African & Mediterranean AGRICULTURAL JOURNAL AL AWAMIA

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Evaluation of the planting method, and auxin hormone for vegetative multiplication of *Opuntia spp*. varieties resistant to *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Morocco

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Abstract

Cactus *Opuntia spp*. is a perfectly appropriate crop for land rehabilitation in the arid and semi-arid regions of Morocco. Unfortunately, the sustainability of this extremely resilient crop has become seriously threatened by the appearance of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), a cochineal specific to *Opuntia spp* cacti that was introduced in Morocco in 2014. Thus, eight *Opuntia spp*. varieties were identified by National Institute of Agricultural Research in Morocco as resistant to the cochineal, to provide a solid basis for the national emergency program of rehabilitating the devastated *Opuntia spp*. ecosystems throughout the country. To produce large quantities of planting material, a rapid *Ex-situ* propagation method for prickly pears have been developed. For each of the eight varieties, cladodes were harvested in march, 2019 and 2020 in the Experimental Station of the Regional Office of Agricultural Development of Doukkala (ORMVAD). They were cut into 6 pieces of about 140 cm^2 with at least two bud areolas. After 25 days of drying at a temperature range from 17 to 28 °C, the cuttings were soaked for 5 min in 2.5 g/l of a Rhizogen product (containing 0.3% IBA), which represents for us the Auxin, or in talc powder or in talc powder and a rapid soaking in auxin solution (mix 1) or soaking in water then in talc powder (mix 2) or in water. The cuttings were planted in normal polarity in black plastic cylindrical containers (24.6 cm long and 14.1 cm wide), filled with a mixture of fine sand (2/3 w/w) and peat (1/3 w/w) and placed at an average ambient temperature of 28°C/17°C (day/night) in field for growth. In general, auxin (2.5 g/l) and mix 1 treatments were found to be the most effective regarding rooting (20-73 roots number) and growth of cuttings. This research has also allowed us to demonstrate that, in general, for all the varieties studied, basal cuttings planted in orientation (N-S) and normal polarity show very interesting results under optimal conditions concerning rhizogenesis and caulogenesis.

Keywords: Cactus, Varietal resistance, scale pest, Auxin, Talc powder, Rooting of cuttings.

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Évaluation de la méthode de plantation et de l'auxine pour la multiplication végétative des variétés de de figuier de Barbarie (*Opuntia spp.*) résistantes à *Dactylopius opuntiae* (Hemiptera : Dactylopiidae) au Maroc

Résumé

Le cactus *Opuntia spp*. est une culture parfaitement adaptée à la réhabilitation des terres dans les régions arides et semi-arides du Maroc. Malheureusement, la pérennité de cet écosystème extrêmement résilient est devenue sérieusement menacée par l'apparition de *Dactylopius opuntiae* (Cockerell) *(*Hemiptera: Dactylopiidae), une cochenille spécifique à l' *Opuntia spp*. qui a été introduite au Maroc en 2014. Ainsi, dans un premier temps, l'Institut National de la Recherche Agronomique du Maroc a identifié huit variétés de d'Opuntia cactus comme résistantes à la cochenille qui constitueront une base solide pour le programme national de reconstitution des *Opuntia spp*. décimés à travers le pays. Pour produire le matériel végétal nécessaire à ce programme, une méthode de multiplication rapide *Ex situ* des cactus a été développée. Pour chacune des huit variétés, les cladodes collectées en mars 2019 et 2020 à partir de la Station Expérimentale de l'Office Régional de Mise en Valeur Agricole du Doukkala (ORMVAD). Elles ont été coupées en 6 morceaux d'environ 140 cm^2, comportant au moins deux aréoles de bourgeons dans l'une de ses bases. Après 25 jours de séchage à une température comprise entre 17 et 28 °C, ces boutures ont été trempées pendant 5 min dans 2.5 g/l d'un produit rhizogène (contenant 0.3% d'AIB) et qui représente pour nous l'auxine, ou dans le talc, ou dans le talc suivi par un trempage rapide dans une solution de l'auxine (mélange 1), ou un trempage dans l'eau puis dans le talc (mélange 2) ou dans l'eau. Les boutures ont été plantées en polarité normale dans des récipients cylindriques en plastique noir (24,6 cm de long et 14,1 cm de large), remplis d'un mélange de sable fin (2/3 w/w) et de tourbe (1/3 w/w) et placés à une température ambiante moyenne de 28°C/17°C (jour/nuit) dans le champ pour la croissance. En général, les traitements à l'auxine (2,5 g/l) et le mélange 1 se sont avérés les plus efficaces pour l'enracinement (20-73 nombre de racines) et la croissance des boutures. Egalement, et pour toutes les variétés étudiées, les boutures basales plantées en orientation (N-S) et en polarité normale ont donné des résultats très appréciables de rhizogenèse et de caulogenèse dans les conditions optimales.

Mots clés : Cactus, Résistance variétale, *Dactylopius opuntiae*, Auxine, Talc, Enracinement des boutures

تقييم كفاءة طريقة الزرع وهورمون الأوكسين على التكثير الخضري لأصناف صبار الكمثرى (*Opuntia spp.*) المقاومة لـ *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) في المغرب

محمد العلاوي ومحمد سباغي

ملخص

يعتبر نبات الصبار من المحاصيل المناسبة لإعادة تأهيل الأراضي في المناطق القاحلة وشبه القاحلة في المغرب. لسوء الحظ، أصبحت استدامة هذا النظام البيئي الشديد المرونة مهددة بشكل خطير بسبب ظهور الحشرة القرمزية *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) التي ظهرت لأول مرة في المغرب سنة 2014. لذلك، وكخطوة أولى، شكلت الأصناف الثمانية التي تم تحديدها من طرف المعهد الوطني للبحث الزراعي بالمغرب على أنها مقاومة للحشرة أساسًا متينًا للبرنامج الوطني الاستعجالي لاستعادة الصبار المتضرر في جميع أنحاء البلاد. لإنتاج الكميات الضرورية لهذا البرنامج من شتلات الصبار المقاوم، تم تطوير طريقة للتكثير السريع في الظروف الطبيعية. في كل صنف، تم حصد الالواح في مارس 2019 و 2020من ضيعة التجارب التابعة للمكتب الجهوي للاستثمار الفلاحي لدكالة، وتم تقطيعها إلى 6 قطع مساحتها حوالي 140 سم مربع، بها على الاقل برعمين في احدى الجهتين. بعد 25 يومًا من التجفيف تحت درجة حرارة بين 17 و 28 درجة مئوية، تم نقع القطع لمدة 5 دقائق في 2.5 غرم / لتر من الاوكسين أو في غبار التلك ، أو التلك ونقعها سريعًا في محلول أوكسين (الخليط 1)، أو نقعها في الماء ثم في التلك (الخليط 2) أو في الماء. زرعت القطع في قطبية عادية في عبوات بلاستيكية سوداء أسطوانية (طولها 24.6 سم وعرض 14.1 سم) ، مملوءة بمزيج من الرمل الناعم (3/2 وزن / وزن) وخث (3/1 وزن / وزن) وتم وضعها عند متوسط درجة الحرارة المحيطة 28 درجة مئوية / 17 درجة مئوية (نهارًا / ليلًا) في الحقل للنمو. بشكل عام، أثبتت معاملات الأوكسين (2.5 غرم/لتر) والخليط 1 أنها الأكثر فعالية من حيث تجذير (عدد الجذور 20-73) ونمو عقل الصبار. كما تمت ملاحظة أنه، بالنسبة لجميع الأصناف التي تمت دراستها، كل العقل القاعدية المزروعة في الاتجاه شمال جنوب (NS) و حسب قطبية عادية تعطي نتائج ملحوظة للغاية في الظروف المثالية فيما يتعلق بتكوين الجذور وتكوين الجلفة.

الكلمات المفتاحية: الصبار، الأصناف المقاومة، الحشرة القرمزية ، الأوكسين، التلك، تجذير العقل

Introduction

Cactus pear (*Opuntia spp.*) is an important drought-tolerant crop (It's a Crassulacean Acid Metabolism (CAM) plant, with nocturnal stomatal opening and CO_2 uptake occurring typically at night) that has various beneficial uses. The fruit is used in human consumption, or as a dye, and in cosmetic products as well (Sheehan and Potter, 2017; El Aalaoui *et al*., 2019a). The pads are used as animal fodder predominantly in the arid zones on degraded lands where shortages of water and fodder resources are limiting factors for animal production (Sheehan and Potter, 2017).

Unfortunately, the sustainability of this cactus pear resilient ecosystem has become seriously threatened by the appearance of an invasive and devastating scale pest: *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae). This cochineal which is specific to *Opuntia spp*. cacti was introduced in Morocco in 2014, in Sidi Bennour-Doukkala region, where it has caused enormous damages in several cactus areas. Similar cases have been reported by Lopes *et al*. in (2009), when *D. opuntiae* attacked a fodder species of cactus, *Opuntia ficus indica* (L.) Miller, 1768), in Brazil where 100,000 ha were damaged, estimated to about $ 25 million losses.

To prevent further spread of this scale pest, the Moroccan Agriculture Department, established a major emergency control plan since 2016, that included, among other aspects, a research program covering the most important management components such as biopesticides, beneficial insects (Bouharroud *et al*., 2018,2019; El Aalaoui *et al*., 2019a,b,c), and host plant genetic resistance (Sbaghi *et al*., 2019). In this respect, eight varieties were identified as resistant to the cochineal, and should be massively propagated to provide a solid basis for launching the national emergency program for the rehabilitation of devastated cactus ecosystems throughout the country (Sbaghi *et al*., 2019). Indeed, the use of cactus varieties resistant to *D. opuntiae* is an encouraging, economic, and ecological control strategy that minimizes production costs and does not require particular technical skills from the farmers.

Cactus can be propagated by different techniques, most commonly through vegetative propagation, either by cladodes (aged one year), or by *in vitro* tissue culture techniques (organogenesis or somatic embryogenesis), as well as by seeds. The most widely used vegetative propagation method concerns rooting whole cladodes, or small segments of mature cladodes, or by using fruits as propagules (Stambouli-Essassi *et al*., 2015). Soil humidity and structure, as well as fertilization with N and P, are very important elements for good rooting (Mondragón-Jacobo *et al*., 2001). Plant growth regulators may also be used to improve rooting capacity of the plant material (Mulas *et al*., 1992). Shehu *et al*. (2016) reported that rooting of cladodes is most effective when treated with 2.5 g/l of IBA.

The current increasing demand for the eight resistant *Opuntia* cactus varieties by agricultural enterprises and farmers, as well as the great need of having sufficient plant materials to be used for both, in *Opuntia* cactus management programs and in genetic improvement research programs, requires research activities for efficient, rapid, and economic propagation methods. The dual purpose of the present study is therefore to (a) quantify the response of *Opuntia* varieties to photosynthetically active radiation (PAR) under the effect of orientation and (b) evaluate the effects of auxin and talc powder on rooting and growth of *Opuntia* cactus cuttings.

Materials and Methods

Study area and studied species

One-year-old cladodes used as biological material in this study were obtained from the woodlot established at the ORMVAD Experimental Station in Zemamra, Doukkala region, Morocco in 2017 (The plants were cultivated under intensive fertilization and irrigation). This collection was mainly established from eight *Opuntia* cactus varieties (Karama, Ghalia, Belara, Marjana, Melk Zhar, Cherratia, Angad, and Aakria) reported by Sbaghi *et al*. (2019) as resistant to *D. opuntiae* (Table 1), where mother plants have been pruned each year in March for propagation. Some of the pruned one-year-old cladodes are used for rooting and growth experiments. Each cladode had a surface of about 600 cm^2 and weighed about 700 g. To avoid fungal diseases during planting, we avoided cladodes wounding during harvest and transport. Experiments were repeated for two years: 2019 and 2020.

The Experimental Station is located in khemis Zemamra, Doukkala (Latitude N 32° 21, longitude W8° 22, and altitude 168m). It is a semi-arid area where rainfall varies between 112.6 and 607 mm/year, with mean annual precipitation of 330 mm (mean of the last 30 years). The temperature varies from -1°C (Dec-Jan) to 40-45°C (July-August). The Station soil presents a sandy-clay hydromorphic structure with an alkaline pH (Khattabi *et al*., 2004). According to Eljebri *et al*. (2019), the values of pH, percent silt and sand, clay, CEC (Cation Exchange Capacity), P_2O, K_2O, and organic matter of the whole irrigated plain of Doukkala area, are 8.15, 14.25, 56.40, 29.31, 22.40, 45.27 ppm, 90.40 ppm and 1.46%, respectively.

Table 1. List of *Opuntia* cactus pears varieties resistant to *D. opuntiae* registered in the Official Catalogue of Cactus in Morocco (Sbaghi *et al*. 2019).

Varieties	Species	Origin
Marjana	*Opuntia engelmannii* Salm-Dyck, 1850	Dchira- Inezgane - Morocco
Belara	*Opuntia ficus indica* (L.) Miller, 1768	Dchira - Inezgane - Morocco
Karama	*Opuntia engelmannii* Salm-Dyck, 1850	Dchira - Inezgane - Morocco
Ghalia	*Opuntia engelmannii* Salm-Dyck, 1850	Dchira - Inezgane - Morocco
Angad	*Opuntia robusta* (Haw.) Haw., 1812	Oujda - Morocco
Aakria	*Opuntia dillenii* (Ker Gawl.) Haw., 1819	Bouknadel - Morocco
Melk Zhar	*Opuntia robusta* (Haw.) Haw., 1812	Irradiation *O. robusta* - Morocco
Cherratia	*Opuntia robusta* (Haw.) Haw., 1812	Bouznika-Morocco

Experimental design

Two experiments were carried out in this study:

Trial 1: Effect of auxin and talc powder on rooting and growth of *Opuntia* cactus plants. For each of the eight *Opuntia spp*. varieties tested, cladodes harvested in March in the ORMVAD experimental station plantation were cut into 6 pieces of about 140 cm^2 with each piece always having at least two bud areolas in one of the bases (Fig. 1) and have been allowed to air dry for 25 days. After 25 days of drying at a temperature ranging from 17 to 28 °C, the right cuttings were soaked for 5 min in 2.5 g/l of a Rhizogen product (containing 11% N, 55% P205 and 0.3% IBA), which represents the Auxin treatment, or in talc powder ($Mg_3Si_4O_{10}(OH)_2$ at a concentration of 2.7 g/cm^3) or in talc powder and a rapid soaking in auxin solution (mix1) or soaking in water then in talc powder (mix 2) or in water (control).
The *Opuntia spp*. cuttings, of not more than 140 cm^2 surface, were planted in normal polarity in a cylindrical black plastic container (Mulas *et al*. 1992), with an average length of 24.6 cm and an average width of 14.1 cm, filled with a mixture of fine sand (2/3 w/w) and peat (1/3 w/w) and placed at an ambient mean temperature of 28°C/17°C (day/night) in field. The orientation of the long axis of each cutting was towards (North-South). The cuttings were watered weekly with tap water according to Stambouli-Essassi *et al*. (2015). A completely randomized experimental block design with ten replicates was used for each combination variety-treatment (eight cultivars). Rooting was observed through careful uprooting of the cuttings without damaging the roots. The number and length of roots were recorded every 5 days during the first 30 days after plantation. Roots dry weight was obtained at the 30th day after plantation, through root drying in laboratory conditions at 25°C for 72 h, using three cuttings for each variety/treatment combination. After 4 months of the experiment, destructive measures were carried out on cactus plants concerning vegetative growth (number of secondary cladodes initiated per cutting, height and width of the plant), evolution of cladode surface, number of areoles per cladode, number of areoles per plant, number of spines per areole, and date and duration of first budding.

Trial 2: Effect of the cutting zone and planting orientation on rooting and growth of *Opuntia spp.* plants. For each of the eight varieties, ten cladodes were harvested, divided (6 pieces of about 140 cm^2), and dried as described above. A total of 480 cuttings were obtained. Each of the basal (B), median (M), and apical (A) cladode zones were cut into 2 parts. The right and left cuttings were planted in normal polarity so that the orientation of the long axes were N-S or E-W, in cylindrical black plastic container (24.6 cm length and 14.1 cm width) filled with the same substrate mixture and conditions as mentioned above. A completely randomized block design was adopted. Assessment of the effect of cladode zones and planting orientation on the rooting and growth of cactus cuttings was carried out as described in Trial 1 above.

Figure 1. Cladode cuttings used in the experiments.

Statistical analysis

The statistical analysis was carried out using the STATISTICA Software (Ver.6). The differences in rooting and bud growth rates among treatments were analyzed using the factorial analysis of variance ANOVA and the means comparison was performed using Tukey's HSD test (alpha= 0.05). Percentages data were subjected to an arcsine transformation in order to approximate the normal distribution before analysis.

Results

Trial 1: Effect of Auxin, talc powder and variety on rooting and growth of *Opuntia* cactus cuttings

Number of roots

According to the *Opuntia spp*. variety/treatment interactions, it appeared that the number of roots per cutting was the highest in Cherratia variety under Auxin treatment ($F= 8.12$, $df = 49$, $P< 0.0001$). However, the lowest number of roots (0.2) was found in Cherratia variety, with control and mix 2 treatments (Fig. 2). Measurements of root number for the eight studied *Opuntia spp.* varieties showed that Cherratia and Aakria varieties produced the highest root number, with maximum values of 72 for Cherratia and 63 for Aakria after 30 days of the experiment (Fig. 2). However, treatments with Auxin, talc powder, or mix 1 had a significant effect on rooting (Fig. 2). Considering the root number per cutting, it was determined that Cherratia variety produced the maximum root number. The Auxin treatment produced the highest root number (72) while the lowest number of roots (0.2) was recorded in control and mix 2 treatments.

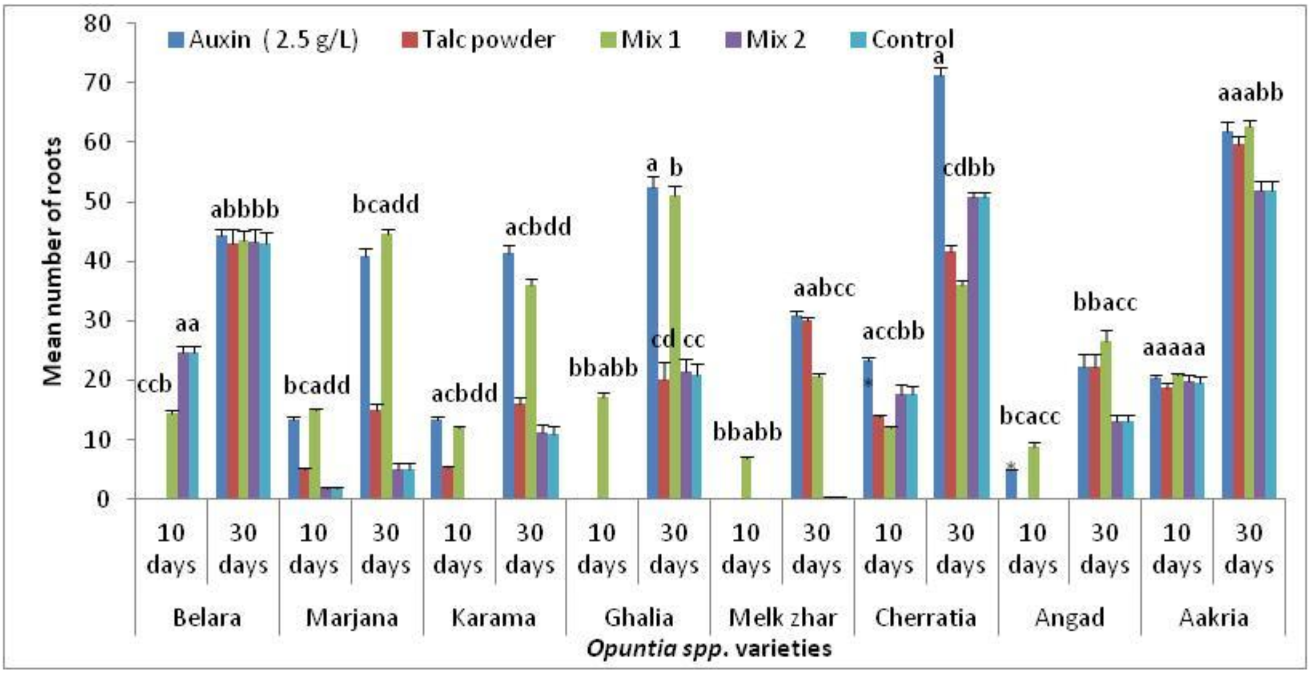

Figure 2. Effect of Auxin, talc powder and genotype on mean root number of *Opuntia spp*. cuttings. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Root length

The analysis of variance of the treatments-*Opuntia spp*. varieties interactions effect on roots length, showed that the highest root length values (60.7 cm) were obtained in Aakria cuttings treated with mix 1 treatment, while the lowest root length values were obtained in Melk Zhar cuttings under control and mix 2 treatments ($F= 15.86$, $df = 49$, $P< 0.0001$) (Fig. 3). The highest root length (60.3 cm) was recorded in Aakria variety cuttings, while Melk Zhar cuttings produced the shortest roots with a length of 0.2 cm (Fig. 3). Concerning the treatments effects on root length, the longest roots (60.7 cm) were obtained in mix 1 treatment, whereas the lowest ones (0.2 mm) were in control treatment. However, for Angad and Melk Zhar varieties, cuttings treated with talc powder had the lengthiest roots compared to mix 1 treatment.

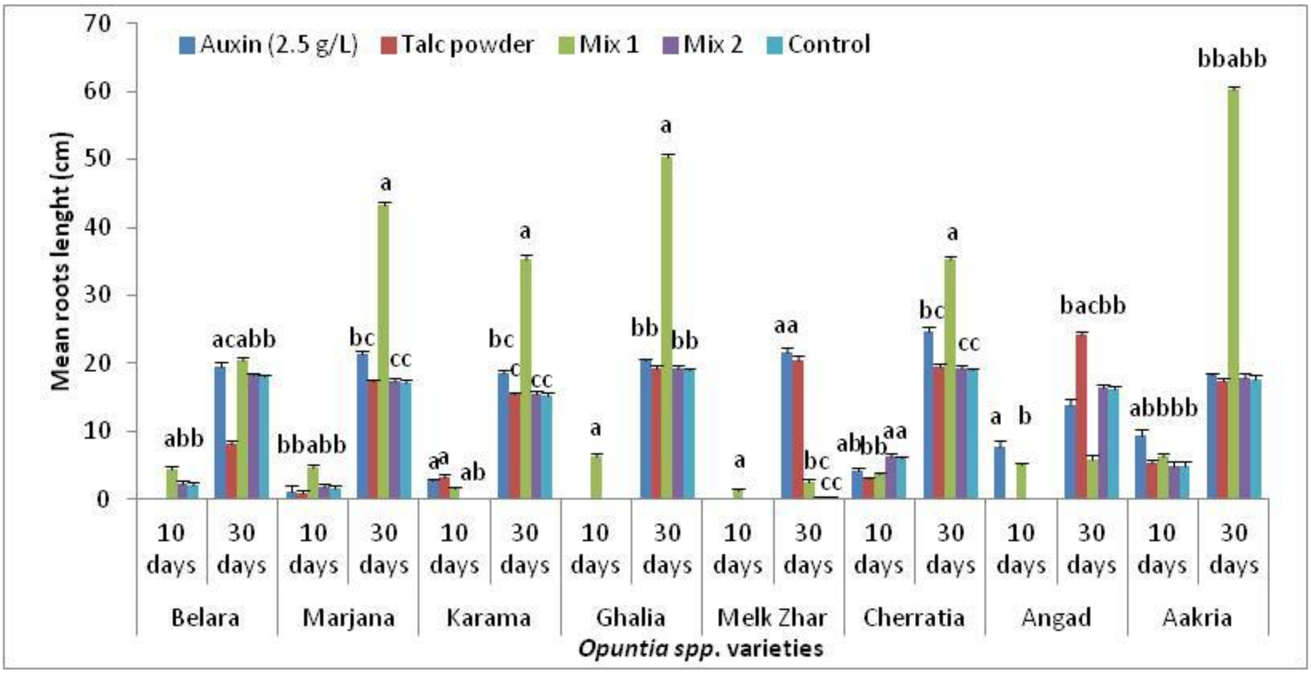

Figure 3. Effect of Auxin, talc powder and genotype on mean root length of *Opuntia spp*. cuttings. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test (p ≤ 0.05).

Root dry weight

Analysis of *Opuntia spp*. varieties/treatments interaction showed that the highest value (9.6 g) was recorded in case of Cherratia variety cuttings under Auxin treatment (F= 14.24, df = 49, P< 0.0001). It was observed also that Melk Zhar variety/mix 1 treatment interaction gave the lowest value of 0.1 g. For Melk Zhar and Angad varieties, no significant difference was observed between Auxin and talc powder treatments (Fig. 4).

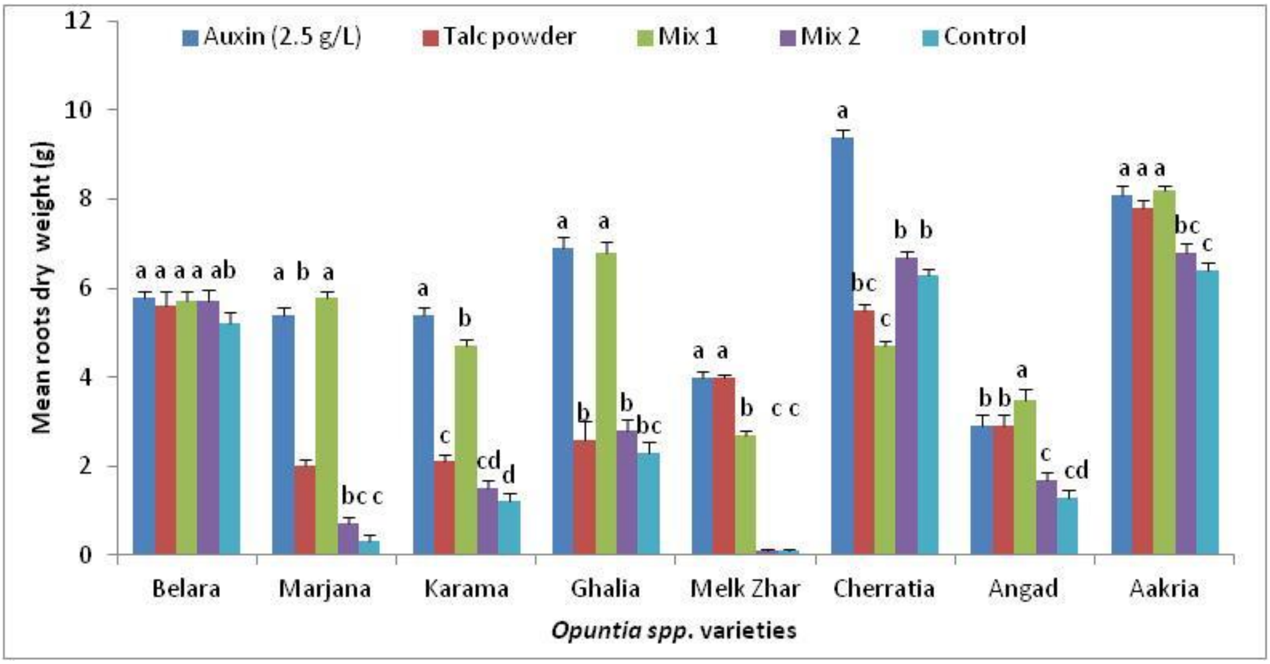

Figure 4. Effect of Auxin, talc powder and genotype on mean roots dry weight of *Opuntia spp*. cuttings. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Percentage of secondary cladodes initiated per cutting

The analysis of *Opuntia spp*. varieties/treatments interaction showed that 100% of secondary cladodes initiated per cutting in the case of Aakria variety with mix 1 treatment ($F= 15.14$, $df = 49$, $P< 0.0001$) (Fig. 5). It was noted also that Melk Zhar cuttings treated with Auxin, talc powder, mix 2, and control treatments presented the lowest value of 20 %. No significant difference was observed between Auxin and mix 1 treatments in the case of Marjana variety (Fig. 5). Aakria variety gave 100% of secondary cladodes initiated per cutting. Whereas, the other tested varieties reached a percentage of between 20%- 60% as shown in figure 5.

Concerning the treatments effect on the percentage of secondary cladodes initiated per cutting, it was found that Marjana, Karama, Melk Zhar, and Aakria varieties, treated with mix 1, provided the highest values compared to the other treatments. Whereas for Belara variety, it is Auxin treatment that provided significant results, while no significant difference among treatments was recorded for the other varieties (Fig. 5).

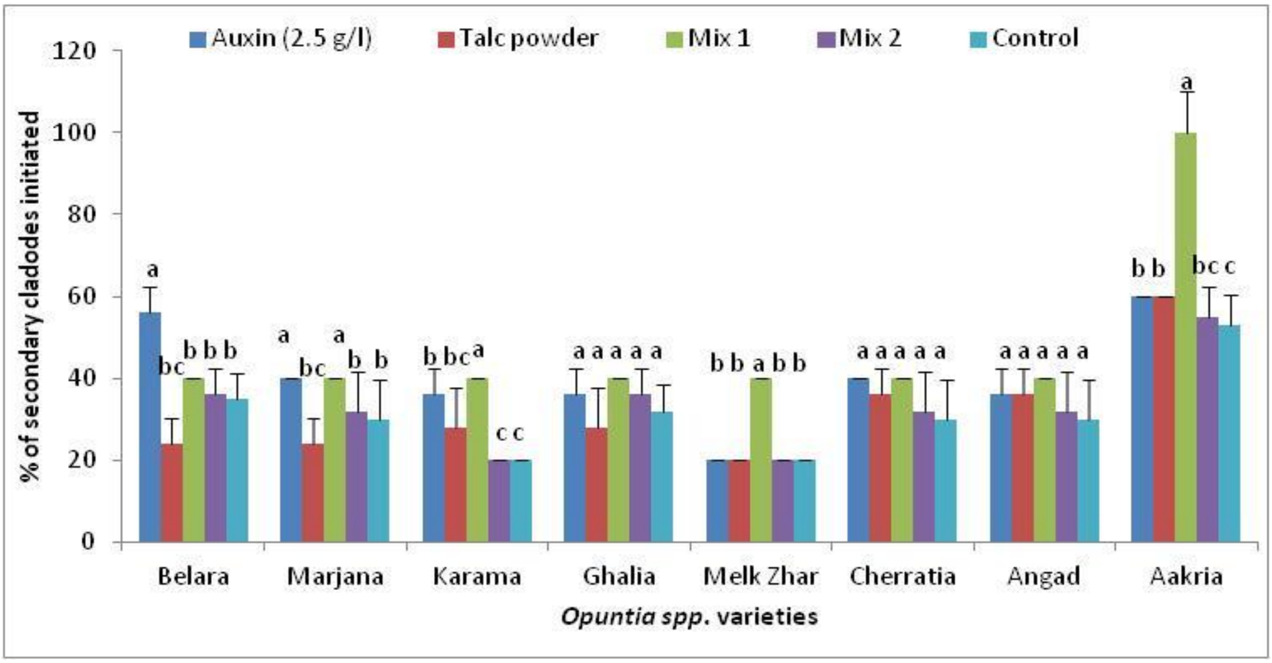

Figure 5. Effect of Auxin, talc powder and genotype on percentage of secondary cladodes initiated per cutting. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Duration for first budding

Among the *Opuntia spp.* varieties/treatments interaction, the longest duration for first budding (32.2 days) was recorded in Melk Zhar variety with Auxin treatment, while the lowest duration (2.3 days) was recorded in Aakria variety with all the tested treatments as shown in figure 6 ($F= 18.25$, $df = 49$, $P< 0.0001$) (Fig. 6). The longest duration for first budding (32.2 days) was recorded in Melk Zhar variety and the lowest value (2.3 days) was found in Aakria variety (Fig. 6).
Analysis of the treatments effects on duration for first budding for all the tested varieties showed that, generally, mix 1 treatment increases this duration compared to the other treatments. No significant difference was recorded among treatments, for Aakria and Karama varieties

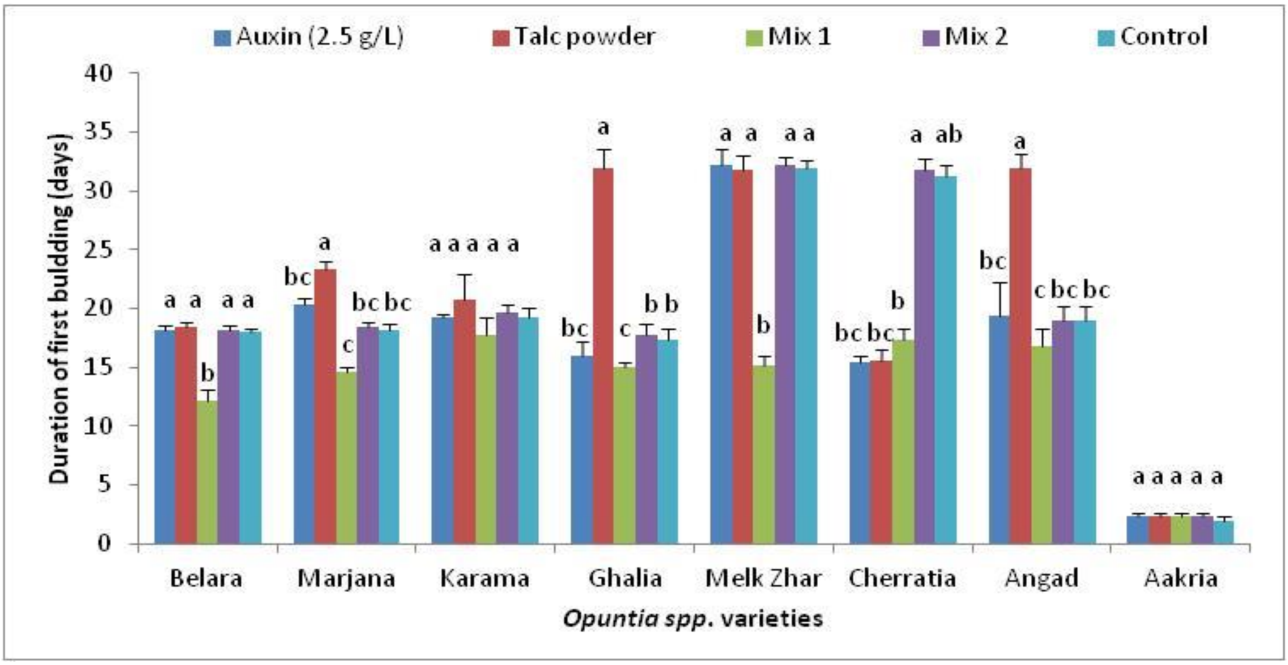

Figure 6. Effect of Auxin, talc powder and genotype on duration for first budding of *Opuntia spp*. cuttings. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Cladode surface

Concerning *Opuntia spp*. varieties/treatments interaction, the highest cladode surface (305.2 cm^2) was recorded in Angad variety with mix 1 treatment while the lowest surface value (76.5 cm^2) was recorded in Aakria variety without Auxin treatment (control) as shown in figure 7 ($F= 9.86$, $df = 49$, $P< 0.0001$) (Fig. 7). The highest value of cladode surface (307 cm^2) was found in Angad variety and the lowest one (78.8 cm^2) was found in Aakria variety. When analyzing the effects of treatments on cladode surface in all the studied varieties, it was found that, Auxin and mix 1 treatments induced the highest cladode surface values while control and mix 2 treatments showed the lowest values (Fig. 7).

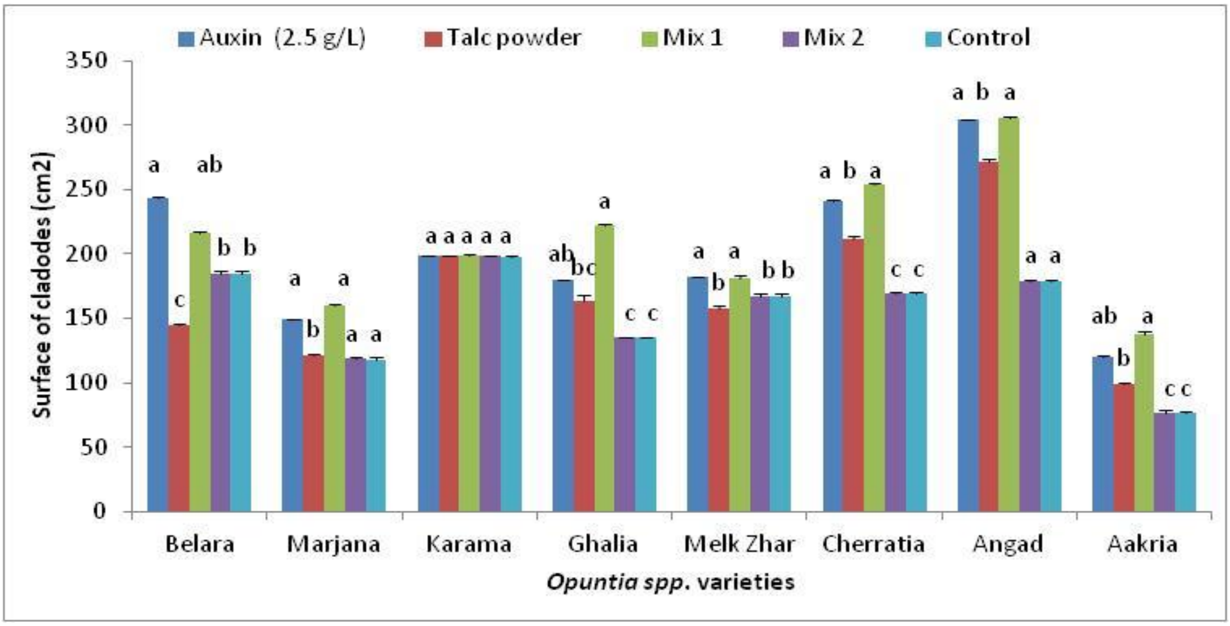

Figure 7. Effect of Auxin, talc powder and genotype on cladode surface of *Opuntia spp*. varieties. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test (p ≤ 0.05).

Number of areoles per cladode

Among the *Opuntia spp*. varieties/treatments interaction, the highest number of areoles per cladode (156.8) was obtained in Karama variety with Auxin treatment while the lowest value (35.0) was recorded in Aakria variety without Auxin treatment (control) as shown in figure 8 (F= 29.04, df = 49, P< 0.0001). It was also shown that the average number of areoles per cladode in Karama variety (156.8) was the highest compared to the other tested varieties (Fig. 8).

Regarding the treatments effects on number of areoles per cladode, it was found out that generally, the values obtained from Auxin and mix 1 treatments were significantly the highest than those of the other treatments. For Marjana variety, no significant difference was recorded among treatments (Fig. 8).

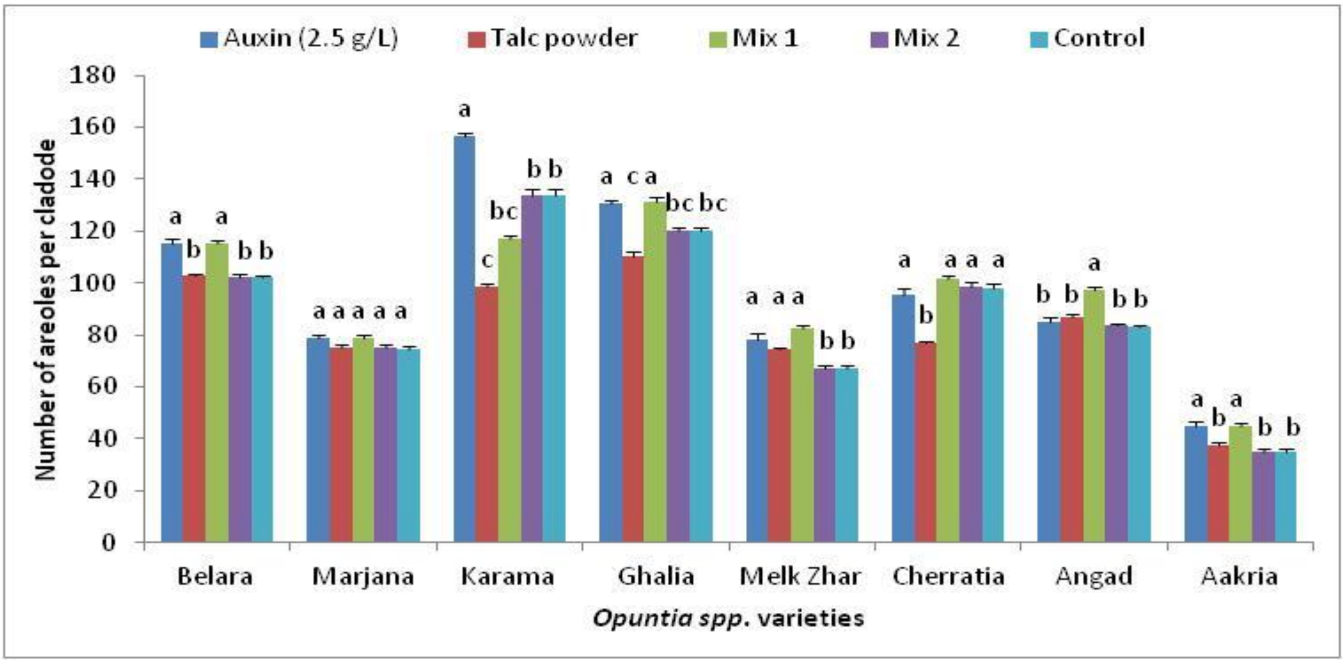

Figure 8. Effect of Auxin, talc powder and genotype on number of areoles per cladode of *Opuntia spp*. varieties. Data are from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test (p ≤ 0.05).

Number of areoles per plant

Analysis of the interactions, the highest average number of areoles per plant (323.2) was obtained in Belara variety with Auxin treatment while the lowest value (67.2) was obtained in Aakria variety with mix 2 and control treatments (F= 7.50, df = 49, P< 0.0001). Also, it was found that the average number of areoles per plant in Belara variety (323.2) was the highest than in other tested varieties. Concerning the treatments effects on number of areoles per plant, it was generally found out that the values induced from Auxin and mix 1 treatments were significantly the highest than those of talc powder, mix 2 and control treatments. The lowest areoles number (67.2) was found in control treatment (Fig. 9).

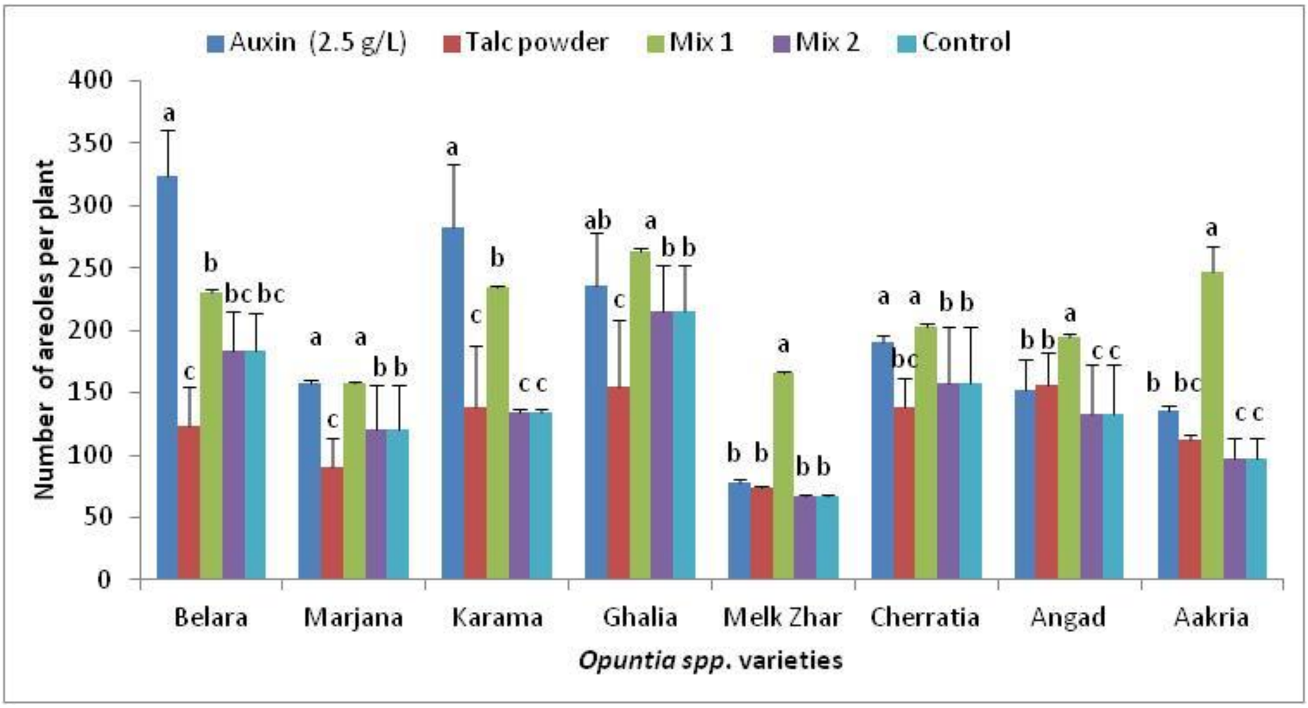

Figure 9. Effect of Auxin, talc powder and genotype on number of areoles per plant of *Opuntia spp*. varieties. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test (p ≤ 0.05).

Trial 2: Effect of cuttings genotype, zone and planting orientation on rooting and growth of *Opuntia* cactus plants

Average number of roots

The obtained results are summarized in figure 10, which shows that the highest number of roots (52.5) was obtained in Aakria variety with basal (North-South) cuttings. When examining the treatments effects on the mean number of roots, it was found out that for all the tested varieties, the values derived from basal (North-South) treatment were significantly the highest than those obtained with other treatments. No significant difference was observed among treatments in number of roots of Marjana variety for which, nevertheless, the number of roots was less important (4.6-5).

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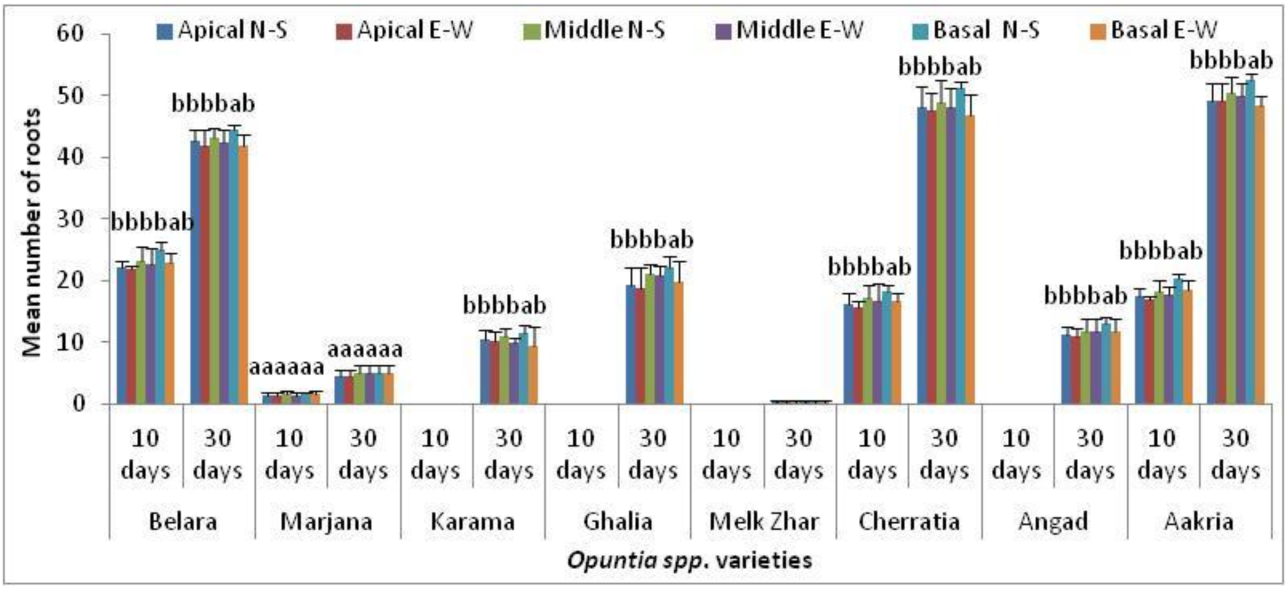

Figure 10. Effect of cutting genotype, zone and planting orientation on mean roots number of *Opuntia spp*. varieties. Data are from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Mean root length

As reported in figure 11, the mean root length values of Ghalia variety with basal (North-South) cuttings were the highest ones (19.6 cm), while for all apical (East-West) cuttings, the mean root length per cutting was less important despite it reached an interesting length varying from 1.5 cm to 17.5 cm. When analyzing the treatments effects on mean root length, it was found out that for all the tested varieties, the values of basal (North-South) treatment were significantly the highest than those obtained with other treatments, 30 days after planting. For all the tested varieties, no significant difference was observed among treatments 10 days after planting.

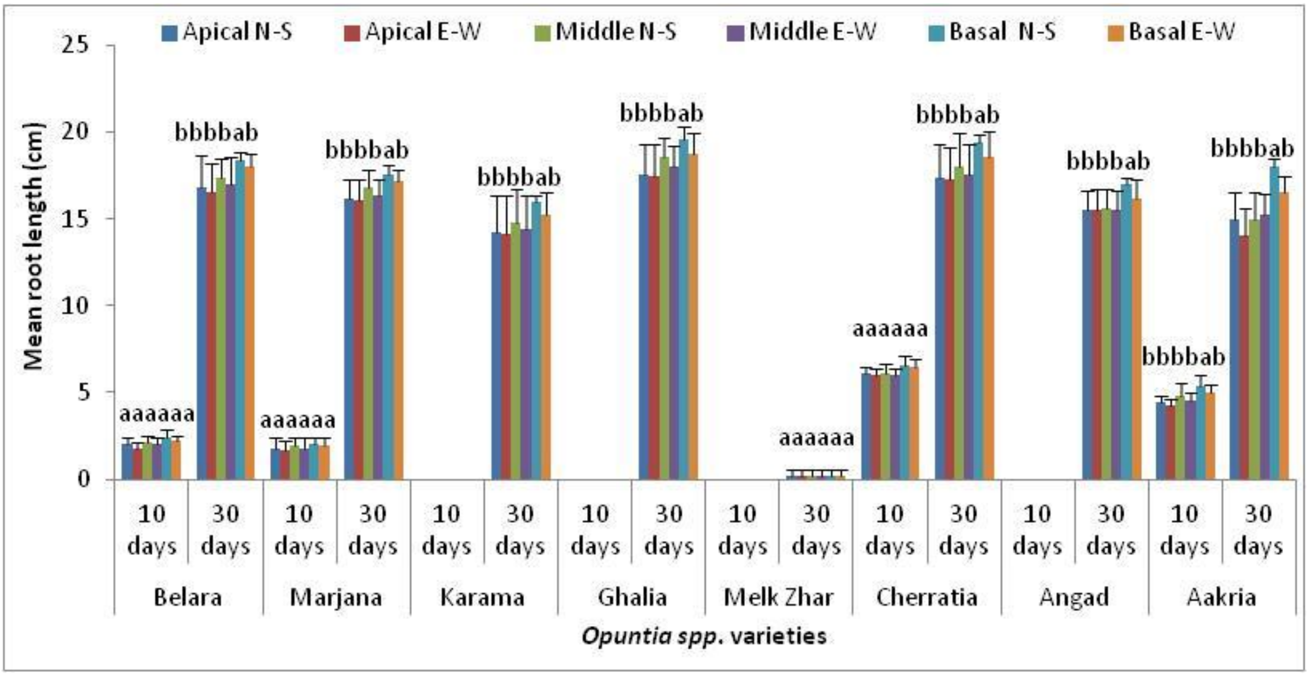

Figure 11. Effect of cutting genotype, and zone, and planting orientation on mean root length of *Opuntia spp*. varieties. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$). DAT: Days after treatment.

Mean root dry weight

The highest values of mean root dry weight were obtained from Aakria and Cherratia varieties with 6.9 g, and 6.7 g respectively, while the lowest values were found in Melk Zhar variety. Analysis of the treatments effects showed that the varieties Aakria and Cherratia with basal (North-South) cuttings provided the highest mean root dry weight values, while no significant difference was observed among the other treatments. For the other tested varieties, no significant difference was observed among the treatments (Fig. 12).

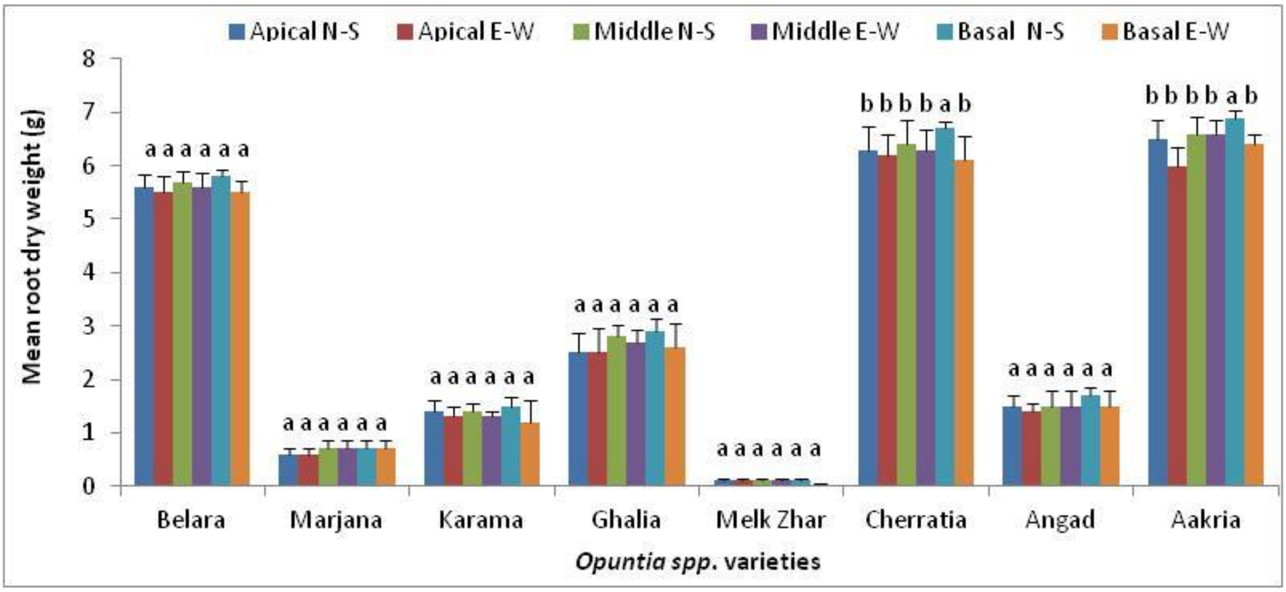

Figure 12. Effect of cutting genotype, zone, and planting orientation on mean root dry weight of *Opuntia spp*. varieties. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test ($p \leq 0.05$).

Percentage of secondary cladodes initiated per cutting

After 4 months of cultivation, the most important percentages of initiating secondary cladodes were obtained from Aakria variety with basal (N-S) (66.5%), basal (E-W) (65.0%), middle (N-S) (65.0%), and middle (E-W) (65.0%) cuttings (Fig 13).

The most significantly highest ($p<0.0001$) percentages of secondary cladodes initiation were generally obtained using basal (N-S) quarter cuttings. There was no significant difference among treatments on Karama, Melk Zhar, and Cherratia varieties (Fig 13).

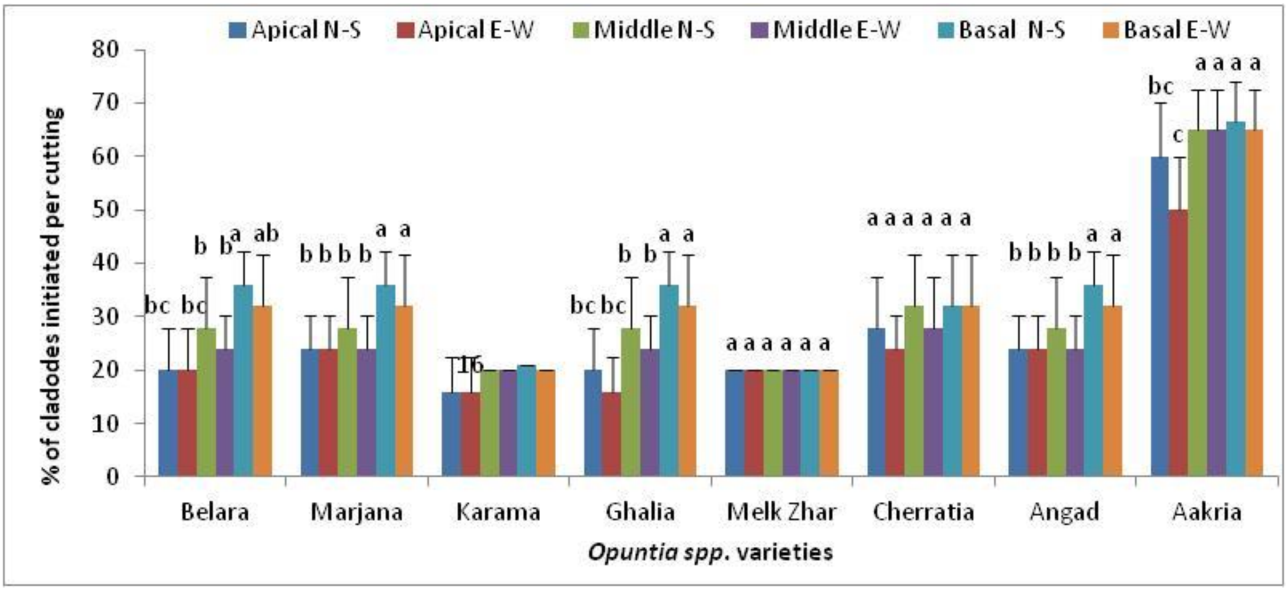

Figure 13. Effect of cutting genotype, zone, and planting orientation on percentage of cuttings initiating secondary cladodes of *Opuntia spp*. varieties. Data are averages from experiments carried out in 2019 and repeated in 2020. Unlike letters above vertical columns indicate significant difference by HSD Tukey's test (p ≤ 0.05).

Discussion

Trial 1: Effect of Auxin product and cactus genotype on rooting and growth of *Opuntia* cactus plants

Over a two years' experiment, destructive measurements related to rooting and vegetative growth were carried out on eight *Opuntia* cactus varieties resistant to the specific *Opuntia* cactus scale pest *D. opuntiae* with or without Auxin treatment. Additional observations and non-destructive measurements were also carried out mainly related to plant height and width that make it possible to calculate the volume of the vegetative system, which is considered as an overall stand growth indicator that can be related to the evolution of the cladode surface and to yield components. The selection of cactus varieties for fodder or fruit production requires taking into account both their rooting capacity and subsequent performance in the field. It has long been recognized that the genotype of the plant has a profound effect on the rooting capacity of cuttings (Haissig and Riemenschneider, 1988). Many studies indicate that different genotypes of the same species have very different rooting capacities, e.g. clones of various cultivars of *Eucalyptus globulus* (Negishi *et al*., 2014), olive trees (*Olea europea* L.) (Wiesman and Lavee, 1995), poplar species (Populus) (Zhao *et al*., 2014), and scots pine (*Pinus taeda*) seedlings (Foster, 1990). The present study showed that the tested *Opuntia* cactus varieties differ greatly in their ability to rooting. Similar results were reported for three important prickly pear cactus cultivars (*Opuntia ficus-indica*) propagated in vitro in Morocco by El Finti *et al*. (2013). The mechanism responsible for the genetic effect on rooting is not clear yet (Izhaki *et al*., 2018). One possible explanation is the accumulation of different levels of rooting inhibitors or promoters. Recent observations indicated that auxin metabolism and the ratio of auxin and cytokinin may be involved in the genetic effect on rooting (Negishi *et al*., 2014). Also,

the ontogenetic stage of development, i.e. juvenility and maturation, of the mother plants from which cuttings are harvested has a central effect on rooting ability (Hartmann *et al*., 2014).
Treatment of cuttings with auxins to promote rooting can be carried out using a range of methods, the most common of which is the application of talc powder and rapid soaking in hormone solution (Blythe *et al*., 2007). The results obtained in the present research showed a very significant effect of the application of exogenous auxin (coming from a product containing 0.3% IBA), on the measured traits. The highest values of mean number of roots per cutting, mean root length, mean root dry weight, percentage of secondary cladodes initiated per cutting, cladode surface area, number of areoles per cladode, and number of areoles per plant were observed in the Auxin (auxin) and mix1 (application of talc powder and rapid soaking in auxin solution) treatments. Hartmann *et al*. (1997) reported that auxin concentrations significantly higher than those normally found in plant tissue can play an inhibitory role in growth and root formation. Ramdayal *et al*. (2001) and Gupta *et al*. (2002) found the maximum root number at 1000 ppm IBA. Owais (2010) and Sharma *et al*. (2009) found that the longest roots were derived from hormone-rich plants. The number of shoots increased with the use of hormones, following the conclusions of Janick (1972) and Hartmann and Kester (1983) who concluded that the use of natural hormones increases the percentage of survival as well as all the vegetative development of the seedlings. Babaie *et al*. (2014) carried out similar studies on some *Opuntia* cactus species using IBA as a hormone and found that the highest numbers of cladodes were obtainable at doses of 2000 and 4000 ppm. Mehraj *et al*. (2013) found that maximum vegetative growth was achieved with 1000 ppm IBA. Literature data indicated that the best method for applying auxin differs among species (Dirr and Heuser, 2006; Hartmann *et al*., 2014). In this study, we also observed that the effects of auxin on vascular differentiation and the success of cuttings depended entirely on method of application and treatment durations. The presence of auxin, a plant hormone, regulates many developmental processes, and depending on its content it can affect cell division, growth, and cell differentiation (Wilmoth *et al*., 2005). The results obtained in the present research showed also that the activation and increasing number of areoles takes place under the influence of auxin. The highest number of areoles was observed in the Auxin treatment group for all the *Opuntia* cactus varieties tested.

Trial 2: Effect of cutting genotype, zone and planting orientation on rooting and growth of *Opuntia spp*. plants

Nobel (1982a) reported that the orientation of *Opuntia* cactus cladodes occurs to maximize the reception of solar radiation during the growing season, and the direction of orientation is determined by regional factors such as latitude, and topography. Drezner (2020) suggested that plant response to PAR reception is strongly controlled by local-scale factors. Our finding in this study, showed that for all the studied *Opuntia spp*. varieties, the greater root number, root length, root dry weight, percentage of secondary cladodes initiated per cutting, cladode surface, number of areoles per cladode, and number of areoles per plants were obtained with the basal (N-S) cuttings. Stambouli-Essassi *et al*. (2015) have conducted some studies on evaluation of the efficacy of *Opuntia ficus-indica* cladodes cuttings for vegetative propagation and found that the number of roots was the highest on right tenth basal cuttings; the percentage of secondary cladodes initiated was the highest on right apical cuttings (74%), and the

longest roots were initiated on both left and right tenth basal cuttings (13-14 cm). Exposure to sunlight during planting did not significantly affect the growth rate of *O. ficus-indica* plants (Homrani *et al*., 2016).

According to Nobel (1982a) and Drezner (2020), orientation is not a mechanism to reduce high surface temperatures, but to maximize the reception of PAR. Photosynthesis is inhibited by low temperatures, as has been documented for *Opuntia stricta* (Barker *et al*., 1998). *Opuntia* cactus plants oriented to receive a higher PAR have a greater accumulation of dry matter in autumn (Rodriguez *et al*., 1976; Nobel, 1981). Nobel (1982b) suggested that each successive cladode adjusts its orientation to the direction for maximum PAR reception, thus exhibiting a phototropic response, which is very local. In our case, the cladode type of Karama, Melk Zhar and Cherratia varieties, had no significant effect ($p > 0.05$) on the percentage of secondary cladodes developed. A similar observation was reported by Gibson and Nobel (1986) in some *Opuntia* cactus species. Newly initiated cladodes result from the differentiation of areolar meristems from cladodes called specialized buds (Gibson and Nobel, 1986; Bowers, 1996). These areoles are homologs of the axillary buds of Dicotyledons (Boke, 1944) which have a dormant vegetative point protected by bristles or trichomes (Feugang *et al*., 2006). The appearance of new secondary cladodes is mainly localized in the apical zone (crown top) of all cuttings and less frequently on the faces of the cuttings. The initiation of adventitious roots from areolar meristems, preferably located in the basal cuttings, could be correlated with the availability of high levels of auxins concentrated in the basal zone of the cladodes. The transport of polar basipetal auxins in higher plants is a controlled and directional process. In stems, auxin is transported and moves from the shoot apex to the base (Lomax *et al*., 1995; Bohn-Courseau, 2010). Size and conditions of the cladodes did not affect the vegetative growth of the *Opuntia boldinghii* cactus (Padrón Pereira, 2013).

Based on the collected data and observations made on a number of cladodes, regarding growth of plants, phases of the cycle where the plant population expresses signs of dysfunction, and areolar complex, our study showed that the polyvalent meristematic axillary complexes are able to differentiate themselves in any specialized structure (root or secondary cladode) according to their topographical position on the cladode in relation to internal morphogenetic correlations. These observations are in line with those mentioned by Nobel (1994) and Stambouli-Essassi *et al*. (2015). In general, cuttings planted in normal polarity and respecting their position on the mother plant gave more interesting results than those obtained with cuttings planted in horizontal polarity. For whole cladode planting, Homrani et al. (2016) demonstrated that horizontally planted cladodes showed high productivity, high photosynthetic area and the best rooting compared to the vertically planted ones. The basic meristematic units in Opuntia are areoles (Gibson and Nobel, 1986). They are helically positioned on cladodes, and can develop either in branches, flowers, or roots (Boke, 1980; Sudzuki, 1995; Bowers, 1996). It is also noted that cladodes can begin the rooting process few days (2-3 days) after planting for some varieties (e.g. Aakria variety). Rooting of cuttings can be induced at any time of the year, in contrast to the initiation of axillary cladodes, which was more important in the spring, mainly from the areoles in the apical zone (Stambouli-Essassi *et al*. 2015). According to Nobel (1982a, b), the initiation of secondary cladodes can occur at any time of the year, or in two periods, September-November and February-April (Escobar *et al*., 1986).

Conclusion

Over the years, farmers and agricultural societies in North Africa and especially Morocco have developed methods of asexual propagation of *Opuntia ficus-indica* using cladodes. The obtained data indicate that the eight *Opuntia* cactus varieties investigated in this study show remarkable differences in terms of rooting, growth, and interaction with the application or not of exogenous auxin. Each variety had superiority to the others in different aspects. Ghalia and Cherratia varieties had the highest values in term of roots number, roots length, and roots dry weight than the others. Aakria variety had the highest value of % secondary cladodes initiated per cutting. Ghalia, Melk Zhar, Cherratia and Angad varieties had the highest values of duration for first budding. Angad, Ghalia and Belara varieties had the highest value of cladode surface, and Belara, Karama, and Ghalia had the highest values of number of areoles per cladode and number of areoles per plants than the other tested varieties. In general, Auxin and mix 1 treatments were found to be the most effective regarding rooting and growth of cuttings, while Angad and Melk Zhar varieties cuttings treated with talc powder had the highest root length values than those treated with mix 1 treatment. It can therefore be concluded from the present study that the treatment of cuttings with Auxin (2.5 g/l) has a positive effect on rooting and growth capacity for all tested varieties. This research has also allowed to demonstrate that, for all studied varieties, basal cuttings planted in orientation (North-South) and normal polarity show very interesting results concerning rhizogenesis and caulogenesis.

Further researches are needed to achieve high rooting and development potential of *Opuntia* cactus cuttings and to overcome their genotype dependency by adjusting rooting and development conditions, mainly the optimization of auxin concentration and its application method and the effect of environmental factors, especially temperature, as we noticed during our field work that *Opuntia* cactus is very sensitive to changes in environmental factors, especially temperature.

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