POLYAMINES AND ADVENTITIOUS ROOT FORMATION IN *VITIS VINIFERA* L.

RÔLE DES POLYAMINES DANS LE DÉVELOPPEMENT DES RACINES ADVENTIVES DE *VITIS VINIFERA* L.

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Abstract :The effects of polyamines were examined for growth and polyamine contents in cuttings, callus and primary adventitious roots of *Vitis vinifera* L. Variations in free, conjugated and wall-bound polyamines in cuttings were observed during rhizogenesis. The main polyamines in cuttings were conjugated polyamines while in callus and primary adventitious roots they were free polyamines. Exogenous polyamine addition did not modify the total number of roots per cutting but increased the mean size and number of long roots. Moreover, exogenous polyamines increased polyamine levels in callus and roots, particurlarly wall-bound and conjugated polyamines. The involvement of these classes of polyamines in morphogenic processes is discussed.

Résumé : Le rôle des polyamines sur la formation des cals et la croissance des racines adventives de boutures de *Vitis vinifera* L. est étudié. Les teneurs en polyamines libres, liées aux macromolécules et liées aux acides hydroxycinnamiques varient au cours de la rhizogenèse et la répartition des polyamines dans les différentes parties de la boutures est différente. Les principales polyamines dans les bois sont les polyamines conjuguées aux acides hydroxycinnamiques alors que dans les cals et les racines adventives les polyamines libres sont majoritaires. L'apport exogène de polyamines dans le milieu de rhizogénèse ne modifie pas le nombre total de racine par bouture mais augmente leur taille moyenne ainsi que le nombre de longues racines. L'apport exogène de polyamines entraîne également une accumulation de polyamines dans les cals et les racines.

Mots clés : polyamines, boutures, cals, racines adventives, rhizogénèse, *Vitis vinifera* L.

Key words : free, conjugated and wall-bound polyamines, cuttings, callus formation, rhizogenesis, adventitious roots, *Vitis vinifera* L.

INTRODUCTION

The proper formation of adventitious roots at the base of stem cuttings is an important developmental phenomenon in their growth and survival. With respect to plant development, it involves the initiation of several new meristematic areas in the differentiated tissues of stem cuttings and their subsequent development into mature root structures. The process appears to be governed by an array of endogenous physiological factors (GASPAR *et al.*, 1997). Among the endogenous factors, polyamines seem be involved in the rooting of stem cuttings but there is much controversy regarding their exact role played by polyamines in adventitious root formation at the base of stem cuttings (FRIEDMAN *et al.*, 1982, 1985 ; BALLESTER *et al.*, 1999).

During rhizogenesis, an increase in free putrescine and spermidine levels, and in their biosynthetic activity has been reported in different plant tissue cultures (FRIEDMAN *et al.*, 1982 ; CHRIQUI *et al.*, 1986). In addition to free polyamines, there are two classes of polyamines bound either to low-molecular-weight, principally hydroxycinnamic acids (conjugated PA) or to high-molecular-weight compounds (wall-bound PA), whose respective physiological roles are probably completely different (SERAFINI-FRACASSINI et MOSSETI, 1986).

The study of the polyamine pattern in *Vitis vinifera* L. cuttings is particurlarly interesting because of the presence, at certain stages of development, i.e. during flowering or ovary development after flowering, of a large amount of polyamines conjugated to hydroxy-

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cinnamic acids and wall-bound polyamines, whose significance is still far from understood (GENY *et al.*, 1997). Moreover, cuttings are the main form of regeneration vineyard.

The present study was therefore undertaken to investigate the role of polyamines in adventitious root formation at the base of *Vitis vinifera* L cuttings. The study included the analysis of free, conjugated and wall-bound polyamines required by cuttings for the development of callus (root primordia) and primary adventitious roots. In addition, we tried elucidate the involvement of polyamines in root initiation and development using exogenous polyamines.

MATERIALS AND METHODS

I - PLANT MATERIAL AND ROOT FORMATION

Dormant cuttings were obtained from one-year-old pruned Cabernet-Sauvignon cane collected in a vineyard at Léognan near Bordeaux (France). After re-hydration (12 hours in water at 30°C), the bases of cuttings were planted in sand and transferred to the cold room under controlled conditions (26°C in sand at the base of cuttings in a cold room at 4°C). The culture was grown for 31 days in continuous darkness.

Water was provided by drip irrigation (50 ml/plant/day), and solutions of polyamines (putrescine and spermidine at 10-3M.l^{-1}) were added in water for treatment throughout the culture.

TABLE I

Effect of exogenous polyamine treatment on rhizogenesis and adventitious root development

Values are the mean of triplicate ± SD

Effet de l'apport de polyamines exogènes sur la rhizogénèse et le développement de racines adventives.

Les valeurs correspondent à la moyenne de trois réplications ± SD

	Control	PUT+SPD
Date of appearance (days of culture) :		
Calli	11 ± 0.74	11 ± 0.68
First roots	19 ± 0.64	19 ± 0.52
No. of calli / cutting	3.2 ± 1.23	3.1 ± 1.52
No. of roots / cutting	11.3 ± 4.18	12.8 ± 5.82
Mean size of roots (cm)	4.5 ± 0.85	8.8 ± 1.88
No. of long roots (> 2 cm) / cutting	4.2 ± 1.17	5.5 ± 1.29
No. of mid-sized roots (0.5 and 2 cm) / cutting	3.6 ± 1.78	4.5 ± 2.25
No. of small roots (<0.5 cm) / cutting	2.5 ± 1.23	1.8 ± 1.28

II - SAMPLING PROCEDURE

The adventitious roots were analyzed periodically for growth (number of calli : primordia roots), number and length of primary adventitious roots and polyamine contents during the culture.

For each treatment (control and exogenous polyamine media), batches of 10 cuttings were harvested on different days of culture and weighed ; samples (roots, callus and wood) were taken from cuttings, fixed in Hcl N and stored at -20°C until use.

Each sample consisted of 5 cuttings and was analyzed in triplicate.

III - POLYAMINE EXTRACTION AND ANALYSIS

Wood, calli and roots for polyamine analysis were extracted according to the method of FLORES et GALSTON (1982). HPLC in combination with fluorescence spectrophotometry were used to separate and quantify polyamines as their dansyl derivates from plant tissue according to the method of SMITH et DAVIES (1985).

RESULTS

I - ROOT FORMATION

In the control, calli appeared 11 days after the beginning of culture and the first roots appeared after 19 days. Treatment had no significant effect on time appearance of calli and roots (table I).

Exogenous polyamine treatment did not promote root formation (number of calli and total number of roots not modified), but did increase root length and size (mean size of roots and number of long and mid-length roots increased) (table I).

II - POLYAMINE CONTENT DURING RHIZOGENESIS

1) Control

Polyamine contents in cuttings :Total polyamine content in cuttings changed during rhizogenesis (figure 1). Total polyamine content increased at 11, 18 and 29 days of culture. These accumulations corresponded to the appearance of calli at 11 days, to that of the first roots at 18 days and to root rowth at 29 days. These polyamine accumulations were principally due to conjugated polyamines at 11 and 29 days of culture.

Polyamine contents in calli : Calli were the richest parts in polyamines (8-fold more than in cuttings and 2.5-fold more than in roots) (figure 1). During their development (between 15 and 22 days of culture), total

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polyamine contents were very high and the main polyamines were free polyamines with spermidine (data not show). The maximum polyamine content corresponded with the stage of callus differentiation to roots at 18 days.

Polyamine contents in roots : Polyamine contents were analyzed in roots of different size (size<0.5 cm : small roots – 0.5<size<2 cm : mid-sized roots – size>2 cm : long roots) and in different parts of long roots (base of roots, middle of roots and apex).

Total polyamine content decreased with size of roots (figure 2) : small roots were 2-fold more rich in polyamines than mid-sized and long roots. There are no difference between the two latter.

In roots, free polyamines and particularly free putrescine were the main polyamines (data not show).

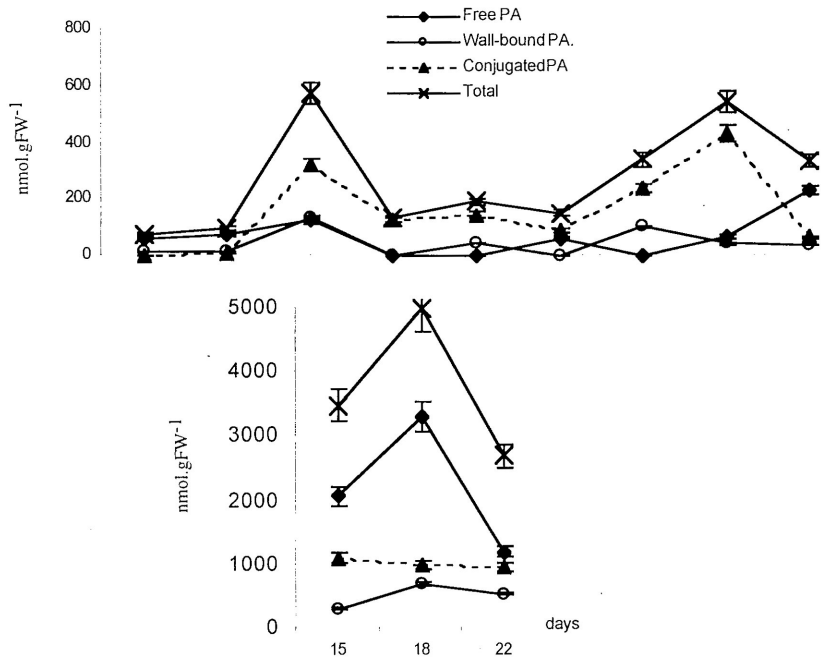


Fig. 1 – Total of free, conjugated and wall-bound polyamines in wood and calli of cuttings during rhizogenesis.
Vertical bars are the mean of triplicate ± SD.

Polyamines totales libres, liées aux macromolécules et liées aux acides hydroxycinnamiques des bois et des cals de boutures au cours de la rhizogénèse.
Les barres verticales représentent la moyenne de 3 répétitions ± SD.

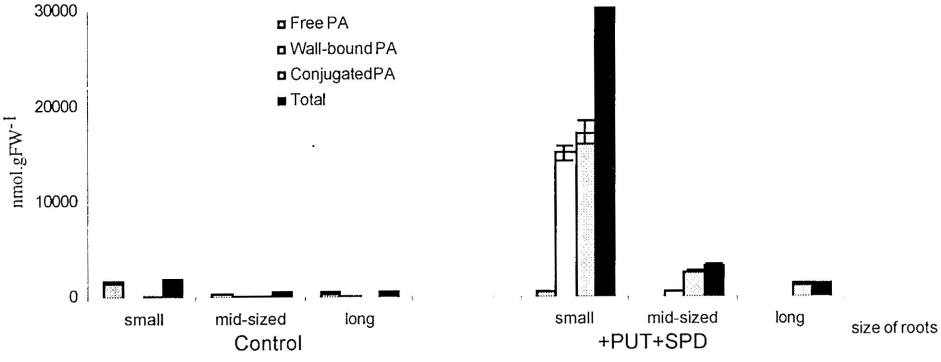


Fig. 2 – Total of free, conjugated and wall-bound polyamines in roots of cuttings. Effect of treatment.
Vertical bars are the mean of triplicate ± SD.

Polyamines totales libres, liées aux macromolécules et liées aux acides hydroxycinnamiques des différentes racines adventives. Influence de l'apport exogène de polyamines.
Les barres verticales représentent la moyenne de 3 répétitions ± SD.

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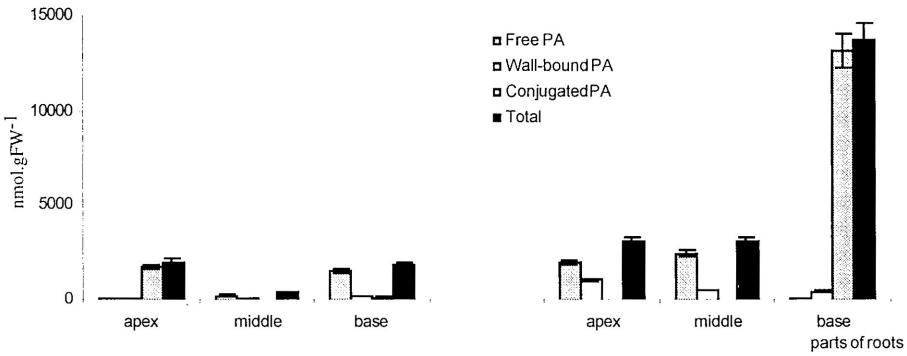


Fig. 3 – Total of free, conjugated and wall-bound polyamines in different parts of roots of cuttings. Effect of treatment.

Vertical bars are the mean of triplicate ± SD.

Polyamines totales libres, liées aux macromolécules et liées aux acides hydroxycinnamiques dans les différentes parties des racines adventives. Influence de l'apport exogène de polyamines.

Les barres verticales représentent la moyenne de 3 répétitions ± SD.

The base and apex of roots were the parts richest in polyamines (figure 3) and the contents did not differ. Nevertheless, their distribution was different (figure 3) : in apecis, the main polyamines were conjugated polyamines with diaminopropane ; in bases, free polyamines (putrescine and spermidine) predominated. In the middle part, the total polyamine content was very low.

2) Effects of treatment

Polyamine contents in roots : Exogenous polyamine treatment drastically increased total polyamine content in small roots (figure 2). It increased wall-bound and conjugated polyamines (figure 2).

In all parts of the roots, treatment changed polyamine distribution (figure 2). Exogenous polyamines considerable increased conjugated polyamine levels in the bases of roots while in apices and the middle parts, free polyamines were more abundant than in the control. In apices and the middle parts, the main polyamines were free and wall-bound putrescine while in the bases, the main polyamine was conjugated-diaminopropane.

DISCUSSION

While a few papers have discussed the role of free polyamines during the rhizogenesis in cuttings, no work has been published to our knowledge concerning changes in free, conjugated and wall-bound polyamines in this morphogenic process.

Free and bound polyamines appear to be strongly involved in the induction and development of adventitious roots in *Vitis vinifera* L. Polyamine contents were higher during this morphogenic process and lower when tissues weree either completely differentiated or when rhizogenesis and callogenesis had ceased to occur. In cuttings, free putrescine and spermidine rose concomitantly at 11 and 31 days of culture, while conjugated diaminopropane increased much more than conjugated putrescine and spermidine at 11 and 29 days of culture (data not show). Free polyamines increased in calli concomitantly with the differentiation of primary roots (figure 1) and were the main ones in roots (figure 2). Small roots were richer in polyamines (figure 2) than mid-size and long roots and the distribution of polyamine differed between parts of roots (free polyamines were more numerous in bases while wall-bound polyamines were more abundant in apices) (figure 3).

This suggests a different role and/or utilization of free polyamines than that of bound ones during these morphogenic process, but an interconversion between free and bound polyamines is also a possible hypothesis.

The function of conjugated and wall-bound polyamines during cell division and cellular differentiation is still a matter of debate. The question is whether they are storage forms for the polyamines or biologically active substances by themselves. SLOCUM et GALSTON (1985) suggested that there is only limited exchange between free and conjugated polyamines, at least in tobacco. However, FLORES et FILNER (1985) claimed that conjugation to cinnamic acids might be a way of regulating the free polyamine pools in plant cells. It appears that during germination of rice, conjugates of putrescine, spermidine and tyramine act as storage form of amines which, upon hydrolysis, could supply the cell with additional amines that could in turn influence cell division and/or cell suspension (BONNEAU *et al.*, 1994). Conjugation reactions could regulate polyamine functions e.g. by affecting their

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binding and interaction with nucleic acids. Moreover, polyamine conjugation to a hydroxycinnamoyl moiety might be important in the regulation of the free polyamine titers and/or detoxication phenolic compounds known to inhibit growth.

High levels of free polyamines have been reported to influence growth by cell division and low levels with cell expansion (EGEA-CORTINES et MIZRAHI 1991). Moreover, the endogenous concentration of polyamines can be growth-limiting, as reported by SMITH (1982). Exogenous incorporation of polyamines, and putrescine in particular, have been shown to stimulate growth of several higher plants. In our experiments, exogenous polyamines increased the mean size of roots, conjugated and wall bound polyamines contents in callus and roots (figures 2 and 3). Furthermore, conjugated and wall-bound polyamines are believed to stimulate growth.

A considerable increase in polyamine levels accompanying the growth and induction of roots was observed in apple (WANG et FAUST 1986). Thus it can be concluded that the early phase of adventious root formation in *Vitis vinifera* L. requires high levels of polyamines and particularly conjugated and wall-bound polyamines. At the beginning of rhizogenesis, calli and small roots were richer in polyamines than the other parts, but during growth polyamine levels decreased. Although FRIEDMAN *et al.* (1982) found no promotive effects of polyamines on root cuttings, our observations lend support to those of JARVIS *et al.* (1983) who reported promotive effects of putrescine, spermidine and diaminopropane in roots of cuttings.

It is probable that the role of polyamines in many morphological changes occurring in plant tissues such as somatic embryogenesis, the formation of floral and vegetative buds, or rhizogenesis is to promote or assist in cell division. For example, addition of polyamine-biosynthesis inhibitors to thin cell layers of tobacco caused cell expansion and an inhibition of cell division and rhizogenesis, effects which could be reversed by the addition of exogenous polyamines (ALTAMURA *et al.*, 1991).

CONCLUSION

The present data show that the size of roots can be changed by the exogenous polyamines, and that polyamine contents (particularly conjugated and wall-bound polyamines) in roots are modified at the differents stages of rhizogenesis. This suggests that conjugated and wall-bound polyamines are required for the production of adventitious roots in *Vitis vinifera* L. The mechanism of this effect, and indeed of the involvement of polyamines in many other development process, is unclear, but the techniques of molecular genetics are now providing a new impetus to polyamine research. This may lead to an improved understanding of experiments based on the use of metabolic inhibitors.

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