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## IMPORTANCE OF COMPATIBILITY BETWEEN ROOTSTOCK AND SCION AND ITS RELATIONSHIP

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### ABSTRACT

Numerous studies have been conducted to try to understand the causes underlying graft incompatibility in order to better understand how grafts form. These publications discuss the effects of these occurrences on the subsequent graft response as well as the early cytological and biochemical reactions that occur in response to grafting. According to certain theories, cellular recognition may play a role in the formation of a functional vascular link ever since the first calluses appear. Callus development can, however, be a passive reaction to a wound with no consequences for the compatibility responses in the future. We list several factors that could affect graft success, including the presence of growth regulators and peroxidases, the development of plasmodesmata, connections between vascular tissue, and an intrinsic system of cellular incompatibility. Additionally, it has been noted that when graft bridging is established and functioning, phloem-mobile proteins traverse the graft interface. Overviews of recent developments in the understanding of the mechanisms behind the early reactions to grafting for the establishment of cohesiveness between the stock and scion during graft ontogeny are included in this review.

**Key words:** Graft, compatibility, scion, rootstock, scion- rootstock relationship, growth.

### Introduction

Grafting plants is a common method of plant multiplication and growth management that is crucial for the adaptation of interesting cultivars in suitable habitats. The grafted partners can be from the same species or genus, but more genetically diverse components are typically employed. In these situations, the stock and scion don't always make a good graft and exhibit their incompatibility as a sign of discord. process of fusing two components so they grow as one plant. Natural or intentional fusion of plant parts that results in the establishment of vascular continuity between them and the functioning of the resulting genetically composite organism as a single plant (David *et al.*, 2003). more than two thousand years ago, when it first appeared. While it was, grafting methods have been consistently refined and enhanced over a long period. Grafting is a prevalent practise today for the routine asexual multiplication of many different plant species, including fruit trees,

vegetables, and flowers. used to boost production, improve quality, and strengthen resistance. Scion is the upper portion, which is the shoot system, Stock is the lower portion, which is the root system, and Interstock is the stem piece in between the two.

The order of structural occurrences during the healing of grafts in woody and herbaceous plants has been identified by a number of writers. (Hartmann *et al.*, 2002) overview in their examination of the timeline.

1. Cut scion in order to join through the callus bridge, tissue capable of meristematic activity is brought into secure, intimate contact with similarly cut stock tissue in such a way that the cambial areas of both are in close proximity. The callus tissue that soon intermingles and interlocks, filling the spaces between the two components, connecting the scion and the rootstock, is produced once the two parts of the graft, stock and scion, are in close contact. New parenchymatous cells

proliferate from both the stock and the scion at this point.

2. New cambial cells separate from the freshly created callus, creating a continuous cambial connection between the rootstock and scion. Furthermore, early xylem and phloem may be distinguished before the binding of vascular cambium across the callus bridge. The wound-repair xylem is generally the first differentiated tissue to bridge the graft union, followed by wound-repair phloem.
3. The newly generated cambial layer in the callus bridge starts typical cambial activity creating new vascular tissues as the final step of the graft establishment process. The vascular link between the scion and rootstock is thus made possible by the production of new xylem and phloem. This is regarded by the majority of authors as being a necessary condition for a successful graft (Moore and Walker, 1981a, b; Yeoman, 1984; Tiedemann, 1989).

Uncertainty surrounds the method by which incompatibility manifests, and a number of hypotheses have been put out in an effort to do so. The bulk of early-stage development-related hypotheses have involved herbaceous systems. However, there are few research on early establishment in woody plants, where incompatibility is frequently seen. The breaking of the trees at the point of the union particularly when they have been growing for some years (apricot on *Prunus* grafts, pear on quince grafts).

### Physiology of grafting

Graft union creation includes the formation of the isolation layer, the first adhesion, and the rootstock callus formation on the wound. Reestablishing the vascular link between the scion and rootstock:

1. Formation of an isolation layer (1 day) Cutting: causes the isolation layer (Necrotic layer) to develop; Early stages: The entire graft interface is covered with an isolation layer (Stoddard *et al.*, 1979);
2. Callus formation in the wound (2-3 days): Parenchyma cells were created by the division of cambium, phloem, xylem, and pith cells close to the injured surface. Only a few callus cells are present in the pith, where callus cells first develop at the vascular bundle and cortex;
3. Formation of the wound callus (2-3 days): During callus formation, the isolation layer in the vascular

area is disturbed, subsequently extended progressively. Rootstock and Scion can communicate directly. Because of the growth pressures brought on by the callus plunging at both ends of the graft union, the isolation layer vanishes. The two graft partners may become even more connected if the wound callus cells are mixed together. In the thinning zone of the isolation layer, plasmodesmata develop between scion and rootstock cells. They are regarded as secondary plasmodesmata because they develop in non-dividing cell walls. Cells between the rootstock and scion are connected by secondary plasmodesmata to create a continuous symplast. These are crucial to the continued growth of the graft union.

4. The establishment of wound-healing vascular bridges between the scion and rootstock is a crucial phase in suitable grafting. Divergence of xylem components can be 3 days after grafting, elements can be seen in the wound callus. Newly generated xylem elements typically immediately develop from callus cells at the graft union or parenchyma cells surrounding vascular bundles. They have an uneven shape. (1983; Jeffree and Yeoman) A freshly created wound cambium may eventually form between them and give rise to new phloem and xylem strands as the xylem and phloem portions of the graft union gradually differentiate. (Moore, 1981). Many times, the completion of the graft union is indicated by the formation of wound cambium spanning the graft union. (Kollmann *et al.*, 1990)

### Callus formation: the beginning of the graft process

Following the callus' initial creation, when the callus cells first contact, it appears that the subsequent processes are crucial in determining how future vascular connections will grow. Yeoman described a cell recognition system in which opposing cells of graft partners touch as the first to record structural events associated with the alterations in wall cells occurring during graft formation in Solanacea (Yeoman *et al.*, 1978; Yeoman, 1984). This recognition system's fundamental mechanism is based on the fact that protein molecules released from plasmalemmas combine to create a complex with catalytic activity, which then starts a developmental sequence that results in the production of a successful graft. Up until now, neither the makeup of these proteins nor their functions have been described. A special type of protein called lectin causes the opposing graft cells to mutually reject each other in the absence of this complex, which results in the

**Table 1:** The productivity of and quality of cuttings from non-grafted and grafted chrysanthemum “Jinba”.

Batch	Treatment	Yield (no. of cuttings per 10 plants)	Mean length (cm)	Mean node number	Mean diameter (mm)	Mean fresh matter (g)	Mean dry matter (g)
H1	Non-grafted	37	8.1 ± 0.40a	5.2 ± 0.19b	3.43 ± 0.091a	1.98 ± 0.137a	0.244 ± 0.022a
	Grafted	42	8.1 ± 0.36a	6.3 ± 0.29a	3.14 ± 0.054b	1.98 ± 0.127a	0.236 ± 0.012a
H3	Non-grafted	98	5.1 ± 0.26b	5.9 ± 0.23a	2.95 ± 0.043b	0.99 ± 0.040b	0.163 ± 0.019b
	Grafted	150	6.1 ± 0.22a	6.3 ± 0.19a	3.35 ± 0.029a	1.48 ± 0.072a	0.203 ± 0.028a
H5	Non-grafted	72	6.9 ± 0.28b	7.3 ± 0.21b	2.50 ± 0.054b	1.09 ± 0.061b	0.191 ± 0.012b
	Grafted	83	8.6 ± 0.49a	9.1 ± 0.35a	2.72 ± 0.058a	1.42 ± 0.088a	0.246 ± 0.016a

creation of incompatible grafts (Yeoman and Brown, 1976).

Additional research on the development of callus tissue suggests that some of these substances play a role in the graft partners' adhesion mechanism. Callus cells near the graft union have been found to have wart-like projections on the cell wall surface (Jefree and Yeoman, 1983; Barnett and Weatherhead, 1988). In Sitka spruce, a “cement” or binding substance that projects bead-like projections from the callus and consists of a homogeneous matrix made up of a mixture of pectin, carbohydrate, protein, and fatty acids as well as a fibril/vesicular component primarily composed of carbohydrate and pectins aids in the adhesion between cells of the scion and rootstock (Miller and Barnett, 1993). In addition to serving as binding or cementing cells, these bead-like projections may play a more active role in cell recognition and the successful fusion of tissues between the graft partners.

The first adhesion of stock and scion is a passive action that happens in response to injury, therefore Moore and Walker (1981a,b) looked at the relationship between the structural events related with graft formation in Crassulacea and discovered that no recognition events are required. However, at least during the differentiation of the procambium between the stock and scion, some form of cellular communication is necessary (Moore and Walker, 1981a). This procambial development may not have occurred in incompatible grafts because the graft partners lacked a second, more direct method of cellular communication (Moore and Walker, 1981b). Therefore, even in the absence of blood, grafting surfaces can cohere. As proposed by Moore (1984) in compatible autografts in *Sedum telephoides*, where the initial

**Table 2:** Number of structural shoots 50 cm above ground, diameter of flowers, length and diameter of roots on *Rosa floribunda* cultivars.

Cultivars/ <i>Floribundas</i>	Number of structural shoots	Diameter of flowers, cm	Length of roots, cm	Diameter of roots, mm
Efill Tower	5.8	12.3	32.2	6.78
Queen Mother	4.6	11.3	34.5	7.26
Kings Ranson	6.7	10.5	30.5	7.23
Papa Meiland	5.3	11.4	35.6	6.12
Summer Fashion	4.9	9.8	29.2	8.12
Bourbon Queen	6.3	9.8	33.5	7.87
Fragrant Delight	5.6	10.4	37.4	7.21
Cristophor Colombo	6.2	11.1	29.8	7.65

**Table 3:** Structural shoots 50 cm above ground, diameter of flowers, length and diameter of roots on Polyantha Rose cultivars.

Cultivars/ <i>Polyantha Rose</i>	Number of structural shoots	Diameter of flowers, cm	Length of roots, cm	Diameter of roots, mm
Anne Denneke	5.7	11.3	29.6	6.12
Ingrid Bergman	6.2	10.5	31.20	7.43
Charles de Gouille	5.9	10.6	34.33	8.23
Double Delight	6.6	11.2	32.1	6.78
Mister Lincoln	5.9	9.7	34.4	7.8
Norita	6.3	10.4	32.1	8.23
Vaj Vicend	5.8	9.3	37.2	7.78
Summer Nights	5.9	10.4	32.8	7.21

cohesiveness of stock and scion is mediated by some extracellular connection between the graft partners, of direct cellular contact and protoplasmic continuity. The graft union contains a large number of the cell wall polymers suggested for cellular recognition reaction. These would be pectic pieces used to determine graft compatibility as chemical messengers (Jefree and Yeoman, 1983). It has been extensively researched that oligosaccharides generated from cell walls can control how plants grow and develop (for review, see Ridley *et al.*, 2001; Creelman and Mullet, 1997; John *et al.*, 1997; Fry *et al.*, 1993).

Extracellular materials linking adjacent cells at the surface of intact, growing callus cells have also been discovered in other studies. These findings most likely represent pectic material involvement in the creation of the first mechanical union across cell surfaces (Moore, 1983; Kollmann and Glockmann, 1991).

Despite the fact that callus formation happens as a result of a wound reaction and that it can occur in both compatible and incompatible grafts, the makeup and characteristics of the cells involved in the first stage of graft formation can have a significant impact on the events that cause a strong and successful union to form. In this regard, some variations in cell organisation, stain intensity of cellulose and lipid, and cell phenol content have been noted in the callus fusion of *Prunus* combinations developing *in vitro* (Errea *et al.*, 2001). The alterations in the callus system and the modifications in the graft systems at a later stage are connected. In order to prevent a strong union in *Prunus* systems, the cellular anomalies found in callus tissue appear to be responsible for the absence of cambial and vascular development. Some theories suggested that herbaceous plants may explain the callus differentiation mechanism by mediating local interactions between the opposing cells of the graft union (Yeoman, 1984; Jefree *et al.*, 1987).

The question of what sort of cellular communication and substance creation might be involved in this differentiation mechanism concerns why some callus tissue is capable of forming cambium and vascular connections, while a significant amount of the callus generated is unable to differentiate.

**Table 4:** The effect of interaction between propagation methods and cultivars on measured parameters.

Cultivars	African Dawn		Ilios		Maroussia		Soprano	
	Cutting <sup>a</sup>	Stenting <sup>a</sup>	Cutting <sup>a</sup>	Stenting <sup>a</sup>	Cutting <sup>a</sup>	Stenting <sup>a</sup>	Cutting <sup>a</sup>	Stenting <sup>a</sup>
Flowering stem number	9.75abc <sup>b</sup>	8.38cd	10.75a	9.38abcd	10.13abc	8.75bcd	9.75abc	8.13d
Flowering stem length (cm)	53.25b	51.38b	53.25b	50.47b	54.79b	51.89b	62.38a	62.25a
Flowering stem fresh weight (g)	28.67e	49.59a	27.67f	41.22c	20.51g	38.01d	29.06e	44.91b
Quality index (g cm <sup>-1</sup> )	0.54d	0.97a	0.49de	0.82b	0.38f	0.73c	0.46e	0.73c
Flowering stem diameters (mm)	4.30c	6.32a	4.07c	4.92b	3.98c	6.00a	5.26b	6.20a
Node number	11.88ab	11.25ab	11.59ab	11.23ab	10.34b	10.63b	15.00a	14.44ab
Internode length (cm)	4.25c	3.60d	4.79b	4.28c	6.64a	6.30a	4.11c	4.04cd
Leaf number of flowering stem	13.00b	11.88cd	12.34c	12.42bc	10.44e	11.63d	15.50a	14.94a
Leaf chlorophyll content (mg g <sup>-1</sup> fw)	0.75c	0.82ab	0.62d	0.72c	0.61d	0.76c	0.81b	0.86a
Leaflet number of flowering stem	57.25e	55.13e	63.25c	63.65c	57.54e	60.00d	78.75a	72.60b
Petal number	29.13e	32.13d	46.38c	46.21c	86.90b	97.44a	31.88d	31.94d
Flower fresh weight (g)	6.56f	10.91b	9.27d	9.87c	7.85e	17.95a	6.16f	7.78e
Flower dry weight (g)	1.19d	2.21b	1.69c	1.94bc	1.19d	2.71a	1.21d	1.74c
Flower diameter (cm)	2.30de	2.39de	2.59bcd	2.91b	2.41cde	3.28a	2.16e	2.75bc
Flower length (cm)	3.58cd	5.73a	3.70cd	3.94c	3.88c	3.28d	4.55b	4.87b

### Plasmodesmata and its role in cellular communication

Plasmodesmata, which come in a variety of shapes and are extremely dynamic, provide a special route for symplastic cell contact and could provide a route for cells in the graft bridge. Studies on the plasmodesmata's mechanism have demonstrated their significant contribution to cellular communication (Salisbury and Ross, 1991; Lucas *et al.*, 1993; Schulz, 1999), despite the long-standing controversy about the existence of symplastic connections in grafts. According to Jefree and Yeoman (1983), when callus cells interact, the cell walls dissolve, holes occur in the cell walls, the plasmalemma come into touch, and plasmodesmata form. They symplastically link the grafting partners, allowing the interacting cells to interact metabolically (Strasburger, 1901). To produce a strong cohesiveness between rootstock and scion, this calls for the leaching or diffusion of certain substances from one side of the union to the other, diffusing into the cell walls (Yeoman and Brown, 1976).

With particular focus on the establishment and modification of primary plasmodesmata as in the novo formation or modification of secondary plasmodesmata, research on the mechanism of plasmodesmata formation has revealed prominent differences in the development of interspecific plasmodesmata between graft partners, suggesting that cell recognition and functional coordination may be involved in graft formation (Kollmann and Glockmann, 1985; Kollmann *et al.*, 1985). (Ehlers and Kollmann, 1996, 2001). The ER's smooth domains bind to the plasmalemma and determine where and how the nascent plasmodesmal strands will take shape. This is the first step in the creation of secondary plasmodesmata. Then, a process of plasmalemma invagination surrounding the ER cisternae takes place, which is explained as the result of wall material secretion by a plasmalemma/Golgi

vesicle fusion process. The plasmalemma that lines the plasmodesmal connections is made up of the membranes of the fusing Golgi vesicles (Kollmann and Glockmann, 1991). Since the opposing ER-plasmalemma contact is formed before any symplastic cell-to-cell contact is created, the coordination may include the exchange of informational signals across the cell wall (Jefree and Yeoman, 1983; Moore and Yeoman, 1989; Kollmann and Glockmann, 1991, 1999). Because insufficient coordination between adjacent cells results in the development of mismatched, half-plasmodesmata through the wall cell only in one cell partner, continuous secondary plasmodesmata must therefore be formed with the perfect cooperation of both cell partners. Utilizing species-specific cell markers to identify the graft interface, discontinuous-half plasmodesmata have been seen in graft unions between several types of cells in incompatible heterografts (Kollmann *et al.*, 1985). These reports suggest that, although they might not be specific to graft compatibility, plasmodesmata may contribute to graft failure due to a misalignment of the graft partners.

The physiological control of plasmodesmata can now be studied experimentally because to advancements in studies on caged probes, which eliminate experimental manipulations such pressure changes or cells that have been injured by microinjection (Schulz, 1999; Martens *et al.*, 2004). Recent research employing caged fluorescein has suggested that *P. munsoniana*'s *in vitro* callus cells may contain plasmodesmal connection (Pina *et al.*, data unpublished). The response thus far has been encouraging, and it is anticipated that incompatible combinations won't show a lot of uncaged fluorescein, which would indicate the presence of non-functional plasmodesmata. These investigations offer a foundation for understanding callus tissue's early reactions to

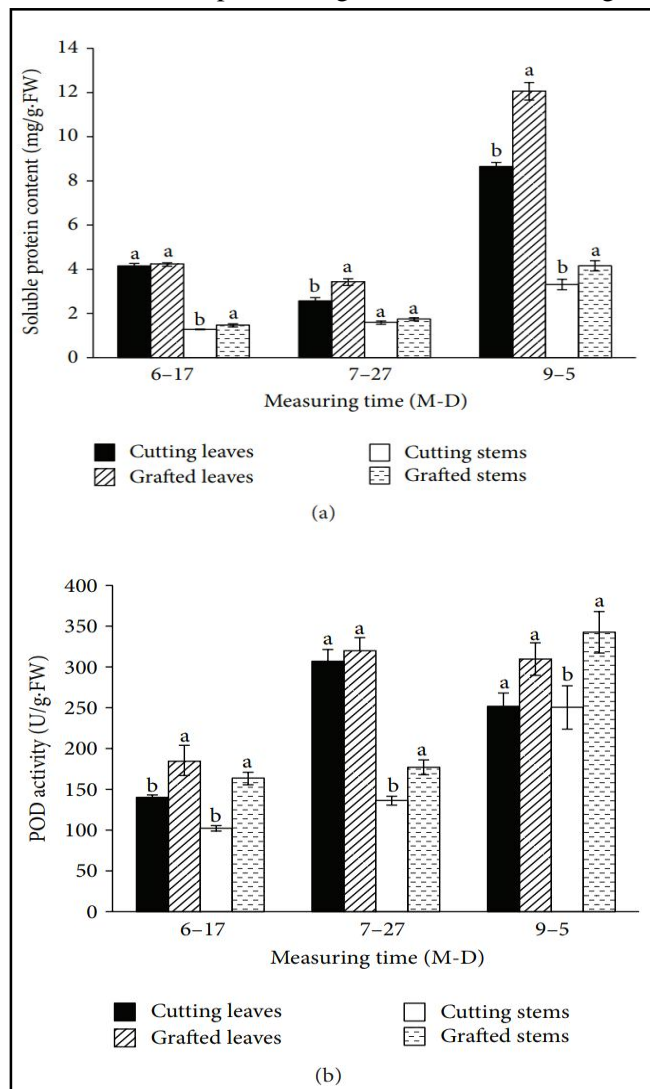
mixtures of apricot and plum. There needs to be further experimental proof of its potential involvement in the compatibility process and it is a subject of further researches.

### Vascular connections in the graft process

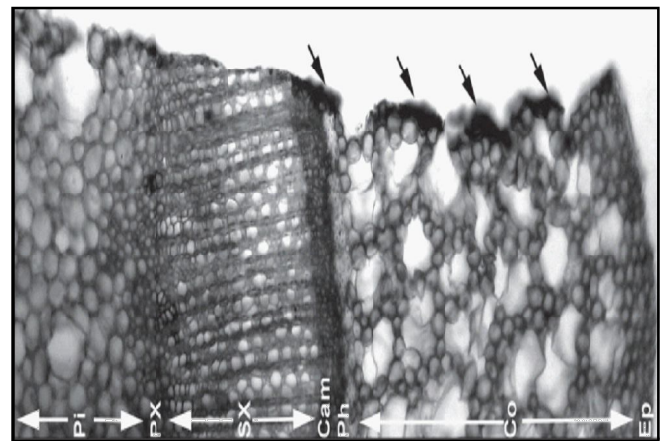
The primary cause of incompatibility in woody plants has been hypothesised to be the possibility that the new vascular connections may not be effectively differentiated or may be weakly formed (Mosse, 1962; Errea *et al.*, 1994a,b). In woody plants, incompatible grafts can grow for several years without any external signs of incompatibility, indicating the presence of functional vascular connections in incompatible grafts. This is contrary to the appearance of herbaceous plants, where the formation of functional vascular connections appears to be necessary for successful grafts (Mosse, 1962; Hartmann *et al.*, 1997). Some herbaceous experiments have focused on phloem regeneration across the graft

interface (Stoddard and McCully, 1979; Tiedemann, 1989; Kollmann and Glockmann, 1990; Golecki *et al.*, 1998). According to Schöning and Kollmann (Schöning and Kollmann, 1995, 1997), <sup>14</sup>C-labelling techniques showed that assimilated transport occurs from the apical callus of the scion to the basal callus of the stock in the compatible system *Lycopersicon esculentum* (L) on *Solanum tuberosum* (S) as well as in the autografts. As predicted, it was possible to show a close relationship between transport and phloem restitution in the graft union. In contrast, there was no link between phloem regeneration and assimilation transfer through the graft interface in the less compatible system of *Vicia faba* (V) on *Helianthus annuus* (H), and there was no increase in <sup>14</sup>C-transport to the stock during any stage of graft union formation. The non-functional phloem connections in the V/H-heterograft have been confirmed by 5-6 carboxyfluorescein (CF) translocation studies (Schöning and Kollmann, 1997). The activity of the newly formed xylem and phloem may be affected by this lack of cambial activity in some areas of the graft union, leading to discontinuities in the cambium and the formation of a parenchymatous line interrupting the vascular connection (Hartmann *et al.*, 2002), resulting in a mechanically weak union. Expression of important proteins should be induced by the urgent need to repair damaged vascular bundles, possibly at decreased levels due to shortened developmental times (Schulz, 1990).

Grafting techniques have been used to explore the long-distance translocation of proteins (Golecki *et al.*, 1998, 1999) and RNA (Ruiz-Medrano *et al.*, 1999; Go'mez and Palla's, 2004; Go'mez *et al.*, 2005) via the phloem due to the significance of the phloem as a nutrient and information transport system. According to research



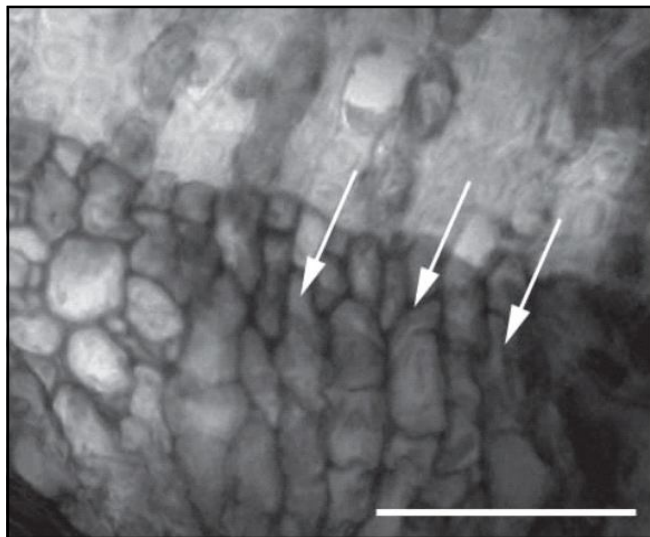
**Fig. 1:** The contents of soluble protein and POD activity in the nongrafted and grafted cuttings.



**Fig. 2:** Transverse section through a 3-day-old graft union shows scion anatomy and layer of necrotic tissues (black arrows). Ep, epidermis; Co, cortex; Ph, phloem; Cam, cambium; SX, secondary xylem; PX, primary xylem; Pi, pith. Bar = 100  $\mu$ m.

by Clark *et al.*, (1997) and Golecki *et al.*, (1999), when sieve elements are created and functional, P-proteins (PP1, PP2) are translocated into companion cells of developing sieve element-companion complex (Leineweber *et al.*, 2000). According to Dannenhoffer *et al.*, (1997), the expression of PP1 and PP2 is developmentally tied to specific stages of phloem differentiation, and they are intended to be phloem-mobile (Tiedemann and Carsens-Behrens, 1994). It is possible that the PP2-gene plays a significant role in the growth and operation of the vascular system given the conservation of the pattern of PP2-gene expression in the sieve element-companion cell complex in many angiosperm species (Dinant *et al.*, 2003). Although it is also known that the majority of phloem proteins play a role in both direct and indirect stress and defence responses in addition to maintaining sieve tubes (Walz *et al.*, 2002, 2004). Furthermore, it has been identified and characterised some melon phloem proteins with RNA-binding activity (Go´mez *et al.*, 2005)

Growth regulators also have an impact on the connections between scion and stock, and it has been hypothesised that graft incompatibility may also exist. For instance, auxin, which is released from vascular strands of the stock and scion and causes the differentiation of vascular tissues, serves as a key ingredient in the establishment of suitable unions (Moore, 1984; Aloni, 1987; Mattsson *et al.*, 2003). In addition, other substances, such as polyphenols, also significantly contribute to the formation of graft unions by influencing lignification processes and by their capacity to precipitate proteins (Haslam, 1979). According to a theory put up by Van Sumere *et al.*, (1985), stress conditions can result in the accumulation of flavanols as well as their oxidative



**Fig. 3:** Callus cell formation from recent cambial derivatives (arrows) in a 6-day-old graft union. Bar = 50 µm.

breakdown, which can have significant effects on the growth and metabolism of tissues. One such effect is the blockage of the lignin pathway (Buchloh, 1960). Several studies detail findings about the characterization of monomeric and oligomeric flavan-3-ols in apricot cultivars and rootstocks and their accumulation in apricot combinations with varying degrees of compatibility (Errea *et al.*, 1994a) (Errea *et al.*, 1992, 2000). ABA and GA can induce the synthesis of flavanones, which can be used to test compatibility in *Prunus*, such as prunasin (Moing *et al.*, 1987; Moing and Carde, 1988). (Treutter and Feucht, 1988). Growth regulators have an impact on how prunin levels are regulated. All of these macromolecules, which are found in sap phloem and include RNA, hormones, and phloem proteins, can seem significant from our perspective during the process of vascular development in plants.

More research should be done on the grafting process in fruit trees to learn more about the mechanisms involved in graft incompatibility reactions. This will help us choose a rootstock early on, before we notice any external incompatibility symptoms.

#### **Graft compatibility: successful union of the scion and its rootstock**

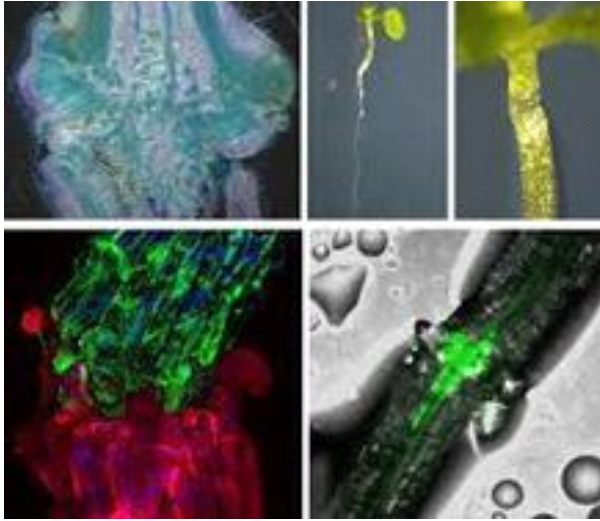
**Reasons for graft incompatibility:** incompatibility of the two species being grafted: Intra- specific grafts- more successful than interspecific grafts. Intra- generic grafts- more successful than intergeneric grafts, other factors: Toxicity between the rootstock and scion, phenolics that inhibit proper union formation, hormonal abnormalities (Hatmann *et al.*, 2010)

**Grafting and Translocation:** uptake, as well as the translocation of various substances such as: Ions, Photosynthates, Plant hormones, Alkaloids- can be influenced by rootstock or by grafting. Basic purposes for grafting are the utilization of the vigorous root systems of the rootstocks, grafted plants usually show increased uptake of water and minerals as compared to the self-rooted plants. Grafting influences the absorption and translocation of ions, that is, phosphorus, nitrogen, magnesium and calcium (Kim and Lee, 1989). Microelements such as iron and boron are influenced by rootstock (Gomi and Masuda, 1981)

#### **Factors influences in graft compatibility**

Success is influenced by genetics, structure, growth characteristics, physiological and biochemical factors. Genetics factors are critical for compatibility. Closer the taxonomic relationship between scion and rootstock, the higher the grafting success rate. Physiological factors may be important reasons for graft incompatibility.

**Phenolic compounds:** Phenolic compounds in involved in stress and wounding response (Cohen *et al.*, 2007). Normal wound reaction, an intense production of new phenolic compounds has been reported during the establishment of a graft union (Kueger *et al.*, 2012). Small quantities of phenols can be enough to produce locally limited disfunctions in the interphase between two or more cells (Fiehn *et al.*, 2008). In incompatible graft union, phenols move from the vacuole into the cytoplasm, causing stress that results in growth dysfunction (Hartmann *et al.*, 2002)



**Hormonal signalling on graft union:** it is now known that some mRNA signals change just 24hr after grafting, and 48hr after grafting auxins increase at the union and stimulate cell division and differentiation (Koepke and Dhingra, 2013)

**Plant hormones:** Involved in the development of the graft union. Auxins are released from vascular strands of both stock and scion to induce the differentiation of vascular tissues (Koepke and Dhingra, 2013). Lack of compatibility has been associated with a pronounced accumulation of polyphenols above the graft union (Feucht *et al.*, 1992), which are known to affect auxin transport (Errea, 1998)

**Robots in grafting:** the first robot development was the “Cutting-off Cotyledon Grafting” (CCG) system to graft cucurbit vegetables. It took three seconds to make a grafted plant with 95 percent survival rate. In Korea, (Hwang *et al.*, 1997) of Sungkyunkwan University developed a grafting robot that used approach grafting. In Taiwan, (Lee *et al.*, 2001) developed an automatic grafting robot for passion melon that is able to graft 114 seedlings per hour, has a grafting success rate of 70% and a survival rate of 95%.

## Review of Research Work

Zhang *et al.*, 2013, compared the vigour, rooting ability, and some physiological parameters between cuttings harvested from nongrafted “Jinba” (non-grafted cuttings) with those collected from grafted “Jinba” plants onto *Artemisia scoparia* as a rootstock (grafted cuttings). The yield, length, node number, stem diameter, fresh weight, and dry weight of the grafted cuttings were superior to the non-grafted cuttings. Also grafted cuttings “Jinba” rooted 1 day earlier, but showing enhanced rooting quality including number, length, diameter, and dry weight of roots, where compared to the non-grafted. The physiological parameters that indicated contents of soluble protein, peroxidase activity, soluble sugar, and starch, ratios of soluble sugar/nitrogen ratio, and carbohydrate/nitrogen (C/N), as well as contents of indole-3-acetic acid (IAA) and abscisic acid (ABA), and IAA/ABA ratio were significantly increased in the grafted cuttings. This suggested their important parts in mediating rooting ability. Results from this study showed that grafting improved productivity and rooting ability related to an altered physiology, which provide a means to meet the increasing demand.

Significant differences of different treatments are compared in the same batch. H1: cuttings harvested on June 17; H3: cuttings harvested on July 27; H5: cuttings harvested on September 5; values (mean  $\pm$  S.E.) labelled with a different letter suffix differ significantly from one another at the same time point according to Duncan’s multiple range test ( $P < 0.05$ ).

The contents of soluble protein and POD activity in the nongrafted and grafted cuttings. H1: cuttings harvested on June 17; H3: cuttings harvested on July 27; H5: cuttings harvested on September 5; means and standard errors calculated from three replicates. Significance ( $p < 0.05$ ) indicated by lower case lettering ((a), (b)), derived using the Duncan’s multiple range test Izadi *et al.*, 2013, The influence of two grafting techniques on the success of stenting (simultaneous cutting and grafting) and some ensuring growth parameters of stentlings were studied under glasshouse conditions. The Avalanch and Peach Avalanch glass-house rose varieties were utilized as scion and grafted on *R. manetti* as rootstock. Two grafting techniques namely, splice and omega grafting methods were practiced and graft combinations were inserted in cocopeat-perlit (1:2) medium under mist system. The number of roots, longest root size, shoots and leaf numbers and successful grafting percentage were evaluated after grafting. In Avalanch/*R. manetti* and Peach Avalanch/*R. manetti* combinations, higher percentage of successful

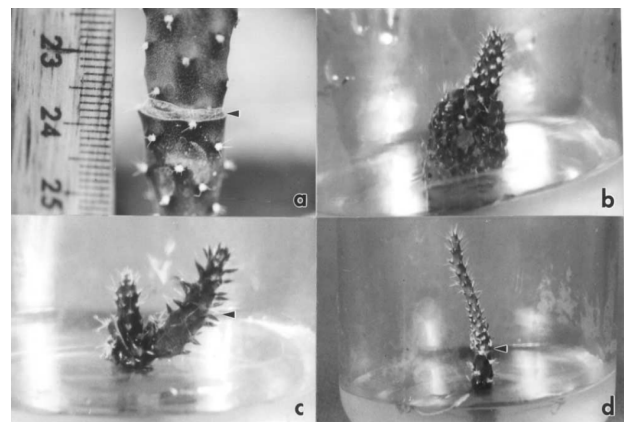
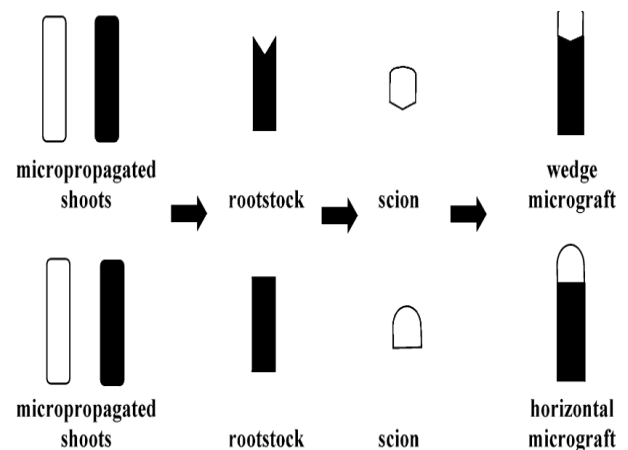
grafting was observed in the stentlings propagated via omega grafting technique. Furthermore Avalanch/R. manetti stentlings prepared by omega grafting were found to produce a greater number of roots, shoots and leaves and longest root size as compared to those propagated through splice grafting method. In case of Peach Avalanch/R. manetti combinations propagated via omega grafting more number of shoots was observed as compared to those propagated by splice method. However, these were not significantly different with respect to their leaf numbers. The results showed the superiority of omega grafting procedure.

Megre *et al.*, 2004, Light microscopy was used to study the graft union formation of splice graft in the elepidote rhododendrons cultivar 'Cunningham's White'. The first visible reaction after grafting was the appearance of a necrotic layer between graft partners consisting of fragmented and compressed cells. Callus was visible six days after grafting and was formed from recent cambial derivatives and phloem rays cells. New cambium and tracheary elements were differentiated from callus cells. Newly differentiated cambium was slightly curved or had an S-shape, and it depended on rootstock and scion tissue matching. When initially the gap between the graft partners remained wide, callus produced more new cambiums: one of them emerged between pre-existing cambium of graft component and an uncommon one - in the pith region.

Izadi *et al.*, 2014, current study supports the claim that the productivity and quality of stentlings of greenhouse roses are influenced by the balance between the aerial parts and roots. The above obtained results have emphasized the improvement of propagation by stenting in late September for obtaining successful grafted plants. It was clearly demonstrated that Rosa canina was the best rootstock for Avalanch cultivar. Other observations did not lead to prominent result as it varied with time of grafting, scion and rootstock cultivars.

Esrada-Luna *et al.*, 2001, the histology of the unio formation in *in-vitro* grafted PPC is comparable to that observed in graft formations with other plant species, including five general stages : formation of the union, development of a necrotic layer and proliferation of callus bridge at the graft interface, differentiation of new vascular cambium, restoration of the continuity of new vascular tissue, and restorations of the continuity of the epidermis at the union zone. Restoration of the continuity of new vascular tissue was observed 28 days after grafting. After *ex vitro* transplanting, the development of scion was affected by the type of Esrada-Luna *et al.*,

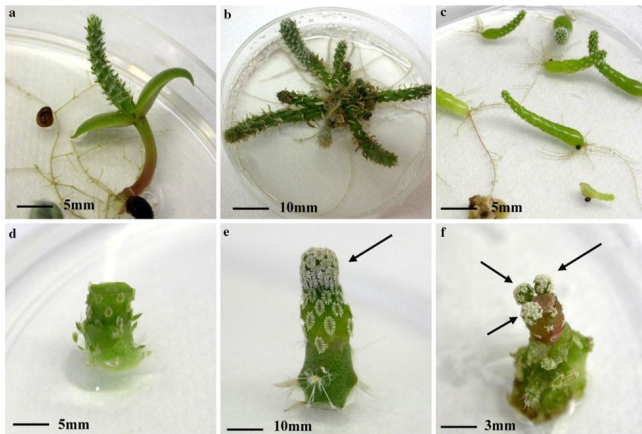
2001, the histology of the unio formation in *in-vitro* grafted PPC is comparable to that observed in graft formations with other plant species, including five general stages: formation of the union, development of a necrotic layer and proliferation of callus bridge at the graft interface, differentiation of new vascular cambium, restoration of the continuity of new vascular tissue, and restorations of the continuity of the epidermis at the union zone. Restoration of the continuity of new vascular tissue was observed 28 days after grafting. After *ex vitro* transplanting, the development of scion was affected by the type of combination during grafting, which was favoured when homograft combinations were formed. Finally, this micrografting technology has the potential for larger scale production of PPC plants and might be extended to the propagation of other micro propagated cacti species.



Bayat *et al.*, 2015, results showed that the quality of the grafting was different in the spring and autumn together. There was a significant difference in terms of sprouting between grafting in the spring and autumn. In the spring buds were formed at the bottom and end of the scions but in the autumn, sprouting occurred only in the end of scions. The results showed a significant effect of rootstock on the grafting success and efficiency in



cactus. Thus, the selection of appropriate rootstocks and scions is one of the possible solutions to improve the vegetative propagation of cactus.



Badalamenti *et al.*, 2016, study describes the protocol for micrografting of an endangered Cactaceae, *Pelecypora aselliformis*. Our results indicate that it is possible to start massive propagation with just a few seeds without the need to find the optimal in vitro requirements, which are species-specific, and to some extent genotype-specific. This technique could further be useful for the massive production of ornamental plants reducing the overharvesting of endangered species from the wild. Moreover, the production of micrografted plants may contribute also to ex situ conservation of this threatened species.

Kumar *et al.*, 2002, effect of rootstock-scion relationships on growth and flowering in rose was studied with four rootstocks budded with four cultivars. Budtake percentage was maximum in *R. indica* var. *Odorata* budded with Super Star and these plants attained significantly higher height. The cv. Happiness, when budded on *R. indica* var. Briar produced longest flower stalk. Neck length, buds size (length and diameter) also varied significantly in different rootstock-scion combinations.

Balaj *et al.*, 2022, Analysis of variance confirms that there are statistically differences regarding the length of flowering shoot and their diameter. There was found a significant level of compatibility between rootstock *Rosa canina* var. *Laxa* with all tested cultivars of groups Floribundas and Polyantha rose. Rootstock *Rosa canina* var. *Laxa*, had an impact on the growth of flowering shoots, diameter of shoots, flower diameter for all tested roses cultivar. Rootstock has a great effect in adaptation to certain pH value and drainage conditions of the soil, climatic factors, disease resistance, plant longevity, productivity and flower quality.

Izadi *et al.*, 2014, current study demonstrated that the application of auxin plays an important role in success of *Hibiscus rosa-sinensis* propagation through stenting method. The highest root number, dry weight of root, healing percentage and leaf number were obtained with auxin treatment (3000 ppm and 5000 ppm). Although effect of rootstock's cultivar just on root number was significant but its interaction with auxin was significant on the produced root number, root dry weight, mean of healing percentage and leaf number. One of the best combinations was "Jeanne d'Arc" \*3000, therefore the application of auxin with 3000 ppm is advisable for stenting propagation of *Hibiscus rosa-sinensis* when grafted on "Jeanne d'Arc" rootstock. However, "Blue Stain" treatment's result was not consistent but using 5000 ppm of IBA caused good achievements at least in a few measured parameters.

Nazari *et al.*, 2009, four *Rosa hybrida* L. cultivars ('African Dawn', 'Ilios', 'Maroussia' and 'Soprano') was evaluated. They were grown either on their own roots or grafted (stenting) onto *Rosa canina* L. 'Inermis' rootstock in a polyethylene greenhouse with hydroponics system. Parameters of plant growth and flower quality were investigated for two successive harvesting years (2005 and 2006). Results indicated that, all the cultivars were superior for most of the parameters studied when grafted onto rootstock compared to being on their own roots. Flowering stem fresh weight and diameters, flower fresh and dry weight, flower diameter and length, petal number, leaf chlorophyll content and quality index were higher in grafted plants compared to those propagated by cuttings. However, highest flowering stem length and number were observed in plants propagated by cutting, although not significant as compared with stenting method.

Phenotypic parameters	Plant type	Expression level	Graft effect
Flower yield	Scion	Low	Weak
	Rootstocks	High	
	Grafted plant	Middle	
Stem length	Scion	Small	NO
	Rootstocks	Large	
	Grafted plant	Small	
No. of leaves	Scion	Few	NO
	Rootstocks	Many	
	Grafted plant	Few	
Electron transport rate	Scion	Low	Strong
	Rootstocks	High	
	Grafted plant	High	
Photosynthetic rate	Scion	Low	Strong
	Rootstocks	High	
	Grafted plant	High	
Transpiration rate	Scion	Low	Strong
	Rootstocks	High	
	Grafted plant	High	
Stomatal conductance	Scion	Low	Strong
	Rootstocks	High	
	Grafted plant	High	
Root activity	Scion	Low	Strong
	Rootstocks	High	
	Grafted plant	High	
Stomata size	Scion	Middle	Strong
	Rootstocks	Small	
	Grafted plant	Large	
No. of stomata	Scion	Middle	Weak
	Rootstocks	Many	
	Grafted plant	Few	

Kwon *et al.*, 2022, First, although there was a difference depending on the type of rootstock, the photophysiological response, the root activity, and the yield of grafted cut rose flowers were all improved under high temperatures in the summer season in the greenhouse. Second, the morphological characteristics, such as number of leaves, number of stomata, and stem length, of the grafted plants were hardly affected by the rootstock. Finally, the stomatal size of the grafted cut rose flowers was completely different from that of the scion or rootstocks and was in a form adapted to the high-temperature environment. The grafted plant is considered to have evolved into a new type of plant different from the scion and the rootstock with regard to specific characteristics related to environmental adaptation.

Chen *et al.*, 2018, results revealed that the growth declines in the grafted chrysanthemums were relatively lower than those of the non-grafted plants under drought stress, and net photosynthetic rate, stomatal conductance, water use efficiency, and transpiration rate in the non-grafted chrysanthemums were significantly decreased. Moreover, the intercellular CO<sub>2</sub> concentrations were significantly increased compared with the grafted plants at 5 and 6 d following drought stress. The grafted plants exhibited higher relative expression of the *CmrbcL*, *CmrbcS*, *CmpsaB*, and *Cmcab* genes, as well as higher Rubisco activity and chlorophyll content under the drought treatment. Sugar accumulation also increased under drought stress, particularly in the non-grafted plants. This result suggested that non-grafted chrysanthemums were less able to resist dehydration, and repressed the genes encoding the expression of photosynthetic components. In conclusion, using *A. annua* rootstock could alleviate drought stress in chrysanthemums by improving gas exchange capacity and maintaining *CmrbcL*, *CmrbcS*, *Cmcab*, and *Cmpsa B* gene expression, thereby increasing Rubisco activity and improving photosynthetic performance.

### Conclusion

The growth of the graft union has received a lot of attention, and a lot of data has been acquired about it. Several researchers have made observations about structural occurrences that might be in charge of the growth of cohesiveness between the stock and scion during graft ontogeny. Since the basis of an incompatibility response could be determined by local interactions between the opposing cells of the graft union itself, these reports specifically focus on the possibility that the phenomena of cellular recognition could be involved in the development of functional vascular connections. Contrary to this theory, it has been proposed that the

cohesiveness of graft partners is caused by the deposition and subsequent polymerization of cell wall components that take place in response to graft establishment's intrinsic injury, which is unrelated to the compatibility reaction. Although callus formation can be thought of as a typical plant wound healing response, recent developments in the study of plasmodesmata as highly dynamic structures that provide a pathway for symplastic cell communication have opened the door to their significant role in cell recognition and the compatibility and incompatibility response. More experimental evidence will be required to fully understand their involvement in phloem function. Additionally, other lines of research using grafting techniques revealed the existence of soluble proteins in phloem exudates that have been developmentally related to defined stages of phloem differentiation. Future research should incorporate a much larger range of similarities on woody responses to obtain a better understanding of the mechanism of incompatibility in these graft combinations as the mechanisms involved in all of these processes have been related to herbaceous graft combinations.

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