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Stem and Canopy Architecture Differences and Their Relationship to Productivity In *Capsicum Annuum* Lines

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Architecture of stem and canopy, defined as the number of branches of different categories and size of the canopy, affects leaf distribution and total leaf area. Hence, the architecture might play an important role in photosynthesis, carbon accumulation and fruit yield. The objective of this study is to characterize stem and canopy architecture differences among sib-lines of *Capsicum annuum* inbreds derived from using the single seed descent method. After progeny plants were derived from a cross between 'Jalapeno' and 'Round of Hungary', specific lines were selected based on fruit quality traits. At the F_5 stage, a line RJ107(6)A3 was noted to be morphologically different in terms of branching and canopy architecture from either of the parents. To document the stem architecture and its effects, we counted the number of branches acropetally, measured the height of the stem below the first node, and estimated the leaf areas and the canopy volume for each plant. We found no significant difference among the sib-lines and parents of the pepper varieties in terms of the branch numbers of the first four levels. However, the RJ107(6)A3 line had a significantly greater number of branches of the fifth to the tenth order. There was no significant difference of fruit yield between plants with this branched stem and canopy architecture and the sib-lines without such architecture.

Pepper (*Capsicum annuum*) is a globally important vegetable crop with multiple uses. Plant architecture of pepper plants has significant roles in determining the horticultural traits of the crop, such as determinate nature of stem growth, compactness of the canopy, leaf size, flower position and fruit yield (Elitzur, et al., 2009; Mutlu and Kurtulan, 2015) Branch type and node location have been shown to have influence on the fruit yield of bell peppers (Gaye et al., 1992) and inheritance of traits related to stem architecture have been studied in *Capsicum*. One mutation first described as 'bunchy habit' (Deshpande, 1944) was later described as *fasciculate* (fa), and this mutant had a clustered fruit bearing habit (Bergh and Lippert, 1975; Elitzur et al., 2008). The fasciculate gene is the pepper orthologue of the tomato gene self pruning. Mutations in *Capsicum* resulting in altered canopy architecture known as 'umbrella' branching habit were on three major recessive genes, one of which was fasciculate (McCammon and Honma, 1984). Jeifetz et al. (2011) identified CaBLIND as a regulator of axillary meristem initiation and transition to flowering. A trait called 'pre-bifurcation shooting' was a result of the development of multiple shoots before the first bifurcation of the central stem. This was a quantitative trait controlled by a few genes (Shifriss and Hakim, 1977).

For breeding purposes, we want to know more about the inheritance of the plant architecture related genes and to select lines with favorable canopy architecture. In one of our breeding experiments, we noted a plant with multiple branching stem architecture associated with smaller leaves comparable to fasciculate but with single fruit per axillary meristem. In this study the pepper cultivars 'Round of Hungary' (ROH) and 'Jalapeno' (JAL) were crossed and plants were selected for several generations using single seed descent method. , The F_5 progeny line coded as RJ107(6)A3 had the highly branched canopy while its sister line RJ107(4)B5 lacked it (Fig. 1). The objective of this study is to quantify the stem architectural differences between RJ107(6)A3 and its sister line RJ107(4)B5 and the parental lines ROH and JAL, and test whether architectural differences affected yield under field conditions.

Materials and Methods

PLANT MATERIAL. Seeds of 'Round of Hungary' and 'Jalapeño" were purchased from Park Seed (Hodges, SC). RJ107(6)A3 and RJ107(4)B5 were lines developed by crossing ROH and JAL and following single seed descent method of breeding for five generations.

FIELD TRIALS. Field trials were conducted at the University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) Plant Science Research and Education Unit (PSREU), Citra, FL. The soil was a sandy loam. Pre-plant fertilizer was applied in beds using granular 10–10–10 [(nitrogen:phosphorus:potassium (N:P:K)] with minors at 400 lbs. per acre. Twenty seedling plants of each accession ROH, JAL, RJ107(6)A3, and RJ107(4)B5 were transplanted in the field in Oct. 2017. A spring field trial was planted in Feb. 2018. RJ107(6)A3C and RJ107(4)B5C, the F_6 derivatives, were planted with ROH and JAL.

Fertilizers at the rate of 10 lb/acre of N and 13 lb/acre of K were applied once a week for eleven weeks via drip irrigation after transplanting. Right after transplanting, insecticides were applied through drip injection for whitefly control, after which more
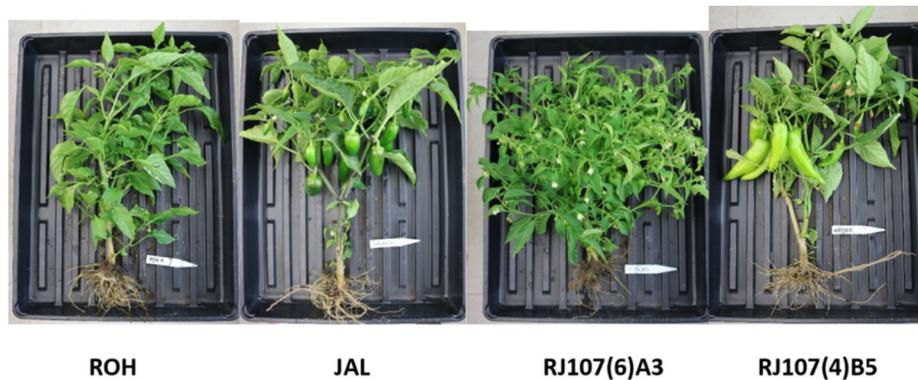
Fig. 1. Difference of plant architecture phenotype. Plants typical of each line, grown in the same field and season were photographed. Note that the RJ107(6)A3 has a denser canopy than its sister line RJ107(4)B5.

pesticides were applied for controlling whiteflies and caterpillars. The irrigation was done twice a day at 30-min intervals with 5 gal water/min/100 inches of row.

For the fall field trial, three plants of each of the four accessions were randomly chosen and evaluated for stem and canopy architecture, leaf area and leaf dry weight, and fruit yield. The evaluations were repeated with four plants of each of the four accessions for the spring field trial in June 2018.

EVALUATION OF STEM AND CANOPY ARCHITECTURE. Height, length, and width of the plant canopy were measured using a ruler and the canopy volume was computed from those values.

Branches were categorized as follows: the main stem is the primary branch, branches developed from the first node are the secondary branches, branches developed from the nodes of the secondary branches are tertiary branches, and so on until the last level of branches on the tip. Branches of each category were labeled with different color-coded tapes for ease of counting. The primary to fourth branches were added together, and the fifth and higher branches were added together.

Stem height was measured from the start of the root to the first main branch node using a ruler. Stem diameter was evaluated from the middle of the main stem of each plant using a digital caliper.

LEAF AREA AND LEAF DRY WEIGHT. Leaf-related parameters were measured in the fall trial. Leaves of each plant were collected and scanned by using a scanner (Cannon) into image files (jpg). Images were later analyzed using Tomato Analyzer (Version 3.0, Brewer et al., 2006) for total leaf area. For the images that could not be analyzed by Tomato Analyzer, Canopeo (Patrignani and Ochsner, 2015) was used to analyze the leaf area in each scanned page. Total leaf area per plant, was calculated by deriving the image's leaf areas to the proportion of the image to real size ratio. These leaves were then dried for a week in an incubator set at 60 °C and were weighed.

FRUIT YIELD. During the spring trial, fully ripe fruit of marketable quality were evaluated following a single harvest.

STATISTICAL TREATMENT OF DATA. All quantitative data were analyzed using analysis of variance using SAS software and significant differences between means were deciphered using Duncan's test at $P \leq 0.05$.

Results

When the progenies of a RJ107 F_3 plant were analyzed, an unusual phenotype with multiple branches was observed. Upon selfing, this canopy phenotype was inherited over multiple gen-erations. Because a larger number of stem branches may be of interest for potentially improving photosynthesis, increasing fruit yield, improving fruit placement in the canopy, and mechanical stability of the branches, we chose to analyze the architecture of RJ107(6)A3 plant, (one of the F_5 progeny plants of RJ107F$_3$ following self-pollination) in comparison to its sister line RJ107(4) B5 and the parent lines ROH and JAL (Fig. 1). Note that RJ107(6) A3 canopy is morphologically different from its parents and its sister line RJ107(4)B5 as it appears to have smaller leaves and denser stem branches (Fig. 1).

CANOPY VOLUME. When plants grown in the field during spring were analyzed, three lines RJ107(6)A3C, JAL and RJ107(4)B5C had significantly greater canopy volume than ROH (Fig. 2).

NUMBER OF BRANCHES. In the fall trial in the field, there were no significant differences between the parents and progenies in terms of the number of the primary to quaternary branches per plant (Fig. 3A), though the parents JAL and ROH were significantly different from each other. RJ107(6)A3's architecture resembled JAL, the parent with a higher number of primary to quaternary branches. For the number of fifth to tenth branches, RJ107(6)A3 had a significantly greater number of branches than the three other lines compared (Fig. 3B). For the spring field trial, RJ107(6)A3C had significantly more branches for the sum of the first to fourth branch number (Fig. 3C) and the fifth and higher branch numbers than RJ107(4)B5C, JAL, and ROH (Fig. 3D).
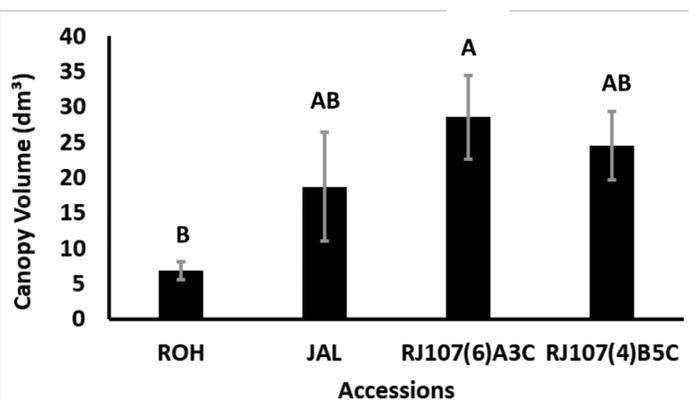

Fig. 2. Canopy volume of plants in the spring field trial. The canopy volumes of plants were calculated by multiplying the plant height, canopy length and width. The bars represent the mean and standard error from four values each from four individual plants. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.
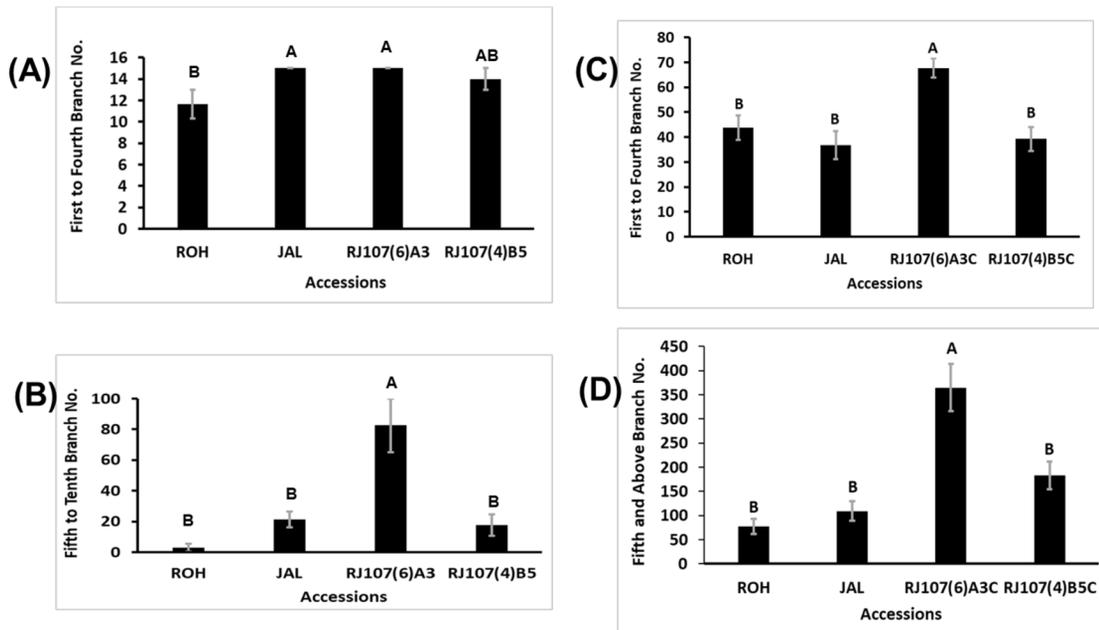
Fig. 3. Mean branch number per plant of plants grown in the field during fall (**A, B**) and spring (**C, D**). Branches of each pepper plant were categorized and counted. The central main stem is regarded as the first branch, branches developed from the primary branch are regarded as the secondary branches, and branches from the secondary branches are regarded as the tertiary branches and so on. The numbers of the first to fourth branches were added together (**A** and **C**), and the numbers of the fifth to tenth branches were added together (**B** and **D**). The bars represent the mean and standard error from three (**A, B**) or four (**C, D**) values each from an individual plant. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.

TOTAL LEAF AREA AND LEAF DRY WEIGHT. For the fall field trial, leaf area per plant was evaluated. Leaf area of RJ107(6)A3 was significantly higher than those of the parents but not that of RJ107(4)B5 (Fig. 4A). The dry weight of leaves of RJ107(6)A3 was significantly greater than ROH, but was comparable to those of RJ107(4)B5 and JAL (Fig. 4B).

STEM HEIGHT. For fall field trial, there were no significant differences in stem height among the four accessions of pepper
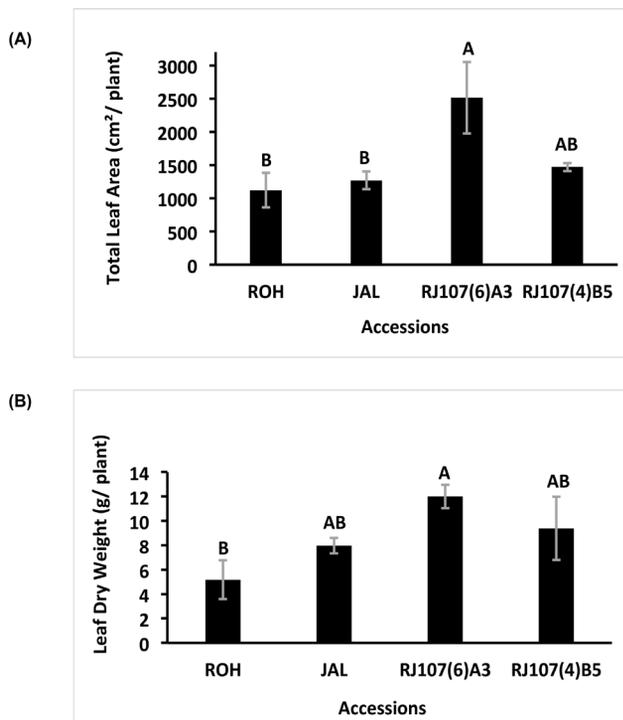

Fig. 4. (**A**) Total leaf area per plant of fall field trial. The bars represent the mean and standard error from three values each from an individual plant. (**B**). Leaf dry weight per plant for plants grown in the field during fall. The bars represent the mean and standard error from three values from three individual plants. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.
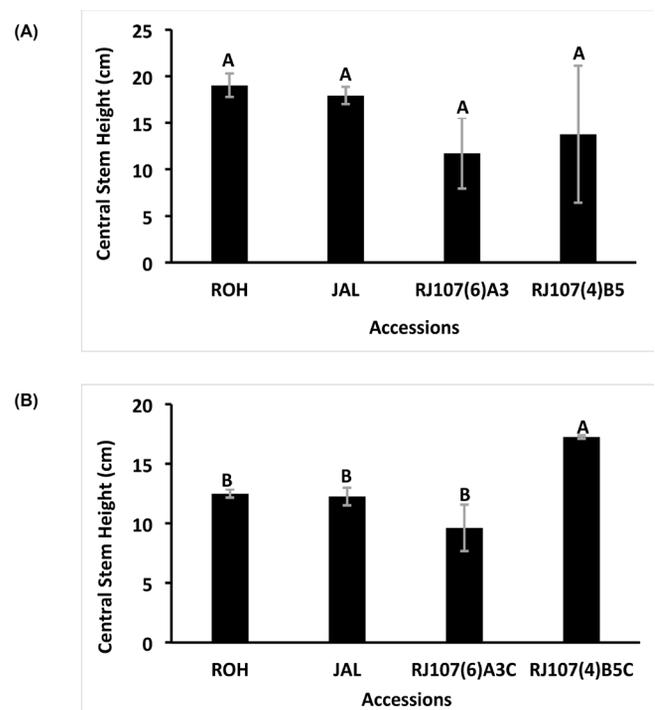

Fig. 5. Central stem height of plants from fall (**A**) and spring (**B**) field trials. Stem height was measured from the start of the root to the first branch node in the main stem using a ruler. The bars represent the mean and standard error from three (**A**) or four (**B**) values each from three or four individual plants. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.
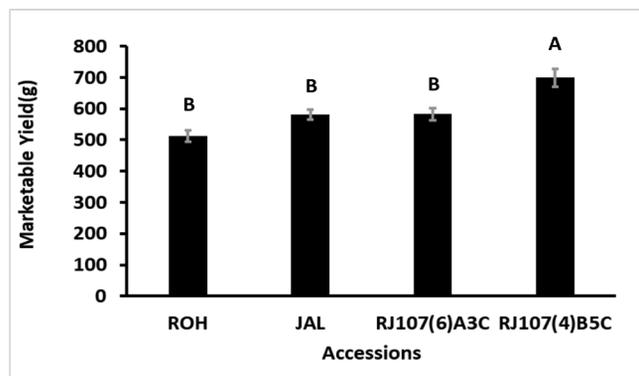
Fig. 7. Fruit yield of spring field trial. Marketable fruit were measured for yield on representative plants of 'Jalapeño' (JAL), 'Round of Hungary' (ROH) and two F7 lines of RJ107 derivatives. The bars represent the mean and standard error from four values each from individual plants. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.
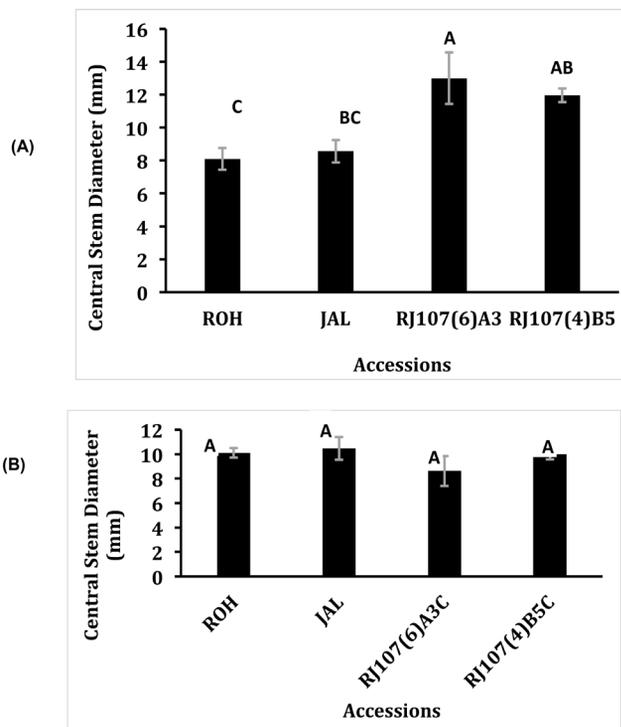
Fig. 6. Central stem diameter of plants grown during fall (**A**) or spring (**B**) field trial. Stem thickness was measured at the midpoint of the main stem using a digital caliper. The bars represent the mean and standard error from three (**A**) or four (**B**) values each from individual plants. Bars marked by the same letters are not significantly different from each other using Duncan's test at $P < 0.05$.

plants, though the progenies RJ107(6)A3 and RJ107(4)B5 have a higher standard error than their parents ROH and JAL (Fig. 5A). For spring field trial, RJ107(4)B5C had a significantly longer central stem than other lines, but there was no significant difference among JAL, ROH and RJ107(6)A3C (Fig. 5B).

Stem diameter. For the fall field trial, RJ107(6)A3 had the greatest stem diameter compared with both parents JAL and ROH. The stem diameter of RJ107(4)B5 was also significantly larger than that of ROH (Fig. 6A). For the spring field trial, there were no significant differences among all lines for this trait (Fig. 6B).

Yield. For fall field trial, there was no significant difference in pepper fruit yield per plant among the accessions based on two early fruit harvests (data not shown). For the spring trial, RJ107(4)B5C had significantly higher yield than RJ107(6)A3C, ROH, and JAL, but there were no significant differences among all the latter lines (Fig. 7).

Discussion

We confirmed the unique stem and canopy architecture of RJ107(6)A3 (Fig. 1), as it and its progeny plants showed this phenotype over several generations. While we observed this canopy phenotype to be inherited, we do not know whether this is controlled by a single gene or multiple genes or is allelic to previously known genes controlling plant architecture.

Among the two parents, JAL had a greater canopy volume than ROH (Fig. 2) and the RJ107(6)A3C and RJ107(4)B5 lines appear to have inherited this trait. The most remarkable aspect of RJ107(6) A3 is that it had a significantly greater number of total branches compared to the parental lines and RJ107(4)B5 as documented in

both the fall and spring field trials (Fig. 3). Plants grown during the spring had a greater number of branches than those grown in the fall (Fig. 3). It is to be noted that spring growing conditions in North Central Florida are warmer especially during flowering and fruit development while during fall, the average temperatures are lower. We hypothesize that levels or responses to auxin and cytokinins might be different in RJ107(6)A3C compared to its parents, as plant growth hormones are known to regulate the development and growth of axillary buds (Janssen et al., 2014). Temperature difference between fall and spring may influence root and apex activity, which is likely responsible for cytokinin and auxin production and transport respectively (McSteen and Leyser, 2005). Our results about greater branch numbers during the spring compared to the fall season are comparable to observations by others who showed that during high temperatures in summer, the carbohydrate is used for maintaining vegetative growth in branches of higher categories (Jovicich et al., 1999).

Further research needs to be done to investigate the relevant genetic background of branch initiation and the lines identified in this study will be useful for that.

We tested whether an increase in the number of branches, in RJ107(6)A3 could influence total leaf area and leaf dry weight. However, RJ107(6)A3's leaf area and leaf dry weight were not significantly greater than its sister line RJ107(4)B5C (Fig. 4), suggesting that the stem branching phenotype did not alter biomass productivity. For the stem diameter (Fig. 5) and stem height (Fig. 6) traits, the derived lines RJ107(4)B5C and RJ107(6)A3 did not differ significantly from each other except during spring when RJ107(4)B5C had a significantly greater stem height (Fig 5B). This suggests that the novel phenotype of increased stem branches of RJ107(6)A3 has little influence on stem diameter and central stem height.

We grew all the four lines in the field without stakes for support. The larger stem diameter, higher branch numbers and smaller fruit of RJ107(6)A3 constituted a more stable and denser plant architecture than the other lines tested (data not shown). This stable plant architecture gives a hint for future breeding for plants with no need for stakes, which could save labor and cost of production. The denser canopy trait is also desirable in breeding ornamental peppers (Mutlu & Kurtulan, 2015) and hence we could use RJ107(6)A3 as a parent to transfer the stem branching phenotype to ornamental pepper varieties.

When we tested whether the RJ107(6)A3 had significantly improved marketable fruit yield, we noted that it was not different than the parental lines but RJ107(4)B5C had significantly greater yield (Fig. 7). The vigorous canopy architecture of RJ107(6)A3 did not however compromise fruit yield, compared with its parents. This confirmed a previous study that showed that the fruit traits and plant growth traits of pepper are mostly inherited separately (Barchi et al., 2009). This could be used in breeding for ideal plant architecture without interfering with yield. The genetic and physiological mechanism behind the dense canopy phenotype of the line are not understood. The lines developed in this research will be useful for future research to examine the dense canopy phenotype's role in yield, growth and potential partitioning of photosynthates between leaf and fruit.

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