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Effect of Auxin and Wounding on Adventitious Root Formation of Prickly-pear Cactus Cladodes

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SUMMARY. Mature cladodes of prickly-pear cactus (*Opuntia amyclaea* Tenore, 'Reina') were treated with five wounding methods and four concentrations of potassium salt indole-3-butyric acid (K-IBA) to stimulate adventitious root formation. The wounding method and

K-IBA had highly significant effects on root number and root dry mass of cladodes. Interaction between K-IBA and wounding methods showed that greater root number was obtained at the higher auxin concentrations and with wounding methods that had the greatest cut surface area. K-IBA concentrations from 4,144 to 41,442 μM (1,000 to 10,000 $\text{mg}\cdot\text{L}^{-1}$) increased root dry mass. Only the wounding method affected rotting of cladodes. Treatments allowing suberization had a higher percentage of nonrotted cladodes. This research validates the commercial practice of allowing cladodes to suberize early in the propagation cycle. K-IBA altered rooting polarity and stimulated adventitious root formation along the wounded cladode surfaces. The vertical nonsuberized wounding methods and auxin treatments are an excellent classroom demonstration for manipulating rooting polarity. Auxin application and wounding could be of commercial benefit for enhanced rooting in the clonal regeneration of new selections for prickly-pear cactus orchards.

Prickly-pear cactus (*Opuntia amyclaea* Tenore) is native to the American hemisphere and is cultivated in other areas of the world, including Brazil (300,000 ha), Italy (100,000 ha), and Chile (50,000 ha) [Bravo, 1978; Pimienta, 1994]. Prickly-pear cactus is primarily cultivated for its fruit production, which includes a wide variety of fruit flavors and colors (Pimienta, 1994). Young cladodes are also used as spring vegetables or consumed as relish, while older cladodes are used for animal consumption. Due to its low water requirements and high adaptability, prickly-pear cactus has become important as an alternative fruit and vegetable crop for other

world regions (Pimienta, 1990). Valuable byproducts include wine, candies, and the pigment, acetocarmine (Barbera, 1995).

Prickly-pear cactus can be propagated from seed, cladodes and other asexual methods such as grafting and tissue culture (Estrada-Luna et al., 1994). Whole cladodes are most commonly used for orchard establishment. However, other alternative methods include the division of the cladodes into small sections, which increase propagule numbers and reduces production costs (Barrientos and Brauer, 1964). Suberization of the wounded area where the cladodes are detached from the stock plants is a common practice to prevent fungal infections.

There is an increased need to integrate studies on prickly-pear cactus growth systems and improve the efficiency of the propagation process (Fabbri et al., 1996). IBA has been reported to produce more roots on *O. ficus-indica* (L.) Mill. (the Indian fig or tuna) under in vitro conditions (Escobar et al., 1986; Mohamed-Yasseen et al., 1995). Naphthalene acetic acid (NAA) has been reported to increase rooting of young shoots and increase cladode weight of *O. ficus-indica* (Mulas et al., 1992). Though not commercially done, treatment with auxins may be an advantage for enhancing rooting of prickly-pear cladodes over a shorter propagation period to improve field establishment.

The objective of this current study was to evaluate the effect of auxin, different wounding methods and suberization on the rooting of cladodes of prickly-pear cactus. These findings are important for developing techniques that enhance propagation efficiency and lead to faster orchard establishment. Because asexual propagation practices vary widely among growers, traditional and nontraditional methods of wounding were analyzed.

Materials and methods

Mature cladodes of 'Reina' prickly-pear cactus were obtained from commercial stock plants in the state of Guanajuato, Mexico, and used in a factorial experiment to evaluate four concentrations of indole-3-butyric acid potassium salt ($\text{C}_{12}\text{H}_{12}\text{NO}_2\text{K}$, Sigma Chemical Co., St. Louis, Mo.) K-IBA [0; 4,144; 12,433 and 41,442 μM (0; 1,000; 3,000; 10,000 $\text{mg}\cdot\text{L}^{-1}$)] and five wounding methods. The cut surfaces of the cladodes were either presuberized or nonsuberized, and then dipped for 5 s in an aqueous solution of K-IBA concentrations. The wounding methods

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Fig. 1. Wounding techniques for prickly-pear cactus (*Opuntia amyrlaea* 'Reina'). HN = horizontal cut, nonsuberized, in which a horizontal cut was made through the middle of the cladode; VN = vertical cut, nonsuberized, in which a vertical cut was made through the middle of the cladode; HS = horizontal cut, suberized, in which a horizontal cut was made and the wounded surfaces of the cladodes allowed to suberize for 72 h before planting; BS = basal cut, suberized, made by using cladodes that had 2 cm removed from their basal portions, then suberized for 72 h before inserting into the propagation medium; and PS = presuberized, in which the cladode was left in its original suberized shipped condition.

included 1) horizontal cut, nonsuberized (HN), in which a horizontal cut was made through the middle of the cladode, followed immediately by basal treatment of the apical portion with K-IBA, and planting in the propagation medium; 2) vertical cut, nonsuberized (VN), in which a vertical cut was made through the middle of the cladode, with each half subsequently treated with the K-IBA and inserted into the propagation medium; 3) horizontal cut, suberized (HS), in which a horizontal cut was made, the cladode dipped in K-IBA and the cut surfaces of the cladodes allowed to suberize for 72 h at a maximum/minimum temperature of 32/25 °C

before planting; 4) basal cut, suberized (BS), made by using cladodes that had 2 cm removed from their basal portions, dipped in K-IBA and suberized for 72 h before inserting into the propagation medium; and 5) presuberized (PS), in which the cladode was left in its original suberized shipped condition, treated with the K-IBA and immediately planted (Fig. 1). All the cladodes were stuck 4.5 cm into the rooting media after K-IBA application.

Plastic trays [24.35 × 15.6 × 8.13 cm] Jiffy brand, Anderson Brothers, San Antonio, Texas] containing a steam pasteurized mixture of 3 sphagnum peat : 1 coarse horticultural grade perlite (by volume) were used. Cladodes were planted 20 Oct. 1994 and cultured for 34 d (24 Nov. 1994) to allow sufficient root development under glasshouse conditions. Maximum irradiance was 660 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with minimum and maximum temperatures of 20/24 ± 2 °C, and relative humidity of 75/95% ± 4 %, respectively. Trays were overhead irrigated with reverse osmosis (RO) treated water every fifth day. Cladodes were drenched every 10 d with a Benomyl-Captan fungicide mixture (1:1 ratio; 1 g a.i./L).

At the termination of the experiment, root number, root dry weight and percentage of nonrotted cladodes were assessed. The 4 × 5 factorial experiment was distributed as a completely randomized block design, which included four concentrations of K-IBA and five wounding techniques. There were 15 replications per treatment (n = 15). Data collected throughout the study were subjected to analysis of variance (ANOVA), least square means obtained for the interaction, and regression analysis for root number versus K-IBA concentrations (SAS Institute Inc., 1995).

Results and discussion

The only interaction between wounding and K-IBA occurred with root number (Table 1). Wounding method and K-IBA had highly significant effects on root number and dry mass. Only the wounding method affected percentage of rotted cladodes.

ROOT NUMBER. Least square means for root number were obtained for the interaction wounding method and K-IBA concentrations (Table 2). In general, root number was higher in the treatments when K-IBA concentrations were linearly increased with wounding methods that had the greatest cut surface area. The promoting effect on adventitious root formation with K-IBA using concentrations higher than 4,144 μM was evident along the wound area exposed to the hormone. Mulas et al. (1992) reported enhanced rooting with auxin, but reported no rooting differences when cuttings were basally soaked for 4 h with 100 and 200 $\text{mg}\cdot\text{L}^{-1}$ IBA. Results from our study show that *O. amyrlaea* 'Reina' cladodes dipped with 4,144 μM K-IBA and higher concentrations can produce similar root number in 34 d compared with the four month propagation period used with 4-h basal soaked cuttings (Mulas et al., 1992).

Despite the positive effect of the auxin on root number, root length (visual observation) was reduced with increasing concentrations of K-IBA, particularly at 41,442 μM (Fig. 2a and b). K-IBA at 4,144 and 12,433 μM promoted a more balanced root system in terms of root length and development (visual observation) (Fig. 2a and b).

The best fit regression equation was obtained for K-IBA concentration versus root number. The equation ob-

Table 1. Analysis of variance for effects of wounding method and potassium salt indole-3-butyric acid (K-IBA) concentrations on root number, root dry mass, and percentage of nonrotted cladodes of prickly-pear cactus (*Opuntia amyrlaea* 'Reina').

Source of variation	df	Mean square		
		Roots (no.) ^z	Root dry mass (g) ^z	Nonrotted cladodes (%) ^y
Wounding method	4	5230.06***	0.239***	3733.33***
K-IBA	3	40682.57***	0.137***	524.44 ^{NS}
Wounding × K-IBA	12	1609.54***	0.010 ^{NS}	457.77 ^{NS}
Error	224	215.16	0.007	282.05
R ²		0.78	0.48	0.67

^zData analyzed excluded rotted cladodes.

^yData analyzed with rotted cladodes.

^{NS},***Nonsignificant and significant at $P = 0.0001$.

Table 2. Least square means for the interaction of wounding method $\times$ potassium salt indole-3-butyric acid (K-IBA) concentrations on the root number per cladode of prickly-pear cactus (*Opuntia amyrlaea* 'Reina').

Wounding method ^a	K-IBA (μM)	LS mean ^b	Treatment no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
BS	0	7.6	1	+	*	***	***	NS	**	***	***	NS	**	***	***	NS	NS	***	***	NS	***	***	***
BS	4,144	20.3	2	*	+	***	***	*	NS	***	***	*	NS	***	***	*	*	NS	**	**	**	***	***
BS	12,433	43.8	3	***	***	+	***	***	***	**	***	***	***	**	***	***	***	***	NS	***	ns	ns	ns
BS	41,442	67.0	4	***	***	***	+	***	***	NS	***	***	***	NS	***	***	***	***	***	***	***	*	*
HS	0	7.7	5	NS	*	***	***	+	**	***	***	NS	**	***	***	NS	NS	*	***	NS	***	***	***
HS	4,144	23.8	6	**	NS	***	***	**	+	***	***	**	NS	***	***	**	*	NS	**	***	*	***	***
HS	12,433	69.0	7	***	***	**	NS	***	***	+	*	***	***	NS	***	***	***	***	***	***	***	**	*
HS	41,442	81.0	8	***	***	***	***	***	***	*	+	***	***	***	NS	***	***	***	***	***	***	***	***
HN	0	4.5	9	NS	*	***	***	NS	**	***	***	+	**	***	***	NS	NS	*	***	NS	***	***	***
HN	4,144	27.1	10	**	NS	***	***	**	NS	***	***	**	+	***	***	**	*	NS	***	***	*	***	***
HN	12,433	58.6	11	***	***	**	NS	***	***	***	**	***	***	+	***	***	***	***	*	***	**	NS	NS
HN	41,442	87.0	12	***	***	***	***	***	***	***	NS	***	***	***	+	***	***	***	***	***	***	***	***
PS	0	8.3	13	NS	*	***	***	NS	**	***	***	NS	**	***	***	+	NS	*	***	NS	***	***	***
PS	4,144	12.3	14	NS	*	***	***	NS	*	***	***	NS	*	***	***	NS	+	*	***	*	***	***	***
PS	12,433	21.0	15	***	NS	***	***	*	NS	***	***	*	NS	***	***	*	*	+	***	*	***	***	***
PS	41,442	41.0	16	***	**	NS	***	***	***	***	***	***	***	*	***	***	***	***	+	***	NS	NS	*
VN	0	1.6	17	NS	**	***	***	NS	***	***	***	NS	***	***	***	NS	*	**	***	+	***	***	***
VN	4,144	39.0	18	***	**	NS	***	***	*	***	***	***	*	***	***	***	***	**	NS	***	+	*	*
VN	12,433	50.6	19	***	***	NS	*	***	***	**	***	***	***	NS	***	***	***	***	NS	***	*	+	NS
VN	41,442	53.8	20	***	***	NS	*	***	***	*	***	***	***	NS	***	***	***	***	*	***	*	NS	+

^aBS = basal suberized; HS = horizontal suberized; HN = horizontal nonsuberized; PS = presuberized and VN = vertical nonsuberized wounding methods.

^bData analyzed excluding rotted cladodes.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

Fig. 2. Effect of potassium salt indole-3-butyric acid (K-IBA) and wounding techniques on rooting of prickly-pear cactus (*Opuntia amyrlaea* 'Reina') cladodes. Quick-dips of K-IBA were applied at 0, 4144, 12,433, and 41,442 μ M (0, 1,000, 3,000, and 10,000 mg·L⁻¹). See Fig. 1 and the materials and methods for an explanation of the wounding treatments.

tained was highly significant at $P > 0.001$ ($y = 0.0052X + 17.791$) (Fig. 3).

K-IBA was highly significant for root number and root mass (Table 1). Regression for K-IBA concentrations was not a good predictor ($R^2 = 0.479$)

of root number per cladode, thus surface application area must be considered. These results suggest that when using a specific wounding method, root number will increase linearly and could be predicted.

ROOT DRY MASS. Data were analyzed excluding the rotted cladodes to determine root dry mass. Vertical nonsuberized, horizontal suberized and horizontal nonsuberized wounding methods were significantly lower in root dry mass compared to basal suberized and the control presuberized wounding method (Table 3). The K-IBA control treatment of 0 μ M was significantly low-

est in comparison with 4,144, 12,433, and 41,442 μ M, respectively (Table 3). These results showed that the combination of basal suberized or presuberized cladodes dipped into 4,144 or 12,433 μ M give the best rooting quality in terms of root length and number (Fig. 2).

PERCENTAGE OF NONROTTED CLADODES. Basal suberized, presuberized, and horizontal suberized wounding methods were significantly higher in the percentage of nonrotted cladodes (Table 3). These results suggest that suberization for at least three days before planting reduces black rot infection, and validates commercial suberization practices. K-IBA had no effect on the per-

Fig. 3. Slope of the linear regression between potassium salt indole-3-butyric acid (K-IBA) concentration and root number per cladode of prickly-pear cactus. Data collected across wounding methods.

centage of nonrotted cladodes (Table 3).

POLARITY. The initiation of root formation in prickly pear cactus after wounding was polar as shown in the control treatment (Fig. 2e), which may be attributed to endogenous levels of auxins at the base of the cladode (Hartmann et al., 1997). The application of exogenous K-IBA reduced the polarity at the proximal end and the rooting process occurred along larger areas of the wounded surfaces in vertically wounded and nonsuberized (VN) cladodes (Fig. 2e).

Conclusions

Our findings show a positive effect of K-IBA at higher rates (4,144 to 41,442 μM) and wounding methods on the root number and root dry mass of prickly-pear cactus cladodes. We conclude that increasing K-IBA concentrations linearly increased root number in all wounding methods. Root length decreased when increasing K-IBA concentration. Treatments with 4,144, and 12,433 μM of K-IBA in basal suberized and presuberized wounding methods had the best root system in terms of root number and length (Fig. 2a and b). Basal suberized and presuberized treatments had the higher root dry mass and

K-IBA application increased root dry mass per cladode of prickly pear cactus.

Suberization for at least 3 d increased the percentage of nonrotted cladodes. Increasing K-IBA concentrations with the vertical nonsuberized wounding method, make an excellent classroom demonstration for manipulating rooting polarity. Treatment with 4,144 μM K-IBA and wounding at the basal surface followed by suberization are alternative techniques for enhancing root system development that should be commercially tested for the establishment of prickly pear cactus orchards.

Table 3. Effect of wounding method and potassium salt indole-3-butyric acid (K-IBA) concentrations on root dry weight and percentage of rotted cladodes of prickly-pear cactus (*Opuntia amyoclaea* 'Reina').

Wounding method ^y	Observations ^z (no.)	Root dry mass (g) ^x	Nonrotted cladodes (%) ^w
BS	60	0.275 a ^v	100 a
PS	60	0.240 a	100 a
HN	40	0.145 b	67 b
HS	52	0.143 b	87 a
VN	38	0.123 b	63 b
K-IBA concn (μM) ^u			
0	57	0.128 b	76 ^{NS}
4,144	61	0.198 a	81 ^{NS}
12,433	65	0.210 a	87 ^{NS}
41,442	67	0.235 a	89 ^{NS}

^xData analyzed excluded rotted cladodes.

^yData for wounding method is presented as the average across all K-IBA concentrations.

^wData analyzed with rotted cladodes.

^vMean separation within columns followed by the same letter are not significantly different according to Tukey's standardized range test at $P = 0.05$.

^uData for K-IBA concentrations is presented as the average across all wounding methods.

^{NS}Nonsignificant.

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