of plant height and width of chili pepper in AVPP9813, AVPP0303, and AVPP0512 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.



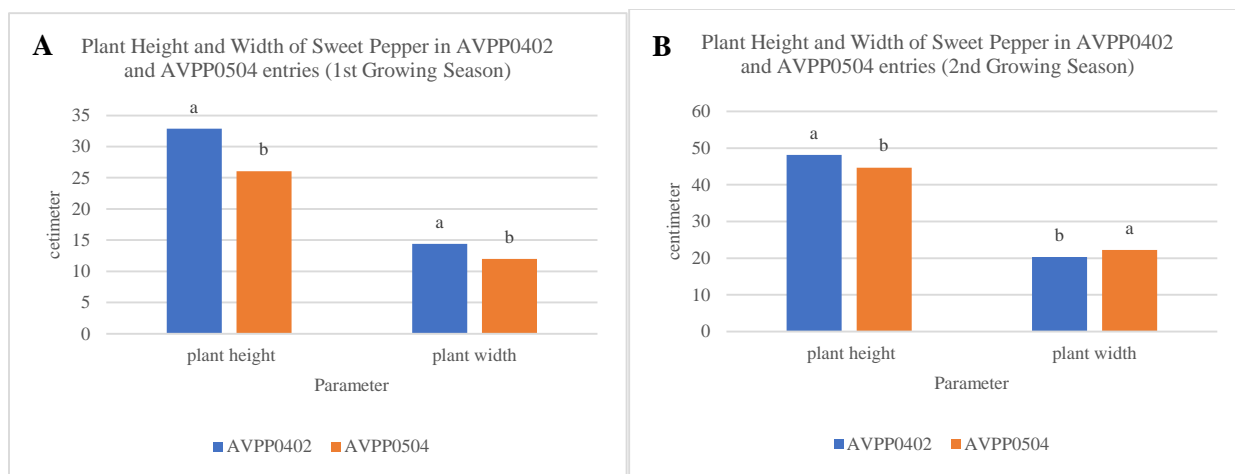


Figure 7. The chart of plant height and width of sweet pepper in AVPP0402 and AVPP0504 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

Between pot sizes, there are significant differences in plant growth and width of chili and sweet pepper (Figs. 8 and 9). The 7" pot treatment resulted in higher and wider plant compared to 3" and 5" pots (Fig. 9). Plants that were grown in second growing season had greater height and width compared to the first growing season (Figs. 8 and 9).

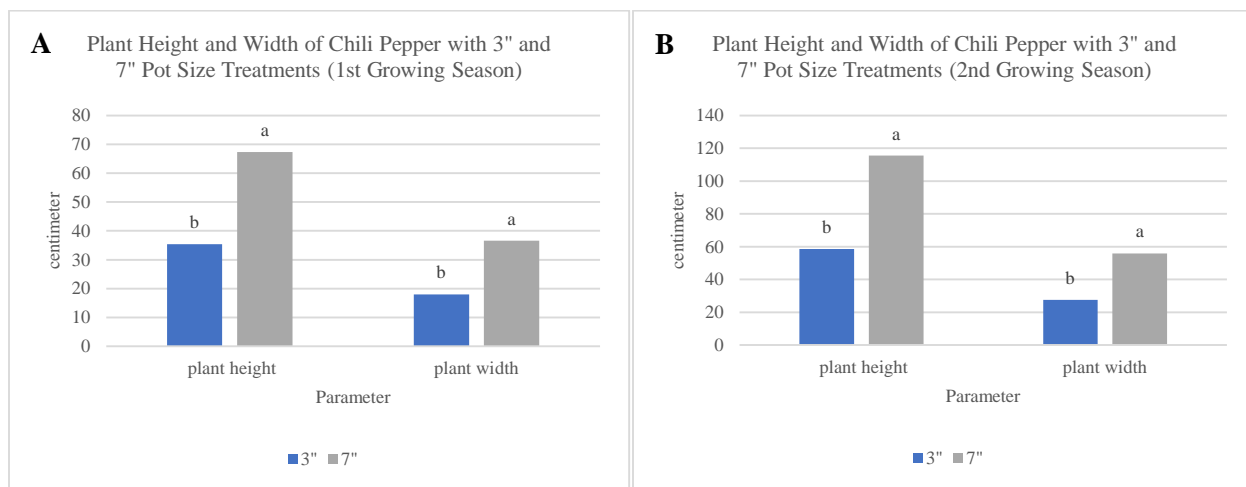


Figure 8. The chart of plant height and width of chili pepper with 3" and 7" pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.



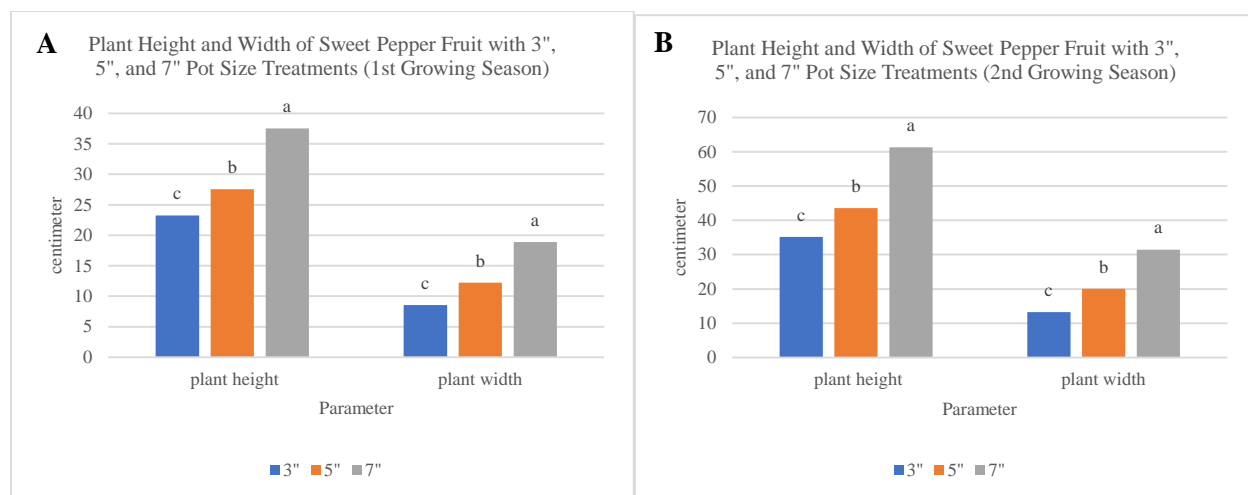


Figure 9. The chart of plant height and width of sweet pepper with 3", 5", and 7" pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

- **Day After Transplanting to Flowering (Anthesis), Fruit Setting, and Maturity**

We compared all parameters, with WorldVeg accessions and pot size as the factor. Comparison among WorldVeg accessions effect of pot size to early, medium, and late maturing plant growth rate. In case of pot size is affecting plant growth rate, we expect significant differences among the results of pot size treatments and similar results of different entries in 2 growing seasons demonstrates that different pot size treatment is indeed affects the plant growth rate in every season.

The number of days after transplanting to flowering (anthesis), fruit setting (set) and maturity among chili pepper entries of AVPP9813, AVPP0303, and AVPP0512 were different between the 2 growing seasons. In the first growing season, early, medium, and late maturing plants had significant difference in each parameter. In second growing season, early, medium, and late maturing plants did not show significant difference, mainly on day after transplanting to fruit setting (days after transplanting set) (Fig. 10). We suspect this result was caused by the weather in second growing season that did not support reproductive growth of AVPP9813, despite the fact that the weather in second growing stage could be supportive for vegetative growth. The time when most of the plants had started to enter reproductive stage, the rainfall intensity and relative humidity was high (Fig. 2).

The growth rate between sweet pepper accessions is more predictable. The overall trend in the first season is similar to the second season (Fig. 11), with the exception in days to fruit set. In second growing season, days to set of AVPP0504 and AVPP0402 had significant difference, compared to the first growing season with no significant difference. Nonetheless, the day after transplanting to maturity for both accessions were not significantly different.

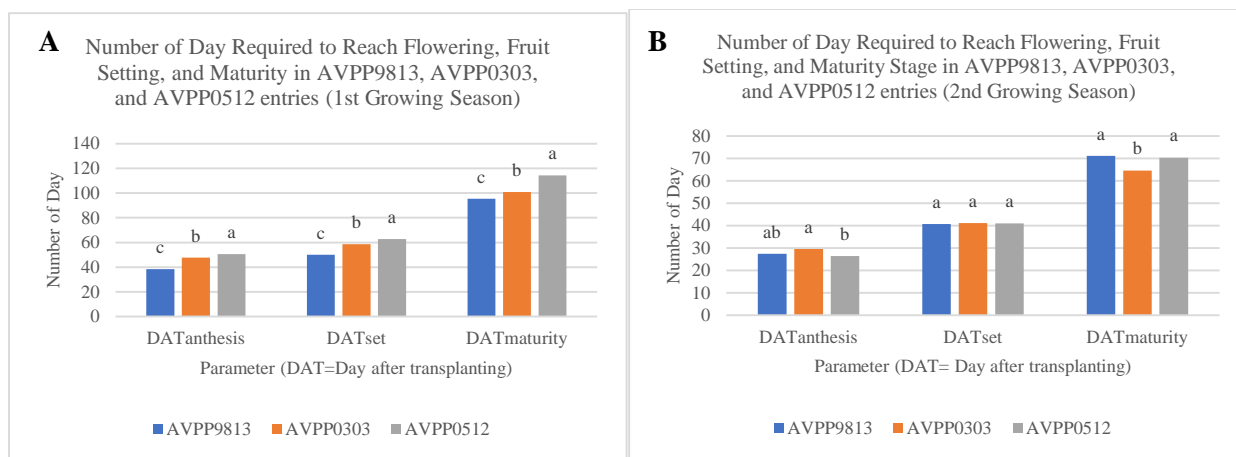


Figure 10. Number of days required to reach flowering, fruit setting, and maturity stage in AVPP9813, AVPP0303, and AVPP0512 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

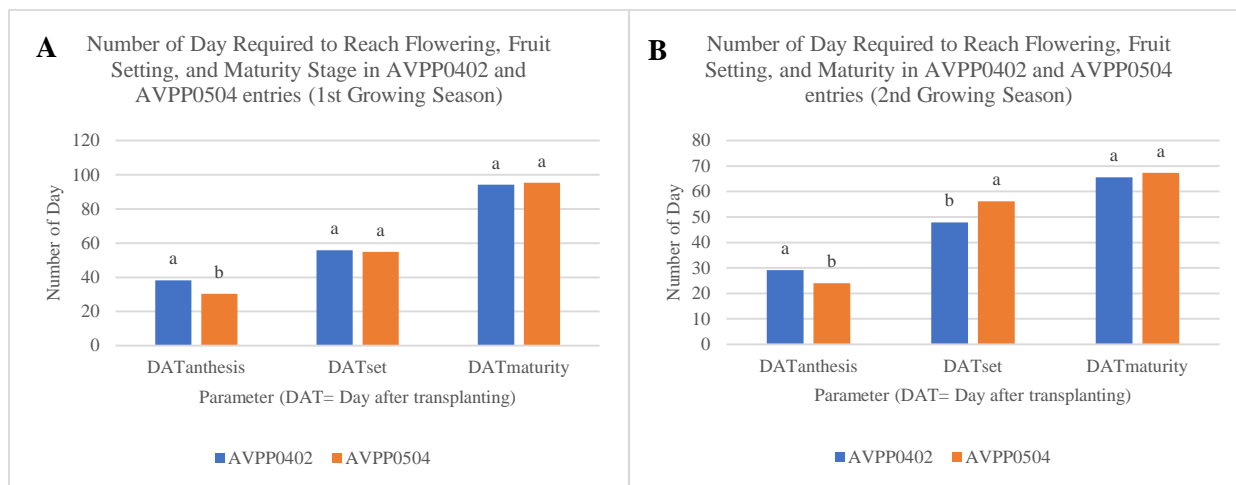


Figure 11. Number of days required to reach flowering, fruit setting, and maturity stage in AVPP0402 and AVPP0504 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

In our observation, pot size did not significantly affect the growth rate of chili and sweet pepper plant. Based on HSD result, day after transplanting to flowering (anthesis), fruit setting (set), and maturity stages among 3 pot sizes were not significantly different (Figs 12 and 13). Despite the fact that 3", 5", and 7" pot sizes were significantly different in days to flowering stage of sweet pepper (Fig. 13), but number of days after transplanting to maturity for all pot sizes remained similar.

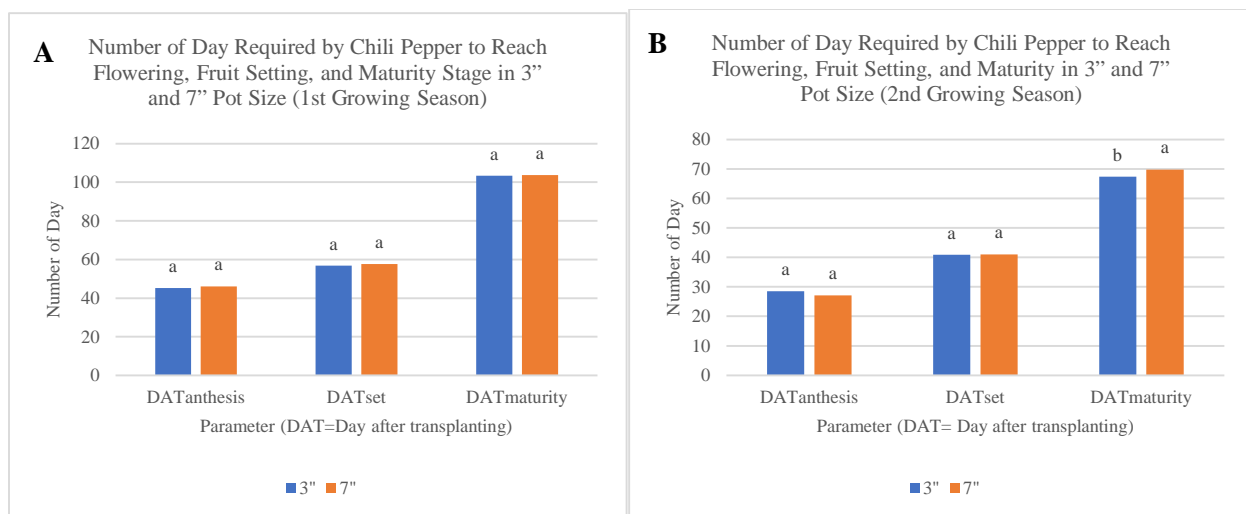


Figure 12. Number of days required for chili plant to reach flowering, fruit setting, and maturity stage in 3'' and 7'' pot sizes. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

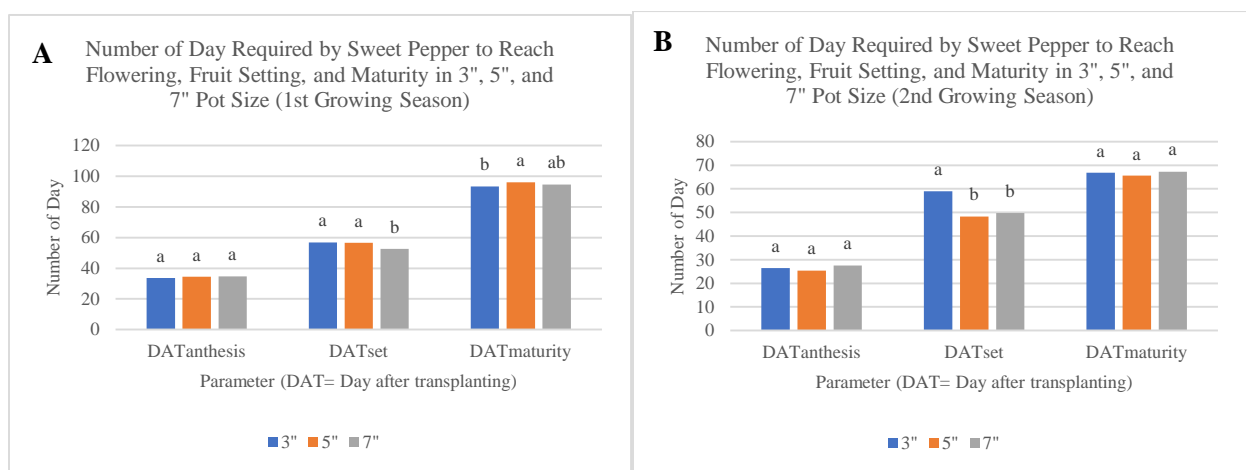


Figure 13. Number of days required for sweet pepper plant to reach flowering, fruit setting, and maturity stage in 3'', 5'', and 7'' pot sizes. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

### • Fruit Length, Width, and Weight

Overall, fruit length and width of AVPP9813, AVPP0303, and AVPP0512 entries grown in different growing seasons were not significantly different. However, in second growing season, fruits length of AVPP0303 were significantly larger compared to first growing season (Fig. 14). Meanwhile, sweet pepper fruits that were produced in second growing season were showing decrease in length and width compared to previous season (Fig. 15). We presume this difference might happen because of different weather condition and had no correlation with pot size treatment.

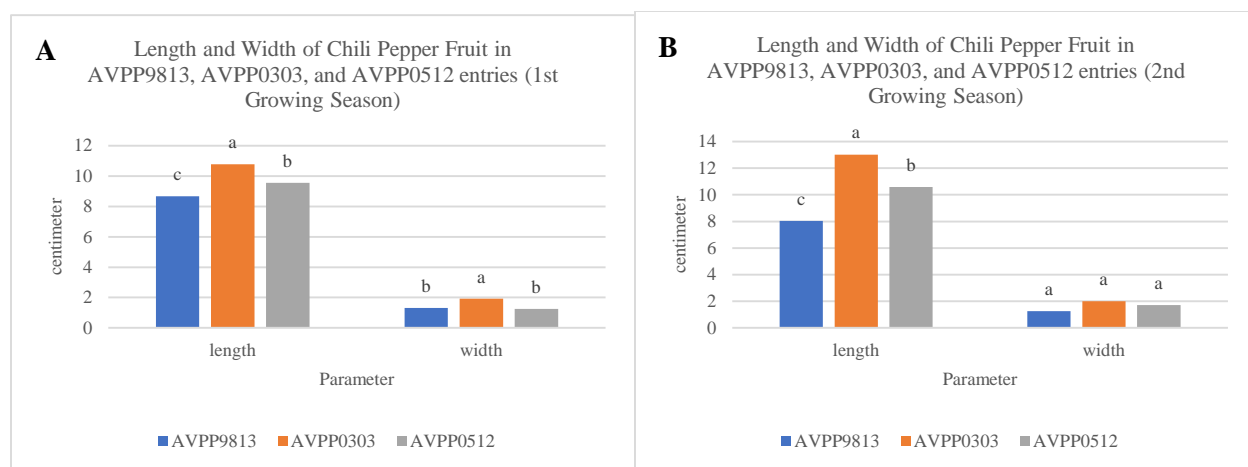


Figure 14. Length and width of chili pepper fruit in AVPP9813, AVPP0303, and AVPP0512 entries. (A) in first growing season, (B). in second growing season. Letters above every bar represent the data group.

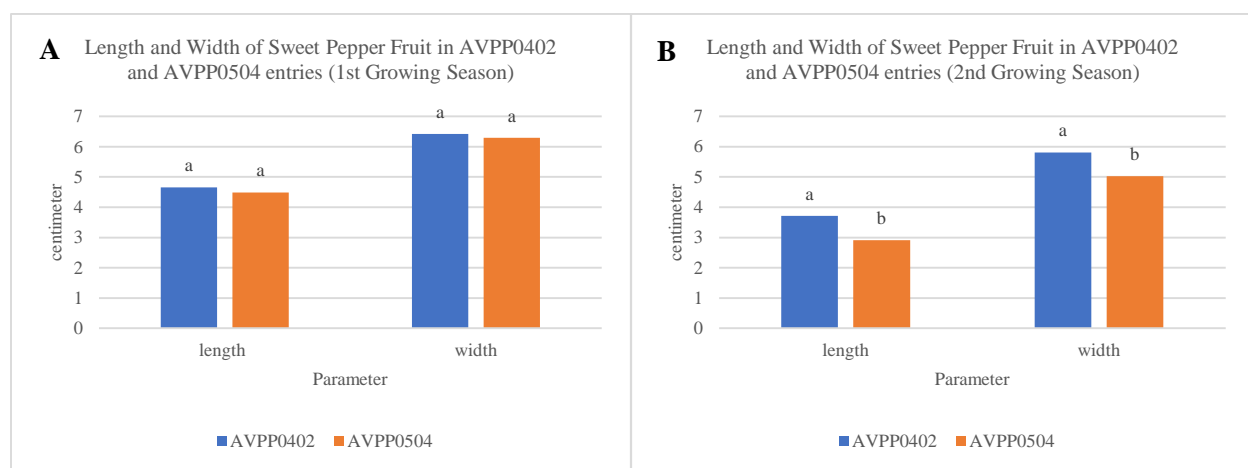


Figure 15. Length and width of sweet pepper fruit in AVPP0402 and AVPP0504 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

Fruit length of chili pepper was significantly different among 3” and 7” pot treatments (Fig. 16). The second growing season resulted in longer chili pepper fruits in both pot sizes (Fig. 16). Different pot size treatments in sweet pepper significantly affected fruit size, with bigger pot size resulting in bigger fruits (Fig. 17). In contrast with the result of chili pepper in different growing season, sweet pepper fruit length and width were lower than first growing season (Fig. 17). These results indicate that pot size, along with weather of different season, will affect fruit size.

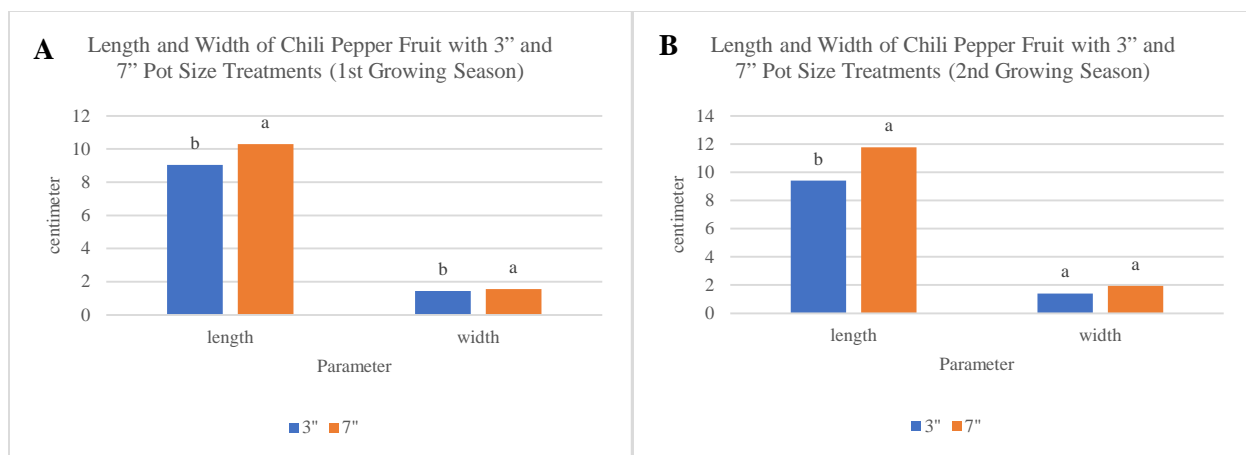


Figure 16. Length and width of chili pepper fruit with 3'' and 7'' pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

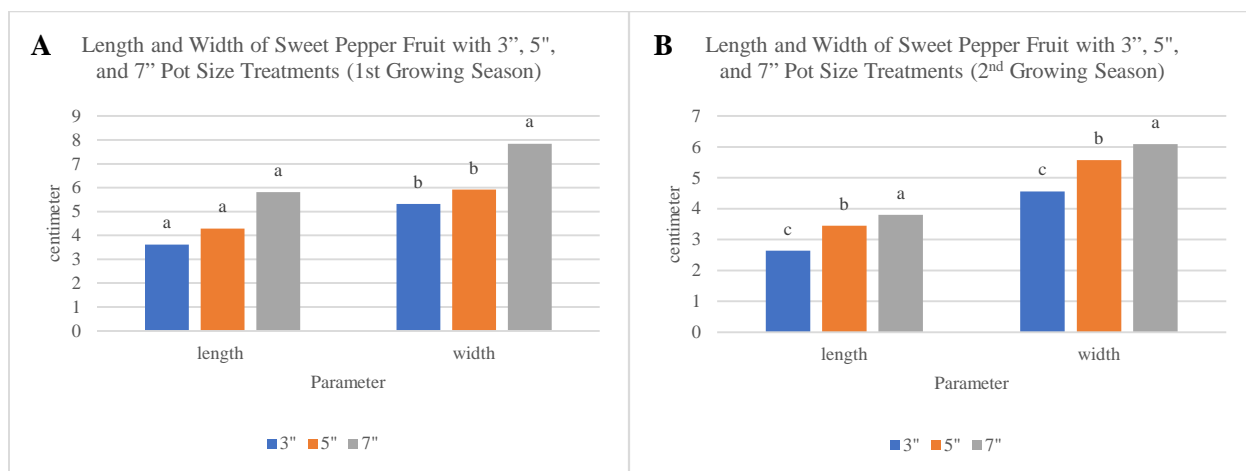


Figure 17. Length and width of sweet pepper fruit with 3'', 5'', and 7'' pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

Weight of AVPP0303 was significantly different from AVPP9813 and AVPP0512 (Fig. 18). Fruits that were produced in second growing season was lighter than in first growing season (Fig. 18). Fruit weight between sweet pepper entries of AVPP0402 and AVPP0504 were significantly different in both growing seasons, with lighter fruit weight in second growing season (Fig. 19). Therefore, weather of different seasons also contributed to fruit weight.

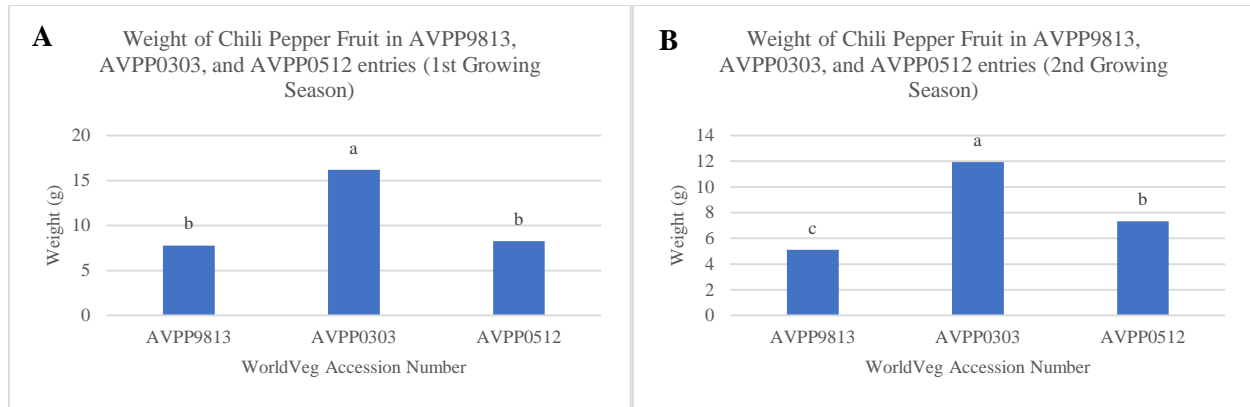


Figure 18. Weight of chili pepper fruit in AVPP9813, AVPP0303, and AVPP0512 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

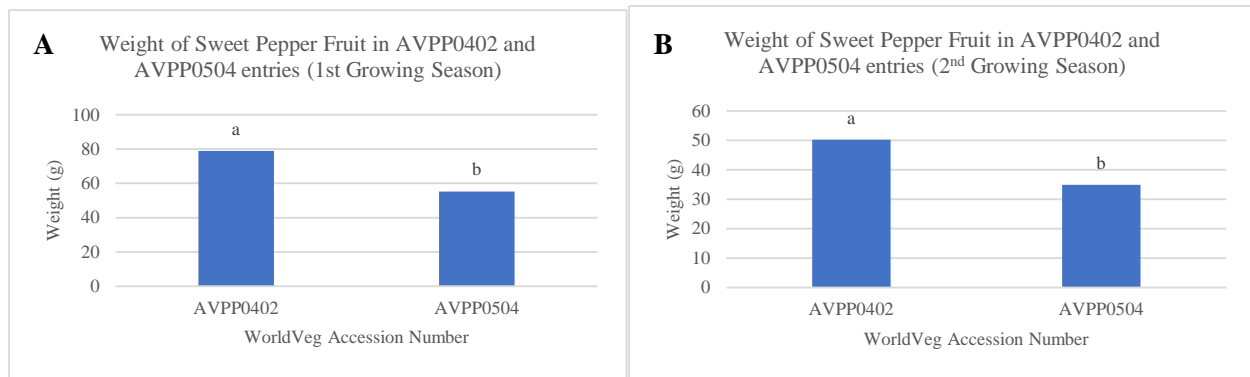


Figure 19. Weight of sweet pepper fruit in AVPP0402 and AVPP0504 entries. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

For the different pot size treatments, chili peppers with 7" pot treatment produced significantly less fresh weight than 3" pot (Fig. 20). This phenomenon could happen because most of the fruits from 7" pot treatment was not ripened at the time we harvested the yield. Therefore, the weight of chili pepper fruit in second growing season cannot be compared to first growing season. There were significant differences in fruit weight between sweet pepper plants with different pot size treatments. Second growing season produced lighter sweet pepper fruit than first growing season (Fig. 21). Therefore, pot size and growing season could affect fruit weight of sweet pepper.

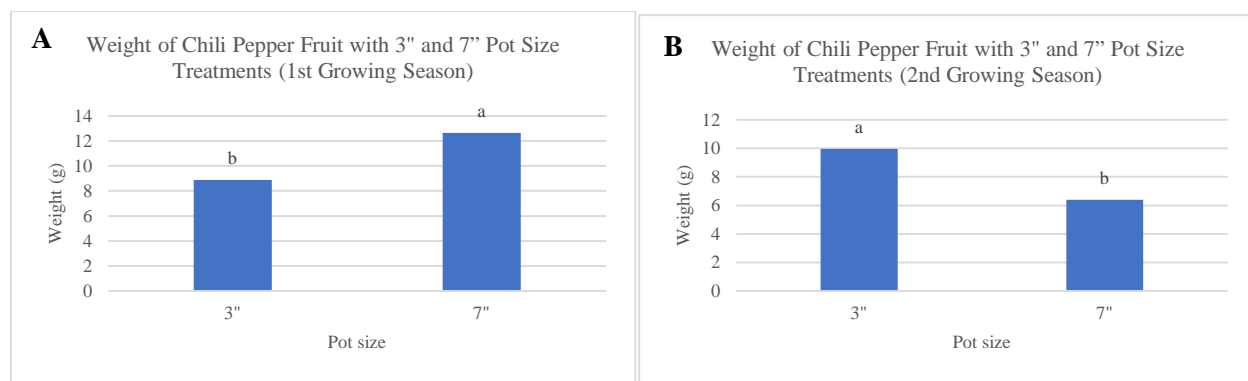


Figure 20. Weight of chili pepper fruit with 3" and 7" pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

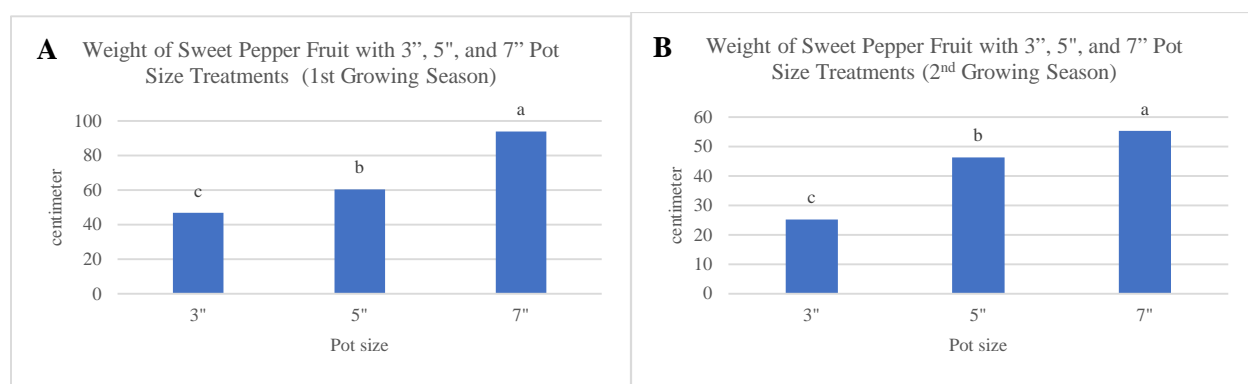


Figure 21. Weight of sweet pepper fruit with 3", 5", and 7" pot size treatments. (A). in first growing season, (B). in second growing season. Letters above every bar represent the data group.

All data for the entries and treatments for days to flowering and fruiting and fruit size for the chill pepper (Table 2) and sweet pepper (Table 3) were compared. In general, season 2 resulted in early flowering and maturity and larger chili fruits but smaller sweet pepper fruits. It is anticipated that the influence of growing environment was the greatest contributed to the variability, but this analysis was not done here.

Table 2. The data comparison of chili pepper grown in 3" and 7" pots. Number 1 and 2 represents first and second growing season, respectively.

Pot size	DAT to anthesis 1 (day)	DAT to anthesis 2 (day)	DAT to fruit setting 1 (day)	DAT to fruit setting 2 (day)	DAT to maturity 1 (day)	DAT to maturity 2 (day)	Fruit Length 1 (cm)	Fruit Length 2 (cm)	Fruit Width 1 (cm)	Fruit Width 2 (cm)	Fruit Weight 1 (g)	Fruit Weight 2 (g)
3"	45.2 a	28.6 a	56.8 a	40.9 a	103.4 a	67.4 b	9 b	9.4 b	1.4 b	1.4 a	8.9 b	10.0 a
7"	46.0 a	27.1 a	57.7 a	41.0 a	103.7 a	69.8 a	10.3 a	11.8 a	1.6 a	1.9 a	12.6 a	6.4 b



Table 3. The data comparison of sweet pepper grown in 3", 5", and 7" pots. Number 1 and 2 represents first and second growing season, respectively.

Pot size	DAT to anthesis 1 (day)	DAT to anthesis 2 (day)	DAT to fruit setting 1 (day)	DAT to fruit setting 2(day)	DAT to maturity 1 (day)	DAT to maturity 2 (day)	Fruit Length 1 (cm)	Fruit Length 2 (cm)	Fruit Width 1 (cm)	Fruit Width 2 (cm)	Fruit Weight 1 (g)	Fruit Weight 2 (g)
3"	33.7 a	26.4 a	56.9 a	59.0 a	93.4 b	66.8 a	3.6 a	2.6 c	5.3 b	4.6 c	46.8 c	25.2 c
5"	34.4 a	25.4 a	56.7 a	48.2 b	96.1 a	65.6 a	4.3 a	3.4 b	5.9 b	5.6 b	60.4 b	46.2 b
7"	34.7 a	27.5 a	52.6 b	49.7 b	94.7 ab	67.3 a	5.8 a	3.8 a	7.8 a	6.1 a	94.0 a	55.4 a

### Seed Length and Weight

Seed size is a widely accepted measure of seed quality and large seeds have high seedling survival growth and establishment (Ambika, Manonmani, & Somasundaram, 2014). Therefore, we did additional step to the seeds collected from previous experiment by measuring seeds length and weight.

Based on the HSD test result, there is no significant difference between seed length and weight of chili grown in different pot size (Fig. 22). This result suggests chili pepper grown in 3" pot and 7" pot will produce the similar seed size. Sweet pepper seeds produced from 3" pot has significant difference in length to the 5" and 7" pot (Fig. 23), but are significantly different in weight among 3 treatments (Fig. 23). By these results, we can conclude that bigger pot size will produce bigger seed in sweet pepper.

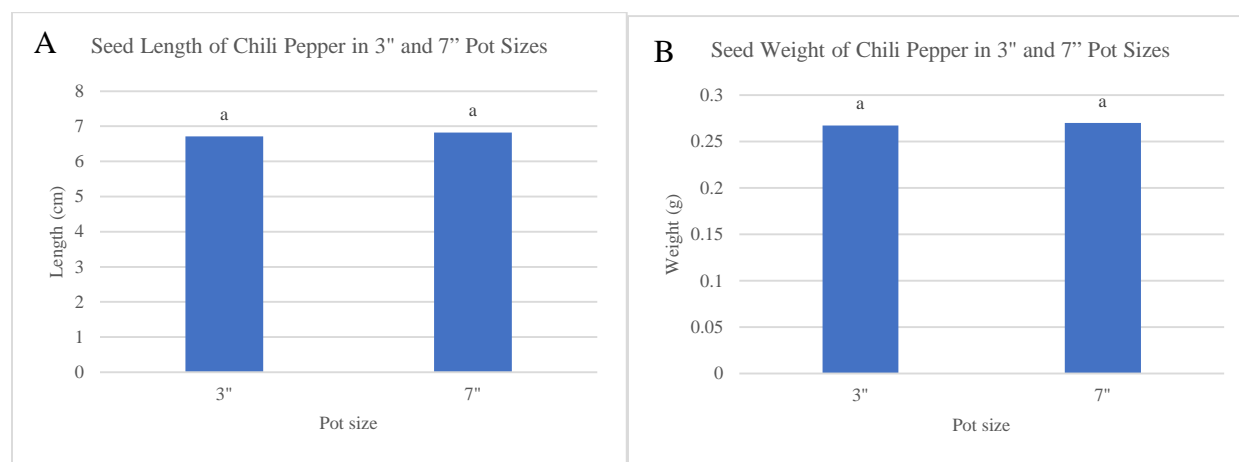


Figure 22. HSD test result of (A). seed length and (B). seed weight of chili pepper grown in 3" and 7" pots. Letters above every bar represent the data group.

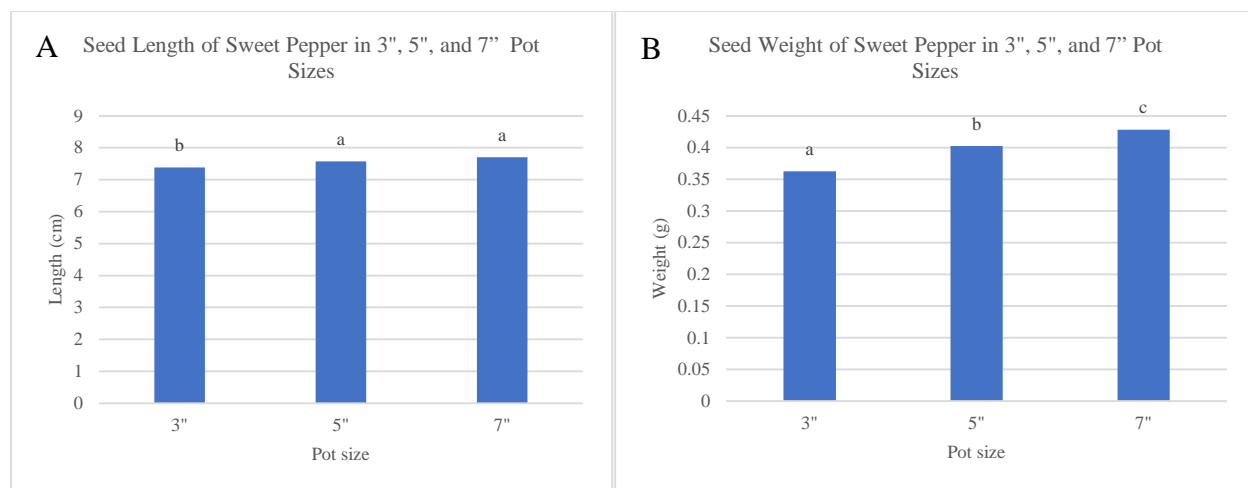


Figure 23. HSD test result of (A). seed length and (B). seed weight of chili pepper grown in 3", 5", and 7" pots. Letters above every bar represent the data group.

### Seed Germination rate

Seed germination is one of the important stages for successful seedling establishment, efficient plant growth and development (Mangena, 2021). Hence, we did the seed germination rate test to inspect the vigor of seeds produced in the previous experiment. In this experiment, we ensured the hygiene of seeds by disinfecting seeds and using disinfected tweezers. Even though seeds used in this experiment had been disinfected with trisodium phosphate (TSP) and hydrochloric acid (HCl) before, but these chemicals still could not prevent mold infection.

Mold started to grow in several petri dishes on the first week after sowing and we changed the filter paper on all petri dishes at the same time. However, mold was still spread to all petri dishes on the second week. We suspect this condition was caused by the growth chamber shared with mycology division of World Vegetable Center. During this experiment, the mycology division was also growing petri dishes with mold inside the petri dish. Seeds with severe infection were discarded and did not included in the data, thus affecting the calculation of seed germination rate.



Figure 24. The example of petri dish with mold infection.

High humidity, along with limited space and low light penetration, made seeds inside the petri dish were struggled to survive and became infected with fungi. Seeds with rot damage were counted as germinate on condition that the embryo has emerged.



Figure 25. The example of petri dish with rotten seeds.

To obtain the germination rate, we divided the number of germinated seeds with the number of discarded seeds (seeds with severe damage and seeds that were not germinating). Despite of the difference between chili and sweet pepper, germination rate calculation of chili and sweet pepper are combined together because we only require the germination rate of every accession and seeds with different pot size treatments.

HSD test result of germination rate data shows no significant difference among sweet pepper entries of AVPP0504 and AVPP0402 and two of the chili pepper entries of AVPP0512 and AVPP9813. However, AVPP0303 of chili pepper is significantly different from the rest accessions. Based on different pot size, there is no significant difference among germination rate of three different treatments (Fig. 26). This result explains that pot size will not affect germination rate.

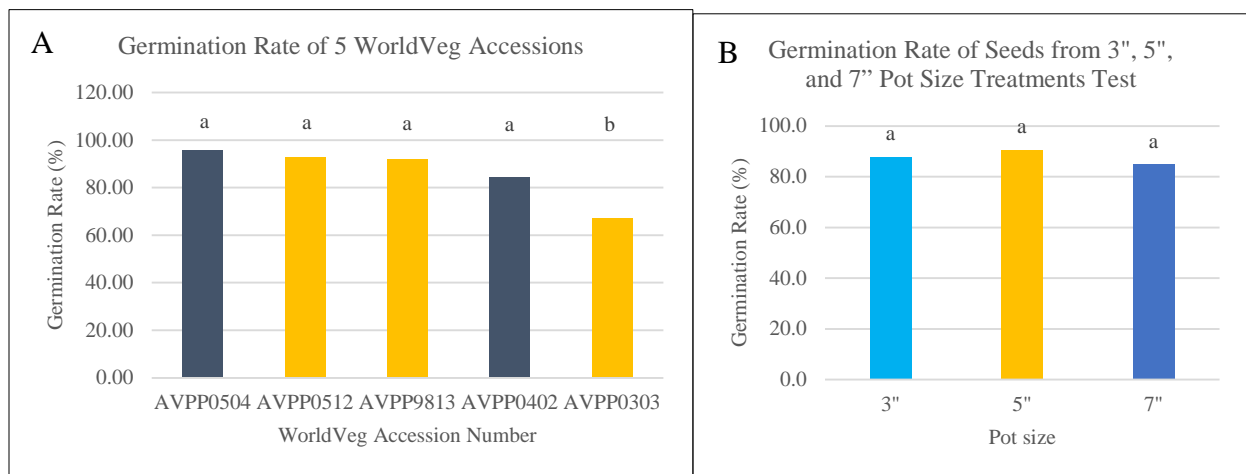


Figure 26. HSD test result of seed germination rate in percentage. (A). Germination rate of five WorldVeg entries. Dark blue bars represent sweet pepper entries, and orange bars represent chili pepper entries. (B). Germination rate of seeds from 3", 5", and 7" pot size treatments.

### Drought Stress to Speed Breeding Protocol

Stress condition, especially water-related stress, is believed to hasten the growth of plant because plant will produce the growth hormone called abscisic acid (ABA) (Muhammad Aslam et al., 2022). For that reason, we conducted the drought stress experiment to provide the basic concept for implementing speed breeding protocol in the future.

All plants grew later than estimation, making the experiment start later than projected. We suspect that cool and overcast weather during this experiment was the cause, with rain fell every afternoon, making sunlight penetration low.

On the second day of the test, we moved all the plants to another side of the same greenhouse. This movement brought an effect on evaporation rate and making the soil become less drought than expected. Therefore, we changed the standard to 70 mL and 35 mL of water and water all plants when the soil of drought treatment plants was dry.

As of this writing of this report, no plant has opened its flower and no data can be collected for this report. This experiment will be continued by pepper breeding team.

### **Conclusion**

The effect of pot size in pepper plant growth rate and seed vigor are not significant. Bigger pot size brings positive effect on plant and fruit morphology (height, length, and width). Therefore, it is recommended to use 3" pot in future experiment because 3" pot could save space and materials. In 100 pot cultivation, the area required for 3", 5", and 7" are 0.8, 2.25, and 4.2 m<sup>2</sup>, respectively. By using 3" pot, we could save up to 3.4 m<sup>2</sup> per 100 pots. The implications of this work is that it will provide a basis for future studies and the development of a protocol for speed breeding in pepper, as speed breeding cultivation is in limited space.

The second growing season (late April to mid-August) is better for speed breeding. Pepper cultivation for harvesting is better in first growing season (October to early March). Higher temperature in April to August can increase the growth rate of chili and sweet pepper. Therefore, we recommend that in addition to drought the next treatment to evaluate for the development of a speed breeding protocol in pepper is temperature. For future work we suggest that an experiment be conducted to identify the optimal temperature to maximize early flowering and maturity without inhibiting pollen viability and germination.

As for seed germination rate test, re-disinfection with 0.5 or 1% bleach solution is required after TSP and HCl disinfection. Moreover, environment around the seeds is important to retain the germination rate to the maximum value. It is recommended to grow seeds for germination rate test in one growth chamber specialized for germination test and not to share with other division.

### **Acknowledgement**

The author would like to thank World Vegetable Center for the summer internship opportunity. During this summer internship, uncountable knowledge was transferred to the author. Furthermore, the author recognizes the hard work of Dr. Derek Barchenger and Hank Lin and would like to thank them for the guidance during the WorldVeg summer internship program. This paper can be finished because of their support.

Last but not the least, the author would like to thank everyone that is involved in this project and summer internship program. The author is deeply grateful for the assistance that all workers had given and the memories that the author has been through with all interns.

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