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Stem diameter for Italian tomato grafting

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ABSTRACT

The adoption of grafting in vegetable production is an innovative technique that presents challenges to be overcome, which one of the main ones is the need to determine the appropriate diameter of the plant at the time of grafting. Therefore, the objective of this work was to evaluate the development of seedlings and production of tomato plants, grafted with different stem diameters, under protected environmental conditions. The experiment was divided into two phases, seedling development and field production. The experimental design was completely randomized with five treatments, composed of the following seedlings stem diameters at the time of grafting: 1.8; 2.0; 2.2; 2.5 and 2.8 mm. Morphophysiological and productive characteristics of the grafted plants were evaluated. The results demonstrated that Italian tomato seedlings, grafted with 2.2 mm stem diameter, showed better development. However, the production rates were similar for the different stem diameters (1.8 to 2.8 mm) adopted at the grafting moment. Estimated yields of around 200 t/ha, much higher than the national average, demonstrate the success of the grafting technique in Italian tomatoes, under protected environmental conditions.

Keywords: *Solanum lycopersicum* L., *Lycopersicon esculentum* Mill, clip, gauge.

RESUMO

Diâmetro do caule para enxertia do tomate italiano

A adoção da enxertia na produção de hortaliças é algo inovador, que apresenta desafios a serem superados, destacando-se dentre esses, a necessidade de determinação do adequado diâmetro da planta no momento da enxertia. Dessa forma, objetivou-se com o presente trabalho avaliar o desenvolvimento de mudas e produção do tomateiro, enxertado com diferentes diâmetros de coleto, sob condições de ambiente protegido. O experimento foi dividido em duas fases: desenvolvimento das mudas e produção de frutos. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos, compostos pelos diâmetros do coleto das mudas no momento da enxertia, sendo: 1,8; 2,0; 2,2; 2,5 e 2,8 mm. Foram avaliadas características morfofisiológicas e produtivas das plantas enxertadas. Os resultados demostraram que mudas de tomate italiano, enxertadas com 2,2 mm de diâmetro de coleto, apresentaram melhor desenvolvimento. Contudo, os índices de produção foram semelhantes para os diferentes diâmetros de coleto (1,8 a 2,8 mm) adotados no momento da enxertia. As produtividades estimadas em torno 200 t/ha, muito superior à média nacional, evidenciam o sucesso da técnica de enxertia no tomate italiano, em condições de ambiente protegido.

Palavras-chave: *Solanum lycopersicum* L., *Lycopersicon esculentum* Mill, clipe, calibre.

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The global production of tomatoes increased by more than 35% over the past ten years, positioning this vegetable among the most consumed worldwide. Brazil stands out in the global scenario not only as a major consumer of tomatoes but also as an efficient producer, being the 9th largest producer (FAO, 2022).

Tomato cultivation can be carried out in open fields or in protected environments, which have been expanding in the pursuit of higher productivity, fruit quality, reduced pest and disease attacks, and partial control of some environmental factors. In both cultivation environments, it is common to observe variations in productivity and fruit quality, usually associated with prevailing biotic and abiotic factors.

Among the abiotic factors, cultural practices are quite important. These practices include the seedling production phase and the productive phase, such as staking, suckering, apical pruning, thinning of fruits on the clusters, leaf removal, irrigation, and phytosanitary management. These procedures are performed in tomato cultivation to achieve higher productivity and plant longevity (Fontes & Silva, 2002; Berrueta *et al*., 2020). The use of high-quality seedlings can be considered one of the most important stages of tomato production and can be identified by appropriate morphological characteristics such as shoot height, root length, leaf area, and stem diameter, which will ensure high survival rates and good fruit production. Thus, the characteristics of the seedlings are highly related to higher tomato fruit productivity (Rysin & Louws, 2015).

Among the technological innovations in tomato seedling production is grafting, which was initially introduced in the Asian continent. The grafting technique, already used for several decades in other species, has gained space in tomato cultivation (Lee *et al*., 2010). Currently, in Korea, Japan, and China, about 65% of tomatoes are produced by grafted plants, while in the Netherlands, all tomatoes in soilless cultivation conditions are produced by grafted plants (Maurya *et al*., 2019). In Brazil, the adoption of grafting has stood out as a relevant technique to avoid disease incidence, increase productivity, and improve the quality of the produced fruits (Pedó *et al*., 2022).

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The positive response of grafting on the productivity and fruit characteristics of tomato plants is attributed to various physiological and biochemical changes that occur in the interaction between the rootstock and the scion (Mogy *et al*., 2022). To generate knowledge about the rootstock/scion interactions that determine growth, development, tolerance, and fruit quality is a constant pursuit (Huang *et al*., 2014), especially for the Italian tomato group, for which the adoption of grafting is still recent. Among the challenges are determining the appropriate combination of age and stem diameter of the grafted plants and rootstocks at the time of grafting (Singh *et al*., 2017). Linked with this information is the need to adjust the stem diameter to be adopted to the grafting clips available on the market. Guan & Hallet (2016), working with various clips available in the North American market, observed that the minimum diameter should be 1.5 mm.

In the Brazilian market, grafting clips of various diameters are available, commonly ranging between 1.8 to 2.8 mm. However, it lacks about knowledge of the effect of the caliber of the rootstock/scion at the time of grafting on the morphophysiological characteristics of the seedlings and the subsequent fruit production, especially in protected environments.

Therefore, this study aimed to evaluate the development of seedlings and the production of Italian-type tomato plants grafted with different stem diameters under protected environment conditions.

MATERIAL AND METHODS

The experiments were conducted in partnership with the Grupo Empresarial Plantec and the Peter Fruit Company, based in the Alto Caxixe district, in the municipality of Venda Nova do Imigrante, in southern Espírito Santo (20º24'6"S; 41º4'58"W, 1210 m altitude). The Köppen classification is tropical humid with an average annual temperature of 24°C (Pezzopane *et al*., 2012).

The rootstock used was Green Rise cultivar from Takii Seeds®, characterized by its resistance to Bacterial Wilt (*Ralstonia*), *Verticillium*, *Pyrenochaeta*, Fusarium Wilt (*Fusarium oxysporum* f.sp. *lycopersici* races 1, 2, and 3), *Fusarium oxysporum* f.sp. *radicis-lycopersici*, Tomato Mosaic Virus (*Tm-2a*), and nematodes (*Meloidogyne arenaria*, *M. incognita*, *M. javanica*). For the scion/graft, the Benedetti cultivar, an Italian type with indeterminate growth, from Enza Zaden® was used.

Seedling stage

The experiment was conducted in a high tunnel greenhouse, at the Top Mudas nursery (Grupo Plantec), following a completely randomized experimental design with five treatments, consisting of seedling stem diameters at the time of grafting, corresponding to the grafting clips available on the market, being: 1.8; 2.0; 2.2; 2.5 and 2.8 mm, with fifteen repetitions. The clip was made of silicone material recommended in the market for grafting solanaceous plants (tomato, pepper, and eggplant), cucurbits (cucumber, melon, and watermelon), and flowers (rose).

For caliber adjustment, the rootstock was sown ten days after the scion/graft sowing, both in disposable plastic trays filled with commercial substrate Carolina Soil® (Composition: Peat and Expanded Vermiculite).

The management in the greenhouse was carried out according to the nursery practices, with irrigation two to three times a day to keep the substrate close to field capacity, weekly application of a complete nutrient solution with electrical conductivity (EC) of 1.2 mS/cm to 1.5 mS/cm, according to the seedling age, and spraying when necessary for pest control. The applications were made automatically via bar spraying.

The grafting method adopted was simple cleft grafting, when the seedlings had the desired diameter according to the treatments. The following steps were performed: a) Double bevel cut on the scion and cleft on the rootstock (for better contact between scion and rootstock inside the grafting clip); b) Fixation with clip after joining the parts; c) Healing chamber: immediately after grafting, the seedlings were placed in a humid chamber with relative humidity above 80% and temperature around 25°C.

After seven days, considering the seedlings healed, development evaluations were carried out, including: morphological characteristics: height (measured with a ruler from the collar to the tip of the youngest fully expanded leaf); stem diameter (measured with a caliper at the seedling collar, 2 cm above the substrate surface); seedling leaf area (determined with a photoelectric meter, Licor Area Meter 3100); root length (measured from the collar to the tip of the longest roots); fresh and dry mass of the shoot and root system (dry mass was determined after samples were dried in a forced-air oven at 65°C until constant weight); Physiological characteristics through nitrogen balance index (NBI), chlorophyll, flavonoids, and anthocyanins, measured in the fully developed leaf. All these measurements were made between 8:00 AM and 10:00 AM, using the Dualex device (Force-A, Orsay, France) (Milagres *et al*., 2018).

The obtained data were subjected to analysis of variance, adopting the F-test at 5% probability. For the variables that showed significant differences, the Tukey test at 5% was used for mean comparison. The R Development Core Team (2018) software with the ExpDes.pt package was used.

Production stage

Upon reaching the transplant stage, considering a size of 10 to 12 cm and with four to six leaves (38 days after sowing), a portion of the seedlings was taken to the production phase, with the experiment conducted in a protected environment, a high tunnel greenhouse with dimensions of 60 m in length, 7.5 m in width, and 6.0 m in maximum height. The area belongs to Peter Fruit, and is located on land adjacent to the nursery.

The soil in the area was classified as Red-Yellow Latosol, medium texture (Santos *et al*., 2018). Soil samples were collected (0-20 cm depth layer), and upon analysis, showed the following characteristics: pH (H_2O): 5.58; Mehlich 1 phosphorus (P): 139.90 mg/dm^3; potassium (K): 127.41 mg/dm^3; aluminum (Al): 0.20 cmolc/dm^3; calcium (Ca): 4.26 cmolc/dm^3; magnesium (Mg): 1.12 cmolc/dm^3; sum of bases (SB): 5.71 cmolc/dm^3; V%: 62.68; cation exchange capacity (CEC): 9.11 cmolc/dm^3.

Soil preparation was carried out by plowing to a depth of 40 cm, followed by harrowing. After harrowing, ridges approximately 30 cm high were raised and covered with plastic film. The spacing adopted was 1.0 x 0.3 m. All crop management was carried out according to the Company's planning, which included spontaneous plant control and daily drip irrigation throughout the cycle. Plant training was done with polypropylene strings and two main stems. After defining the main stems, a weekly removal of lateral shoots was carried out; apical pruning (topping) of the two main stems was carried out when the plant had 18 clusters; the removal of lower leaves when dry and artificial pollination were carried out once a week using blowers or manually by shaking the plants.

Monthly fertilizations were performed, and at the end of the 180 day cycle, the total amounts reached were 33, 32, 51, 11, 8, 10, and 1 kg of N, P_2O_5, K_2O, CaO, Mg, S, and B, respectively, applied per 1000 plants (300 m^2). The following fertilizer sources were used: N: ammonium nitrate; P_2O_5: single superphosphate; K_2O: potassium chloride (considering the potassium used in sulfates); S: potassium sulfate and magnesium sulfate (considered when providing Mg); Ca: calcium nitrate; Mg: magnesium sulfate; B: boric acid or borax.

The experimental design used was randomized blocks with the same five treatments (seedling grafting diameters of 1.8; 2.0; 2.2; 2.5; and 2.8 mm), with five repetitions. Each plot consisted of three planting rows, with five plants in each row, using the five plants in the central row as the useful area.

Harvesting began at 71 days after transplanting (DAT) and was carried out weekly, thus starting the productive evaluations with fruit characterization by weight (grams), diameter (mm), and length (mm). At the end of the cycle, the number of fruits and production per plant were determined, and the productivity per area (kg/ha) was estimated.

The data were subjected to analysis of variance, adopting the F-test at 5% probability. For variables that showed significant differences, the Tukey test at 5% was applied for mean comparison. The R Development Core Team (2018) software with the ExpDes.pt package was used.

RESULTS AND DISCUSSION

Seedling development

All grafts, regardless of the adopted diameter, showed a healing (engraftment) rate of 80%. Differences in the physiological characteristics of the seedlings were observed according to the stem diameter at the time of grafting. The nitrogen balance index (NBI) was higher only in seedlings grafted with 1.8 mm. The opposite result was found for the flavonoid and anthocyanin indices, with the lowest values observed in seedlings grafted with 1.8 mm. The chlorophyll index, however, was not affected by the stem diameter at the time of grafting (Table 1).

The higher NBI in the leaves of seedlings grafted with 1.8 mm indicates a better state of available nitrogen, potentially meaning free amino acids available for seedling growth. When this occurs, it may lead to carbohydrate accumulation in their tissues, according to the carbon/nutrient balance hypothesis (Zheng *et al*., 2015).

It is worth noting that the available nitrogen levels in the leaves of the other treatments, although lower, might still be sufficient for the good development of the seedlings. Supporting this reasoning, the lack of difference in the chlorophyll index result demonstrates similar photosynthetic capacity (Streit *et al*., 2005) among seedlings grafted with different diameters.

TThe result for the flavonoid index, opposite to the NBI, was expected, considering that flavonoids are secondary metabolites of the polyphenol class, generated when plants are under N deficiency (Demotes-Mainard *et al*., 2008). Therefore, lower flavonoid indices might indicate that the plant is in a non-limitation N condition.

Anthocyanins, on the other hand, are secondary metabolites with various functions in plants, such as regulating cell growth and protecting the leaf against stresses, like grafting. Therefore, the higher value observed with the larger diameter (2,8 mm) suggests that this diameter may induce stress in the plant (Milagres *et al*., 2018).

There is a difference in the morphological development of the seedlings, regarding height, leaf area, and stem diameter, depending on the stem diameter adopted at the time of grafting. It is noted that the tallest plants were observed with diameters of 2.2 to 2.5 mm. The largest leaf areas were observed in seedlings grafted with diameters from 2.2 mm. About the final stem diameter of the seedlings, it is noted that seedlings grafted with 2.2 mm also stood out, although statistically similar to 1.8 and 2.5 mm. Root length was not affected by the stem diameter of the seedlings at the time of grafting (Table 2).The results indicate the influence of the stem diameter at the time of grafting, with a diameter of 2.2 mm resulting in better seedling development, providing better heights, leaf area, and final stem diameter, without affecting root length. This result may indicate a good interaction between the scion and rootstock when grafting is performed at this diameter, possibly reflecting the better recovery of conductive vessels, contributing to greater nutrient absorption that directly influences plant growth (Taiz & Zeiger, 2013). The larger leaf area indicates an increased capacity of the plant to utilize solar energy, which is used in the photosynthetic process (Gonzales-Sanpedro *et al*., 2008), potentially directly reflecting on the growth and development of the seedlings. Thus, in addition to the significant interaction between acclimation environment and grafting methods (Zeist *et al*., 2020), the ideal diameter for grafting should also be observed to achieve success.

Regarding biomass accumulation, higher values for fresh and dry aerial biomass were noted in seedlings grafted with a stem diameter of 2.2 mm, not differing from the 2.5 mm treatment (Table 3). These results reflect the better development of the aerial part (greater heights and leaf area), demonstrating the capacity to produce vigorous seedlings with the adoption of these stem diameters at the time of grafting.

Dry root biomass, again, stands out for seedlings grafted with a stem diameter of 2.2 mm. This result is not noted in fresh biomass (Table 3). This is also an interesting result, as the presence of larger root biomass demonstrates that these seedlings would better distribute photoassimilates between the plant's source and sink organs (Aumonde *et al*.,2011; Pedó *et al*., 2022).

Overall, the results demonstrate that Italian tomato seedlings, Benedetti® cultivar, grafted with a stem diameter of 2.2 mm, on the rootstock, Green Rise® cultivar, would show better development.

Production indices

The stem diameter adopted at the time of grafting did not influence the fruit quality indices evaluated in average weight, length, and diameter of the fruits (Table 4). The standard of produced tomatoes was medium, which would meet the special class, with an average fruit weight of around 90 grams, 55 mm in diameter, and 75 mm in length, regardless of the adopted graft diameter (CEASA-DF, 2023).

The stem diameter adopted at the time of grafting did not influence the production indices evaluated in the

Table 1. Nitrogen balance index, chlorophyll, flavonoids and anthocyanins levels in Italian tomato seedlings leaves, adopting different stem diameters at the grafting moment. Venda Nova do Imigrante, UFES-UFV, 2020.

Stem diameter (mm)	Nitrogen balance	Chlorophyll	Flavonoids	Anthocyanin
	Absorbance indices			
1.8	86.29a[1]	27.73^{ns}	0.35b	0.09c
2.0	57.83b	27.60^{ns}	0.50a	0.11b
2.2	46.85b	25.87^{ns}	0.52a	0.13ab
2.5	57.71b	26.30^{ns}	0.47ab	0.12ab
2.8	57.57b	27.61^{ns}	0.52a	0.13a
CV (%)	24.59	8.67	23.39	8.04

[1]Means followed by the same letter in the column did not differ significantly from each other according to the Tukey test at 5% probability level; ns: not significant according to the F test at 5%.

number of fruits, production per plant, and productivity (Table 5). In general, individual plant production was high, with an average of 140 fruits/plant and production around 12 kg/plant. This result leads to practical production estimates of 1.6 plants/box or 600 boxes/1000 plants, which are considered high, given the average of 2 plants/box or 500 boxes/1000 plants (Trento *et al*., 2021).

Table 2. Plant height (PH), root length (RL), leaf area (LA) and stem diameter (SD) of Italian tomato seedlings, adopting different stem diameters at the grafting moment. Venda Nova do Imigrante, UFES-UFV, 2020.

Stem diameter (mm)	PH (cm)	RL (cm)	LA (cm²)	SD (mm)
1.8	10.58b[1]	11.28[ns]	26.22b	3.18abc
2.0	10.86b	12.03[ns]	28.27b	3.13bc
2.2	12.08a	11.38[ns]	38.39a	3.34a
2.5	11.44ab	11.53[ns]	37.34a	3.22ab
2.8	11.16b	11.87[ns]	37.87a	3.03c
CV (%)	5.44	19.05	13.95	3.29

[1]Means followed by the same letter in the column did not differ significantly from each other according to the Tukey test at 5% probability level; ns: not significant according to the F test at 5%.

Table 3. Fresh mass of the aerial part (FMAP), fresh mass of roots (FMR), dry mass the aerial part (DMAP) and dry mass of roots (DMR) of Italian tomato seedlings adopting different stem diameters at the grafting moment. Venda Nova do Imigrante, UFES-UFV, 2020.

Stem diameter (mm)	FMAP (g)	FMR (g)	DMAP (g)	DMR (g)
1.8	1.74d[1]	0.64ab	0.16b	0.02c
2.0	2.03cd	0.51b	0.18b	0.03b
2.2	2.56a	0.52b	0.22a	0.04a
2.5	2.35ab	0.53b	0.19ab	0.03b
2.8	2.24bc	0.67a	0.17b	0.03b
CV (%)	10.72	17.07	15.79	14.87

[1]Means followed by the same letter in the column did not differ significantly from each other according to the Tukey test at 5% probability level; ns: not significant according to the F test at 5%.

Table 4. Average fruit weight, length and diameter of Italian tomato fruits, adopting different stem diameters at the grafting moment. Venda Nova do Imigrante, UFES-UFV, 2020.

Stem diameter (mm)	Average weight (g)	Fruit length (mm)	Fruit diameter (mm)
1.8	86.90a[1]	74.13a	55.34b
2.0	86.40a	75.96a	55.34b
2.2	88.70a	77.53a	55.80ab
2.5	86.50a	76.66a	56.59a
2.8	90.00a	75.71a	55.39ab
CV (%)	4.6	2.33	1.37

[1]Means followed by the same letter in the column did not differ significantly from each other according to the Tukey test at 5% probability level; ns: not significant according to the F test at 5%.

As a result of the high individual production, high productivities were obtained, with an estimated average above 200 t/ha (Table 5), which is much higher than the national average productivity of 65 t/ha in the field (Conab, 2019). These results highlight the success of the grafting technique in Italian tomatoes, using a highly productive scion on a rootstock with disease resistance and vigor.

In addition to the grafting technique, adequate cultivation conditions were also present, conducted in a protected environment with pest and disease control, excellent fertilization, and irrigation. It is believed that this condition allowed seedlings with inferior performance to recover and generate plants with productive indices similar to those of plants originating from seedlings grafted with a stem diameter of 2.2 mm, which showed the best performance in the seedling phase.

Thus, it was demonstrated that the choice of stem diameter at the time of grafting can influence the quality of the produced seedling. However, the reflection of this better performance in production indices can be influenced by management during the productive cycle, compensating for a possible poorer development in the seedling phase. Rodica *et al.* (2018) emphasized that cultivation conditions in a protected environment positively reflect the final productivity of tomatoes. Maurya *et al.* (2019) highlighted the factors influencing the success of the grafting process, among them the compatibility of the grafted materials and optimal cultivation conditions.

Therefore, it is concluded that Italian tomato seedlings, Benedetti® cultivar, grafted with a stem diameter of 2.2 mm, on the rootstock, Green Rise® cultivar, showed better development. However, production indices were similar for the different stem diameters (1.8 to 2.8 mm) adopted at the time of grafting.

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Table 5. Number of fruits, yield per plant and productivity of Italian tomato adopting different stem diameters at the grafting moment. Venda Nova do Imigrante, UFES-UFV, 2020.

Stem diameter (mm)	Number of fruits/plant	Yield (kg/plant)	Productivity (t/ha)
1.8	140a[1]	13.53a[1]	240.67a
2.0	146a	12.59a	224.88a
2.2	137a	12.20a	217.91a
2.5	144a	12.49a	223.06 a
2.8	146a	13.18a	235.40 a
CV (%)	11.4	9.1	9.1

[1]Means followed by the same letter in the column did not differ significantly from each other according to the Tukey test at 5% probability level; ns: not significantly according to the F test at 5%.

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