agronomy

MDPI

Article

Pitaya, an Attractive Alternative Crop for Mediterranean Region

Alice Trivellini [1,*], Mariella Lucchesini [2], Antonio Ferrante [3], Daniele Massa [4], Matteo Orlando [1], Luca Incrocci [2] and Anna Mensuali-Sodi [1]

1 Institute of Life Sciences, Scuola Superiore Sant'Anna, Pz. Martiri della Libertà 33, 56127 Pisa, Italy; m.orlando@santannapisa.it (M.O.); a.mensuali@santannapisa.it (A.M.-S.)
2 Department of Agriculture, Food and Environment, Università degli Studi di Pisa, Via del Borghetto 80, 56124 Pisa, Italy; mariella.lucchesini@unipi.it (M.L.); luca.incrocci@unipi.it (L.I.)
3 Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milano, Italy; antonio.ferrante@unimi.it
4 CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Via dei Fiori 8, 51017 Pescia, Italy; daniele.massa@crea.gov.it
* Correspondence: alice.trivellini@gmail.com

Received: 15 June 2020; Accepted: 21 July 2020; Published: 23 July 2020

Abstract: While the ongoing global warming and the economic crisis threaten horticultural and ornamental products production in the Mediterranean region, new challenges and opportunities for renewing plant material occur. Pitaya has great potential as a new crop for Mediterranean growers: it consumes little water and it adapts well to the high temperatures present in the greenhouse. Moreover, the market shows increasing demand of new, healthy exotic fruits and the fruit of these crops are worldwide appreciated as a super fruits. The aim of this study was to investigate the potential of introducing a new type of product that in addition to the aesthetic value can combine functional nutritional characteristics, through a whole chain approach, in order to develop basic knowledges and technical information that may lead to the commercial production of pitaya by small-scale farmers in Italy. The results of this study report and discuss various aspects to support the establishment of pitaya cultivation, such as its propagation by cuttings and micropropagation, greenhouse cultivation practices (through the clarification of the sprouting and flowering phenology as well as the fruit maturation period), while providing also information on the sensory properties and the antioxidant levels which naturally occurring in these fruits.

Keywords: hylocereus; dragon fruit; propagation; micropropagation; cuttings; antioxidant level; sensory evaluation; greenhouse cultivation

1. Introduction

Horticulture is a dynamic sector where a wide variety of crops and their products are continuously innovating, and new market opportunities are considered and explored. It includes the cultivation of fruits, vegetables, nuts, seeds, herbs, sprouts, mushrooms, algae, flowers, seaweeds, and non-food crops such as grass and ornamental trees and plants. However, this sector faces many challenges, from retaining economic competitiveness, achieving full economic and environment sustainability and responding to climate change.

The Italian ornamentals market is currently undergoing a deep transformation due to the worldwide competition. The number of smaller and less specialist producers is falling or is losing considerable market share from competing countries. In fact, new production areas have been expanding, leading to a gradual delocalization towards countries with favorable climatic conditions, plenty of natural resources and with lower production costs [1].

The ornamentals trade has been further exacerbated by the global climate changes leading to water deficits and temperature increase. Italy is a Mediterranean country, located at the interface between arid African and Arabian deserts that are very short of water, and temperate climates with relatively abundant and consistent water resources. Water resource availability in the Mediterranean has already been affected by a combination of effects varying from global climate change and anthropogenic pressures. Horticultural crops are more susceptible to changing conditions than arable crops and the effect of increased temperatures and water scarcity is expected to rise in the next decade [2].

While the ongoing global warming and the economic crisis threaten horticultural and ornamental products production around the Mediterranean region, it also opens new challenges and opportunities for renewing plant material as well as developing new agricultural systems to stand the global competitiveness. Some species from tropical dry areas might be considered for the diversification of cultivated species around the Mediterranean, thus creating new profitable alternatives/opportunities [3].

Pitaya or Dragon Fruit (*Hylocereus* spp.) meets these requirements and has emerged as a viable ornamental and fruit crop alternative with great profit potential for small-scale agricultural producers in Italy. Basically, unknown twenty years ago, except in its areas of origin, pitaya is the fruit produced by the plant with the same name belonging to the genus *Hylocereus* and *Selenicereous* of the *Cactaceae*. The plant, native to tropical America, adapts very well to various climatic conditions in its native habitat, where it grows at nearly sea level in the arid coastal plains of the Pacific Coast and in more humid, cloud forests conditions at higher elevations. Pitaya is a non-facultative CAM-type plant, characterized by a metabolism with enhanced water use efficiency and maintains photosynthesis under stress conditions [4–6]. This photosynthetic adaptation allows gas-exchange to occur at night, and during the day, stomata close to save water, thereby showing a high tolerance to drought and an adaptation to dry and hot environments [7,8]. Pitaya is also considered a potential ornamental plant for garden or public gardens in the Southern Mediterranean regions. Pruning and cultivation managements can allow the vertical cultivation or maintained as shrubs. The ornamental traits are represented by plant morphology, flowers, and fruits.

These species produce an edible fruit that has generated considerable consumer interest because of its nutraceutical components, high nutritional value and positive health benefits. These fruits are source of essential micro- and macro-nutrients as well as contain high levels of essential minerals and vitamins [9–11]. Several studies have demostrated the health benefits of peel, flesh and edible seeds of pitaya, such as chemoprevention of cancer, anti-inflammatory and antidiabetic effects, and a reduction in the mortality risk of cardiovascular disease, as well as antioxidative properties [9,12–14]. Fruits from most *Hylocereus* species have red-purple pigmented skins, while the pulp color ranges from white (in *H. undatus*) to red and purple (in *H. polyrhizus* and *H. costaricensis*) depending on the concentration of two betalain pigments, namely, the red−violet betacyanins and yellow−orange betaxanthins [15]. Betalains in pitaya flesh and peel have been shown to have strong antioxidant activity [16], antimicrobial effect [17] as well as anticancer properties [18] and have been proposed as a promising source of natural colorants offering preparations with a broader color spectrum [19]. The interest to develop new sources for natural pigments has been increasing because the official EU and USA regulations have restricted the use of synthetic colorants as additives in food, due to their toxicity and carcinogenic effects [20]. The presence of these natural bioactive compounds in pitaya have the potential to be exploited in food, pharmaceutical and cosmetic industries.

Dragon fruit has been cultivated in Vietnam for over 100 years and much longer in their native locations. Currently it is widely cultivated in tropical and warm subtropical regions, including Israel, Australia, and, most recently, the United States (U.S.). Pitaya today occupies a growing niche in Europe's exotic fruit market [21] showing high potential as an ornamental-agrifood commodity as well as industrial source of natural additives. The production of pitaya in Italy is in its infancy but its demand is presently gaining popularity among the consumers and the small farmers.

The aim of this study was to investigate the potential of introducing a new type of product that in addition to the aesthetic value can combine functional nutritional characteristics, through a whole

chain approach, from propagation to the greenhouse cultivation of species belonging to the genus *Hylocerius* spp. in order to develop basic knowledges and technical information that may lead to the commercial production of pitaya by small-scale farmers in Italy. The overall experimental approach is reported in Figure 1.

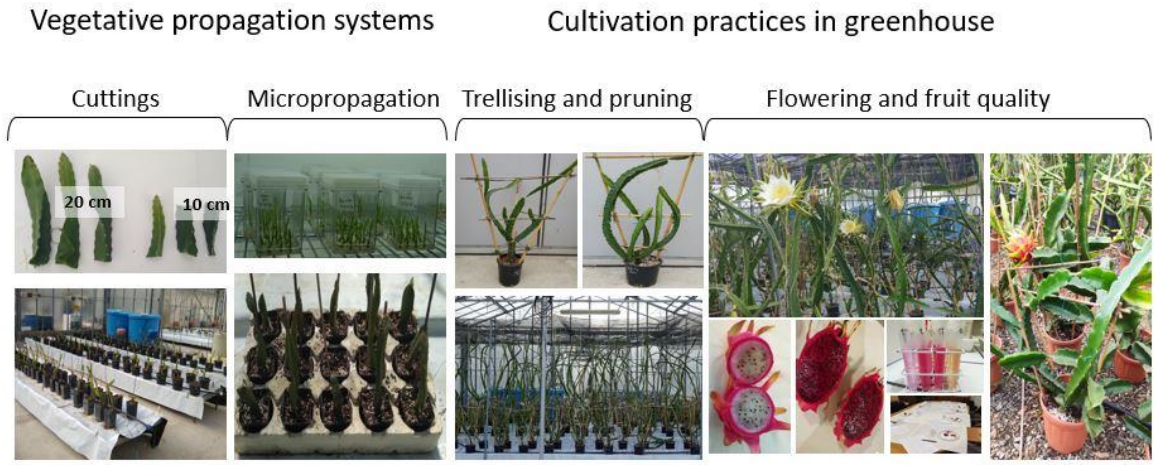

Figure 1. Overview of the whole chain approach, from propagation to the greenhouse cultivation of species belonging to the genus *Hylocerius* spp.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

Two different dragon fruit (*Hylocereous* spp.) commercial clones were introduced as rooted or unrooted cuttings and they were studied in 2017–2020. Clone 1 (*H. hundatus*) introduced from Canary Island has red skin with white flesh. Clone 2 (*H. hundatus* × *H. polyrhizus*) introduced from Thailand has red skin with red-purple flesh. The cuttings were individually potted and planted in a greenhouse located in Pisa, Italy (latitude, 43°43′ N; longitude, 10°23′ E), under natural environmental conditions during spring summer. During colder months, the greenhouse was set to be heated at 14–15 °C. Since the ideal substrate is rich in organic matter, slightly acidic and well-drained, a multi-layer substrate for the pots was used consisting of pumice, perlite and then a mix 1:1 (*v*/*v*) of peat moss:perlite.

Drip irrigation was carried out using a nutrient solution with an electrical conductivity (EC) of 1.45 dS m^{-1} and pH 5.7. The composition of the nutrient solution was as follows (concentrations are expressed in mol m^{-3}): 8.3 N-NO^{3-}, 1 P-PO, 4.6 K^{+}, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 0.6 Na^{+}, and 1.3 S-$SO_4{}^{2-}$. Micronutrients were added at Hoagland's concentration (in mmol m^{-3}: B 20, 20 Fe, 2.4 Cu, 16 Zn, and 10 Mn).

2.2. Vegetative Propagation Systems

2.2.1. Propagation by Cuttings

Cuttings used in the experiments were taken from shoots of mother plants grown in the greenhouse. To identify the best period to propagate Pitaya in Italy, the cuttings were taken from a healthy mother plant during spring-summer (SS) and autumn- winter (AW) months. A slanted cut was made at the stem base. Cuttings of 10 (small) and 20 (medium) cm length were prepared either from an entire shoot with the apical bud or from stem sections. Then the cuttings were left to cure (dry and heal) in a dry, shady location (greenhouse) for about 7 days. This was to avoid at the time of transplant the possible entry of telluric pathogens thought a fresh cut. After this time the cuttings with a dry cut were ready to be transplanted. About 100 pots of 30 cm in diameter, 50 for each size of cuttings, were filled to about two-third full with a substrate characterized by a high drainage. The substrate was irrigated

with water and left to drain excess water. Cuttings were inserted into the medium for one third of their length under the surface. The pots were then filled up to one-thirds full with a substrate and were placed on a greenhouse bench. The cuttings were watered every week using micro-irrigation. Cuttings performances was assessed after 2 and 4 weeks for number and length of the developed roots and after 2, 4 and 6 weeks for rooting percentage.

2.2.2. In Vitro Propagation

Preparation and disinfection of explants—Young joints (10–20 cm in length) were collected from greenhouse plants weekly sprayed three times with Benomyl (1.0 g L^{-1}; Du Pont Agricultural products, Wilmington, Delaware, USA), cut into 4–5 cm sections and subjected to different disinfection procedures (Table 1). Experiment 1: Explants were washed with tap water supplemented with one drop of Tween 20 (Sigma, Italy) for 30 min. Pre-treatment to disinfection started with immersion in a water solution (1% *v*/*v*) of Antibiotic Antimycotic Solution (AAS, Sigma, Italia) for 10 min, followed by 15–30 s in ethanol (70% *v*/*v*). Disinfection as such was conducted with 15% *v*/*v* aqueous solution of NaOCl (8% active chlorine) supplemented with one drop of Tween 20 (Sigma, Italy) for 15 min. Finally, the explants were washed three times with sterile distilled water in a laminar flow cabinet. Experiment 2: The experiment 1 was modified only by carrying out the sterilization with 1% (*v*/*v*) AAS (Sigma, Italia) under vacuum for 5 min. All other steps were the same as previously described above in Experiment 1. Experiment 3: The explants, after rinsing in tap water with one drop of Tween 20 (Sigma, Italy) for 30 min, pre-treatment to disinfection started with immersion in a fungicide solution (Benomyl 1g L^{-1}) for 5 min, followed by air-dry under flow cabinet (about 20–30 min), then 15–30 s in ethanol (70% *v*/*v*) and, followed again by washing in 3% (*v*/*v*) NaOCl and subsequently three washing in distilled water. Then, the explants were sterilized with 4% (*v*/*v*) Plant Preservative Mixture (PPM, Micropoli, Italy) under vacuum for 5 min. Finally, the explants were surface-sterilized in 0.2% (*v*/*v*) NaOCl for 1 min and then rinsed 3 times with sterile distilled water in a laminar flow cabinet. Explant borders, usually damaged by the disinfection procedure, were removed by trimming, leaving individual areoles with little surrounding tissue (section of 1 cm). These primary explants were placed vertically in 30-mL polycarbonate UryTM vials (PBI, Milano, Italy) with 5 mL culture medium. MS medium [22] without plant growth regulators (PGRs), containing 30g L^{-1} sucrose, MES 500mg L^{-1}, GSH 300mg L^{-1} and Gelrite 2.5g L^{-1} was employed (basal medium). pH was adjusted to 5.8 with 1N KOH and the medium was autoclaved at 121 °C (ca. 0.12 MPa) for 15 min. Basal medium of explants resulting from disinfection procedures reported in experiment 2 and experiment 3 was supplemented with a 1ml of 200mg L^{-1} ultra-filtered cefotaxime. Explant cultures were placed under a 8:16 h, dark:light photoperiod (100 μmol^{-1} m^{-2} s^{-1}) at 22–24 °C. The explants sterilization performances (e.g., contamination percentage) after 15 days and the explants survival/death percentages after 45 days of culture were assessed. Thirty explants for each disinfection experiments (experiment 1, experiment 2a minus cefotaxime, experiment 2b plus cefotaxime, experiment 3 plus cefotaxime) were used.

Shoot proliferation—Six weeks after establishment of aseptic culture), new formed shoots, were used as secondary explants. These shoots were cut (15–20 mm) and cultured on fresh medium for shoot proliferation. To induce axillary shoot formation, basal media were enriched with plant growth regulators (PGRs): an auxin, Indole−3-butyric acid (IBA, 0.25 mg L^{-1}) and two different cytokinin, 6-benzylaminopurine (BA, 0.5 mg L^{-1}) and zeatin (ZEA, 3 mg L^{-1}), (Table 2). Then, explant cultures were placed under a 8:16 h, dark:light photoperiod (100 μmol^{-1} m^{-2} s^{-1}) at 22–24 °C. After 4 weeks the new developed shoots were subcultured for further growth on the same media. Shoot proliferation performances were assessed after 4 weeks in the two treatments, as number of shoots per explant, shoot height and diameter.

Rhizogenesis of in vitro shoots—After 5 successive subcultures (4–5 weeks each one) shoots were continuously maintained 6–7 weeks on the same two media employed for shoot proliferation and growth to allow roots formation.

Table 1. Disinfection strategy applied in different experiments (EXP 1, 2a, 2b and 3) to Pitaya stem explants to start the micropropagation process.

Disinfection Strategy	EXP 1	EXP2a	EXP2b	EXP3
Pretreatment				
1% AAS 10 min	X			
1% AAS 5 min vacuum		X	X	
1g L^{-1} Benomyl 5 min vacuum				X
70% Ethanol 15–30 s	X	X	X	X
I disinfection				
15% NaOCl 15 min	X	X	X	
3% NaOCl 15 min				X
II disinfection				
4% PPM 5 min vacuum				X
0.2% NaOCl 1 min				X
Culture medium				
200mg L^{-1} ultra-filtered cefotaxime			X	X

Table 2. Composition of media used for proliferation stage.

Proliferation Medium	ZEA + IBA	BA + IBA
Macroelements	MS	MS
Microelements	MS	MS
Vitamins	MS	MS
Sucrose	30 g/L	30 g/L
MES	500 mg/L	500 mg/L
GSH	300 mg/L	300 mg/L
IBA	0.25 mg/L	0.25 mg/L
Zeatin	3 mg/L	
BA		0.5 mg/L
Gelrite	2.5 g/L	2.5 g/L
pH	5.9	5.9

Abbreviations–Murashige & Skoog Medium (MS); 2-(N-morpholino) ethanesulfonic acid (MES); Glutathione (GSH); 6-benzylaminopurine (BA); zeatin (ZEA); Indole−3-butyric acid (IBA).

Acclimatization of plants - Agar was carefully removed from roots with tap water and the plants were placed in 165 cm^3 plastic pots filled with autoclaved mix 1:1 (*v*/*v*) of soil:perlite and covered with plastic lid for 7–10d under growth chamber condition. Then, the plants were transferred in 24-well trays (each well measuring 4 × 4 cm) under mist irrigation (4 s every 15 min) in greenhouse (Temperature: 20 to 25 °C; RU 70–80%). After 60 days in the greenhouse, the number of surviving plants was evaluated.

2.3. Greenhouse Cultivation Practices

The pitaya plants were grown in pots containing a pumice, perlite, perlite:peat mixture. The plants were fertirrigated ones a week with drip irrigation during spring and early summertime. During hot summer months (end of July and August) the plants were irrigated twice a week. In autumn and winter months the plants were irrigated every 10–15 days. The optimal amount of water worked out was increased during the second year as the plants have grown considerably reaching a height of 2.5/3 m. Drip irrigation was adopted applying 240 g/plant of standard nutrient solution instead of 120 g/plant.

Both espalier and inverted supports were chosen to ensure a suitable and compact ornamental habitus of pitaya potted plants. The espalier and inverted pyramid vertical supports developed are shown in Figure 1. In both type of vertical supports pruning was important, and the stems were selected in such a way to force the plant to climb over the entire support used. All lateral growth and parts of the plant facing the ground were removed, while the main stems and branch stems were kept, except those that touched the ground. In the espalier support the main stem as well the branch stems were attached to the vertical support with clips. In the inverted pyramid support only the branch stems

were forced to climb over the different sides of the pyramid. During the second year, 33% (n = 40) of the plants were pruned at the level of the two previously made supports (Figure 1). The remaining 66% (n = 80) instead was supported and linked to vertical supports (cords) to ensure a vertical growth of the canopy and possibly allow the development of the flowers/fruits (Figure 1).

2.3.1. Trellising and Pruning

Pitaya are semi-epiphytic plants, which crawl, climb and attach naturally to any natural or artificial support they meet, thanks to their aerial roots. In this research, two different types of vertical support were developed (espalier and inverted pyramid) and the appropriate structural pruning, to make a structure on the trellis was performed. During the second year, 33% (n = 40) of the plants were pruned to be maintained at the level of the two employed supports). The remaining 66% (n = 80) instead was supported and linked to vertical supports (cords) to ensure a vertical growth of the canopy and possibly allow the development of the flowers/fruits.

2.3.2. Phenology of Sprouting and Floral Bud Emergence

The number of new shoots, floral buds, and aborted floral buds were recorded at 3-week intervals in two consecutive years (January 2018–January 2020).

2.3.3. Hand-Pollination and Assessment of Fruit Set

The pollination treatments were: (i) Hand self-pollination. The flowers were bagged before anthesis. In the early morning after the night anthesis, the flowers were emasculated. To carry out self-pollination, pollen from the same emasculated flower was applied to the stigma with the aid of a brush. (ii) Hand cross-pollination. Anthers of mature flower buds were removed six hours before anthesis, and the flowers were bagged. On the morning following anthesis, emasculated flowers were hand-pollinated with pollen from random neighboring plants of the same clone. (iii) Automatic self-impollination. The flowers were covered with bags throughout anthesis. The influences of pollination treatments were monitored using the following parameters: (i) Fruit set (%) = (Number of fruit/Number of flowers) × 100. (ii) Fruit fresh weights (FWs) were measured using an electronic balance to an accuracy ± 0.01 g. Assessment of fruit set was made three weeks after pollination.

2.4. Fruits Nutraceutical Characteristics

Fruits were harvested from November to December 2019, when the peel turned bright pink-purple. The nutraceutical potential and antioxidant capacity of white-flesh and red-flesh pitaya fruits were assessed measuring the total phenols and the antioxidant capacity. For both analytical methods the pulps of pitaya fruits (1 g) were soaked with 5 mL extraction solvent, ground with mortar and pestle, and transferred in 10-mL test tubes. The tubes were sonicated 15 min in ice bath four times, stored overnight at −20 °C and centrifuged 5 min at 2700× g. After separation of the supernatant, the extraction was repeated on the pellet with 5 mL fresh extraction solvent. The two supernatant aliquots were pooled and used for the subsequent analyses. All the parameters were expressed on a fresh weight (FW) basis. (i) The determination of total phenols was carried out by the Folin-Ciocalteu reagent, and by absorbance readings at 320 nm, as reported by Kang and Saltveit [23]. The concentration of total phenols was determined by measuring the absorbance of the solutions at 765 nm, using standard gallic acid (0–500 mg L^{-1}) for calibration, and expressing the results as mg gallic acid g^{-1} FW. The results were expressed as absorbance units of the pure extract at 320 nm per gram leaf tissue, A (320 nm) g−1 FW. (ii) The antioxidant capacity was determined by both the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) assays. The FRAP determination was carried out according to Benzie and Strain [24]. A calibration curve was prepared with standard solutions containing ferrous ion (Fe(II); 0–1000 μM), obtained from ferrous ammonium sulfate. The absorbance was read at 593 nm and the results were expressed as μmol Fe(II) g^{-1} FW. The DPPH assay was performed following Dudonné et al. [25] with slight modifications. The percentage

inhibition of the DPPH radical per gram tissue was read at 515 nm and calculated from the absorbance values of the blank (A blank) and of the sample (A sample) as follows: % Inhibition g^{-1} FW = 100 × [(A blank − A sample)/A blank]/g FW.

2.5. Sensory Evaluation of Harvested Fruits

Twenty free-tasters (12 males and 8 females) were recruited by adverts among the academic community (students, teachers, other staff, etc.) from the Life Science Institute (Scuola Superiore Sant'Anna, Pisa, Italy) and the Department of Agriculture, Food and Environment (University of Pisa, Pisa, Italy). Fruits used for sensory evaluation were freshly harvested, longitudinally cut, placed onto a little white plastic tray and used the same day for the panel. Seven different identifiable attributes (color, overall appearance, flavor, sweetness, tartness, flesh texture and overall liking) were included in the evaluation scheme and were expressed using a 9-point hedonic ratings where 1 = extremely dislike, 5 = neither like nor dislike, and 9 = extremely like. At least three pooled fruits were used for the two fruit varieties (*Hylocereous* sp. and *H. undatus*, purple flesh and white flesh, respectively).

2.6. Statistical Analysis

The data were subjected to statistical analysis using PRISM 6 software (GraphPad Software, San Diego, CA, USA). To stabilize variance and normalize percentage data, the arcsine transformation was used. Analysis of variance (one-way or two-way ANOVA) was used and means values were separated using the Bonferroni multiple comparison test ($p < 0.05$). Student's t-test was used to compute the pair-wise comparisons between group means.

3. Results

3.1. Propagation of Pitaya Cuttings Depends on Their Size and Season

During the spring-summer season, rooting frequency was found to be significantly influenced by cutting size. After two weeks, 87% of the medium size cuttings and 65% of the small size cuttings developed roots (Figure 2A). After four weeks 100% of the medium cuttings developed roots, while after six weeks the rooting percentage was 100% also for the small cuttings. Furthermore, the number of roots developed on cuttings with different lengths differed dramatically (Figure 2B): after two weeks medium cuttings developed a number of roots that is more than twice compared to the small ones, and this ratio was maintained even after 4 weeks. Root length increased linearly with time during the first four weeks (Figure 2C). However, root length was also affected by the cutting size: medium cuttings developed the longest roots. Similarly, to SS period, in AW months the medium cuttings developed a better radical apparatus in terms of rooting frequency, number and length of roots. The seasonal period significantly affected the entire process of rooting. Both small and medium cuttings shown a significant improvement of rooting percentage (Figure 3A) and a significant enhancement of root number and root length during the SS time, compared to AW season (Figure 3B,C).

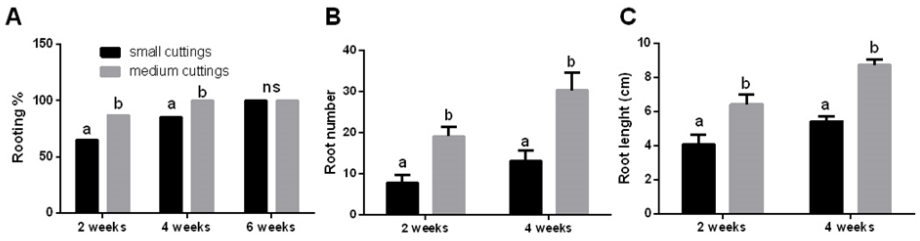

Figure 2. Effect of cutting size on the rooting percentage (**A**), root number (**B**) and length in pitaya (**C**) (summer-spring season). For each parameter and each group considered, columns labeled with different letters are significantly different (at $p < 0.05$). Student's *t*-test was used to compute the pair-wise comparisons between group means.

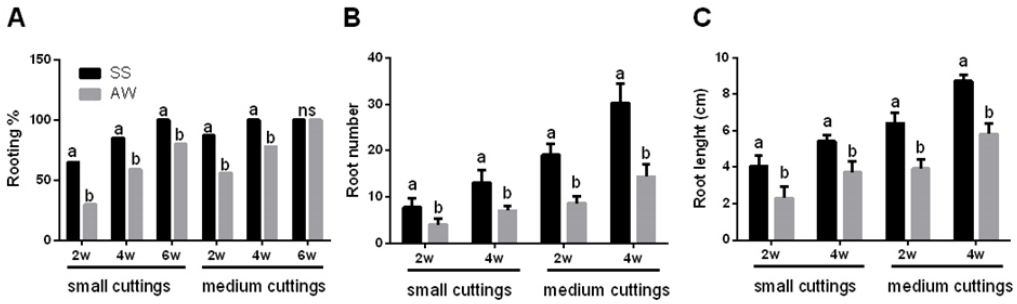

Figure 3. Effect of seasonal period on the rooting percentage (**A**), root number (**B**) and length in pitaya small and medium-sized cuttings (**C**). For each parameter and each group considered, columns labeled with different letters are significantly different (at $p < 0.05$). Student's *t*-test was used to compute the pair-wise comparisons between group means. SS: spring-summer season; AW: autumn-winter season. Abbreviations: 2 week (2w); 4 week (4w); 6 week (6w).

3.2. Efficiency of Micropropagation System in Pitaya (or Efficient Pitaya Propagation Methodology)

3.2.1. Establishment of Aseptic Cultures

Explants sterilization performances under different experimental sets are reported in Figure 4. In the experiment 1, 100% contamination was observed when the explants were pretreated with AAS for 10 min, irrespective of the NaOCl concentration (15%) employed for subsequent disinfection. The contamination observed, was mainly an endogenous contamination. Conducting the pre-treatment disinfection with AAS under vacuum in the experiment 2a provided a better sterilization efficiency showing a contamination percentage of 70%. In the experiment 2b, the addition of an ultra-filtered water solution of cefotaxime (a broad-spectrum antibiotic with activity against numerous gram-positive and gram-negative bacteria) on the surface of culture media significantly reduced the bacterial contamination (40%). However, in order to improve the sterilization efficiency obtained in experiment 2b, a different disinfection protocol was performed (experiment 3). The immersion of explants in a systemic fungicide solution followed by their natural drying under the flow and the use of a more effective sterilizing agent (PPM) under vacuum combined to cefotaxime on the surface of basal media resulted in an almost complete disinfection of the explants (11% of contamination). The green pigmentation and the discoloration of the not contaminated explants was recorded after 45 days of culture to assess the survival percentage after the sterilization procedures. Green colored or discolored explants was considered as survival and death explants, respectively. No significant differences were observed in the survival % between experiment 2a and 2b (Figure 4B). In both experiment this was low (from 25 to 34%) compared to experiment 3 (75%), and presumably due to higher NaOCl concentration employed for the disinfection process (in experiment 2a and 2b).

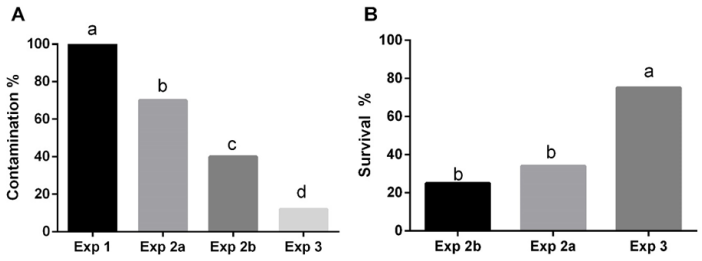

Figure 4. Establishment of pitaya sterile coltures. (**A**) Contamination percentage of different sterilization protocols after 15 days of culture: Experiment 1 (Exp 1), Experiment 2a (Exp 2a), Experiment 2b (Exp 2b), Experiment 3 (Exp 3). (**B**) Survival percentage of different sterilization protocols after 45 days of culture: Experiment 2a (Exp 2a), Experiment 2b (Exp 2b), Experiment 3 (Exp 3). Values are expressed as mean ($n = 30$). Data were subjected to analysis of variance and differences were analyzed by a Bonferroni posttest. Different letters denote significant differences at $p < 0.05$.

3.2.2. Factors Affecting Shoot Proliferation, Rhizogenesis and Acclimatization Process

In this study, a non-expensive aromatic (BA), and an isoprenoid costly (ZEA) cytokinins, in combination with an auxin (IBA) were used in the shoot proliferation media (Table 1). The combination of ZEA + IBA and BA + IBA on shoot number per explant and shoot diameter did not show any statistical differences (Figure 5A,C). On the other hand, by BA + IBA combination, shoot height was reduced significantly, (Figure 5B). The rhizogenesis in shoots proliferated in ZEA + IBA and BA + IBA media, containing IBA at the same concentration, was induced in the 5th subculture. The plants were maintained on the same medium for an additional 2 weeks (six weeks in total) and this was sufficient to trigger the rhizogenesis process despite the low dose of auxin and an unbalanced relationship to the cytokinin, which resulted in >98% rooting, a very satisfactory result (Figure 5D). Rooted plantlets from the media were transferred to plastic pots containing a 1:1 (*v*/*v*) mixture of autoclaved soil:perlite and hardened for 7–10 d in in-vitro growth chamber. After this period, the plants were transferred in trays under mist irrigation and acclimatized to greenhouse environment. After 60 days in the greenhouse, more than 87% of the in vitro-derived plants had survived, showing no significantly differences between treatments (ZEA+IBA and BA+IBA).

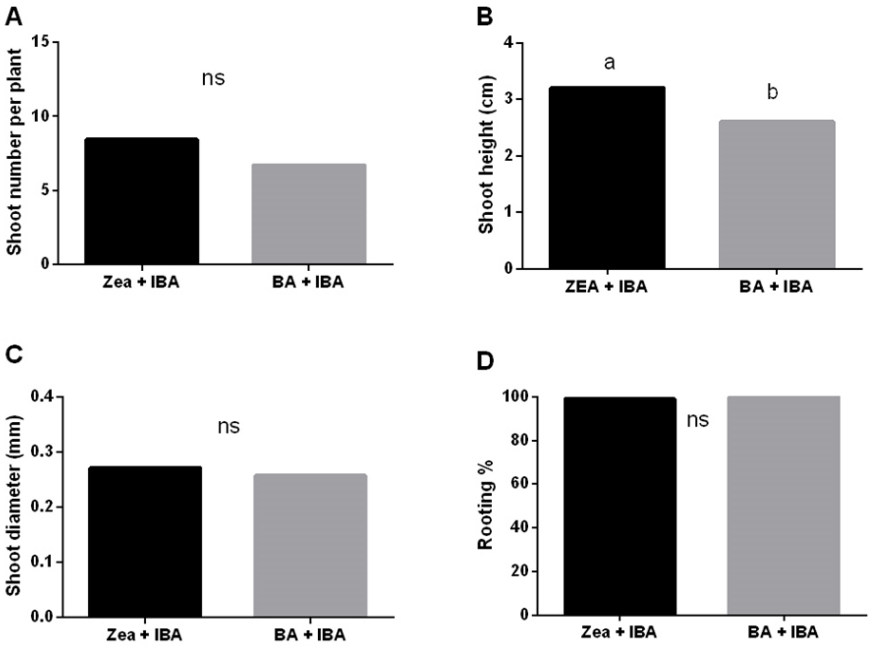

Figure 5. Effects of plant growth regulators on shoot propagation of pitaya micropropagated shoots. The parameter considered are: Shoot number per plant (**A**); Shoot height (**B**); Shoot diameter (**C**); Rooting percentage (**D**). For each parameter and each group considered, columns labeled with different letters are significantly different (at $p < 0.05$). Student's *t*-test was used to compute the pair-wise comparisons between group means. Abbreviations: 6-benzylaminopurine (BA); Indole−3-butyric acid (IBA).

In Figure 6 is reported a schematic representation of the overall pitaya micropropagation procedures.

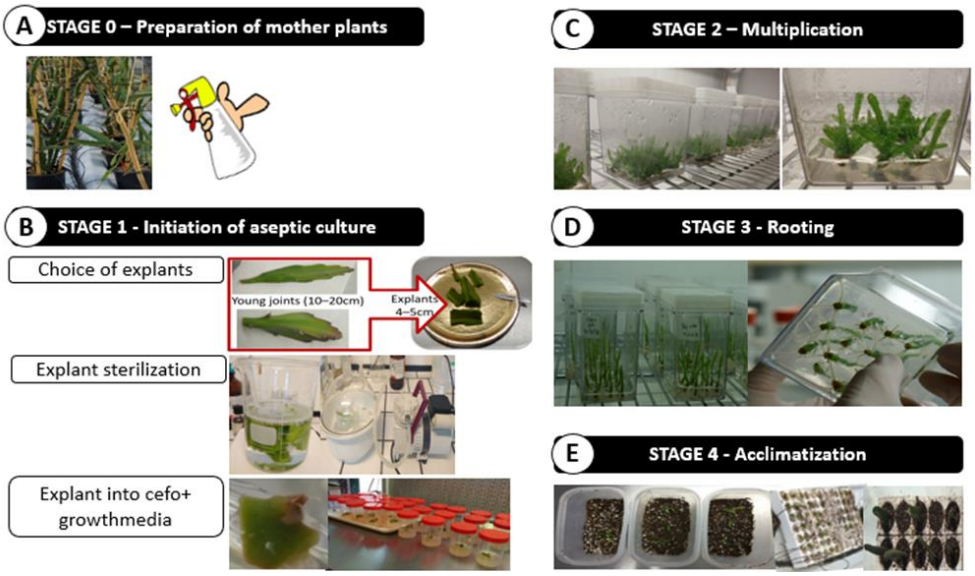

Figure 6. A schematic representation of pitaya in vitro propagation procedure. Preparation of mother plants, stage 0 (**A**); Initiation of aseptic culture, stage 1 (**B**); Multiplication, stage 2 (**C**); Rooting, stage 3 (**D**); Acclimatization, stage 4 (**E**).

3.3. Growing Potted Pitaya in Greenhouse

3.3.1. Phenology of Sprouting and Floral Bud Emergence

The phenology of sprouting and floral bud emergence was observed during two consecutive years. Sprouting in the red pitaya (*H. undatus*) showed two peaks (Figure 7A,D). Data collected from January 2018 to January 2019 showed that the major peak occurred between March and July and accounted for 83% of the yearly sprouting, whereas the minor one occurred between October and November. The beginning of bud development with a percentage of 8% of yearly flower bud formed was observed in July, at the end of the major sprouting wave. Seventy-seven percent of the yearly floral buds emerged lately in August, and the remaining 15% emerged sluggishly in September (Figure 7B). However, most of the flower bud aborted throughout the flowering season and accounted for the 77% of the yearly flower bud (7C). The flower abortion was most severe at the end of August and beginning of September, when the temperature in greenhouse reached 40–42 °C (Figure 7C).

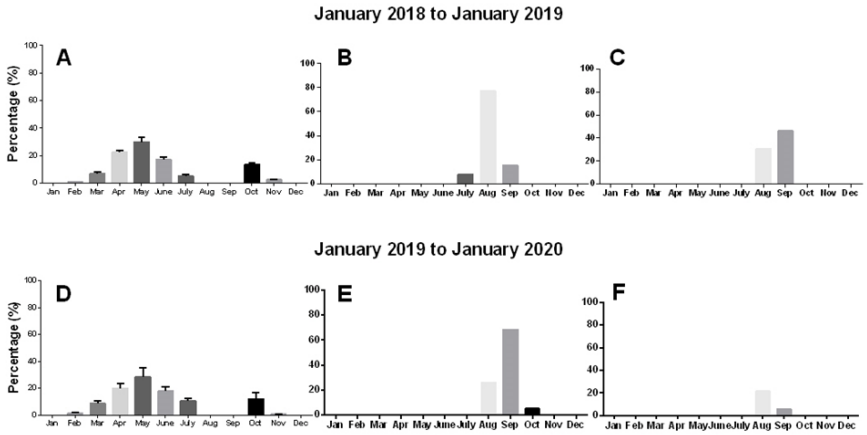

Figure 7. (**A–C**) Phenology of sprouting and floral bud formation in *H. hundatus* in Pisa (Italy). Data from January 2018 to January 2019 (**A–C**) and data from January 2019 to January 2020 (**D–F**). Total sprouting rate (represented as means of n = 30 plants. (**A,D**), floral bud formation rate (**B,E**), and floral bud abortion rate (**C,F**) were 100% within the period considered.

Data collected from January 2019 to January 2020 confirmed the phenological sprouting trend observed in 2018–2019. On the other hand, the beginning of bud development occurred in August, a month later than previously observed in 2018–2019 period, with a percentage of 26.3% of yearly flower bud formed at the end of the major sprouting wave (7D, E). Then, 66% of the yearly floral buds emerged lately in September, and the remaining 5% emerged sluggishly in October (Figure 7D). Twenty-six percent of the floral buds aborted throughout the flowering season, most severely in mid-August, and accounted for the 21% of the yearly flower bud (7F). The flower bud abortion was most severe in August, when the temperature in greenhouse reached 40–43 °C.

3.3.2. Pollination and Assessment of Fruit Set

Seventy-four percent of the yearly (2019) flower buds developed in fully opened flowers. *H. hundatus* flowers opened successfully with sepals and petals completely separated, anther dehiscence and receptive stigma positioned well above stamen as reported in Figure 8C. Flowers are nocturnal and open only for one night. In greenhouse conditions, due to the absence of natural pitaya flower pollinators, a hand self or cross pollination were performed within 12 h after the flowers have opened. After 3 weeks, the fruits were successfully pollinated, pericarpel was grown, as well as the endocarp was developed (Figure 8E). The beginning of fruit maturation and color development with basal portion of bracts turns pink was observed after 5 weeks from self-pollination event (Figure 8F). The fruit reached the maturity about 40–45 days after flowering and pollination (Figure 8G,H). A similar phenology of sprouting and floral bud formation was observed for the clone *H. hundatus* × *H. polyrhizus*. The white-fleshed cultivar (*H. undatus*) and the red-fleshed cultivar (*Hylocereus* spp.) were both self-compatible, setting fruit with hand self-pollination but cannot produce fruit by automatic self-pollination. In both cultivars hand cross-pollination, as well as hand self-pollination led to a 100% fruit set rates (Table 3), but in hand cross-pollination were observed heavier fruit weight than hand self-pollination ones. In *H. undatus* (white-flashed cultivar), the heaviest fruits obtained from hand cross-pollination ranged from 232–311 g, whereas hand self-pollination produced fruits ranged from 60–140 g (Table 3). Similarly, the weight of fruits in *Hylocereus* spp. (red-flesh cultivar) obtained from hand self-pollination was significantly lower (ranging from 80–134 g) than from hand cross-pollination (ranging from 210–465) (Table 3).

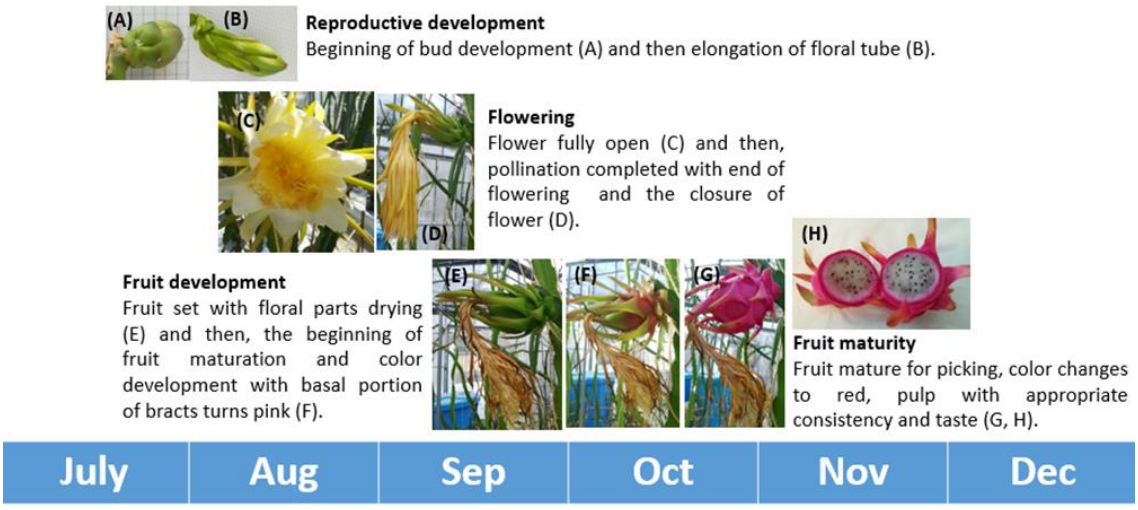

Figure 8. (**A**–**H**) Sequential illustrations progression of reproductive development, flowering, fruit development and maturation of dragon fruit (*H. undatus*) according to the extended BBCH scale from Kishore et al. (2016). Time elapsed in each stage (horizontal bar), (2018–2020). Reproductive development (**A**,**B**); Flowering (**C**,**D**); Fruit development (**E**,**F**); Fruit maturity (**G**,**H**).

Table 3. Effect of pollination method on fruit set percentage (%) and fruit weight (g) in the two Hylocereous spp. clones grown in greenhouse in Italy.

Hylocereous spp. Clones	Hand Self-Pollination		Hand Cross-Pollination	
	Fruit Set %	Fruit Weight (g)	Fruit Set %	Fruit Weight (g)
White flesh	100	140 a	100	273 a
Purple flesh	100	113 b	100	315 a

Values represent means (n = 6–7) fruits per treatment. Within rows, mean values followed by the same letter do not differ significantly ($p < 0.05$). Student's t-test was used to compute the pair-wise comparisons between group means.

3.4. Nutraceutical Potential and Antioxidant Benefits of Pitaya

The two dragon fruits (*Hylocereous* spp.) clones were harvested 40 to 45 days after anthesis (DAA). Clone 1 (*H. undatus* × *H. polyrhizus*) has red skin with red-purple flesh and clone 2 (*H. undatus*) has red skin with white flesh. The antioxidant power of these fruits was determined by means of both the FRAP and the DPPH assays (Figure 9A,B, respectively), and the results obtained with the two independent methods were linearly correlated (correlation coefficient r^2 = 0.9816; Figure 9C). In purple-flesh pitaya fruits the ferric-reducing capacity of antioxidants measured with FRAP method and the ability of antioxidants to scavenge the DPPH radical determined by DPPH assay were significantly higher compared to those observed in white-flesh pitaya fruits (Figure 9A,B). The Folin-Ciocalteu method was used to evaluate the total content of phenols. It was found that purple-flesh pitaya showed the highest phenolic concentration of 65.12 mg GAE/100 g FW followed by white-flesh pitaya (30.9 mg GAE/100 g FW), (Figure 9D). To correlate the phenolic compounds concentrations with the antioxidant capacities, the linear correlation coefficients (r) were calculated for the pitaya fruits extracts. The r between the antioxidant capacities obtained from FRAP assay and phenolic contents was 0.9180 (Figure 9E) and the r between the antioxidant capacities obtained from DPPH assay and phenolic contents was 0.9195 (Figure 9F).

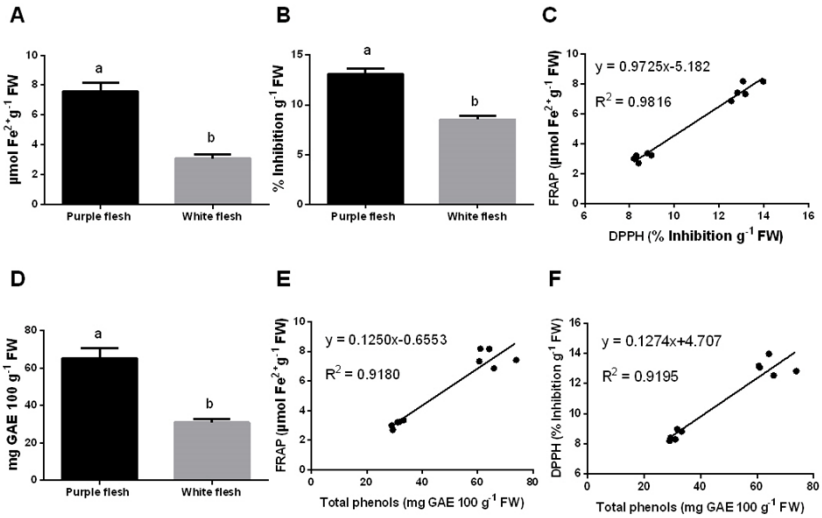

Figure 9. (**A**) Reducing capacity of extracts from purple and white flesh pitaya determined by FRAP test. (**B**) Radical-scavenging activity of extracts from purple and white flesh pitaya determined by DPPH test. (**C**) Linear correlation between the results of two antioxidant capacity assays: FRAP and DPPH methods (n = 10 samples by extract) (**D**) Total phenolic contents of extracts from purple and white flesh pitaya determined by Folin Ciocalteau method; (**E**) Linear correlation between the amount of total phenols and antioxidant capacity measured by the FRAP method (n = 10 samples by extract); (**F**) Linear correlation between the amount of total phenols and antioxidant capacity measured by the DPPH method (n = 10 samples by extract). (**A**,**B**,**D**) The results shown are the means ± SD (n = 5). Pair-wise statistical comparisons were calculated with a two-tailed Student's t-test and different letters denote significant differences at $p < 0.05$.

3.5. Fruit Quality Evaluation by Sensory Panel

The sensory results in this work was based on hedonic ratings from a small consumer population (20 participants), thus this might be considered merely a preliminary investigation on how consumers rate the liking of these fruits. The sensory profiles of purple- and white-fleshed pitaya fruits following the panel test were reported in Figure 10. Visual assessment evaluations by the sensory panelists indicated that the different pulp colors of the two pitaya varieties examined did not influence the overall liking appearance of the fruit. In fact, there were no significant differences in any parameters related to visual quality (i.e., color and the overall appearance).

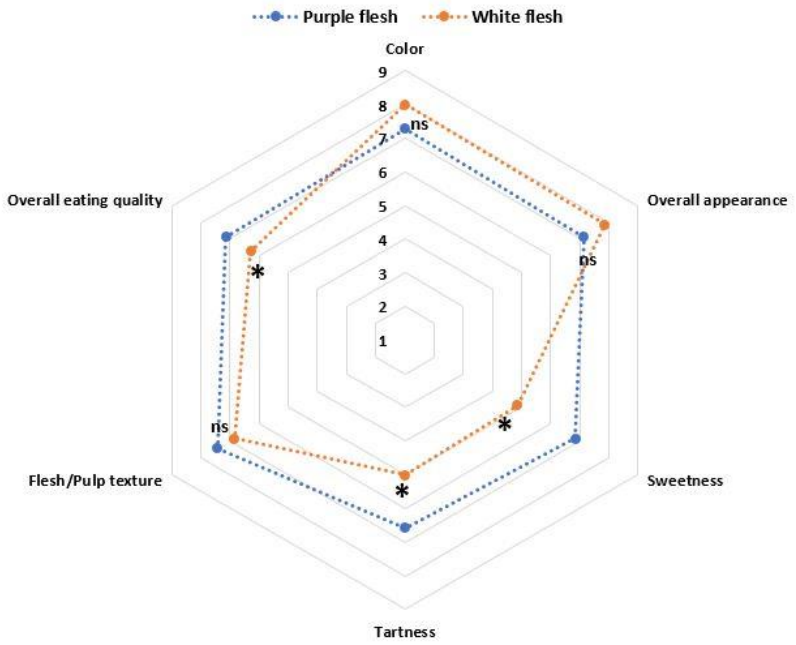

Figure 10. Sensory profiles (color, overall appearance, flavor, sweetness, tartness, flesh texture and overall eating quality) of two varieties of pitaya immediately after harvest. Sensory scores were expressed using a 9-point hedonic ratings where 1: extremely dislike, 5: neither like nor dislike, and 9: extremely like. The results shown are the means ±SD (n = 20). Pair-wise statistical comparisons were calculated with a two-tailed Student's t-test and asterisk indicates statistical significance ($p \leq 0.05$) while ns indicates a lack of significance.

On the other hand, flavor evaluations indicated that the sweetness and tartness were the dominant factors that were significantly different between the two pitaya varieties and thus these were closely related to those of overall eating quality (Figure 10).

4. Discussion

The present study is the first to investigate the potential of pitaya species as a new niche crop for the diversification of cultivated species around the Mediterranean to create new profitable alternatives/opportunities for small-scale agricultural producers in Italy and stand the global competitiveness while playing an important role in the future climate scenario.

Pitaya has great potential as a new crop for Mediterranean growers: it consumes little water (high efficiency of water use), it adapts well to the high temperatures present in the greenhouse [6]. Moreover, the economic market shows increasing demand of new, healthy exotic fruits and the fruit of this crops is worldwide appreciated as a super fruits, for its nutraceutical components, high nutritional value and positive health benefits [21,26–28].

In this study we mainly focused on the development of the whole production chain from propagation to the greenhouse cultivation of pitaya species to offer basic knowledges and technical

information that may lead to the commercial production of these new commodities in Italy, providing also information on the antioxidant properties which show these fruits.

To facilitate the establishment of pitaya (*Hylocereus* spp.) cultivations in new areas, different variables affecting its propagation by cuttings and micropropagation were studied.

Pitaya may be propagated from seeds; however, fruit and stem characteristics are variable. Additionally, this plant is characterized by a long juvenility phase so the use of seeds entails longer time to grow and produce fruit, the time from planting to fruit production may be up to 6 years.

The results reported in this study show a significant effect of cutting size on the overall rooting performances. Medium cuttings developed a better radical apparatus in terms of rooting frequency, number and length of root compared to the small cuttings and this trend was maintained in the two seasonal periods assessed (Figures 2 and 3). The data reported are consistent with previous results obtained in *Hylocereous undatus* [29,30] and other plants, such as *Eucalyptus* sp. [31], *Lavandula dentata* [32], *Duranta repenes* [33] and *Tinospora crispa* [34]. Plant are dependent on for its carbohydrate supply on the photosynthetic activity of shoot and stem [35] and longer pitaya cuttings have a higher surface dedicated to photosynthesis, thus higher carbohydrate reserves stored to initiate and sustain the develop and the growth of roots.

The overall rooting process of both small and medium cuttings were positively affected during spring-summer season (Figure 3) which resulted in more and longer plant roots, and faster root growth. This phenomenon might be caused by temperatures increase, which significantly accelerates the root meristem cell division [36], as well as cell expansion and differentiation by increasing nuclear auxin signaling and root growth [37]. This study demonstrated that rooting of 20 cm cuttings was superior to that of 10 cm, therefore the longer cuttings might be recommended for direct field planting to maximize the survival which ultimately result in an earlier reproductive phase. On the other hand, when propagation material is limited (i.e., elite clones) small cuttings may need to be used to satisfy large plant demand and the choice of the best seasonal period of propagation could make small sized cuttings ideal for propagation.

To support the expansion of pitaya crop in Italy, the establishment of an efficient propagation methodology is essential for the pitaya cultivars (*Hylocereous undatus* and *Hylocereous* sp.) which have demonstrated good adaptation and produced quality fruits in Mediterranean region. Several protocols were developed for propagation of pitaya [38–42]. However, tissue culture responses of micropropagated explants are controlled by diverse genetic mechanisms and dragon fruit cultivars with diverse genetic backgrounds respond differently to culture medium conditions [40–42]. Thus, an efficient method for micropropagation of the two genetically different pitaya cultivars object of this study was developed to produce multiple adventitious shoots directly from preformed areole buds while minimizing somaclonal variation. A schematic representation of the pitaya in vitro propagation procedure is reported in Figure 6.

The disinfection of initial explants is a major problem in the micropropagation. According to previous studies, bacterial contamination and the high tissue sensitivity to disinfection treatments with NaOCl are recurrent problems in cacti tissue culture [40,43]. To reduce these issues frequently faced during the establishment of in vitro cultures of pitaya, in this work the synergistic use of a systemic fungicide in the early step of disinfection was combined with a broad-spectrum preservative and biocide, for plant tissue culture. Conducting the sterilization process under vacuum allowed a better penetration of sterilizing agent. Moreover, a significant reduction in the level of damage (higher survival %) was achieved using a two-step procedure with relatively low NaOCl concentration instead of a single severe treatment [44]. Yet, the addition of cephotaxime, a broad-spectrum antibiotic, into the culture media was helpful to get rid of endogenous contamination and might have a beneficial effect also in the proliferation of explants during the successive induction phase. The role of this antibiotics is not yet clear: although a positive effect of the inclusion of cefotaxime in the culture media [45] was observed in other growing systems. The metabolism of this compound by plant cell

esterases may produce metabolites which exert growth regulator-like activity as well as influence plant development [46].

Combining concentrations of cytokinins and auxins, maintaining a favorable proportion to the cytokinins, was reported in pitaya micropropagation studies to both sustain shoot multiplication and promote rhizogenesis in in vitro-derived shoots [38,41]. In the present study, the use of either 0.5 mg L^{-1}BA + 0.25 mg L^{-1}IBA, or 3 mg L^{-1}ZEA + 0.25 mg L^{-1} IBA did not affect the shoot number per explant and the shoot diameter, whilst BA decreased significantly the height of newly regenerated shoots in comparison with ZEA as also observed in other in vitro cultured species [45]. Among cytokinins, BA is the cheapest cytokinin, and can be easily autoclaved [47]. Considering the ease of handling and the cost, the use of the combination of BA+IBA was proposed in this study as an optimum medium for multiplication of pitaya cultivars for commercial micropropagation establishment.

Spontaneous rooting (>98% rooting) was observed when the explants were cultivated for two weeks in cytokinin and auxin depletion media. Many catcti species (including *Hylocerous* sp.) develop roots in absence of growth regulators [48–51]. Moreover, the spontaneous formation of roots is important to speed up acclimatization of plants in the greenhouse, allowing to obtain high success rate of survival (87%), in agreement with previously reported studies on *Hylocereous* species [40].

In Italy, sprouting of *Hylocereous* spp. cultivated in greenhouse, occurred in two peaks, a major peak was observed between spring and early summer and the minor in early autumn. Moreover, the sprouting behavior shown consistency over a period of two years (January 2018 to January 2019 vs. January 2019 to January 2020, Figure 7).

Over the two years of observation, the flower bud initiation began in summer, with a slightly different timing between the two years. The phase of flowering in 2018 was characterized by high temperatures from August–September when the temperature in greenhouse reached 42 °C. As previously reported [52,53], high summer temperatures during the most abundant floral bud emission phase seem to be clearly linked to the severe abortion of flower buds observed during the first year. During the second year of observations the floral bud emission and flowering, occurred a month later compared to 2018 (Figure 7E) and this was probably due to differences in climatic conditions which were characterized by high temperatures in greenhouse (43 °C) from June–July leading to the abortion of early formed flower buds as well as more general delay in bud emission. The climatic condition observed in the second year might positively affected the flower differentiation and development, while delaying the flowering process at the end of summer, when the temperatures were not so extreme.

Hylocereous sp. flowers are hermaphroditic, nocturnal and open only for one night [54]. They are pollinated by night bats and large hawkmoths and during the day, immediately after their anthesis, by bees [54,55]. To determine the requirements for pollination of the two pitaya cultivars grown under greenhouse conditions in Italy, thus in absence of natural pitaya flower pollinators, a hand-self or -cross pollination were performed.

The two cultivars, *H. undatus* (white-flesh cultivar) and *Hylocereus* sp. (red-flesh cultivar) were self-compatible, producing both high fruit set percentages after hand self-pollination (Table 3). However, hand cross-pollination gave larger fruits than hand self-pollination, when both cultivars were hand-crossed with pollen from a different, concurrently flowering *Hylocereus* clones (Table 3). Some previous studies reported that self-compatible or self-incompatible species had largest fruit after a specific cross-pollination [56–59]. Because of the low fruit weight obtained after hand self-pollination, out-crossing should be carried out for both cultivars to guarantee high yields when natural pollinators are not available or may be ineffective [56]. Moreover, manual pollination is effective if conducted within 12 h after the flower has opened [58].

Commonly, the maturity indices used to assess the harvest point are the color skin changes and the days after flowering (DAA) [60]. *Hylocereous* fruits are typically harvested once the color of the skin changes from green to full red because the best eating quality are attained [52,61,62]. Several studies have been conducted to examine the optimal physiological maturity of pitaya fruits among different

production areas, providing recommendations on the optimal time to harvest these non-climateric fruits to ensure that quality is maintained. In Vietnam the optimal harvest time for *Hylocereous* fruits ranging from 28 to 32 DAA [61,63], in Brazil from 34 to 42 DAA [64], in California from 40 to 45 DAA [65], in Hawaii from 35 to 50 DAA [66] and in Israel from 32 to 35 DAA [67]. In this study conducted in Italy, the fruit maturation period of *Hylocereous* cultivars grown in greenhouse extended from September to early-December and it was approximately 40–45 days long (Figure 8).

To provide information on the potential benefits deriving from the consumption of red-flesh and white-flesh dragon fruit cultivars grown in Italy, their total phenolic content, their antioxidant activities as well the correlations between the strength of these scavenging capacities and the total phenolic content were measured. In agreement with previous studies, the red-flesh fruits showed the highest total phenolic contents and total antioxidant capacities than white-flesh fruits [68–70]. The higher the total phenolic content, the higher the antioxidant activities (for both DPPH and FRAP assays), and these observations were confirmed by the strong positive correlation between total phenols and antioxidant activities reported in fruits of both *Hylocereous* clones (Figure 9E,F). Such high r values suggested that the scavenging activity and the reducing power were highly correlated with phenolic compounds and the phenolic compounds in *Hylocereous* fruits were responsible for their antioxidant capacity. However, red-flesh fruits showed a better antioxidant activity that may be due to the higher amount of phenolic compounds in the pulp extract. In addition, the red-flesh fruits showed a better appreciation level (overall eating quality) by the panelists compared to the white-flesh (Figure 10). Flavor evaluations indicated that the most variation between the two cultivars was in perceived intensity of tartness and sweetness; a lower tartness together with a higher sweetness were the key sensory terms linked to the higher overall eating quality of red-flesh fruits. A previous published sensory analysis of *Hylocereous* fruits reported that the strongest linking was associated with the highest rating of sweetness [70].

5. Conclusions

The results of this studied report various aspects to support the introduction of pitaya cultivation in Italy through a whole chain approach, from propagation to greenhouse cultivation of species belonging to the genus *Hylocereous* spp.

(i) Efficient vegetative propagation methodologies were developed for both pitaya cultivars (*Hylocereous undatus* and *Hylocereous* spp.) to support the cultivation and expansion of pitaya crop in Italy.

(ii) A detailed analysis of the greenhouse cultivation practices and the effect of controlled environment for *Hylocereous* cultivars were provided. The clarification of the sprouting and flowering phenology as well as the fruit maturation period of pitaya crop grown in Italian greenhouse will enable the grower to define the agronomic management required to create a new profitable alternative horticultural/ornamental product.

(iii) From the results obtained in the sensory and nutraceutical properties evaluations of *Hylocereous* fruits it can be concluded that the red-flesh fruits represent a promising source of natural antioxidants with a superior overall eating quality perceived by the panelists.

Author Contributions: Conceptualization, A.T., A.F. and A.M.-S.; methodology A.T., M.L., M.O., D.M.; data curation, A.T., M.L. and A.F.; writing—original draft preparation, A.T., A.F. and A.M.-S.; writing—review and editing, A.T., A.F., D.M., L.I., and A.M.-S.; supervision, A.M.-S. and L.I.; project administration, A.M.-S.; and funding acquisition, A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Fondazione Caript (Italy), Bando Pistoia Giovani & Ricerca scientifica 2017–2019.

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

References

1. Ferrante, A.; Trivellini, A.; Scuderi, D.; Romano, D.; Vernieri, P. Post-production physiology and handling of ornamental potted plants. *Postharvest Biol. Technol.* **2015**, *100*, 99–108. [CrossRef]
2. Iglesias, A.; Garrote, L. Adaptation strategies for agricultural water management under climate change in europe. *Agric. Water Manag.* **2015**, *155*, 113–124. [CrossRef]
3. Costes, E.; Khadari, B.; Zaher, H.; Moukhli, A.; Morillon, R.; Legave, J.; Regnard, J. Adaptation of Mediterranean fruit tree cultivation to climate change. In *The Mediterranean Region under Climate Change*; Moatti, J., Thiébault, S., Eds.; AllEnvi, IRD Éditions: Marseille, France, 2016; pp. 503–510.
4. Nobel, P.S.; Barrera, E.D.L. Stem water ralations and net CO_2 uptake for a hemiepiphytic cactus during short-term drought. *J. Exp. Bot.* **2002**, *48*, 129–137. [CrossRef]
5. Keiichi, N.; Mai, I.; Takuya, A.; Yoshimi, Y. Seasonal differences in diurnal patterns of metabolities and enzyme activities in pitaya (*Hylocereus undatus*) grown in a temperate zone. *J. Jpn. Soc. Hortic. Sci.* **2010**, *79*, 135–140.
6. Wang, L.; Zhang, X.; Ma, Y.; Qing, Y.; Wang, H.; Huang, X. The highly drought-tolerant pitaya (Hylocereusundatus) is a non-facultative CAM plant under both well-watered and drought conditions. *J. Hortic. Sci. Biotechnol.* **2019**, *94*, 643–652. [CrossRef]
7. Lüttge, U. Ecophysiology of crassulacean acid metabolism (CAM). *Ann. Bot.* **2004**, *93*, 629–652. [CrossRef]
8. Herrera, A. Crassulacean acid metabolism and fitness under water deficit stress: If not for carbon gain, what is facultative CAM good for? *Ann. Bot.* **2009**, *103*, 645–653. [CrossRef]
9. Stintzing, F.C.; Carle, R. Betalain-emerging prospect for food scientist. *Trends Food Sci. Technol.* **2007**, *18*, 514–525. [CrossRef]
10. Nurliyana, R.; Syed Zahir, I.; Mustapha Suleiman, K.; Aisyah, M.R.; Kamarul Rahim, K. Antioxidant study of pulps and peels of dragon fruits: A comparative study. *Int. Food Res. J.* **2010**, *17*, 367–375.
11. Wichienchot, S.; Jatupornpipat, M.; Rastall, R.A. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chem.* **2010**, *120*, 850–857. [CrossRef]
12. Cos, P.; Bruyne, D.T.; Hermans, N.; Apers, S.; Berghe, D.V.; Vlietinck, A.J. Proanthocyanidins in health care: Current and new trends. *Curr. Med. Chem.* **2004**, *11*, 1345–1359. [CrossRef]
13. Wybranice, S.; Mizrahi, Y. Fruit flesh betacyanin pigments in hyloceruus Cacti. *J. Agric. Food Chem.* **2002**, *50*, 6086–6089. [CrossRef] [PubMed]
14. Kim, H.; Choi, H.; Moon, J.; Kim, Y.; Mosaddik, A.; Cho, S. Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. *J. Food Sci.* **2010**, *76*, C38–C45. [CrossRef] [PubMed]
15. Esquivel, P.; Stintzing, F.C.; Carle, R. Pigment pattern and expression of colour in fruits from different *Hylocereus* sp. Genotypes. *Innov. Food Sci. Emerg. Technol.* **2007**, *8*, 451–457. [CrossRef]
16. Wu, L.C.; Hsu, H.W.; Chen, Y.C.; Chiu, C.C.; Lin, Y.I.; Ho, J.A. Antioxidantand antiproliferative activities of red pitaya. *Food Chem.* **2006**, *95*, 319–327. [CrossRef]
17. Strack, D.; Vogt, T.; Schliemann, W. Recent advances in betalain research. *Phytochemistry* **2003**, *62*, 247–269. [CrossRef]
18. Guimarães, D.A.B.; De Castro, D.D.S.B.; de Oliveira, F.L.; Nogueira, E.M.; da Silva, M.A.M.; Teodoro, A.J. Pitaya extracts induce growth inhibition and proapoptotic effects on human cell lines of breast cancer via downregulation of estrogen receptor gene expression. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 7865073. [CrossRef]
19. Gengatharan, A.; Dykes, G.A.; Choo, W.S. Betalains: Natural plant pigments with potential application in functional foods LWT. *Food Sci. Technol.* **2015**, *64*, 645–649. [CrossRef]
20. Tsuda, S.; Murakami, M.; Matsusaka, N.; Kano, K.; Taniguchi, K.; Sasaki, Y.F. DNA damage induced by red food dyes orally administered to pregnant and male mice. *Toxicol. Sci.* **2001**, *61*, 92–99. [CrossRef]
21. Le Bellec, F.; Vaillant, F.; Imbert, E. Pitahaya (*Hylocereus* spp.): A new fruit crop, a market with a future. *Fruits* **2006**, *61*, 237–250. [CrossRef]
22. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
23. Kang, H.M.; Saltveit, M.E. Antioxidant capacity of lettuce leaf tissue increases after wounding. *J. Agri. Food Chem.* **2002**, *50*, 7536–7541. [CrossRef] [PubMed]

24. Benzie, I.; Strain, J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power: The FRAP Assa. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
25. Dudonné, S.; Vitrac, X.; Coutière, P.; Marion Woillez, M.; Mérillon, J.M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem.* **2009**, *57*, 1768–1774. [CrossRef]
26. Dembitsky, V.; Poovarodom, S.; Leontowicz, H.; Leontowicz, M.; Vearasilp, S.; Trakhtenberg, S.; Gorinstein, S. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food Res. Int.* **2001**, *44*, 1671–1701. [CrossRef]
27. Mizrahi, Y. Vine-cacti Pitayas the New Crop of the World. *Rev. Bras. Frutic.* **2014**, *36*, 124–138. [CrossRef]
28. Cornara, L.; Xiao, J.; Smeriglio, A.; Trombetta, D.; Burlando, B. Emerging Exotic Fruits: New Functional Foods in the European Market. *EFood* **2020**, *1*, 126–139. [CrossRef]
29. Obeidy, A.A.E. Mass propagation of pitaya (dragon fruit). *Fruits* **2006**, *61*, 313–319.
30. Kakade, V.; Dinesh, D.; Singh, D.; Bhatnagar, P.R.; Kadam, D. Influence of length of cutting on root and shoot growth in dragon fruit (Hylocereus undatus). *Indian J. Agric. Sci.* **2019**, *89*, 1895–1904.
31. Naidu, R.D.; Jones, N.B. The effect of cutting length on the rooting and growth of subtropical Eucalyptus hybrid clones in South Africa, Southern Forests. *J. For. Sci.* **2009**, *71*, 297–301.
32. Bona, C.M.; Biasetto, I.R.; Masetto, M.; Deschamps, C.; Biasi, L.A. Influence of cutting type and size on rooting of *Lavandula dentata* L. *Rev. Bras. Planta Med. Botucatu* **2012**, *14*, 8–11. [CrossRef]
33. Okunlola, A.I. The effects of cutting types and length on rooting of Duranta repens in the nursery. *Glob. J. Hum. Soc. Sci. Geogr. Geo-Sci. Environ. Disaster Manag.* **2013**, *13*, 380–390.
34. Aminah, H.M.; Ahmad Fauzi, S.; Tariq Mubarak, H.; Hamzah, M. Effect of Hormone and Cutting Length on the Rooting of Tinospora crispa. *Int. J. Sci. Res. Publ.* **2015**, *5*, 1–4.
35. Philipson, J. Root growth in Sitka spruce and Douglas-fir transplants: Dependence on the shoot and stored carbohydrates. *Tree Physiol.* **1998**, *4*, 101–108. [CrossRef]
36. Francis, D.; Barlow, P. Temperature and the cell cycle. *Symp. Soc. Exp. Biol.* **1988**, *42*, 181–201.
37. Feraru, E.; Feraru, M.I.; Barbez, E.; Waidmann, S.; Sun, L.; Gaidora, A.; Kleine-Vehn, J. PILS6 is a temperature-sensitive regulator of nuclear auxin input and organ growth in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 3893–3898. [CrossRef]
38. Mohamed-Yasseen, Y. Micropropagation of pitaya (Hylocereus undatus Britton et rose). *In Vitro Cell Dev. Biol-Plant.* **2002**, *38*, 427–429. [CrossRef]
39. Dahanayake, N.; Ranawake, A.L. Regeneration of dragon fruit (Hylocereus undatus) plantlets from leaf and stem explants. *Trop. Agric. Res. Ext.* **2011**, *14*, 85–89. [CrossRef]
40. Vinas, M.; Ferna'ndez-Brenes, M.; Azofeifa, A.; Jime´nez, V.M. In vitro propagation of purple pitahaya (Hylocereus costaricensis [F.A.C. Weber] Britton & Rose) cv. Cebra. *In Vitro Cell Dev. Biol. Plant* **2012**, *48*, 469–477.
41. Fan, Q.J.; Zheng, S.C.; Yan, F.X.; Zhang, B.X.; Qiao, G.; Wen, X.P. Efficient regeneration of dragon fruit (Hylocereus undatus) and an assessment of the genetic fidelity of in vitro: Derived plants using ISSR markers. *J. Hortic. Sci. Biotechnol.* **2013**, *88*, 631–637. [CrossRef]
42. Hua, Q.; Chen, P.; Liu, W.; Ma, Y.; Liang, R.; Wang, L.; Wang, Z.; Hu, G.; Qin, Y. A Protocol for Rapid in Vitro Propagation of Genetically Diverse Pitaya. *Plant. Cell Tissue Organ. Cult.* **2015**, *120*, 741–745. [CrossRef]
43. Pérez-Molphe-Balch, E.; Santos-Díaz, M.d.S.; Ramírez-Malagón, R.; Ochoa-Alejo, N. Tissue culture of ornamental cacti. *Sci. Agric.* **2015**, *72*, 540–561. [CrossRef]
44. Teixeira da Silva, J.A.; Kulus, D.; Zhang, X.; Zeng, S.; Ma, G.; Piqueras, A. Disinfection of explants for saffron (Crocus sativus L.) tissue culture. *Environ. Exp. Biol.* **2016**, *14*, 183–198. [CrossRef]
45. Lucchesini, M.; Mensuali-Sodi, A. Influence of medium composition and vessel ventilation on in vitro propagation of *Phillyrea latifolia* L. *Sci. Hortic.* **2004**, *100*, 117–125. [CrossRef]
46. D'Utra Vaz, F.B.; dos Santos, A.V.P.; Manders, G.; Cocking, E.C.; Davey, M.R.; Power, J.B. Plant regeneration from leaf mesophyll protoplasts of the tropical woody plant, passionfruit (Passiflora edulis fv flavicarpa Degener.): The importance of the antibiotic cefotaxime in the culture medium. *Plant Cell Rep.* **1993**, *12*, 220–225. [CrossRef]
47. Zaerr, J.B.; Mapes, M.O. Action of growth regulators. In *Tissue Culture in Forestryl*; Bonga, J.M., Durzan, D.J., Eds.; Martinus Nijhoff: Dordrecht, The Netherlands; Boston, MA, USA, 1982; pp. 231–255.

48. Moebius, K.G.; Mata, M.; Chávez, V.M. Organogenesis and somatic embryogenesis in *Ariocarpus kotschoubeyanus* (Lem.) K. Schum. (Cactaceae), an endemic and endangered mexican species. *In Vitro Cell. Dev. Biol.-Plant* **2003**, *39*, 388–393. [CrossRef]
49. Burdyn, L.; Luna, C.; Tarrago, J.; Sansberro, P.; Dudit, N.; González, A.; Mroginski, I. Direct shoot regeneration from leaf and internode explants of *Aloysia polystachya* [Gris.] Mold. (Verbenaceae). *In Vitro Cell. Dev. Biol.-Plant.* **2006**, *42*, 235–239. [CrossRef]
50. Drew, R.A.; Azimi, M. Micropropagation of red pitaya (*Hylocereous undatus*). *Acta Hortic.* **2002**, *575*, 93–98. [CrossRef]
51. De Medeiros, L.A.; de Ribeiro, R.C.S.; Gallo, L.A.; de Oliveira, E.T.; Demattê, M.E.S.P. In vitro propagation of *Notocactus magnificus*. *Plant Cell Tissue Organ Cult.* **2006**, *84*, 165–169. [CrossRef]
52. Nerd, A.; Sitrit, Y.; Kaushik, R.A.; Mizrahi, Y. High summer temperatures inhibit flowering in vine pitaya crops (*Hylocereus* spp.). *Sci. Hortic.* **2002**, *96*, 343–350. [CrossRef]
53. Raveh, E.; Nerd, A.; Mizrahi, Y. Responses of two hemiepiphytic fruit crop cacti to different degrees of shade. *Sci. Hortic.* **1998**, *73*, 151–164. [CrossRef]
54. Grant, V.; Grant, K.A. The pollination spectrum in the southwestern American cactus flora. *Plant. Sytematics Evol.* **1979**, *133*, 29–37. [CrossRef]
55. Valiente-Banuet, A.; Santos-Gally, R.; Arizmendi, M.C.; Casas, A. Pollination biology of the hemiepiphytic cactus *Hylocereus undatus* in the Tehuacán Valley, Mexico. *J. Arid Environ.* **2007**, *68*, 1–8. [CrossRef]
56. Weiss, J.; Nerd, A.; Mizrahi, Y. Flowering behavior and pollination requirements in climbing cacti with fruit crop potential. *HortScience* **1994**, *29*, 1487–1492. [CrossRef]
57. Lichtenzveig, J.; Abbo, S.; Nerd, A.; Tel-Zur, N.; Mizrahi, Y. Cytology and mating system in the climbing cacti Hylocereus and Selenicereus. *Am. J. Bot.* **2000**, *87*, 1058–1065. [CrossRef]
58. Tran, D.H.; Yen, C.R.; Chen, Y.K.H. Effect of pollination method and pollen source on fruit set and growth of red-peel pitaya (*Hylocereus* spp.) in Taiwan. *J. Hortic. Sci. Biotechnol.* **2015**, *90*, 254–258. [CrossRef]
59. Ha, T.D.; Oanh, L.T.K.; Yen, C.R. Flowering phenology and mating system of a red skin pitaya (*Hylocereus* spp.) germplasm collection in Taiwan. *Asian J. Adv. Agric. Res.* **2018**, *7*, 1–8. [CrossRef]
60. Freitas, S.T.D.; Mitcham, E.J. Quality of pitaya fruit (*Hylocereus undatus*) as influenced by storage temperature and packaging. *Sci. Agric.* **2013**, *70*, 257–262. [CrossRef]
61. To, L.V.; Ngu, N.; Duc, N.D.; Trinh, D.T.K.; Thanh, N.C.; Mien, D.V.H.; Hai, C.N.; Long, T.N. Quality assurance system for dragon fruit. *ACIAR Proc.* **2000**, *100*, 101–114.
62. Ortiz, T.A.; Takahashi, L.S.A. Physical and chemical characteristics of pitaya fruits at physiological maturity Genet. *Mol. Res.* **2015**, *14*, 14422–14439. [CrossRef]
63. Hoa, T.T.; Clark, C.J.; Waddell, B.C.; Woolf, A.D. Postharvest quality of dragon fruit (*Hylocereus undatus*) following disinfesting hot air treatments. *Postharvest Biol. Technol.* **2016**, *41*, 62–69. [CrossRef]
64. Magalhães, D.S.; da Silva, D.M.; Ramos, J.D.; Pio, L.A.S.; Pasqual, M.; Boas, E.V.B.V.; Galvão, E.C.; de Melo, E.T. Changes in the physical and physico-chemical characteristics of red-pulp dragon fruit during its development. *Sci. Hortic.* **2019**, *253*, 180–186. [CrossRef]
65. Merten, S. A review of Hylocereus production in the United States. *J. Prof. Assoc. Cactus Dev.* **2003**, *5*, 98–105.
66. Zee, F.; Yen, C.-R.; Nishina, M. Pitaya. In *Fruits and Nuts*; Cooperative Extension Service; College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa: Honolulu, HI, USA, 2004.
67. Nerd, A.; Gutman, F.; Mizrahi, Y. Ripening and postharvest behaviour of fruits of two Hylocereus species (Cactaceae). *Postharvest Biol. Technol.* **1999**, *17*, 39–45. [CrossRef]
68. Choo, W.S.; Yong, W.K. Antioxidant properties of two species of Hylocereus fruits. *Adv. Appl. Sci. Res.* **2011**, *2*, 418–425.
69. Senadheera, S.P.N.M.K.; Abeysinghe, D.C. Bioactive Compounds and Total Antioxidant Capacity of Different Tissues of Two Pitaya (Dragon Fruit) Species Grown in Sri Lanka. *J. Food Agric.* **2015**, *8*, 33–40. [CrossRef]
70. Obenland, D.; Cantwell, M.; Lobo, R.; Collin, S.; Sievert, J.; Arpaia, M.L. Impact of storage conditions and variety on quality attributes and aroma volatiles of pitahaya (*Hylocereus* spp.). *Sci. Hortic.* **2016**, *199*, 15–22. [CrossRef]