

The relationship between changes in lignification and the mechanical strength of rose flower peduncles

B. CHABBERT*, B. MONTIES*, N. ZIESLIN† and R. BEN-ZAKEN‡

Laboratoire de Chimie Biologique, INRA, INA-PG, 78850 Thiverval-Grignon, France and

‡The Hebrew University of Jerusalem, Faculty of Agriculture, P.O. Box 12, Rehovot, 76-100 Israel

SUMMARY

The resistance to bending of rose flower peduncle is important to the post-harvest quality of rose flowers. The content and composition of lignin was determined in respectively strong and weak peduncles of *Rosa × hybrida* cv. Mercedes and Nubia. Xylem tissues of the peduncles of the stronger cv. Mercedes were more lignified than in the weaker cv. Nubia. However, phloem lignin content was increased only in the basal parts of the peduncles of cv. Mercedes. Differences in the monomeric composition of lignins were apparent between cultivars. In both the phloem and xylem fractions, lignin in the peduncles of cv. Mercedes was less condensed than in the weaker cultivar Nubia. Furthermore, lignin rich in syringyl monomeric units occurred in the distal parts of the strong peduncles, accounting for the higher S/G ratio observed in the stronger peduncles compared to the weaker ones. Despite lignin from both phloem and xylem tissue showing a similar S/G ratio, the phloem fraction must synthesize a more condensed lignin network than xylem tissue. Such qualitative and quantitative lignin differences suggest the possible role of lignification in the resistance of rose peduncles to bending.

Key-words: Bent-neck, guaiacyl monomers, lignin content, monomeric composition, non-condensed monomeric units, *Rosa × hybrida* cv., syringyl monomers, thioacidolysis.

INTRODUCTION

Bending of rose stems (bent-neck) during ageing is one important characteristic determining the quality of rose cultivars (Burnett 1970). The tendency to bend varies between cultivars and stages of flower bud development but is less pronounced in the basal (lower) part than in the distal (upper) part of the peduncle (Zieslin *et al.* 1989a,b).

Bent-neck is a physiological phenomena; it seems very likely that lignification plays a role in preventing bending in cut flowers exposed to water stress. The resistance to bending of rose peduncle is correlated with highest peroxidase activity and with anatomical differences (Zamski *et al.* 1991; Zieslin & Ben Zaken 1991).

Peroxidases have been implicated in cinnamyl alcohol polymerization during lignin biosynthesis (Higuchi 1981). Among other functions, lignin is further assumed to affect

*To whom correspondence should be addressed.

This paper is dedicated to Professor Dr M.M.A. Sassen on the occasion of his retirement.

the mechanical properties of the plant cell wall (Cleland 1981; Northcote 1989; Lewis & Yamamoto, 1990). Such an effect could occur by formation of molecular associations and phenolic network rearrangement between lignin polymer and the other cell-wall components (Monties 1991a).

Lignin polymer exhibits large differences between plants both in the content and the structure (Lewis & Yamamoto 1990; Monties 1991b). Complexity of the polymer is related to differences in:

- (1) the relative content of monomeric units, mainly guaiacyl and syringyl units;
- (2) the frequency of intermonomeric linkages,
 - (a) β -O-4 and α -O-4 alkyl-aryl ether linkages which are the most frequent ones,
 - (b) Aryl-aryl ether C-O-C bonds and carbon-carbon C-C bonds also referred to as 'condensed' linkages, which are more resistant to chemical degradative processes (Lai & Sarkanen 1971).

Differences in the lignin content and composition are found between plant species and also in the cell type at the tissue and cell-wall layers level (Musha & Goring 1975; Terashima *et al.* 1986). The relative content of the guaiacyl and syringyl monomer typically changes also according to plant species (Sarkanen & Hergert 1971).

Furthermore, lignification can be modified by internal or external factors such as mechanical stress (Timell 1981), microbial attack (Moerschbacher 1989) and the application of plant growth regulators (Aloni *et al.* 1990; Tollier *et al.* 1991).

The aim of the present study was to compare the content and composition of lignin in the xylem and phloem isolated from distal and basal parts of the peduncles of rose cultivars bearing strong and weak peduncles, respectively cv. Mercedes and cv. Nubia.

The monomeric composition of lignin was characterized by depolymerization of the phenolic network using thioacidolysis (Lapierre *et al.* 1986). Due to selective breakage of aryl-alkyl ether linkages, thioacidolysis allows the analysis of the 'non-condensed' monomeric units which are characterized by the phenylpropane (C₆-C₃) skeleton of the lignin monomers corresponding to the guaiacyl (G) and the syringyl (S) substitution pattern of their C₆ aromatic cycle (Monties 1989). In addition, 'condensed' oligomeric units, mainly dimers, are solubilized during thioacidolysis. It is assumed here, that the lowered S+G thioacidolysis yield in non-condensed monomeric units is due to the occurrence of a larger proportion of condensed structure in the lignin network. Increases in the content of, for example, diphenyl ether, diaryl ether, diarylpropane and resinol intermonomeric linkages could account for such differences, as shown in the case of spruce lignin fractions (Lapierre *et al.* 1991). Until now however, the analysis of the corresponding condensed dimeric structures has not been applied to the complex case of the guaiacyl-syringyl lignin of rose stems.

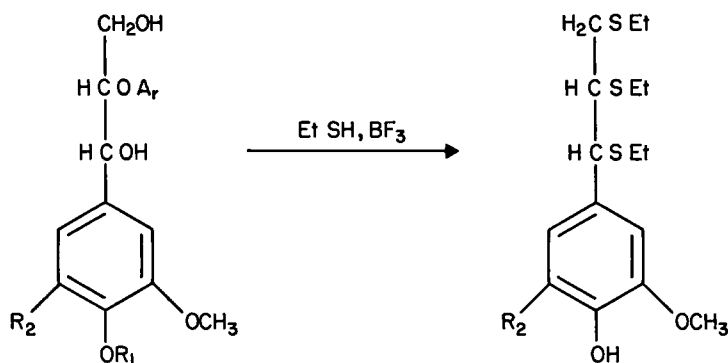
MATERIALS AND METHODS

Plant material

Rose (*Rosa × hybrida*) cultivars Nubia and Mercedes were grown in a greenhouse as previously described (Zieslin & Ben Zaken 1991). The phloem and xylem fractions were separated from the distal (upper) and basal (lower) parts of the peduncles collected at aged flower bud development (Zieslin *et al.* 1991). Tissue samples were freeze-dried and ground in a vibratory mill. The ground samples were successively extracted in a Soxhlet apparatus with toluene-ethanol azeotrope obtained from (1/1, v/v) mixture, followed by ethanol and

Table 1. Lignin content of xylem and phloem CWR from rose peduncles (as %CWR \pm SD)

Part of the peduncle	Xylem		Phloem	
	Nubia	Mercedes	Nubia	Mercedes
Basal	24.5 \pm 1.0	29.4 \pm 1.3	10.7 \pm 0.4	13.8 \pm 0.4
Distal	18.1 \pm 0.1	21.4 \pm 1.0	10.5 \pm 0.4	10.7 \pm 0.4

**Fig. 1.** Thioacidolysis of β -O-4 alkyl-aryl ether linkages allows recovery of guaiacyl (G) and syringyl (S) units with preservation of their phenylpropane skeleton.

water to give an extractive free-cell wall residue (CWR) which was freeze-dried before analysis.

Lignin determination and analysis

The lignin content of CWR was estimated spectrophotometrically at $A = 280$ nm after digestion with acetyl bromide according to Iiyama & Wallis (1990). Spruce milled wood lignin prepared according to Lapierre *et al.* (1986) was chosen as reference.

The monomeric composition of lignin in CWR was determined by thioacidolysis (Lapierre *et al.* 1986). Reaction was performed on 10 mg CWR with reagent consisting of a solution of BF_3 etherate (0.2 N) in 8 ml of a mixture of dioxane-ethanethiol (9/1, v/v). After 4 h at 100°C , monomeric products released from lignin were analysed by gas chromatography as TMS derivatives (Lapierre *et al.* 1986).

In both determinations, at least three replicates were used in the calculation of the means in the tables.

RESULTS AND DISCUSSION

Lignin content

A higher lignin content was found in the basal and distal xylem tissues of the stronger cv. Mercedes peduncles than in corresponding tissues of the weaker cv. Nubia peduncles

Table 2. Monomeric composition of lignin in xylem CWR from rose peduncle (as $\mu\text{mol G or S g}^{-1}$ lignin \pm SD)

Part of the peduncle	Cultivar	Guaiacyl G	Syringyl S	Total S+G	Ratio S/G
Basal	Nubia	306 \pm 4	453 \pm 2	759 \pm 6	1.48
	Mercedes	432 \pm 25	745 \pm 46	1177 \pm 71	1.70
Distal	Nubia	96 \pm 12	75 \pm 7	171 \pm 19	0.78
	Mercedes	266 \pm 16	580 \pm 34	846 \pm 50	2.18

Table 3. Monomeric composition of lignin in phloem CWR from rose peduncle (as $\mu\text{mol G or S g}^{-1}$ lignin \pm SD)

Part of the peduncle	Cultivar	Guaiacyl G	Syringyl S	Total S+G	Ratio S/G
Basal	Nubia	53 \pm 4	93 \pm 7	146 \pm 11	1.75
	Mercedes	152 \pm 8	253 \pm 4	405 \pm 12	1.66
Distal	Nubia	14 \pm 4	11 \pm 2	25 \pm 6	0.78
	Mercedes	25 \pm 2	66 \pm 6	91 \pm 8	2.64

(Table 1). In the case of phloem, such a difference was found only in the basal parts. In both cultivars, lower lignin contents were found in the distal parts of the peduncles compared with the basal parts. The distal parts are also the most resistant to bending. The differences in the lignin contents of the peduncles correspond to the differences in their tendency to bend and in peroxidase activity (Zieslin & Ben Zaken 1991). In addition, tissue fractions showed different lignin contents; xylem fractions being the most lignified.

Lignin composition

Thioacidolysis allows selective cleavage of the β -O-4 alkyl-aryl ether bonds, the most frequent linkages in lignin, (Fig. 1), and thus provides an estimate of the content of these 'non-condensed' lignin structures as expressed, here, by the differences in the total yields (syringyl + guaiacyl) recovered by thioacidolysis from CWR. The monomeric composition, defined by the molar ratio syringyl/guaiacyl, can be also determined by this method.

Evaluation of the composition of xylem lignin in the rose peduncles showed a clear-cut increase in the (S+G) recovery yields from lignin in the stronger peduncles of cv. Mercedes compared to the weaker cv. Nubia (Table 2). Higher yields of monomers involved in β -O-4 bonds were found in the basal parts than in the distal ones.

The recovery of syringyl monomers was much reduced in the distal parts of cv. Nubia compared with the stronger cultivar cv. Mercedes. This accounted for the much lower

value of the molar ratio S/G determined in the distal parts of xylem tissues from the weak peduncle. Lignin in the basal parts showed a similar S/G ratio in both cultivars.

Similar results were also found for the phloem tissue (Table 3). That is, there was a higher recovery of (S + G) from the strong peduncles compared with the weaker ones and in the basal parts compared with the distal parts, and a lower lignin S/G ratio from distal parts of the weak peduncle, explained by a lower recovery of syringyl monomers. However, it was observed that phloem tissue had a reduced content of β -O-4 structure than xylem fractions as evidenced by the reduced (S + G) yield.

The lower (S + G) yields in the weak peduncles indicated that a lower content of β -O-4 'non-condensed' structures was present in the polymer. Lignin from weak peduncles appeared therefore to be a more condensed polymer than in strong peduncles. Similarly, the distal parts contained a more condensed lignin network than the basal parts. The general increase in β -O-4 lignin structure in strong peduncles corresponded with an enrichment of syringyl monomer in the distal parts. Such syringyl-rich lignin fractions have been reported in fibre-secondary cell wall in different plant materials such as poplar (Terashima *et al.* 1986), birch (Saka & Goring 1988), and Japanese beech (Takabe *et al.* 1992). On the other hand, fibre-cells represent the main supporting tissues in plants (Esau 1977). The above presented data therefore suggest that accumulation of lignin enriched in syringyl monomers could occur in fibre-secondary wall, accounting for the high S/G ratio in the stronger peduncles. Such lignin may contribute to the mechanical properties of the fibres and corresponding stem tissues.

The suggestion of a correlation between the chemical quality of the lignin network and the bending strength of the rose peduncles is open to several alternative mechanistic explanations. For example, the three-dimensional structure of the lignin network deposits and their possible associations with the other cell-wall polymers, 'reticulation', could have significant effects on the bending strength of the peduncles via differences in the fractal dimensions and in the auto-organization properties of phenolic networks as previously discussed (Monties 1991a, 1991c). Until now however, experimental evidence indicating the occurrence of such effects has been lacking. Furthermore, taking into account the linear, end-wise, molecular model structure suggested by Sarkanen (1971) for lignin fractions rich in syringyl monomeric units, the strengthening of peduncles could also be correlated with the deposition, in the secondary walls, of end-wise linear syringyl-rich lignin fractions along the cellulosic microfibrils. According to the growth-stress hypothesis of Boyd (1985), such topologically-controlled deposition of lignin could allow the generation of forces ensuring adequate strength and maintenance of lignified organs near the optimum position. An alternative theory—that growth-stress generation is the result of continuing crystallization of cellulosic microfibrils after their initial deposition in fibre wall layers—has been promoted by Bamber (1987) and also deserves attention in this respect.

That growth-stress generation is primarily the result of dimensional changes imposed on the fibre wall by 'intussusception', the interweaving of new lignin material within previously formed microfibril layers (Esau 1977), is the central concept supporting the theory of growth stress generation by lignin swelling. The fact, that more cells with thicker cell walls were found in the xylem elements and in the phloem fibres of the stronger cv. Mercedes peduncles than in those of cv. Nubia (Zamski *et al.* 1991) seems thus to corroborate the lignin swelling theory, in the case of rose stem.

Furthermore, even if corresponding increases in lignin content and peroxidase activity occurred in cv. Mercedes, one cannot yet state the precise function of peroxidase in the

preferential syringyl unit deposition in the non-condensed lignin structures of this strong peduncle. Likewise, the various isoperoxidases occurring in rose peduncles have not been studied for their substrate specificity towards guaiacyl or syringyl units.

Although lignin variations of the same kind were found in phloem and xylem tissues, within the cultivar or part of the peduncle, these two tissues contained quite different amounts and compositions of lignin. Phloem lignin appeared thus more condensed than xylem lignin.

The quantitative and qualitative changes in lignification of rose peduncles have been demonstrated here in the case of aged flower bud development. Similar variations in the lignification process were found however during ageing of the flower bud (Chabbert *et al.* 1993).

CONCLUSION

Rose cultivars bearing strong and weak peduncles differ in their lignification pattern for both lignin content and monomeric composition. Peduncles of the stronger cv. Mercedes, while containing more total lignin, must synthesize a lower condensed lignin polymer. Furthermore, differences in the composition of lignin (S/G ratio) are clearly demonstrated in the distal parts of the peduncles which exhibit syringyl lignin fractions in the strong cultivar.

This study underlines the possible involvement of lignification in the mechanical properties of rose peduncles. The hypothesis of a special fibre differentiation and of lignin swelling effects in the stronger peduncles needs further investigation. The specific involvement of peroxidases in the preferential syringyl deposition in the lignin of cv. Mercedes, also requires further investigation. This is also the case for the enzymes involved in lignin monomer biosynthesis such as methyltransferases, cinnamoyl-ligases and reductases. In this respect, one can suggest the occurrence of genetic variations in the spatial and temporal expression of the enzymatic systems involved in the metabolism of the phenylpropanoid precursors of lignin, between the two rose cultivars. Very recently such variations have been reported in the case of chemically mutagenized arabidopsis by Chapple *et al.* (1992) which can compare to the natural quantitative and qualitative variability in lignification previously described in the case of trees and crops (Monties 1989, 1991b).

Structural changes in the lignin polymer may thus be of great importance in the post-harvest quality of rose flower cultivars. Further studies should thus concern possible lignin modification in response to external factors. Plant growth regulators which could alter both tendency to bending of rose peduncles (Zieslin *et al.* 1989a) and lignification of coleus or kenaf (Aloni *et al.* 1990; Tollier *et al.* 1991) may thus represent a way of improving the post-harvest quality of rose cultivars.

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