

# CactusNet Newsletter

Special Issue 15. May 2021

## CACTI AND SUCCULENTS ECOPHYSIOLOGY



Editor  
Giorgia Liguori



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**Editor**

Giorgia Liguori



# in memory of Nicolas Franck

*The first time that I met Nicolas was during the VII<sup>th</sup> International Congress on Cactus Pear and Cochineal held in Agadir (Morocco), in 2010. I was very impressed by his passion for cactus pear ecophysiology, we talked about our recent researches regarding the carbon balance in *Opuntia ficus-indica* and we were in touch until we met again during the IX International Congress on Cactus Pear and Cochineal “CAM crops for a hotter and drier world” held in Coquimbo in 2017, where he was co-convener. In Coquimbo I found the same passion that I knew in Agadir when I met him for the first time, we talked about our idea to make a Research Project on cactus pear ecophysiology to develop together and we enjoyed the time spent together during the Congress and the field trips. I was hoping that we could share other cactus experiences together but it was not possible, I’m very sad about that, I will miss you Nicolas.*

*Giorgia Liguori*

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# Foreword

It has been 30 years since, as Enrique Arias pointed out in the Foreword of the last Newsletter, "a group of selfless researchers, convinced of the benefits of cactus pear, decided to share their experiences (First International Congress of Nopal and Cactus Pear in Lagos de Moreno, Jalisco, Mexico, 1991), which led to the creation of the International Network of Technical Cooperation in Cactus Pear or (currently FAO-ICARDA CACTUSNET), which was formalized under the auspices of FAO in 1993, with the slogan: an effort to produce and conserve the environment in arid and sub-arid regions".

We have now reached this new anniversary trying to leave behind a pandemic, with all its pain and suffering, but also with important learning. One of them, the great importance of friends and face-to face meetings, that allow us to share knowledge, professional and humans experiences, and from where we draw the strength to face the challenges of the day to day, and of having lost very good colleagues and friends in 2020.

Among those good friends was Enrique Arias, tireless promoter and driving force of CactusNet, who we will remember with affection and gratitude. Sergio Uhart, great professional and generous member of our Network. Alejandro Ayres, businessman who participated and also supported some of our activities... All of them, people of great human value that have left us a very high standard in the activities of CactusNet as well as in their dedication to try to improve the welfare of people living in dry areas.

Before them, in 2017, Nicolas Franck left us, to whom we dedicate this special issue of the Newsletter about the *Opuntia ficus-indica* physiology. Nicolas passed away being a young and enthusiastic academic of the University of Chile, convener of the International Congress of cactus pear and cochineal, held in Coquimbo, Chile in 2017. He left to young researchers and to all of us an example on how to work in science and to share this knowledge with all kinds of people.

The example and dedication of all of them imposes new goals and challenges to CactusNet, which continues to grow, aspiring to be an important contribution to the sustainability, conservation and development of arid and semi-arid areas. The multiple properties and versatile forms of consumption and uses of the cactus pear can take a more important role in the dry areas and can be seen as a plant with future for their inhabitants.

I would like to thank Prof. Giorgia Liguori who generously led and coordinated this Newsletter and all the authors who contributed to make available to the community new knowledge about the physiology of this wonderful plant.

*Professor Carmen Saenz*

*General Coordinator FAO-ICARDA CactusNet*



# POSTHARVEST HANDLING OF CACTUS PEAR TO REDUCE DECAY AND PHYSIOLOGICAL DISORDERS

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## Harvest

The stage of physiological development at which cactus pear are harvested can vary greatly, depending on destination. When fruit are intended for distant markets are picked in the earlier stages of ripening (Figures 1, 2) to maintain a longer postharvest life, whereas when are intended for self-consumption or local markets, harvesting can be done in more advanced stages of maturity, even when fruit start to age (Figure 3).

Several objective and subjective parameters can be used as maturity indexes, among which TSS content, firmness, peel color, flower cavity depth and fall of the glochids are the most popular (Yahia and Saenz, 2011; Potgieter and D'Aquino, 2017). All these parameters at different degrees correlate well with the ripening process, but according to the cultivar and pedo-climatic conditions the rate of change of individual parameters is not always consistent with changes occurring to the others, being able to progress faster or slower. For example, TSS can accumulate rapidly and reach high levels with the beginning of maturity, when the peel begins to color, and undergo slower increases thereafter, just when color develops rapidly.

Eating quality in terms of sweetness, acidity and flavor does not vary too much during fruit coloration, whereas flesh firmness can decline very rapidly in this phase, especially in fruits that ripen during the hottest months. In most cases the maturity index of choice is peel color, which besides being fairly reliable is also

practical and ease to apply for pickers (Cantwell, 1995; Potgieter and D'Aquino, 2017).



Figure 1 – Cactus pear cv 'Gialla' at the earlier stages of maturity.



Figure 2 – Fruit destined to long distant markets are harvested when peel begins to color.



Figure 3 – Over ripe fruit showing clear signs of ageing.

Harvest operations need be conducted accurately to prevent or reduce disorders and microbiological decay that can appear several days after harvest but that can dramatically reduce fruit quality and postharvest life.

In many countries fruit are picked by rotating the base until separate from the cladode. This method is not advisable because the base of the fruit, irregularly wounded, can be easily invaded by pathogens causing decay. When fruit are separated from the cladode with a clean cut done with well sharpened knives or scissors, the wounded surface is even and tissue can rapidly heal forming an efficient physical barrier to pathogens (Cantwell, 1995).

As fruit ripen, glochids tend to fall and during the hottest and dry hours of the day in windy conditions they can even fly in the air. Floating glochids are fastidious and dangerous for pickers

because can enter the eyes and the respiratory tract. To make safer and more comfortable pickers' work, fruit are generally harvested early in the morning when air humidity prevents glochids to spread from fruit surface to air. Harvest is a critical step of the entire market chain and employees should be properly trained and provided with suitable devises (gloves, overalls, glasses) that should protect the hands from injuries caused spine and in the same time help to overcome the instinctive tendency to avoid spines by using only two fingers to grasp the fruit in order to prevent compression injuries (Cantwell, 1995; Inglese et al., 2002).

The practice of picking the fruit in the early hours of the day when air humidity is high, if in one hand is good for pickers and for the fruit lower metabolic activity thanks to the lower temperatures, on the other hand humid or wet conditions can be cause of serious phytosanitary issues as spores of



Figure 4 – Physical damages caused by improper handling operations during harvest and transportation to packing house. Symptoms can become evident after several days the stresses had occurred.



pathogenic microorganisms entering the fruit tissues through micro-wounds largely present on fruit surface, would easily germinate and rapidly growing mycelium would invade sound tissues (Potgieter and D'Aquino, 2017).

High care should also be paid when dropping the fruit into buckets and from buckets to boxes or bins, to prevent impact bruising (Fig. 4). It is important

## Postharvest Physiology

Cactus pears are non-climacteric fruit and do not contain starch, thus the ripening process goes on until fruit are attached to the tree (Cantwell, 1995). Nevertheless, relevant changes involving important compounds that affect flavor, odor and nutritional quality take place even after harvest. For example, color, which is mainly due to betaxanthins (yellow-orange) and betacyanins (red color), generally increases after harvest even in fruit stored for long time in refrigerated conditions (Cru-Bravo et al., 2019). Metabolic activity of cactus pear shows a wide range of variability not only among species but also within the same cultivar. Besides genetic diversity this may depend on several other factors such as horticultural management, pedo-climatic conditions, maturity stage, fruit size and water content. In *Opuntia* spp. respiratory activity ranges between 8 and 40 mL CO<sub>2</sub>/kg/h; the lowest values are reported for *O. ficus-indica*. Unless physiological stresses or microbiological infections happens after harvest, respiratory activity gradually tends to decrease, thus depletion of sugars and organic acids occurs gradually. Nevertheless, a continue exposure to warm temperatures hastens the consumption of respiratory substrates and cause a loss of firmness, which greatly alters the sensory quality. Refrigeration is the most powerful means to slow

that while waiting for transport, harvested fruit are not exposed to direct sun light to avoid detrimental increases of fruit temperature, but are placed in shadow and, if possible, ventilated sites. Improper handling during transport from the field can also cause compression injuries and abnormal increases of temperature within boxes or bins, especially when fruit are covered with tarpaulin.

down the tissue metabolism and to extend the postharvest life. Theoretically, the lower is the storage temperature above the freezing point the higher is the reduction of metabolic activity and the potential postharvest life. However, being cactus pear as most fruits of tropical and subtropical origin sensitive to chilling temperatures, the lowest safe storage temperature at which fruit can be exposed during storage is that not causing chilling injury, which generally is in the range of 8-12 °C.

Ethylene production rates in cactus pears is very low and generally decrease lowering the temperature. Indeed, a slight increasing trend has been detected over the ripening and the following senescent processes. Similarly, negligible increasing rates have also been detected in postharvest studies, unless fruit are affected postharvest disease or physiological disorders. A transient rise in ethylene as well as in respiratory activity is normal to occur when fruit from low temperatures are moved to warm conditions, whereas a high respiratory intensity coupled with high rates in ethylene production lasting for several days following transfer from cold to warm temperatures are clues of altered metabolism or incipient microbiological infections (Wang, 2016).

## Physiological Disorders

Despite cactus pears grows in harsh climates, the fruit are very delicate, primarily for the structure of the mesocarp that, differently than other fruits, is fleshy, juicy and spiny; all factors that make tissue particularly susceptible to physical injuries.

Bruises (Figure 4) are very common in cactus pears and occur as a result of impact and compression stresses during harvesting, the subsequent transportation to the packing house and postharvest operations. Damages involve the parenchymatous cells underneath the epidermis whose physical integrity is compromised. Depending on damage severity, bruises can appear even after several days the causal events had occurred. When bruised tissues are severely damaged and cells' organelles compartmentalization is compromised, several enzymes may come in contact with healthy surrounding tissue increasing the portion of altered fruit, with the final overall results of reduced postharvest life, quality and fruit marketability.

Chilling injury is a physiological disorder that affect most of fruits and vegetables of tropical and subtropical origin when exposed for several days at temperatures below a safe threshold (generally between 12 and 10°C) but above the freezing point (Wang, 2016). Besides the genetic background, several factors such as weather conditions over the growing season, cultural practices, blooming periods, maturity stage, can affect tissues susceptibility to low temperatures. Indeed, there is no specific metabolic pathway that uniquely is associated with chilling injury, but abnormal increases in respiratory activity, ethylene production or electrolytic leakage alongside with a juice build-up of ethanol and acetaldehyde

almost always are associated with chilling injuries. Visual symptoms of chilling injury include superficial scald of fruit surface, extending to the underneath tissues in case of severe damages (Figure 5). Physical damages of the peel caused by glochids or excessive dehydration of the tissue surrounding the crown may be severely enhanced by continuous exposure to low temperatures. In these cases, tiny necrotic pitting or shrink-wilted portion of the crown edge may evolve in brownish depressed spots or stains (Figure 6). Despite primary events of chilling injury start while fruit are exposed to low temperature, metabolic imbalances and damages develops mainly when fruit are moved to warm temperature. Chilling injury progress from the outer tissues to the center of the fruit. So when slight symptoms of chilling injury develop only fruit appearance is altered, while chemical composition and organoleptic characteristics of the edible portion of the fruit are not affected. Chill injured tissues are very susceptible to decay development as fruit are moved to room temperature.

Water loss is another important postharvest problem of cactus pear. Besides causing a direct loss of income proportional to the reduction of weight, water loss hastens the senescent process by changing the tissue hormonal balance and conferring the fruit a shrink and wilt appearance (Ben-Yehoshua and Rodof, 2003). Water loss of 6-8 % can prejudice fruit marketability. However, in cactus pears, transpiration does not occur evenly on fruit surface; the crown area and the stem end are the fruit portion where transpiration occurs at higher rates (Figure 7). Thus, according to fruit size and shape, fruit losing the same percentage of weight may appear more or less aged than others.



The simplest means to slow down the transpiration rate is to rise the environment level of humidity, however excessive humidity can cause condensation on fruit surface for the inherent continuous fluctuation of temperature of storage systems and predispose the fruit to pathogens' growth; on the other hand, high levels of humidity

alleviate chilling injury severity. So the best solution is to set humidity at the highest level that does not cause condensations; this depend on the efficiency of the refrigeration systems, the storage temperature, storage room load and other factors, but a relative humidity of 85-90 % normally is a good choice.



Figure 5 – Chilling injury symptoms overlapping with necrotic pits caused by glochids.



Figure 6 – Necrotic pits spread on fruit surface caused by glochids whose severity increases with storage and low environmental humidity.



Figure 7 – Fruit showing severe water loss and chilling injury.

## Microbiological Spoilage

The fleshy consistence of cactus pear peel makes the fruit highly susceptible to microbiological decay. Indeed, the high juice pH and the numerous micro-wounds caused by glochids spread all over the fruit surface makes the fruit an optimum substrate for filamentous fungi as well as bacteria. Nevertheless, the number of pathogens that cause economic loss in cactus pear is quite limited (Inglese et al., 2002).

Penicillium molds (Figure 8), caused by several fungi belonging to the *Penicillium* genus, is probably the most important postharvest disease of cactus pear world-wide. The main sites of infections are represented by micro-wounds caused by glochids. However, prolonged storage periods, fruit in advanced stage of maturity, chilling injury, transfer from cold to warm temperatures, improper storage conditions, are all factors that predispose fruit to penicillium decay (Granata et al; 2017; Potgieter and D'Aquino, 2017).



Figure 8 – Soft rots caused by *Penicillium digitatum* (left) and *Penicillium polanicum* (right).



*Botrytis cinerea*, the causal agent of grey mold (Figure 9, left), is another pathogen that can actively or through wounds infect cactus pear.

Losses can be very high even during cold storage, as, unlike penicillium decay, grey mold develops well even at temperature as low as 0°C (Granata et al., 2017).

Another important pathogen that induces soft rot is *Macrophomina phaseolina* (Figure 9, right). This fungus can be particularly dangerous when harvesting is not done properly and tissue at the base of fruit separated from the cladode are severely damaged and not able to heal. Losses can be very high in fruit harvested in advanced stage of maturity (Granata et al., 2017).

Other soft rots showing no molds are those caused by several bacteria, which grow well in over-ripe fruit for the low acidity of the juice.

Several species of *Alternaria* can infect cactus pear, but differently than the other filamentous fungi, *Alternaria* spp. develop slowly on fruit surface and cause necrosis of the tissue that are dry and brown in color (Figure 10, left). Initially, infected areas appear as tiny pitted necrotic brown spots which, as the disease progresses, involve the neighboring cells and evolve in depressed black patches. *Alternaria* dry rot can be confused with symptoms of chilling injury or other physiological dermatosis. Frequently the fungus develops on damaged tissues (Cantwell, 2004; Potgieter and D'Aquino, 2017).



Figure 9 - Soft rots caused by *Botrytis cinerea* (left) and *Macrophomina phaseolina* (right).



Figure 10 - Dry rot caused by *Alternaria* spp. (left) and fruit infested by *Ceratitis capitata* (right).

## Postharvest Handling

More than any other species postharvest handling of cactus pear represents a critical phase along the chain from the farm to consumers. Any physical stress and damage caused by glochids result in detrimental physiological responses that drastically reduce fruit quality and marketability. The presence of spines on fruit surface represents the main constrain limiting cactus pears popularity among consumers, and their removal is a basic prerequisite for consumers' acceptance.

The tiny spines that form the glochids, on the other hand, can severely damage the epidermis causing diffused pits on fruit surface that reduce fruit freshness, but also may increase weight loss and the risk of infections by wound pathogens. Their removal is not an easy operation and need be managed properly.

Unlikely, in some countries spines elimination is done roughly by sweeping the fruit on the ground

with brooms, that, besides causing severe injuries, is little effective, leaving too many spines on fruit (Cantwell, 2004).

The most efficient way to remove glochids is achieved more efficiently by devises that minimize damages by conveying the fruit through a series of rotating brushes formed by long bristles that combine stiffness with compliance (Figure 11, left). As fruit advance, the bristles remove the glochids and increase peel shine. Hard brush should be avoided as they could remove too much waxes and cause scratches on fruit surface. To replace natural epicuticular waxes removed during despination and enhance fruit shining, in some countries fruit are waxed. However, their application does not give important benefits from the commercial point of view, conferring the fruit an artificial gloss not much appreciated by consumers.



Figure 11 - Fruit moving through rotating brushes for despination.



After despination, fruit are calibrated and sorted to remove those with defects. In small packing houses calibration and sorting can be done manually by operators who place the fruit in cartons and remove those damaged (Figure 12, left). In large packing houses after despination fruit are delivered through a calibration line (Figure 12, right). In this case along the line fruit of the same size are conveyed in the same sector from where workers place sound fruit in boxes or trays and remove those damaged.

All these operations are conducted in dry conditions and fruit are held for several hours in ventilated conditions in the packing house before being delivered to the market chain or set for short periods in refrigerated conditions. From the phytosanitary point of view this is a good procedure because micro-wounds occurring over the processing chain have enough time to heal in most cases, reducing the risk of infection thereafter (Potgieter and D'Aquino, 2017).



Figure 12 – Calibration, sorting and packing done manually on a rotating table (left); calibration through a packing line (right).

## Postharvest Treatments

Besides despination cactus pears rarely are subjected to other postharvest treatments. Some packing houses apply wax to replace natural waxes removed together with glochids during despination. Indeed, opinions on the beneficial effects of this treatment are controversial, because if in one hand it contributes to a reduction of weight loss, on the other hand the unnatural gloss that artificial waxes confer the fruit is not always appreciated by consumers. Besides, the reduced gas exchange of the peel could lead to anaerobic conditions and an unexpected decline of the

eating quality for the build-up of off-flavor and unpleasant odor compounds.

The practice of cold storage to prolong the market window is not a normal practice for cactus pears, apart short periods while fruit are kept in stock in packinghouse before being shipped to markets, and the time interval between harvest and consumption is quite short to justify postharvest treatments to prevent decay development or loss of freshness.

However, the demand for quality fruit in countries

long distant from producing areas is increasing and the availability of out of season fruit can in part be satisfied by prolonged storage periods. For this reason, there is a demand by the cactus pear industry of a guidance for postharvest treatments and storage conditions capable of prolonging postharvest life for several weeks while maintaining overall quality.

Scientific works have shown that the storage potential and the response to low temperature of cactus pear varies greatly with cultivar, field conditions, growing season and pre-harvest treatments (Inglese et al., 2002; Yahia and Saenz, 2011). In some areas fruit are stored at ambient temperature for several weeks. In Sicily, for example, farmers used to store cactus pears, harvested with the whole cladode, on the roofs of sheds or stables for self-consumption from late autumn through the winter season. Nevertheless, despite cactus pear is not a climacteric fruit, to effectively prolong the postharvest life while maintaining market quality, cold storage is the "conditio sine qua non".

As stated above, sensitivity of cactus pear to low temperatures does not allow to expose the fruit for long time to temperatures that would induce chilling injury. Storage at 8-10 °C and 85-90 % RH are generally considered optimal conditions (Cantwell, 2004; Yahia and Saenz, 2011; Potgieter and D'Aquino, 2017). Exposure to lower temperatures may further prolong the overall postharvest life only while fruit are in refrigerated conditions, but would reduce the shelf-life when fruit are moved to room temperature.

However, as happens for other horticultural products, as storage time increases fruit age and gradually preformed natural compounds able to

prevent decay development and well as the overall defense mechanisms decline. As a result, the risk of postharvest diseases together with the development of physiological disorders become important issues to face, especially when fruit from cold storage are moved to retail conditions. Thus, postharvest treatments that can control pathogens' growth or capable to maintain high the natural resistance of tissues to invasion of pathogens, typical of young tissue, are very important.

A number of scientific reports have proven that prestorage treatments with world-wide registered synthetic fungicides to control decay in other fruit species, such as thiabendazole, imazalil, fludioxonil, can effectively control decay also in cactus pear stored for long periods (Schirra et al., 2002; D'Aquino et al. 2015, 2019). Fungicides can be sprayed on fruit surface along the processing line of the packing house or by immersing the fruit for a few minutes into the fungicides solutions. As a general rule fungicides are more effective when given by dips rather than sprays. In both cases their activity can increase by several fold when fungicidal solutions/emulsions are heated (Schirra et al., 2009). In dip treatments the solution temperature can be raised till 40 and 50 °C with immersion times of 2-5 minutes; higher temperatures or longer exposure times may cause heat injuries (Schirra et al., 1996). In sprays treatments, as the contact between fruit surface and the fungicide solution lasts only a few seconds, the tissue can withstand temperature as high as 60-65 °C (Dimitris et al., 2005).

Beside directly damaging pathogens organs and enhancing the fungicides activity, pre-storage heat treatments have a number of beneficial



effects that indirectly contribute to reduce decay and some physiological disorders. For example, heat can slow down the rate of ripening, sustain wounds healing, delay the loss of antifungal compounds naturally present in the rind, induce the production preformed antifungal compound and of enzymatic and non-enzymatic compounds capable of increase resistance to chilling injury (Barkai-Golan, 2001; Rodriguez et al., 2005). Induction of resistance to chilling injury can also be achieved by exposing the fruit from several hours to 2 days at temperature of 30-38 °C. Heating also induce a partial melting of the epicuticular waxes, which, in turn, fill the cracks that separate individual wax platelets (Schirra et al., 1999). This effect is very important because by closing the cracks interrupt potential entry avenues for pathogens. Besides, the continuous layer of wax can contribute to a significant reduction of water loss. The positive effect heat treatments gradually decrease with storage as epicuticular waxes when fruit are cold stored tend again to become brittle, to break and to reform platelets separated by cracks. The induction of resistance to chilling injury can be exploited in fruit subjected to cold quarantine treatments, a treatment required by importing countries that want to prevent the introduction of some pests like medfly (*Ceratitidis capitata*) (Figure 10, right) (D'Aquino et al., 2020).

Prestorage conditioning of cactus pears at 38 °C for several hours has shown to confer the fruit the capability of withstanding continuous exposure to temperatures as low as 2 °C for 3 weeks without significant damages (D'Aquino et al., 2014).

Intermittent warming (Chessa and Schirra, 1992), consisting of periodical interruption of cold storage with short periods of 12-24 hours at warm or room temperatures, has proved to improve fruit tolerance to low temperatures and to prolong the postharvest life, especially when fruit are moved to warm temperatures.

Another postharvest treatment that can greatly prolong the storage life of cactus pear is the application of polymeric films as low-density polyethylene (LDPE) or polypropylene polymers (OPP). The use of plastic film to wrap the fruit individually or in trays with 4-6 fruit (Figure 13) has shown an impressive effect in reducing senescence and physiological disorders associated with prolonged cold storage periods.

One of the more evident effect of film wrapping is the reduction of weight loss that can be of only 1-2 % even after several weeks of storage. However, the barrier to gases and water vapor provided by the film, if the package is not properly designed, can be dangerous for the potential risk of in-package anaerobic conditions. Yet, the high humidity within the packages increase the risk of decay if proper fungicidal treatments are not done before packaging (Piga et al., 1997).



Figure 13 – Cactus pears individually wrapped using a heat-shrinkable film (left), or wrapped with a LDPE film in groups of four in trays.

## Concluding remarks

Although cactus pear still represents a means of subsistence for poor farmers that contribute to survival of their families and animals, it is a fruit of great marketable potential for its exotic appeal, nutritional value and phytochemicals compounds. However, more research is needed to explore new potential markets and improve postharvest technologies along the distribution chain.

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# EFFECTS OF ENVIRONMENTAL CONDITIONS, CULTIVARS AND GROWTH-STAGE OF CLADODES ON THE PHYSICO-CHEMICAL, TECHNOLOGICAL AND FUNCTIONAL PROPERTIES OF CACTUS PEAR MUCILAGE

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## Introduction

Limited water resources, climate change and expected population increases require crops which can contribute to more resilient, more productive, more sustainable and climate-smart food systems. Cactus pears are an emerging crop which could provide a sustainable food source in hot and dry regions, while at the same time provide mucilage as a functional product, which could be profitable as the demand is increasing worldwide (Saenz et al., 2010). In an established, sustainable cactus pear orchard, the fruit develops from spring to high summer. It is only after the fruit harvest, that farmers need to diversify their income by harvesting cladodes for the extraction of mucilage. Cactus pears have high a commercial potential for cultivation in hot regions to produce mucilage, as high environmental temperatures may increase yields and therefore offer new opportunities to farmers in harsh and dry regions as a sustainable, drought-resistant and multi-purpose crop (Du Toit et al., 2020).

Cactus pear mucilage is a novel, inexpensive hydrocolloid that is used to add nutrients, body and texture to functional food products. The mucilage molecules have complex structures that absorb and hold large amounts of water (Trachtenberg and Mayer, 1981; Sepulveda et al., 2007). Mucilage molecules are unbranched, negatively charged, water-soluble hetero-polysaccharide chains that repel each other, causing them to stretch throughout the solute, resulting in increased viscosity. These properties might be retained in freeze-dried mucilage, which could make it a useful ingredient in food products. Cactus pear mucilage has various applications in the food and packaging (edible coatings and films) industry. It is eco-friendly, economical (cheap), safe, nutrient (fiber) rich, functional and has multiple health benefits (prevent cholesterol, diabetes, ulcers and cancer (Nazareno, 2013)). Mucilage consists mostly of indigestible, soluble fiber and contains minerals and antioxidants, which qualifies it as a low-calorie nutraceutical ingredient. Mucilage has been described as food stabilizers, thickeners, emulsifiers, fat replacement agents, suspension agents and for encapsulations used in the pharmacological industry. It possesses texture-modifying capabilities that can improve or repair the textural characteristics of products (Saenz et al. 2010). This fiber's water holding capacity, fat-emulsification, viscosity and texturizing capacity depend on its chemical



composition, physico-chemical properties (porosity, particle size, pH, conductivity) and technological properties (hydration and oil-emulsification capacities) (Cui and Roberts, 2009; Elleuch et al., 2011). The viscosity desired by the industry will vary according to the specific end-use. The food products themselves, the storage conditions and the preparation methods of food products have to be taken into consideration when using cactus pear mucilage.

Food developers search for new, innovative, inexpensive, natural, non-caloric hydrocolloids to use in functional food products, which not only have health benefits but also improve the physical and structural properties of food (Smith and Charter, 2010; Elleuch et al., 2011). Fiber-rich ingredients are incorporated in functional foods because they are effective in correcting or improving viscosity, texture, appearance and sensory characteristics. The physico-chemical and technological characteristics of freeze-dried mucilage powders may be affected when cladodes are harvested from different cultivars, species, growth stage (post-harvest/dormant stages) and weather conditions (seasons). However, a problem with extracted mucilage is the inconsistency of its characteristics as the yield and viscosity constantly vary, making it difficult to standardize formulas and make predictions in terms of yields (Du Toit, 2016). It has been speculated that these differences occur as a result of hydration of cladodes as a consequence of the abundance of rain or extended periods of drought, and the maturity stages of cladodes have been suggested as an influencing factor on mucilage yields. It is known that climatic conditions influence the quality of the fruit. The demand for cactus pear mucilage is increasing worldwide; thus, it is necessary to understand and predict its physico-chemical characteristics in order to produce a profitable functional product. The cultivar with the most optimal nutrient content and the preferred harvest times are still yet unknown. In this study, we aimed to examine the effect of weather conditions, growth-stage and cultivar on the physico-chemical, technological and functional characteristics of cactus pear cladodes and mucilage over seasons to understand these observed variations in mucilage characteristics.

## Materials and Methods

As part of a larger research project, cladodes were harvested over three years (2013–2015) and the mucilage were extracted and analyzed. In De Wit et al. (2019) the findings from 42 cultivars harvested in 2013 in the dormant stage (winter) were documented and it was proposed that the environmental conditions should be investigated, as the differences in mucilage yield and viscosity could be influenced by the weather. In 2014, eight cultivars were harvested over two growing seasons, namely the dormant stage (winter) and

the post-harvest stage (summer). Again, significant variations in mucilage yield and viscosity were observed, not only between cultivars, but also when cladodes from a single cultivar were harvested at different times of the year (Du Toit, 2016). In the third study, daily weather data were obtained, cladode weight and moisture contents determined, while yield, viscosity, pH, conductivity and nutritional composition was determined on mucilage. Pearson correlation coefficients were calculated between the weather conditions,

cladode properties, and mucilage properties extracted from mature cladodes harvested in 2015 over a six-month period (February to August). The data from two species and four cultivars, namely *Opuntia ficus-indica* cultivars Algerian, Morado and Gymno-Carpo and *Opuntia robusta* cultivar Robusta were used to correlate environmental temperatures and rainfall with the yield and viscosity of mucilage extracted over the six-month period. In particular, the relationships between temperature, rainfall, cladode size, cladode moisture content, mucilage conductivity and mucilage viscosity were investigated. Mature cladodes (cladodes that grew from spring 2014) were collected from the north side (maximum exposure to the sun in the Southern hemisphere) of the plant.

The cladode had to be north/south orientated for maximum sun exposure, hip height ( $\pm 1\text{m}$ ) and of good quality (without bearing any fruit) (Du Toit et al., 2018; 2019; 2020). A simple, economical, water and chemical-free, but effective process for the extraction of mucilage was developed and patented by Du Toit and De Wit (2011) (Figure 1).



Figure 1- Extracted mucilage (Photo: M de Wit).

## Results and discussion

The cultivar and harvest month significantly influenced the physico-chemical and technological properties of cactus pear mucilage. Varied physico-chemical and technological properties of mucilage powders, when cultivar and harvest month of cladodes differ, could lead to differentiation in the application of mucilage powders. February (hot summer) mucilage powders were the most porous with highest oil absorption and oil holding capacity, lowest water holding and swelling capacity, and lowest ability to increase viscosity. August (still cold after winter) mucilage powders had the smallest impermeable particles, highest water holding and hydrophobic properties, as well as the best emulsifying capacity, stability and ability to increase viscosity (Du Toit et al., 2018; 2019). (Figure 2).

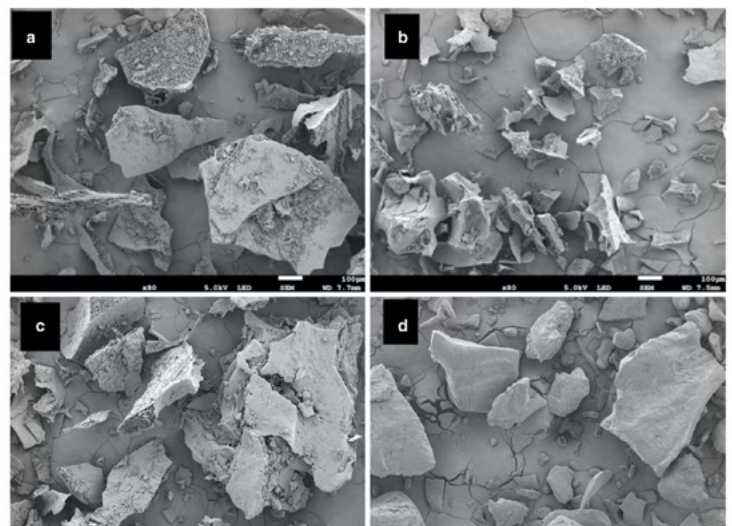


Figure 2 - Magnification of February mucilage powder to demonstrate average size difference between a) *Opuntia ficus-indica* (Gymno-Carpo) and b) *Opuntia robusta* (Robusta) ( $\pm 100\text{lm}$  at 9 80) as well as particle magnification to demonstrate the porosity of February powder particles c) Algerian ( $\pm 100\text{lm}$  at 9 80) and the solidity of August powder particles d) Algerian ( $\pm 100\text{lm}$  at 9 80) (Du Toit et al. 2018).



Mucilage powders obtained from cladodes harvested in hot and dry conditions (post-fruit harvest in February) are low in kilojoules and rich in minerals (Du Toit et al., 2018). Nutrients that contribute energy were low. The mineral content was high, particularly calcium and phosphorous. Low insoluble acid-detergent fibre and neutral-detergent fibre values indicated that mucilage was mostly soluble fibre. *Opuntia robusta* powders had higher protein content. The protein found in the mucilage powders may be too low to be nutritionally important, yet it provides mucilage with the ability to act as a functional ingredient. The change in climate from February to August significantly influenced the energy and mineral content. Optimal harvest time for mineral content was directly after the fruit harvest in February, as the energy content is the lowest and the dietary minerals the highest. The protein fingerprint, fibre, K and Mg content and the shape and occurrence of calcium oxalate crystals were significantly different between the four cultivars. *O. robusta* powders had the highest content of crude protein and K, while the fresh cladode tissue had smaller and fewer calcium oxalate crystals (Du Toit et al., 2018; 2019).

Interestingly, neither increasing cladode weight, nor rainfall had an effect on mucilage inconsistencies. However, the correlations showed a relationship between environmental temperatures, cladode pH and conductivity, and mucilage viscosity and yields. In hot summer weather, the pH was lower, which led to an abundance of H<sup>+</sup> in cladodes. The H<sup>+</sup> ions neutralize the negative charges along the outstretched mucilage molecule, thereby reducing repulsion and extension of the molecule, causing it to coil up and reduce the viscosity of the

mucilage. As such, a physical change in the molecular shape and configuration occurs since fewer water molecules would be bound along the molecule. Mucilage of lower viscosity was also more readily separated from the cladode solids during extraction; thus, the mucilage yield was higher during warmer months. Therefore, environmental temperatures, rather than rainfall or cladode maturity, influenced the physico-chemical characteristics of mucilage. Neither cladode weight nor rainfall content was found to be relevant to the moisture content of cladodes or the viscosity or yield of mucilage. However, the abundance of electrolytes, which occurred in warmer weather (lower mucilage pH and higher conductivity), had a strong correlation with higher yields and lower viscosity. In fact, warmer weather conditions were positively correlated to higher cladode moisture content, lower mucilage viscosity and higher mucilage yields (Du Toit et al., 2018; 2019).

High and low viscosity mucilage have the potential to stabilize, thicken, emulsify and restore textural properties in functional food products. The selection of specific cultivars for their most beneficial components is recommended, depending on the purpose of mucilage powder application in functional food products. High viscosity *Opuntia robusta* mucilage is recommended for highly viscous emulsified products such as mayonnaise while the low viscosity mucilage obtained from *Opuntia ficus-indica* (Algerian, Morado and Gymno-Carpo) cladodes harvested in high summer is recommended for low-viscosity, emulsified liquid products. Thus, the specification of the product predetermines the cultivar and the harvest time for optimal mucilage viscosity. Du Toit et al. (2019)

used low viscosity mucilage for ice-cream and sorbet products to replace dairy or fats. In contrast, higher viscosity mucilage was preferred to replace egg or fats in mayonnaise formulations. *Opuntia robusta* produced brighter, darker green, more viscous mucilage while *Opuntia ficus-indica*

powders were dull, light yellow-green with a lower viscosity and emulsifying capacity. Overall, the mucilage powders were easy to pour and free-flowing. Robusta mucilage was successfully applied in mayonnaise products to replace up to 50% egg yolk and 30% oil (Figure 3).



Figure 3 - Mayonnaise: 50% egg yolk replacement (left); mayonnaise: 30% oil replacement (right).

## Concluding remarks

It was concluded that mucilage powders might contribute to the textural and nutritional quality of food products. However, the careful selection of cultivar and harvest month was recommended depending on the purpose of application. The highest-quality mucilage was obtained from cladodes harvested in hot and dry conditions; therefore, it may present a rare opportunity for the commercial cultivation of cactus pears in arid and semi-arid areas. *O. robusta* harvested directly after the fruit harvest in February is recommended for the production of mucilage powders. The acceptance of mucilage powder as an active functional and nutraceutical food ingredient will also lead to the development of cactus as a commercially viable crop in arid and semi-arid areas where few other crops can survive. Demand for mucilage powders could stimulate the commercial cultivation of the cactus pear as a crop, which could be a valuable prospect for regions with limited farming opportunities. This is a functional ingredient that is produced from an easily cultivated crop, as cactus pears grow in areas with poor soil, extremely high daytime temperatures and limited water supplies.



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# IMPLICATIONS OF CACTUS ECOPHYSIOLOGY ON FODDER PRODUCTION AND UTILIZATION

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## Introduction

Cactus has a unique physiology that confers tolerance to drought, making these plants adapted to semiarid regions. In this review, we will focus on *Opuntia* and *Nopalea*, which are the cactus genera most used for fodder production. These fast-growing cacti are adapted to semiarid regions and have the potential to produce great amount of fodder biomass. Anatomical and physiological traits combined, result in these productive plants. Anatomical traits include waxy cladodes with few stomata (Mauseth, 2006), reducing the evapotranspiration (ET). Cacti present the crassulacean acid metabolism (CAM), which allows the plant to keep the stomata closed during the day when under water deficit, reducing water losses. During the night, stomata open and CO<sub>2</sub> uptake occurs; however, because there is no sunlight, CO<sub>2</sub> is fixed by phosphoenolpyruvate carboxylase (PEPC) resulting in the formation of malate, which is accumulated in the vacuole overnight as malic acid. During the daytime, malate will be decarboxylated and the released CO<sub>2</sub> will follow the Calvin cycle. With this mechanism, CAM plants are much more efficient using water than C<sub>3</sub> and C<sub>4</sub> plants (Garcia et al., 2014). This CAM mechanism has direct implication on cactus ecophysiology, and consequently, fodder production and utilization.

## Ecological adaptation

Cactus adapts well in semiarid regions; however, it grows best at highlands with cooler temperatures during the night when under water deficit. Cooler air temperatures reduce the atmospheric potential to retain water vapor, increasing air relative humidity during the night. Nobel (1995) reported that the capacity of the atmosphere to retain water vapor decreases from 39.7 g m<sup>-3</sup> at 35°C to 6.8 g m<sup>-3</sup> at 5°C. Altitude affects temperature, and for every 100 m elevation there is a decrease in 0.65°C on the average temperature (Rocha, 1991).

In lowlands, with warm temperatures during the nights, cactus adaptation and productivity is reduced, and in some cases, it is necessary supplemental irrigation to sustain productivity (Lima et al., 2015).

Cacti (*Opuntia* and *Nopalea*) need improved soil fertility to reach its productive potential. Several studies have been performed in the past, indicating the positive response to fertilizer application, especially organic amendments



(Dubeux et al., 2017). In NE Brazil, *Opuntia ficus-indica* cv. 'IPA-20' when in dense plantings (> 40,000 plants/ha) responded linearly up to 80 t of cattle manure applied per hectare every two years, leading to productivities greater than 50 t DM ha<sup>-1</sup> yr<sup>-1</sup> (Silva et al., 2016). In this same research, the efficiency of cattle manure utilization ranged

from 0.5 up to 5 t DM per ton of manure, varying with plant population and level of manure application. Cactus (*O. ficus-indica*) are tolerant to acidity and typically grows well in a range of soil pH from 4.5 up to 8.5 (Berry and Nobel, 1985), however, they are not tolerant to salt stress (Freire et al., 2018). The light environment might also affect cactus productivity and nutritive value (Miranda et al., 2019; Dubeux et al., 2021).

### Fodder production and utilization as affected by ecophysiology

The foremost important aspect of the cactus ecophysiology is to be able to produce and thrive under water deficit conditions in drylands (Figure 1). In fact, cactus is the basis of some livestock systems in semiarid regions by supplying great

amount of biomass rich in digestible energy, water, vitamins, and minerals (Dubeux et al., 2017). This is all possible because of the CAM physiology, which allows cactus to be productive under these conditions.



Figure 1 - Cactus (*Opuntia* sp.) in intensive rainfed cultivation system in the semiarid of Northeast Brazil. This variety ('Orelha de elefante Mexicana') is resistant to *Dactylopius opuntiae*.

One question that has been arising in recent decades is whether planting orientation and planting season affect light interception and cactus growth. Santos et al. (2019) addressed this

question by testing two planting seasons (dry and rainy seasons), three cladode orientations (North-South, East-West, and 45° angle between these), and three cactus varieties. They found no

difference of season of planting and cladode orientation on dry matter accumulation and concentrations of crude protein, neutral detergent fiber, and acid detergent fiber. These results reflect the random orientation taken by new cladodes emerging from the planted cladodes. Therefore, it is advisable to prioritize contour lines and prevention of soil erosion, with cladodes perpendicular to the slope to reduce soil erosion.

Because of reduced stomata in terms of size and numbers, waxy epidermis, and ability to survive under water deficit, cladodes can be stored under a dry shade and not losing its nutritive value for at least two weeks, and in sometimes up to four weeks (Santos et al., 1992). This has significant implications to cactus harvesting and utilization, reducing harvesting and transportation costs.

## Concluding remarks

Cactus unique ecophysiology allows it to grow in drylands. If managed well (i.e., organic fertilizer application, weed management, improved varieties), cactus can reach outstanding biomass productivity, supplying digestible energy, vitamins, water, and minerals. Cactus ecophysiology also facilitates storage for longer periods without losing its nutritive value. Highlands in the tropics, with mild temperatures overnight, offer among the best conditions for cactus growth and development.

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# A LOOK AT WATER RELATIONS OF CACTI AND SUCCULENTS: BASES FOR A SUSTAINABLE DRYLAND AGRICULTURE

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## Introduction

Arid and semi-arid zones characterized by high temperatures and frequent incidence of drought represent one-third of the continental area (Malagnoux et al., 2007). In Chile, these areas represent 41% of the national area (Benítez et al., 1994) concentrating an important part of fruit culture. Due to the water scarcity and the high atmospheric demand that characterize these environments, water reservoirs and efficient irrigation technologies to sustain fruit species with high productivity have been implemented. The fruit production species have high water consumption and relatively low tolerance to water stress, e.g. avocado, grape and citrus fruits (Franck and Muñoz, 2014).

Climate change events, such as the mega-drought that occurred in central Chile from 2010 through 2019 (water deficit of 25-45%), have been catastrophic for agriculture in arid and semi-arid areas of the country (Garreaud et al., 2018). There were vast areas of null production, as well as the uprooting of orchards due to massive drought-induced plant death and the abandonment of land. (Franck and Muñoz, 2014). Drought and the few fruit growth alternatives encourage abandonment. The selling of agricultural land for different uses promotes migration from rural areas to the cities. Latin America is expected to have 17 million internal migrants due to climatic reasons in 2050 (Rigaud et al., 2018).

In this context, the high investment costs for fruit production, and its economic and social importance in arid and semi-arid zones, demand the design of systems capable to withstand drought and recover their function in the post-drought period. Both processes are integrated into the concept of resilience. Cacti and succulents have been proposed as groups of plants with great potential to sustainably produce food under the climate change (Yang et al., 2015), so increasing knowledge about their water relations is essential for future food security.

## Plant water relations and peculiarities of cacti and succulents

Plants require water to maintain various turgor-dependent functions, e.g. cell elongation, which constitutes a major dimension of plant growth and mechanical stability. Photosynthesis is a most

important biochemical processes since it constitutes the entry of carbon and chemical energy into the biosphere. In itself, photosynthesis as a chemical reaction consumes one mole of



water per mole of fixed carbon dioxide. The water requirements concerning plant carbon gain is however substantially higher. The flow of CO<sub>2</sub> from the atmosphere to the carboxylation sites occurs through the stomata, which are pores formed by two occlusive cells present on the surface of the leaves. As the atmospheric air is quite dry (especially during the day), when the stomata are open, the water vapor flows from the intercellular spaces of the leaves into the atmosphere thanks to a favorable partial pressure gradient of water vapor (the leaves are virtually saturated with water), a flow is known as transpiration. Thus, for each mole of CO<sub>2</sub> assimilated by a plant with C<sub>3</sub> photosynthetic metabolism, a transpiration between 100 and 2000 mol of H<sub>2</sub>O occurs (Nobel, 1991). It is estimated that close to 40% of the water that precipitates on the earth's surface is returned to the atmosphere as water vapor through transpirative flow (Schlesinger and Jasechko, 2014), making this function a relevant hydrogeological rate.

In simple terms to assimilate CO<sub>2</sub>, C<sub>3</sub> and C<sub>4</sub> plants lose water in abundance, which translates into low water use efficiency. Cacti and succulents are peculiar in this sense, they have developed a rather particular and effective strategy from the point of view of the economy of water. Unlike the C<sub>3</sub> and C<sub>4</sub> photosynthetic metabolism where CO<sub>2</sub> capture occurs simultaneously with photosynthesis, cacti and succulents evolved a crassulacean acid metabolism (CAM), CO<sub>2</sub> capture occurs during the night and photosynthesis during the day (Figure 1). They keep the stomata closed during the day, when transpirative demand is high, and open them at night when evaporative demand is minimal (Caird et al., 2007). It is suggested that the most feasible theory for a nocturnal response of stomata in CAM plants is photoperiodic circadian rhythm (Lee, 2010). This offers comparative advantages increasing the water use efficiency that explain the high degree of adaptation of these groups of species to arid conditions.

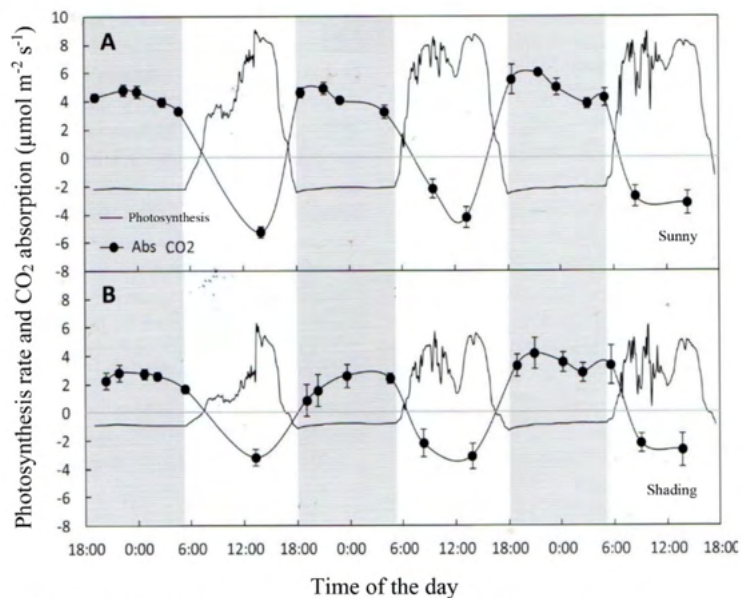


Figure 1 - Measured CO<sub>2</sub> flux and modeled photosynthesis of growing cladodes of *Opuntia ficus-indica* over three days under sunny and shady conditions. The gray stripes indicate the length of the night; vertical bars indicate  $\pm$  one standard deviation. Franck and Muñoz.(2014).

## Use and water use efficiency of cacti and succulents of agricultural interest

The ability to transpire at night gives cacti and succulents relatively low water requirements compared to other cultivated species. Species such as prickly pear, aloe, and pitahaya achieve commercial yields in arid and semi-arid zones of northern Chile with seasonal amounts of water of

between 1500 and 4000 m<sup>3</sup> ha<sup>-1</sup> (Table 1). The range is explained by variables such as local conditions in terms of atmospheric water demand and the need for irrigation water to wash salts from the soil.

**Table 1: Reference values of water requirement and yield (in fresh matter) of three CAM cultivated species in arid and semi-arid zones of northern Chile.**

Species	Harvested organ	Water requirement (m <sup>3</sup> ha <sup>-1</sup> year <sup>-1</sup> )	Yield (Mg [FM] ha <sup>-1</sup> )
<i>Aloe vera</i>	Leaves	1500-3000	50-70
<i>Hylocereus undatus</i>	Fruit	2500-3500	10-15
<i>Opuntia ficus-indica</i>	Fruit	1500-4000	20-30

Source: Data obtained from trials in arid and semi-arid zones of northern Chile, Center for Arid Zones Studies, University of Chile.

The ability of CAM species to open the stomata at night when temperature and atmospheric demand are low and keep them closed during the day, allow them to incorporate a greater amount of carbon per unit of water transpired than species of photosynthetic metabolism C<sub>3</sub> and C<sub>4</sub>. CAM species have nocturnal primary carbon assimilation by the enzyme phospho-enol-pyruvate carboxylase (PEPC), producing malic acid that is stored in mesophyll cell vacuoles and subsequently decarboxylated during the light period to provide CO<sub>2</sub> for refixation by Rubisco (Winter and Smith, 1996). This implies that the transpiration efficiency of CAM species is between 3 to 5 times higher than those of C<sub>3</sub> or C<sub>4</sub> metabolism species. For *Aloe vera*, transpiration efficiencies of between 24.5 and 15.2 g [DM] kg [H<sub>2</sub>O]<sup>-1</sup> have been reported (Silva et al., 2014), like cactus pear, and

substantially higher than other fruit species, including species known for their high efficiency and tolerance to water stress, such as pomegranate and fig (Figure 2).

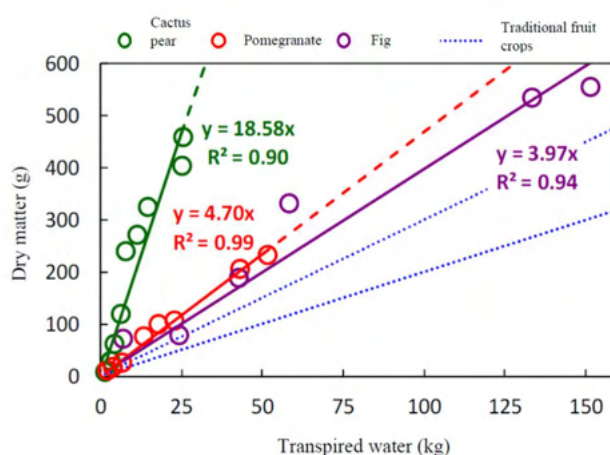


Figure 2 - Transpiration efficiency of reference species (C<sub>3</sub> metabolism). Two species of high efficiency (fig and pomegranate) and traditional fruit crops compared to a cactus pear. Modified from Franck and Muñoz. (2014). The slope of the curves is the transpiration efficiency.



## Anatomical-functional traits associated with the water relations of cacti and succulents

Although the nocturnal stomatal opening is key to understanding the low water requirement and high transpiration efficiency of cacti and succulents, associated with CAM plants (Ogburn and Edwards, 2010), these stand out for having a series of functional anatomical characteristics that give them advantages in arid areas in terms of drought resistance. These traits may be relevant in their agronomic management and their function and genetic variability should be considered in breeding programs.

Compared to  $C_3$  and  $C_4$  species, CAM plants characteristically have lower stomatal density, reported in *O. ficus indica* 20 stomata  $\text{mm}^{-2}$ , much lower than the density reported in  $C_3$  and  $C_4$  photosynthetic metabolism species of between 150 and 300 stomata  $\text{mm}^{-2}$ . This trait would be highly plastic, reducing the number of stomata per unit area under conditions of water deficit as observed in *Aloe vera* (Silva et al., 2001).

Cacti and Succulents tend to have dense and shallow root systems (Ogburn and Edwards, 2010) that manage to take advantage of low-intensity rainfall, characteristic of arid and semi-arid zones. In the case of *Opuntia*, root growth occurs rapidly in response to increased soil moisture (Nobel et al., 2002). These active roots are efficient in absorbing water, and their hydraulic properties are

segmented concerning the stem or cladodes. Root-Stem junction functions as a hydraulic safety valve that can maximize water uptake in axial and radial directions at limited rainfall. This junction can also prevent the stem from leaking water to the soil under drought conditions (Kim et al., 2018).

Other traits of interest are the presence of thick cuticles that modify the optical properties of the surface, increasing reflectance. The high cuticular resistance minimizes residual conductance, and therefore, the loss of water through the cuticle during the day. Also, the presence of thorns and other epidermal structures maintaining the boundary layer by controlling latent and sensible heat flux (several cacti require high temperatures for proper function), as well as collecting water droplets from wet air or fog (Ju et al., 2012), and high capacitance of the tissues, i.e. a high capacity to release or absorb water per unit change of the water potential of the tissue. This last feature allows to maintain the transpirative flow, and therefore productivity, in conditions of water deficit, and allows the individual to hydrate efficiently when water is available. This means that the water potentials of cacti and succulents remain relatively high (-1 MPa) even when rainfall is low (Ogburn and Edwards, 2010).

### Concluding remarks

Cacti and succulents have various traits that make them suitable for the design of agricultural systems adapted to climate change, particularly drought. This lies mainly in its photosynthetic metabolism, where

carbon capture and photosynthesis are temporarily out of phase, substantially improving its water use efficiency. This remarkable strategy of evasion of water stress makes them low-requirement species. Other anatomical-functional adaptations give them complementary escape routes, which, added to their water storage capacity, make them highly resilient species.

The intrinsic capacity of cacti and succulents to grow in arid areas must be complemented with adequate agronomic management that maximizes their yield. Unfortunately, in countries like Chile, the cultivation of cacti and succulents is seen as a low-input agricultural activity, so the development of technologies to maximize their performance is not promoted, e.g. the study of its water requirement, tolerance to water and saline stress, formation pruning, and adaptation to high-density orchard configurations (Figure 3). In the same way, its wide genetic variability must prospect for the development of genetic improvement programs within CAM species.



Figure 3 - High-density prickly pear orchard formation system. Vicuña, Coquimbo Region, north of Chile. Red lines represent the target shape of the plant (fan) after formation pruning. Source: Franck and Muñoz.(2014).

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# CO<sub>2</sub> UPTAKE OF *OPUNTIA FICUS-INDICA* (L.) MILL. WHOLE TREES AND SINGLE CLADODES

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## Introduction

The ecological and agricultural success of platyopuntias such as *Opuntia ficus-indica* (OFI) or similar cacti, is due largely to their photosynthetic pathway that results in about 5- to 10-fold higher water use efficiency and an annual dry matter productivity that can exceed that of nearly all cultivated C and C plants (Nobel, 1988, 2002). This group of species have a nocturnal stomata opening with CO<sub>2</sub> uptake occurring, primarily, from dusk to dawn. During the nighttime, through phosphoenolpyruvate (PEP) carboxylation, malate is formed in the cytosol and then actively transported and stored in vacuoles, leading to a noticeable increase of acidity. During the following daytime, while stomata are closed, malate is decarboxylated in the cytosol by Rubisco, and the released CO<sub>2</sub> is refixed via Rubisco, following the same pathway as in C<sub>3</sub> plants (Nobel, 1988). Cladode daily and seasonal CO<sub>2</sub> exchange patterns have been measured, using IRGA portable systems with leaf chambers adapted to cladode morphology, since the early 1980s, when Nobel and Hartsock (1984) measured CO<sub>2</sub> uptake on single cladodes grown in growth chambers. Since then, gas exchange has been measured on single portions of both the cladode planar surfaces, at specific intervals (2-4 h) during the daytime, and especially during the night, whether in the field or in growth chambers, to understand the response to environmental factors, such as air temperature, light intensity, water availability, and elevated CO<sub>2</sub> (Nobel, 2002). All this information was summarized to propose an environmental productivity index (EPI) able to predict plant performance under different ecological conditions (Nobel, 1988).

Cladodes are the stem-like flattened organs responsible for CO<sub>2</sub> uptake in OFI, while fruit accounts for a very limited carbon gain (Nobel, 1988, 2002; Inglese et al., 1994). Under optimal conditions (25/15 °C day/night) and light saturation (PPF of about 30 mol m<sup>-2</sup> day<sup>-1</sup>) *O. ficus-indica* may assimilate up to 344–600 mmol m<sup>-2</sup> day<sup>-1</sup> CO<sub>2</sub>, (Nobel and Hartsock, 1984; Pimienta Barrios et al., 2005). Instantaneous values of net CO<sub>2</sub> uptake of 1-year-old cladodes of this species may exceed 15 μmol m<sup>-2</sup> s<sup>-1</sup> (Nobel, 1988; Inglese et al., 1994; Pimienta-Barrios et al., 2005), the highest rate among platyopuntias (Nobel, 1988). It is, therefore, not surprising that OFI is the most widely cultivated cactus species.

Lower or higher temperature ranges result in a consistent reduction of net CO<sub>2</sub> uptake. Cladode carbon gain is, indeed, 50% less when day/night temperatures are 35°/25°C, because of stomata closure and a shorter



period of nocturnal gas exchange. In Italy, fruit of the spring flush, which ripen in August, have a shorter development period than those of the summer flush, ripening in October, because of the shorter duration of the last stage of fruit growth (stage III) and a lower absolute growth rate (Inglese et al., 2012). This may be related to the high temperature regime in August (35°/28°C day/ night), which reduces resource availability to the fruit while late-ripening fruit reach stage III when prevailing temperatures are optimal (25°/15°C day/night) for CO<sub>2</sub> uptake (Inglese et al., 1999). OFI nocturnal CO<sub>2</sub> uptake is related to total daily PAR (Photosynthetic Active Radiation), with a compensation level, on a total daily PAR basis, of about 3 mol m<sup>-2</sup> day<sup>-1</sup>, and a saturation point of about 30 mol m<sup>-2</sup> day<sup>-1</sup> (Nobel, 1988, 2002). Fully exposed terminal cladodes may have a net CO<sub>2</sub> uptake two-fold higher than sub-terminal cladodes that receive 65% of total PAR. The other underlying older cladodes account for a marginal contribution to whole tree photosynthesis (Nobel, 1988). The decrease in PAR interception accounts, also, for a delay in fruit ripening time and a reduction of cladode fertility, fruit size, and sugar content (Liguori et al., 2006). Shading reduced the PAR intercepted by the cladodes to one-third, which consequently reduced gross CO<sub>2</sub> assimilation and respiration also by half, due to lower cladode temperature and growth, in young potted plants, whose CO<sub>2</sub> assimilation rates were estimated by different methods (IRGA, ΔTitratable acidity, and a model based on the Electron Transport Rate light response) (Franck et al., 2010).

Water availability plays a primary role in carbon gain. Nobel and Hartsock (1984) have shown how, after 17 days of drought, net CO<sub>2</sub> uptake decreases by about 50%. Even though net CO<sub>2</sub> uptake over a 24 h period is around zero after 50 days of drought, nocturnal acidity may remain around 40% of maximal accumulation (Acevedo et al. 1983), due to incorporation of CO<sub>2</sub> released within the tissue by respiration. In either case, total assimilation is lower in dry conditions rather than in irrigated trees (Pimienta Barrios et al., 2005).

Continuous gas exchange measurements of entire single cladodes, branches, or trees have been recently reported (Liguori et al., 2013) using open system chambers, made up of polyethylene balloons (Corelli Grappadelli and Magnanini, 1993) that cover the entire organ/tree. This method can significantly increase our knowledge on whole plant reaction to environmental constraints as well as on interaction between different cladodes of the same tree.

The objective of our experiment was to measure CO<sub>2</sub> fluxes in a plot made by *O. ficus-indica* 3-year-old trees, cv Gialla. Measurements were carried out in whole trees in relation to water status, and individual cladodes differentiated by age. All observations were made at the experimental site, located in Palermo (38°06' N, 13°21' E, 40 m a.s.l.), from May to October. Net CO<sub>2</sub> uptake was measured using an open system for gas exchange applied to single trees and cladodes (Liguori et al., 2013).

## Chamber construction and operations

Open chambers were made of low density (95% transparency) polyethylene plastic sheets, closely fixed around the whole canopy or single cladodes of *O. ficus-indica* (chambers were built up 10-15 min before the beginning of the measurement and until the end of them). To keep the CO<sub>2</sub> concentration, between chamber and outside air, much higher than the infrared gas analyzer precision, a constant airflow through the chambers was generated by a ventilation system formed by 6 centrifugal fans powered by electric motors and with PVC pipes of 5.4 cm internal diameter (Miller *et al.*, 1996). Airflow rate within the chambers was 8-10 l s<sup>-1</sup> for those built up around the whole trees, and 3-4 l s<sup>-1</sup> for those fixed on single cladodes. Differences in the flow rate were due to the different volume of the chambers needed to cover single trees or a single cladode. A foam rubber gasket material sealed the chamber to trunk, or single cladode, and pipes. Air capacity was measured at hour intervals in the middle point of the pipes, using a hot wire digital anemometer; air velocity was measured positioning inside the pipe the sensing head of the anemometer at two different distances, along its diameter, from the pipe wall, and assuming a velocity of zero at the edge: the fan air capacity was calculated by multiplying the tube sectional area time the sectional air velocity into the pipe. Air capacity generated by pipe fans was also verified against the same parameter measured by a Venturimeter, built by GMR Strumenti (model FMD-01, Firenze, Italy) (Liguori *et al.*, 2013).

CO<sub>2</sub> concentrations of the reference (external) air and the chambers outlet airflow were measured using an IRGA (CIRAS-1, PP-Systems, Hitchin Herts, UK) (Figure 1A). Temperatures inside the chambers

were continuously measured, throughout the sampling periods, being 2.3±0.3°C and 0.2±0.01°C higher than the outside air, respectively during the daytime and at night. Measurements were taken over a 24 h period, starting at 02 p.m. Differences in CO<sub>2</sub> concentration were alternately taken in each of six chambers with a 10' interval (in one hour, all six chambers were measured) using a home-made 3-channels solenoid valve controller commanded by a data-logger (model CR1000, Campbell Scientific Ltd., Logan, USA) (Figure 1A) (Liguori *et al.*, 2013).

Measurements were taken when steady-state conditions were reached, about 10-15 min after the plastic sheets-made chambers were fixed to the trees and the fans were switched on to blow air into the chambers (Figure 1B, 1C). Whole-tree CO<sub>2</sub> assimilation A (photosynthetic rate, mmol s<sup>-1</sup>) was calculated using following equation (Alterio *et al.*, 2006):

$$A = F * \Delta\text{CO}_2$$

where  $\Delta\text{CO}_2$  is the difference in CO<sub>2</sub> concentration between the outlet and the inlet (mmol mol<sup>-1</sup>) air flows and F is the molar air flow blown by the fan. Time reading of the CIRAS-1 was set to one minute: in each 10 min interval, differences between the external and the outlet air CO<sub>2</sub> concentration were measured 10 times and averaged in order to calculate the hourly concentration for each chamber.

Total daily net CO<sub>2</sub> uptake was obtained by integrating (using the hourly average) the instantaneous rates over the 24 h measurement period (Nobel, 1988). On the dates of gas exchange measurements photosynthetic photon



flux density (PPFD) was measured hourly from dawn to dusk on both sides of each sampled cladode, with a LI-190 portable quantum photometer (LI-

COR, Lincoln, NE, USA) and then integrated to get the total daily PPFD expressed as  $\text{mol m}^{-2} \text{d}^{-1}$  (Liguori et al., 2013).

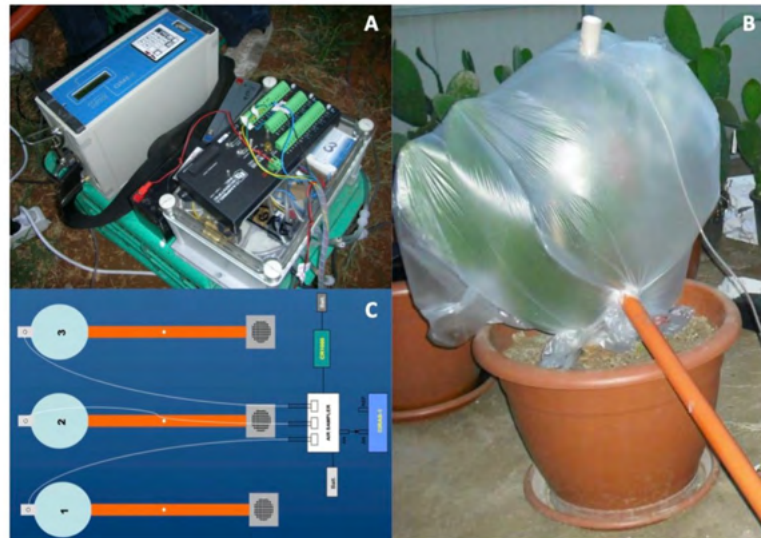


Figure 1 - CO<sub>2</sub> fluxes monitoring system: A) home-made 3-channels solenoid valve controller, CR1000 and CIRAS-1; B) balloon; C) CO<sub>2</sub> monitoring system scheme

## CO<sub>2</sub> uptake of potted trees in relation to water status

CO<sub>2</sub> exchange rates of three irrigated and three non-irrigated *O. ficus-indica* trees were measured 0, 1, 3, 6, 8, weeks after the onset of the dry period (started on June 2nd). Measurements were carried out from June 4th until August 2nd. Six 3-year-old trees were used, placed in 250 liters polyester pots filled with a sandy-loam soil with a field capacity of 26.3% and a wilting point equal to 12.3%. Trees were used as a single replicate (Figure 1B) (Liguori et al., 2013).

Three of the six trees were continuously watered throughout the season, starting from 15 April, to maintain soil water content above 20% (75% of available water). In the remaining trees irrigation was suspended on June 2nd, and the trees were re-watered 10 weeks afterwards. To assess their recovering capacity, CO<sub>2</sub> uptake of re-watered and continuously watered trees was measured

2 weeks after the rewatering (29 August) (Liguori et al., 2013).

At the onset of the experiment, all trees had no fruit and  $11 \pm 1.0$  1-year-old mother cladodes; the number of current-year daughter cladodes was set, early in June, to  $3 \pm 1$  and all new buds were removed soon as they appeared. Older cladodes had a similar surface area. At the beginning and at the end of the season, for each tree, the planar surface area of 1-year-, 2-year- and 3-year-old cladodes was measured, using paper silhouettes of each cladode (Garcia de Cortazar and Nobel, 1992), successively measured by a leaf area meter (Delta-T Devices LTD, Cambridge, UK); cladode thickness was measured at three locations, and total surface area was expressed as  $(2 \times \text{projected cladode area}) + (\text{circumference} \times \text{thickness})$ .

Air temperature and relative humidity (RH) were continuously measured using meteorological sensors (HOBO H-08, Onset, Bourne, MA, USA). Soil moisture was detected, every three days, from soil samples (2 samples in any pot at 15 cm of depth)

placed in an air-forced oven (M-250-VF, MPM Instruments srl, Bernareggio, Italy) until constant mass. PPFD was measured as described before (Liguori et al., 2013).

## CO<sub>2</sub> uptake of single cladodes differentiated by age

Seven gas exchange measurements were carried out from May to September on six single, sun-exposed terminal cladodes differentiated by age (three 1-year-old and three 2-year-old cladodes), growing on each of three watered, potted cactus pear trees. The planar surface of the sampled 1- and 2-year-old cladodes was measured and the

total cladode area was calculated as previously described. Air temperature and RH were continuously measured using meteorological sensors, placed within and above the canopies and PPFD during the day-time was recorded as previously described (Liguori et al., 2013).

## Results

### CO<sub>2</sub> uptake of potted trees in relation to water status

At the beginning of the experiment, 1-year- and 2-year-old cladodes surface area was similar in irrigated and non-irrigated trees (7974 cm<sup>2</sup> and 7536 cm<sup>2</sup>, respectively). At the end of the drought period (end of August), the surface area of current-year cladodes was 4644 cm<sup>2</sup> in well-watered trees and 2036 cm<sup>2</sup> in the non-irrigated ones, 37% and 21% of whole-canopy surface area, respectively.

Average day/night temperatures increased from 22/14°C early in June, to 26/19°C in late June, 28/20°C in mid-July, and 29/23°C in early August. Soil water content never changed in watered pots and decreased, continuously, in non-watered pots. PPFD also increased gradually from 25 mol m<sup>-2</sup>

d<sup>-1</sup> early in June, to 27.3, 35.1 and 39.2 mol m<sup>-2</sup> d<sup>-1</sup>, in July and August. At the first measurement date (June 4th) all trees were at field capacity; mean daily net CO<sub>2</sub> uptake was 138.6±6.57 mmol m<sup>-2</sup> d<sup>-1</sup> and a variability between the trees not higher than 15% (data not shown). Maximum instantaneous rates occurred from midnight until 2.00 am with an average value (n=6) of 6.5±0.88 mol m<sup>-2</sup> s<sup>-1</sup>. One week after the onset of the drought period, when soil moisture in non-water pots was 18%, there still were no differences among the trees (Liguori et al., 2013). Three weeks afterwards, soil moisture in non-irrigated pot was 7.5%. Highest instantaneous rates reached 6.2±0.91 mol m<sup>-2</sup> s<sup>-1</sup> in non-irrigated trees



and  $7.0 \pm 0.21 \text{ mol m}^{-2} \text{ s}^{-1}$  in irrigated ones.

However, instantaneous  $\text{CO}_2$  uptake rates differed during part of the night and the early hours of the morning, which correspond to Phase I and II of the CAM circadian rhythm (Nobel, 1988). As a consequence, daily net  $\text{CO}_2$  uptake was 25% lower in non-irrigated than in irrigated trees:  $130.7 \pm 7.92 \text{ mmol m}^{-2} \text{ d}^{-1}$  and  $174.6 \pm 8.58 \text{ mmol m}^{-2} \text{ d}^{-1}$ , respectively (Figure 2A).

Six weeks after the onset of the drought period, soil moisture in dry pots was 5.5%. The nighttime instantaneous  $\text{CO}_2$  assimilation showed a peak of  $7.8 \pm 1.12 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $10 \pm 0.48 \text{ mol m}^{-2} \text{ s}^{-1}$  in non-irrigated and irrigated trees, respectively. Significant differences occurred throughout the night (Phase I) and until sunrise. Non-irrigated trees had almost 40% less carbon uptake than irrigated ones, since daily net  $\text{CO}_2$  uptake was  $146.8 \pm 6.12 \text{ mmol m}^{-2} \text{ d}^{-1}$  in non-irrigated and  $242.0 \pm 8.54 \text{ mmol m}^{-2} \text{ d}^{-1}$  in irrigated trees (Figure 2B) (Liguori et al., 2013).

Eight weeks after the onset of the drought period, at 5% soil moisture, maximum instantaneous  $\text{CO}_2$  uptake rates were  $11.1 \pm 0.94 \text{ mol m}^{-2} \text{ s}^{-1}$  in irrigated

trees and  $8.2 \pm 0.46 \text{ mol m}^{-2} \text{ s}^{-1}$  in the non-irrigated ones. Differences occurred from sunset to sunrise, along Phases IV, I and II of the CAM circadian rhythm (Nobel, 1988). Non-irrigated trees fixed 50% less carbon than irrigated ones, with daily net  $\text{CO}_2$  uptake of  $302.9 \pm 9.51 \text{ mmol m}^{-2} \text{ d}^{-1}$  for irrigated trees and  $153.8 \pm 6.33 \text{ mmol m}^{-2} \text{ d}^{-1}$  for non-irrigated ones (Figure 2C) (Liguori et al., 2013).

During the season, total daily net  $\text{CO}_2$  uptake doubled in well-watered trees and did not change in non-irrigated trees (Figure 2A, 2B, 2C). The pattern of net  $\text{CO}_2$  uptake did not change with treatments, and well-watered trees had higher assimilation rates during most of the night. Non-irrigated trees were re-watered on August 12; two weeks later (August 27) they had a daily net  $\text{CO}_2$  uptake of  $263 \pm 56.28 \text{ mmol m}^{-2} \text{ d}^{-1}$ , while well-watered trees had an assimilation of  $314.8 \pm 7.41 \text{ mmol m}^{-2} \text{ d}^{-1}$ , with an average day/night temperature of  $23/16^\circ\text{C}$ , indicating an almost complete recovery of their potential photosynthetic capacity (Liguori et al., 2013).

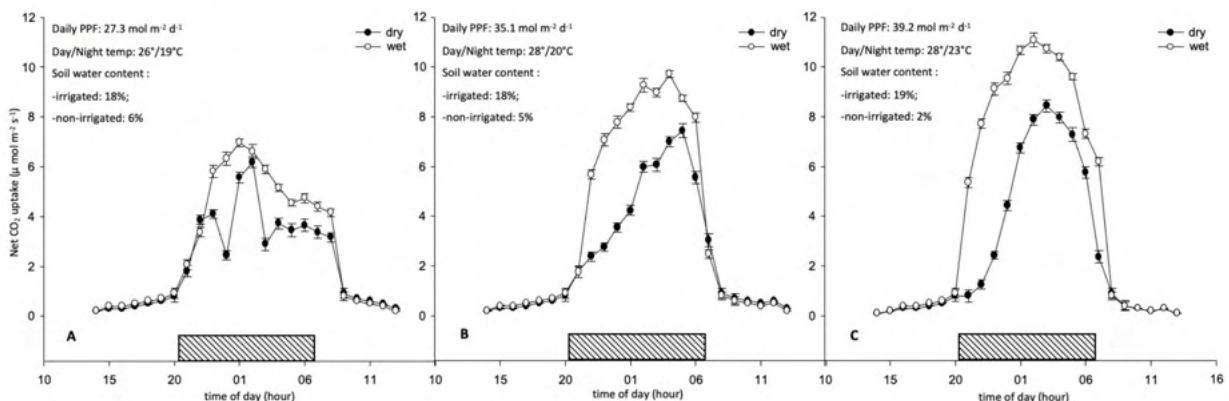


Figure 2 - Daily net  $\text{CO}_2$  uptake in irrigated and non-irrigated *Opuntia ficus-indica* trees. Data are means ( $\pm$ SE) of 60 min measurements made with open chambers covering three single trees for each treatment (dry and wet), used as single replicates (A: 25 June 2010, B: 16 July 2010, C: 1 August 2010). Dotted bar coincides with the nighttime period.

## CO<sub>2</sub> uptake of single cladodes differentiated by age

Net CO<sub>2</sub> uptake of 1- and 2-year-old cladodes averaged, throughout the season, 436.6 mmol m<sup>-2</sup> d<sup>-1</sup> and 382.3 mmol m<sup>-2</sup> d<sup>-1</sup> respectively (Table 1), accounting for a carbon sequestration of 5.2 g C m<sup>-2</sup> d<sup>-1</sup> in 1-year-old cladodes and 4.5 g C m<sup>-2</sup> d<sup>-1</sup> in 2-year-old cladodes. Differences related to cladode age occurred only from the second week of July to the first week of August, when net CO<sub>2</sub> uptake of 1-year-old cladodes was almost twice as much than for 2-year-old ones (Table 1) (Liguori et al., 2013).

Correlation (regression) between daily net CO<sub>2</sub> uptake and total daily PPFD showed a higher value ( $R^2=0.72$ ) in 1-year-old cladodes than in 2-year-old cladodes ( $R^2=0.32$ ) (data not shown), indicating higher photosynthetic efficiency of the former ones at the highest PPFD (Table 1). The nocturnal pattern and the highest instantaneous maximal assimilation rates of net CO<sub>2</sub> did not change with cladode age, but differences occurred at the beginning of the night (data not shown).

**Table 1. Daily net CO<sub>2</sub> uptake over 24 h period for 1-year- and 2-year-old whole-cladodes of *Opuntia ficus-indica* during summer 2010 (data are means of 3 cladodes).**

Date	Total daily net CO <sub>2</sub> uptake		Total daily Photon Flux (PPF mol m <sup>2</sup> d <sup>-1</sup> )	Mean daily Temperature (°C)
	1-year-old cladode (mmol m <sup>2</sup> d <sup>-1</sup> )	2-year-old cladodes (mmol m <sup>2</sup> d <sup>-1</sup> )		
<b>21 May</b>	314	349	25.5	17
<b>4 June</b>	510	502	27.3	18
<b>8 June</b>	485	519	31.2	21
<b>16 July</b>	554	418	35.2	23
<b>5 August</b>	554	344	39.0	24
<b>2 September</b>	228	246	28.2	19
<b>17 September</b>	311	298	28.5	18



## Concluding remarks

Surprisingly, our measurements integrating whole-tree gas exchange proved that *O. ficus-indica* trees continued their photosynthetic activity 60 days after irrigation was stopped and when soil water content was lower than 5%. At this stage, current-year and 1-year-old cladodes of non-irrigated trees had become flaccid, but, still, daily net CO<sub>2</sub> uptake of the whole tree kept the same rate as at the beginning of the experiment while, along with the season, well-watered trees had doubled their net CO<sub>2</sub> uptake (Liguori et al., 2013). Eventually, the whole-tree daily CO<sub>2</sub> uptake, which integrates cladodes of different age and photosynthetic capacity, was much lower than values measured for individual cladodes under similar, wet or dry, conditions (Nobel and Bobich, 2002; Pimienta-Barrios et al., 2005), indicating the different contribution of single cladodes to whole tree photosynthesis. The highest instantaneous CO<sub>2</sub> uptake rates, 11.1 mol m<sup>-2</sup> s<sup>-1</sup> and 8.2 mol m<sup>-2</sup> s<sup>-1</sup> for well-watered and non-irrigated trees respectively, occurred in August 2010, 60 days after the onset of the dry period. Similarly, Pimienta-Barrios et al. (2000) measured the highest maximal CO<sub>2</sub> assimilation rates in single cladodes of *O. ficus-indica*, grown in a commercial orchard in Mexico, 60 days after the end of the rainy period, when soil moisture was <10%.

As a result of a much lower seasonal assimilation rate and a lower surface area development of current-year cladodes, trees subjected to drought had, over the season, a lower net productivity and C accumulation than well-watered ones. In fact, averaged over the six measurements dates, from June to August, total daily net CO<sub>2</sub> uptake was 275.6 mmol m<sup>-2</sup> d<sup>-1</sup> for well-watered trees and 175.6 mmol m<sup>-2</sup> d<sup>-1</sup> for non-irrigated trees. Considering a cladode surface area of 12,618 cm<sup>2</sup> for well-watered and 10,572 cm<sup>2</sup> for trees under drought, a daily amount of 4.2. g C d<sup>-1</sup> was accumulated in well-irrigated trees in that period, while non-irrigated trees had 50% lower values. The capacity of *O. ficus-indica* trees to substantially maintain their photosynthetic activity, though they lost most of the water from the parenchyma, is likely to be related to a water movement from mother to daughter cladodes and inter-tissue water transfer from parenchyma to chlorenchyma that reduced water loss of the photosynthetic tissue (Nobel, 2006; Pimienta-Barrios et al., 2000).

Few data are available on cladode net CO<sub>2</sub> uptake, according to cladode age. Samish and Ellern (1975) noted that titratable acidity decreased linearly with increasing age, and 1-year-old cladodes had three times higher level of acidity than 2-year-old cladodes, located in a basipetal position. Our data, taken on sun-exposed, apical 1- and 2- year-old cladodes, show that over seven measurement dates in summer differences between net CO<sub>2</sub> uptake for 1-and 2-year-old cladodes occurred only twice, corresponding to the highest PPFD and temperatures. Nevertheless, net CO<sub>2</sub> uptake of 2-year-old cladodes, averaged throughout the season, was 40% lower than for 1-year-old ones. This value could further decrease, considering their natural basipetal position within the tree that would reduce PPFD availability (Nobel, 1988); seasonal differences of C accumulation, according to cladode age, must be considered to set the optimal stem area index (SAI) in cactus pear fruit orchards (Inglese et al., *in litteris*).

In summary, the open gas exchange chamber was successfully applied to measure whole tree or single organ net CO<sub>2</sub> uptake in cactus pear particularly to understand the response of the whole tree to environmental stress. Differently than on single cladode measurements, in our case it was possible to demonstrate how, after 60 days of drought, whole cactus pear trees were still keeping a same level of net CO<sub>2</sub> uptake, though a substantial water loss in the parenchyma of most photosynthetically active cladodes occurred. Our data confirm that OFI trees and orchards are able to stock large C quantities with low input requirements. OFI proves once more to be a sustainable crop, coupling the high C supply with a low input demand (water, fertilizers, pesticides), with its particular multi-functional role both for human and animal feeding and for landscape preservation.

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# PERFORMANCE OF CACTUS PEAR UNDER WEST ASIA CONDITIONS

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## Introduction

Arid and semi-arid regions cover approximately 30% of the world's continental surface and support more than 20% of the world's population (Wu, 2001). These areas are less suitable for crop production due to inherent soil constraints like low water retention, sandy texture, shallow depth, occurrence of rocks and stones. Under these conditions, certain species such as spineless cactus pear (*Opuntia ficus-indica* L. Mill.) can grow well and help to enhance the livelihood of the poor farmers. Cactus pear is one of the most important species of the cactaceae family which records approximately 1600 species distributed worldwide (Griffith, 2004; Bárcenas et al., 2011). In fact, cactus pear plays an important economic role and provides good quality foods (fruits, juices, marmalades), cosmetics, and medicinal products and plays an important economic role as a forage and fodder provider (Todaro et al., 2020; Louhaichi et al., 2018; FAO and ICARDA, 2017; Inglese et al., 2002). Moreover, cactus pear has proven potential to alleviate soil erosion and increase carbon sequestration (Hassan et al., 2018). Under semiarid conditions, carbon sequestration was estimated at 0.3 t/ha and 1.9 t/ha respectively for young plantation and for old plantation (Daly-Hassen et al., 2019).

From its native distribution area and in the late 15<sup>th</sup> century, the species *O. ficus-indica* have spread into different parts of the world, particularly in the Mediterranean Basin, Northern and Southern Africa, the Middle East, Australia and Northern India (Osuna-Martinez et al., 2014). Now, this species is cultivated in over 20 countries for its fruits and as feed for livestock (Inglese et al., 2002). The reasons behind the diffusion of *O. ficus indica* species around the world are many, they are related to the simple cultivation practices required to grow the plant; the easy vegetative propagation practices that favour speedy planting material exchange among different areas, the ability to grow in unfavourable environmental conditions, multifunctionality and industrialization of different parts of the *O. ficus indica* in addition to the potential of this plant to combat desertification and waste and Rangeland improvement (Nefzaoui et al., 2014; FAO and ICARDA, 2017).

Despite the importance of cactus pear, there has been very little research reported on that quantified areas and production of cactus pear crop in most of the countries (FAO and ICARDA, 2017). In West Asia, cactus pear is cultivated for fruit production which have a good market value. Also, it is planted at the edges of farms and gardens as a biological fence. For instance, in Jordan the planted cultivar is the local spiny and spineless Khadri cactus area is estimated at 300 ha mainly in the Jordan Valley, Madaba area and Irbid area (FAO and ICARDA, 2017; Katbeh Bader and Abu-Alloush, 2019). More recently, farmers are becoming



increasingly interested in growing cactus to produce fruits as a mean to generate income (Liguori and Inglese, 2015; FAO and ICARDA, 2017).

The lack of diversity of cactus pear cultivars in West Asian countries is one of the limiting factors that affect the adoption rate and out scaling of this technology. Thus, introduction of new cactus cultivars will provide opportunities to increase the cultivation of cactus pear under local conditions and to help small holders to diversify their products and increase their income.

During the last decade, the International Center for Agricultural Research in the Dry Areas (ICARDA) with the collaboration with the National Agricultural Research Center (NARC) and support of the CactusNet facilitated the transfer of selected material from different locations such as Italy, Brazil, Mexico and Tunisia and for both fruit and fodder production to establish a cactus pear germplasm in Jordan (Figure 1). Following their introduction, the new cultivars were evaluated in order to assess their adaptation and performance to specific local habitats. This work has resulted in the characterization and identification of most promising traits of the available cactus cultivars which will be promoted at farm level.



Figure 1- Cactus pear germplasm collection at Muchaqqer research station, south Amman, Jordan.

## Methodology

Thirty cultivars introduced from Italy, Tunisia, Brazil and Mexico in addition to the local cultivar were selected and planted in 5 replications to compare their performance under the Jordan environmental conditions (Table 1). These cultivars were planted at Mushaqqar research station, (31°46'37.50"N, 35°47'51.06"E; 797 m a.s.l), located in the Madaba Governorate, 25 km south of Amman. The climate is semi-arid, characterized by a highly variable and fluctuating rainfall with a mean annual value of 360 mm. Plant

Morphological Traits (plant vigour, number of cladodes per plant, plant height (cm), plant diameter east-west (cm), plant diameter north-south (cm)); cladode morphological traits (Cladode: length (cm), width (cm), shape index (length/width), thickness (mm) and surface area (cm<sup>2</sup>)) and were recorded using the "Descriptor for Cactus Pear" published by the FAO-ICARDA Cactusnet (Chessa and Nieddu, 1997). Fruits color, fruits crunchiness, fruits sweetness, seed softness and preference were evaluated by farmers.

**Table 1. Cactus pear cultivars planted in Muchaqqer station and the country of origin.**

Accession name	Country of Origin	Accession name	Country of Origin
Trunzara Red San Cono	Italy	46_Mornag B_74076	Tunisia
Red Roccapalumba	Italy	20_Sbeitla_74071	Tunisia
Yellow Santa Margherita	Italy	34_Caref 58_69219	Tunisia
Yellow San Cono	Italy	31_Burbank Azrou_69223	Tunisia
Yellow Roccapalumba	Italy	15_Sicile Le folin_73063	Tunisia
Red Santa Margherita Belice	Italy	30_Mdjez El Bab_73952	Tunisia
Red San Cono	Italy	29_Matmata_69242	Tunisia
Trunzara yellow San Cono	Italy	26_Djebel Bargou_68247	Tunisia
10_ FOZA10	Mexico	32_Matmata_69242	Tunisia
2_17_21	Mexico	37_Thala_69241	Tunisia
2_11_85	Mexico	6_Ain Boudriess_96245	Tunisia
2_25_15	Mexico	22_El Borouj_75018	Tunisia
2_21_68	Mexico	13_Bab Toza_74115	Tunisia
F1_COPENA F1	Mexico	Muchaqqer	Jordan
IPA – 90 - 115	Brazil		
COPENA V1	Brazil		
Jalpa – F23	Brazil		

## Results

The dendrogram resulted from the Hierarchical Ascending Classification of the cactus pear cultivars based on morphological traits allowed as identifying four major clusters (Figure 2). Group (I) with better performance included 14 cactus pear cultivars majority from Tunisia: 10\_ FOZA10, 6\_Ain Boudriess\_96245, 26\_Djebel Bargou\_68247, 2\_21\_68, 46\_Mornag B\_74076, 20\_Sbeitla\_74071, 30\_Mdjez El Bab\_73952, 2\_17\_21, V1\_ COPENA V1, 34\_Care F 58 C, 37\_Thala\_69241, 31\_Burbank Azrou\_69223 29\_Matmata\_69242, 15\_Sicile Le folin\_73063. The second cluster included 6 cactus

pear cultivars (one from Brazil, three from Italy and two from Tunisia) namely: IPA\_90\_156, Red Roccapalumba, Turunza Yellow San Cono, Yellow Roccapalumba, 32\_Matmata\_69242, 13\_Bab Toza\_74115. Third group consisted of five cultivars: 2\_23\_15, Jalpa – F23, Trunzara Red San Cono, 22\_El Borouj\_75018 and Muchaqqer. Last last cluster continues six Italian and Mexican cultivars: F1\_COPENA F1, 2\_11\_85, Yellow Santa Margherita Belice Red Santa Margherita Belice, Red San Cono, Yellow San Cono.



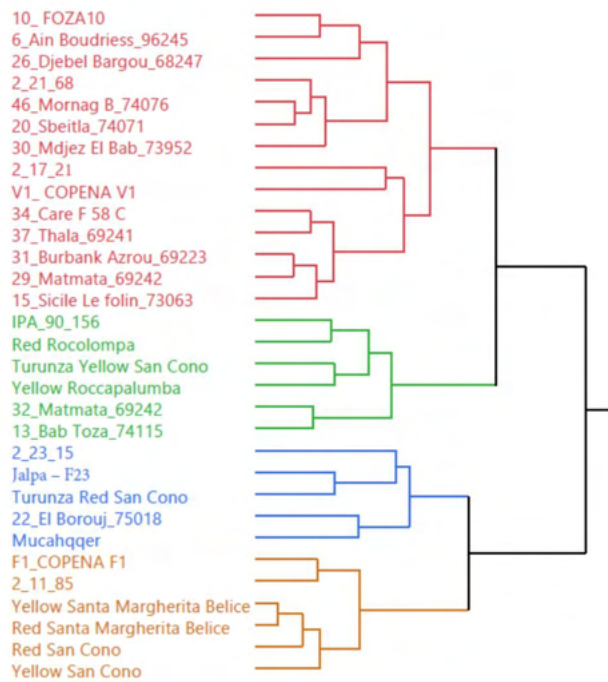


Figure 2 - Dendrogram constructed by Euclidean distance using Ward's method, to study the relationships among the 31-cactus pear cultivars based on their morphological characteristics.

Farmers were very excited to explore the huge diversity of cactus pear, the common fruit type in Jordan is the yellow one therefore they were very excited to test the other types. In general, farmers were in favour of the white fruits type had the

highest preference of the farmers, it was more attractive, crunchier and contained the softest seeds among the three types, both red and white type fruits were sweeter than the yellow type (Figure 3).

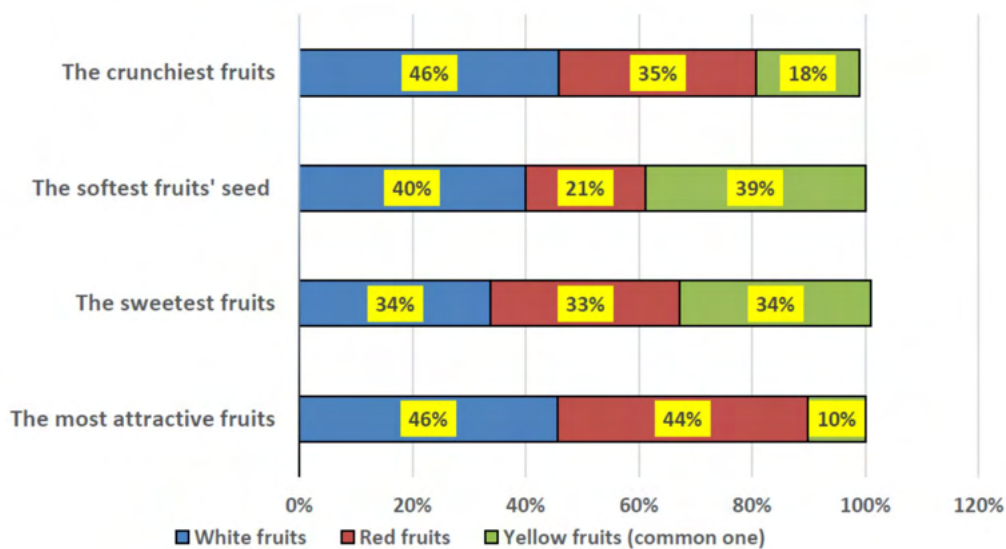


Figure 3 - Cactus pear fruits preference.

Among the white fruiting type, IPA – 90 – 115 was ranked first, while Red Roccapalumba and 2\_17\_21 recorded the highest score among the red type cultivars. Yellow San Cono and Yellow Roccapalumba were the best among the yellow fruiting type including the local cultivar. Farmers

think that new types are better than the common ones and they showed big interest in growing the new accessions in order to produce the new fruits. Farmers expressed the high economic value of the new cactus pear accessions as they are willing to pay higher prices to get the new cactus fruits.

## Concluding remarks

- The introduction, evaluation, and dissemination of new cactus cultivars for fruit production is very important to boost consumer preference and increase commercialisation of these cactus fruits
- Awareness-raising programmes are necessary to inform decision makers, government officials and farmers about the importance of cactus using local TV and social media
- Cactus pear offer an opportunity to help poor farmers in west Asia diversify their products and increase their income
- The diversity of cactus pear cultivars can be a mean to create the interest of many farmers to start growing cactus pear
- Cactus pear cultivars at Mushaqqar research station in Jordan have been playing an important role for the high adoption rate. The demand for cactus pear plant materials (cladodes) is higher than what is available at the local nurseries.

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# PITAYA (*HYLOCEREUS*) CULTIVATION IN DRYLANDS TOWARDS IMPROVING TOLERANCE TO HIGH TEMPERATURES

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Conventional crops are generally best farmed in areas with low environmental stress, plentiful fresh water for irrigation, and low input costs. However, global warming and a reduction in water resources are already affecting developed and less developed nations alike in both untold and overt ways, including a marked impact on food production. One of the means at the disposal of the agriculture sector to deal with this problem is to expand its range of produce to include new crops that offer specific advantages over traditional crops. This notion is particularly relevant to dryland agriculture, which is facing a vast R&D challenge that can be met only by increasing crop biodiversity through the development of germplasm resources. In line with this agenda, the development of new crops intrinsically adapted to stressed environmental conditions is a prerequisite for achieving livelihood security – at both the financial and nutritional levels – for farmers in drylands.

Suitable crop candidates are the hemi-epiphytic *Hylocereus* species (Cactaceae), collectively known as pitaya. According to Bauer's nomenclature (Bauer, 2003), 14 species constitute the genus *Hylocereus*. These species can grow in poor and marginal soils (Mizrahi and Nerd, 1999), and they exhibit a range of specific adaptations to extreme drought conditions, such as branches with aerial roots that attach themselves to trees, spines instead of leaves, and succulent stems. The plants are characterized by elongated, three-angled stems and large, beautiful hermaphrodite flowers. The flowers open during the night and are receptive for only a few hours (Nerd and Mizrahi, 1997). The fruits, which command excellent prices in international and local markets, are large and decorative, with various peel colors (from yellow to pink/red), a sweet and juicy flesh of various colors (from white through pink to red/purple), and edible black small seeds (Tel-Zur et al., 2011).

The ability of pitaya plants to retain water under severe drought conditions, such as those prevailing in drylands, derives from their Crassulacean acid metabolism (CAM) pathway of photosynthesis, which is characterized by open stomata during the night and closed stomata during the day (Raveh et al., 1995; Nobel and La Barrera, 2004). Pitaya cultivation in dryland regions is, however, very challenging, since these species are native to the shaded and warm humid regions of the Americas, with regional temperatures ranging between 20 and 30 °C (Nobel and La Barrera, 2004). In their native geographic zone as well as in tropical areas, pitaya is cultivated in the open (Barbeau, 1990), but in dryland regions this is not possible, since



the extremely high temperatures, frequently exceeding 40 °C, adversely impact pitaya growth, survival and productivity (Mizrahi, 2014).

*Hylocereus* species grown under extremely high solar radiation exhibit stem damage-ranging from yellowing (bleaching) of the green tissues to total "liquefaction" of large parts of the whole plant (Mizrahi, 2014; Nobel and La Barrera, 2004), depending on the sensitivity of the species and the genotype. The damage to the green tissues reduces yield and can even cause plant collapse (Mizrahi and Nerd, 1999; Tel-Zur, 2017). Consequently, outside natural environment of these species, orchards are frequently planted in net-houses or greenhouses to prevent photo-inhibition damage (Mizrahi and Nerd, 1999; Raveh et al., 1998), where the degree of net density required (from 20% to 60%, expressed as a percent of full solar irradiation) varies for the different cultivars (Mizrahi and Nerd, 1999; Mizrahi, 2014).

One of the means used to improve tolerance to heat stress in *Hylocereus* species and hybrids is grafting, an important agro-technique used in perennial crops to confer tolerance to various stressors. Grafting constitutes a low-cost technique (not labor intensive) that may be used as an alternative or an addition to conventional breeding for improving tolerance traits. To evaluate the utility of this technique for improving heat tolerance in *Hylocereus*, our group (de Oliveira et al., 2021) conducted a study of *H. undatus*, *H. megalanthus* and its di-haploid gamete-derived line 2719, and an elite hybrid (designated Z-10), all grafted onto *H. undatus* (Figure 1). *H. undatus* was chosen as the rootstock on the basis of its rapid and vigorous root system growth and its known resistance to soil nematodes (Mizrahi, 2014). Physiological, biochemical and molecular studies showed that overall, the grafted plants performed better, and, importantly, that the grafted plants recovered more rapidly from heat stress and suffered less stem damage (de Oliveira et al., 2021). Thus, we concluded that for *Hylocereus* species grafting is an excellent agro-technique for improving tolerance to the stress induced by extremely high temperatures.

Another way to confer tolerance to extremely high temperatures is through long-term breeding and selection programs. Classic breeding and selection strategies have therefore been applied by our group to develop improved heat-tolerant hybrids yielding beautiful exotic-looking fruits of excellent quality (Tel-Zur et al., 2017; de Oliveira et al., 2020). These hybrids exhibit improved survival rate, enhanced vegetative development and good yields under extremely high temperatures. In a recent study, the degree of tolerance to high temperatures of the parental species *H. undatus*, *H. monacanthus*, and *H. megalanthus*, and nine hybrids was evaluated (de Oliveira et al., 2020). The plants were exposed to heat stress (45/35 °C) or control conditions (25/20 °C) for eight days, and their physiological, morphological and molecular performance was evaluated (de Oliveira et al., 2020). The results showed a range of tolerance to heat stress, with *H. megalanthus* being the most sensitive species in that it showed severe stem damage, along with a marked reduction in total chlorophyll content (de Oliveira et al., 2020). *H. undatus* and *H. monacanthus* exhibited slight stem "liquification" and were classified as low-tolerance species. Several hybrids exhibited good performance under heat stress and almost no stem damage (de Oliveira et al., 2020). One of the hybrids, designated Z-16, exhibited the best performance under heat stress and a total absence of stem damage (de Oliveira et al., 2020). In general, it was found that most of the hybrids performed better than their parental species, and we

thus, concluded that long-term breeding and selection programs can indeed provide suitable hybrids for cultivation under extreme conditions to heat stress.

Currently, several new hybrids are being evaluated in commercial orchards in Israel, including areas in the extreme south of the country that are subject to extreme heat stress. The new hybrids will increase the range of varieties available to pitaya farmers, in the expectation of establishing profitable farming enterprises in dryland areas.

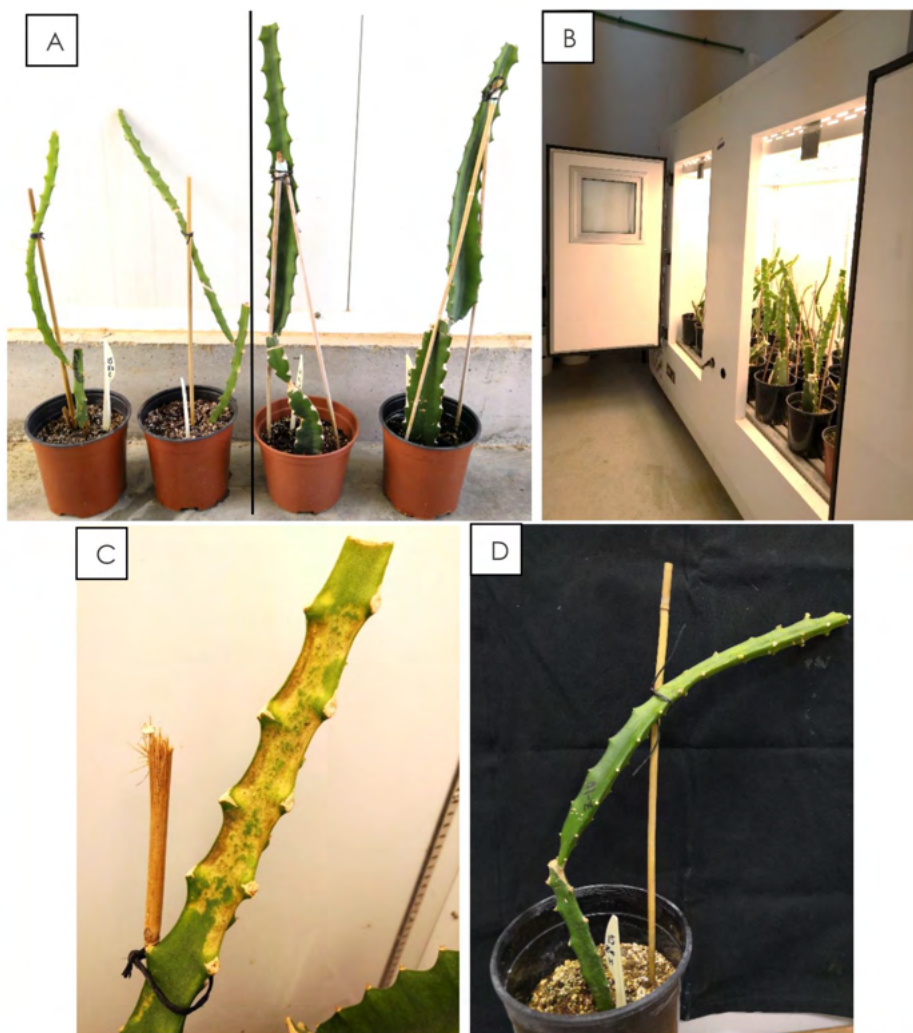


Figure 1- A) *H. megalanthus* non-grafted (left) and grafted (right) on *H. undatus* rootstock. Grafted plants developed better than non-grafted plants. B) Growth chamber - grafted and non-grafted plants during the heat-stress treatment. C) Grafted *H. megalanthus* after one day under heat-stress (45/35 °C). D) Grafted hybrid Z-10 after 3 days under heat-stress (45/35 °C).



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## **X INTERNATIONAL CONGRESS ON CACTUS PEAR AND COCHINEAL**

### **Welcome to the X International Congress on Cactus Pear and Cochineal!**

Dear colleagues, on behalf of the Paraíba Agriculture and Livestock Federation (FAEPA) and the National Rural Learning Service (SENAR/PB), in partnership with the International Society for Horticultural Science (ISHS) and the FAO-ICARDA International Network for Technical Cooperation on Cactus Pear is pleased to invite you to participate in the **X INTERNATIONAL CONGRESS ON CACTUS PEAR AND COCHINEAL**, which will be held in João Pessoa, Paraíba, Brazil, from 28 to 31 March 2022.

The Cactus Pear is a cactus native to the arid regions of the American continent, more specifically, Mexico. Today it is found in all the continents of the world, being cultivated for the production of fodder, fruits and food, cosmetics and medicinal products.

The Paraíba Federation of Agriculture and Livestock (FAEPA) organized the VI International Congress on Cactus Pear and Cochineal, held in João Pessoa in 2007. The opportunity to host the X Congress allows us to bring you back to the Brazilian territory, country which is in a prominent position in the production of Cactus Pear as animal feed, generating local economic development through its cultivation, either as fodder or for its fruit, the Tuna. Many things have changed during these 13 years. The Brazilian Society of Cactus Pear and other Cactaceae was founded, as well as the Brazilian Congress of Cactus Pear and other Cactaceae, held every two years. In addition, there was a great expansion of the new varieties of Cactus Pear tolerant to Carmine Cochineal (*Dactylopius* sp.) throughout the Northeast region, notably in the semi-arid region of Paraíba. In addition, the area planted with Cactus Pear expanded into new areas, such as in the States of Ceará and Bahia, as well as in the North of Minas Gerais.

We propose through the X Congress to share the advances mentioned with the international community, in the stunning city of João Pessoa, capital of the State of Paraíba, a city along the Atlantic Ocean coast, which has the most eastern point of the Americas in its location. It will be a great pleasure to welcome you back to the city and to Brazil.



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