STENTING OF ROSES, STARCH DEPLETION AND ACCUMULATION DURING THE EARLY DEVELOPMENT

P.A. van de Pol, M.H.A.J. Joosten and H. Keizer
Agricultural University
Department of Horticulture
P.O. Box 30
6700 AA Wageningen
The Netherlands
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Abstract

Stenting of roses is a propagation technique, based on simultaneously cutting and grafting (Van de Pol et al., 1982). In the present study whip grafting was introduced with stock and scion held together with a clothes-peg. Microscopical investigation of the stem above and below the graft union showed a marked increase of the starch content of the scion, particularly during the first week after grafting. Starch in the rootstock shows a sharp decrease, directly after grafting, reaches level 0 on day 3 and subsequently increases until after 12-15 days it approaches the level of the scion. At day 12 from grafting 100% of the plants had formed a xylem vessel connection as shown by colouring with acid fuchsine, and 15 days from grafting 75% were rooted. The increase of percentages of rootstocks with starch from day 3 onwards preceeds the increase of percentages of stentlings with xylem vessel connection by about 2 days. The CO_2 concentration in the propagation bench decreased from more than 1500 ppm during the night to less than 100 ppm during the day. This change in CO_2 concentration and the pattern of the starch accumulation in the stem prove the activity of the scion leaf. This is a necessity for the development of roots and shoots on the young stentling.

1. Introduction

In some fruit trees inhibition of growth will stimulate the generative development. Jones et al. (1983) showed that in apple the graft union can reduce the amount of minerals and cytokinins passing through, and therefore may play a role in the dwarfing effect of a rootstock. Skene et al. (1983) showed that the grafting technique influences height and lateral development of fruit and ornamental trees. Because in roses flowering is terminal, a more vigorous growth implies a higher production. From this follows the need for invigorat rootstocks and firm graft unions.

In the present program to improve plant material of roses, technique of stenting is used in most experiments (Van de Pol et al., 1982). Stenting, based on grafting unrooted cuttings is a quick propagation technique, applicable the year around and therefore ideal for studying scion-rootstock interrelations and wound healing. From a physiological point of view a stentling is a complicated plant because photosynthesis, wound healing, root formation and bud development occur simultaneously, moreover, these processes are interacting. Several authors have reported on the role of starch in rooting (Nanda et al., 1972) and rose production (Zieslin et al., 1975). In the present study vascular development at the graft union and starch accumulation and depletion around the graft union during the early development of a rose stentling were investigated.

2. Materials and methods

2.1. Plant material

In order to simplify the system 'Ilona' was grafted on 'Ilona' without using auxin and compared with 'Ilona' on 'Indica Major'. The plant material consisted of flowering stems of 'Ilona', grown in greenhouses, with full-grown leaves and just opening flowers. After normal harvesting, each individual stem was kept apart and cut into sections with 1 leaf with at least 5 leaflets and a dormant bud, with ½-1 cm of stem above it and as much as possible underneath. Within each stem the sections were equally divided into future scion and rootstock material, numbered and put in moistened plastic bags to prevent wilting, after the apex leaflet of the scions and the complete leaf of the future rootstocks had been removed. The plastic bags, containing the sections of separate stems, were kept in the dark until microscopical examination and grafting on the same day.

Rootstock material of 'Indica Major' was grown in greenhouses. The soft-wood material must be at a stage where leaves are well developed and thorns can be broken off easily.

2.2. Determination of starch content prior to grafting

Preliminary tests showed that the levels of starch of internodes of a single stem appeared to vary less than internodes of different stems. This may be due to differences in age or variation between bushes. In order to use graft partners with more or less equal levels of starch, graft combinations were made between internodes of the same stem. Before grafting, the starch content of each scion and rootstock was determined according to a slightly modified version of the technique described by Brandon (1939). From the basal zone of each section transverse slices were cut all around the circumference of the stem with a sharp razor blade, stained with KJ-iodine, mounted in glycerine and visually judged on starch distribution and content in the tissues as mentioned in table 1, at a microscopical magnification 100 to 250 x. In preliminary tests it was found that the process of starch accumulation and depletion involves various tissues in a more or less sequenced pattern. Thus, operating an arbitrary scale became feasible, marking the inner cortex and medullary rays as the two tissues of major importance.

2.3. Stenting

The grafting technique used was a whip grafting (Hartmann et al., 1975). As rootstocks were used pieces of stem consisting of single internodes without buds. The top of the rootstock internodes and the basal part of the scions were cut at an angle of 30°. Scion and internode were combined and held together with a clothes-peg (Fig. 1). For a good development of the graft union it is necessary that at least on one side the cambia of the graft partners are in close contact.

To prevent wilting the leaves were moistened. In case auxin was used, the rootstock internodes were dipped in talc powder with indole butyric acid (IBA) 0.4%, to accelerate root formation. The plants were placed in a propagation bench, covered with double layered polyacrylic. The rooting substrate was a mixture of sand and peat at a ratio of 1:1 V/V at pH 5.5. The planting density was about 100 plants/m^2, with the scion leaves not overlapping. CO_2 measurement in the propagation bench pointed to considerable variations with less than 100 ppm during the day and more than 1500 ppm during the night.

The relative humidity of the atmosphere was kept at about 100% by misting a few times a day and the temperature of medium and atmosphere was kept at about 25°C.

The sequence of processes proved to be: growing together of the grafted partners, root formation, and growth of the axillary bud. To prevent premature release of the axillary bud of the scion and to stimulate wound healing and root development during winter, the natural day light was extended to 24 h by blended light MLL 250 W Philips lamps, giving at least 3 W/m^2 at plant level. After 2-3 weeks scion and stock have grown together, the roots have been formed and the plant can be hardened off (Fig. 2).

2.4. Observations after grafting

From the stock material of 100 stentlings of 'Ilona' on 'Ilona' representative samples of 8 stentlings were taken daily during 10 days after grafting and after 12 and 15 days. These samples were taken corresponding to the average score rating of the rootstocks at the time of planting (day 0). Starch content was determined just above (scion) and below (rootstock) the graft union.

Vascular contact between scions and rootstocks was investigated by colouring the connected xylem vessels. For this test the basal part of the rootstock internode was cut off and placed in acid fuchsine 1%. Ten minutes later the coloured vessels are clearly visible after removal of the bark. By this technique xylem contact can be proved quantitatively as well, by estimating the percentage of connected vessels.

The leaf surface of each stentling was measured and found to be about 42 cm^2. The remainder of the stems were subsequently stored in liquid nitrogen for a quantitative starch determination of the daily samples afterwards, following the prescriptions of Hassid et al. (1964).

3. Results

The starch content of 'Ilona' stems at different stages of development of the flower is presented in table 2, showing a significant increase with stem age. Because for use as rootstocks a minimum stage of ripeness of the stem is required to avoid infections and because starch depletion had to be observed it was decided to use stems with a flower that was just opening (between II and III with a starch mark around 2). No variation in starch content was found on different heights in the internodes of an intact stem (Table 3). Starch accumulation in the scion at first mainly occurred on the leaf's side of the stem, however, implying the necessity for localized starch assessment. This phenomenon could not be found in intact stems. For the rootstock - a bare piece of internode - this is not of interest until phloem vessels are connected, also causing a more or less localized starch accumulation. In this case starch levels were also locally judged.

Changes in the starch content of stentlings during 15 days after grafting are presented in Fig. 3, indicating remarkable differences between the pattern of scion and rootstock. The starch content of the scion stem increases steadily, particularly during the first week after grafting. After grafting, day 0, the starch of the rootstock shows a sharp decrease, reaches zero at day 3 and subsequently increases again, approaching the level of the scion 10 to 15 days after stenting. These results, obtained in June, corresponded with previous data collected in December.

Preliminary results of the quantitative determination of starch of the rootstock indicated low concentrations of 1 to 0% of the dry weight, fluctuating in the same pattern found by microscopical investigation (Fig. 3).

At day 6 one out of 8 stentlings showed xylem vessel contact. This number increased to 100% on day 12. Figure 4 shows that the increase of the percentages of rootstocks with starch from day 3 onwards preceeds the increase of percentages of stentlings with xylem vessel connection with about 2 days. The percentages of rooted plants were 12.5 at day 12 and 75 at day 15. All stentlings of the combination 'Ilona' on 'Indica Major' proved to have xylem vessel connection within 12 days. The percentage of rooted plants was then already 29. This implies that the development of this combination is as fast as the combination 'Ilona' on 'Ilona'.

4. Discussion

Buck (1954) studied the course of histological events in the healing of a bud graft of roses. He described the processes leading to the formation of a continuous ring of cambium through the scion and around the stock between the tenth and fourteenth day after budding. Von Rupprecht (1954) also described the wound development after grafting rose cultivars on different rootstocks using the technique of whip grafting. He reported that vessel connections were formed from 35 days after grafting onwards, depending on the rootstock. In the present work the scion-rootstock interactions and the earliest development of vessels were investigated.

The increase of starch content starting 4 days after grafting points to phloem vessel contact, while the transmission of acid fuchsine starting after 6 days points to xylem vessel contact. The differences in time to vessel connection between Von Rupprecht and the present work may be caused by differences in grafting technique and method of observation. In the system of Von Rupprecht an active growing rootstock was cut off and whip grafted. This can have caused a severe dying off of roots (Fuchs, 1985) influencing wound healing. The grafting technique of T-budding used by Buck permits normal root activity. Observations on the process of vascularization by Von Rupprecht were of anatomical kind, while we investigated transmitted substances. Scions with 4 leaflets were used, while Von Rupprecht used scions with only the small 2 basic leaflets. The patterns of starch accumulation and CO_2 content of the propagation bench prove the photosynthetic activity of the leaves. This activity of the leaf possibly promotes wound healing directly or indirectly. According to Shinninger (1979) the role of carbohydrates in the control of vascularization is not clear. Some effects of sugars in in vitro cultured tissue are mentioned. Molnar et al. (1976) stated that sugar supply is probably the most important factor in delaying senescence of detached plant parts of roses. Starch might play an important role in the early development of a good productive rose bush. Several authors reported the role of starch and carbohydrates during the rooting processes, see Nanda et al., 1972 with *Populus nigra* L.; Vieitez et al., 1980 with *Castanea sativa* Mill. and Van de Pol et al., 1983 with carnation. The stored starch will provide the later developing shoots with carbohydrates (Zieslin et al., 1975). Understanding the processes involved during the start of a rose plant may contribute to the development of treatments which will raise its subsequent quality and productivity.

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Table 1 - Scale used for determination of starch in scion and rootstock of 'Ilona' roses. Score ratings ranked from 0-8.

score ratings	inner cortex	medullary rays	o.p.	phl.	i.p.	coll.	chl.
0	-	-	-	-	-	-	-
1	+	-	-	-	-	-	-
2	+	+	-	-	-	-	-
3	++	++	+	-	-	-	-
4	++	++	++	+	+	+	-
5	++	+++	++	++	+	+	+
6	++	+++	+++	++	++	++	++
7	+++	++++	++++	+++	+++	+++	+++
8	++++	++++	++++	++++	++++	++++	++++

o.p. = outer pith
phl. = phloem
i.p. = inner pith
coll. = collenchyma
chl. = chlorenchyma (outer cortex)

\- = starch absent
\+ = starch occasional present
++ = starch frequent present
+++ = starch abundant present
++++ = tissue packed with starch

Table 2 - Mean starch values (scored according to Table 1) of flowering stems of 'Ilona' at different stages of development in June (N = 10).
(Starch determined in the middle part of the shoot).

	stage of flower development	mean starch value
I	very tight closed bud	1.1 a*
II	coloured petals just visible	1.6 b
III	petals unfolded, open flower	2.4 c
IV	bloomed out, petals fallen	3.3 d

*Significant differences according to Student's T-test p = 0.05

Table 3 - Mean starch value (scored according to Table 1) on different heights in internodes of 5 intact stems of 'Ilona' with just opening flowers (N = 36)

Part of internode	mean starch value
base	2.1 a*
middle	2.0 a
top	2.0 a

*Significant differences according to Student's T-test p = 0.05

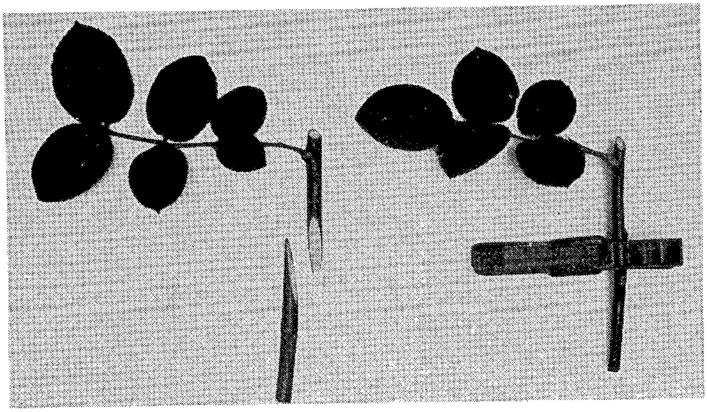

Figure 1 - Stenting by whip grafting and fastening by a clothes-peg.

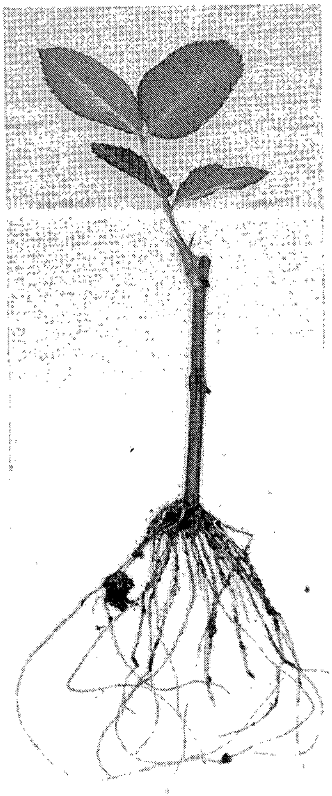

Figure 2 - Stentling of 'Sonia'/'Indica Major' 3 weeks after grafting.

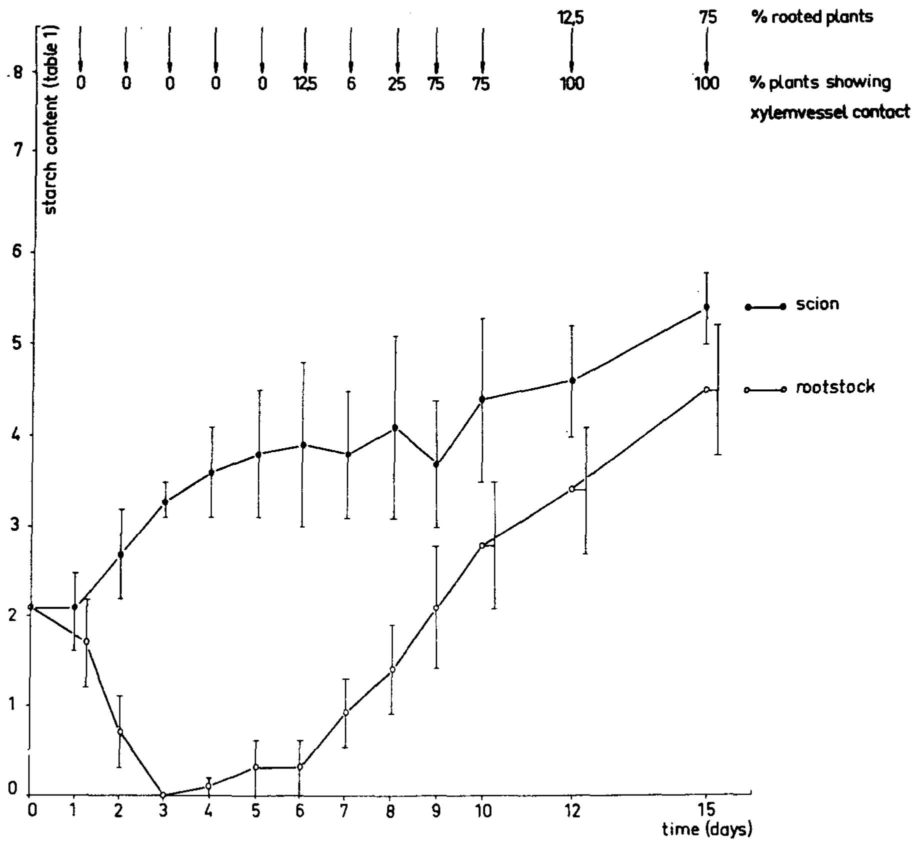

Figure 3 - Changes in starch content of stentlings during 15 days after stenting. Starch of the stock material (100 plants) is presented on day 0. Each other mark represents the average of 8 stentlings with 95% confidence intervals (vertical bars). Arrows indicate the percentage of stentlings with xylem vessel contact. Combination: 'Ilona'/'Ilona'. Period: June.

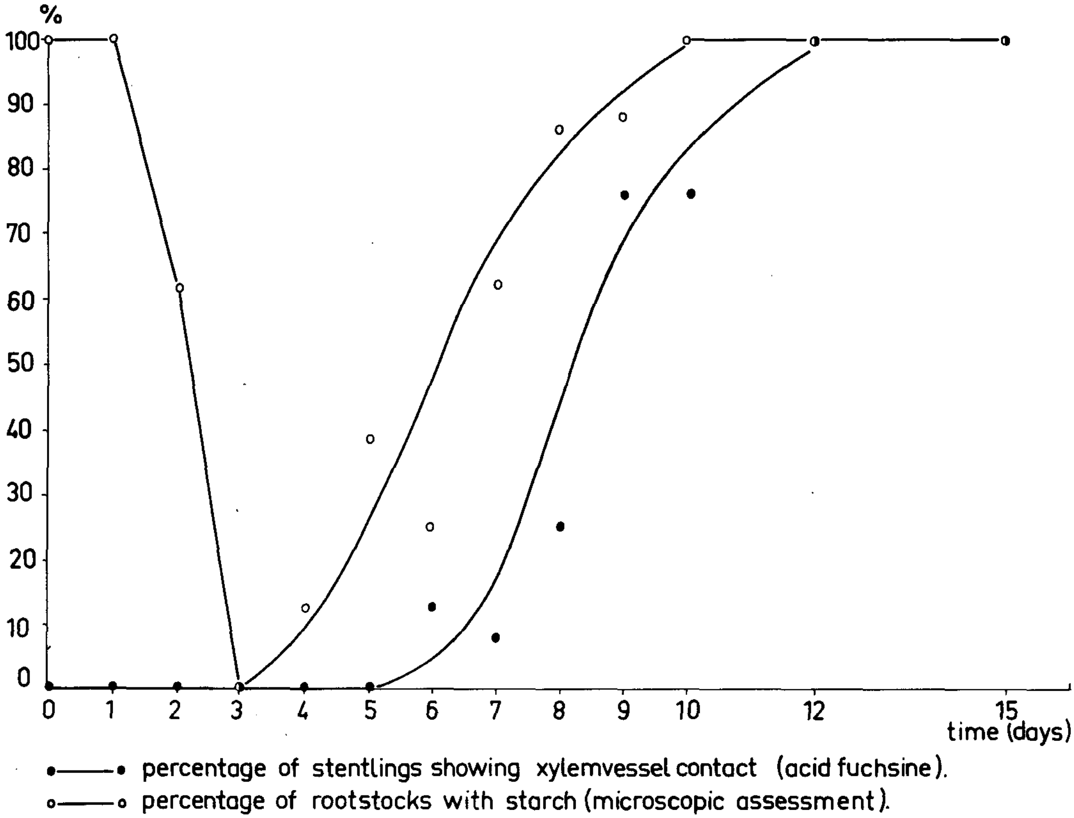

Figure 4 - The percentages of stentlings showing xylem vessel contact and the percentages of rootstocks with starch, during 0 to 15 days after grafting. Combination: 'Ilona'/'Ilona', period: June.