PHYSIOLOGICAL FACTORS INFLUENCING THE ROOTING OF PLUM ROOTSTOCKS' HARDWOOD CUTTINGS PLŪMJU KOKSNAINO SPRAUDEŅU APSAKŅOŠANOS IETEKMĒJOŠIE FIZIOLOĢISKIE FAKTORI

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Kopsavilkums

Izmēģinājumā tika pētīta dažādu tehnoloģisko, fizioloģisko un bioķīmisko faktoru ietekme uz triju veidu koksnaino spraudeņu apsakņošanos. Tehnoloģiskie faktori – spraudeņu griešanas laiks, un ISS optimālā koncentrācija. Augstākā apsakņošanās novērota galvenokārt miera sezonas sākumā vai īsi pirms tās grieztiem spraudeņiem, pielietojot 2000 ppm ISS. Apkārtējās vides ietekme – vidējā temperatūra, aukstuma vienības, kas ietekmēja apsakņošanos netieši. Konstatēta vāja apsakņošanās sakarība ar fizioloģiskajiem faktoriem (lapu nokrišana un plaukšana). Fenolu savienojumu ietekme lapu biršanas laikā un peroksidāzes aktivitātes palielināšanās lapu plaukšanas laikā korelē ar apsakņošanās intensitāti. Polifenolu oksidāzes aktivitāte neietekmēja spraudeņu apsakņošanos.

Abstract

Rooting is a complex process that is determined by the relation of many factors. Rooting rate is highly influenced by the time of cutting collection, which includes the state of the mother plant and the environmental factors on that day.

In our experiment three rootstocks' rooting ability was examined, in relation to the different technological, physiological and biochemical factors. The technological factors were the time of cutting collection, and the optimal dose of IBA. We gained the higher rooting rates mostly in the first half of the dormant season, or before, with applying 2000 ppm IBA. The environmental circumstances that we examined (daily mean temperatures, Cold Units) influenced rooting indirectly. We found a relatively loose connection between rooting and the physiological factors, leaf fall and sprouting. From the state of leaf fall the values of the phenolics that has close correlation to rooting can be suggested, also the peroxidase enzyme activity from sprouting. The third biochemical factor that we examined, the polyphenol oxidase activity did not show any correlation with rooting.

Keywords: rooting, leaf fall, sprouting, water content, temperature

Introduction

Vegetative propagation by hardwood cuttings is a simple and cheap method of the multiplication of plum rootstocks, which has not been used with great success in Hungary so far. The reason for that can be found partially in our climate, and in the Hungarian nurseries' practice. Though applying it to cultivars with more than 50 % rooting rate can be economical (Hartmann *et al.*, 1997). An improvement in propagation technology is needed where the investigations on environmental factors and some biochemical features of mother plants at the time of cutting collection could contribute to progress.

According to literature data, the three rootstock that we examined can root in relatively high percentages, the establishment of 'Sainte Julien GF 655/2' (*Prunus insititia* JUSL.) can be around 70-80 % (Kracikova, 1996; Uzunov, 1987), and 'Marianna GF 8-1' (*Prunus munsoniana* WIGHT & HEDRICK x *Prunus cerasifera* EHRH.) can give almost 100% rooting according to the practice in France (Demol, 2001). The growth reducing apricot rootstock, 'Fehér besztercei' (*Prunus domestica* L.) is a Hungarian selection, a softwood cutting is advised for its propagation; no satisfying results are known when using hardwood cuttings.

In the 1980s, the many studies concerning rooting also focused on almost each of the determining factors of rooting. Besides the environmental and technological factors, however several works studied the roles of the internal processes inside the mother plant, which can be even more

determining. The phenological state and biochemical features of the mother plant influence rooting (Bassuk and Howard, 1981, Loreti *et al.*, 1985, Gur *et al.*, 1988, Guskov *et al.*, 1988). It is known that certain materials forming in the plant are related to rooting, but their exact chemical comparison, way of action, and the relation among them have not been clearly defined yet.

Our aim was to examine the phenological state of the mother plant, the environmental conditions on the collecting day, and the different biochemical parameters of the cuttings and buds, in relation to rooting.

Materials and Methods

The open field experiments were carried out in the Experimental Station of Corvinus University of Budapest at Soroksár, where the soil is slightly humic and sandy. The laboratory experiments were performed at the Applied Chemistry Department of the Food Science Faculty of Corvinus University of Budapest. Hardwood cuttings were collected from three rootstocks: 'Marianna GF 8-1', 'Sainte Julien GF 655/2', and 'Fehér besztercei', from September to March during four dormant seasons, then prepared to the length of 25 cm, from the basal part of shoots 7-8 mm in diameter. After collection the cuttings were dipped to fungicide solution, and put to cold storage for one day. Hormone treatment was applied, dipping the base of the cuttings to 50 % alcoholic solution of indole-butyric acid for 5 seconds, using 2000 ppm dose. After the treatment the cuttings were put into wet perlite and stored at 2°C, until planted to open field the next spring.

We examined the ratio of leaf fall, with counting the leaves on marked shoots every week until total leaf fall. For the examination of sprouting cuttings were put to room temperature on each collection date. For measuring the free water content of the cuttings at collection, cuttings were dried at 120°C for three days. The daily maximum and minimum temperature data were used for calculating the daily mean temperatures and cold units, the later was counted by the method of Richardson *et al.* (1974), starting from 23 September. The samples for the biochemical assays were prepared from the cuttings and from the buds of shoots used for the preparation of cuttings. The activity of peroxidase enzyme (POD) was measured by the method of Srivastava (1983), that of polyphenol oxidase (PPO) by the method of Bassuk *et al.* (1981), the determination of the protein content was accomplished by the spectrophotometric way of Bradford (1976). The results are given in Unit^{-mg} protein. The phenolics were assayed by the method described by Singleton and Rossi (1965), the results are given in µg equal to gallic acid^{-mg} fresh sample. Open field experiments were carried out between 1997-2002 during four dormant seasons, while the biochemical investigation between 2000-2003 during three dormant seasons. The results were analysed with one- and two-factor analysis of variance, regression analysis and partial correlation analysis.

Results and Discussion

The comparison of the rooting ability of hardwood cuttings collected on different dates shows a strong effect of timing, which differs among rootstocks. Based on our results, the optimal hardwood cutting collecting period for 'Fehér besztercei' rootstock is the end of October, but in some years cuttings collected in the middle of December can give satisfactory results as well. The hardwood cuttings of 'Sainte Julien GF 655/2' give the best results if collected in the middle of October. For the easily-rooting rootstock 'Marianna GF 8-1' the optimal period for propagation with this method is from the beginning of October until December, but this period can be longer, in some years cuttings collected in January also can root in high percentages (Figure 1.).

The cuttings collected in fall rooted better, this period can be described also with the extent of leaf fall. The quadratic figure fitted on five data pairs at all the three rootstocks reaches its peak at 40-60 % leaf fall. Rooting is supposedly influenced by the weather, the temperature of the collecting days or the period before, through influencing leaf fall. By our point of view, the materials formed in the separating zone of the leaves during leaf fall – first of all phenolics and polyphenol oxidase enzyme – can have an effect on rooting. We could not find clear correlations between the PPO activity measured in the rootstocks' buds and cuttings, but the phenolics content of the buds had square regression relation to rooting in all of the rootstocks. For 'Fehér besztercei' the phenol content in buds over 10 μ g/mg, for the Sainte Julien between 10-30 μ g^{-mg}, and for the Marianna 10-40 μ g^{-mg} resulted a relatively good rooting. In the literature the lower phenol content of the mother plants is said to be

favourable (Haissig, 1986), but on the contrary the total phenolics values measured by us were favourable in relatively wide intervals but it is true, that near the measured maximum the rooting dropped (Figure 2.-3.).



Figure 1. Rooting of plum hardwood cuttings on different dates during four dormant seasons (1997-2002)



Figure 2. The correlation between leaf fall of mother plants and rooting of plum hardwood cuttings



Figure 3. The correlation between phenol content of the buds and rooting of Marianna GF 8-1 hardwood cuttings

According to our results, the water content at collection influences rooting. Though the strength and features of the relation was varying in the years, based on three years' data, higher water content showed to be favourable in all of the three rootstocks. The higher (above 50 %) rooting percentages usually reached when the water content of the cuttings was over 50 %, though lower establishment rates occurred as well. The 50 % water content on collection probably is a limit that under it high rooting cannot be expected.

Statistical analysis did not show significant relation between the rooting of hardwood cuttings and the daily mean temperature at the day of collection in any of the rootstocks. Among the years differences can be seen in the relation between rooting and the cold units (CU) accumulated until the day of collection. The figure fitted to all of the 'Marianna GF 8-1' rootstock's data pairs can be seen that CU under 250 is the best for cutting collection, during this period rooting was over 50%. The material collected later can give good results as well, but the establishment of the cuttings is less certain. The strength of the relation was differing in years for the two other rootstocks, the effect of the year was strong so we cannot use CU data for confident determination of the optimal collecting period (Figure 4.).



Figure 4. The correlation between Cold Units and rooting of plum hardwood cuttings



Figure 5. The correlation between the rooting and the number of weeks needed for reaching maximal bud sprouting in 'Fehér besztercei' hardwood cuttings

The dormant state of the mother plants can be described with the capacity of sprouting of their buds. We kept the cuttings for nine weeks at room temperature after collection, recording every week the rate of sprouting buds. The number of weeks needed for reaching maximal sprouting was in linear connection with rooting rate, while the maximal rate of sprouting showed a varying relation. From the correlations analysed we suggested that the extent of sprouting is less important concerning rooting than the time required for sprouting. So the deepest the dormant state of the buds is, the higher rate of rooting can be expected. In the case of 'Fehér besztercei' if sprouting reached its maximal extent in more than five weeks (including when sprouting was not significantly different from 0 after nine weeks), than rooting could be expected above 20 %. In every season examined this need of time decreased less than six weeks in January, the dormant period of European plums ends at this time (Figure 5.).

We found significant correlation between the rooting of all the three rootstocks and the POD enzyme activity measured in one of the organs. The lower activity values were favourable, for 'Fehér besztercei' and 'Sainte Julien GF 655/2' this was under 4 U^{-mg} protein, while for the Marianna rootstock it meant activity under 3 U^{-mg} protein. We have found similar results in the literature, when the cuttings of cultivars with lower IAA-oxidase activity (which is mostly due to peroxidases) rooted better (Guskov *et al.* 1988). But example for the contrary can be found also (Ryugo and Breen 1974). As the POD activity and sprouting had strong correlation, we suggest that POD is one of the biochemical factors that take part in processes resulting sprouting (Figure 6.).



Figure 6. Correlation between POD activity and rooting in Marianna GF 8-1 plum hardwood cuttings



Figure 7. The possible relations between the factors influencing rooting of plum hardwood cuttings (Bold arrows show strong correlations)

Conclusions

Rooting is a complex process that is determined by the relation of many factors. Emphasizing only one factor can be misleading. That is why to the determination of optimal cutting date is complicated, many factors should be considered, and the correlation of factors is variable year by year. The physiological factors have an important role in rooting, but are determined through the biochemical parameters. Rooting rate is highly influenced by the time of cutting collection, which includes the state of the mother plant and the environmental factors on that day. From the statistical analyses among the different parameters measured, the following complex relation system can be suggested, influencing rooting (Figure 7.):

The daily mean temperature and Cold Units derived from it have an effect on almost all examined parameters. Leaf fall is correlated to rooting, but strongly related to phenolics and PPO-activity. These three factors have big influence together on rooting. Water content has only a limiting role, but related to this PPO-phenolics system. Sprouting is a determining parameter, supposedly acting through POD enzyme activity.

References

- 1. Bassuk N.L. and Howard B.H. (1981) A positive correlation between endogenous root-inducing cofactor acivity in vacuum-extracted sap and seasonal changes in rooting of M26 winter apple cuttings. Journal of Horticultural Science, 56 (4), 301-312.
- 2. Bassuk N.L., Hunter L.D. and Howard B.H. (1981) The apparent involvement of polyphenol oxydase and phloridzin in the production of apple rooting co-factors. Journal of Horticultural Science, 56 (4), 313-322.
- 3. Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248-257.
- 4. Demol P. (2001): Oral communication.
- 5. Gur A., Gad A.E. and Haas E. (1988) Rooting of apple rootstock clones as related to phenols and their oxdation. Acta Horticulturae, 227, 160-166.
- 6. Guskov A.V., Tikhominov I.A. and Polikarpova F.Ya. (1988) Peroxidase activity in clonal apple rootstocks differing in capacity for rhisogenesis. Fiziologija-Rastenii, 35 (5), 945-954.
- Haissig B.E. (1986): Metabolic processes in adventitious rooting of cuttings. In: JACKSON M.B. (Ed.) New root formation in plants and cuttings. Dordrecht, Martinus Nijhoff Publishers, 141-189
- 8. Hartmann H.T. et al. (1997) Plant propagation. New Jersey: Prentice-Hall, 770.
- 9. Kracikova M. (1996) Selection of plum rootstocks for economic propagation by hardwood cuttings. Vedecke Prace Ovocnarske. 15, 41-49.
- 10. Loreti F., Morini S. and Grilli A. (1985) Rooting response of 'P.S. B2' and 'GF 677' rootstock cuttings. Acta Horticulturae, 173, 261-269.
- 11. Richardson E.A., Seeley S.D. and Walker D.R. (1974) Model for estimating the completion of rest for "Redhaven" and "Elberta" peach tres. Horticultural Science, 9 (4), 331-332.
- 12. Ryugo K. and Breen P.J. (1974) Indoleacetic acid metabolism in cuttings of plum (Prunus cerasifera x Prunus munsoniana cv. Marianna 2624). J. Amer. Soc. Hort. Sci., 99, 274-251.
- 13. Singleton V.L. and Rossi J.A. (1965) Colorymetry of total phenolics with phosphomolytdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16, 144-158.
- 14. Srivastava S.K., Vashi D.J. and Naik B.I. (1983) Control of senescence by poliamines and guanidines in young and mature barley leaves. Biochemistry, 22 (10), 2151-2154.
- 15. Uzunov A. (1987) Effect of growth regulator treatment on GF655/2 rootstock propagation by hardwood cuttings. Rastenievdni Nauki, 24 (7), 62-66.