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Rooting behaviour, polyphenol oxidase activity, and biochemical changes in grape rootstocks at different growth stages

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Abstract: Four grape rootstocks belonging to different Vitis species were planted in September. Rooting behaviour, polyphenol oxidase (PPO; EC 1.14.18.1) activity and biochemical parameters were studied at different growth stages after planting the cuttings in polythene bags. Significant differences were recorded for rooting success among the different rootstocks, with the maximum sprouting percentage determined in the Freedom rootstock. The highest variation in PPO activity was also recorded in Freedom. High PPO activity was recorded in rootstock 140 Ru, while the highest root length was recorded in Dog Ridge. In the rootstock 110 R, the PPO activity was the lowest during the initial stage, though it increased up to 60 days after planting (DAP) and was then reduced up to 90 DAP. Increases in the number of rooting primordials were recorded in all the different rootstocks at different stages of growth. A higher number of rooting primordials was recorded in Freedom, and it was followed by 110 R. The present study suggests the differences in the rooting behaviour of the different rootstocks are based on PPO activity at regular time intervals up to 90 DAP.

Key words: Biochemical constituents, grape rootstocks, growth parameters, polyphenol oxidase activity, rooting behaviour

Introduction

Grapes are a temperate fruit crop grown successfully in different agro-climatic zones of the world. The world production of grapes is presently 65.48 million tonnes, of which India accounts for 1.2 million tonnes, giving it a 1.83% share of world production and representing 3% of total fruit production in the country. Grapes hold an enviable position as a staple fruit crop as well as a cash crop.

Propagation by cuttings is one of the most important practices in viticulture. It is well known that in comparison to softwood cuttings, grapes are generally propagated through hardwood cuttings due to the higher success rate. In traditional viticulture, commercial varieties of grapes were grown on their own roots. However, such viticulture practices suffered from a number of increasing problems including soil salinity, drought and poor fruiting. Owing to this problem in Indian viticulture, the use of rootstocks for the cultivation of table grapes was given added importance. Grape rootstocks play a major role in Indian viticulture to combat the adverse effects of water scarcity, and soil and water salinity. Many different rootstocks are being used to establish vineyards, and of these Dog Ridge is considered to be one of the most important commercial rootstocks known for its drought tolerance. Other rootstocks that are gaining popularity in India include 110 R, 140 Ru, and Freedom, all of which are known for their tolerance to drought and salinity, aside from their improved bud fruitfulness due to their moderate vigour.

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Rootstocks vary in their ability for rooting based on the biochemical constituents of the mother vines and also the Indole Butyric Acid (IBA) concentrations used in the treatment of cuttings. Rooting ability depends on the genetic characteristics of a plant (De Klerk and Brugge 1992), environmental conditions (Levitt 1980; Sakai and Larcher 1987; Moe and Anderson 1988), and the exogenous and endogenous supply of biochemical constituents. In some plant species, it is also possible that the ability to root can change over time (Haissig and Davis 1994). Many experiments have indicated that in addition to auxins, carbohydrate, protein, phenolic, and enzyme sources, among others, are critical for rooting. Veierskov et al. (1982) and Veierskov (1988) studied the rooting response in *Pisum* cuttings by changing the light cycle and thus indirectly changing carbohydrate concentrations. Altman and Wareing (1975) and Okoro and Grace (1976) showed the influence of leaves on the rooting process as a result of their interference with the carbohydrate metabolism.

In addition to the biochemical constituents, many researchers (Hahlbrock and Grisebach 1979; Gonazales et al. 1991; Dalet and Cornu 1998) have established the role of a number of enzymes during the rooting process, and it has been suggested that some of these enzymes are involved in rooting. Among these, polyphenol oxidase (PPO) is of much interest. PPO can directly regulate the synthesis of phenolics and plays a role in the organisation and development of a primordial root. Considering this, an experiment was conducted to study the influence of the initial biochemical reserve of the mothervine, as well as the biochemical reserve of the plant over a predetermined period of time on rooting behaviour and polyphenol oxidase activity in the rootstocks 110 R, 140 Ru, Freedom and Dog Ridge under Indian conditions.

Materials and methods

An experiment was conducted at the nursery of National Research Centre for Grapes, Pune (India), during 2008-09 with the objective of studying the rooting behaviour, biochemical status and polyphenol oxidase activity of grape rootstocks. Four rootstocks, Dog Ridge, 110 R, 140 Ru and Freedom, belonging to different *Vitis* species were selected for the study. The mother vines of these rootstocks were maintained in the nursery block. Pune is situated in the mid-west of Maharashtra state, at an altitude of 559 m and lies at latitude 18°32′N and longitude 73°51′E. Standard cultural practices were followed to maintain the mother vines of the rootstock in the nursery. Hardwood cuttings from the previous season of 8-10 mm in diameter and having 4 buds were selected from the mother vines. From these, 5 cuttings were used to estimate the initial status of starch, reducing sugars, protein, phenols, and nitrogen.

The prepared cuttings were then kept in running water for 24 h to leach out the rooting inhibitors. A slanting cut at the basal end was then taken on each cutting so as to absorb the rooting hormone properly and to ensure the maximum area for rooting. The cut portion was dipped in 2 mg L^{-1} indole - 3 - butyric acid (IBA) for 60 before planting in polythene bags. The polythene bags of 12.7 × 17.8 cm in size were filled with a soil:sand:FYM medium at 2:1:1 proportions. The planting of the cuttings in the bags was then done after dipping these cuttings in IBA. The cuttings were inserted in the polythene bags containing the potting mixture in such a way that 2 buds were inside the media while 2 buds were above the soil.

All the cuttings were irrigated at regular intervals and maintained in the nursery following standard cultural procedures. Observations on the percentage of sprouting were recorded 30 days after planting (DAP). Observations on the number of primordial roots were also recorded at 30, 60, and 90 DAP.

Biochemical analysis

The total content of phenolics and protein was determined using the Folin-Ciocalteu method (Singleton and Rossi 1965) and Lowry's method (Lowry et al. 1951), respectively. Reducing sugar was estimated by the dinitrosalicylic acid method (Miller 1972) and total carbohydrates were estimated by the Anthrone method (Hedge et al. 1962). Also, the amount of total chlorophyll in the leaves was measured at each growth stage by using Arnon's method (1949). The results obtained for each biochemical parameter analysed were expressed as mg g^{-1} of the lyophilised leaf and cane sample. The micro-Kjeldahl method was followed for the estimation of total nitrogen percentage.

For the estimation of PPO activity, the destruction method of sampling was followed wherein the basal portion of the shoot along with the callus was taken. PPO activity was measured periodically at 30, 45, 60, 75, and 90 DAP. One gram of the meristematic part of each rootstock was taken at different growth stages and was then homogenised in a chilled mortar in 5 mL of 0.1M phosphate buffer (pH 6.00). The extract was centrifuged in a cold centrifuge at 15,000 rpm for 20 min and the supernatant was used as the enzyme source. Polyphenol oxidase activity was assayed by the method of Esterbaner et al. (1977). The oxidation of catechol was measured from a reaction mixture containing 2 mL of phosphate buffer (pH 6.5), 0.5 mL of enzyme extract, and 1 mL of 0.01 M catechol at 495 nm. Initial absorbance was read at 495 nm and then absorbance was measured at 30-s intervals on a UV-visible spectrophotometer (Shimadzu-1601). Enzyme activity was expressed as EU mL^{-1} min^{-1}.

The standard reference chemicals used, including D-glucose, 4-methylcatechol, and bovine serum albumin, were obtained from S.D. Fine-Chem Ltd. (Mumbai, India). All other buffers and chemicals were of AR grade and obtained from Merck Pvt. Ltd.

Statistics

Data recorded from the 3 replicates were pooled together after passing a normality test. Standard deviation was used as a measure of variability. The overall significance of the treatments was determined by one-way and two-way ANOVA test, as applicable, followed by Duncan's LSD comparison as a post-hoc test. Data analysis was carried out using SPSS (version 9.0) and a P value of 0.05 was taken to be significant.

Results

The observations recorded on sprouting, percentage success, and total root length are presented in Table 1. The sprouting percentage recorded at 30 DAP showed that more sprouts were recorded on Freedom, whereas 110 R recorded the lowest sprouting percentage. Significantly, the rooting percentage was higher for Dog Ridge, while it was lower for Freedom. Total root length after 90 DAP varied among the different rootstocks with the maximum root length found in Dog Ridge, followed by Freedom, while the minimum root length was recorded in 140 Ru.

The data recorded for the number of root primordials are presented in Table 2. The differences recorded for the number of root primordials in the rootstocks were mostly non-significant except in 110 R, which showed significant variance from Freedom and 140 Ru at 30 DAP; and 140 Ru from Freedom and Dog Ridge at 90 DAP. Although the differences were non-significant, the maximum number of primordial roots was recorded in Freedom at 90 DAP, while the

Table 1. Sprouting and rooting behaviour of different rootstocks at a particular growth stage.

Rootstocks	Percentage sprouting 30 DAP	Percentage rooting 90 DAP	Total root length (cm) 90 DAP
Dog Ridge	83.75 ± 1.27 ab	92.59 ± 8.07 a	123.87 ± 3.46 a
Freedom	88.88 ± 12.45 a	68.23 ± 3.06 c	84.11 ± 3.08 b
140 Ru	74.62 ± 2.18 b	89.16 ± 0.96 ab	62.13 ± 0.91 d
110 R	60.81 ± 0.61 c	82.52 ± 2.49 b	76.15 ± 0.03 c

Letters indicate the rootstock-wise significant difference at the indicated time course independently. Rootstocks followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 2. Number of root primordials in the rootstocks after specific time intervals.

Rootstocks	Number of root primordials		
	30 DAP	60 DAP*	90 DAP
Dog Ridge	9.00 ± 2.00 ab	10.00 ± 3.00	11.00 ± 1.00 a
Freedom	7.00 ± 2.00 b	14.00 ± 1.00	18.00 ± 1.00 a
140 Ru	8.00 ± 1.00 b	12.00 ± 0.00	16.00 ± 1.00 b
110 R	11.00 ± 0.00 a	13.00 ± 4.00	17.00 ± 1.00 ab

* Non-significant data. Letters indicate the rootstock-wise significant difference at the indicated time course independently. Rootstocks followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 3. Biochemical constituents of rootstock cuttings (canes) at the time of planting.

Rootstocks	Starch (mg g^{-1})	Reducing sugar (mg g^{-1})*	Carbohydrate (mg g^{-1})	Proteins (mg g^{-1})	Phenols (mg g^{-1})	Nitrogen (%)
Dog Ridge	10.71 ± 0.43 b	22.79 ± 0.68	36.79 ± 1.47 c	47.33 ± 1.42 b	22.42 ± 1.57 a	0.89 ± 0.03 a
Freedom	11.96 ± 0.60 b	23.35 ± 1.63	51.97 ± 3.64 b	56.70 ± 2.84 a	11.10 ± 0.44 b	0.91 ± 0.05 a
140 Ru	15.57 ± 1.09 a	22.94 ± 1.38	51.29 ± 2.56 a	48.45 ± 1.44 b	12.55 ± 0.63 b	0.53 ± 0.03 c
110 R	9.00 ± 0.27 c	21.16 ± 1.06	32.16 ± 1.29 a	37.85 ± 2.27 c	12.97 ± 0.93 b	0.65 ± 0.03 b

*Non-significant data. Letters indicate the rootstock-wise significant difference. Rootstocks followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

lowest number was seen in 140 Ru. Changes in the number of root primordials for each rootstock were found to increase as DAP rose from 30 DAP onwards.

The data obtained for the biochemical constituents of the cuttings estimated before planting are presented in Table 3. The highest starch content was recorded in 140 Ru, followed by 110 R. The differences recorded for reducing sugar content were non-significant for all the rootstocks. Freedom was superior over all the other rootstocks for carbohydrate content. The highest protein content was recorded in Freedom followed by 110 R. The highest content of phenolics was recorded in Dog Ridge. Similarly, a higher nitrogen percentage was recorded in 110 R cuttings, followed by 140 Ru. Significantly, a higher chlorophyll concentration was recorded in Freedom at all the growth stages after planting (Table 4).

The results demonstrated that Dog Ridge had the highest PPO activity during the initial stage of the rooting process, and that these differences were significant at different growth times (Figure). The PPO activity of Dog Ridge at 90 DAP was comparable with that of Freedom. A reduction in the activity of PPO was recorded in 140 Ru from 60 DAP. However, the rootstock Dog Ridge recorded higher PPO activity at 90 DAP. In 110 R, it was lower compared to the other rootstocks at the initial stage; however, PPO activity in 140 Ru increased from 30 to 60 DAP and was again reduced up to 90 DAP. The lowest PPO activity was recorded in 140 Ru at 90 DAP.

Table 4. Total chlorophyll concentration (mg g^{-1}) in the leaves of grape rootstocks after specific time intervals.

Rootstocks	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Dog Ridge	0.96 bc, l	0.62 b, n	0.86 c, m	1.03 a, l	0.86 c, m
Freedom	1.17 a, mn	0.88 a, o	1.24 a, m	1.11 a, n	1.66 a, l
140 Ru	0.55 c, n	0.81 a, n	0.79 c, m	1.02 a, l	0.79 c, m
110 R	0.50 c, n	0.62 b, m	0.97 b, l	1.02 a, l	0.97 b, l

*There is a significant difference in the rootstocks, DAP and rootstocks x DAP interaction ($P < 0.001$) by two-way-ANOVA. The letters a, b, c denote significant difference between the rootstocks within DAP, whereas l, m, n, o denote a significant difference between DAP within the rootstocks by Duncan's Multiple Range test at $P = 0.05$.

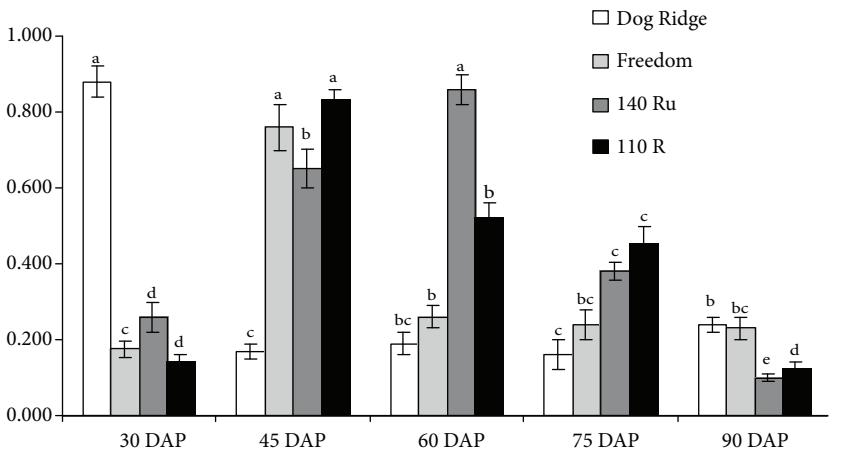

Figure. PPO activity (EU mL^{-1} min^{-1}) in grape rootstocks during rooting.

Discussion

The biochemical constituents present in the cuttings play an important role during their rooting process by acting as a reserve energy source. The lower root length, percentage sprouting and rooting in 140 Ru, 110 R, and Freedom, respectively, might be due to the availability of a higher percentage of rooting inhibitors. Sturve (1981) reported that a higher C:N ratio in the tissues of cuttings promotes rooting, though did not predict the degree of rooting response. From the results obtained it can be said that the reserve material may have helped the cuttings of each rootstock to produce rootlets. The higher number of root primordials in the Freedom rootstock was found to be associated with the increased amounts of reducing sugar and carbohydrates. The presence of sugar in the cuttings might have helped the cuttings to produce more rootlets and thus the increased root length in the rootstock Freedom. The results obtained in this study are in accordance with the results obtained by Sadhu and Bose (1982), who reported that the level of soluble sugars and the C:N ratio correlated positively with rooting in guava.

The chlorophyll content was found to vary among the different rootstocks. It was higher in Freedom at all growth stages after planting. The increase in rooting primordials and also increased shoot length helps plants grow at a faster rate. The presence of starch, protein and carbohydrates in the rooted plants acts as an energy source for further plant growth. In the

Freedom rootstock, higher amounts of starch, protein and carbohydrates were recorded when compared to the other rootstocks. The increase in root length and also shoot growth in the Freedom rootstock might have been due to the availability of these biochemical constituents at higher rates, which helped the plant to grow via the production of more chlorophyll, which is required for photosynthesis. Zachariakis et al. (2001) reported that total chlorophyll content increased the total carbohydrate concentration in grapevine shoots.

The sprouting recorded 30 DAP revealed that more sprouts were recorded in Freedom and the lowest number were found on 110 R. The PPO activities in the rootstocks increased over time after sprouting up to 60 DAP. The increase in metabolic activities in the plant after sprouting may help the plants enhance the physiological process, and in turn encourage the production of secondary metabolites like phenols (Satisha et al. 2007). There may be a direct correlation between the number of root primordials and the increased PPO activity in the different rootstocks. This study indicated that rootstocks vary in their ability at rooting and in generating more root primordials. Coban (2007) also reported the increased activity of PPO in Sultana, Round Seedless and Yalova Incisi during the early stages after planting, followed by a decrease in activity after some time.

It is clear from the results that phenolic compounds varied significantly among the different rootstocks. In Freedom, PPO activity started increasing from 30 to 45 days. Significantly, the reduction in PPO activity correlated with reduced phenol levels in the same rootstock. The present results are in accordance with the results obtained by Satisha et al. (2008), who reported that there was a corresponding reduced phenolic content in the cuttings of St. George during the initial stage. PPO is involved in the oxidation of phenols and thus contributes energy for cell division and cell differentiation in the organogenesis of explants in the tissue culture. Since the level of phenolic compounds was lower, the PPO activity was also reduced in the rootstocks studied. The increased phenol content in the rootstock Dog Ridge at the initial stage also correlated with increased PPO activity at the initial stage of rooting. The availability of phenolic compounds in the plants is also known to act synergistically with auxin and thus stimulate root initiation (Hartman et al. 1993). Rios et al. (1997) in their studies on rooting response in relation to peroxidase, PPO and IAA oxidase in walnut explants reported a preferential increase in PPO and peroxidase activity during the induction stage. The reduction in the peroxidase activity in Freedom suggests that peroxidase and PPO activities are not involved in the initiation of the rooting. The rootstock Dog Ridge had higher root length and this was also correlated with increased phenol content after 90 days of planting. Also, phenols may play an important role in the rooting process of the cuttings. This study indicates that the presence of phenol in the cuttings helped the plants to develop more root primordials and greater total root length. In the present study, the Dog Ridge rootstock had a higher phenolic content and longer root length. Several researchers have reported that phenolics are negatively related to seed germination and in vitro proliferation (White 1994; Prasad 1989). In contrast, Thomas and Ravindra (1999) have reported a positive correlation between phenolics and totipotency while working on shoot tip culture in the mango plant. Phenolic compounds sometimes have an inhibitory or stimulating effect on plant growth, which varies from species to species (Ozyigit et al. 2007). A slightly enhanced level of PPO during the first 3 weeks in brown and non-brown callus tissues of the Virginia pine was also reported by Tung and Newton (2004). Thus, from the study it can be concluded that the rooting behaviour and PPO activity of the rootstocks varies from cultivar to cultivar. Rootstocks with higher phenolic content showed heightened PPO activity at an earlier stage of development (i.e. up to 30 DAP), and this seems to be associated with the formation of primordial roots by accelerating the process of cell division. Also, the initial biochemical status of the cuttings was found to be associated with plant growth and development during the earlier stages.

References

Altman A Wareing PF (1975) The effect of IAA on sugar accumulation and basipetal transport of ^{14}C-labelled assimilates in relation to root formation in *Phaseolus vulgaris* cutting. Physiol Plant 33: 32-38.

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant Physiol 24: 1-15.

Coban H (2007) Determination of polyphenol oxidase activity during rooting in cuttings of some grape varieties (Vitis vinifera L). Asian J Chem 19: 4020-4024.

Dalet F, Cornu D (1998) Lignification levels and peroxidase activity during *in vitro* rooting of Prunus avium. Can J Bot 67: 2192-2186.

De Klerk GJ, Ter Brugge J (1992) Factors affecting adventitious root formation in microcuttings of Malus. Agron 12: 747-755

Esterbaner H, Schwarzl E, Hayn M (1977) A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. Analy Biochem 77: 486-494.

Gonazales R, Sanchez R, Rodriguez R, (1991) Ethylene in relation to proteins, peroxidase and polyphenol oxidase activities during rooting in hazelnut cotyledons. Physiol Plant 83: 611-620.

Hahlbrock K, Griesbach H (1979) Enzymatic controls in the biosynthesis of lignin and flavonoids. Annu. Rev. Plant Physiol 30: 105-136.

Haissig BE, Davis TD (1994) A historical evaluation of adventitious rooting research to 1993. In: Biology of Adventitious Root Formation (Eds. TD Davis, BE Haissig). Plenum Press, NY, pp. 275-331.

Hartmann HT, Kester DE, Davies FT (1993) Plant propagation - principles and practices. Prentice Hall, New Delhi, India.

Hedge JE, Hofreiter BT (1962) In: Carbohydrate Chemistry 17 (Eds. Whistler RL and Be Miller JN) Academic Press, New York.

Levitt J (1980) Responses of Plants to Environmental Stresses, 2nd ed. Academic Press, NY, USA.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-Phenol reagents. J Biol Chem 193: 265-275.

Miller GL (1972) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31: 426-428.

Moe R, Anderson AS (1988) Stock plant environmental and subsequent adventitious rooting. In: Adventitious root formation cuttings (Eds. Davis TD, Haissig BE, Sankhla AN). Dioscorides Press, Portland, pp. 214-234.

Okoro OO, Grace J (1976) The physiology of rooting Populus cuttings: I. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138.

Ozyigit II, Kahramanz MV, Ecranz O (2007) Relationship between explants age, total phenols and regeneration response in tissue cultured cotton (Gossypium hirsutum L.). Afr J Biotechnol 6: 3-8.

Prasad R (1989) Effect of nonyl phenol adjuvant on macrophytes. In: Adjuvant and agrochemicals: mode of action and physiology activity (Ed. PNP Chow). CRC Press, Boca Raton FL. Chap 6. Rios DG, Sanchez O, Gea ME, Revilla MA, Rodrigues R (1997) Rooting response in relation to PO, PPO, IIA O activities in walnut explants. In: Proceedings of III International Walnut Congress, Alcobaça, Portugal, pp. 235-442.

Sadhu MK, Bose S (1982) Propagation of Tropical and Subtropical Horticultural Crops. In: Proceedings of 21st Horticultural Congress, Hamburg, Germany p. 1379.

Sakai A, Larcher W (1987) Frost survival of plants: responses and adaptation to freezing stress. Springer-Verlag, Germany, p. 321.

Satisha J, Ramteke SD, Karibasappa GS (2007) Physiological and biochemical characterisation of grape rootstocks. South Afr J Enol Vitic 28: 163-168.

Satisha J, Raveendran P, Rokade ND (2008) Changes in polyphenol oxidase activity during rooting of hardwood cuttings in three grape rootstocks under Indian conditions. South Afr J Enol Vitic 29: 98-101.

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am J Enol Vitic 16: 144-158.

Sturve DK (1981) The relation between carbohydrates, nitrogen and rooting of stem cuttings. Plant Propagat 27: 6-7.

Thomas P, Ravindra MB (1999) Shoot tip culture in mango: influence of medium, genotype, explants factors, season and decontamination treatments on phenolic exudation, explants survival and axenic culture establishment. J Hort Sci 72: 713-722.

Tung W, Newton RJ (2004) Increase of polyphenol oxidase and decrease of polyamines correlate with tissue browning in Virginia pine (Pinus virginiana Mill). Plant Sci 167: 621-628.

Veierskow B (1988) Relations between carbohydrates and adventitious root formation. In: Adventitious root formation in cuttings (Eds. TD Davis, BE Haissig, AN Sankhla). Dioscorides Press, Portland, pp. 70-78.

Veierskov B, Anderson AS, Stumann BM, Henningsen KW (1982) Dynamics of extractable carbohydrates in Pisum cuttings. II. Carbohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperature and genotype. Physiol Plant 55: 174-178.

White R (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinol 135: 175-182.

Zachariakis M, Tzorakakis E, Kritsotakis I, Siminis CI, Manios V (2001) Humic substances stimulate plant growth and nutrient accumulation in grapevine rootstocks. International Symposium on Composting of Organic Matter. pp. 193-202.