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The Effects of Gibberellic Acid and Emasculation Treatments on Seed and Fruit Production in the Prickly Pear (*Opuntia ficus-indica* (L.) Mill.) cv. "Gialla"

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Abstract: Prickly pear (*Opuntia ficus-indica* (L.) Mill. 1768) is cultivated in several dry and semi-dry areas of the world to produce fresh fruit, bioenergy, cosmetics, medicine, and forage. One of the main production constraints is the presence of many seeds within the fruit, which can negatively influence both the fresh-fruit market price and industrial transformation processes. In this study, different gibberellic acid (GA_3) concentrations were tested for their ability to produce well-formed and seedless fruits. Different application methods (injection and spraying) and concentrations of GA_3 (0, 100, 200, 250, and 500 ppm) combined with floral-bud emasculation were applied to a commercial plantation in southern Italy to evaluate their effects on the weight, length, and diameter of the fruits, total seed number, hard-coated viable seed number, and seed weight per fruit. The results indicated that the application of 500 ppm GA_3 sprayed on emasculated floral buds was the most effective method for reducing seed numbers of prickly pear fruits (−46.0%). The injection method resulted in a very low number of seeds (−50.7%) but produced unmarketable fruit. Observed trends suggest the need to investigate the impact of higher GA_3 concentrations and the applicability of a maximum threshold. Further studies are needed to increase our understanding of the physiological effects of the gibberellic acid pathway through productive tissue in terms of organoleptic and fruit quality.

Keywords: cactus pear; GA_3; injection application; spraying application; lignification

1. Introduction

Prickly pear (*Opuntia ficus-indica* (L.) Mill.) is the most cultivated plant species in the Cactaceae family due to edible fruit production [1]. It is a bushy-shaped, xerophytic, and crassulacean acid metabolism (CAM) plant originating from dry areas of Mexico [2]. The annual global production of prickly pear is approximately 500,000 tons, and Italy supplies 12% of the total market, preceded by Mexico and followed by Israel [3,4]. According to the Food and Agriculture Organization (FAO), in the last few years, prickly pear cultivation has gained a considerable amount of interest in relation to coping with food security issues in mostly dry and semi-dry regions, such as in South America, Africa, and the Mediterranean basin, thanks to its high resistance to drought and the important nutritional compounds present in the fruits [2,5]. Despite numerous species of the *Opuntia* genus being mainly cultivated to produce fresh fruit, this cultivation can play a key role in other contexts, such as environmental defense, forage and bioenergy production, the medicine and cosmetics sectors, and human health [1,6–8]. For instance, in some tropical agroforestry systems, *Opuntia elatior* (Mill.)

and *O. ficus-indica* are cultivated in association with other crops [9,10], as a productive living fence guarding against desertification [11,12]. In Africa and South America, the association of *Opuntia robusta* (J.C. Wendl) and *O. ficus-indica* var. *inermis* provide both living fences and livestock fodder [7,13]. Species such as *Opuntia maxima* (Mill.), *Opuntia heliabranoava* (Scheinvar), and *O. ficus-indica* are currently studied for biogas and fertilizer production, especially when associated with domestic plants in rural areas that are situated off of the energy grid [8,14–16]. *O. ficus-indica* has also been recently studied for medical and nutritional purposes since its juice has been found to show nutraceutical activities [17] and beneficial properties against specific types of cancer cells (bladder, ovarian, and leukemia) [18–20].

The *Opuntia ficus-indica* fruit is a false berry with an average weight of approximately 100–120 g, of which 2–10% is seeds and 60–70% is pulp [21]. *Opuntia ficus-indica* fruits present polyembrionatic seeds and 40–45% of them are aborted [22,23], while the remaining 60–55% are viable hard-coated seeds. This represents one of the main production challenges, which can influence the market price, because fruits with few or abortive seeds are more appreciated by consumers [24]. Abundant hard-coated seeds also complicate industrial processes, negatively impacting the transformation of fruit into such products as juice, nectars, jam, and food coloring [25–27], and potentially affecting consumer health by causing constipation [28–30]. However, whilst in other species such as citrus, the pulp development is not strictly linked with seed presence, in *Opuntia ficus-indica*, fruit pulp development depends on the funiculus of the seeds [31,32]. The funiculi are needed to produce a commercially acceptable pulp volume; however, a higher number of abortive seeds, characterized by their smaller size, would be more acceptable to consumers. The creation of hybrid types characterized by a good balance between these two latter aspects (i.e., high volume pulp—less number of seeds) would be feasible primarily through genetic breeding programs and agronomic techniques. The application of the first approach [31,33,34] is not economically viable and has resulted in poor fruit performance. By contrast, agronomic techniques, such as spring flushing or growth regulator treatments to inhibit seed growth, especially with auxin and/or gibberellins, may easily and more quickly provide well-formed and seedless fruits [2]. In the last decades, a few studies investigated the use of a phytoregulator on prickly pear [35–38]. For instance, Gil et al. [36] showed that the treatment of emasculated floral buds using gibberellic acid (GA_3) at 200 ppm increased both the development of ovular tissue and the funiculus, but also the hard-coated abortive seeds. Barbera et al. [35] indicated that at least 200 ppm of GA_3 injected into *Opuntia*'s stem (cladode) underneath the fruit was able to decrease the percentage of regular seeds. Mejía et al. [38], comparing the use of GA_3 by injection and spray application at different maturation stages, indicated the best performances using a 100 ppm GA_3 injection in pre- and postblooming. Kaaniche-Elloumi [23] also reported that the number and timing of GA_3 applications can affect fruit and seed development.

In this study, we tested the application of two methods (injection and spraying) of gibberellic acid (GA_3) on cactus prickly pear both at pre- and postblooming in order to obtain well-formed seedless fruits in emasculated flowers. Increased GA_3 concentrations and floral-bud emasculation techniques were also applied to evaluate fruit weight, length, and diameter; and seed weight, the total number of seeds, and the number of hard-coated viable seeds per fruit.

2. Materials and Methods

2.1. Study Area

The experiments were conducted in the spring–summer of 2016 in a prickly pear orchard located in the Apulia region, southern Italy (41°35′58″ N, 15°45′25″ E). The soil is sub-alkaline and shallow, with a calcareous bedrock substrate [39]. The regional climate is typically Mediterranean, with dry summers and mild winters. Average yearly rainfall is approximately 400 mm, with the lowest precipitation occurring in July and August. Air temperature maximums occur in August and July (~30 °C) and the minimum in January and February (~3 °C). During the experiments, the recorded maximum daily temperature was 39.1 °C, while the lowest rainfall (21 mm) was observed in July [40].

The field experiment was a 4-hectare, 10-year-old orchard with a density of 2.000 plants/ha, characterized by globe-shaped growth and a north–south row-oriented axis. The orchard was under organic management, with no irrigation and a permanent grass cover between rows.

2.2. Experimental Design

The experiment was conducted on *O. ficus-indica* cv. "Gialla", an Italian cultivar [41]. Combination treatments consisted of two randomized blocks to investigate both the effect of flower emasculation and the application of different concentrations of GA_3. Floral buds (N° = 360) were treated and examined, considering five floral buds for each plant. Fifty percent of the floral buds were emasculated (EM), while the rest were left intact (IN). Emasculation was performed 24 h before the first gibberellic acid application, in the morning (6:00–8:30 a.m.), by cutting stamens with a scalpel and then isolating the flower buds with a non-woven fabric cover to prevent natural pollination [38]. Both EM and IN floral buds were then subdivided into two groups and exposed to the two different application methods of gibberellic acid (Berelex® 40SG—Sumitomo Chemical, Saint Didier au Mont d'Or, Lion, France (GA_3)).

The GA_3 was injected (INJ) or sprayed (SPY) on floral buds using 1 mL of GA_3 solution in the following concentrations: for INJ, 0, 100, and 200 ppm; for SPY, 0, 250, and 500 ppm. Control floral buds were injected and/or sprayed with distilled water. The different dose regimes of INJ and SPY were selected because injection treatment is more efficient than spraying [38]. Doses will hereafter be indicated as control level (0 ppm for both INJ and SPY), low level (100 ppm for INJ and 250 ppm for SPY), and high level (200 ppm for INJ and 500 ppm for SPY). Following the methodology proposed by Mejía and Cantwell [38] and De La Barrera and Nobel [42], the GA_3 was applied twice on each bud at two different times corresponding to different phenological stages: 1–2 days before blooming (i.e., floral-bud diameter of 1.3–1.5 cm), and 20 days after blooming. Other management options (i.e., irrigation and fertilization) were not applied during the experiment.

2.3. Fruit and Seed Analyses

All fruits were harvested at the end of August when control fruits reached commercial maturity. At harvest time, fruit weight, diameter, and length were measured, and fruits were immediately stored in plastic bags at −20 °C. While frozen, each fruit was peeled and centrifuged (Girmi il Naturista mod. CE25 500W, Omegna, Italy) to separate pulp and seeds. Seeds were collected, washed with tap water, dried at 30 °C for 24 h, and then weighed, counted, and separated by seed type (hard-coated viable seeds or soft-coated aborted seeds) [38]. The fruit parameters considered were length, weight, and diameter, while the seed parameters considered were number, weight, and presence of viable seeds.

2.4. Statistical Analyses

Analysis of variance (ANOVA) was carried out by applying a mixed model on a complete factorial design to evaluate both the effects of each factor and all interactions, considering the GA_3 levels between INJ and SPY as equivalent. In the model, blocks were considered as random factors, while emasculation (EM/IN), application methods (INJ/SPY), and GA_3 levels were considered as fixed factors. Data that did not fulfill ANOVA assumptions were square-root transformed before running the model. All the analyses were performed using SPSS v.25 software (IBM Corp., New York, NY, USA). Tukey's post-hoc test was also calculated with Bonferroni correction ($p \leq 0.05$).

3. Results

3.1. Fruits Characterization

In general, the lowest average values for diameter, length, and weight were those of EM plants (Table 1). Considering the combined effect of only INJ/SPY and EM/IN (not GA_3 level) on fruit diameter, the highest mean value was found in the IN+SPY group. No significant difference in diameter was found between IN+SPY and IN+INJ combined treatment (−3%), while lower diameters were detected

using the combined treatments EM+SPY (−16%) and EM+INJ (−18.5%). The GA_3 levels somewhat affected the results. Higher diameters were found in IN+SPY under all GA_3 levels and the IN+INJ control, while the lower values were found in the EM group control using both methods (i.e., INJ and SPY). However, the highest statistical significance was observed between control levels of emasculated fruits (INJ: 3.35 ± 0.33 cm, SPY: 3.29 ± 0.33 cm) and control levels of intact fruits (INJ: 4.95 ± 0.24 cm, SPY: 4.74 ± 0.32 cm).

Table 1. Effect of gibberellic acid (GA_3) application treatments on fruit variables.

Method		Fruit Diameter (cm)		Fruit Length (cm)		Fruit Weight (g)	
	GA_3 Level	EM [z]	IN	EM	IN	EM	IN
INJ	control [y]	3.35 ± 0.33 f [x]	4.95 ± 0.24 a	5.10 ± 0.58 de	6.65 ± 0.47 a	23.84 ± 6.51 e	82.24 ± 9.71 a
	low	4.07 ± 0.38 de	4.27 ± 0.49 de	5.78 ± 0.62 bc	6.21 ± 0.74 ab	54.93 ± 14.28 cd	59.42 ± 17.25 cd
	high	3.95 ± 0.37 de	4.38 ± 0.34 cd	5.63 ± 0.55 cd	6.27 ± 0.51 ab	49.41 ± 11.95 d	65.34 ± 12.76 bc
SPY	control	3.29 ± 0.33 f	4.74 ± 0.32 ab	5.00 ± 0.53 e	6.43 ± 0.52 a	24.11 ± 7.26 e	74.46 ± 12.97 ab
	low	4.10 ± 0.33 de	4.60 ± 0.28 bc	5.79 ± 0.67 bc	6.66 ± 0.42 a	53.14 ± 14.9 d	72.67 ± 10.87 ab
	high	4.34 ± 0.31 cd	4.61 ± 0.33 bc	6.23 ± 0.54 ab	6.64 ± 0.68 a	60.35 ± 8.89 cd	73.39 ± 16.79 ab
INJ	mean	3.79 ± 0.06 B	4.53 ± 0.05 A	5.50 ± 0.08 B	6.38 ± 0.07 A	42.70 ± 2.10 B	68.97 ± 1.75 A
SPY	mean	3.91 ± 0.06 B	4.65 ± 0.04 A	5.67 ± 0.08 B	6.58 ± 0.06 A	45.83 ± 2.01 B	73.43 ± 1.57 A
All	mean	3.85 ± 0.06	4.59 ± 0.04	5.59 ± 0.08	6.48 ± 0.07	44.27 ± 2.06	71.20 ± 1.67

[z] EM: emasculated fruits; IN: intact fruits. Application methods were injection (INJ) and spraying (SPY). [y] The levels of GA_3 were control, low levels (100 ppm and 250 ppm) and high levels (200 ppm and 500 ppm). [x] Different letters indicate significant differences using Tukey's post-hoc test with Bonferroni correction ($p \leq 0.05$). Lower case letters (a–f) are for comparison of individual treatment means and upper case (A,B) are for main effect means.

Considering the combined effect of only INJ/SPY and EM/IN (without GA_3 levels) on fruit length, the highest mean was found within the IN+SPY combined treatment, while the lowest was observed using EM+INJ. The application of GA_3 resulted in statistically significant differences in fruit lengths between intact and emasculated fruits. Specifically, greater fruit lengths were found in the intact fruits under all GA_3 levels and application methods. In contrast, low statistical significance was observed in emasculated fruits among all GA_3 treatments and methods, and the only exception was for the highest level of the SPY method in line with the results of intact fruits.

Considering the combined effect of INJ/SPY and EM/IN (without GA_3 level) on fruit weight, the highest mean was found using the IN+SPY combined treatment, while the lowest was observed using EM+INJ. No significant difference in weight was found between EM+INJ and IN+INJ combined treatment (−6%), while with respect to the remaining combinations (EM+SPY and EM+INJ), considerable weight differences were observed (−38% and −42%, respectively). The application of GA_3 resulted in statistical significance between intact and emasculated fruits. The higher values were found using IN+SPY at all GA_3 levels, with similar weights for all treatments, and in the IN+INJ control group. In contrast, the lowest values were found specifically for the control of the emasculated fruits using both INJ and SPY. The highest statistical significance was observed, indeed, between the control group of the IN+INJ and control group of EM+INJ combination treatment.

3.2. Fruit Defects

At harvest time, 39% of total fruit displayed defects. These defects were defined as (Table 2, Figure 1) (a) lignification on pulp tissue; (b) lignification of ovular tissue; (c) recalcitrant fruits (i.e., fruits that have not reached maturity).

Table 2. Relative frequencies of harvested fruits and the related defects per combination treatment. Frequency is based on the observation of thirty floral buds per combination treatment.

Method	GA_3 Level	Lignification on Pulp Tissue (%)		Lignification on Ovary Tissue (%)		Recalcitrant Fruits (%)		Healthy Fruits (%)	
	---	EM	IN	EM	IN	EM	IN	EM	IN
INJ	control	-	-	20.00	-	80.00	-	-	100.00
	low	53.30	63.90	13.30	6.70	3.00	13.30	30.40	16.10
	high	60.00	43.30	3.30	-	20.00	-	16.70	56.70
SPY	control	-	-	30.30	-	53.30	-	16.40	100.00
	low	-	-	-	-	3.00	-	97.00	100.00
	high	-	-	-	-	-	-	100.00	100.00
INJ	mean	37.77	35.73	12.20	2.23	34.33	4.43	15.70	57.61
SPY	mean	-	-	10.10	-	18.77	-	71.13	100.00
All	mean	18.88	17.87	11.15	1.12	26.55	2.22	43.42	78.79

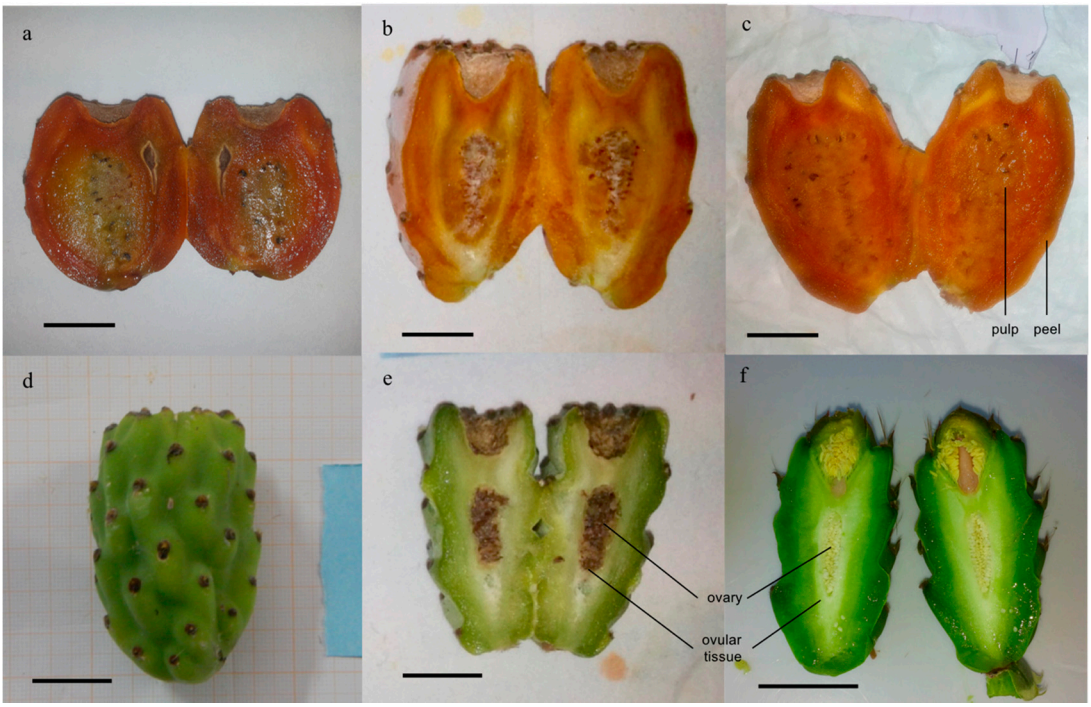

Figure 1. Main defects of harvested fruits: (**a**) lignification on pulp tissue; (**b**) lignification of ovary tissue; (**c**) healthy fruits; (**d**) recalcitrant fruits and (**e**) its longitudinal section; (**f**) longitudinal section of floral bud. Scale bar = 2 cm.

Lignification on pulp tissue was only found in EM+INJ and IN+INJ combined treatments, while under EM+SPY and IN+SPY, this defect was not observed. Specifically, this defect was observed when GA_3 treatments were applied. In particular, the highest and lowest defect percentages were found in the intact fruits using the injection method for low and the high GA_3 levels, respectively.

Lignification of ovular tissue was observed in three of the four combined treatments, specifically EM+INJ, EM+SPY (10.10%), and IN+INJ. Only within the IN+SPY group was this defect not found. This defect was observed to a greater extent in the EM fruits, with the highest percentage observed within the control group of both the INJ (20.00%) and SPY methods. In contrast, in intact fruit, lignification of ovular tissue was observed only for the INJ method for the low GA_3 level (6.70%).

Finally, recalcitrant fruits were found under EM+INJ, EM+SPY, and IN+INJ, while no defect was found using IN+SPY. Fruit recalcitrance was observed in the EM group, with the highest percentage observed in the control groups of both the injected and sprayed methods. The lignification of ovular tissue in IN fruits was observed only in the low GA_3 group using the INJ method (13.30%).

The only group free of defects was IN+SPY under all GA_3 levels. In contrast, the highest presence of defects was observed using the EM+INJ combined treatment, where only 15.70% of harvested fruits were healthy.

3.3. Seed Characterization

In all groups, the EM flowers showed the lowest average values for all seed variables: the number of seeds was 83.83 ± 8, the number of hard seeds was 12.17 ± 2, and the weight of seeds was 0.16 ± 0.02 g (Table 3).

Table 3. Effect of GA_3 application treatments on seed variables.

Method		Seeds Per Fruit (N°)		Hard Seeds Per Fruit (N°)		Weight of Seeds Per Fruit (g)	
	GA_3 Level	EM [z]	IN	EM	IN	EM	IN
INJ	control [y]	16.00 ± 43 f [x]	232.00 ± 58 a	1.00 ± 1 d	220.00 ± 55 a	0.02 ± 0.03 e	2.81 ± 0.56 a
	low	118,00 ± 81 cd	140.00 ± 86 bcd	25.00 ± 15 d	71.00 ± 44 c	0.28 ± 0.20 de	0.61 ± 0.44 d
	high	103.00 ± 73 de	185.00 ± 65 ab	16.00 ± 8 d	167.00 ± 59 b	0.24 ± 0.21 de	1.01 ± 0.55 c
SPY	control	45.00 ± 50 ef	195.00 ± 55 ab	0.00 ± 0 d	185.00 ± 53 ab	0.04 ± 0.04 e	2.47 ± 0.78 a
	low	106.00 ± 57 d	174.00 ± 47 abc	21.00 ± 10 d	166.00 ± 44 b	0.23 ± 0.32 de	2.05 ± 0.58 b
	high	115.00 ± 42 d	181.00 ± 51 ab	10.00 ± 4 d	177.00 ± 50 b	0.15 ± 0.07 e	1.99 ± 0.67 b
INJ	mean	79.00 ± 9 B	185.67 ± 9 A	14.00 ± 2 C	152.67 ± 9 B	0.18 ± 0.02 C	1.48 ± 0.11 B
SPY	mean	88.67 ± 7 B	183.33 ± 7 A	10.33 ± 1 C	176 ± 6 A	0.14 ± 0.02 C	2.17 ± 0.08 A
All	mean	83.83 ± 8	184.5 ± 8	12.17 ± 2	164.33 ± 8	0.16 ± 0.02	1.82 ± 0.1

[z] EM: emasculated fruits; IN: intact fruits. Application methods were injection (INJ) and spraying (SPY). [y] The levels of GA_3 were control, low levels (100 ppm and 250 ppm) and high levels (200 ppm and 500 ppm). [x] Different letters indicate significant differences using Tukey's post-hoc test with Bonferroni correction ($p \leq 0.05$). Lower case letters (a–f) are for comparison of individual treatment means and upper case (A,B) are for main effect means.

Without considering the GA_3 levels, the combination treatment IN+INJ showed the highest average number of seeds. Only a slight difference was found between IN+INJ and the IN+SPY combined treatment (−1.3%), while, on average, a considerably lower number of seeds was found with EM+SPY (−52%) and EM+INJ (−57.5%). The GA_3 levels resulted in statistical significance between intact and emasculated fruits. Specifically, more seeds were found in the IN fruits under most GA_3 levels and application methods, with the only exception being the low GA_3 level using the INJ method. In contrast, low values were observed using EM fruits under all GA_3 treatments and methods, particularly in the control groups.

Regardless of GA_3 levels, the highest number of hard seeds per fruit was in the IN+SPY combined treatment. Few differences were observed between IN+SPY and IN+INJ (−13.3%), while large differences were found under EM+SPY (−94.1%) and EM+INJ (−92%) combined treatments. The GA_3 levels resulted in statistical significance between intact and emasculated fruits. In particular, the highest level of significance was found in the control groups of both injection and sprayed methods.

Finally, the average highest seed weight per fruit, without considering GA_3 levels, was within IN+SPY. The lower average weight of seeds per fruit was found using IN+INJ (−32%) with respect to IN+SPY, while large differences in seed weight were observed using EM+SPY (−93.5%) and EM+INJ (−91.7%). Similarly, the GA_3 levels for the seed weight per fruit resulted in statistical significance between intact and emasculated fruits. In particular, the highest level of significance was found under the control of both the INJ and SPY methods.

3.4. ANOVA Results

ANOVA assumptions showed that only the seed variables needed to be transformed. Afterwards, the statistical analysis showed the significant effects of fixed factors and their interactions on both fruit and seed variables (Table 4). Emasculation treatment significantly influenced each variable for both fruits and seeds. The combined treatment SPY/INJ influenced fruit size, seed weight, and viability, but not the number of seeds per fruit. GA_3 levels strongly influenced all variables and only moderately impacted the seed number per fruit. Interaction between all factors (EM/IN × SPY/INJ × GA) revealed that there was a strong effect among factors for all the variables.

Table 4. ANOVA significance results of the single and combined effects on fruit and seed dimensions.

		Fruit Diameter	Fruit Length	Fruit Weight	Seeds	Hard Seeds	Seed Weight
Source	**Abbr.**	**Significance**					
Emasculation	EM/IN	** [z]	**	**	**	**	**
Application methods	SPY/INJ	**	*	*	ns	*	**
GA_3 levels	GA	**	**	**	*	**	**
EM/IN × SPY/INJ × GA	-	**	**	**	**	**	**

[z] "ns" means not significant ($p \geq 0.05$); "*" low significance ($0.01 < p < 0.05$); "**" high significance ($p \leq 0.01$). These results were obtained from data presented in Tables 1 and 3.

4. Discussion

The use of different application methods and GA_3 concentrations in prickly pears to obtain well-formed and seedless fruits in *O. ficus-indica* (L.) Mill. "Gialla" provided several curious results.

The objective of obtaining well-formed fruits with few seeds was only partially achieved in this study. More well-formed fruits were obtained from the IN rather than the EM treatment, but the IN treatment produced a higher seed content. This was also observed in the aforementioned study by Mejía and Cantwell [38], which found the emasculated fruits were generally smaller and had lower numbers of hard seeds (viable seeds) than the intact ones. This difference could be explained by the lack of stamens (emasculation), which contributed to the lack of or low development of the flower tissues. The external tissue of the anthers is the main gibberellin biosynthesis site, and thus the main regulating factor for the development of the remaining floral parts. This was deduced from Inglese et al. [37], who observed that *Opuntia ficus-indica* flowers with removed anthers show lower levels of endogenous gibberellin than pollinated ones. This behavior has been observed in other crops such as rice [43] and *Arabidopsis* [44].

The emasculated fruits showed a general decrease in all the analyzed variables (i.e., diameter, length, and weight) compared to the intact ones, regardless of the GA_3 treatment applied. In the prickly pear, pulp development originates from the funiculus, which connects the seed to the ovular tissue, and thus seeds are needed for fruit development [32,38,45]. On this basis, if the development of the ovular tissue and funiculus was inhibited, the *Opuntia*'s fruit may have difficulty in developing properly [23]. Despite the plausibility of the hypothesis, the gibberellin transport mechanism in the developing organs of the flower—from the male part (stamen) to the female part (ovary tissue and funiculus)—is not currently fully understood [46,47].

Generally, it has been observed that treatment with GA_3 may improve pulp development and reduce seed numbers in emasculated prickly pear fruits as a result of the replacement of endogenous gibberellins. This was also suggested by the fact that in *Opuntia ficus-indica*, the highest levels of GA_3 in the flowers were found during blooming [37], and these, in turn, are responsible for the development of fruits and natural pollination [42]. This has also been observed in other plant species, i.e., the *Citrus* genus [48], where although gibberellic acid is not the only factor emulating the effects of natural pollination, the contribution of both pollination and exogenous GA_3 application can improve fruit development.

However, the response of fruits and seeds to growth regulator treatments in this study depended on the specific application method. More specifically, while the spraying of GA_3 (both low and high levels) generally enhanced the performance of all the analyzed variables of the treated fruits, the injection method showed the opposite pattern, especially for the intact fruits. One of the effects caused by gibberellic acid is control over the elongation of cellular tissues in plants [49–51]. Generally, the exogenous sprayed GA_3 is able to spread through the elongation of cells in plant tissues, thus facilitating the absorption and avoiding the direct negative effect of gibberellic acid within the parenchyma tissue [42,52].

Whilst several studies have demonstrated that GA_3 application can increase the presence of defects in *Arabidopsis* [44,53], *Coriadrum sativum* L. [54], *Oryza sativa* L. [46], *Zea mays* L. [52], and *Daucus carota* L. [55], to our knowledge, only a few studies have investigated the effects of GA_3 on *Opuntia* [35,56]. In this study, the main defects were lignification of the ovular and pulp tissue, and the presence of recalcitrant fruits. Lignification on pulp tissue was observed in both EM and IN fruits, particularly in the INJ groups. Specifically, most pulp tissue lignification was found corresponding to the needle entry hole for the GA_3 injection. This condition may have been caused by two different factors. First, the needle was not able to reach the ovary, thus spreading the GA_3 solution into the pulp. It is also possible that the injected compounds returned to the entry hole. This was partially deduced by Nobel et al. [56], who observed that in *Opuntia ficus-indica* tissues, injected gibberellic acid likely came into contact with expanding parenchyma tissue, thus leading to an excessive accumulation of dry matter in the tissue around the needle entry hole. This has been confirmed in several studies [42,52], which proposed that the gibberellic acid in prickly pear fruits can cause a sink effect promoting dry matter accumulation in parenchyma tissue. This pattern was also indirectly supported by Jedidi Neji et al. [57], in which injection of gibberellic acid into the ovary of the *Opuntia* flower through the stigma and not the pulp did not result in lignification effects on the pulp tissue in fruits.

The lignification on ovular tissue, mostly recorded in EM fruits, was highest when the GA_3 was sprayed rather than injected. Ortiz Hernandez et al. [58] reported a similar behavior in an *O. amyclaea* study: when the flowers were emasculated and treated with different growth regulators, the fruits showed ovarian tissue lignification. This result was likely driven by the flower emasculation rather than the method of GA_3 application since the absence of stamens can cause a lack or little development of the remaining flower tissues. Gupta et al. [47] suggested that, generally, even a short-distance movement of GA_3 from the stamen to the other floral organs and the pedicel may be sufficient for flower development.

Recalcitrant fruits were found mostly in EM fruits. The highest incidence was observed within the control groups for EM+INJ (80%) and EM+SPY (53%). This result was likely because emasculation does not allow for the development of full fruit maturity, thus creating smaller fruits. These findings are consistent with a similar study carried out by Kaaniche-Elloumi et al. [23], in which prickly pear emasculated fruit reached maturity after GA_3 treatment. Besides, when comparing the IN control group fruits with the different GA_3 treatments, an overall decrease in the number of seeds, the number of hard-coated seeds, and the weight of seeds was observed. This may confirm that exogenous GA_3 on *Opuntia* can reduce the number of hard-coated seeds [35,36,38]. Furthermore, seed abortion could be related to the effect of GA_3 on chromosomal DNA, which may lead to the incomplete development of the endosperm [57].

5. Conclusions

Prickly pear cultivation is important in several dry and semi-dry areas of the world owing to its diverse uses (e.g., as fresh fruit, in bioenergy, cosmetics, and medicine production, and as forage). The results of GA_3 application on fruits indicated that 500 ppm of GA_3 sprayed on emasculated floral buds was the most effective technique for reducing the number of seeds within prickly pear fruits. The spraying of the GA_3 (both low and high levels) enhanced the growth performance of all the analyzed variables of the treated fruits, while the injection method, though capable of reducing the

number of seeds, can increase the presence of defects, making the fruit unmarketable. The results suggested the need to further investigate the impact of higher GA_3 concentrations on fruit production, and particularly, GA_3 application methods, especially regarding industrial production. The GA_3 spraying method would indeed be easier to apply in large-scale production than the injection method, whilst manual emasculation may be better replaced by chemical emasculation, which provides similar results. Given the scarcity of studies on prickly pear cultivation and the repercussions of its industrial processes, future studies should focus on these aspects by conducting experiments that directly address the application of these treatments in industrial-scale processes. Moreover, further studies should focus on the maximum thresholds of GA_3 applicability and the physiological effects of the gibberellic acid pathway through productive tissue, thus elucidating the economic viability of this cultivation technique and the changes in fruit quality and organoleptic properties.

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