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Article

pH, dilution and alcohol concentr ations of IBA in *Ficus carica* rooting

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Abstract

Vegetative propagation with stem cuttings requires the origination of shoots and roots to form a complete plant. Factors involved in root generation have been researched, but little work has been done on pH. The research was conducted at the College of Postgraduates, Montecillo Campus, Texcoco, Mexico in 2021. In *Ficus carica* cuttings, the following were evaluated: pH values (4, 7, and 11) in solution with or without auxin, dilution (alcohol or NaOH) of indole butyric acid (IBA), concentration of alcohol to dissolve IBA (10, 15, and 20%), in the percentage of rooting, number of roots, length of the longest root, percentage of sprouted buds at 21 days, date of establishment and anatomical changes at 0, 3 and 6 days after the cutting. Meristematic cells and root primordia were found up to day 6. One hundred percent rooting was obtained in all treatments. pH was not shown to have an effect on the auxin solution, but it did influence the percentage of rooting assessed individually. The way auxin was diluted influenced the number of roots due to the concentration of alcohol used, lower concentration (10%) had a positive effect.

Keywords:

adventitious rooting, ethanol concentrations, H^+.

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Introduction

Cutting uses parts of a plant's stem, leaf, or root to multiply it, thus relying on the formation and development of adventitious roots and new shoots (Hartmann *et al*., 2014). Adventitious root formation (ARF) includes three phases: induction, initiation, and differentiation. The first deals with molecular and biochemical events; the second (visible phase), cell divisions that will give rise to root primordia and the last, organized growth of root primordia (Guan *et al*., 2015). It is a physiological process influenced by endogenous and exogenous factors that need to be understood (Druege *et al*., 2019).

Auxins are the plant growth regulators most closely related to adventitious rooting. Their accumulation at the base of the cuttings after their cut is important for root induction as it makes this place carbohydrate-demanding (Agulló-Antón *et al*., 2011). On the other hand, the application of auxins in the cutting accelerates divisions in the vascular cambium and increases sucrolytic activity, thus favoring ARF (Ahkami *et al*., 2009; Agulló-Antón *et al*., 2014).

The application of auxins to promote rooting is done in two ways, powder or liquid solution (Hartmann *et al*., 2014); nevertheless, when applied in solution, the following is not considered: 1) the pH of the final solution; 2) the solvent of the auxin (alcohol, water or NaOH); and 3) if alcohol is used as a solvent, the concentration of alcohol in the solution. pH should be considered in research addressing chemical compounds to promote ARF (Jin-Hao *et al*., 1993) as it is important in: cell growth and elongation by acidifying the apoplast, softening the rigid structure that is the cell wall.

In addition, it also influences the formation of ATP through the driving force of the proton, the functionality of proteins, and as a cellular messenger (Rengel, 2002). A slightly acidic pH has been reported to increase rooting, sometimes independently of auxin application, perhaps because it promotes auxin transport (Khosh-Khui and Tafazoli, 1979; Jin-Hao *et al*., 1993). Acidic pH in rooting substrate also favors ARF (Abdulkadir and Muharrem, 2018).

In relation to the type of solvent, alcohol, or sodium hydroxide (NaOH), no differences have been found (Oliveira *et al*., 2009; Pereira *et al*., 2021); however, some authors point out that low alcohol concentrations have a positive effect on rooting (Middleton *et al*., 1978; Bhattacharya *et al*., 1985). Therefore, it is necessary to find appropriate concentrations of the auxin solvent to promote ARF.

In various studies of *Ficus carica* related to rooting of cuttings, four aspects can be noted, 1) the pH of the auxin solution or its individual effect has not been studied; 2) the media (NaOH or ethanol) and concentrations for dissolving auxin (ethanol) have not been evaluated; 3) there is a lack of anatomical studies that evaluate the changes in the tissue during the rooting process; and 4) most do not have efficient propagation protocols as they do not reach 100% rooting (Hiral *et al*., 2017; Aghera and Makwana, 2018; Kaur *et al*., 2018).

Therefore, the present research aimed to evaluate the effect of the pH of the auxin solution, the form of dilution of auxin, the pH as an independent factor at the time of rooting and the concentrations of alcohol used to dilute auxin, in addition to obtaining an anatomical sequencing of the three rooting phases and an efficient protocol for propagating figs.

Materials and methods

The research was carried out in the forest nursery of the Postgraduate Degree in Forestry Sciences, Montecillo *Campus*, College of Postgraduates, Texcoco de Mora, State of Mexico (19° 27' 38.182" north latitude, 98° 54' 23.898" west longitude and at an altitude of 2 250 m). The plant material of *Ficus carica* var. 'Nezahualcóyotl' was obtained from plants in intensive production of the 'Víctor Manuel Mendoza Castillo' protected area, of the Experimental Field of the Department of Phytotechnics, Chapingo Autonomous University (19° 29' 29.531" north latitude, 98° 52' 25.041" west longitude).

The collection took place between May and August 2021. Semi-woody cuttings of a month and a half of development, 18 to 23 cm in length, 12 to 14 mm in basal diameter and with 4 to 5 buds were cut from lateral branches of eight-year-old plants that are guided to six stems and pruned to

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the main trunk in August of each year. The cuttings were washed and left to dry for 30 min. They were then immersed for 15 min in a metalaxyl (Ridomil Gold®) solution, 5 g L^{-1} of water.

Treatments

Auxin dilution medium and pH of auxin solution. Three treatments were applied with different pH (4, 7, and 11), with 2 000 mg of IBA (indole-3-butyric acid, Sigma I-5386) L^{-1} dissolved in alcohol (acidic and neutral pH) and sodium hydroxide (alkaline pH). With alcohol, 0.2 g of IBA was dissolved in 15 ml of alcohol and then 85 ml of distilled water was added.

Because the pH fluctuated between 3.43 and 3.46, it was adjusted to pH of 4 and 7 with 1 N NaOH. With NaOH, 0.2 g of IBA was dissolved in 10-15 drops of 1 N NaOH and immediately 100 ml of distilled water was added at 60-65 °C, the pH was adjusted to 11 with 1 N NaOH. The length of the cuttings was adjusted to 15-20 cm and at their base, four vertical wounds about 2 cm long and 3 mm deep were made with a penknife. The experiment was replicated four independent times on the dates: May 21 to June 11 (E1), June 7 to June 29 (E2), June 30 to July 22 (E3), and July 29 to August 19 (E4).

Along with E3, two independent experiments were also conducted. In these additional evaluations, the number of buds per cutting ranged from 3 to 5. pH levels without auxin application. The pH of the solutions was modified with 15% alcohol (pH 4 and 7). For pH 4, after mixing the 15 ml of alcohol with the 85 ml of water, drops of HCl prepared at 1 N were applied.

With respect to pH 7 and pH 11, drops of 1 N NaOH were also applied. Alcohol concentration. The described procedure was used to dilute auxin in 15% of alcohol, but also in 10% and 20%, as the pH was close to 4, it was modified to 4 by applying drops of 1 N NaOH.

The basal 4-6 cm of the cuttings were then immersed for 5 s in the respective solutions. In experiments with pH levels without IBA and alcohol concentrations, the number of buds per cutting ranged from 3 to 5. Finally, the cuttings were placed in plastic boxes, 30 cm high x 50 cm long x 35 cm wide, with a wet mixture of peat and agrolita (expanded perlite) (1:1, v/v).

The relative humidity was 100% and the minimum, average and maximum temperatures in E1 and E2 were 11.3, 21.2 and 26.4 °C and 12.5, 18.7 and 22.9 °C. Due to the decrease in ambient temperature during experiments E3 and E4, which is not recommended for rooting, an oil heater with a thermostat was used, which turned on from 7 pm to 9 am, and the temperatures were 17.4, 23.3 and 36.4 °C.

Variables evaluated

Anatomical changes

Three cuttings were randomly selected at 0, 3 and 6 days after treatment with pH 4 (time in which induction occurs), this pH was chosen because no differences in rooting time were observed between treatments. The base of the cutting (2 cm) was cut and placed in formaldehyde fixative solution: acetic acid: 96% ethanol: water (10:5:50:35 v:v), for 24 h in a vacuum hood. Dehydration lasted 8 h, in ethanol solutions of 50, 60, 70, 80, 90 to 100% (2 times); ethanol: xylene (3:1) three times and xylene 100% (twice).

Samples were soaked in Paraplast®, 10 µm thick cross-sections were made with an 820 rotary microtome (American Optical USA®), they were placed on slides removing excess paraffin, and stained with safranin-fast green (0.05% safranin, 2% NaCl in 50% ethanol and 0.12% fast green in 95% ethanol). Groups of meristematic cells, their tissue of origin and root primordia with a defined shape and meristematic apex were identified.

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Rooting variables

The following was characterized: rooting percentage, as a result of the number of rooted cuttings divided by the number of cuttings placed by 100, number of roots per cutting, length of the largest root, percentage of sprouted buds per cutting (not including the base bud).

Experimental design and statistical analysis

The experimental design used for E1, E2, E3, E4, pH without IBA and ethanol concentrations was completely randomized with three treatments and with 10, 19, 19, 10, 9 and 7 repetitions per treatment, respectively. The experimental unit in all experiments was a cutting and the source of variation, in E1-E4, 2 pH levels (4 and 7), two ways to dissolve IBA (NaOH and alcohol), in rooting without IBA, 3 pH levels (4, 7, and 11), and in alcohol concentrations, 3 alcohol concentrations (10, 15 and 20%). The results of the treatments were analyzed independently of the other experiments using Anova, Tukey's mean test ($p< 0.05$) and for variables with non-homogeneous variances, the Kruskal-Wallis test was performed with the SAS statistical package (Statistical Analysis System 9.4).

Results and discussion

Anatomical changes during rooting of *F. carica* cuttings

The cuttings showed anatomy similar to that of a dicotyledon with secondary growth. From the periphery to the center of the stem, the following were identified: epidermis (Ep), cortical parenchyma (CP), phloem fibers (PF), phloem (Ph), vascular cambium (VC) and xylem (Xy). On days 0 and 3, no cell divisions were observed. The first meristematic cells and root primordia (with well-defined apical meristem) appeared until the sixth day in the phloem cells adjacent to the cambium (boxes c and d of the Figure 1).

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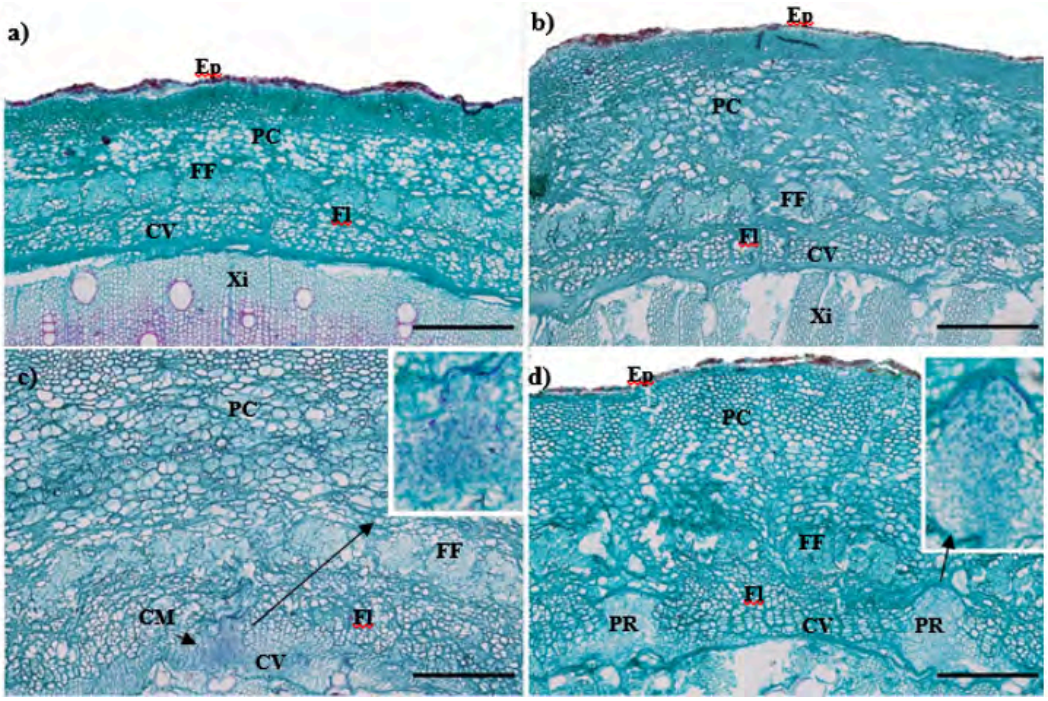
Figure 1. Cross-sec on of fig (Ficus carica) cu ngs treated with 2 000 mg L $^{-1}$ IBA at 15% alcohol and pH 4. a) day 0 (at the me of obtaining the cu ng); b) day 3; c and d) day 6 where epidermis (Ep), cor cal parenchyma (PC), phloem fibers (FF), phloem (Fl), vascular cambium (CV) and xylem (Xi) are observed. Boxes within the images show close-up of meristematic cells and root primordia. Cut made 0.5-1.5 cm above the basal cut site. Scale bars = 300 µm.



Also on the sixth day, the images showed clusters of shapeless, dividing meristematic cells, box (Figure 1c), and primordia with a typical root-shaped, box (Figure 1d), with visible nuclei. This differs from those reported in *Carnation* and *Rosa* as small groups of meristematic cells or divisions near the vascular cambium or in the vascular cambium occurred from the second and third day (Costa *et al*., 2003; Agulló-Antón *et al*., 2011). On the contrary, the results coincide with what was observed in Petunia, where the first root primordia were visualized from day 6 (Ahkami *et al*., 2009).

The above shows that the time of induction, initiation, and differentiation of roots depends on the species, so it is important to determine them in each one to define the time in which the cuttings should be 'harvested', to avoid too long roots and difficulties in transplanting. In this study, cuttings were 'harvested' at 21 days, they had 69 roots, an average length of the longest root of 5.24 cm, and a survival and rooting percentage of 100%, considered suitable for transplanting, while Sivaji *et al*. (2014); Aghera and Makwana (2018); Kaur *et al*. (2018) transplanted at 90 days and Becker *et al*. (2010) at 60 days with longer root lengths, but lower rooting percentage and number of roots. This shows that the protocol used in this research is more efficient.

pH of auxin solution and dilution medium

Except for the pH 4 treatment in E2, where 73% rooting was obtained, in the rest of the experiments and treatments the result was 100% (Table 1).

Table 1. Percentage of *F. carica* rooting by applying 2 000 mg L $^{-1}$ of IBA in aqueous solution with different pH on four establishment dates.

Treatments	E1- May 21 (%)	E2- June 7 (%)	E3- June 30 (%)	E4- July 29 (%)
pH 4 (ethanol)	100 ^{NS}	73 ^{S}	100 ^{NS}	100 ^{NS}
pH 7 (ethanol)	100	100	100	100

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Treatments	E1- May 21 (%)	E2- June 7 (%)	E3- June 30 (%)	E4- July 29 (%)
pH 11 (NaOH)	100	100	100	100

E1= experiment 1; E2= experiment 2; E3= experiment 3; E4= experiment 4. Non-homogeneous variances. ^{NS}= not significant; ^{S}= significant; for medians compared using the Kruskal-Wallis test, $p \leq 0.05$.

Regarding the number of roots (Table 2), in E1 and E3 there was no statistically significant difference between the three pHs. In contrast, in E2 the neutral and alkaline pH statistically exceeded the acidic pH; on the other hand, in E4 the best treatment was the alkaline pH followed by the acidic pH.

Table 2. Average number of roots per *F. carica* cutting by pH treatment on four establishment dates.

	E1- May 21	E2- June 7	E3- June 30	E4- July 29
	E1- May 21	E2- June 7	E3- June 30	E4- July 29
pH 4	76.8 ±12.1 a	12 ±2.9 b	56.1 ±17.7 a	108.6 ±18.2 ab
pH 7	79.3 ±7.9 a	44.1 ±12.3 a	59.2 ±20.8 a	101.2 ±11.1 b
pH 11	68.1 ±14.6 a	36.9 ±14.1 a	60.1 ±12.1 a	126.2 ±21 a
HLSD	16.2	13.8	15.3	21.8
Average	74.7 B	31 D	58.5 C	112 A

Different lowercase letters in a column indicate statistically significant differences. E1= experiment 1; E2= experiment 2; E3= experiment 3; E4= experiment 4. Non-homogeneous variances. ±= standard error; Tukey, $p \leq 0.05$. HLSD= honest least significant difference.

The differences in the average length of the longest root per cutting were not significant between the three pH evaluated in E1, E3 and E4; in contrast, in E2 the best treatment was the one that included IBA diluted with ethanol and with pH adjusted to 7 with NaOH (Table 3).

Table 3. Average length (cm) of the longest root per *F. carica* cutting in the treatment of pH of the IBA solution on four establishment dates.

Treatments	E1- May 21	E2- June 7	E3- June 30	E4- July 29
T1- pH 4	5.4 ±0.7 a	1.5 ±0.4 b	3.4 ±0.9 a	7.9 ±0.6 a
T2- pH 7	7.3 ±1.2 a	3.7 ±0.9 a	3.5 ±1.1 a	8.7 ±1.5 a
T3- pH 11	5.4 ±1.9 a	2.5 ±1.1 b	3.9 ±1 a	9.3 ±2.4 a
HLSD	1.9	1.1	0.9	2.1
Average	6 ^{S}	2.2	3.5	8.5

Different letters in a column indicate statistically significant differences. E1= experiment 1; E2= experiment 2; E3= experiment 3; E4= experiment 4; ±= standard error, Tukey, $p \leq 0.05$. HLSD=honest least significant difference; ^{S}= significant, for medians compared using the Kruskal-Wallis test, $p \leq 0.05$.

The generation of shoots from the buds is important because they will generate the structure where the leaves will be housed so that the plant provides chemical energy and secondary metabolites necessary for the plant (Hartmann *et al*., 2014). All the cuttings evaluated emitted shoots, similar to what was documented by Barcelos *et al*. (2016) in the species. The differences in the percentage of sprouted buds per cutting, by treatment, were not significant (according to the Kruskal-Wallis test) between E1, E2 and E3; only E4 showed statistically significant differences between treatments (Table 4), where the best treatment was IBA at pH 7, then pH 11 and finally pH 4.

Table 4. Percentage of sprouted buds per *F. carica* cutting by treatment on four establishment dates.

Treatments	E1- May 21	E2- June 7	E3- June 30	E4- July 29
T1- pH 4	66.7 ^{NS}	83.3 ^{NS}	100 ^{NS}	50 ^{S}

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Treatments	E1- May 21	E2- June 7	E3- June 30	E4- July 29
T2- pH 7	66.7	66.7	100	100
T3- pH 11	66.7	87.5	100	83.3

E1= experiment 1; E2= experiment 2; E3= experiment 3; E4= experiment 4. Non-homogeneous variances. ^{NS}= not significant; ^{S}= significant; for medians compared using the Kruskal-Wallis test, $p \leq 0.05$.

Research on the species has not analyzed pH in the ARF (Hiral *et al*., 2017; Aghera and Makwana, 2018; Kaur *et al*., 2018), so it is difficult to compare the results obtained, therefore, we will compare with other species where the effect of pH has been evaluated. The results of rooting percentage in E1, E3 and E4 (Table 1), in E2 and E3 with number of roots (Table 2) and length of the largest root differ from those reported by Kumar *et al*. (2011) in *Gmelina arborea* since in this species the acidic pH (5.5) with IBA increased the rooting percentage compared to neutral or slightly alkaline pH (8.5).

On the other hand, they are similar in number of roots and length of the largest root since they also found no difference. The results obtained with *F. carica*, *G. arborea*, show that the response to the pH of the solution depends on the species and that these are factors that should be considered when doing research with rooting of cuttings to try to better understand the process, they also suggest that in addition to the pH, the cutting time is important.

The substrate probably plays a role in the response obtained since, although we modify the pH of the medium that transports auxin, it retains its pH. This has been proven in *Camellia sinensis* by Abdulkadir and Muharrem (2018), who found that slightly acidic pH in the substrate promotes a higher percentage of rooting.

Perhaps to inhibit the effect of the substrate, it would be necessary to use pH buffer solutions in the auxin solution, or to increase the exposure time of the auxin solution, similar to what was described by Khosh-Khui and Tafazoli (1979); Jin-Hao *et al*. (1993). Regarding the medium in which auxin was diluted, the results in E1, E3 and E4 are similar to those found in *Olea europaea* and *Varronia curassavica* as no differences were noted in the percentage of rooting and root length when diluting IBA with NaOH or ethanol (Oliveira *et al*., 2009; Pereira *et al*., 2021).

The causes of the lower rooting percentage of pH 4 in E2 have not been identified at present. The higher rooting in E2 and E4 with pH 11 does not agree with what was described by Pereira *et al*. (2021) in *Varronia curassavica* since IBA diluted with ethanol had a higher average number of roots compared to IBA diluted with NaOH. It does not coincide either with what was described by Jin-Hao *et al*. (1993) in *Helianthus annus*, who observed that acidic pH increased the number of adventitious roots and suggest that this is because they promote the movement of auxins to the formation zone.

Our results may be related to what was described by AL S'ady *et al*. (2018), who found that alkaline pHs alter the stability and activity of the peroxidase enzyme, which oxidizes IAA. Becker *et al*. (2010) showed that *F. carica* cuttings can have 100% rooting by applying 1 000 mg of IBA L^{-1} and making a wound at their base, but the percentage decreases to 94% with 2 000 mg of IBA L^{-1}. In other studies, rooting was less than 100% with 1 000, 2 000, 2 500, 3 000, and 3 500 mg of IBA L^{-1} (Lajús *et al*., 2007; Aghera and Makwana, 2018).

The results can be influenced by the substrate, moisture, and metabolite content in the cuttings. Nonetheless, Pipattanawong *et al*. (2008) reported that almost hermetic environments increase rooting percentages. This factor could explain the difference between the results of the present study and most of those documented in the literature in this species, thus being an option to consider for future research on rooting cuttings.

The number of roots counted in E1, E3 and E4 was higher than that reported for the varieties 'Poona' and 'Brown Turkey' at concentrations of 2 000 mg of IBA L^{-1} (Becker *et al*., 2010; Sivaji *et al*., 2014; Kaur *et al*., 2018) and also for concentrations of 800, 3 000 and 4 000 indicated as best for rooting *F. carica* (Araújo *et al*., 2006; Hiral *et al*., 2017; Kaur *et al*., 2018).

The results in E1 (Table 3), are similar to those indicated by Hiral *et al*. (2017) in two treatments, even though they performed the evaluation nine days later, as they documented that the average length of the longest root was 5.5 cm with 4 000 mg of IBA L^{-1}. The results in E1, E2, E3 and E4

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are higher than those reported by Becker *et al*. (2010), who evaluated at 60 days and reported an average length of 1 cm with 2 000 mg of IBA L^{-1}. The difference in the evaluation time of the studies makes it necessary to define it so that other studies can compare the results obtained.

The differences between the results of this study and those documented could be due to the physiological conditions of the cuttings and to the fact that in this study, they came from mother plants with management and cultivated in protected areas, characteristics pointed out by Saya *et al*. (2008) as adequate to increase rooting percentage and number of roots. This suggests that the time of rooting and management of mother plants is important for the success of *F. carica* propagation.

With the rooting characteristics and conditions of this study, the anatomical analysis indicated that root initiation occurred in the first six days, which suggests that: 1) future research evaluating rooting in *F. carica* should reduce the evaluation time; and 2) the transplantation of cuttings can be done in less time than documented in the literature (which can make us make mistakes) in order to provide space and proper handling for better and rapid development.

Regarding bud sprouting (Table 4), the results differed from those observed by Abdulkadir and Muharrem (2018) in *C. sinensis*, where IBA together with acidic pH in substrate led to higher percentages of sprouting compared to cuttings treated with slightly acidic and neutral pH.

The results of E4 are similar to those reported by Pereira *et al*. (2021) in *V. curassavica* and *M. alternifolia*, where cuttings treated with auxin diluted with NaOH presented higher percentages of sprouting compared to ethanol. Sprouting occurred one week after the experiments were established, and although it does not depend on the treatments, it is advisable that the cuttings sprout after the beginning of root differentiation, which, according to the observations in the anatomical sections, occurs before the sixth day.

Studies such as that of Martínez-Alcántara *et al*. (2015) mention that the growth of one organ requires metabolic energy and at the same time can limit the growth of another, this could explain the results obtained in E4, where the treatment with pH 7 with more sprouting was the one with the lowest number of roots. These results coincide with those reported by Souza *et al*. (1986) in woody cuttings in *F. carica*. Regarding the above, in general, E3, which obtained 100% sprouting, had a lower average number of roots (58.5) and a shorter average length of the longest root (3.63 cm) than E1 (74.7 and 6.07 cm) and E4 (112.04 and 8.68 cm) (Tables 2 and 3).

Rooting with different pH levels without auxin

pH affected rooting percentage; with pH 7, a higher rooting percentage was obtained; on the contrary, differences in the number of roots, longest root length, and sprouted buds per cutting were not significant (Table 5). Although there were differences in the rooting percentage, it is observed that these values are lower than those obtained when auxin is added (100%) (Table 1), the values of number and length of roots are also lower, 44 roots and 3.7 cm as maximum values in E2 (Tables 2 and 3), reaffirming the importance of this regulator in propagation by cutting.

Table 5. Rooting percentage (RP), number and length of roots (NR and RL), and percentage of sprouted buds (SB) in *F. carica* at the three pH levels without IBA. (June 7- June 29).

pH	RP (%)	NR	RL (cm)	SB (%)
pH	RP (%)	NR	RL (cm)	SB (%)
pH 4	55.5 ^{S}	9 a	1.2 a	75 ^{NS}
pH 7	77.7	4 a	1.7 a	66.6
pH 11	55.5	6 a	1.3 a	75

Different letters in a column indicate statistically significant differences. ±= standard error, Tukey, $p \leq 0.05$. Non-homogeneous variances. ^{NS}= not significant; ^{S}= significant, for medians compared using the Kruskal-Wallis test, $p \leq 0.05$.

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Since there is currently no information in *F. carica* on rooting cuttings at different pH with or without IBA, we will compare them with other species where pH without auxin has been used. This differs from what was reported by Kumar *et al*. (2011) in *Gmelina arborea* in percentage of rooting, since pH 5.5 generated higher rooting compared to 7 and 8.5, but it is similar in the number of roots since they did not find differences either. It also differs from what was observed in *Camellia sinensis*, where they obtained better results at pH of 5.5 (Lima *et al*., 2013).

Perhaps the difference between our results and those observed by Kumar *et al*. (2011) for *G. arborea* and by Lima *et al*. (2013) for *C. sinensis* is due to the fact that one of the pH concentrations evaluated was 5.5, which favors transport through the influx of the protonated form of IAA (Taiz and Zeiger, 2010).

Alcohol concentrations

Usually, the aqueous ethanol solution used to dilute the auxins that are applied to promote rooting is ethanol: water 1:1 (Pereira *et al*., 2021), perhaps taken from what is recommended by Hartmann *et al*. (2014). The only variable in which statistical differences were observed is root length (Table 6), where 10% exceeded the other concentrations, this value even exceeded the maximum value obtained 3.95 cm (Table 3), in the three pHs in E3, at 15% of ethanol (experiment that was carried out at the same time), which means that the induction, Initiation and differentiation of roots occurred first in this concentration.

Table 6. Rooting percentage (RP), number and length of roots (NR and RL), and percentage of sprouted buds (SB) in *F. carica* cuttings treated with 2 000 mg dissolved in three concentrations of ethanol (June 30- July 22).

Ethanol (%)	RP (%)	NR	RL (cm)	SB (%)
10	100 ^{NS}	74.5 ±37.9 a	5.94 ±2 a	81.5 ^{NS}
15	100	63.3 ±18.3 a	3.8 ±0.8 b	77.8
20	100	57.5 ±25 a	4.1 ±1.5 b	71.3
HLSD	NA	33.3	1.8	NA

Different letters in a column indicate statistically significant differences. ±= standard error, Tukey, $p \leq 0.05$. Non-homogeneous variances. ^{NS}= not significant; ^{S}= significant, for medians compared using the Kruskal-Wallis test, $p \leq 0.05$.

Considering that the three concentrations used in this experiment are lower than those used in most rooting studies, we can point out that this factor could be affecting the response of 'other research', so it is necessary to establish appropriate concentrations of ethanol to dilute IBA.

The results differ from those reported in *Phaseolus vulgaris* in number and length of roots; in these, 0.2% increased the number of roots compared to 2%, which increased the length (Middleton *et al*., 1978). In *Vigna radiata*, the number of roots decreased due to high ethanol concentrations (Bhattacharya *et al*., 1985). This may be due to the fact that ethanol can be toxic to plants and inhibit their development and growth (Kern *et al*., 2009). Perhaps the difference between what was reported and the present study is the ethanol concentrations, which vary between 0.5 and 2% and contrast with our lower concentration, 10%.

Conclusions

These findings provide anatomical knowledge about when the stages of induction, initiation, and differentiation of adventitious roots might be occurring in *F. carica* and suggest modifying, not only in this species but in many others, the dates of evaluation of treatments that influence the rooting of cuttings. The pH of the auxin solution is not an important factor to consider when rooting cuttings of *F. carica*, contrary to being evaluated individually, it could influence the rooting percentage as in other species.

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The protocol used in this research, since it proved to be efficient, could be used in future fig propagation practices. The way auxin is diluted seems to have an effect on the number of roots in the species, which implies that at the commercial and research level, protocols that use adventitious rooting as a means of propagation could be improved.

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