horticulturae

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Communication

Aeroponic Cloning of *Capsicum* spp.

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Received: 9 March 2019; Accepted: 10 April 2019; Published: 16 April 2019

Abstract: Aeroponic cloning is a great strategy to maintain desired genotypes by generating a whole new plant from cuttings. While this propagation technique has been demonstrated for tomatoes (*Solanum lycopersicum*) and potatoes (*Solanum tuberosum*), no protocol has been developed for peppers (*Capsicum* spp.). The ability to clonally propagate different *Capsicum* holds promise for domestic and industrial growing operations since elite cultivars with desirable traits (e.g., high capsaicin levels, nutrient content, and striped fruit) can be perpetuated without the need of planning a nursery. We tested six *Capsicum* species for their feasibility of aeroponic cloning by stem cuttings. All domestic species were successfully regenerated under aeroponic conditions but not for *Capsicum eximium*, a wild species. Of the species analyzed, *Capsicum annuum* peppers had the fastest node formation (11.6 +/− 0.89 days, $P \leq 0.01$) and obtained a larger volume of roots ($P \leq 0.01$) after node formation as compared to *C. baccatum*, *C. frutescens*, and *C. pubescens*. This study presents a cost-effective strategy to clonally propagate peppers for personal, industrial, and conservation purposes.

Keywords: pepper; propagation; domestic; wild

1. Introduction

The genus *Capsicum* consists of more than 30 species, five of which are the result of domestication dating back to 6000 BC: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* [1,2]. Commercially-cultivated pepper is an important vegetable and spice consumed daily by nearly a quarter of the world's population [3,4]. Domesticated in South-central Mexico (*C. annuum*; [5]) or Peru (*C. baccatum* and *C. pubescens*; [6]), peppers spread rapidly across the world after European contact due to their multitude of culinary uses, high nutritional content, and unique chemistry (e.g., capsaicin) [4]. Peppers are grown extensively worldwide with about 32 million metric tonnes produced in 2014 [7]. In 2016, the United States pepper production was valued at approximately 700 million USD [8]. In addition to the fresh market, there are many other high value products such as red pepper, chili flakes, and hot sauce that have distinct niche markets [9,10]. The most common pepper species in cuisine often depends on local cultural traditions. For example, in northerly temperate

latitudes sweet bell peppers (*C. annuum*) are favored, while in many tropical regions fiery types (*C. chinense*) are preferred [11]. Furthermore, *Capsicum* spp. cultivars produce varying levels of capsaicin, provitamin A, vitamin C, and folate, which has sparked interest in developing varieties to combat human malnutrition [12,13].

While pungency is an important characteristic in peppers [14], there are several roadblocks to propagating pungent plants. Often pungent varieties have poor and inconsistent germination, require longer time to germinate, and are expensive (more than 1 USD per seed). Additionally, many boutique growers make their own novel hybrids, both within and between species, and select for specific ideotypes [15].

Traditionally, pepper seed production is achieved by the self-pollination of promising plants to develop true breeding lines. In recent years, there has been an increase in the use of hybrid cultivars because of their higher yields and often have unique characteristics. However, seeds from hybrid plants cannot be saved for the next generation since self-progeny would show segregation of desirable traits. Therefore, a clonal propagation protocol could be implemented to enhance profitability of these novel germplasm types.

Clonally propagated pepper hybrids would provide an effective way to immortalize favored hybrid genotypes. Additionally, cloning protocols could be used to preserve pepper lineages that exhibit medicinal qualities or that are threatened by extinction [16]. While a major domestication trait across the plant kingdom has been a reduction in seed dormancy [17], which leads to easier use of plants in farming systems, it is unclear if this has also been selection for an increase in the ability of domestic species to be cloned, which would lead to a similar ease of use in cultivated systems.

Tissue culture is a method that is often used to clonally propagate recalcitrant, unique, or valuable samples by taking seed, embryos, or somatic tissue and placing it on a nutrient growth media. Tissue culture based methods have been developed to propagate successfully various *Capsicum* spp. [18] in laboratory conditions. However, tissue culture has limited utility for boutique or home growers as they do not have access to the sterile facilities or the necessary materials and supplies to carry out this task. Aeroponic cloning promises to be a great technique that can be used by pepper enthusiasts since it consists of soilless culture that allows for plant growth in a controlled environment, such as a greenhouse or growth chamber that can be readily available [19]. However, peppers have not been evaluated using this technique. The objective of this study was to evaluate the suitability of an aeroponic cloning protocol by using five domestic *Capsicum* spp. as well as one closely related wild species. Two key phenotypes that measure the success of this process are: time to node formation to judge the start of active growth (analogous to germination) and biomass accumulation, which is an important marker of plant vigor and a good predictor of future yield.

2. Materials and Methods

2.1. Propagation of Mother Plants

Pepper types were sourced from various heirloom seed producers across North America and represent six different species (unless otherwise noted; Table 1). Seeds from each pepper type were planted 0.635 cm deep using Master Garden premium potting mix (1.25N/0.21P/0.30K) (Premier Tech Horticulture, Ltd., Olds, AB, Canada) and sown in covered 32 cell deep flats for 7–14 days. Once plants emerged from soil, covers were removed from flats and plants were grown indoors for 18 h of light and 6 hours of a dark photoperiod under Sunblaze T5 fluorescent lights 5000 lumens (Sunlight Supply, Inc., Vancouver, WA, USA). All plants were fertilized with MotherPlant Nutrients (0.2N/0.3P/0.2K) (Hydrodynamics International, Lansing, MI, USA) following the manufacturer's recommendations.

Table 1. Species, cultivar and seed source for cultivars tested in this study.

Species	Cultivar	Seed Source
Capsicum annuum	Ruby King	Seed Savers
Capsicum annuum	Sweet Big Daddy Hybrid	Burpee
Capsicum baccatum	Dedo De Moca	PepperLover.com
Capsicum baccatum	Aji Limon	Chile Pepper Institute
Capsicum chinense	Tobago Yellow Scotch	PepperLover.com
Capsicum chinense	Trin. Scorpion Sweet	PepperLover.com
Capsicum chinense	Hot Zavory	Burpee
Capsicum eximium	CGN 19198	PepperLover.com
Capsicum eximium	CGN 24332	PepperLover.com
Capsicum frutescens	Bradley	PepperLover.com
Capsicum frutescens	Tobasco Heirloom	Burpee
Capsicum frutescens	Tobasco	Seed Savers
Capsicum pubescens	Red Rocoto	Seed Savers

2.2. Cloning Experiments

Once plants formed stems with a diameter greater than 1 mm, branches were cut for cloning experiments and placed into an aeroponic cloning chamber. Cuttings ranging from 7.62–15.24 cm in length were taken from top growing tips or hearty side shoots. Each cutting had 2–4 sets of leaves to aid photosynthetic activity. The lower two nodes and any flower buds were trimmed from cuttings. Sterile clean blades were used to make a 45-degree angle cut 0.635 cm below a set of nodes, and cuttings were placed in stem collars, allowing a minimum of 2.54 cm of the stem to be exposed under the collar. For all aeroponic cloning experiments, as recommended by the manufacturer, 50 mL of Power Clone Advanced Liquid Formula (Botanicare, AZ, USA) was mixed per 3.79 L of distilled water and the solution was placed in the reservoir of a Turbo Klone T96 cloning machine (Everything Green Hydroponics, LLC. Las Vegas, NV, USA) and run with continuous spraying to help increase the onset of node formation (Figure 1). Two Sun Blaze Fluorescent Strip Lights were placed 26.67 cm apart from each other and 30.48 cm above pods in the growing chamber. Plants were grown at 21.0 °C. Each clone was inspected for; (1) time to node formation, identified as time from stem cutting to the initiation of node formation, (2) whole plant dry weight 14-days post node formation, and (3) root dry weight 14-days post node formation.

Figure 1. *Capsicum* spp. cuttings in aeroponic cloning machine.

2.3. Statistical Analysis

The experimental design was a randomized complete block with three blocks, where treatments consisted of different *Capsicum* species. Stem width and height at the time of cloning were treated as covariates. Analyses were conducted in the R statistical software package [20]. Treatment means were

separated using a Fisher's protected least significant difference with a significance level of $P < 0.05$. When treatments did not grow, they were treated as missing data.

3. Results and Discussion

There were significant differences in vigor and cloning success between species in time to node formation and the robustness of subsequent cloned material (Figure 2). As expected, *C. annuum* was the fastest species to form nodes. *C. annuum* is the most highly cultivated of the species tested in this study and has the longest domestication history. Unexpectedly, *C. chinense* had the slowest time to node formation, as its growth habit suggested that it would be amenable to cloning, given that stem diameter is a highly significant covariate to cloning success. *Capsicum eximium,* the only non-domestic species, failed to resume active growth after cloning (Figure 2). This is likely due to small stems of *C. eximium* (Figure 3E,F), as well as the wild nature of the plant. Future studies should use larger cuttings with thicker stems to evaluate if aeroponic cloning may be suitable for exotic (non-domestic) species. If this is successful, aeroponic cloning may aid in the preservation of rare species, some of which are endangered and poorly conserved in ex situ collections. One such example is *C. galopengense*, which is no longer present in its native Galapagos Islands due to the introduction of goat herbivory [21].

It took an average of 15.6 days for all species to form a node, similar to what previous studies reported for germination time in pepper [22]. *Capsicum chinense* seeds are known to take longer to germinate and obtain shorter heights than *C. annuum* plants [23]. This trend is similar to the findings from this study for node formation and root emergence. The range of days to growth initiation was much smaller for cloned material in this experiment (10–28 days) as compared to previously reported seed germination ranges of 10–47.4 days [22]. Our data supports previous research showing that somatic regeneration of pepper is feasible [24,25]. Plant growth proceeded normally in all cloned material. Initial stem width and stem height at the time of cloning (Figure 3) were significant covariates ($P < 0.01$). There were initial differences in growth at seven days after node formation for both total dry weight and ratio of above to below ground biomass (Figure 2). However, after 14 days these differences were minimal. Dissimilarities in plant vigor were indicative of ease of cloning and provided an opportunity to assess which species are most amenable to this procedure.

Aeroponic cloning chambers are highly accessible to small-scale growers, requiring only $300 USD to purchase the chamber, nutrient solution, and lighting, as compared to the tissue culture approach that can have operating costs of approximately $24,600 USD per year (numbers based on an estimate for St. Paul, Minnesota, 2016). There was an increased labor requirement relative to traditional crossing, but the cloning methodology provides an opportunity to preserve genotypes that cannot be maintained as seed stocks. Additionally, aeroponic cloning may help small scale boutique growers by allowing them to increase production of novel genotypes without the need for true breeding varieties. In this study the success of this method depended on propagation material being disease and insect free, as plants treated with insecticide or fungicide showed a lack of node formation, root formation, and overall growth. Therefore, cuttings should be taken from plants either grown indoors and disease free, or from outdoor plants pretreated for pests prior to taking cuttings for cloning.

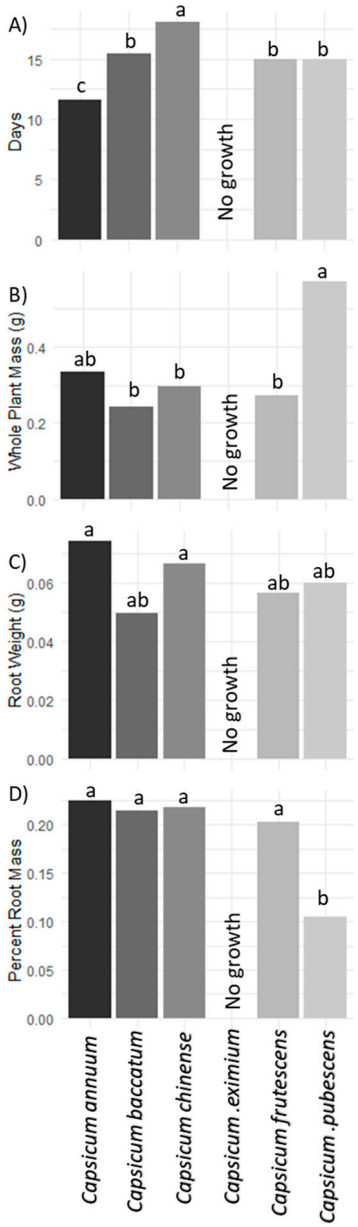

Figure 2. Vigor measurements from different *Capsicum* spp. tested in aeroponic conditions. (**A**) Time to node formation in days, (**B**) Total Dry Weight in grams (g), (**C**) Root Dry Weight in grams (g), (**D**) Ratio of Root Dry weight to Shoot Dry Weight. Letters indicate significant differences between means based on a Fischer's protected LSD.

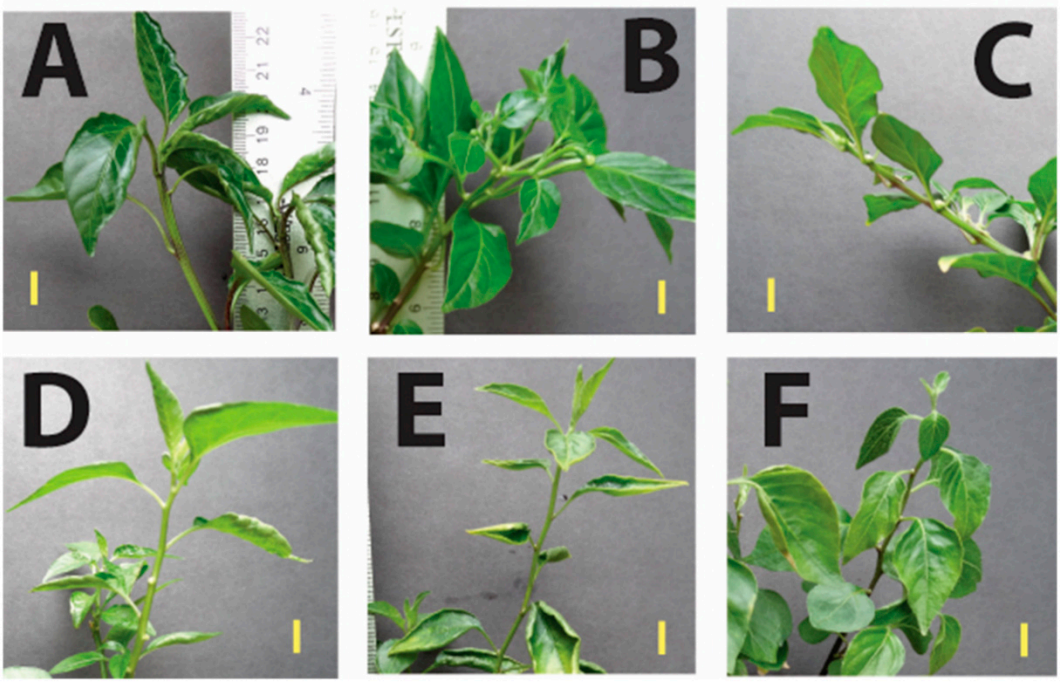

Figure 3. Variation of stem thickness for *Capsicum* spp. evaluated in this study: *C. annuum* (**A**), *C. baccatum* (**B**), *C. chinense* (**C**), *C. frutescens* (**D**), *C. eximium* strain CGN 19198 (**E**), and *C. eximium* strain CGN 24332 (**F**). Yellow scale bar = 1 cm.

4. Conclusions

The success of cloning in all five domesticated *Capsicum* spp. in aeroponic conditions illustrates that it is a viable option for increasing populations of plants with desirable phenotypic traits for both home and boutique growers. Aeroponic cloning offers a cost-effective solution for increased propagation, as some seeds of highly sought after varieties are expensive and are available only in limited quantities. Future experiments should explore the yield and quality of cloned material in both the field and the greenhouse.

Author Contributions: Conceptualization, D.J.B., T.H.; methodology, D.J.B. analysis, M.B.K., S.M., A.R.D.V.-E.; investigation, J.B., M.K.F., L.H.; resources, D.J.B.; data curation, D.J.B., J.B., M.K.F., L.H.; writing—original draft preparation, M.B.K., S.M., A.R.D.V.-E. writing—review and editing, D.J.B., M.B.K., S.M., A.R.D.V.-E.; visualization, M.B.K., D.J.B., A.R.D.V.-E.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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