University of Ankara
Publications of Faculty of Agriculture : 1074

Scientific Research Reports : 577

ABSCISIC ACID AND ROOTING INHIBITORS IN GRAPEVINE CUTTINGS

Assoc. Prof. Dr. Hasan ÇELİK
University of Ankara
Faculty of Agriculture
Department of Horticulture

ANKARA
1988

CONTENTS

A. Ü. Ziraat Fakültesi Ofset Basım Ünitesi ANKARA - 1988

I. INTRODUCTION

It has been known for quite a while that difficult-to-root vine cuttings contain endogenous rooting inhibitor(s) which may be removed by leaching (Spiegel 1954, Saraswat 1973, Chapman 1976).

The nature of these inhibitors has not been established so far, except for few studies which indicated the presence of "growth inhibitors" in various bioassays (Spiegel 1954, Tizio et al. 1968, Kracke et al. 1981). The relationships of the rooting inhibitors with ABA also have not been examined so far, although the presence of ABA in grapevine buds and seeds was investigated with respect to dormancy (Iwasaki and Weaver 1977, Emmerson and Powell 1978, Broquedis et Bouard 1985), and no data on the removal of ABA by leaching or on the effect of exogenous ABA, are available.

The role of ABA in adventitious root formation appears to vary in different plant systems, both with respect to the effect of exogenous treatments, and to changes in endogenous ABA. Thus, several ABA concentrations have been found to promote root formation in mung bean and pea cuttings, while in several other cases ABA treatments were inhibitory (Chin et al. 1969, Basu et al. 1970, Batten and Goodwin 1978). Only limited information is available as to changes in endogenous ABA in cuttings (Batten and Goodwin 1978), and as to the adequacy of the mung bean rooting bioassay for detecting the presence of rooting inhibitors from other plant species.

In the present study, the hypothesis that ABA may

regulate root formation in cuttings of easy and difficult-to-root grapevine cultivars has been examined by determination of endogenous ABA levels, and through the mung bean rooting bioassay (Heuser and Hess 1972, Blazich and Heuser 1979). The effect of soaking on endogenous ABA content and the effect of exogenous ABA treatments on rooting were compared in various grapevine cuttings.

2. MATERIALS AND METHODS

These experiments were carried out at the Department of Horticulture, Faculty of Agriculture in Rehovot (ISRAEL) in 1980-1981.

2.1. Plant material

Cuttings of two easy-to-root cultivars, 'Perlette' (Vitis vinifera L.) and '1613 C' (Solonis x Othello), and the difficult-to-root cultivar 'Salt Creek' (Vitis champini L.) were used throughout. Dormant canes were collected from mature vines in the Yizra'am Center for distribution of virus-free propagation material, and uniform single bud cuttings, 8-9 mm in diameter, were sampled from the 4th to 11th node position on the cane. Cuttings were used as such, or soaked in aerated tap water, or in 25 or 250 $mg.l^{-1}$ ABA solutions for 48 h at 25°C, in the dark, as mentioned.

2.2. Extraction and ABA determination

Extraction and purification of ABA was carried out according to Beardsell & Cohen (1975). The 'bound' fraction was obtained by incubation of the aqueous residue at pH 11.0, 60°C, for 1 h, followed by reacidification to pH 4.0 with conc. formic acid and

reextraction of the acid fraction with chloroform. Gas-liquid chromatography (GLC) with an electron capture detector was used for determination of ABA as previously described (Goldschmidt et al. 1973).

2.3. Mung bean rooting bioassay

Extraction of vine cuttings, chromatography, and the mung bean rooting bioassay was performed according to Blazich and Heuser (1979), with some modifications. Five g fresh weight of control and water-soaked cuttings of the 3 cultivars were ground, boiled for 1 min in 25 ml absolute methanol, homogenized, filtered, and the residue extracted 2 more times in methanol, overnight and for 1 h. All extracts were combined, centrifuged, filtered, and the clear extract dried at 45°C, dissolved in 80% v/v ethanol, and streaked on 5 cm strips of 3 MM Whatman paper. Descending chromatography in isopropanol:water (8:2, v/v) was performed to a distance of 30 cm. The chromatograms were divided into ten 3 cm sections which were placed in glass vials with 10 ml autoclaved distilled water containing $5x10^{-6}$M IBA and 5 ppm boric acid. Mung bean (Viga radiata (L.) Wilczek) seeds were surface-sterilized with calcium hypochrolite, rinsed, soaked in running tap water for 18 h, and sown in vermiculite. The seedlings were grown for 8 to 9 days at 27 ± 1°C with a 16 h photoperiod of cool-white fluorescent light at ca. 4000 lux, and were then cut 3 cm below the cotyledonary node. Five cuttings were introduced into a vial with the appropriate test solution for 18-24 h (under the same temperature and light regime) until most of the solution was taken-up, autoclaved water was then added, and the roots were counted after 5 additional days. Three such replicates were used throughout. In some experiments, as mentioned, mung bean

cuttings were placed in solutions which contained only 5 ppm boric acid and different combinations of IBA and ABA.

2.4. Rooting experiments

Single-node cuttings of the 3 cultivars were soaked in tap water of ABA solutions, as mentioned, and planted, 5x7 cm apart, in a peat:volcanic scoria, 1:1 (v/v), medium with bottom heating at 25 ± 1°C. The cuttings were grown in the greenhouse for 2 months after which the rooting percentage, the number of main roots formed, and the total dry weight of the roots were determined. In addition, bud break was recorded at 2 day intervals during the entire rooting period. Three replicates of 21 cuttings each were used for all treatments.

3. RESULTS

3.1. ABA in grapevine cuttings

Control cuttings, which have not been soaked, contained considerable amounts of ABA (Table 1), and ABA concentration was highest in the difficult-to root Salt-Creek. Soaking for 48 h in water reduced endogenous ABA content of Perlette cuttings, while little or no difference was found in the other two cultivars. The soaking solution contained measurable amounts of ABA which, however, could not fully account for the differences between the ABA content of control and soaked cuttings.

3.2. Mung bean rooting bioassay

Comparisons of the biological activity of extracts from control (non-soaked) and soaked cuttings of the 3 grapevine cultivars in the mung bean rooting bioassay are presented in Fig.1.

Considerable differences in rooting promoters of all three varieties are evident, while an inhibitory zone could be detected in Salt-Creek cuttings only. This inhibitory zone was not washed out by soaking. Soaking, however, partially removed several promoters but emphasized the presence of a promoter at Rf 0.8-1.0 in '1613 C' cuttings. In no case did an inhibitory zone correspond to the Rf of synthetic ABA marker, whereas some promoting zones coincided with authentic ABA.

The sensitivity of the mung bean bioassay to ABA was further studied by evaluating the effect of IBA combinations on root formation (Table 2). Whereas 10 $mg.l^{-1}$ ABA slightly promoted root formation, 100 $mg.l^{-1}$ was inhibitory. Only this high ABA concentration counteracted the IBA-induced root formation.

3.3. Rooting experiments

Rooting experiments with 3 Vitis cultivars revealed that soaking of cuttings indeed resulted in better root formation, as judged by the number and dry weight of roots, while ABA treatments inhibited it, particularly at 250 $mg.l^{-1}$ (Table 2, Fig.1). Similarly, bud burst was inhibited by the high ABA concentrations and promoted by soaking of cuttings. It should be mentioned, however, that 100% rooting of cuttings was observed in all cases at the end of the rather long rooting period (2 months).

4. DISCUSSION

Soaking of grapevine cuttings for 48 h in tap water resulted in improved rooting in the present study (Fig.1, Table 3), confirming previous reports (Spiegel 1954, Saraswat 1973, Chapman 1976). Indeed, soaking reduced endogenous ABA content, and measurable amounts of ABA were released into the soaking medium (Table 1).

There appears to be some discrepency, however, between the small amounts of ABA found in the soaking solution and the effectiveness of the soaking treatment with regard to rooting. This may be related to incomplete leaching of ABA from the cuttings, or to rapid breakdown of the released ABA in the soaking solution (Manos and Goldthwaite, 1975). Whereas soaking and ABA treatments did not affect the rooting percentage, possibly due to the rather long rooting period, both the growth of adventitious roots (dry wt) and bud burst were affected by soaking and ABA treatments in the 3 cultivars (Table 3). The contribution of buds to adventitious root formation, presumably via supply of rooting promoters and/or inhibitors, is well established (Roberts and Fuchigami 1973, Hartmann and Kester 1983), though the present experiments do not allow to conclude whether there is a direct effect of soaking and ABA treatment on root formation or it is an indirect one, via bud activity. It is interesting to note that in the ABA-treated cuttings, roots were restricted to the area beneath the bud, in contrast with the profuse root formation in control cuttings (data not shown). The presence of ABA in vine cuttings and its removal by soaking (Table 1) could not be detected in the standard mung bean rooting bioassay (Fig.1). The inadequacy of the mung bean bioassay in revealing inhibition by ABA from the grapevine extracts may be explained by the fact that root formation in mung bean cuttings is not inhibited by low ABA concentrations (Table 2). In fact, 10 $mg.l^{-1}$ of ABA slightly promoted rooting, similar to other findings (Basu et al., 1970, Chin et al., 1969) and the same ABA concentration was ineffective also in counteracting the IBA-induced root formations for the mung bean and grapevine cuttings, or a different response to rooting inhibitors others than ABA. Although the mung bean test is considered a standard rooting bioassay it is not

Table 1 : ABA content of non-soaked and soaked grapevine cuttings and of the leachate. Cuttings were soaked in tap water for 48 h, and cis ABA (free and bound) was determined in extracts of the cuttings and in the leachate. Data are average of 5 separate cuttings (non-soaked and soaked cuttings) and 3 separate leachates.

Variety	Cis ABA ($ng.g^{-1}$fw)		
	Non-soaked cuttings	Soaked cuttings	Leachate
Perlette	0.37	0.19	0.013
1613 C	0.20	0.17	0.017
Salt Creek	0.85	0.85	n.d.[1]

[1] not determined

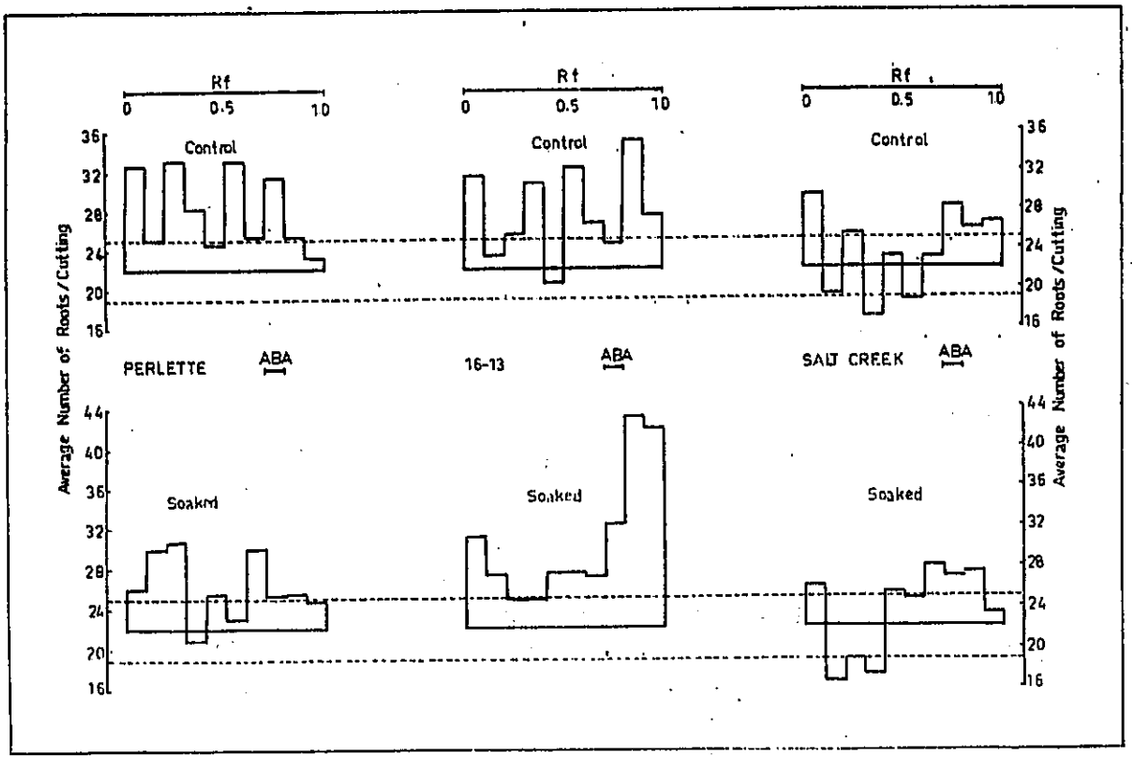

Fig.1 Biological activity of extracts from non-soaked and soked cuttings of 3 grapevine cultivars, in the mung bean rooting bioassay. Extraction and chromatography is described under Materials and Methods. The position of synthetic ABA marker and the standard error of the mean (broken lines) is indicated.

Table 2 : Effect of combination of IBA and ABA on the number of roots per cutting in the Mung bean rooting bioassay. Data are average of 3 separate experiments, 5 cuttings per treatments in each, and the standard error of the mean is indicated.

	ABA ($mg.l^{-1}$)		
IBA ($mg.l^{-1}$)	0	10	100
0	7.2 ± 0.9	11.0 ± 0.7	2.2 ± 0.3
0.5	32.0 ± 1.5	36.1 ± 3.2	8.1 ± 1.6
1.0	42.2 ± 1.7	34.6 ± 5.7	3.9 ± 1.5

Table 3 : The effect of water-soaking and ABA treatments of grapevine cuttings on rooting and bud burst parameters. Cuttings of 3 different grapevine cultivars were soaked in tap water for 48 h, or treated with ABA for 48 h (basal application), and rooted as described under Materials and Methods. Data are average of 3 replicates of 21 cuttings each ± standart eror, per cutting. Bud burst index: the average number of days to 50% bud burst.

Treatment	Perlette			1613 C			Salt Creek		
	No.main roots	Root dry wt(mg)	Bud burst index	No.main roots	Root dry wt(mg)	Bud burst index	No.main roots	Root dry wt(mg)	Bud burst index
Control	11.3± 0.7	59± 6	34	6.5± 0.4	54± 7	32	8.0± 1.1	89± 4	15
Soaking	15.5± 1.3	87± 5	30	7.5± 0.3	50± 4	30	11.3± 0.4	100± 7	16
ABA,25 mg.l^{-1}	13.1± 0.7	78± 3	32	2.8± 0.2	26± 7	45	9.3± 0.5	107± 6	15
ABA,250 mg.l^{-1}	7.3± 0.3	38± 7	40	3.1± 0.4	18± 5	48	5.9± 0.4	53±14	22

surprising that various plant species should respond differently with respect to adventitious root formation. The different response of the mung bean and grapevine cuttings to ABA, and possibly also to other rooting inhibitors and promoters, should be taken into account in further studies.

5. SUMMARY

Water-soaking of dormant cuttings slightly improved root formation in 3 different grapevine cultivars (Perlette, 1613 C, Salt Creek) and measurable amounts of ABA (0.013-0.017 ng.g^{-1} fresh wt) were found in the soaking solution. Considerable levels of ABA (0.17-0.85 ng.g^{-1} fresh wt) were retained, however, in the cuttings even after soaking. The difficult-to-root Salt Creek had the highest ABA content. Soaking of cuttings in 250 mg.l^{-1} synthetic ABA inhibited root formation, budburst, and shoot growth in all three varieties.

The presence of ABA in grapevine cuttings was not revealed in the mung bean rooting bioassay and only one inhibitory zone, which did not correspond to ABA, was found in extracts of Salt Creek cuttings. Synthetic ABA inhibited control as well as IBA-induced root formation of mung bean cuttings only at the high concentration (100 mg.l^{-1}).

The adequacy of the mung bean rooting bioassay for detection of rooting inhibitors and promoters in grapevine cuttings is discussed.

6. ÖZET

Asma çeliklerinde köklenme ile ABA arasındaki ilişkilerin araştırıldığı bu çalışmadan elde edilen bulgulara göre, suda bırakma uygulaması (48 saat) üzerinde çalışılan asma çeşitlerinde (Perlette, 1613 C, Salt Creek) kök oluşumunu ve gelişmesini olumlu yönde etkilemiş, ayrıca uygulamadan sonra ıslatma suyu içinde önemli sayılabilecek miktarlarda (0.013-0.017 $ng.g^{-1}$ tane ağırlık) ABA saptanmıştır.

Diğer yandan, suda bırakma uygulamasından sonra bile asma çeliklerinde yüksek düzeylerde ABA (0.17-0.85 $ng.g^{-1}$ taze ağırlık) bulunduğu belirlenmiştir. Zor köklenen Salt Creek çeliklerinin ABA kapsamı, çelikleri kolay köklenen diğer iki çeşite göre çok daha yüksek bulunmuştur. 250 $mg.l^{-1}$ sentetik ABA, uygulanan her üç çeşitte de kök oluşumu, sürme ve sürgün gelişmesini engellemiştir.

Mung fasülyesi biyolojik testi ile sadece Salt Creek çeliklerinden elde edilen ekstraklarda,ABA ile çakışmayan,yalnızca bir engelleyici bölge belirlenebilmiştir.

Sentetik ABA'in uygulanan en yüksek dozu (100 $mg.l^{-1}$) hem kontrol hem de IBA uygulanmış Mung fasülyesi çeliklerinde, kök oluşumunu engellemiştir. Bu bulgulara göre Mung fasülyesi testinin, asma çeliklerindeki köklenmeyi uyarıcı ve engelleyicilerin teşhisinde duyarlı bir yöntem olduğunu söylemek oldukça zordur.

ACKNOWLEDGEMENT

I am always grateful to Prof.Dr.Arie Altman, Prof.Dr. Eliezer E.Goldschmidt,Prof.Dr, Ben Ami Bravdo and Prof.Dr,Shaul P. Monselise at the Department of Horticulture, Faculty of Agriculture in Rehovot (ISRAEL) for their invaluable supervisions, supports and discussions.

7. REFERENCES

Basu,R.N., Roy,B.N. and Base,T.K. 1970. Interaction of abscisic acid and auxins in rooting of cuttings. - Plant & Cell Physiol. 11: 681-684.

Batten,D.J. and Goodwin, P.B. 1978. Phytohormones and the induction of adventitious roots. In: Letham, D.S. et al., eds. Phytohormones and Related Compounds - A Comprehensive Treatise, Vol. II pp. 137-145.

Beardsell,M.F. and Cohen,D. 1975. Relationships between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. - Plant Physiol. 56: 207-212.

Blazich,F.A. and Heuser,C.W. 1979. The mung bean rooting bioassay: a re-examination. - J.Amer.Soc.Hort.Sci. 104: 117-120 .

Broquedis,M. et Bouard,J. 1985. Influence de l'incision annularie sur la qulité des pépins de raisin: Conséquenses sur leurs teneurs en acide abscissique et leurs possibilités de germination. Connaissance de la Vigne et du Vin 19: 137-147.

Chapman,A.P.A. 1976. A method for rooting Salt Creek and Dog Ridge grapevine cuttings. - Agric. Records. 3: 24-25.

Chin, T.Y., Meyer,M.M. and Beevers, L. 1969. Abscisic acid stimulated rooting of stem cuttings.-Planta 88: 192-196.

Emmerson,J.G. and Powell, L.E. 1978. Endogenous abscisic acid in relation to rest and bud burst in three Vitis species. J.Amer. Soc.Hort.Sci. 103: 677-680.

Goldschmidt,E.E.,Goren,R.,Even-Chen,Z. and Bittner,S. 1973. Increase in free and bound abscisic acid during natural and ethylene-induced senescence of citrus fruit peel.- Plant Physiol. 51: 879-882.

Hartmann,H.T. and Kester,D.E. 1983. Plant Propagation. Prentice-Hall, Inc., N.J. ISBN 0-13-68 1007-1.

Heuser,S.W. and Hess,C.E. 1972. Endogenous regulators of root initiation in mung bean hypocotyls. - J. Amer.Soc.Hort. Sci. 97: 392-396.

Iwasaki,K. and Weaver,R.J. 1977. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting, and inhibitor content of buds of "Zinfandel" grape (Vitis vinifera L.). - J.Amer.Soc.Hort.Sci. 102: 584-587.

Kracke,H., Cristoferi,G. and Marangoni,B. 1981. Hormonal changes during the rooting of hardwood cuttings of vinegrape rootstocks. - Amer. J.Enol. Viticulture 32: 153-157.

Manos,P.J. and Goldthwaite, J. 1975. A kinetic analysis of the effects of gibberellic acid, zeatin and abscisic acid on leaf tissue senescence in Rumex. - Plant Physiol. 55: 192-198.

Roberts, A.N. and Fuchigami,L.H. 1973. Seasonal changes in auxin effect on rooting of Douglas-fir stem cuttings as related to bud activity. - Physiol.Plant. 28: 215-221.

Saraswat,K.B. 1973. Studies on the effect of time of planting, soaking in water and precallusing on rooting capacity of grapevine cuttings. - Prog.Hortic. 5: 57-65.

Spiegel,P. 1954. Auxin and inhibitors in canes of Vitis. - Bull Res. Coun. Israel, 4: 176-183.

Tizio,R., Moyano,J.S. and Morales,H. 1968. Inhibitor-like substances in vine cuttings and their possible relationship to the rooting process. - Phyton, 25: 123-128.