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Incorporación de tecnología robótica industrial convencional al proceso de injerto de plántulas hortícolas

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Incorporation of conventional industrial robotic technology to the grafting process of horticultural seedlings

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A la educación pública,
a quien se lo debo todo.

Al regalo más grande que he tenido en esta vida, mis hijas Carolina y Ana, por ser fuente de alegría constante, una gran inspiración y motor para seguir adelante. A María José, mi mujer, compañera y mejor amiga, por su incondicional apoyo y sacrificio, y por alentarme en este proyecto y aventura. A mis padres Pedro y María Ángeles, por ser para mí, verdaderos ejemplos de vida. A mis hermanos Pedro y Javier, por estar siempre ahí.

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A todos ellos, y a otros muchos de los que no me olvido, GRACIAS.

Resumen

El injerto es una técnica de mejora de producción vegetal, comúnmente aplicada al cultivo intensivo de hortalizas, y cuyo objetivo principal es la lucha contra enfermedades causadas por patógenos de suelo. La producción de plántulas injertadas es un trabajo repetitivo e intensivo, donde la tarea es habitualmente realizada a mano. La tasa de éxito depende en la mayoría de los casos, no sólo de las variedades empleadas, condiciones de cultivo y técnica de injerto utilizada, sino que también se ve influenciada por el grado de concentración del trabajador, la velocidad de trabajo, su experiencia, destreza y habilidad. La demanda de plantas injertadas para cultivos protegidos bajo invernadero es cada vez mayor, y para cultivos de solanáceas, tales como el tomate (*Solanum lycopersicum*), se ha generalizado el uso de la técnica de injerto de empalme.

La automatización mediante robotización de este proceso es más que una necesidad, y la presente tesis de investigación analiza y desarrolla, de modo experimental, como hacer frente a este desafío mediante el uso de robótica industrial convencional apoyada por sencillos equipos auxiliares que ayuden a la consumación de la tarea del injerto. Durante las últimas tres décadas la automatización del proceso ha evolucionado lentamente, y el desarrollo de máquinas semiautomáticas o completamente automatizadas de injerto de plántulas se ha materializado en unos pocos modelos comerciales con dispar índice de aceptación. En oposición a estos desarrollos, nuestra investigación utiliza como base de trabajo robots industriales convencionales, como tecnología asentada y madura en otros campos, aplicada al área de injerto de plántulas.

El sistema de estudio está constituido por dos brazos industriales funcionando de manera coordinada y apoyados por unidades auxiliares sencillas, que consuman la tarea de injerto mediante la técnica de empalme, sustituyendo por completo la labor humana. En el proceso, ambos robots toman con precisión las plántulas patrón y variedad desde bandejas de plántulas madre. Cada robot opera de manera independiente, manejando cada una de las plántulas, y portándolas hasta el punto donde se efectúa una disección a bisel del tallo por dos equipos de corte que nos permiten desprendernos de las partes no útiles, es decir, de la parte inferior en la variedad y de la parte superior en el patrón. Una vez realizado el corte, cada uno de los robots porta las partes útiles de las plántulas hacia la zona de unión, donde el injerto quedará consumado cuando las plantas estén ubicadas en contacto íntimo entre ellas, así como el clip de injerto colocado presionando sobre las mismas y garantizando la unión. Finalmente, uno de los robots, deposita el injerto en una nueva bandeja de salida.

A lo largo de la presente tesis han sido estudiadas distintas alternativas de trabajo, de modo que optimizasen le proceso robotizado de injerto. Para ello, en una primera fase se estudió la influencia combinada del ángulo de corte y de la diferencia de diámetros entre plántulas en el éxito de injerto de tomate mediante el uso de la técnica de empalme. Así mismo se estudió la influencia del ángulo de corte en la velocidad de sanación del injerto. Una segunda fase se centró en el estudio del comportamiento de diferentes estrategias de unión usando tecnología automatizada para la técnica de injerto de empalme. Finalmente, y mediante la aplicación sobre el equipo de los resultados anteriores se analizó la tasa de éxito en función de distintas velocidades de trabajo del sistema.

Palabras clave: injerto de tomate, técnica de injerto de empalme, injerto de tubo, injerto de corte oblicuo, injerto japonés de corte superior, robot agrícola, injerto automatizado, maquinaria agrícola, clips de injerto, ángulo de injerto.

Abstract

Grafting is a technique for improving plant production, commonly applied to intensive cultivation of vegetables, and whose main objective is to control diseases caused by soil pathogens. The production of grafted seedlings is a repetitive and intensive work, where the task is done by hand. The success rate depends in most cases, not only on the varieties used, growing conditions and grafting technique used, but it is also influenced by the degree of concentration of the worker, the speed of work, his experience, skills and abilities. The demand for grafted plants for protected crops under greenhouse conditions is increasing, and for solanaceous crops such as tomato (*Solanum lycopersicum*), the use of the splicing technique has become widespread.

Automation by robotization of this process is more than a necessity, and this research thesis analyzes and develops, in an experimental way, how to face this challenge through the use of conventional industrial robotics supported by simple auxiliary devices that helps to consummate the grafting task. During the last three decades process the automation of the process has evolved slowly, and the development of semi-automatic or fully automated seedling grafting machines has materialized in a few commercial models, with disparate acceptance rates. In opposition to these developments, our research uses, as a basis, conventional industrial robots as mature technology in other fields, applied to the seedling grafting area.

The study system is made up of two industrial arms working in a coordinated manner and supported by simple auxiliary units, which take over the grafting task by means of the splicing technique, completely replacing human labour. In the process, both robots take with precision rootstock and scion seedlings from mother seedling trays. Each robot operates independently, handling each of the seedlings, and carrying them to the point where a bevel dissection of the stem is performed by two cutting devices that allow us to remove the non-useful parts, that is, the lower part in the scion and the upper part in the rootstock. Once the cut is made, each of the robots carries the useful parts of the seedlings to the union area, where the grafting will be completed when the cut surfaces of seedlings are in intimate contact with each other, as well as the grafting clip placed by pressing on them and ensuring the union. Finally, one of the robots deposits the graft in a new exit tray.

Throughout this thesis, different work alternatives have been studied, in order to optimize the robotic grafting process. For this, in a first phase, was studied the combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique. The influence of the cutting angle on the healing speed of the graft was also studied. A second phase focused on studying the behaviour of different grafting strategies using automated technology for splice grafting technique. Finally, and by applying to the process the above results, was analyzed the grafting success rate as a function of different working speeds of the system.

Keywords: tomato grafting, splice grafting technique, tube grafting, slant-cut grafting, japanese top grafting, agricultural robot, automated grafting, agricultural machinery, grafting clips, grafting angle.

Índice

1. Introducción

1.1. Antecedentes

La intensificación de la producción de cultivos crea condiciones óptimas para el desarrollo de plagas que potencian el ataque de patógenos del suelo. Enfermedades transmitidas por el suelo generan problemas tales como Fusarium, Verticillium y nemátodos en los nudos de la raíz. Tradicionalmente estos patógenos habían sido controlados mediante la fumigación del suelo con bromuro de metilo (CH_3Br) o dicloropropeno ($C_3H_4Cl_2$). Estos productos en la actualidad están prohibidos y están catalogados como pesticidas tóxicos, suponiendo su utilización un riesgo grave para el medio ambiente y para la salud humana. Las plantas injertadas vienen a resolver este problema [1].

El injerto se puede definir como la fusión natural o deliberada de partes de la planta, mediante la que se establece una continuidad vascular entre ellas [2]. A pesar de que la técnica del injerto se ha practicado en los árboles frutales durante miles de años, el injerto en hortalizas no se desarrolló hasta bien entrado el pasado siglo, y no ha sido hasta el presente siglo, cuando ha crecido y se ha generalizado su uso para la producción de cultivos hortícolas a gran escala en los países occidentales [3,4]. El injerto es una práctica cultural que constituye un importante y rápido mecanismo de lucha integrada frente a plagas en el cultivo protegido en solanáceas y cucurbitáceas, potenciado claramente por el desarrollo de la agricultura intensiva bajo invernadero.

La realización de injertos para producción hortofrutícola está en plena expansión por las ventajas que dicho proceso supone, siendo una cómoda herramienta de selección de características, que no sólo logra aumentar la tolerancia a enfermedades transmitidas por el suelo, sino que lleva asociada una serie de ventajas, tales como un mejor comportamiento ante condiciones de estrés abiótico, un aumento del rendimiento y del tamaño de los frutos, una mejor asimilación de la nutrición mineral, captación y eficiencia de su uso, o un aumento de la vigorosidad de la planta, entre otras [5].

El desarrollo y la utilización de maquinaria en la producción vegetal para reducir la demanda de mano de obra humana, ampliar las capacidades de producción y mejorar la uniformidad de los productos es una necesidad reconocida [6]. En los países agrícolas avanzados se están realizando esfuerzos para desarrollar y utilizar equipos de injerto automático y existe una tendencia importante de que los robots de injerto con potencial de mercado se desarrollen en lugar del injerto manual [7-9].

1.2. Justificación

Tres vectores fundamentales vienen a justificar la investigación de esta tesis:

- **CONTEXTUALIZACIÓN SOCIOECONÓMICA:**

Se estima que hacia el año 2050 se lleguen a superar los 9700 millones de habitantes en el planeta, frente a los 7700 millones de habitantes que se alcanzaron a comienzos del año 2020, produciéndose gran parte de este crecimiento en países en vías de desarrollo, que constituyen por sí mismos potenciales entornos emergentes. Es por todo ello que la producción agrícola deberá aumentar en un 70% para alimentar a la población prevista, y esto debe ser logrado a través de una sostenibilidad socioambiental de los recursos naturales. De dicho aumento de producción, se estima que el 90% se logre con un aumento del rendimiento, la tecnificación y la intensificación de los cultivos [10].

Además, y de manera preocupante, los cultivos agrícolas se ven día a día afectados por factores limitantes y perjudiciales que diezman la producción. Factores restrictivos abióticos, tales como el uso de suelos no propicios para el cultivo, sequía, temperaturas extremas, alta salinidad, inundaciones, baja cantidad de nutrientes, contaminación orgánica y de metales pesados entre otros; así como restricciones bióticas, tales como plagas y enfermedades transmitidas a través del aire o suelo, condicionan la producción agrícola [11]. Esta situación se ve agravada por un aumento en la práctica de cultivos intensivos, que es inevitable para asegurar la alimentación y los ingresos de la mayoría de los agricultores.

La técnica del injerto debe ayudar y es medio de solución para tal fin. El uso del injerto viene asistido por políticas ambientales que apuestan por una eliminación gradual de desinfectantes químicos del suelo como respuesta a una mayor concienciación social, y los efectos negativos sobre el cambio climático. El injerto, por lo tanto, puede ser usado como un vector estratégico más, en respuesta a estas previsiones, ya que es una técnica reconocida de producción de plántulas de enorme importancia.

- **VENTAJAS DE LA ROBOTIZACIÓN DEL PROCESO:**

La investigación y el desarrollo de un equipo automático de injertos, basado en robots industriales convencionales, que dé soporte a la actividad del injerto, y por ende favorezca la tecnificación del proceso, aporta innumerables ventajas frente a una serie de condicionantes, tales como:

Población agrícola cada vez más envejecida: Este es un hecho cada vez más significativo en países desarrollados del este asiático y europeos, zonas donde precisamente, la técnica del injerto está más extendida y es ampliamente utilizada, lo que en ocasiones puede llegar a originar carencia de trabajadores cualificados.

Fuerte estacionalidad: El injerto presenta cargas de trabajo intensas durante cortos periodos productivos, limitados a las fechas de plantación de cada campaña, lo que hace aún más crítica la premura de trabajo y exigencias de personal esporádico que haga frente a una alta demanda productiva.

Trabajo altamente repetitivo: El proceso supone la ejecución cíclica de un injerto tras otro durante largas jornadas laborales. Además de implicaciones de tipo musculoesqueléticas y ergonómicas con consecuencias directas, es importante la aparición de un déficit de atención y de concentración derivado de la monotonía operativa.

Coste de mano de obra dedicada: El precio de plantas injertadas es muy superior al de plantas sin injertar. En parte, este hecho es debido a la importante repercusión de costes que supone la mano de obra en la elaboración de los injertos, llegando según algunos estudios a suponer en torno a un 25% del total de los costes por plántula injertada [12,13].

Necesidad de formación especializada: Injertar es una operación manual intensiva que requiere de cierta cualificación y experiencia. Es muy susceptible a errores humanos, y de la habilidad y destreza del operario dependerá en gran medida el éxito del proceso.

Contagio de patógenos por el contacto humano: Una manipulación directa por parte de operarios siempre engloba una posible contaminación de las heridas y partes expuestas de las plantas. La incompatibilidad del injerto causada por virus y transferencia de patógenos es reconocida como una de las principales causas de fracaso de los injertos.

El precio del injerto: El uso de equipos y sistemas que automaticen la tarea del injerto está directamente asociado al coste de mano de obra invertido en tareas de injerto, pudiendo llegar a suponer un ahorro de dos terceras partes del personal dedicado. Además, a mayor velocidad de trabajo, menores serán los costos por planta [14].

La escala productiva: Debido a las altas exigencias de producción de plántulas injertadas, estas son generalmente adquiridas en viveros especializados, en lugar de recurrir a la autoproducción. Los viveros de media escala suelen realizar injertos por millones. La utilización de plantas injertadas necesita del desarrollo de una industria auxiliar especializada en la manufactura, de modo que, a mayor escala productiva, mayor necesidad de tecnificación del proceso.

Disminución del tiempo de injertado: La velocidad de trabajo media empleada por operarios expertos puede rondar los 150~240 injertos/hora, con unas tasas ente 81% y el 91% de éxito [12,13,15]. Dichas tasas de éxito son susceptibles de mejora tanto en velocidad de trabajo como en tasa de éxito mediante el uso de equipos de injerto automatizados.

Flexibilización del sistema: La flexibilidad de la robótica industrial convencional, frente a sistemas más rígidos y dedicados, permite la reprogramación y adaptación del sistema y una factible reasignación del equipo a otras tareas dentro de un entorno productivo durante periodos de menor demanda.

- **ROBÓTICA INDUSTRIAL:**

Las nuevas generaciones de robots industriales tienden a ser máquinas cada vez menos costosas, a la vez que sus capacidades y prestaciones se incrementan, siendo equipos accesibles y atractivos para una creciente gama de industrias y entornos productivos, como puede ser la manufactura de injertos en viveros.

La elección de un desarrollo basado en robótica industrial convencional puede aportar un mayor control del proceso y una mejora sustancial en cuanto a la calidad de los injertos realizados, así como una reducción de tiempos y de costes de producción, entre otros. Además, la robótica industrial brinda un grado de versatilidad importante en el entorno productivo en viveros, puesto que son equipos abiertos a una fácil reconfiguración ante la variabilidad natural de plántulas de trabajo, e incluso la reasignación a otras tareas productivas. Para ello es necesario contraponer la respuesta del equipo desarrollado frente a otras técnicas de injerto utilizadas, manuales y automatizadas, quedando abierta la verificación de idoneidad de su uso.

1.3. Hipótesis

Habida cuenta de los vacíos detectados y del estado del conocimiento, se postula la siguiente hipótesis de trabajo como conjetura probable, que se ha pretendido demostrar y validar a lo largo del trabajo de investigación de la presente tesis doctoral:

Hipótesis de trabajo (H_i): Es plausible el desarrollo y uso de un equipo para el injerto de tomate (*Solanum lycopersicum*), basado en el trabajo coordinado de dos robots industriales convencionales con el apoyo de dispositivos sencillos de manipulado que logren unos resultados óptimos en cuanto a velocidad de injerto y tasa de éxito.

Hipótesis nula (H_0): No es plausible el desarrollo y uso de un equipo para el injerto de tomate (*Solanum lycopersicum*), basado en el trabajo coordinado de dos robots industriales convencionales con el apoyo de dispositivos sencillos de manipulado que logren unos resultados óptimos en cuanto a velocidad de injerto y tasa de éxito.

1.4. Objetivos

- **OBJETIVO GENERAL:**

El objetivo principal que persigue el presente trabajo de investigación, es, evaluar el grado de adecuación y de éxito de un equipo para el injerto de plántulas hortícolas, y concretamente para el injerto de tomate (*Solanum lycopersicum*), desarrollado a partir de la adaptación y optimización de la técnica de injerto de empalme, y basado en el trabajo coordinado de dos robots industriales convencionales con el apoyo de sistemas y equipos auxiliares de manipulado.

- **OBJETIVOS ESPECÍFICOS:**

El objetivo principal del proyecto de investigación se logrará mediante la consecución de los siguientes objetivos específicos:

- El desarrollo de un prototipo de equipo que plantee una solución tecnológica distinta a las existentes actualmente para la tarea de injerto de tomate, haciendo uso para ello e incorporando tecnología procedente de la robótica industrial convencional, tan eficiente en otros campos productivos.

- El desarrollo de equipos auxiliares complementarios, de funcionamiento sencillo, con los que se logre la consecución de la tarea de injerto: sistemas de corte y preparación de plántulas, y equipo para el conformado y colocación del clip de injerto.

- La optimización y adecuación de las subtareas en el proceso de injerto, de los equipos y de los sistemas implicados; así como el análisis de condicionantes y variantes que garanticen una mayor eficiencia y grado de éxito del conjunto desarrollado en el proceso de injerto.

- La contrastación y comparación de los resultados de trabajo de nuestro desarrollo con otras estrategias, metodologías y técnicas de trabajo de injerto conocidas (injerto manual u otros equipos automatizados o semiautomatizados documentados).

1.5. Estructura de la tesis

La tesis se estructura de la siguiente manera:

En primer lugar, y a lo largo del presente **Capítulo 1**, se exponen los principales problemas y alicientes que motivan la realización del presente trabajo. Se realiza una revisión del estado de la técnica y se realiza una contextualización de la investigación desarrollada a lo largo del presente trabajo de tesis.

La tesis doctoral se presenta como un compendio de trabajos, recogidos a lo largo del **Capítulo 2**. Los títulos de los artículos y trabajos que constituyen el cuerpo de la tesis son los siguientes:

- [A1: Article]: Combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique.
- [C1: Congress]: Effect of cutting angle in the speed of healing under splice grafting method.
- [A2: Article]: Behaviour of different grafting strategies using automated technology for splice grafting technique.
- [A3: Article]: Conventional industrial robotics applied to the process of tomato grafting using the splicing technique.

A lo largo del **Capítulo 3** se realiza un resumen de resultados de cada una de las publicaciones, mostrando los resultados principales y complementarios obtenidos.

En el **Capítulo 4** se exponen conclusiones generales y específicas asociadas a cada una de las publicaciones derivadas de la presente tesis doctoral.

Para finalizar, el documento también ofrece un **Capítulo 5** final, a modo de apéndice, con la bibliografía referida en el presente trabajo de tesis.

2. Publicaciones científicas

2.1. [A1] Combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique.

Título: Combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique.

Tipo de Publicación: Artículo científico (aceptado y publicado)

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Article

Combined Influence of Cutting Angle and Diameter Differences between Seedlings on the Grafting Success of Tomato Using the Splicing Technique

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Abstract: Herbaceous crop yield intensification creates favourable conditions for the development of pests that intensify the attack of soil pathogens traditionally controlled by disinfectant, which are mostly prohibited and unlisted because of their toxicity. The use of grafted plants solves this problem and assists in addressing abiotic stress conditions. Within Solanaceae, specifically tomato crops (*Solanum lycopersicum*), the use of the splicing technique (simple and easily automated) is of special interest. This experiment attempts to present the combined influence of cutting angle and different random diameters on grafting success with the objective of detecting an optimum working range that will be applicable to automated and robotic grafting systems. An increase in the grafting angle is associated with a higher survival of grafted plants despite variations in diameter. Moreover, a threshold cutting angle is observed from which the success rate no longer increases but decreases drastically. Therefore, for a given working range with a significant cutting angle, whether the seedlings of origin are similar in diameter is not important, and this factor is more influential outside the optimal cutting angle range.

Keywords: tomato grafting; splice grafting technique; graft angle; random diameter

1. Introduction

The objective of the experimental study was to determine the combined importance of random rootstock and scion diameters at different cutting angles on splice grafting success. The proposed working hypothesis suggests that both parameters have a statistically significant relationship with grafting success and an optimum working range can be defined to achieve successful grafts.

The experiment was developed as part of a larger study to optimize working conditions for the automation of grafting via the splicing technique. The study is autonomous and independent and presents sufficient and consistent results for the definition and specification of the splice grafting conditions that provide the most optimal results, whether performed manually or automated.

Grafting can be defined as a natural or deliberate fusion of plant parts by which vascular continuity is established [1], so that the resulting organisms function as a single plant [2].

The portion of the upper tissue or crown of a plant, which is also known as the stem or scion, adheres to another portion of the plant, specifically its root and lower part, which is commonly called rootstock, under stock or stock, and both parts come in contact and join with each other so that the resulting composite plant grows and develops as a single organism (graft). The callus corresponds to the mass of parenchyma cells that develop from the plant tissue of the scion and the rootstock around the wound and where the development of vascular connections of the resulting graft union occurs [3].

Reducing the impact of pathogens is a challenge in all agricultural production systems [4], and monocultures are even more vulnerable than more diversified agricultural production systems [5]. Thus, grafting has become a tool of enormous potential to quickly enhance the efficiency of modern vegetable cultivars to promote wider adaptability or resistance to different stress situations [6].

The sequence of structural and biological events that occur in the development of a compatible graft between plants has been described in many studies, and the following development pattern is observed in which three fundamental phases can be distinguished: fusing of the rootstock and scion; proliferation of the callus around the union; and vascular re-differentiation through the interface establishing continuity between rootstock and scion [1,3,7,8]:

1. The meristematic tissues of the stem are placed in direct contact with the tissues of the rootstock. Once both components of the graft are in intimate contact, cambium cells from the rootstock and the scion produce parenchyma cells that fuse forming a callus tissue [9]. This first phase of cohesion that forms the callus is a reaction similar to wound healing, and it does not require recognition between rootstock and scion, occurring in both compatible and incompatible grafts.
2. If the graft is compatible, a differentiation of certain vessels and sieve tube elements of the phloem is observed in the callus, and they are not derived from the cambium and constitute the first transitional and continuous union between the rootstock and the scion.
3. In the last part of the grafting process, the cambium layer newly formed in the bridge of the callus begins its own meristematic tissue activity, thus forming new vascular tissue. Production of these new vascular elements that join xylem and phloem allows establishing a symplastic communication between rootstock and scion [1].

The success of the graft performed with a variety of compatible seedlings is determined by the three events previously described, assuming an important role of the plant hormones related to growth, such as auxin, in the grafting process [10]. Thus, the graft may originate from a combined mechanism of wound healing and conductive vessels formation [11]. Therefore, vascular connection is the last event in a successful healing process and represents the most important event because once such a vascular connection is established, water and solute transport begins from the stock to the scion, and the mechanical strength at the graft union increases [7,12]. One difficulty is to understand when the grafting process is completed [13], since a simple technique for continuous evaluation of graft development is not available [14]. Nonetheless, the assessment can be based on various techniques, including destructive and non-destructive techniques as follows:

(1) Visual estimation of the constituent seedlings and the appearance of graft growth [15]; (2) thermal camera imagery because the temperature of the leaves is 2 to 3 °C lower than that in a failed graft due to the transpiration of a successful graft [16]; (3) vertical cut performed on the graft surface and observation of the curvature of the vascular system formed at the union [17]; (4) measurement of the electrical resistance transferred from the scion to the rootstock through the surface area of its connection, which undergoes variations associated with histological changes during the union of the rootstock and the scion [14]; (5) assay performed to evaluate the tensile strength of the graft and analyse the strength of the graft union between rootstock and scion until breaking [18,19]; (6) displacement transducers used to perform a continuous and non-disruptive evaluation of the functional hydraulic connections within the plant [20]; and (7) NMR-based method (Nuclear magnetic resonance), that reveals water flux vectors inside individual vessels of intact plants [21].

The tomato is one of the world's most important herbaceous crops [22], and grafting of tomato plants is a widespread practice. Grafting methods among seedlings vary greatly and considerably depending upon the type of crops, farmer's experience and preferences, availability of facilities and machines, the number of grafts to perform and even the purpose and destination of the grafts, i.e., whether they are for the farmer's own uses or for sale and commercial distribution [23]. Grafting is a common practice among herbaceous crops, and its use for Solanaceae is highlighted as follows: cleft or split grafting [21,24], splice or tube grafting [25,26], plug-in grafting [27], double-stem grafting [28,29], or pin grafting [15,30], among others.

Splice grafting, also known as tube grafting, top grafting or slant cut grafting, is the most popular [31] and widely used technique for tomato as well as eggplant [32]. The rootstock is cut below the cotyledons, thus eliminating the need to continually eliminate the sprouting of the stock over the plant's life [33]. The scion is also slant cut on a complementary angle above the cotyledons. Both parts are then placed in contact and secured by means of a tube or elastic tube-shaped clip with side slit. This method has the disadvantage of being highly demanding in terms of post-grafting microclimatic conditions, which require meticulous timing and delicate handling after the cut until healing and the maintenance of optimal temperature and humidity conditions to stimulate rooting [34]. As an advantage, the splicing method allows grafting with smaller plants, which reduces the pre-graft cultivation time and takes up less space in cultivation chambers and nurseries [35].

Velasco [12] and Villasana [36] affirmed that successful grafting is contingent upon similar stem diameters and the alignment of the vascular cambium area. However, such claims are dependent on the effect that the seedling diameter variable has on grafting success and do not consider the interrelated influence of other parameters and constraints of the grafting process.

For studying the success or failure of splice grafting, Yamada [37] established three factors of importance for its execution: (a) area of the cut surface; (b) gripping force of the union clip; and (c) smoothness of the cut surface and sharpness of the blade. For the first factor, allusive to the coincidence rate between seedlings, from coincidences of 50% graft successes of over 85% were achieved, and up to 95% success rates were observed for alignments over 80%; all this referred exclusively to a test angle of 30°, so these results were obtained regardless of the effect of the cutting angle on the success of the process.

Furthermore, although the number of vascular bundles does not affect the grafting success, differences in diameters between the rootstock and the scion [38], that mark the alignment between them do have an effect. Thus, when a graft is performed, it is important to increase the chances that the vascular bundles from the rootstock and the scion come in direct contact, maximizing the area of the cut surfaces that are joined by pressing them together [39].

For manual and automated grafting, the alignment of diameter of both seedlings must be identified, classified, and visually paired. This is an expensive and arduous task that applies a series of calibrations and visual comparison criteria based on morphological characteristics, which may be subjective and susceptible to human error [40,41]. The present study analyses the importance of these pre-grafting tasks based on grafting success and whether an optimal working zone can be established that guarantees adequate grafting success without having to pre-sort the seedlings.

2. Materials and Methods

2.1. Definition of Operating Conditions

The experimental study was carried out at the Tenova Technological Center: Foundation for Auxiliary Technologies for Agriculture (Centro Tecnológico Tecnova: Fundación para las Tecnologías Auxiliares de la Agricultura) in Almería between March and June 2017. Almería's surroundings correspond to a model of agricultural exploitation of high technical and economic performance of greenhouse herbaceous fruit crops, especially for the tomato crop (Figure 1).

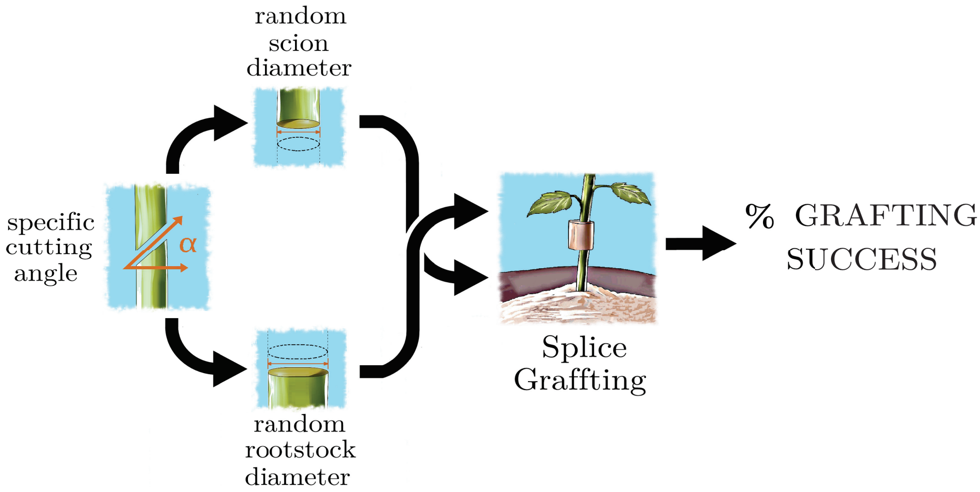


Figure 1. Working procedure of the experimental study to determine the combined importance of random rootstock and scion diameters at different cutting angles on splice grafting success.

For the study, rootstocks of the interspecific hybrid KNVF (*L. esculentum x L. hirsutum*) were used since it is the most used stock for tomato grafting [42–44]. The commercial rootstock Maxifort was used because of its strong roots and vigour and good performance at lower temperatures and in high salinity conditions. It presents high resistance (HR): ToMV: 0–2/Fol: 0,1/For/PI/Va: 0/Vd: 0; intermediate resistance (IR): Ma/Mi/Mj [45]. Likewise, the Ventero variety has been used as a grafting scion as an indeterminate tomato hybrid for truss harvesting, and it presents medium vigour, with good foliar coverage, very uniform fruits, slightly flattened of good red colour and deep shine, very good cracking and micro-cracking tolerance, and compact and well-formed clusters. It presents high genetic resistance (HR): ToMV: 0–2/Ff: B, D/Fol: 0,1/Va: 0/Vd: 0; and intermediate resistance (IR): TYLCV/Ma/Mi/Mj [39]. Both varieties are commonly used for manual grafting using the "tomato on tomato" (ToT) splicing technique, which demonstrated their compatibility prior to the experiment.

The working environment during the study was maintained under stable and controlled environmental conditions throughout all grafting experiments, with temperatures oscillating between 20° and 25°, relative humidity conditions occasionally forced between 75% and 90% and stable brightness conditions of natural in-direct light. Oda [39] indicated that grafting must be performed in the shade in an area protected from the wind and the sun to avoid wilting of grafted seedlings. Grafting was performed at the lowest period of plant transpiration during morning hours [46], between 8 h and 12 h to maintain transpiration similar among the experiments and at the time period when the transport of water from the roots to the leaves is slowest, which makes the graft less susceptible to water stress and therefore to water loss. Other parameters with possible influence, such as atmospheric CO2 and other air contaminants, were not been controlled.

In the nursery, the plants were cultivated and attended to from sowing until 25 to 35 days after, and the scions were sown 2 to 5 days before the rootstock seeds. This variability of days is determined by the growth rate since different plants require different germination periods [47], and such periods are directly related to climatic conditions of the month of growing.

For the experiment, the plants were considered mature and ready for grafting when they had 2 to 4 well-defined true compound leaves [32], preferably with little foliage, thus decreasing the transpiration demand and post-grafting stress. The peat root ball remained wet but not soggy at all times during the grafting process, thus ensuring proper root respiration. The substrate used was 80% black peat with 10% perlite and 10% mulch. The experiments were always conducted with seedlings whose stems were still green and tender (herbaceous and non-woody).

For the splicing method, the seedling stems diameters should be at least 1.5 mm [48], and not too thick but with some natural and random variation. In the study, the diameters in the area close to the cut have varied from 1.5 to 2.5 mm for the scion and 2 to 3 mm for the rootstock.

The matching of the rootstock and scion samples was established randomly among plants that were healthy, had an acceptable anatomy and growth and presented diameters between the established reference limits. Seedlings with anomalous growth and diameters outside the established range were discarded. Diameters were measured using a digital calliper with a resolution of 1 dmm (0.1 mm) and repeatability of 1 dmm in the areas close to where the cut was performed both for the rootstocks and for the scions. The cut in the rootstock was always performed below the cotyledons, whereas the cut in the scion was performed above the cotyledons.

The complete experiment consisted of 10 individual events of 150 grafts each distributed over 4 months. Each graft consisted of 10 series of 15 grafts per tested angle. Therefore, 10 representative angles of the possible cutting range were selected: 0°, 10°, 20°, 30°, 40°, 50°, 60°, 70°, 80° and 85°. Once each of the experiments was performed, grafts were grouped for healing on a single grafting tray of 15 × 10 cells, placing each group of grafts of a given angle in each of the 10 rows of the tray. Each of the grafted plants was rotated within their row for every tested angle eliminating in this way the position factor and its possible effects (ventilation and luminosity among others).

2.2. Device Description

For the experiment, two seedling-cutting machines were implemented to ensure the accuracy of the cutting angle required for each test and the integrity of the dissected seedling. The machines were similar and complementary to each other, where one was designated for cutting the rootstock and the other for cutting the scion. Each of the machines had a double acting dual rod cylinder, model CXSM15-15 by SMC, which operated at 3.5 bars and used dry-pressed air to produce a clean bisection of the seedling via a stainless steel cutting blade (type BA-160-9 mm from NT Cutter) that can be attached as a tip and is interchangeable with an adjustable inclination angle, thus providing optimal sharpness (Figure 2). To ensure a clean cut, the machine has a fitting notch adapted to accommodate the seedling, ensuring the verticality of the stem ahead of the blade and another notch fitted for the blade at each cutting angle. The blade was replaced prior to each experiment (150 grafts per blade), and it was below the limit of 5000 grafts per blade established by Yamada [35], who determined that as the number of cuts per blade increases, the roughness of the cutting area becomes notable, thus reducing the grafting success. Before each use, the blade was cleaned and disinfected as Bumgarner suggests by soaking in alcohol [45], exposure to flame and air drying.

Figure 2. Cutting device. As can be seen, a fitting device is used to guarantee or ensure the verticality of seedling in the cutting process, beside a groove for insertion of the cutting blade. A specific fitting device for each cutting angle was developed. Once inserted, the seedling is disectioned in two by a sharp blow of the sharp blade.

The union of the seedlings cut by the machines was executed manually using the traditional splice grafting technique described by Oda [49] and expanded on by DeMiguel [33]. For this, plastic grafting clips of different lengths were used according to the tested angles. Clips were cut from a continuous flexible transparent plastic roll and outfitted with lateral wings for opening and placement to allow for easy observation of the success or failure of the graft. The clip diameter was less than 3 mm, and the shape was slightly oval, which guaranteed a better grip when the rootstock and scion diameters were unequal. Grafting clips that were too long inhibited the attached graft union, and clips that were too short exerted too much pressure and deformed the graft union [50].

Manual handling of the seedlings was always performed after thorough washing with antibacterial soap. Direct contact with the wounds was constantly avoided. Once grafted, the plants were placed on the tray and introduced into a healing chamber, similar to a small acclimatization tunnel as described by Oda [51]. This chamber was itself placed inside a larger growth chamber that allows for the appropriate basic environmental conditions as follows: 14 h photoperiod and a daily light integral (DLI) of ≈100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR, (~3000 Lux) of indirect diffuse light during the callus development stage [52,53], temperature of 26 °C and relative humidity of 95%.

After the trays were transferred to the chamber, the plants were not manipulated or moved for 3 full days so that the natural healing process was not disturbed. From the fourth day of the graft, the first individual plant by plant visual inspection was conducted inside the chamber. This inspection was routinely repeated during the following days from the 4° DAG (day after the graft) until the 9° DAG to assess changes and the healing process in each plant and therefore the success or failure of the graft. During this period and from the 6° DAG, the environmental conditions of the chamber were gradually relaxed, acclimatising to external environment, and the inside chamber was opened to decrease the humidity and temperature, acording to outside. Between the 10° DAG and the 14° DAG, the plants were eventually removed from the growth chamber and allowed to develop without being transplanted.

While the rootstock and scion establish a vascular connection during the first days [54], at least 14 days are needed for the graft union to heal completely and be considered functional. After 14 DAG of performing daily observations for each experiment, the experiment was ended. Grafts that did not survive the healing process within the stipulated period were considered failures.

The success or failure of the graft has been evaluated by daily visual estimations and observations that evaluated the development of the graft and analysed other external symptoms and evidence, such as physiological abnormalities or signs of wound healing and scarring. Symptoms of internal failure generally precede those of external failure [55]. If the graft is successful, evident progress is generally seen from the wilt stage to greater vigour in the aerial part of the graft, which is reflected in a palpable recovery and associated with a gradual disappearance of signs of dehydration, which implies that adequate vascular continuity has been generated among the elements of the xylem. In addition, this factor is accompanied with the occurrence of axillary buds in the aerial part, thus indicating that the graft is successful and the resulting plant is functional. These factors are used to determine whether the graft is successful. Regardless, the behaviour and subsequent evolution of the grafts continue to be evaluated until 14 DAG to corroborate and validate the evolution of the natural healing process of the graft (Figure 3).

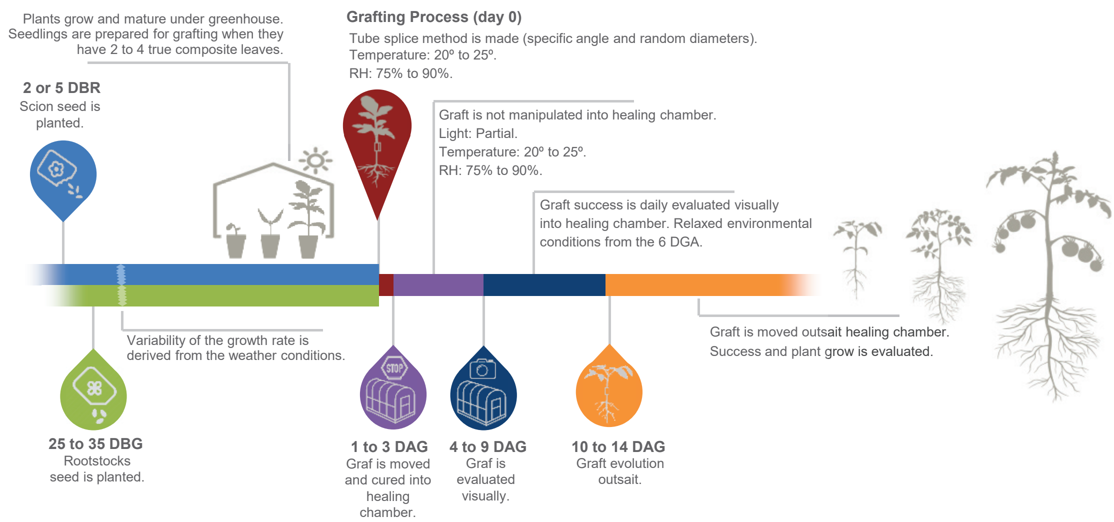


Figure 3. Timeline of the grafting process developed. DBR (days before rootstock has been planted). DBG (days before grafting process). DAG (days after grafting process).

3. Results and Discussion

The results of the 1500 individual experiences were collected and grouped polytomously in 10 sections of equal height to compare the cutting angle and differences in diameter of rootstock and scion and to evaluate the grafted plant survival for each case.

The data analysis process included two stages: the first phase consisted of conducting a descriptive analysis of the data distribution and their correlation through the application of One-way ANOVA for Randomized Complete Block Design (RCBD ANOVA), and the second phase consisted of a two-way analysis of variance in which only one sample per group was run, and the results were then assessed by a post-hoc comparison test, such as Student's *t*-test.

3.1. Data Analysis: Descriptive Statistics

An analysis of the experimental results showed that the effect of variations in diameter on the grafting success decreases as the grafting angle increases, and the differences are nearly negligible in the range between 50° and 80° and between 60° and 80°, where percentage changes between the grafted seedling and the successful graft were maintained at an overall success rate of greater than 90% or even greater than 95%, respectively. This finding confirms that for greater angles, the success probability depends less on the diameter of the seedlings. From 80° onward, successful execution of the graft began to be materially more complicated due to two factors: physical limitations related to the technology used for the cutting and subsequent union of the seedlings; and the exponential increase in the sectioned surface that was directly related to the tangent of the cutting angle, which determined both the exposed surface and the rigidity and firmness of the structure of the dissected and subsequently joined seedlings.

Grouped data confirm that independent of section variation among the seedlings of origin, a working zone between 50° and 80° offers good results in terms of graft success (Figure 4).

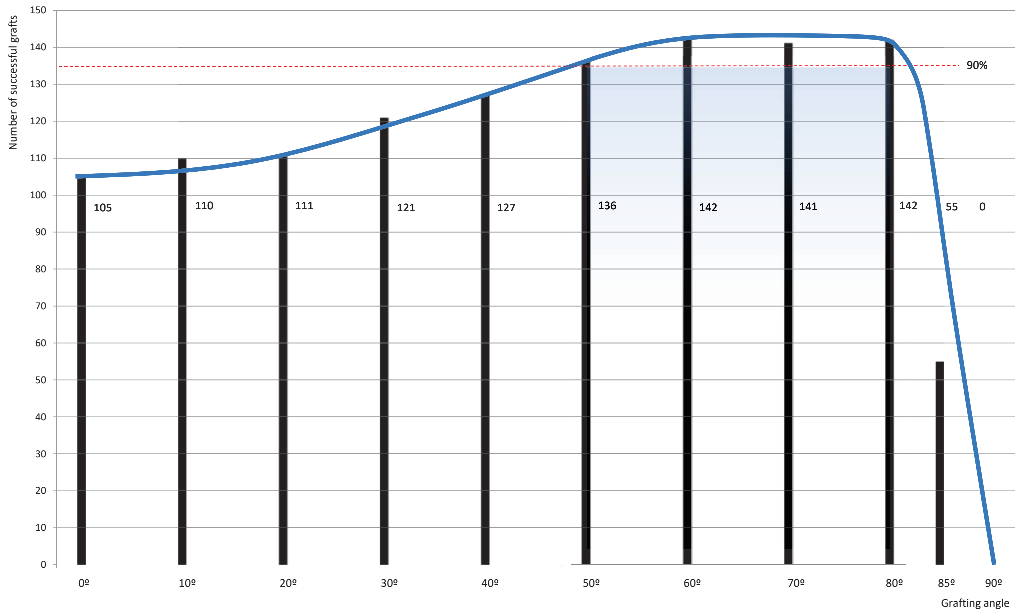


Figure 4. Distribution of successful grafts according to different cutting angles. It can be seen that the zone between 50° and 80° (blue zone), has a success rate higher than 90%, so we can consider it an optimal work zone. This graph represents the absolute number of successes for each cutting angle, without considering the variable of difference between diameters.

Having studied the diametric differences with respect to grafting angles, it is apparent that at small cutting angles and with highly variable diameters, the failure probability is high, and success was not observed, while at larger cutting angles, success associated with high diametric differences was recorded. A slightly greater range between quartiles is observed at angles between 50° and 70°, which indicates a greater tolerance to variable diameters during the grafting process (Figure 5).

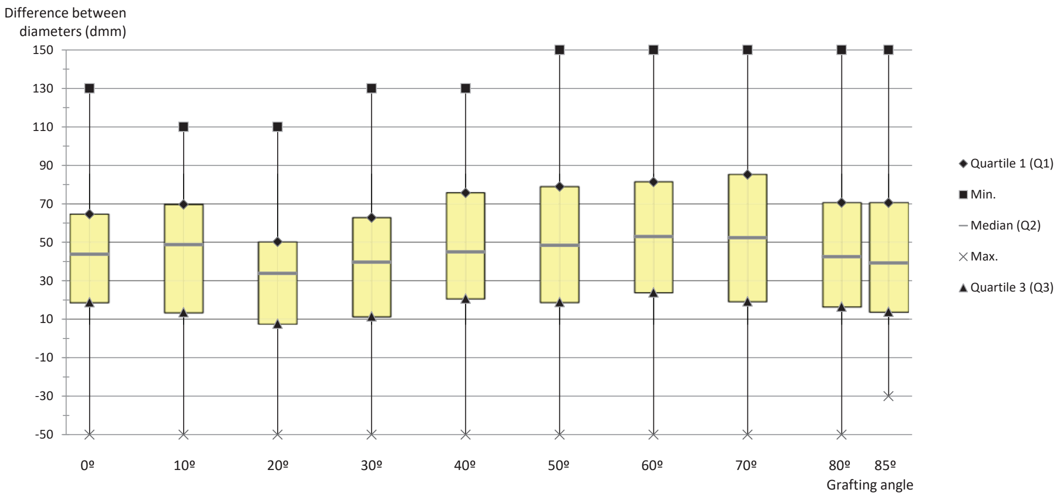
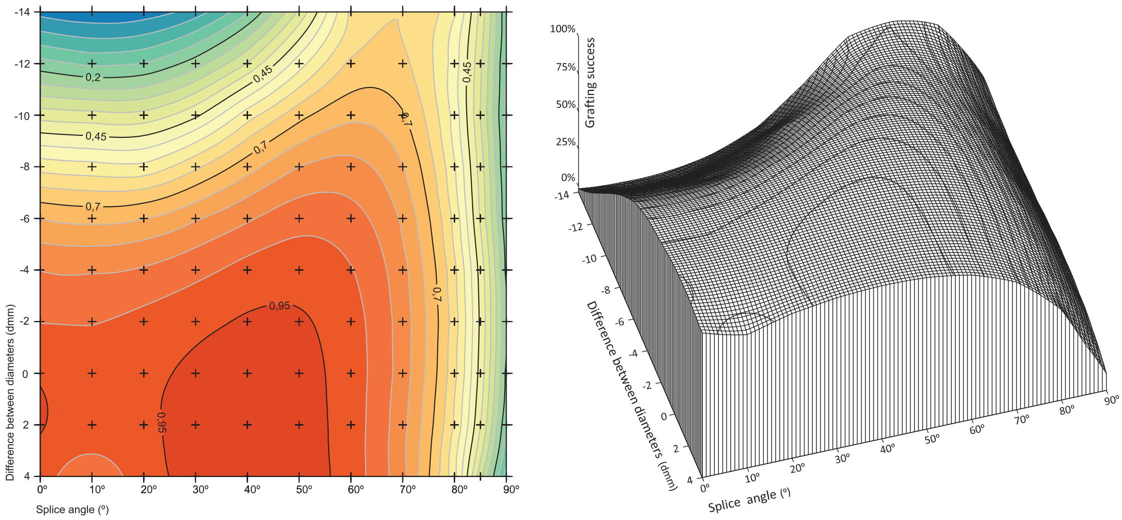


Figure 5. Distribution of successful grafts according to the differences between diameters of plants for each angle of union. This graph represents the density function of successes for each cutting angle (1 dmm is a tenths of a millimeter, 10^{-4} m). A slightly greater amplitude between quartiles is appreciable at angles between 50° and 70° degrees, which indicates a greater tolerance in this range to the disparity of diameters.

The combined representation of cutting angle and diameter differences between plants versus the success of the graft provide evidence of the combined effect that both factors have on the successful execution of the graft. This representation has been developed through the use of the software Surfer12 and the Local Polynomial gridding method for the interpolation of points of the spatial matrix, which only uses points within the defined neighbourhood and adjusts the matrix to a first-order polynomial to the power of two. Polynomial interpolation allows us to create a uniform surface and identify long-range trends in the data set (Figure 6).



(**a**) (**b**)

Figure 6. Graphical representation of the combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splice grafting thecnique. These graphs represent the successes for each concrete difference of diameters, associated to each angle of union (1 dmm is a tenths of a millimeter, 10^{-4} m). Results represented by the Local Polynomial Gridding Method (Polynomial Order 1, Power 2). (**a**) Coloured contour diagram of successful grafts (%), as a function of cutting angle (°) and difference between diameters of grafted (dmm); (**b**) 3D wireframe of successful grafts (%), as function of cutting angle (°) and difference between diameters of grafted (dmm).

At small differences in diameter, the success rate is high for cutting angles between 30° and 60°, whereas when this difference between diameters increases, the maximum values move to values between 50° and 70°, and the percentage of success gradually decreases.

One of the possible causes that justify grafting success within this range of working angles is the exponential increase of the contact surface, which increases in equal measure the possibility of matching between vascular bundles arranged in a circle around the stem [34]. Effective contact depends on the surface and arrangement of the bundles in the two plants that are grafted; therefore, at a larger cutting surface with an appropriate arrangement and matching of the seedlings, a greater area of contact is observed. Thus, this range of graft angles between 50° and 70° is associated with a decrease in failure and substantially less importance and influence of uniformity in stem diameter between both plants on grafting success.

By increasing the difference between the sections at the point of union and at greater graft angles, the area of contact surface increases exponentially, thus increasing the probability of vascular correlation. Moreover, for uncovered surfaces, the area remaining outside the contact area is exposed to pathogens, such as bacteria and fungi, which cause the graft to fail [56]. In addition, greater stress associated with scarring is evident based on the proliferation of a larger callus in response to the wound. As the cutting angle approaches 90°, the successful execution of the graft begins to become more mechanically complicated due to the technology used in the process and the firmness of the dissected seedling themselves. In addition, the uncovered or unmatched surface between the seedlings increases excessively.

Therefore, when significant differences in diameter occur between the rootstock and the scion, the probability of failure is higher at small cutting angles close to horizontal, while the probability of success is higher at similar diameters. This correlation reflects the farmer's own practice and experience and represents a frequently observed factor that is directly related to the success of the graft as observed in the first known publication referring to seedling grafting for herbaceous crops, which indicated that seedlings with similar diameters should be selected [57]. However, subsequent studies have corroborated the direct relationship between the cutting angle and the success of the graft [19], which supports the premise that seedlings should have similar diameters in the cutting zone. Zhao [58] stated that expanding the area of contact between the rootstock and scion is the key to graft survival.

3.2. Data Analysis: ANOVA

The experimental results for grafting success were tested via two-way ANOVA of the cutting angle and diameter difference, where each of these factors has been grouped into 10 blocks, with a single sample or repetition per group (ANOVA). The randomized complete block design (RCBD ANOVA) analysis technique used as the usual standard for agriculture was used, where similar experimental units were grouped into blocks. We consider $\alpha = 0.05$ (95% confidence level). The statistical package Real Statistics Resource Pack 5.8 in Microsoft Excel 2010 was used for the study. Analysis of variance was performed to answer the following general research question (RQ): Are statistically significant differences observed between the means of grafting success for different cutting angles and different diameters between seedlings? (Tables 1 and 2).

Table 1. Analysis of variance of two factors without replication. Factor 1: the difference between the rootstock and scion diameters, where the positive values represent a larger diameter of the rootstock and the negative ones a larger diameter of the scion. Factor 2: cutting angles of the seedlings.

Analysis of Variance of Two Factors without Replication				
Summary	**Count**	**Sum**	**Mean**	**Variance**
15 to 13 dmm	10	3.3333	0.3333	0.1806
13 to 11 dmm	10	3.7242	0.3724	0.1253
11 to 9 dmm	10	5.8789	0.5879	0.1009
9 to 7 dmm	10	7.1258	0.7126	0.0589
7 to 5 dmm	10	8.4487	0.8449	0.0545
5 to 3 dmm	10	9.0306	0.9031	0.0202
3 to 1 dmm	10	9.1009	0.9101	0.0245
1 to (−1) dmm	10	9.2110	0.9211	0.0284
(−1) to (−3) dmm	10	9.2333	0.9233	0.0239
(−3) to (−5) dmm	10	8.6000	0.8600	0.1071
Summary	**Count**	**Sum**	**Mean**	**Variance**
0°	10	6.7621	0.6762	0.1398
10°	10	6.3766	0.6377	0.1391
20°	10	6.0410	0.6041	0.1725
30°	10	6.9445	0.6945	0.1644
40°	10	7.5000	0.7500	0.1405
50°	10	8.5650	0.8565	0.0645
60°	10	9.5815	0.9582	0.0031
70°	10	9.1847	0.9185	0.0117
80°	10	9.4521	0.9452	0.0022
85°	10	3.2792	0.3279	0.0326

Table 2. Combined influence of the cutting angle and the difference betwen diameters in graft success. All statistical analyzes were done using a significance factor of 95% ($p \leq 0.05$). ANOVA summary tables (1 dmm is a tenths of a millimeter, 10^{-4} m). The result of the analysis ANOVA (two factors without replication) indicates that the statistical value of "F" is much higher than the critical value for "F" for both factors: angles and differences between diameters. Therefore, we can assure that the results of our tests are significant.

ANOVA						
Sourface of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean of the Squares**	**F**	**Probability Value**	**Critical Value for F**
Diameter difference	4.7160	9.0000	0.5240	13.6138	0.0000	1.9976
Cutting angle	3.4002	9.0000	0.3778	9.8154	0.0000	1.9976
Error	3.1178	81.0000	0.0385			
Total	11.2340	99.0000				

After running the ANOVA analysis, the null hypotheses H0 were rejected for both cases, and the alternative hypotheses Hi were accepted. Therefore, confirmable cases of significant differences between success means and cutting angles and seedling diametric differences were observed with a 95% confidence. To compare the differences, post-hoc rank tests were conducted to determine which means differ from each other. Student's *t*-type comparison tests (RCBD ANOVA and *t*-test) were performed (Table 3).

Table 3. Comparison of means differences between angles using Student's *t*-test (*t*-test). Use of contrasts to determine whether there is a significant difference ($p \leq 0.05$).

	0°	**10°**	**20°**	**30°**	**40°**	**50°**	**60°**	**70°**	**80°**	**85°**
0°		N	N	N	N	Y	Y	Y	Y	Y
10°			N	N	N	Y	Y	Y	Y	Y
20°				N	N	Y	Y	Y	Y	N
30°					N	N	N	N	N	Y
40°						N	N	N	N	Y
50°							N	N	N	Y
60°								N	N	Y
70°									N	Y
80°										Y
85°										

Significant differences in the mean grafting success values were not observed for similar angles, whereas clearly significant differences were observed when larger angles were compared, especially for angles equal to 20° or less and angles equal to 50° or greater. A cutting angle of 85° produced significant differences in the mean grafting success compared with most angles, including angles close to each other and distant, since the response of grafting success to cutting angle was random and irregular (Table 4).

Variations in the diameters of grafting seedlings greater than 90 cmm produced significant differences in the mean success with respect to the other variations in diameter, which may be due to a random and unpredictable response to the success of the graft from these diametric differences. The remaining variations in diameters below 90 cmm did not produce significant differences between their success means.

Table 4. Comparison of means differences between diameter of seedlings using Student's *t*-test (1 dmm is a tenths of a millimeter, 10^{-4} m). Use of contrasts to determine whether there is a significant difference ($p \leq 0.05$).

	15 to 13 dmm	13 to 11 dmm	11 to 9 dmm	9 to 7 dmm	7 to 5 dmm	5 to 3 dmm	3 to 1 dmm	1 to (-1) dmm	(-1) to(-3) dmm	(-3) to (-5) dmm
15 to 13 dmm		N	Y	Y	Y	Y	Y	Y	Y	Y
13 to 11 dmm			Y	Y	Y	Y	Y	Y	Y	Y
11 to 9 dmm				Y	Y	Y	Y	Y	Y	Y
9 to 7 dmm					N	Y	Y	Y	Y	N
7 to 5 dmm						N	N	Y	N	N
5 to 3 dmm							N	N	N	N
3 to 1 dmm								N	N	N
1 to (-1) dmm									N	N
(-1) to (-3) dmm										N
(-3) to (-5) dmm										

4. Conclusions

A review of the scientific literature suggests that the success of the splice grafting process increases as the cutting angle increases as long as the union is based on similarities between the stems of the grafting seedlings. However, until now, the success rate based on the interaction between the cutting angle and diameter of the seedling has not been reported.

The present study has concluded that the success of a graft depends to a great extent on the cutting angle and diameter of the seedlings, with disparities or similarities between the seedling sections playing an important role in the success of smaller cutting angles, although the stem diameter shows decreasing importance as the cutting angle increases, with the minimum influence of diameter observed within a cutting angle range of 50° and 70°.

Consequently, using the splicing technique with a cutting angle between 50° and 70° can lead to a substantial improvement of grafting conditions and techniques and can eliminate, to some extent, the random factor of differences between diameters as well as the pre-selection and matching requirements for sections between seedlings, thereby simplifying the demands for manual, automated and robotized grafting systems.

Author Contributions: J.-L.P.-A. conceived and designed the experiments, performed the experiments, collected, analyzed the data and interpreted the results, and developed the manuscript. A.C.-O. conceived and designed the experiments, analyzed the data, interpreted the results, and developed the manuscript. C.-C.M.-G. conceived and designed the experiments and performed the experiments. A.-J.C.-F. provided constructive suggestions on experiment analysis.

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Conflicts of Interest: The authors declare no conflict of interest.

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2.1. [C1] Effect of cutting angle in the speed of healing under splice grafting method

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Effect of cutting angle in the speed of healing under splice grafting method

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Abstract

Grafting of tomato plants using the technique of graft by splicing has an increasingly widespread use due to its high success rate, simplicity and ease of automation. This technique allows to control variables such as the cutting angle of the two seedlings to be grafted, among others. The aim of this experiment has been to evaluate the speed of healing, evolution and success of the graft, subject to different angles of union between seedlings, assessing time necessary until an increase of vigor and sturdy of the plants is appreciated, with disappearance of evident signs of wilt of the upper part and the appearance and proliferation of new axillary buds. This inflection point was estimated through a daily visual inspection of the seedlings made between 4 and 9 days after the grafting operation. For its study a total of 1500 grafts were made, distributed in 10 cutting angles, between horizontality and verticality, made with precision thanks to anautomatic cutting device developed for this purpose

As result, it became evident that the greater the graft angle, and under equal environment of maturation, not only the success rate was greater, but the clinical recovery time decreased, contributing with a faster recovery and less time to stay in the healing tunnel. This decrease in cure time continued beyond the worsening of the success rate of the graft, verifying that successful grafts reduced their healing time for a higher cutting angle, between 60º and 80º.

One of the possible causes that justifies this relation of cutting angle and speed of healing is the exponential increase of contact surface between seedlings, that on the one hand increases in equal measure the possibility of correspondence between vascular bundles, while on the other hand, generates greater stress in healing due to the need for proliferation of a greater callus dimensions in response to the wound.

Keywords

Splice grafting for tomato, Solanum lycopersicum, Days of healing, Healing chamber, Grafting angle.

Introduction

Vegetable grafting is considered as a practice that allows to grow one plant with the root of another. On the one hand, the rootstock used provides resistance to some diseases present in the soil to which the cultivated plant or scion is sensitive, and on the other providing greater vigor of the root system (De Miguel, 2005). Cultivated varieties used under greenhouses differ from varieties used for field crops, and are usually adapted specifically to conditions such as lower light, higher humidity and temperature, as well as a better resistance to diseases (Kemble, 2019), (Rouphael, 2018).

Grafting procces is a faster solution than the genetic improvement, obtaining a more successful resulting plant, with advantages such as resistance to the diseases of the rootstok or a better adaptation to water stress and the horticultural characteristics of the variety to be grafted. (Ozores-Hampton, 2010). Advantages provided by the use of grafted plants are greater than the increase in additional costs that derive from their production and handling, such as the purchase of rootstock seed, the percentage of failures in the grafting operation, the indirect costs of the grafting operation or the addition of days of production and cares before and after grafting (Rivard, 2010).

In 2013, the world market for seeds of grafted plants came to represent a global value of more than 370 million dollars, and was estimated that in the past 2018 would be reached 650 million US$dollars, corresponding 50% of this increase to tomato rootstocks (Enzo, 2015). Splice graft thecnique has an increasingly widespread use due to its high success rate, simplicity and ease of automation of the process, being widely used for tomato plants grown in glasshouses, and allowing with few operations and with relative simplicity to achieve plants of greater resistance and productivity. Using graft splicing, growers can graft two to three times faster than with other grafting techniques, optimizing costs, time and space (Johnson, 2011).

One of the most critical factors in the splicing process is post-graft healing, where a callus is formed and the vascular bundles of the stem and the rootstock are reconnected. By means of an optimum acclimatization, the healing of the graft union and the strengthening of the plants is sought before their definitive transplant to the greenhouse, where they will face their production stage (Lee, 2003).

This stage is critical for the survival of grafted plants, consisting on special conditions of care, based on a period of convalescence and recovery under a healing chamber with precise conditions of luminosity, temperature and humidity. The healing process requires initially very restrictive conditions, followed by a final period of re-acclimatization to the external conditions. Acclimatization involves healing of the graft union and hardening of plants before planting in the field or greenhouse; this stage is critical for the survival ofthe grafted plants (Lee, 2003); (Singh, 2017).

Time spent in the healing of the graft is affected by numerous factors, such as are climatic conditios, operational procedure or the seedling conditioning. For example, studies show that shoot removal (SR) or leaf removal (LR) techniques applied to the stem during the grafting process slows down the healing process after grafting, although success rates increase (Loewen, 2017).

With this study, we have evaluated the effect and influence of the cutting angle on the healing speed in the splice graft method, under the same healing conditions and independently of the graft success.

Materials and methods (experimental procedures)

The study was conducted at the facilities of the Technological Center Tecnova: Foundation for the Auxiliary Technologies of Agriculture, located in Almería (36º52'38''N, 2º19'59''W).

The experiment consisted of 1500 tomato grafts made using the splice grafting technique, distributed in 10 cutting angles between horizontality and verticality. These cutting angles was 0º, 10º, 20º, 30º, 40º, 50º, 60º, 70º, 80º and 85º. Grafts made were of intraspecific type. The rootstock used was Maxifort, while the variety used was Ventero, both from De Ruiter Seeds. Tests were performed and distributed in 10 replicatesgroups of 150 grafts, consisting of 15 grafts for each of the angles tested, and conducted during the months of March to June 2017. The experimental study, in addition to value the success of the graft versus the cutting angle (pardo-alonso, 2019), allowed to estimate the recovery and healing speed for each grafting angle.

Seedlings cutting process was carried out by a mechanical device specifically designed for this purpose, and equipped with a blade confronted to the seedling to cut, which makes the cut or dissection by a sharp blow and precise of the blade against the stems of tomato plants. Seedlings are fitted in a groove that forces their verticality in front of the cutting angle, and in this way we can perform a precise split into two the seedlings to graft.

Plants begin to wilt immediately after cutting and grafting, so they were promptly placed on plant growing trays, avoiding direct contact with the wounds in the handling. Graft were placed into a small healing chamber which was composed of a tunnel (0.8 m long x 1.0 m wide and 0.5 m high), covered by a transparent film. This tunnel was introduced inside of a walk-in chamber in which the climatic conditions were controled throughout the healing process. During the first 48h, the plants were maintained without lighting to reduce transpiration and evaporation. In following days light intensity was increased, and a photoperiod of 14 hours of light of value ≈100$\mu mol \cdot m^{-2} \cdot s^{-1}$ of PAR, (~3,000 Lux) of non-direct and diffused light was established, as recommended several studies during the callus formation stage. This lighting corresponds to a value a little above the compensation point. There is also evidence that a high intensity of light prevents callus formation (Hartman, 2011). Lighting level was gradually increased after several days.

Temperature was established with a variable setpoint in the curing chamber between 23° to 30°C, with an average of about 26°C, varying slightly the daytime and nighttime conditions recorded. Relative humidity (RH) was established initially between 75% to 95%, trying to reduce the rate of transpiration of the variety, avoiding high stress and thus preventing it from drying out the graft (Johnson, 2011). Humidity level was gradually reduced on successive days for conditioning to outside.

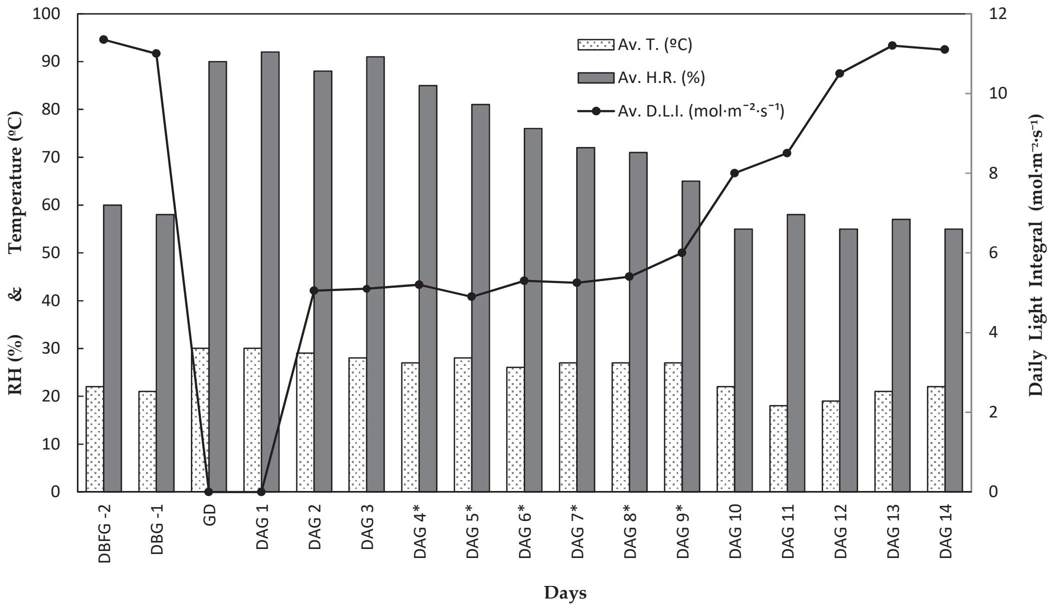
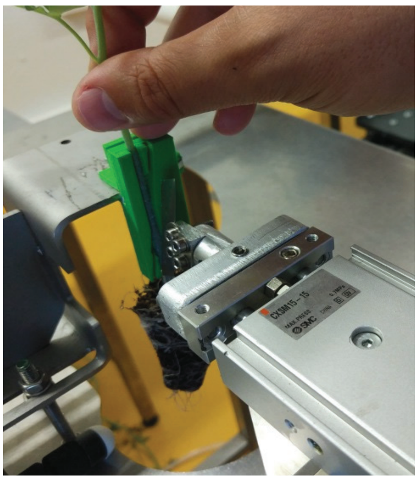


Figure1. Controlled environmental conditions to conduct the experiments. Relative Humidity (RH), Temperature (T) and Daily Light Integral (DLI) recorded during the days before the graft (DBF), the day of the graft (GD) and the days after the graft (DGA). The parameters were measured with adequate instruments.

During the firsts three days the grafts were not manipulated, and after of the fourth day the grafts were subjected to a thorough daily visual inspection, assessing the alterations and the healing process in each of them, and consequently, the success or graft failure. During the first days and due to the loss of water from the aerial part of the graft, linked to the lack of vascular continuity that provides an adequate renewal and flow through the vascular bundles, it was easy to appreciate a state of wilt.

a)



b)

Figure 2. a) Automatic device for cutting seedlings with a precise angle. b) Small healing chamber covered by a transparent film introduced inside of a walk-in chamber for the grafted seedling trays.

There are destructive and non-destructive techniques for the evaluation of graft success. The accurate observation of the appearance and evolution of the grafting junction, the use of thermal cameras, the performing and study of a vertical cut on the graft surface, the measuring the electric wave transferred from the stem to the rootstock, tests evaluating the tensile strength of the graft, the use of displacement transducers, or by NMR (Nuclear Magnetic Resonance) based methods, are techniques usually used. Non-destructive techniques and little aggressive during handling were used.

External symptoms and evidences were estimated, such as physiological abnormalities and withering or signs of healing and reviving of the wound through the formation and firmness of the callus formed at the junction point. For the evaluation, well-defined qualification criteria were used, mostly dichotomous and assessed by visual inspection, such as vigor or sagging on leaves and stems, the consummation of the outer layers of the callus and the proliferation of callus cells until appreciate the filling of all the gaps in the junction, the appearance of necrotic areas in the junction area, significantly substantial differences between rootstock and scion growth, discoloration or yellowing of the leaves and premature defoliation or the progressive appearance of new buds axillary in the variety.

If the graft evolves correctly, an increase of stems vigor begins to be appreciated, accompanied by a gradual decrease of signs of dehydration, the recovery of the leaves color, and even the appearance of new axilliary buds in the scion that certify this recovery and evolution of the graft. All these factors implicitly reveal that an adequate vascular continuity has been generated between the elements of the xylem, and from this moment the grafted plant can be manipulated and transplanted to a greenhouse, considering therefore, consummate the graft and that the resulting plant is functional. Cares and direct observation of grafts continued to be carried out until 14 days after the graft, corroborating and certifying the evolution in the natural process of graft healing.

Junction and healing process are influenced by several factors. In the present study, we evaluated a correspondence between cutting angle and grafting healing, independently of the success associated with the grafting angle, as influencing factor to reduce the stay time post-graft in the healing chambers of nurseries.

Results and discussion

The natural days established for the graft success were evaluated between the fourth and ninth day. It was determined by means of explicit evidences for each graft the day in which the healing process was concluded, there being a suitable vascular continuity and a callosity formation that ensured the graft junction.

Results achieved, regardless of the grafting success with differents union angles, shows that there is a significant relationship between recovery days and the angle of cut made in the graft process. It is observed that the greater the cutting angle made in the junction, the healing and acclimatization process is accelerated, and therefore, a shorter time is used within the culture chamber for the grafting recovery. It can be seen that for small cutting angles, between 0º and 20º, the graft healing time was extended over 7 days, and this was gradually reduced following a decreasing logarithmic tendency to values slightly greater than 5 days for high cutting angles, above 60º. This significant reduction in terms of healing time translates into less critical convalescence, requiring less attention from intensive care.

Table1. Comparison between means of the t levels of a factor after having rejected the null hypothesis of equality of means by means of the ANOVA technique. Grouping of comparisons applying Tukey's HSD test (Honestly-significant-difference). Grouping of comparisons applying Fisher's LSD test (Least significant difference).

Angle	N	*Mean*	*Variance*	St.*Dev*	SS	St.*Error*	95% CI	Grouping (Tukey HDS)	Grouping (Fisher LDS)
0º	105	7,419	1,034	1,017	107,562	0,114	(7,196; 7,642)	A	A
10º	110	7,045	1,402	1,184	152,773	0,111	(6,828; 7,263)	AB	B
20º	110	6,855	1,548	1,233	170,234	0,111	(6,637; 7,072)	B	B
30º	121	6,322	1,654	1,286	198,430	0,106	(6,115; 6,530)	C	C
40º	128	6,078	2,120	1,456	269,219	0,103	(5,876; 6,280)	CD	C
50º	136	5,794	1,854	1,361	250,235	0,100	(5,598; 5,990)	DE	D
60º	142	5,521	1,230	1,109	173,437	0,098	(5,329; 5,713)	EF	DE
70º	141	5,255	0,977	0,988	136,809	0,098	(5,063; 5,448)	F	EF
80º	142	5,232	0,818	0,904	115,331	0,098	(5,041; 5,424)	F	F
85º	55	5,182	0,559	0,748	30,182	0,157	(4,874; 5,490)	F	EF

This factor, associated with the success rate registered for each cutting angle, allows us to assess optimal working conditions, which provide us an adequate success rate, beside a low number of convalescence days, which a lower cost of stay in growing chambers.

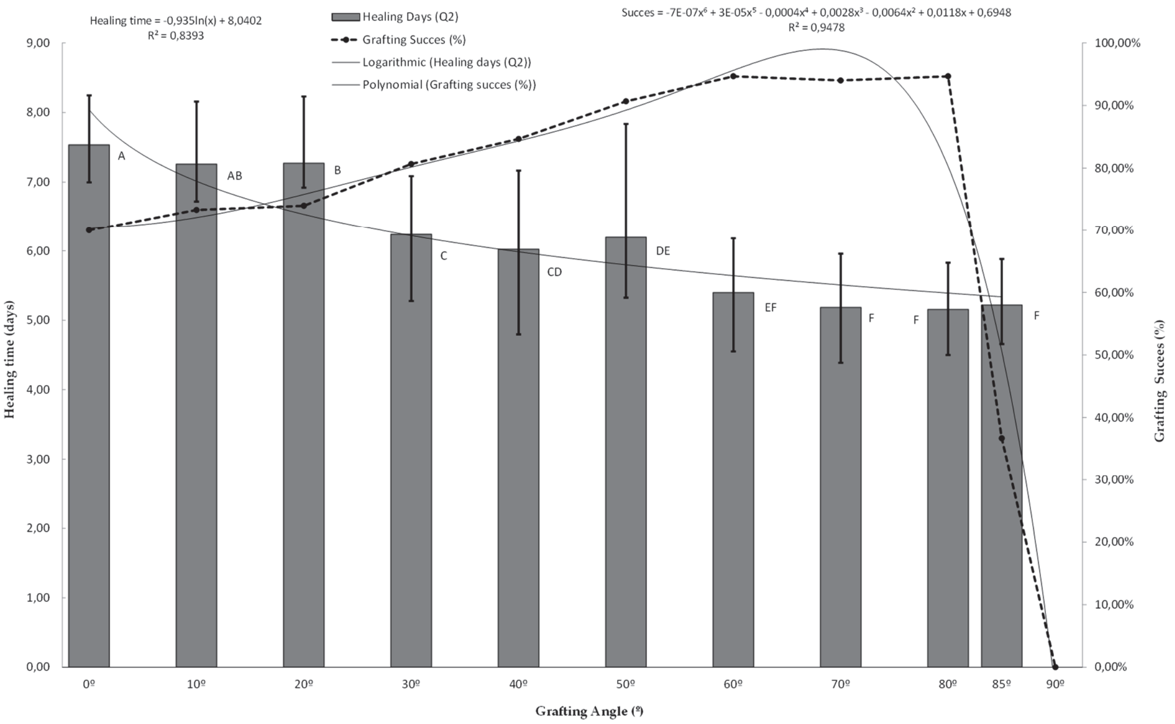


Figure3. Healing days, variance (Q2) and interquartile travel (Q1 and Q3) for different angles of graft cutting and junction. Grouping of comparisons applying Tukey's HSD test for healing days. Approximation by a logarithmic regression for healing days at differents cuttings angles. Grafting success for different angles of cut and junction of grafts. Approximation by a polynomial regression for grafting succes at differents cuttings angles.

At cutting angles between 60º and 80º, the success rate is maximum, producing records of more than 90%. Within this range of successful angles, it can be verified that the healing speed minimizes the period of convalescence at a greater cutting angle, so it will be of interest to work within this range of large cutting angles to reduce the convalence time.

Each cuting angle is associated to a contact surface, which will fluctuate depending on the thickness of the seedlings to be linked together. It is evident that the greater the contact surface, the greater the possibility of contact between vascular bundles, despite the fact that a greater wound surface is established for scarring and callus formation, which supposes a greater stress of callous tissue formation and an increased risk of infection or entry of pathogens that damage the graft, together with greater complexity and dexterity necessity in the grafting operative procedure, alongside the need of a clip of length adapted to the size of the wound.

Conclusion

It is evident that at a higher cutting angle, regardless of its success rate, there is a faster clinical recovery of the grafted plant, which implicitly implies a lower cost in clinical care in healing chamber in production nurseries.

Likewise, successful grafting plants, with a high cutting angle, tend to develop a bigger callus, healing in a smaller number of days, achieving a more robust junction of the callus that allows its manipulation and normal growth in production greenhouses.

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Article

Behavior of Different Grafting Strategies Using Automated Technology for Splice Grafting Technique

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Abstract: Even though the splicing graft technique is relatively recent, it has become the most commonly used grafting method for solanaceae, and in particular, for tomato. Today, almost everyone has standardized the use of plastic or silicone grafting clips, equipped with manipulating wings and a frontal opening, to ensure proper bonding and allow for wound healing. Numerous factors influence the success or failure of the grafting process, factors such as the seedling varieties combined, climatic conditions, pre-graft and post-graft care, cutting point, cutting angle, pressure of the clips, blade edge, or substrate water content, among others. In this work, several alternatives in the graft assembly and coupling protocol were evaluated. Having studied the different working alternatives for grafting using a robotic system, two modes of joining order were analyzed. It has been shown that there are 20% more recorded successes if one first joins the graft seedlings and then places the grafting clip to guarantee their union. In addition, we studied the different orientation alternatives for the cutting line and the seedling union with respect to the clip opening—there were approximately 10% more successes obtained in grafts where the splice-union cutting line between the two plants faced the clip opening.

Keywords: tomato grafting; splice-grafting technique; agricultural robot; automated grafting; agricultural machinery; grafting clips; tube grafting; slant-cut grafting; Japanese top grafting

1. Introduction

Horticultural seedling grafting is a relatively new technique, but its growth and importance are highly significant today [1]. It originated in Japan and Korea in the early 20th century, although it was not documented in a research journal until 1927 [2–5]. Its beginnings in horticulture in the late 1920s and early 1930s are associated with the cucurbitaceae family, where primitive grafting techniques were applied to watermelon, and later to cucumber. The first records collected for the solanaceae family were on eggplant grafts in the 1950s, while its use on tomato was not commercially introduced until the 1960s [3,6,7]. Very slowly but progressively, it was introduced in western countries in the early 1990s, yet it was not until the 21st century that interest in its use for large-scale horticultural crop became widespread [8,9].

The uptake of grafting is motivated and encouraged mainly in response to policies that have opted to gradually eliminate chemical disinfectants from the soil as methyl bromide, which have very negative effects on the environment, and whose use is currently prohibited [10,11]. This prohibition comes in response to increasing social awareness, and also to a growing interest in developing organic and integrated agriculture. Although, today, the fight and resistance to soil pests and viral diseases remains the main objective for applying this technique [12], there are other reasons and particularities justifying its use. It is a faster and more effective tool for selecting desirable crop characteristics than natural screening, selection processes, or species hybridization [13,14]. Grafting increases the health and precocity of the plant, providing stronger and more vigorous root systems than in ungrafted plants, which leads to improved water and nutrient absorption [15–17]. Improved mineral nutrition can result in the significantly lower use of agrochemicals during cultivation [9], as well as better adaptability to environments where there is abiotic stress and potentially harmful and unfavorable conditions, such as water stress, thermal stress, and saline stress; there is also better performance in response to high heavy-metal concentrations [5,12,14,18]. In addition, grafted plants provide higher productivity, with fruit that is significantly larger than that from ungrafted plants [19] while other fruit characteristics, such as their shape, skin color, flesh texture and color, or concentration of soluble solids, are negatively influenced by the rootstock, despite being generally assumed as hereditary stem characteristics [15].

Grafting is a common practice in high value-added cucurbitaceous and solanaceous crops [20]. There are two basic grafting methodologies: One is where the grafting process retains the two root systems, that of the rootstock and of the scion (tongue approach grafting, side grafting); and the other is that in which a shoot from the scion is joined to the rootstock plant (cleft grafting, hole grafting, splice grafting) [21,22]. In the first case, less strict environmental conditions are required during the grafting period and the post-graft phase [23], but its application is complex in solanaceae, mainly because the plants are usually grafted very young when stem thickness is minimal (Figure 1).

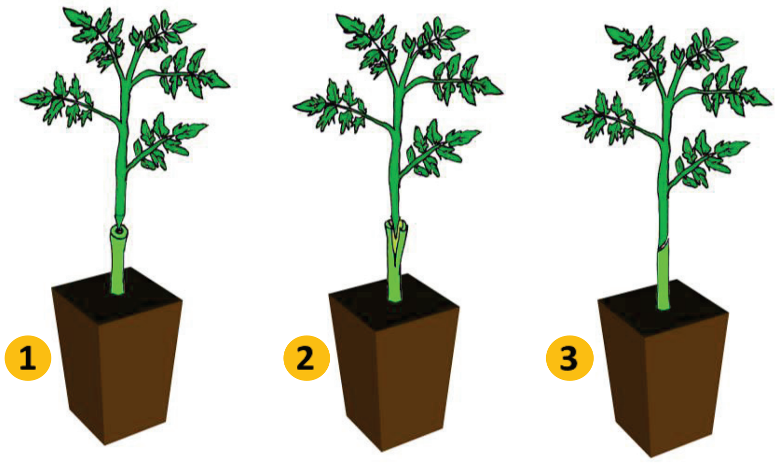


Figure 1. Common grafting methods to join scion and rootstock for solanaceae. Highlights the use of splice grafting technique. It is widely used as it is efficient and easy to execute. ① Hole grafting, ② cleft grafting, ③ splice grafting.

Splice grafting (also called slant-cut grafting, Japanese top grafting, or tube grafting) is currently the most common grafting method for tomato (*solanum lycopersicum*) worldwide [24]. The splice graft has a high success rate, is relatively simple to execute, and is even amenable to automation, so a large number of plants can be grafted in a short period of time [25]. Despite being a relatively recent technique, the splice grafting process has developed exponentially, especially when applied to solanaceae, and has been widely described and defined in much of the literature [25–29].

In the splice graft technique, the rootstock is cut (straight or beveled) below or above the cotyledons. A pronounced cutting angle creates more surface area than a flat cut, allowing more cells on the cut surfaces of the rootstock and scion to be in contact and, thus, fuse better. When cutting above the cotyledons, it is necessary to remove the rootstock regrowth throughout the life of the plant, whereas a graft below the cotyledons can facilitate the rooting of the scion because of its proximity to the ground, so that the grafted scion can bridge the rootstock, casting adventitious roots while completely removing the meristemal tissue from the rootstock [30]. The shoot is also bevel-cut with a complementary angle and a stem height that guarantee a similar diameter at the union. Both parts must be placed in close contact, holding together the union with a grafting clip or other passive device that ensures pressure between the cut surfaces of the rootstock and the shoot. One must make sure that the cambium area between the two seedlings is well aligned, ensuring good contact between the shoot and the rootstock's vascular system [28,31]. One advantage of the splicing method is that it allows grafting with smaller plants, which reduces pre-graft growing times and the space required in the growing chambers [22,32].

The first recorded splice graft tests were developed with graft tubes in the late 1950s, but these tubes were neither elastic nor had the side opening that they have today [33]. In the early 1990s, elastic tubes were developed and applied, very similar to those used nowadays, and, thus, achieved grafting times 2 to 3 times faster than the conventional method used up until then [34–36]. To ensure fusing between the plants, in the graft union, one can use a ceramic or bamboo pin fixed inside the stem, glue or adhesive plastic tape, and a clamp or an elastic clip in the form of a tube with or without a side slit [9,37,38]. In most countries today, there is standardized use of elastic tube-shaped clips with a longitudinal opening and side wings that facilitate handling and clip opening when positioning (Figure 2). Once the clips are in place, they tighten and fasten the seedlings securely during their healing phase.

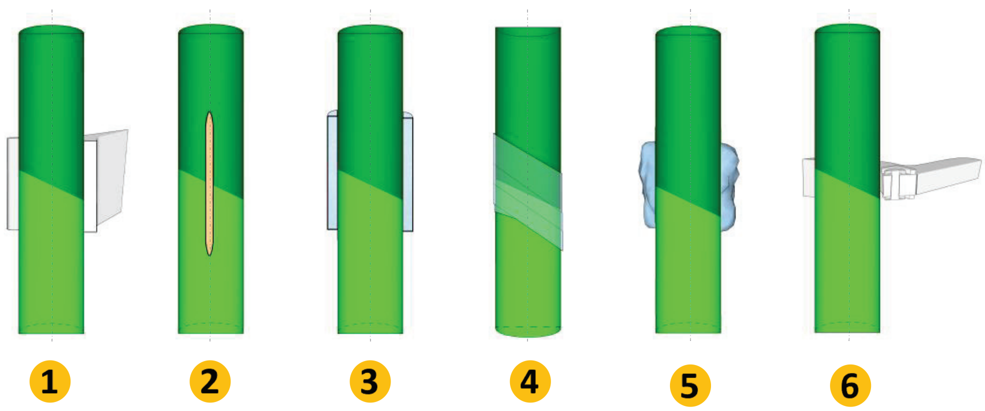


Figure 2. Common bonding and clamping systems used for solanaceae grafting. Highlights the use of clips. A section of the graft junction is showed, where the rootstock is light green and the variety is dark green. ① Clip, ② pin, ③ tube (with or without side opening), ④ adhesive plastic film, ⑤ glue, ⑥ gripper.

The use of graft pins has been common in south-east Asian countries but limited to those areas where the grafting clip use is not possible or they are not available, because of the added difficulty involved in the insertion operation. Using grippers is more common for thick stems such as cucurbitaceae; they are also significantly more expensive elements compared to tube-shaped clips. The grippers also need to be removed manually, whereas the tube clips will release by themselves as the plant grows (if they have a longitudinal opening); if they are closed tubes, they are manufactured using a material that expands as the stem thickness increases and degrades naturally over time [39]. Moreover, using tube-shaped elastic clips ensures a smoother grip and more distributed pressures, as well as minimizing water loss from the graft wounds [40]. The use of transparent film around such thin stems makes handling and placement difficult, especially when grafting is carried out manually. The use of instant glue has been adopted in some automated processes [41], or simply to support other grafting techniques to further secure the union [42].

The grafting techniques developed over the last century for vegetable cultivation have been varied and adapted to particular environments, media, and the technical skills available for their application [10]. Grafting techniques are, therefore, cultural techniques that still seem more an art than a science, born of the experience and dedication of farmers over centuries [43]. The manual grafting task has the problems of a low production ratio, high cost, and sometimes low quality. A robotics-based solution may be a good solution capable of meeting this challenge of vegetable grafting production, aiming to reduce these problems.

Regardless of the varieties chosen, and the treatments and pre-graft and post-graft conditions, different studies have been carried out that evaluate the influence of diverse factors when performing the splicing graft, which, in turn, influence the survival rate. Success depends on factors such as the cutting point (either above or below the cotyledons), the water content in the substrate at the time of cutting [44], the difference and ratio between the grafted seedling diameters, the cutting angle between the rootstock and the scion [45], the influence of the grafting clip pressure around the hypocotyl of the grafted plants, or the influence of cutting the shoots with a somewhat sharp blade [46].

This paper attempts to provide basic documented information on the splicing technique, analyzing the success rates and influence of different joining strategies used in the splicing graft technique. A robotic system is used that coordinates the work of two anthropomorphic robots; these manipulate and manage the displacement and positioning of the seedlings with external auxiliary passive electromechanical devices that condition, prepare, and perform the graft [47]. The placement order between the clip and the seedlings is analyzed, as well as the cutting angle orientation with respect to the side opening of the grafting clip, as the basis for its implementation in automated grafting equipment, or suitability for the hand-executed splice grafting technique using support equipment, or other fully automated equipment.

2. Materials and Methods

2.1. Device Description

To understand the influence of the different seedling grafting strategies on the success of the grafting process, an automated experiment was designed to harmonize the work processes and thereby avoid variation in the obtained results, as far as possible.

The rootstock and scion are cut to a precise angle of 60°; this ensures greater splicing graft success [45]. The cutting is performed using two simple devices, in which the seedling stems are placed in a slit that guarantees verticality. Once the seedlings are set in place, a pneumatic actuator advances a blade that makes the cut with a dry shock movement against the seedling, causing a clean stem bisection and ensuring cutting angle accuracy. The seedlings are placed into the grafting device by two Kuka industrial robotic arms (Agilus model R6 900 developed by KUKA Roboter GmbH). Each robot operates independently in handling each of the seedlings, one responsible for holding the rootstock and the other for the scion.

The grafting equipment on which the various splicing strategies were tested cuts each of the flexible plastic clips from a continuous reel, and then positions the grafting clip, thus ensuring the union between the rootstock and the scion (Figure 3). The process consists of two basic operations: (1) Preparation of the grafting clip by making a custom cut and (2) clamping the clip for optimal opening and placing it on the seedling to be grafted [47].

(1) In the first phase, the device makes a grafting clip from a continuous roll of plastic tube tape through the coordinated and sequential action of several pneumatic actuators and a blade cutting system. To do this, the first microcylinder (SMC CJPB10-5) clamps the continuous tube tape against a second cylinder (SMC CXSM15-15), which vertically pushes through the exact length required for the grafting clip. Finally, the tube is cut by a blade attached to the second cylinder (SMC CXSM15-15), with a clean dry shock cut.
(2) In the second phase, the grafting clip is clamped by a rotary actuator (Festo DM-6-90-P-A), which acts on a lever mechanism that presses the clip's side wings, thus opening the clip to its maximum. As a last step, once the clip is on, a cylinder (SMC CXSM25-70) horizontally scrolls the clip to align with the union point of the two graft seedlings. Once the graft is complete, the rotary

actuator (which holds the clip open) releases the clip and removes itself from the union point, returning to its resting position.

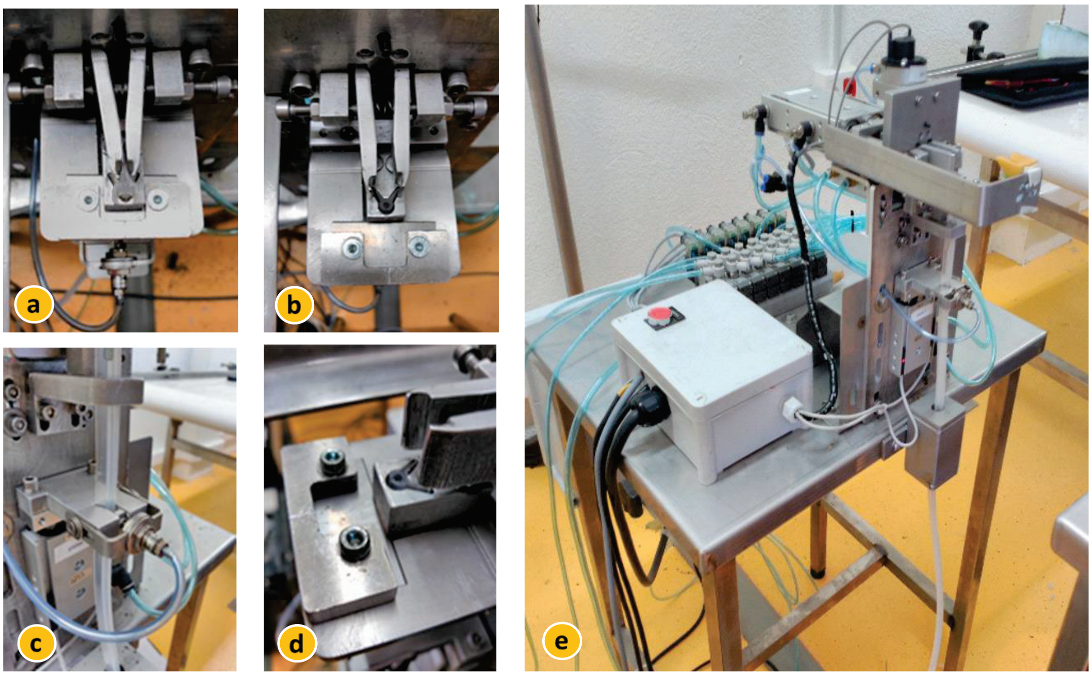


Figure 3. Detailed view of the device that dispenses and places the graft clip. ⓐ Pinching system with clip wings pressed, ⓑ clip clamping system (at rest), ⓒ and ⓓ elements used in the manufacture and clamping of grafting clips from continuous plastic tube, ⓔ overview of the equipment for making, dispensing, and securing the clips.

A passive fitting device is located at the meeting point of both seedlings and the grafting clip, which ensures correct seedling positioning. The seedling stems may suffer slight deviations or inclinations due their natural variability and search for a light source as they grow. The passive device is designed to correct these small deviations. It has two openings, one on top for the scion and one below for the rootstock, which guarantee the correct placement and housing of the seedlings. Both openings are connected by a channel, allowing sufficient space to fit both seedlings and the graft clip and to ensure that both seedlings are perfectly matched up.

Once the clip is positioned, the robot holding the scion graft releases and withdraws while the robot holding the rootstock graft stem likewise withdraws from the grafting point, so that the post-graft healing phase can begin.

The graft clips used in the test were taken from a continuous roll of flexible translucent plastic, equipped with a lateral opening; the 3.00 mm internal diameter is slightly oval to provide better grip and closure when the rootstock and scion stem diameters are not the same, and it is also equipped with side wings for opening and placement. These types of clips, widely used in splice grafting, can be purchased on continuous reels for automation purposes. They can be cut to different lengths, are easy to handle and adapt as the callus heals and the stems thicken, and they eventually fall off by themselves.

2.2. Definition of the Operating Conditions

The research was performed at the Tecnova Technology Centre: Foundation for Auxiliary Technologies in Agriculture, Almería (36°52′38′′ N, 2°19′59′′ W) between March and June 2017. The rootstock used in the experiment was the interspecific hybrid 'Maxifort' (De Ruiter Seed^{TM}, Spain), while the scion used was 'Ventero' (De Ruiter Seed™, Spain). Both seed types are commonly used in seedlings destined for manual grafting using the 'tomato on tomato' (T/T) splicing technique, so their pre-experiment compatibility to the automated grafting system has already been demonstrated.

Prior to the grafting process, the plants were cultivated in the seedbed and nursed until the rootstock and scion achieved a similar growth state; for this to occur, the scion was planted 2 to 5 days before the rootstock so that both seedlings had similar stem diameters. The seedlings were grafted when they had 2 to 4 well-defined true leaves. We tried to pair rootstock and scion stems of similar diameters. The grafting operation should be carried out over a very short period of time. During this process, the environmental conditions in the work environment were regulated with temperatures between 20° and 25°, and relative humidity conditions of between 75% and 95%, with stable lighting conditions of non-direct daylight. Splice grafting requires stringent environmental conditions during the healing process, with high relative humidity (HR) and an optimal temperature in order to reduce stem transpiration until the rootstock and the vascular tissue of the stem are healed and water and nutrient transport is restored [48]. After each of the grafting trays was completed, they were immediately placed in a small healing chamber; this comprised a tunnel slightly larger than the size of each tray, covered by a transparent film. This tunnel was placed in a chamber where climate conditions were controlled throughout the healing process. During the first 48 h, the plants were unlit to reduce transpiration and evaporation. Over the following days, the light intensity was increased, establishing a 14 h photoperiod with a value of 100 μmol-m^{-2} s^{-1} of Photosynthetically Active Radiation (PAR), (x3000 Lux) non-direct and diffuse light, as recommended for the callus formation stage [6,49]. This illumination was gradually increased over successive days while the temperature was controlled between 23 and 30 °C (an average of 26 °C), using a variable setting point in the healing chamber, slightly varying the daytime and night-time conditions. Relative humidity (HR) was initially established between 95% and 75% in the first phase. It was then gradually reduced over subsequent days for outdoor acclimatization.

For the first three days, the grafts were not handled. After the fourth day, the grafts underwent a thorough visual inspection on a daily basis, evaluating the changes and the healing process in each of them, assessing the natural evolution of the grafts and analyzing other symptoms and external signs that might determine their success or failure. This follow-up was performed for 14 days after grafting. Non-destructive techniques were used for the evaluation of graft success, based on direct observation of the evolution of the grafting junction. Visual inspection of evidences was evaluated such as vigor or sagging on leaves and stems, consummation of the callus, appearance of necrotic areas, substantial differences between scion and rootstock part growth, or the progressive appearance of new axillary buds in the scion.

2.3. Experiments

The graft takes place when both the plants are placed in intimate contact with each other, held in position by the grafting clip. To carry out the experiment, 6 grafting strategies or treatments were analyzed. Two alternatives were established regarding the grafting order (Figure 4):

1. One consisted of first placing the grafting clip at the attachment point, and then inserting the rootstock and the scion, with both seedlings being inserted at the same time but from different orientations (Clip and Stems, CS).
2. The other consisted of first placing the seedlings into the passive fitting device and then approximating and positioning the grafting clip onto their attachment point (Stems and Clip, SC).

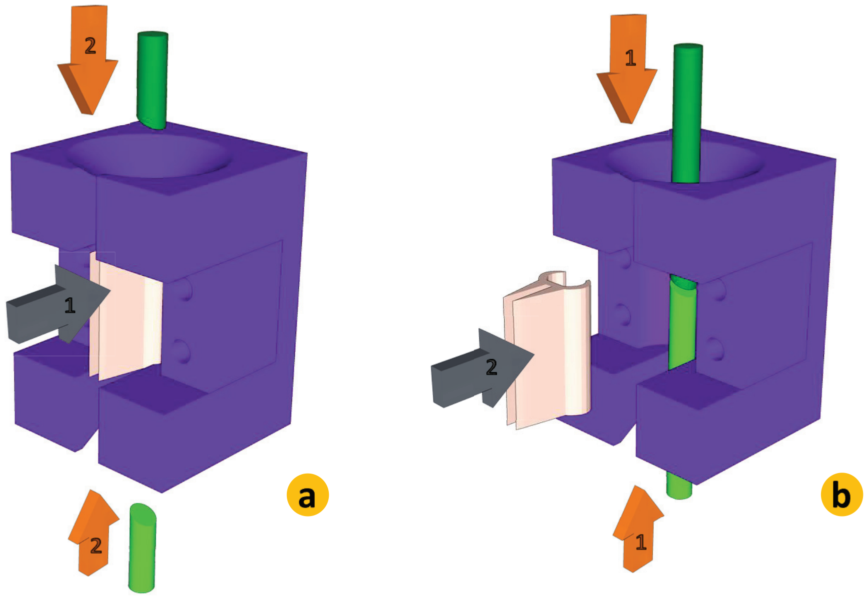


Figure 4. Passive fitting device, with seedlings (rootstock and scion) and the grafting clip at the graft union point. This piece allows the assembly operation, where the two robots and the clip dispensing device consummate the graft. ⓐ First placement of the grafting clip, and then splice of the seedlings (CS). ⓑ First splice of the seedlings, and then placement of the grafting clip (SC).

For each of the grafting-order alternatives, three combinations of the seedling surface orientation were tested with respect to the grafting clip opening (Figure 5):

(1) In the first test option, the stems were attached so that the union line was facing the side opening (side, S).

(2) In the second test option, the stem union was carried out in such a way that the rootstock was to the front, hiding the union surface behind it (front rootstock, FR).

(3) In the third test option, the stem union was carried out with the scion graft facing forward, thus hiding the union surface behind the scion (front scion, FS).

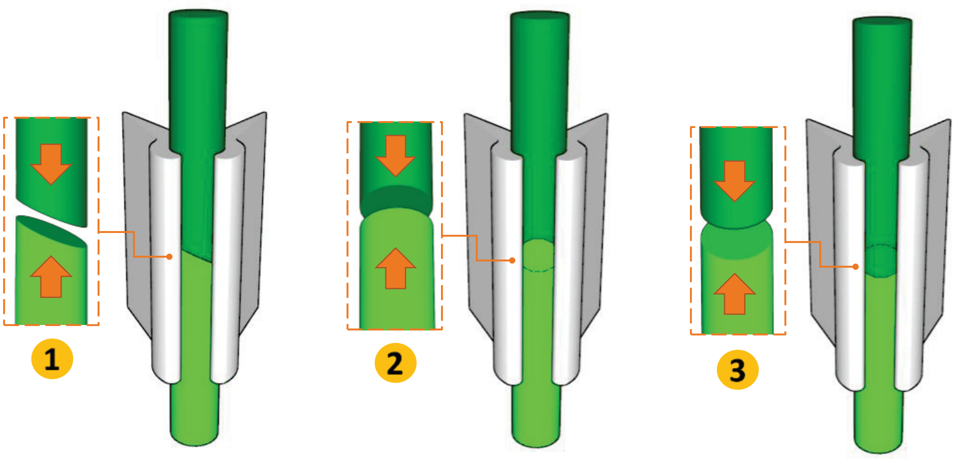


Figure 5. Outline of the orientation combinations between grafted seedlings regarding the grafting clip. ① Profile union surface (S). ② Union surface with the rootstock forward (FR). ③ Union surface with the scion forward (FS).

By combining these variables, we analyzed a total of 6 different grafting alternatives in terms of their success and the influence they had on the grafting process. A total of 900 grafting trials were performed, divided into 6 replicates, with 25 trials for each of the treatments under study per replicate. The sum of the experimental blocks, each of equal size, comprised a total of 150 grafts for each of the 6 alternatives tested, with the aim of determining their influence on grafting success (Figure 6). Each replicate, carried out on a particular date, was treated to identical cultivation preconditions, handling, and post-graft healing. Furthermore, for each of the replicates, the positional order on the tray for each grafting strategy tested was altered, thereby neutralizing the dependence on that factor.

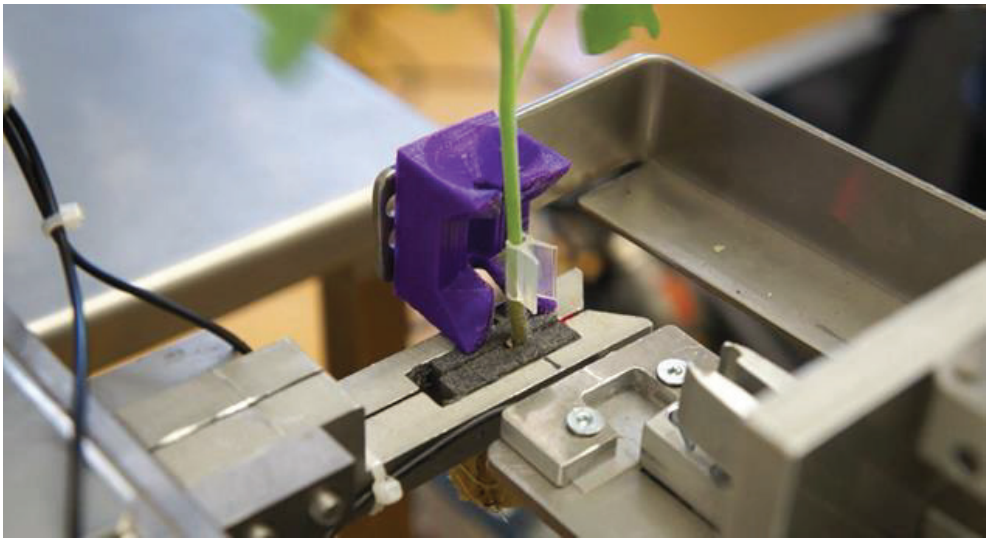


Figure 6. Detailed view of the fitting device and the grafting clip placement. It can be seen that the grafted plant is clamped by the robot that holds the rootstock and that the grafting clip is in place. Demonstrative video of the system: https://youtu.be/CgJE3NxJ0sA (accessed on 1 April 2020).

Statistical analysis of the collected data was performed using MiniTab v.18.1 software. The results underwent variance analysis with confidence levels of 95%, and contrast tests were applied using Tukey's test (the honestly significant difference (HSD) test). Tests that failed for reasons other than the technique used were eliminated from the process.

3. Results and Discussion

The methods described have evaluated six different work alternatives. The data analysis process in the present study included two stages: On the one hand, the evaluation of the trials has involved an analysis of the success of the grafts with different strategies, and on the other, the study of the possible causes that cause said failure. Seedlings were manually selected and matched by stem thickness to avoid dependence on the results with this factor. The seedlings were cut mechanically, and they were located to be gripped by the robots for their guidance of the displacements to the point of completion of the graft. Analyzing the experimental results, it can be seen that both factors, the order of assembly and orientation of the stems within the clip, affect the success of the graft (Figure 7).

The study alternatives allow us to group and study the results of, on the one hand, the order in the linking strategy, and on the other hand, the orientation of the plants inside the clip. Having performed the hypothesis test, we can estimate that there are statistically significant differences between the grafting strategies used to perform the splice grafting depending on the order in which the process is undertaken (Table 1).

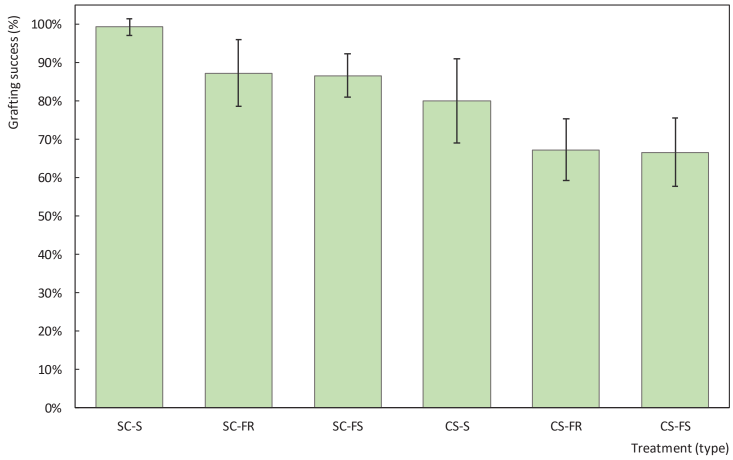


Figure 7. Grafting success for different test techniques. Success rate includes standard deviation, calculated from 6 replications.

Table 1. Comparison between success averages tested for different placement orders. Grouping of the comparisons applying Tukey's honestly significant difference (HSD) test. Significance level $p < 0.05$.

Placement Order	N	Grafts	Mean (Success)	Variance	St. Error	95% CI	Grouping (Tukey's HDS)
Seedlings–Clips (SC)	18	450	91.11%	0.00083	0.0733	(0.8717; 0.9505)	a
Clip–Seedlings (CS)	18	450	71.33%	0.02542	0.0902	(0.6740; 0.7527)	b

Approximating the seedlings until their free surfaces are in contact and then fitting the grafting clip guarantees greater success in the splice-grafting process (SC). This difference in success, around 20% higher, is possibly due to less mistreatment of the seedlings when these are inserted into the clip through their lateral opening, instead of being introduced through their upper and lower holes. Failures were classified and grouped into five categories, according to the origin that gave rise to the failed graft (Figure 8). From analyzing the failures recorded at the time of the grafting, we can see that, for the most part, for the strategy of junction order SC, most failures were due to pinches caused on the stems by the clip, despite being approximate with maximum opening. The total failure rate did not reach 10% for SC. However, for the strategy of junction order CS, these were due to frictional chafing and wounds occurring when the stems were inserted into the clip holes, or because of deformation or damage on the joined surfaces with the stem twisting on itself as it was inserted as a guide inside the clip. The total failure rate was close to 30% for CS.

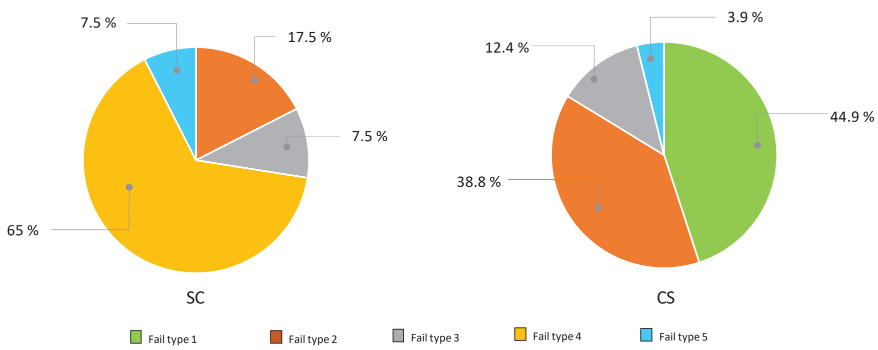


Figure 8. Distribution of unsuccessful grafts according to different causes, depending on the order in which the process is undertaken. Fail type 1: Stem tip bent over on itself. Fail type 2: Scratches in guiding and positioning. Fail type 3: Separation between stems (not well-joined). Fail type 4: Pinching off the clip. Fail type 5: Other non-appreciable failures in the grafting process.

Similarly, we can conclude that there are statistically significant differences between the orientation combinations of the joint surfaces between the seedlings with respect to the grafting clip opening (Table 2).

Table 2. Comparison between the average successes of the different orientations tested. Grouping of comparisons applying Tukey's HSD test (honestly significant difference). Significance level $p < 0.05$.

Orientation	N	Grafts	*Mean* (Success)	*Variance*	St. *Error*	95% CI	Grouping (Tukey's HDS)
Side (S)	12	300	89.67%	0.00135	0.1150	(0.8278; 0.9655)	a
Front R (FR)	12	300	77.33%	0.00267	0.1198	(0.7045; 0.8422)	b
Front S (FS)	12	300	76.67%	0.00075	0.1167	(0.6978; 0.8355)	b

Orientating the seedlings by placing the union surface in front of the vertical clip opening (S) resulted in a greater success rate than orientating the graft by positioning the union surface with either the rootstock or scion in front of the clip opening. This difference in success, around 10% higher, is possibly due to the conventional clips used being prepared to make a clamping force in that determined orientation, compared to the other orientation alternatives. Sometimes, the failures reported were mainly due to the direction of tightening exerted by the clip sides, where the proper directional pressure was not applied, to keep the graft surfaces in intimate contact, or there was not a sufficient level of pressure. Moreover, because of the lack of protection in the clip opening area, the pointed end is free and sometimes loses contact with the other seedling surface. In a similar way, failures were classified and grouped into five categories, according to the origin that gave rise to the failed graft (Figure 9). For the strategy of junction orientation S, most failures were due to the stem tip bent over on itself or scratches and chafing in guiding and positioning the stem into the clip. The total failure rate was slightly higher than 10% for S. For junction orientation FR and FS, these failures are still the most important, but there is an increase in failures due to pinching off the stems by the clip, as these are slightly separated before tightening, and the lateral opening of the graft clip is limited. The total failure rate was higher than 25% for FR and FS alternatives.

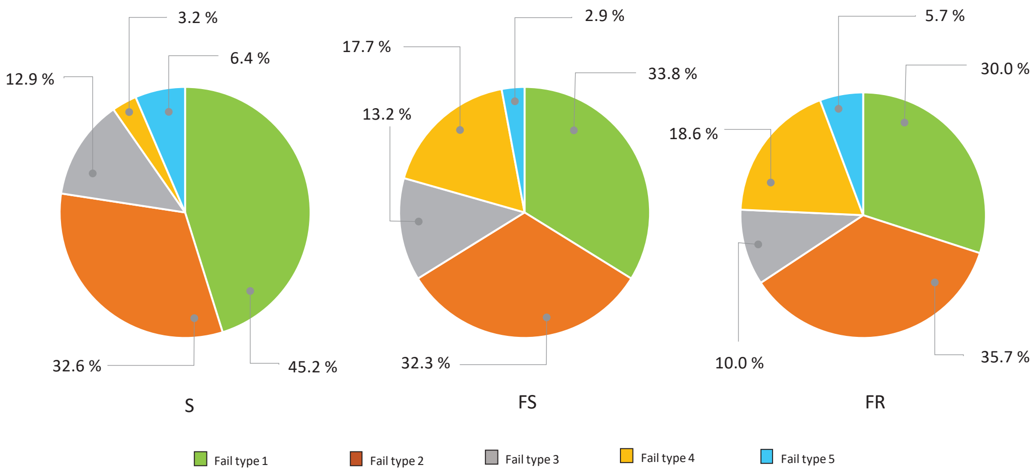
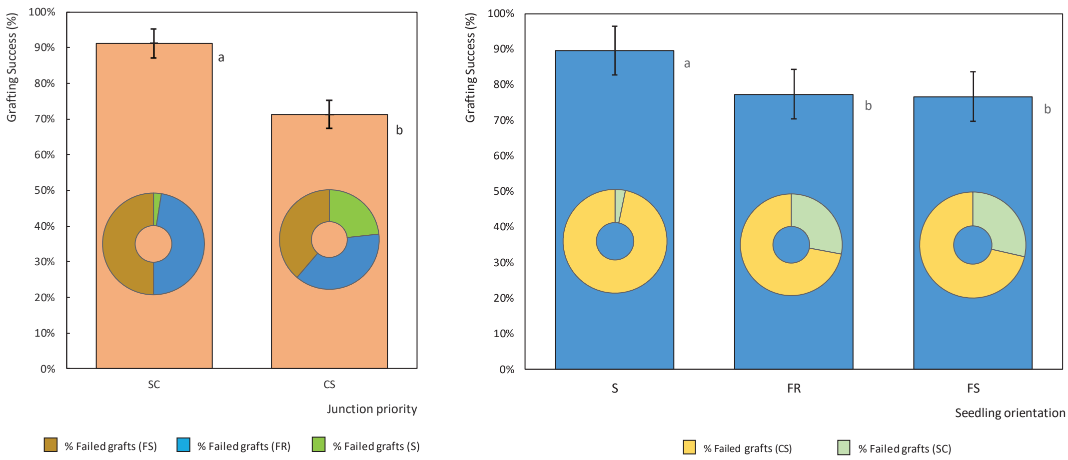


Figure 9. Distribution of unsuccessful grafts according to different cause, depending on the orientation in which the process is undertaken. Fail type 1: Stem tip bent over on itself. Fail type 2: Scratches in guiding and positioning. Fail type 3: Separation between stems (not well-joined). Fail type 4: Pinching off the clip. Fail type 5: Other non-appreciable failures in the grafting process.

Consequently, the best grafting strategy tested to carry out the splicing graft was order-dependent; that is to say, positioning the seedlings first, and then fitting the grafting clip. It also depends on the seedling orientation, placing the union line perpendicular to the clip opening (Figure 10).



(**a**) (**b**)

Figure 10. Grouping of information using Tukey's method with 95% confidence. The success rate includes the typical error. (**a**) Comparison of success for different orders in the graft assembly process. Inside, the ring graphs show the % of failures for each orientation in the junction. (**b**) Comparison between different orientations in the union. Grouping the information using Tukey's method with 95% confidence. The success rate includes integrated circuits. Inside, the ring graphs shows the % of failures for each order in the junction.

It is evident that manual grafting has several problems: (1) Skilled workers are required, which increases the cost of grafted plants [50]; (2) large volumes of labor are required in short periods of time, requiring a large production scale; (3) frequently, grafted plants present a low quality of the union as a result of the manual and repetitive process, under extreme conditions of the working environment (high temperature and humidity), in order to fulfil the objective of an intensive production. Further, when hand grafting is performed in nurseries by specialized personnel, it is common to first insert the cut rootstock stem along the grafting clip halfway, orienting the opening of the grafting clip along the side of the graft cut. Then, the scion is introduced into the middle of the still-open tube at the appropriate orientation and to the necessary depth so that the cut surfaces of both seedlings come into contact [28]. This order in the work method is a consequence of the limitations arising from working with two hands, forcing the graft handler to clasp with one hand and operate with the other.

These problems make it difficult to meet the increasing production requirements and have favored the search for solutions based on automatic grafting machines, whose developments try to fill a growing potential market [3,9,51]. The results of this study aim to highlight the importance of applying an optimal automated work strategy for tomato grafting using the splice grafting technique and using conventional grafting clips.

The success rate for manual graft production, for workers with a certain degree of skill and experience, is around 150–240 grafts/h (with success rates between 81% and 91%) [47,52,53]. In contrast, if we carry out the automated grafting described in the present trial, applying the most successful resulting strategy, consisting of an assembly order comprising a profile union surface of stems (S), and over these to couple the graft clip (SC), which presents 99% success results in the assembly phase to which the other processes that complete the grafting task must be subtracted. These results refer exclusively to evaluating the mechanical operation of the assembly, and can be applied to other varieties of solanaceae, as long as the same criteria of the size of seedlings are used, as well as the same strategy of working.

The system used to develop the method described in this article is based on using two industrial robots for plants handling and an external system responsible for positioning the grafting clip, all held by a passive support that allows the three elements: The rootstock seedling, the scion seedling, and the grafting clip to be positioned. There are, therefore, three manipulative elements acting together and in a coordinated fashion. Such a system cannot be directly extrapolated to grafting carried out exclusively by hand, as the graft handler, however experienced, only has two hands to manipulate and execute the process. However, it can be applied to fully automated systems and even semi-automated systems (based on manual handling by the operator supported by external clip fitting devices). These experimental results were directly extrapolated successfully for application to the complete automated splice grafting process using conventional industrial robotics [47].

4. Conclusions

To summarize, we can say that when performing robotic grafting, the operation is 20% more successful if the grafting plants are joined first, and the grafting clips are then fitted to guarantee their bonding rather than prepositioning the grafting clip and then inserting both plants. Furthermore, when considering the different alternatives for orientating the cutting line of the seedling union with the grafting clip opening, we observe a 10% higher grafting success rate when the bevel cutting line is orientated perpendicular to the clip opening compared to when the rootstock or scion is orientated toward the clip opening. These results for the alternative joining and orientation methods prove to be the same in combination as they are when considered separately, that is to say, that the most successful strategy for performing the graft is to first position the plants and then insert the grafted seedlings into the clip with the cut line perpendicular to the clip opening.

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Conventional Industrial Robotics Applied to the Process of Tomato Grafting Using the Splicing Technique

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Abstract: Horticultural grafting is routinely performed manually, demanding a high degree of concentration and requiring operators to withstand extreme humidity and temperature conditions. This article presents the results derived from adapting the splicing technique for tomato grafting, characterized by the coordinated work of two conventional anthropomorphic industrial robots with the support of low-cost passive auxiliary units for the transportation, handling, and conditioning of the seedlings. This work provides a new approach to improve the efficiency of tomato grafting. Six test rates were analyzed, which allowed the system to be evaluated across 900 grafted units, with gradual increases in the speed of robots work, operating from 80 grafts/hour to over 300 grafts/hour. The results obtained show that a higher number of grafts per hour than the number manually performed by skilled workers could be reached easily, with success rates of approximately 90% for working speeds around 210–240 grafts/hour.

Keywords: tomato grafting; splice grafting technique; agricultural robot; automated grafting; agricultural machinery

1. Introduction

The herbaceous graft is a growing technique that allows two pieces of living plant tissue to be joined together in such a way that they will unite and later grow and develop as a single composite plant [1]. This technique is widespread in Southeast Asia, the Mediterranean basin, and Europe for intensive cultivation in tomato greenhouses. With the use of grafting, plants with properties of agronomic interest are created, fundamentally seeking greater resistance to soil diseases and higher productivity in high-quality cultivars [2]. One of the technique's main disadvantages is its high cost of production. The seeds (of both the scions used and the added cost of the rootstock), the cost of labor, the supplies for each graft, the use of machinery and work tools, and post-graft care in the healing chambers are considered the most important factors in price determination [3–5].

It is estimated that the work of the grafting process itself can amount to approximately a quarter of the total costs associated per grafted plant; a third of these costs represent the total cost of the seeds, and the rest is essentially equally divided between the costs of materials and tools, the cost of the clip and the stay in the healing chamber, the energy costs, and the costs of the work of handling and transplantation personnel [6,7]. Proportionally, and with respect to the cost of the seeds, the scion represents 80% of the cost, compared to the 20% cost of the rootstock (Figure 1).

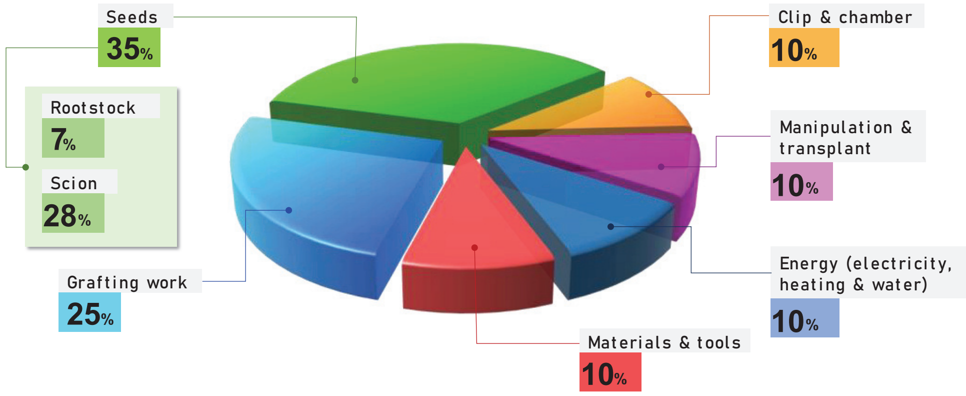


Figure 1. Estimated average cost distribution for a grafted plant. Average data assessed according to data collected in nurseries in Almería (Spain), [6,7].

On the other hand, the average cost of grafted tomato plants versus non-grafted plants varies considerably depending on several factors, mainly the productive scale of the nursery, the cost of labor, the production practices, and the cost of seeds employed, which can sometimes amount to more than 50% of the total costs [7]. In nurseries with a medium–high production volume, the costs of hand-grafted plants are estimated at approximately $0.67 for the USA, compared to $0.15 for non-grafted plants [8–10]. Similar prices are maintained for Asian countries, such as Japan and Korea [5], while for Spain and other European countries, the costs vary between €0.54 for hand-grafted plants compared to €0.18 for non-grafted plants [11]. These data corroborate that grafted plants can accumulate extra costs 3 to 4 times their cost without grafting.

Even so, the advantages of using grafted plants versus non-grafted plants, which include eliminating the common problems of soil pathogens that have traditionally been controlled by fumigation, have made the technique's use widespread and common in large regions of the world. Grafting has become the most effective and economically viable technique to address this problem [12], compared to other alternatives that have failed to provide a convincing ability to control these diseases, such as genetic improvement with resistance genes, greater crop rotation, soil solarization, the use of plastic mulch, biofumigation, the use of water vapor, crops without soil, the fallow technique, the use of trap plants, or the use of integrated biological control [13]. In Japan, Korea, and the rest of Southeast Asia, grafting is a common technique for the production of Solanaceae, especially in greenhouses, which constitute approximately 100% of the cultivated area [14]. Although its introduction in Europe and the Mediterranean basin occurred somewhat later, similar graft percentages are reached today. It is estimated that the cost/benefit ratio is 4.6 for grafted tomatoes, compared to 3.5 for non-grafted tomatoes [15].

Grafting is a task that requires considerable time, concentration, and dexterity, even for skilled workers. The delicate characteristics of the process and the biological requirements of the work seedlings, which need to be specially manipulated in a clean, warm, and humid environment, cause growing concern for plant producers due to the lack of available specialized personnel who are capable of facing intense workloads during short campaigns and with a high productive demand. Grafting requires up to three or four people and dedicated specific tasks within the process [3]. The shortage of skilled workers, along with an ageing agricultural population and an increasing demand for grafted plants, has made it necessary to automate grafting [16].

The need to use machinery in plant production to reduce the demand for human labor, expand production capacities, and improve product uniformity has been recognized for a long time [17]. In advanced agricultural countries, efforts are being made to develop and use automatic graft equipment due to the lack of labor in rural areas [18,19]. An improvement in grafting methods and techniques that reduce the cost of labor in grafting, its subsequent management, and transplants will contribute to the increased use of grafted plants worldwide [5,17].

There is an important tendency towards developing graft robots with a market potential, as opposed to manual grafting [18]. Splice grafting is a widely used method for Solanaceae, with the advantages of being easily mechanized and having well-defined and clear operations. The stem of the rootstock is cut, preferably below the cotyledons, at a specific angle. The scion, cut with the same angle, has a section that is more or less similar to the rootstock. Finally, by means of a special clamp or clip in the form of a tube, the two cuts are joined [1,20–22] (Figure 2).

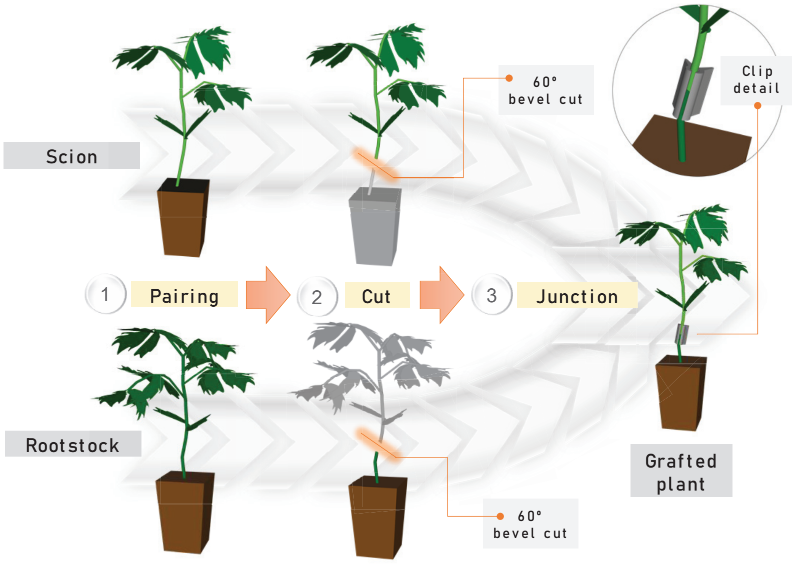


Figure 2. Sketch of the tomato grafting technique known as "tube grafting", "Japanese top-grafting", or "splice grafting".

Since the late 1980s and in the last three decades, there have been numerous attempts to invent equipment that reasonably succeeds in the automated grafting of horticultural plants. In the first two decades, the majority of developments came from Southeast Asia, while in the last decade, developments of mainly European origin have also been added [3,17,23]. This equipment has fundamentally been a semi-automated technology system, which facilitates the grafting task but requires up to two or three operators to function. Other developed systems are completely autonomous but enormously rigid in their performance, while at the same time being complex in their adaptability and operational requirements. Faced with these developments, and based on dedicated and specific automation, a study is presented herein of equipment based on conventional industrial robotic technology, supported by simple auxiliary equipment, which allows the productive requirements of the graft task to be met and can be easily adapted to other tasks and productive needs.

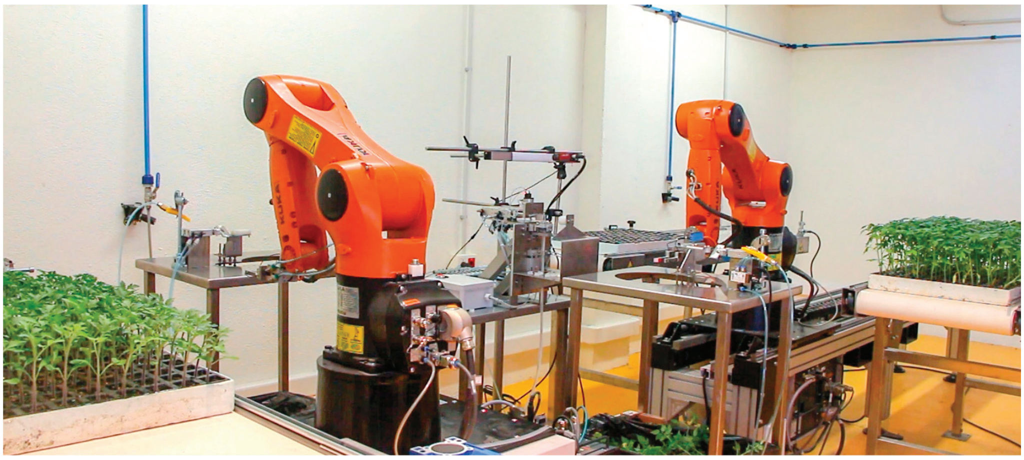
The price trend experienced in recent years in industrial robotics, which allows for the acquisition of small robotic units with similar initial investments at a cost no higher than the biannual cost associated with the minimum wage in developed countries [24,25], together with the use of passive auxiliary units with a low cost for transporting, cutting, and placing the binding clip on the seedlings, would allow for a rapid amortization of investment, which makes the study of this development alternative an area of interest.

The objective of this article was centred on the study and feasibility of automated grafting using a robotic cell based on the use of conventional industrial robotics, which allowed the grafting task to be faced with a greater system configurability and flexibility against the natural biological variability of the seedlings being used. This grafting system is supported by the use of simple and low-cost auxiliary equipment, which allows the task to be completed with tools external to the logistical tasks of the seedling trays, the cutting of the seedlings, and the dispensing and placement of the graft clip.

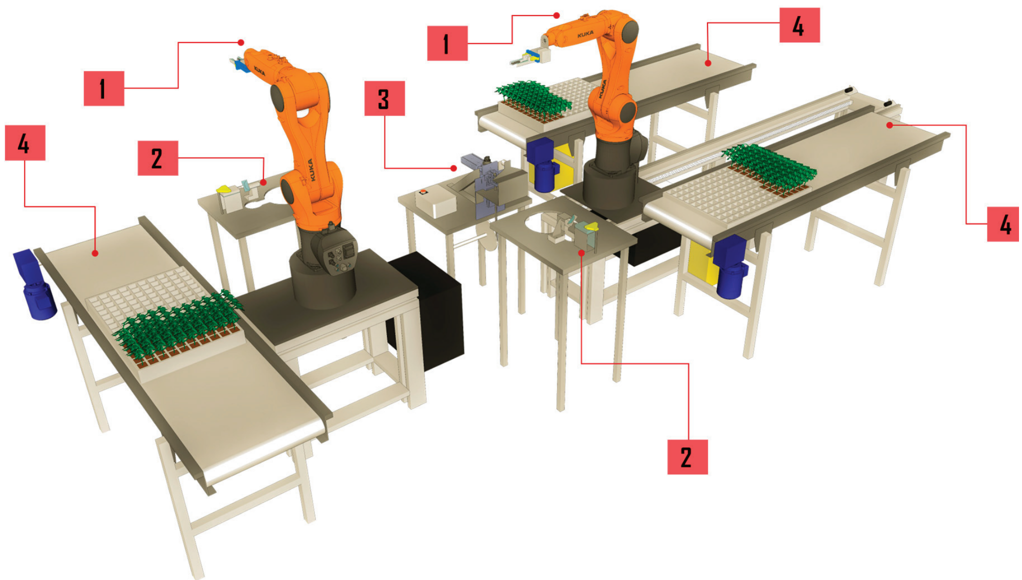
2. Materials and Methods

2.1. Device Description and Productive Process

The robotic equipment for grafting consists of transport devices, the manipulator itself, cutting mechanisms, and devices that facilitate bonding and grip [26]. True to this premise, the study equipment consisted of two anthropomorphic robots equipped with clips adapted for manipulating seedlings, with two seedling bevel cutting devices and a device for the forming, dispensing, and placement of the graft clips (Figure 3).



(a)



(b)

Figure 3. (**a**) General view of the robotic cell developed for grafting plants using the splicing technique. (**b**) Work cell sketch. (1) KUKA KR6 900 robots for manipulating the seedlings. (2) Cutting devices for rootstock and scion seedlings. (3) Forming, dispensing, and placement graft clip device. (4) Conveyor belts for seedling trays (rootstock, scion, and grafted plants). Demonstrative video of the system: https://youtu.be/9GvIDyrBBfo (accessed on 10 December 2019).

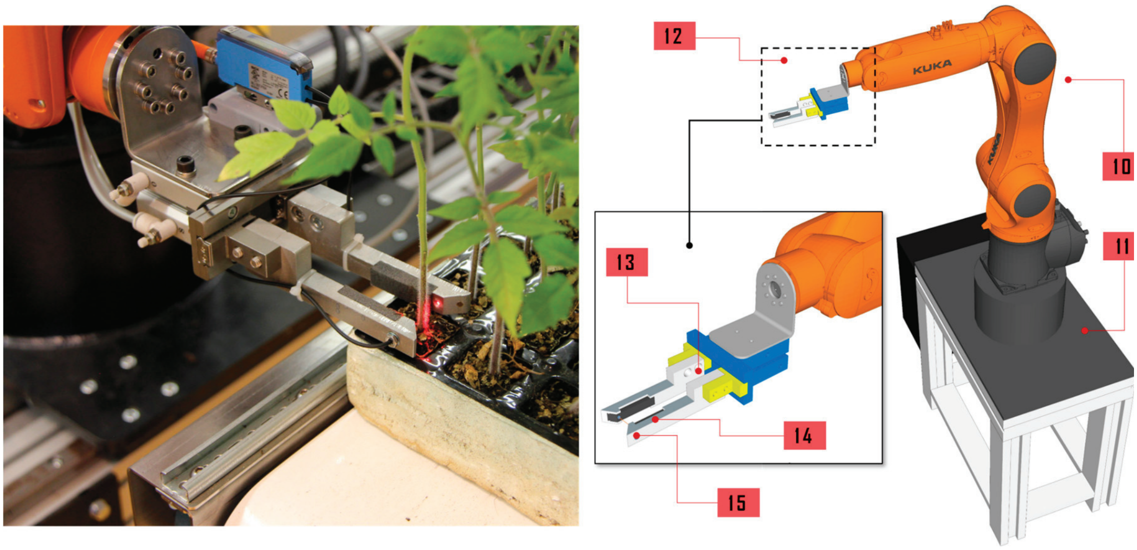
The seedling trays are loaded at the beginning of the process into conveyor belts suitable for this purpose, so that two seedling starter trays, loaded with rootstock and scion seedlings will be properly positioned in front of the working robots.

Each completed row of work is followed by an advance of the belt, which relocates the next row into the appropriate collection area. This process happens until the rows in the seedling starter trays (rootstock and scion) are finished. Likewise, there is a third conveyor belt with an empty output tray for the resulting seedlings to be placed on once grafted. This output conveyor belt also performs partial advances per row while working on that tray.

Once the trays reach the required locations, the two industrial robots, Kuka model Agilus R6 900 (developed by KUKA Roboter GmbH), work from their home position in a coordinated manner on the rootstock and scion to achieve graft completion. These robots have equivalent commercial equipment from other large manufacturers worldwide, such as the robot model IRB1200 (developed by ABB), the model MH5F (developed by Yaskawa), or the model LR Mate 200iD/7 L (developed by FANUC). All of them have similar load capacities, degrees of freedom, speeds, and working spaces, so their replacement would not lead to significant differences.

Each robot operates independently by handling each of the seedlings, which are obtained from the input trays. The rootstock and scion seedlings are approached in a simultaneous and coordinated manner: ① approach the input trays (AIT). This displacement is followed by a precision operation that separates the seedlings from their trays: ② grip and extraction (GE).

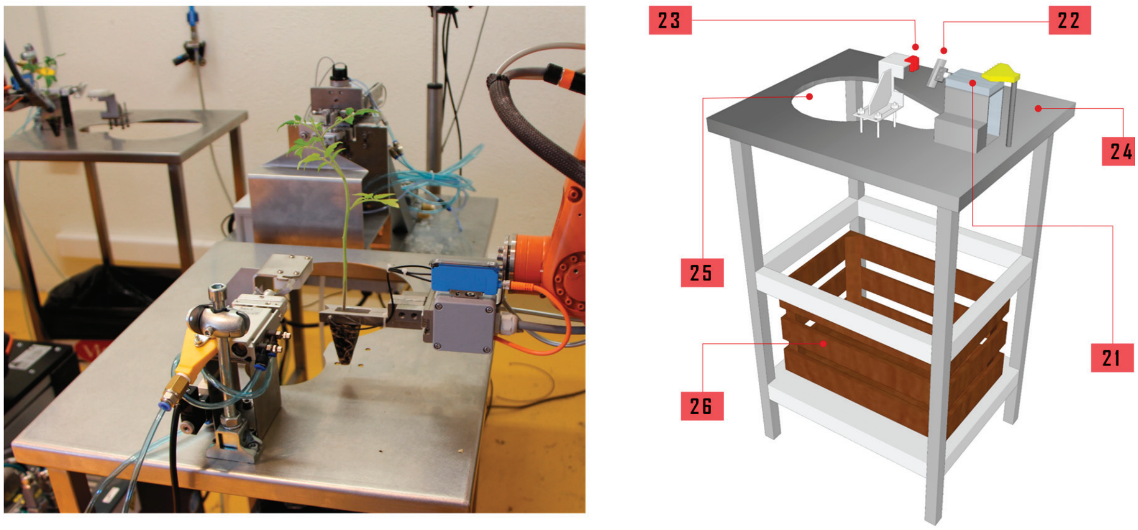
The final elements for the seedling manipulation consist of clamps composed of two fingers with an opening and closing parallel model MHZ2-32D from SMC, equipped with a padded extension zone of low-density (150–200 kg/m^3) polyurethane foam (neoprene) with high resilience for precise and firm seedling attachment. A photocell is located between the ends of these fingers, and a Sick LL3-TB02 optical fibre sensor detects the precise location of the stems of the seedlings, which can emerge at any position within the alveolus of the tray. The individual seedlings have a unique growth morphology, and the alveoli can even be empty (Figure 4).



(a) (b)

Figure 4. (**a**) Detailed view of the clamp or terminal element of the robots. (**b**) Robot and clamp sketch. (10) Industrial robots. (11) Clamping base. (12) Robot clamp. (13) Fingers with parallel opening and closing operation. (14) Padded area for holding seedlings. (15) Sensor for seedling detection.

After extracting the seedlings from their alveoli in the trays, the robotic arms carry the seedlings to the point where the cut will be made: ③ approach the cutting zone (AC). The system uses two equal pieces of equipment for cutting the seedlings to guarantee precision in the required cutting angle and the integrity of the dissected seedling. Both pieces of equipment are responsible for acting, one on the rootstock and another on the scion, allowing the cutting angle to be regulated and complementary between both plants. Prior to cutting, the robots insert the seedlings into a slot or channel located in front of the blade, where the stems are embedded to ensure the verticality of the stems during cutting. The cut is executed by activating a double-shank pneumatic cylinder, SMC model CXSM15-15, coupled to a terminal tool of a sharpened, disinfected, and interchangeable blade of stainless steel with a precise cut angle [27]. The cut is performed by a dry shock stroke of the blade against the seedling, which cleanly bisects the stem and ensures a clean cut: ④ cutting process (C). Meanwhile, an external blower separates the non-useful part of the treated seedling (Figure 5).



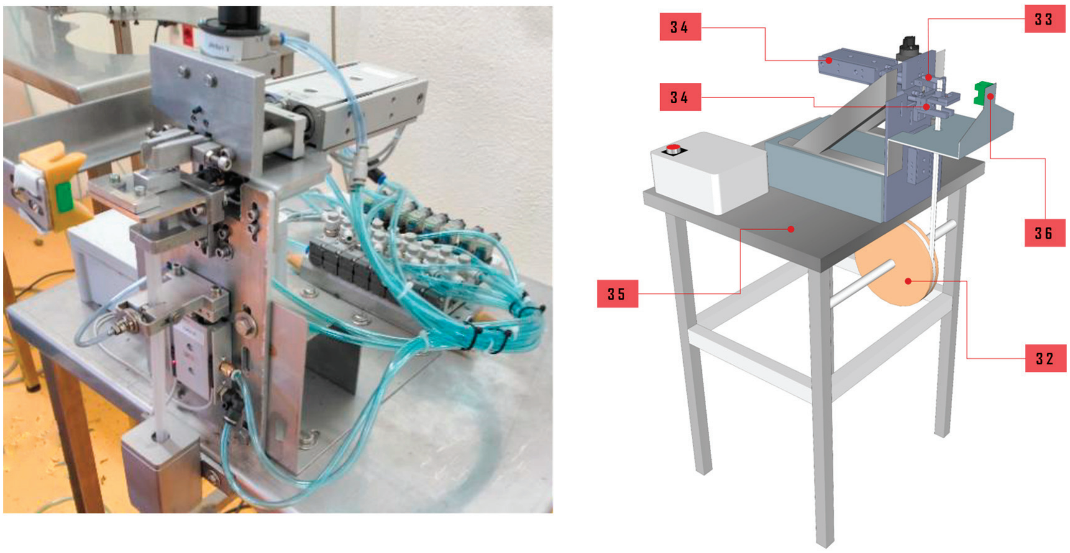
(a) (b)

Figure 5. (**a**) Detailed view of the cutting device. (**b**) Cutting device sketch. (21) Pneumatic cylinder to drive the blade. (22) Cutting blade. (23) Slot for seedling lace. (24) Worktable. (25) Hole for waste disposal. (26) Waste accumulation box. (26) Blower.

After the cut, the robotic arms carry the useful parts of the seedlings for the graft towards the bonding area: ⑤ approach the clip dispensing zone (ACD). The graft equipment that creates the bond is responsible for cutting plastic clips from a continuous roll and then dispensing and placing a clip on the seedlings to be grafted. Thus, this device contains two subsystems: one in charge of preparing the clip and another in charge of placing it.

The first clip preparation subsystem consists of a series of electropneumatic devices that act in a coordinated and sequential manner to perform the clip cutting process from a continuous roll of plastic tube, regulating the advance and thus controlling the length of the cut clips. To obtain a clip with the desired length, an SMC model CJPB10-5 microcylinder presses the plastic tube against the end of a second cylinder, an SMC model CXSM15-15, which advances in the vertical direction the exact length that the clip is desired to have. Finally, an SMC model CXSM15-15 cylinder, equipped with a sharp blade at its end, makes a clean cut with a sharp blow, creating the clip to be used.

The second subsystem contains a rotating cylinder, a Festo model DM-6-90-PA, which grips the cut clip, then tightens its wings and thus clamps and fully opens the clip. Finally, with an SMC model CXSM25-70 cylinder, the clip is brought closer by a precise horizontal movement to the junction point where the two seedlings to be grafted are located. At the point of clip placement, a passive fitting device is adapted to accommodate and locate the seedlings in front of the clip. To ensure a precise bond without unwanted seedling displacement, both parts remain in contact and can thus fasten the bond. Once the clip is placed on the seedlings, the device that kept the graft clip pinched loosens and moves away from the junction point, returning to its resting position (Figure 6).



(a) (b)

Figure 6. (**a**) Detailed view of the device that dispenses and places the graft clip. (**b**) Sketch of the device that dispenses graft clips. (31) Pneumatic cylinders and blade to make the grafting clip. (32) Continuous silicone roll for clip manufacture. (33) Tweezers for the grip and clip opening. (34) Pneumatic cylinder responsible for bringing the grafting clip closer to the junction point. (35) Worktable. (36) Plant placement device and grafting clip insertion point.

The graft is accomplished when both plants are placed in intimate contact with one another and the graft clip is pressed onto them: ⑥ clip preparation and placement (CPP). Once the clip is placed, the robot holding the scion releases the graft and withdraws, leaving the bonding area, while the other robot, which holds the completed graft by its lower part, moves the graft by the rootstock to the tray where the grafts will be left once finished: ⑦ approach the output trays (AOT). The graft is deposited in an alveolus of the output tray: ⑧ insertion on trays and release of plants (ITR).

Once the process is finished, both robots return to their resting positions, either as an end point or as a point of passage from where a new work cycle begins: ⑨ return home (RH). The process is repeated until the work trays are completed. The tray with the grafted seedlings is removed, and the grafted seedlings are subjected to a post-grafting process of healing, where their success is examined over 14 days: ⑩ post-graft losses (PGL). The entire system and grafting equipment described, as well as other secondary elements and auxiliary equipment, are managed and coordinated in a global manner through a central control unit, consisting of a PLC model CJ2 M, with an Omron CPU32.

During the grafting operation, 10 control points were established as singular intermediate points of reference in the process, which allowed us to record the partial times used and a distribution of failures during grafting (Figure 7).

The flowchart describing the operations and process described above allowed us to evaluate the validity and efficacy of conventional industrial robotics applied to tomato seedling grafting using external low-cost passive devices that facilitate grafting completion. The external devices act as a tool both in cutting the seedlings and in the dispensing and placing of the graft clip (Figure 8).

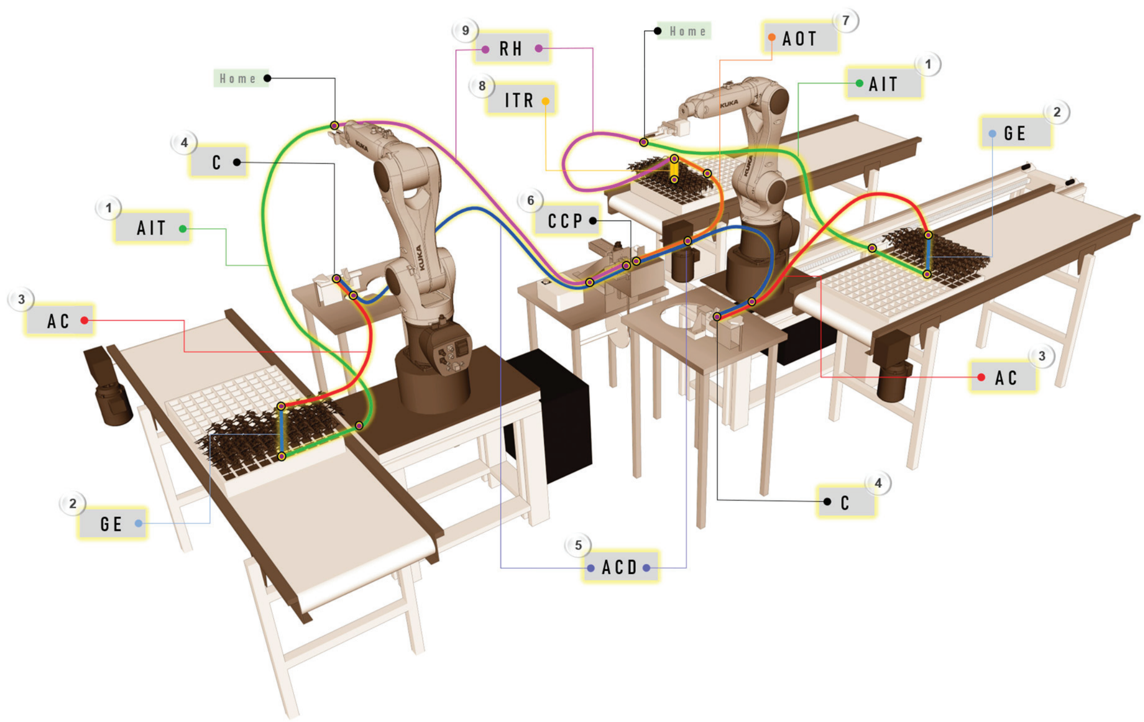


Figure 7. Travel details. Singular points: ① approach the input trays (AIT); ② grip and extraction (GE); ③ approach the cutting zone (AC); ④ cutting process (C); ⑤ approach the clip dispensing zone (ACD); ⑥ clip preparation and placement (CPP); ⑦ approach the output trays (AOT); ⑧ insertion on trays and release of plants (ITR); ⑨ return home (RH).

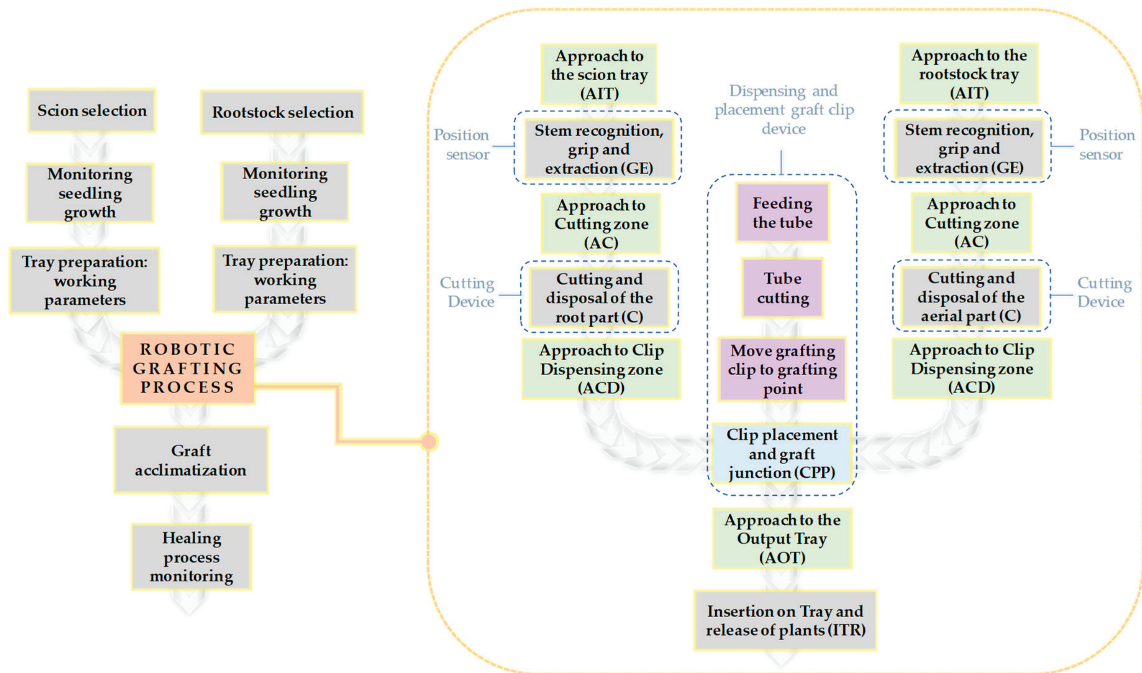


Figure 8. Flowchart of the developed automated grafting process.

2.2. Definition of Operating Conditions

The experiment was conducted at the Tecnova Technological Center (Centro Tecnológico Tecnova): Foundation for Agricultural Technologies of Agriculture, in Almería (36°52′38″N, 2°19′59″W) between the months of April and June 2017. The environment of Almería is a model of agricultural exploitation. Greenhouse growth of arable fruit crops has a high technical and economic performance, with tomato cultivation being of particular importance.

The rootstock used in the experiment was the interspecific hybrid "Maxifort" from De Ruiter Seed^{TM}, which is recommended for crops with better behavior at low temperatures and under high salinity conditions. The "Ventero" variety from De Ruiter Seed^{TM} was used as an indeterminate hybrid tomato for branch harvest. Both types of seeds are routinely used in seedbeds to perform manual grafts using the "tomato on tomato" (T/T) technique, which demonstrates their prior compatibility with the robotic system.

The growth protocol developed in the nursery attempted to obtain plants that were grown and cared for until reaching a similar growth state between the rootstock and scion, with mature plants and those prepared for the graft having two–four well-defined true compound leaves [28] and stem diameters of at least 1.5 mm for the splicing method [29]. Therefore, stems with some natural variability characteristic of the development of each plant (between 1.5 and 2.5 mm in diameter in the area close to the cut for the scion, and between 2 and 3 mm in diameter for the area close to the cut for the rootstock) were worked with. Usually, in the automatic graft, the requirements in terms of growth and uniformity required for the rootstock and scion seedlings are as critical as in the manual graft [30], demanding an arduous previous task of pre-selection and pairing similar diameters between the linked seedlings. In our experiment, this work was eliminated because the seedlings were cut with a bevel at a 60° angle. From a certain cutting angle, between 50° and 70°, and provided that one works within the margins of natural variability between the previously established stems, the success rate of the graft was acceptable and higher than 95%. Therefore, the need to seek equal diameters of the workplaces was of lessened importance [27,31].

Prior to each experiment and for each tray, it was ensured that all the alveoli slots contained seedlings that met the previously established rootstock size criteria. The environmental conditions were regulated during the grafting process, with temperatures between 20 °C and 25 °C, conditions of relative humidity that were sometimes forced and were guaranteed to be above 75%, and stable, non-direct daylight luminosity conditions.

The data were collected for each test via filming and were then timed; the times until reaching each control point of the process until the graft was completed or the point of generation of each failure were evaluated.

Regarding the post-grafting conditions, the plants began to wilt immediately after cutting and grafting, so once each graft tray was finished, it was immediately introduced into a small healing chamber consisting of a tunnel slightly larger than the dimension of each tray and a low height, covered by a transparent film. This tunnel was placed inside a chamber in which the climatic conditions were controlled throughout the healing process. During the first 48 h, the plants were kept without illumination to reduce transpiration and evaporation. On the following days, the intensity of the light was increased, and a 14 h light photoperiod with a value ≈ 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PAR, (~ 3000 lux) of non-direct and diffuse light was established during the callus formation stage, from LED lights, corresponding to a value slightly above the compensation point because there is evidence that a high intensity of light prevents callus formation [1]. The level of illumination was gradually increased after several days. The temperature was established with a variable set point in the healing chamber between 23 °C and 30 °C, with an average of approximately 26 °C, slightly varying between the diurnal and nocturnal conditions. The relative humidity was initially established between 75% and 95% in an attempt to reduce the transpiration rate of the scions, avoiding high stress and thus preventing the drying of the graft [32]. The humidity level was gradually reduced in successive days to condition the grafts to the outdoors. The vapor pressure deficit (VPD), during the critical graft healing phase was around 0.8 kPa in the tunnel inside of the healing chamber, with the aim of decreasing transpiration. This value was gradually increased in the following days.

The success or failure of the final graft was evaluated by estimation and visual assessment performed daily across the 14 days after grafting, assessing the natural evolution of the graft, and analyzing other symptoms and external evidence that would determine its classification as either a success or a failure. In making this determination, key intermediate points were considered to mark an inflection of the task or singularity within the process, so that the successful or unsuccessful completion of this phase of the robotic process could be evaluated.

2.3. Experiments

In the process, different robot working speeds were tested, with the objective of determining the influence on the graft success. The robot speeds were constant within each test and ranged from 100 mm/s to 600 mm/s, with gradual increments of 100 mm/s. In total, six working speeds were tested.

A total of 900 grafts were prepared, divided into three experimental blocks consisting of a total of 300 grafted seedlings each, where a total of 50 grafts were performed for each of the six test rates. The sum of the experimental blocks, equal to one another, therefore consisted of a total of 150 resulting grafts for each of the six tested velocities.

Only the work times of the external processes on the seedlings (manipulations on the trays, cutting, and dispensing of the clip) were kept constant between the tests. It was understood that since the work times are based on pneumatic technology, they were optimized for the corresponding work speed at 3.5 bar (0.35 MPa).

Each experimental block, when was developed on a specific date, was treated under the same cultivation, manipulation, and post-graft healing conditions, with the aim of matching the development conditions between the three experimental blocks. In addition, for each of the experimental blocks, the positional order of each test rate was altered, thereby neutralizing the dependence of said factor.

The statistical analysis of the collected data was performed using the software Minitab v.18.1. The obtained results were subjected to an analysis of variance with a confidence level of 95%, and contrast tests were applied using Tukey's test (honestly significant difference, HSD test).

3. Results and Discussion

After performing the hypothesis test, we estimated that there were statistically significant differences between the grafting times used for the different tested work rates (Table 1).

For each velocity tested (between TS1 and TS6), the variability of time used in each test unit was mainly due to singularities that facilitated the manipulation and the development of the grafting work, to a greater or lesser extent. Singularities included the position of the alveolus in each row of work, the natural variability of the seedling emergence point within each alveolus, and the unique growth morphology of each seedling, among others. At low velocities, the difference for each work velocity was clear, given that the time taken to solve these singularities was less significant compared to the time spent in tasks not affected by these singularities. However, at high speeds, these factors became increasingly important and, to some extent, determined the time spent in each test unit.

Grouping the data by test speed, analyzing the failures associated with each control point that were recorded for the different velocities, and performing the hypothesis test, we estimated that, based on the Tukey's tests, there were statistically significant differences between the groups of different assay speeds (Table 2).

At low test rates, the success rate was higher, greater than 90% for speeds equal to or lower than TS3, and there was a significantly increasing graft failure for operating speeds equal to or greater than TS4. Low speeds, between TS1 and TS3, had similar behavior in terms of failures; therefore, we consider that their differences were derived from chance and not from the working speed itself. Therefore, it is clear that the TS3 production speeds are more attractive, given that they had a higher production ratio associated with a low failure rate.

The relationship linking the number of grafts/hour for each of the tested rates (TS1 to TS6) can be considered practically linear and was only altered by the random parameters derived, to a great extent, from the natural variability of the work seedling growth. Such nuances of correction barely affected the speed, which was largely marked by that established for both robots for each test, although the working speed of the auxiliary devices was kept constant. In addition, the percentage of successes/failures associated with each of the test rates (TS1 to TS6), considering their evolution, allowed us to observe a behavior similar to a quadratic function (Figure 9).

Table 1. Comparison between the mean times spent in grafting task for the six tested speeds. Grouping of comparisons applying Tukey's HSD test (honestly significant difference). Significance level $p < 0.05$.

TS (Test Speed)	*N*	Mean Time (s)	Variance	St. Dev	SS	St. Error	95% CI	Grouping (Tukey's HDS)
TS1	150	40.641	185.078	13.600	27576.61	0.617	(39.43; 41.85)	A
TS2	150	21.521	56.582	7.522	8430.76	0.617	(20.31; 22.73)	B
TS3	150	15.430	27.691	5.262	4125.97	0.617	(14.22; 16.64)	C
TS4	150	13.320	30.922	5.561	4607.40	0.617	(12.11; 14.53)	C
TS5	150	9.416	19.950	4.467	2972.62	0.617	(8.20; 10.63)	D
TS6	150	6.993	22.708	4.765	3383.49	0.617	(5.78; 8.20)	D

The different letters show significative differences.

Table 2. Comparison between the means for grafting failures for the six speeds tested. Grouping of comparisons applying Tukey's HSD test (honestly significant difference). Significance level $p < 0.05$.

TS Test Speed)	Speed (mm/s)	Check Points	Mean (Fails)	Variance	St. Dev	95% CI	Grouping (Tukey HDS)
TS1	100	10	1.50	3.16667	1.780	(0.022; 2.978)	A
TS2	200	10	1.60	3.37778	1.838	(0.122; 3.078)	A
TS3	300	10	1.50	3.16667	1.780	(0.022; 2.978)	A
TS4	400	10	2.20	6.17778	2.486	(0.722; 3.678)	AB
TS5	500	10	2.70	5.37778	2.319	(0.922; 3.878)	AB
TS6	600	10	4.70	11.34444	3.370	(3.220; 6.180)	B

The different letters show significative differences.

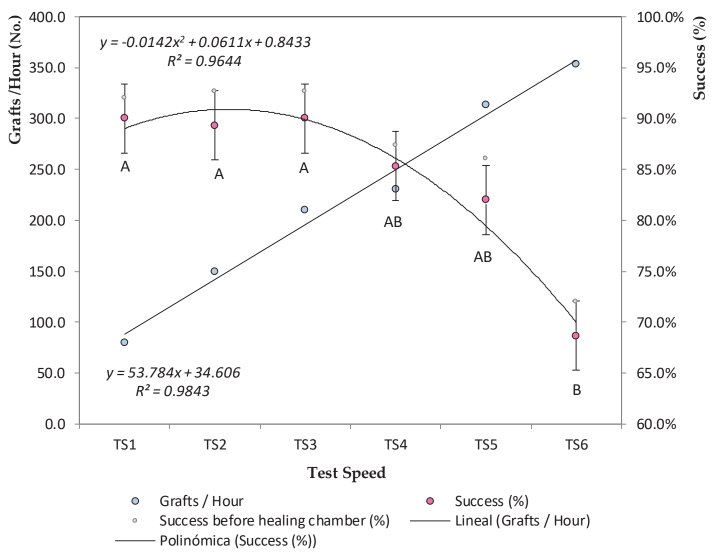


Figure 9. Grafts/hour versus grafting success for different test speeds (TS1 to TS6). Success rate includes the typical error. "Success before healing chamber" has been included to evaluate the influence on the global success of the grafting process.

At the working speeds of the TS3 and TS4 robots (210 and 240 grafts/hour, respectively), the number of grafts estimated for manual expert workers was already surpassed, i.e., a maximum of 150–240 grafts/hour [7,15], and an average of approximately no more than 1000 grafts per person per day [30,33,34]. In addition, the success rates for manual grafting are not usually very high, between 81% and 91% [35]. In part, this rate of failure may come from long hours in a hostile work environment, characterized by high humidity and temperature. The success rates achieved by the robotic graft were quite similar to those achieved by manual workers, reaching 90.0% for the TS3 speed of 300 mm/s and 85.3% for the TS4 speed of 400 mm/s.

For large cutting angles in splicing grafts, the difference between the diameters of the linked stems became less important as long as they stayed within certain a range [31]. For cuts at a 60° bevel, failure in the healing and grafting of the middle graft was between 3% and 6% of failures [27]. The difference between the number of successes considered after grafting and the number of successes recorded after the healing period in the chamber confirmed this parameter (less than 4%), and except for errors not visually detected in the grafting process, we associated the percentage of losses in the chamber to random problems in the healing process itself.

The established isolated intermediate control points allowed us to record the cause for each failed graft. Studying the origin of the graft failures associated with each velocity in detail, we determined that at low test rates, the failures detected in the grafting process in the GE phase (grip and extraction) were slightly more significant. This result may be because the seedling, when extracted from its alveolus at a lower velocity, experiences lower extraction acceleration, with a consequently greater resistance and grip to the alveolus walls. As a result, the roots tend to remain adhered and occasionally incur damage and tears; traction damage to the stem or alterations to the other plants in the tray may also occur, among other problems.

However, it was observed that, as the test speed increased, the number of failures in this phase decreased, as did the number of failures in the operations of adaptation and placement of the seedlings, such as the AC (approach the cutting zone) and ACD (approach the clip dispensing zone) phases, and in the tasks properly conducted by these tools: C (cutting process) and CPP (clip preparation and placement). In part, this was due to seedling management from certain working speeds, where there was a substantial acceleration in the displacements between points, and with it, the inertia experienced on the seedlings, which, together with their root ball (semi-compressed coconut fibre substrate) could suffer greater damage and tearing when experiencing such sudden changes of state. In addition, it was observed that high accelerations led to excessive seedling balance by the ends not held by the robot clamp (root ball and stem), causing the robot to lose its reference point at rest or to not return to it in time, thus spoiling the graft. The development of the grafting process is shown in Figure 10.

As the working speed increased, the times spent in the tasks performed by the external working tools became more important compared to the times spent in the displacement and pre-positioning of the seedlings in front of the tools. This factor is due to the fixed value of the velocities of the pneumatic devices in response to an incremental increase in the robot velocities (Figure 11).

At speeds equal to or lower than TS3, the recorded success rate was relatively good at approximately 90%, but it decreased substantially at higher speeds. The next tested speed, TS4, had a significantly different percentage of recorded failure, five points lower or 85.3%. It is important to assess the success associated with each work rate, because it is the factor that makes it feasible as an alternative to manual grafting, because the system evaluated is scalable in terms of systems and tools operating in parallel. That is, the clamp or end element could be adapted by cloning two or more gripping systems in parallel for the seedlings, and, to the same extent, the auxiliary devices or tools acting on the seedlings could be cloned, thus multiplying the number of plants grafted per hour, maintaining similar success rates. In addition, regarding these working speeds (TS3 and TS4), the number of grafts capable of being developed manually began to be exceeded. Therefore, when evaluating the grafts/success ratio, we estimated a better average behavior for velocities close to TS3 (210 grafts/hour).

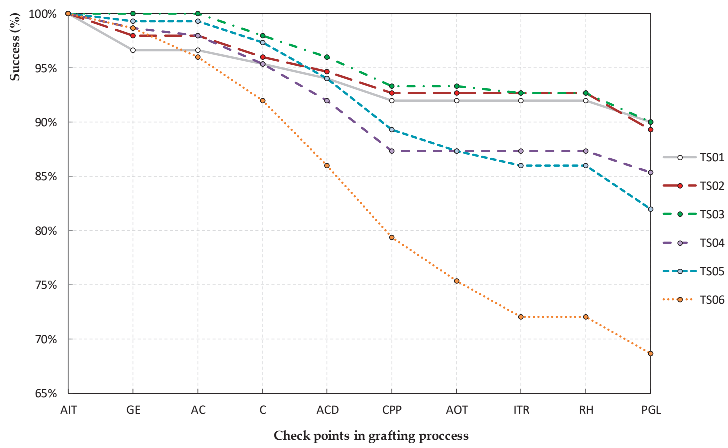


Figure 10. Success associated with each check point for the different test speeds, reflected in a percentage of successful plants in the grafting process. Check Points: approach the input trays (AIT), grip and extraction (GE), approach the cutting zone (AC), cutting process (C), approach the clip dispensing zone (ACD), clip preparation and placement (CPP), approach the output trays (AOT), insertion on trays and release of plants (ITR), return home (RH), and post-graft losses (PGL).

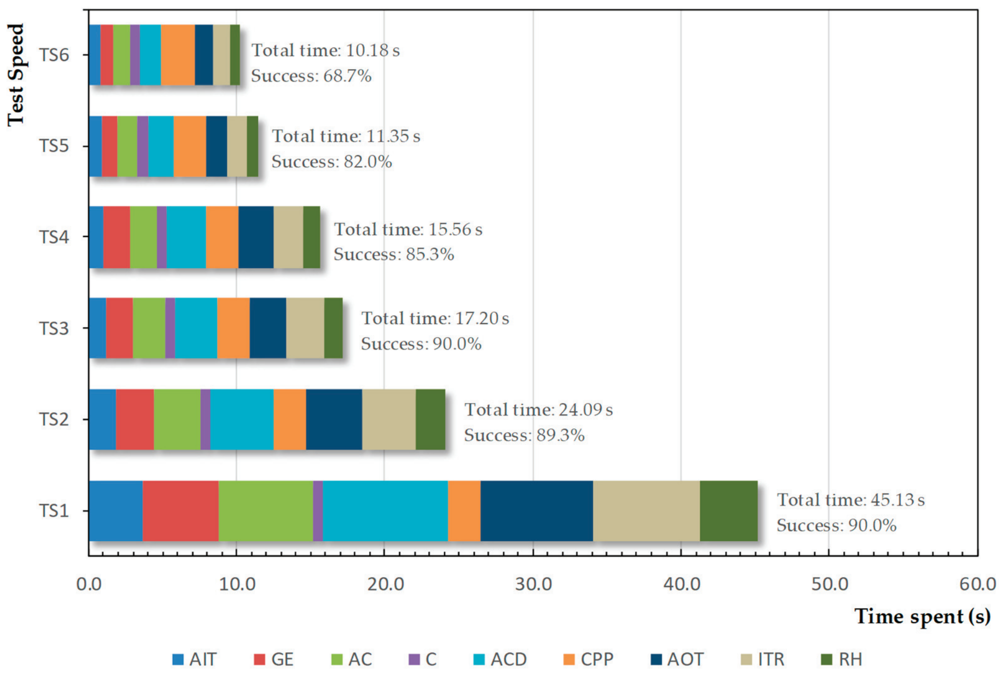


Figure 11. Average time spent in the development of each phase between control points. Check Points: approach the input trays (AIT), grip and extraction (GE), approach the cutting zone (AC), cutting process (C), approach the clip dispensing zone (ACD), clip preparation and placement (CPP), approach the output trays (AOT), insertion on trays and release of plants (ITR), and return home (RH).

Numerous studies have collected the test results of prototypes and commercial devices for the automated grafting of horticultural seedlings over the last three decades [3,5,7,23,36–44], and the results for dozens of pieces of equipment have been collected, mainly from Southeast Asia (Japan, Korea, and China mainly) and Europe.

Comparatively, we can refer to four factors that determine the convenience of and interest in the study equipment compared to other existing equipment: (a) the flexibility in terms of the horticultural family of work and the grafting method developed; (b) the degree of automation and the number of operators involved in the process; (c) system velocity (grafts/hour) and system efficiency; and (d) the price of the equipment.

(a) There are devices prepared exclusively for use with Solanaceae and others that allow working with Solanaceae and Cucurbitaceae. These pieces of equipment are mostly characterized by being inflexible. The study equipment, based on industrial robotics supported by low-cost external equipment, is specifically intended for splicing grafts, but enables easy and economic reconversion and readaptability, as it is able to work with other horticultural families and to apply other grafting methods. It even has the ability to perform other tasks in the seedbeds or productive environments.

(b) The existing prototypes and commercial devices present a different degree of automation, ranging from simple tools to help with grafting (cutting or dispensing the clip), to semi-automated equipment that requires the participation of two to three operators, to fully equipped devices. These are all automated and can be managed by a single operator. Nevertheless, they claim a high homogeneity in the work seedlings, requiring the important tasks of pre-sorting and pairing between seedlings, which to some extent tarnishes the autonomy of the system. The study equipment, by working with a high cutting angle, allowed us to avoid these previous tasks of searching and matching between the diameters of the workplaces and therefore enjoys a high degree of autonomy.

(c) Regarding the number of grafts per hour, the majority of studies present equipment ranging between 200 and 1200 grafts/hour. These values are much higher than those achieved using this equipment (210–240 grafts/hour), but these results are sufficient compared to manual grafting, and improvement is feasible by replicating terminal systems, allowing for working in parallel and over several plants simultaneously. In contrast, the system has a high success rate or efficiency of approximately 90%.

(d) The current price trend of industrial robotics, together with the use of simple, low-cost auxiliary equipment, allows us to estimate that the studied system is a rapidly amortizable system, with an initial investment in robots no greater than the biannual cost associated with the minimum interprofessional wage in countries [25], and an additional base cost of auxiliary and control equipment which is not higher than other interprofessional minimum wages (MW). This implies a total base investment of 5 MW (~€60,000). Faced with this, the estimated costs of high automation systems robotic equipment in high productivity environments (100 million plants per year) are estimated at investments above ~$7,500,000 [45]. Comparatively, and estimating our system working at approximately 230 grafts/hour, we would yield approximately 2 million grafts per year per piece of equipment, which would imply approximately 50 systems working in parallel to reach 100 million grafts. Such scaling would involve an investment of ~€3,000,000, well below the investment necessary for other robotic equipment.

4. Conclusions

The splicing technique, widely used for Solanaceae such as tomato, has the advantage of being simple and methodical, and therefore easily automatable. In the last three decades, there have been numerous attempts to develop equipment to deal with the automated grafting of horticultural plants, and the challenge of using conventional industrial robotics to perform the splicing graft process can provide a great opportunity. The robotic cell tested herein is based on two industrial robots and low-cost passive auxiliary units.

The use of low speeds, between 100 mm/s and 300 mm/s, allows ratios close to a 90% success rate to be maintained. At medium–high velocities, between 400 mm/s and 500 mm/s, success ratios were still acceptable above 80%. However, at a test speed of 600 mm/s, there was a considerable decrease in the success rate to less than 70%.

Consequently, we conclude that it is advisable to use a velocity close to 300 mm/s (90.0% success), which allows working at speeds higher than those estimated for manual expert workers, approximately 150–240 grafts/hour (with success rates between 81% and 91%). Decreasing the work rate below that point did not substantially improve the success rate.

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3. Resumen de las publicaciones científicas

3.1. [A1] Combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique.

El primer artículo que conforma el compendio del presente trabajo de tesis tuvo como objetivo, el determinar la importancia que adquiere sobre el éxito del injerto de empalme la combinación aleatoria de diámetros de patrón y variedad para distintos ángulos de corte. La hipótesis de trabajo planteada sugería que ambos parámetros guardan una relación estadísticamente significativa, capaz de definir una zona óptima de trabajo a la hora de efectuar injertos exitosos.

Hoy día las tareas de injerto de empalme responden a dos premisas de trabajo. Por un lado, es sabido que, tanto para injerto manual como semiautomatizado o totalmente automatizado, se trata de buscar una coincidencia máxima entre los diámetros de ambas plántulas de partida, por lo que estas deben ser identificadas, clasificadas y pareadas visualmente; lo cual es una operación costosa y laboriosa. Para esta vinculación entre plántulas se aplica una serie de criterios de cotejo visual y selección, basados en características morfológicas, y que en ocasiones resultan subjetivos y susceptibles a error humano [6,16]. Por otro lado, y a la hora de efectuar el corte de las plántulas, al ser una tarea habitualmente realizada a mano y carente de precisión, más allá de la que aporta una estimación visual, se opta por realizar la bisección aproximada entre los 30º y los 45º, complicándose el proceso para el injerto a mano el realizar cortes precisos a ángulos de mayor inclinación en tallos tan finos.

El ensayo sobre el que se fundamente el presente artículo consistió en 1500 experiencias de injerto para distintos ángulos de ensayo, siendo estos de 0º, 10º, 20º, 30º, 40º, 50º, 60º, 70º, 80º y 85º. En cuanto a la diferencia de diámetros entre tallos, en el experimento estos fueron testados con una variabilidad entre los 1,5 y 2,5mm de diámetro en la zona próxima al corte para la variedad y entre 2 y 3mm de diámetro para la zona próxima al corte para el portainjerto, siguiendo estos una distribución normal en cuanto a diferencia diametral para los distintos ángulos de ensayo. La combinación de muestras patrón y variedad fue establecida con aleatoriedad, únicamente teniendo en cuenta a la hora de su elección, que las plantas estuviesen sanas, que guardasen una anatomía y crecimiento admisible, y que los diámetros quedasen dentro de las referencias límite establecidas.

Para el experimento se precisó el desarrollo de dos equipos simples de corte de plántulas que garantizasen la exactitud en el ángulo de disección requerido para cada ensayo y la integridad de las plántulas. El equipo garantiza la verticalidad del tallo frente al corte y la precisión y limpieza en el ángulo de corte efectuado. Los equipos, similares y complementarios entre sí, están destinados, uno al corte del patrón y el otro al corte de la variedad, con objeto de poder integrar dichos sistemas sencillos de corte en el entorno final de injerto.

La unión de las plántulas cortadas mediante los equipos descritos, fue ejecutada de manera manual mediante el uso de la técnica habitual de injerto y de curación postinjerto de empalme [17]. Los injertos permanecieron en cuidados intensivos durante los 3 primeros días después del injerto dentro de un túnel de prendimiento, y su consumación y recuperación fue supervisada y valorada rutinariamente durante los 11 días sucesivos dentro de una cámara de cultivo, completando un total de 14 días de observación directa y continuada.

Analizando los resultados experimentales es observable que el efecto de la disparidad en el diámetro reduce su impacto o importancia sobre el éxito del injerto a medida que aumenta el ángulo de injerto, siendo prácticamente despreciable su consideración en una franja comprendida entre el los 50º y 80º, donde la variación porcentual entre injertos efectuados y exitosos se mantuvo en tasas globales por encima del 90% de éxito, e incluso por encima del 95% entre los 60º y 80º, lo que viene a corroborar que en esa franja de altos valores de ángulo las posibilidades de éxito dependen en menor medida de los diámetros de las plántulas de partida.

A partir de los 80º comenzó a ser materialmente más complicada la ejecución exitosa del injerto, debido fundamentalmente a dos factores: por un lado debido a limitaciones físicas, propias de la tecnología empleada para el corte y posterior unión de las plántulas; y por otro lado, debido al aumento exponencial de la superficie seccionada, relacionada directamente con la tangente del ángulo de corte, que condiciona tanto la superficie expuesta como la propia rigidez y firmeza de la estructura de las plántulas diseccionadas y posteriormente unidas.

Atendiendo al estudio del éxito del injerto en función de la diferencia de diámetros, cuando esta fue nula o muy baja, la tasa de éxitos máxima se registró para valores de ángulo comprendidos entre los 30º y 60º de corte, mientras que cuando esta diferencia entre diámetros comenzó a ser sustancial, dicha zona de máximos comenzó a desplazarse a valores de corte comprendidos entre los 50º y 70º, a la par que fue disminuyendo ligeramente el porcentaje de éxito.

3.1. [C1] Effect of cutting angle in the speed of healing under splice grafting method

Con este estudio, complementario y derivado del anterior, se pretendió evaluar el efecto e influencia del ángulo de corte en la velocidad de curación en el método de injerto de empalme, bajo unas mismas condiciones de curación, e independientemente del éxito del injerto, es decir, se obviaron del estudio los injertos fallidos, y solamente se evaluaron aquellos que si consumaron el proceso completo de curación.

El desarrollo de este estudio fue paralelo al anterior, y realizado a partir de las mismas experiencias de injerto efectuadas con los dispositivos de corte descritos, y bajo las mismas condiciones y estrategias preinjerto, de injerto y postinjerto. El experimento, por tanto, fue basado en las 1500 experiencias de injerto realizadas para distintos ángulos de ensayo, siendo estos de 0º, 10º, 20º, 30º, 40º, 50º, 60º, 70º, 80º y 85º, y con una disparidad entre diámetros de 2±0,5mm para la variedad y de 2,5±0,5mm para el patrón.

Para la valoración del punto de inflexión a partir del cual se consideró el injerto como consumado y curado, se estimaron síntomas externos y evidencias fisiológicas, recogidos a partir de la inspección visual del injerto, tales como el vigor o la flacidez de los tallos, la correcta formación y proliferación de células callosas, la aparición de áreas necróticas, la evidencia de diferencias significativas de crecimiento entre patrón y variedad, decoloraciones foliares, defoliaciones o aparición progresiva de nuevos brotes axilares en la variedad, entre otros. Durante los 3 primeros días después del injerto, estos permanecieron sin ser manipulados dentro de un túnel de prendimiento. Los días naturales establecidos para evaluar el éxito del injerto se extendieron entre el cuarto y el noveno día, donde comenzaron a apreciarse evidencias explícitas de una continuidad vascular adecuada, con una mayor vigorosidad de la parte aérea y una formación del callo firme que asegurase la unión del injerto.

Los resultados obtenidos para diferentes ángulos de unión, muestran que existe relación entre los días de recuperación y el ángulo de corte realizado en el proceso de injerto. No se apreció relación significativa entre diferencias diametrales y los días de recuperación del injerto.

Se observa que cuanto mayor es el ángulo de corte realizado en la unión, el proceso de cicatrización y aclimatación se acelera y, por lo tanto, es menor el tiempo de convalecencia crítica en la cámara de prendimiento. Para ángulos de corte pequeños, entre 0º y 20º, el tiempo de curación del injerto se prolongó durante algo más de 7 días, y este se redujo gradualmente hasta situarse en valores próximos a los 5 días para ángulos de corte altos, superiores a los 50º. Pudo comprobarse que la tendencia de tiempo de recuperación clínica del injerto seguía una tendencia próxima a un comportamiento logarítmico decreciente.

3.2. [A2] Behaviour of different grafting strategies using automated technology for splice grafting technique.

El segundo artículo que conforma el compendio del presente trabajo de tesis tuvo por objeto el analizar las tasas de éxito e influencia de distintas estrategias de trabajo bajo la técnica de injerto de empalme. Se analizó el orden de colocación entre clip y plántulas, así como la orientación del corte con respecto a la abertura lateral del clip de injerto, con objeto de servir de base para su uso en el procedimiento de injerto manual con apoyo de equipos externos o para su implementación en equipos automatizados. El fin último del estudio fue la implementación de la estrategia óptima de trabajo en el entorno final de injerto.

Para garantizar la unión entre plantas en el injerto de empalme es muy común el uso de clips en forma de tubo dotados de una abertura longitudinal y con aletas laterales que facilitan su manejo a la hora de la su colocación. Su uso se ha estandarizado a nivel mundial [18]. Una vez colocados los clips, estos ejercen su función de sujeción y apriete sobre las plántulas durante la fase de curación, para después de desprenderse de manera natural.

El ensayo sobre el que se fundamente el presente artículo consistió en 900 experiencias de injerto divididas en 6 estrategias de trabajo distintas. En el experimento, tanto patrón como variedad fueron cortadas a un ángulo de unión preciso de 60º mediante los dispositivos de corte de plántulas definidos en el primer artículo. Para el manipulado, dos robots industriales del fabricante KUKA Roboter GmbH (modelo Agilus R6 900), operaron de forma independiente en el manejo las plántulas, de modo que uno de los robots sujetaba y maniobraba el portainjerto y el otro sujetaba y maniobraba el vástago durante la tarea de dispensado y colocación del clip.

Para la evaluación del ensayo, y con el objetivo de integrarlo en el entorno final de injerto, se desarrolló un dispositivo capaz de realizar las operaciones básicas de preparación y conformado del clip de injerto y de colocación sobre las plántulas a injertar. El dispositivo, en una primera fase, confecciona un clip de injerto a partir de un rollo continuo de cinta de tubo de plástico, cortándolo a la longitud deseada. En una segunda fase, el clip de injerto es sujetado por un mecanismo que presiona las alas laterales, pinzándolo y abriendo así el clip al máximo, En una fase posterior lo aproxima horizontalmente hasta ubicarlo en el punto de unión de las dos plántulas de injerto. Finalmente, el dispositivo de pinzado suelta el clip de injerto, quedando este presionando las plántulas vinculadas.

Se establecieron dos alternativas en cuanto al orden de consumación del injerto. Una de ellas consistente en colocar primero el clip de injerto en el punto de vinculación para, a continuación, introducir por su abertura superior e inferior el patrón y la variedad (CS), y otra, consistente en colocar primero las plántulas en el dispositivo pasivo de encaje, para después aproximar y colocar el clip de injerto en el punto de vinculación sobre las plántulas (SC). Para cada una de las dos alternativas de orden de vinculación fueron ensayadas tres combinaciones de orientación. Una vinculando los tallos de modo que la línea de unión quedase de perfil frente a la abertura lateral del clip (S), otra vinculando los tallos de manera que el portainjerto quedase al frente (FR), y otra vinculando los tallos de manera que la variedad quedase al frente (FS). En consecuencia, y a partir de la comparativa combinada de las 6 estrategias de unión (CS-S, CS-FR, CS-FS, SC-S, SC-FR y SC-FS).

La estrategia de trabajo consistente en aproximar las plántulas hasta que sus superficies libres estén en contacto y luego ajustar el clip de injerto garantiza un 20% más de éxito en el proceso de injerto de empalme (SC). La orientación de las plántulas colocando el perfil de unión frente a la abertura vertical del clip (S) resultó un 10% más exitosa que orientar el injerto colocando la superficie de unión con el portainjerto o el vástago frente a la abertura del clip. En consecuencia, y a partir de la comparativa de las 6 estrategias de unión, la opción de injerto más exitosa es la consistente en colocar las plántulas primero, de modo que la línea de unión quede de perfil frente a la abertura del clip, y luego ajustar sobre ellas el clip de injerto (CS-S), suponiendo un éxito superior en más de 12 puntos porcentuales con respecto a las siguientes estrategias más exitosas.

3.3. [A3] Conventional industrial robotics applied to the process of tomato grafting using the splicing technique

El objetivo de este tercer y último artículo recoge para su aplicación aquellos resultados obtenidos en las anteriores publicaciones. El artículo se centra en el estudio de viabilidad y eficiencia del injerto automatizado mediante una célula de trabajo basada en el uso de robótica industrial convencional, y sustentado en el uso de equipos auxiliares simples, que permitan consumar como herramientas externas las operaciones sobre el injerto. La tendencia de precios que está experimentando en los últimos años la robótica industrial, junto al uso de unidades auxiliares pasivas de funcionamiento sencillo para el corte de plántulas y colocación del clip de unión sobre las plántulas, permitiría una rápida amortización de la inversión, lo cual hace de interés esta alternativa de desarrollo.

El estudio desarrollado hace uso de dos robots industriales del fabricante KUKA Roboter GmbH (modelo Agilus R6 900) para la manipulación y desplazamiento de las plántulas entre los puntos de operación, de dos equipos de corte de plántulas y de un equipo encargado de dispensar y colocar el clip. Cada uno de los robots maneja cada una de las plántulas, patrón y variedad, que son recogidas a partir de dos bandejas de entrada.

Una vez sujetas las plántulas, en primer lugar, estas son posicionadas ante dos equipos de corte, iguales entre sí, y que garantizan la precisión en el ángulo requerido. En dicho punto se efectúa un corte a bisel para ambas plantas a 60º, tal y como quedó definido en el primer artículo de este trabajo de tesis. Realizado el corte, los brazos robóticos portan las partes útiles de las plántulas hacia la zona de unión. El dispositivo que materializa la unión se encarga de elaborar cada uno de los clips de plástico a partir de un rollo continuo, y colocarlo sobre las plántulas a injertar. La estrategia de unión asumida, tal y como quedó determinada en el segundo artículo de este trabajo de tesis, es la consistente en unir primero las plántulas, orientando la línea de unión de perfil a la abertura del clip, para luego colocar y ajustar el clip de injerto. Una vez puesto el clip, el robot que sujeta al injerto culminado, deposita el injerto en la bandeja de salida.

El ensayo sobre el que se fundamente el presente artículo consistió en un total de 900 experiencias de injerto para un total de 6 velocidades ensayadas. En el proceso fueron experimentadas distintas velocidades de trabajo en los robots, con el objetivo de determinar su influencia en el éxito del injerto. Las velocidades de los robots fueron constantes dentro de cada ensayo, y comprendidas entre los 100mm/s y los 600mm/s, con incrementos graduales de 100mm/s. Únicamente se mantuvieron constantes entre ensayos los tiempos de trabajo empleados por los procesos externos sobre las plántulas (dispositivos de corte y dispensado del clip), al basar su funcionamiento en tecnología neumática.

Se evaluó el éxito o fracaso del injerto final mediante una estimación y apreciación visual realizada diariamente durante los 14 días posteriores al injerto. Los injertos permanecieron en cuidados intensivos durante los 3 primeros días después del injerto dentro de un túnel de prendimiento, y los 11 días sucesivos dentro de una cámara de cultivo.

A bajas velocidades de ensayo la tasa de éxito es mayor, situándose por encima del 90% para velocidades igual o inferiores a 300mm/s, y aumentando significativamente el fracaso en el injerto a partir de velocidades de trabajo igual o superiores a 400mm/s. Bajas velocidades, comprendidas entre 100 y 300mm/s, tienen un comportamiento similar en cuanto a fallos, y por tanto podemos considerar sus diferencias derivadas del azar y de la propia variabilidad natural de las plántulas, y no derivada de la propia velocidad de trabajo. Es por tanto evidente que velocidades de trabajo comprendidas en torno a 300mm/s, resultan más atractivas, por ser la de mayor ratio productivo asociado a una baja tasa de fracasos.

Se observa que, a las velocidades de trabajo de los robots entre los 300 y 400 mm/s (210 y 240 injertos/hora respectivamente), ya se supera el número de injertos medio estimado para trabajadores expertos manuales, que ronda máximos de 150~240 injertos/hora [13,19], y produciendo una media en torno a no más de unos 1000 injertos por persona y día [12,15,20]. Además, las tasas de éxito para el injerto manual no suelen ser muy altas, oscilando entre el 81% y el 91% [21]. En parte dicha tasa de fracaso puede venir derivada de largas jornadas en un entorno de trabajo hostil, caracterizado por unas exigencias de alta humedad y temperatura. Las tasas de éxito alcanzadas mediante el injerto robotizado experimentado son bastante similares a las alcanzadas por trabajadores de manera manual, llegándose al 90,0% para la velocidad de 300mm/s, y del 85,3% para la velocidad de 400mm/s.

4. Conclusiones científicas y trabajos futuros

4.1. [A1] Combined influence of cutting angle and diameter differences between seedlings on the grafting success of tomato using the splicing technique.

De este primer artículo podemos extraer como conclusión, y a partir de los resultados obtenidos, que el éxito del injerto de empalme se ve influenciado en gran medida por la combinación de las variables de ángulo de corte y diámetros de las plántulas de trabajo, quedando en evidencia que, la disparidad o similitud entre diámetros de las plántulas juega un importante papel en el éxito a pequeños ángulos de corte, disminuyendo su importancia conforme aumenta dicho ángulo de corte, y siendo mínima su influencia en el intervalo comprendido entre los 50º y 70º, siempre dentro de los rangos de secciones de plántulas de trabajo empleados para el presente estudio.

Consecuentemente, un ángulo de corte situado entre los 50º y 70º en la técnica de empalme puede llegar a suponer una mejora sustancial en las condiciones y técnicas de injerto, eliminando en cierta medida el factor azaroso de desigualdad entre diámetros, y pudiendo eliminar las labores de preselección y emparejado de secciones similares de plántulas, simplificando con ello esta exigencia para sistemas de injerto manuales, automatizados y robotizados.

Los resultados obtenidos a partir de dicho trabajo fueron aplicados con posterioridad a al resto de estudios, jugando con una similar variabilidad natural en el diámetro de los tallos, y con un ángulo de corte de 60º, que ofrece unos resultados óptimos de éxito para el injerto realizado mediante la técnica de empale.

4.2. [C1] Effect of cutting angle in the speed of healing under splice grafting method

Como conclusión puede afirmarse que, para ángulos de corte altos, e independientemente de su tasa de éxito, hay una recuperación clínica más rápida de la planta injertada, lo que implica implícitamente un menor costo en atención clínica en la cámara de curación en viveros de producción. Esta fase de recuperación, es tal vez una de las más delicadas y críticas en cuanto a condiciones climáticas de ambiente y de seguimiento.

Del mismo modo, las plantas de injerto exitosas con un ángulo de corte alto, tienden a desarrollar una respuesta ante la herida más aguda y enérgica, generando una superficie callosa más de mayores dimensiones, y cicatrizando en un menor número de días, logrando con ello una unión más robusta del callo.

4.3. [A2] Behaviour of different grafting strategies using automated technology for splice grafting technique.

Como conclusión, podemos decir que, el éxito en el injerto de empalme realizado mediante el uso de robots industriales para el manipulado de plántulas y el uso de un dispositivo sencillo para el conformado, dispensado y colocación el clip de injerto en sustitución de la tarea humana, se ve influenciado por la estrategia de trabajo desarrollada.

Si acometemos en primer lugar la vinculación de las plántulas a injertar, para posteriormente colocar el clip de injerto que garantice su unión, dicha estrategia es un 20% más exitosa. Además, analizadas las distintas alternativas de orientación de las plantas frente al clip de injerto, se aprecia un 10% más de éxito en el injerto cuando dicha orientación presenta el perfil de corte frente a la abertura del clip.

Dichos resultados presentados por separado, y referidos al orden de vinculación y al modo de orientación, en combinación se comportan de igual manera. Si en primer lugar se colocan las plántulas en contacto, con la línea del perfil de corte ante la abertura del clip, para a continuación colocar el clip de injerto, esto supone al menos un 12% más de éxitos con respecto a las estrategias de trabajo que le siguen en porcentaje de éxito.

4.4. [A3] Conventional industrial robotics applied to the process of tomato grafting using the splicing technique

El uso de bajas velocidades, comprendidas entre los 100mm/s y los 300mm/s permite mantener unas ratios próximas al 90% de éxito. A velocidades medias-altas, aquellas comprendidas entre los 400mm/s y los 500mm/s, continúan teniéndose ratios aceptables de éxito, por encima del 80%, pero, sin embargo, a velocidad de ensayo de 600mm/s se aprecia una disminución considerable en cuanto al porcentaje de éxitos en el proceso de injerto, situándose por debajo del 70%.

Consecuentemente, podemos concluir que es recomendable el uso de una velocidad próxima a los 300mm/s (90,0% de éxito), lo que permite trabajar a velocidades superiores a las estimados para trabajadores expertos manuales, que ronda los 150~240 injertos/hora (con unas tasas ente 81% y el 91% de éxito). El disminuir la velocidad de trabajo por debajo de ese punto no hace mejorar sustancialmente el porcentaje éxito.

4.5. Líneas de investigación para trabajos futuros

El desarrollo y experimentación del presente trabajo de tesis se sitúa dentro del sector de la industria auxiliar de la agricultura, tratando de dar respuesta, mediante el uso robótica industrial convencional y sencillos equipos auxiliares, al proceso productivo automatizado de plántulas hortícolas injertadas de tomate.

El propósito perseguido ha sido el diseño y adaptación de una célula robotizada de trabajo desarrollada, con el fin de optimizar parámetros y metodologías propias de la técnica tradicional de injerto de empalme. El rango óptimo de ángulos de corte de las plántulas, la orientación del corte con respecto al clip de injerto o el orden de vinculación entre clip y plántulas han sido los principales parámetros de estudio testados. Finalmente, y aplicando sobre el equipo los resultados anteriores se analizó la tasa de éxito en función de distintas velocidades de trabajo del sistema para el injerto de tomate.

Posibles líneas de investigación y de trabajo futuro podrían versar sobre:

- La posibilidad de replicado de sus sistemas activos dentro de las distintas unidades auxiliares de manipulado, tales como los elementos terminales de los robots, los dispositivos de corte y el dispensador y colocador de clips, consiguiendo con ello la capacidad de realización de varios procesos de injerto de forma paralela y simultáneamente, multiplicando con ello la capacidad productiva del sistema.

- El estudio de resultados para otras variedades hortícolas de solanáceas o bajo otras estrategias de trabajo, teniendo en cuenta que el desarrollo de la tesis se ha centrado en el ensayo para el injerto de variedad de tomate sobre pie de tomate, como principal variedad demandada.

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