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Istituto di Chimica Agraria, Università di Padova, Italia

# Extraction and preliminary characterization of microsomal ($Mg^{2+}$ + $K^+$)-ATPase activity of grapevine roots

by

Z. VARANINI and A. MAGGIONI

## Extraction et caractérisation préliminaire de l'activité ($Mg^{2+}$ + $K^+$)-ATPasique microsomale des racines de vigne

Résumé: La fraction microsomale extraite des racines de vigne obtenues de boutures possède une activité ATPasique dépendant du $Mg^{2+}$ et stimulée par les cations monovalents. L'addition non seulement de EDTA et DTT mais aussi de BSA, PVPP, choline-Cl, éthanolamine et glycérol-1-P au milieu d'homogénisation est nécessaire pour obtenir les meilleurs résultats de l'extraction.

Quand les microsomes sont extraites selon la procédure d'HODGES et LEONARD (1974), en homogénisant les racines en présence d'EDTA et DTT seulement, on observe l'inhibition de l'activité enzymatique par le $Mg^{2+}$, partiellement allégée par le $K^+$, et des propriétés de phosphatase acide plutôt que d'ATPase.

L'ATPase des microsomes protégés pendant l'homogénisation, montre préférence pour le $Mg^{2+}$ comme cofacteur et une activité optimum à pH 7,0 en présence de $Mg^{2+}$ et $K^+$. Seul le $NH_4^+$ peut substituer le $K^+$ avec une efficacité semblable. La cinétique de la stimulation de l'activité par le $K^+$ est biphasique.

En conclusion, pourvu que les conditions opératives soient telles que l'altération des membranes est ampêchée on peut étudier pour les racines de la vigne les bases biochimiques de l'absorption minérale, qui paraissent être semblables à celles connues pour les plantes annuelles.

Key words: root, cell, enzyme, mineral, magnesium, potassium, additive, acidity, absorption.

## Introduction

Researches on plasma membrane ATPase (ATP phosphohydrolase EC 3.6.1.3) involved in ion movement into cells of higher plants have been carried out mostly using roots or other tissues of annual plants. Results allow to infer that the properties which are peculiar to most plasma membrane ATPases are $Mg^{2+}$ dependence, $K^+$ stimulation, optimal activity at neutral pH (LEONARD and HODGES 1980) and selective inhibition by vanadate (COCUCCI *et al.* 1980). But in microsomes prepared from strawberry (BEN-ARIE and FAUST 1980) and apple fruits (LURIE and BEN-ARIE 1983) inhibition of ATPase activity caused by divalent cations was observed. This was interpreted as a possible expression of the different functioning of enzyme systems which accomplish ion transport. Alternatively, it could be due to changes of enzymes occurring during the process of membrane isolation. Changes of membrane properties and composition caused by phospholipase D and phosphatidyl-phosphatase during the isolation of microsomes were observed and studied (SCHERER and MORRÉ 1978). On the other hand, the importance of the composition of media used for extraction was also widely stressed (GALLIARD 1974).

To the best of our knowledge, there is limited evidence of the presence of $Mg^{2+}$-dependent, $K^+$-stimulated ATPase activity in roots of trees (DOUGLAS and WALKER 1984), and no published information on grapes. It is important to clarify the biochemical basis of ion uptake for these species. In fact, studies on titratable acidity and pH of

grape musts led to hypothesize the presence in grapevines of ATPases able to promote ion fluxes of monovalent cations and hydrogen ions (BOULTON 1980). Moreover, it was shown that roots of entire grape plants are able to extrude hydrogen ions actively and it was hypothesized that ATPases located in the plasma membrane are involved in this process (MENGEL and MALISSIOVAS 1982).

Our preliminary researches showed that the microsomal fraction of grape roots possess ATPase activity which was inhibited by divalent cations. In this paper we show that such inhibition occurs with microsomal preparations inadequately protected during isolation. Furthermore, some properties of ($Mg^{2+} + K^{+}$)-ATPase of microsomes from grape roots are reported.

## Materials and methods

### Plant material

Roots were obtained from single-node woody cuttings of grapevine (*Vitis vinifera* L. cv. Verduzzo trevigiano) by the method already reported (MAGGIONI 1980).

### Isolation of microsomal fraction

Roots (15—20 g f.w.) were washed in cold distilled water and homogenized with mortar and pestle as described by HODGES and LEONARD (1974). Three different media were used: M1 contained 0.25 M sucrose, 3 mM EDTA, 25 mM Tris-MES (pH 7.2) and 8 mM dithiotreitol (DTT) (HODGES and LEONARD 1974); M2 was prepared by adding 3.5 mg bovine serum albumin (BSA) and 350 mg polyvinyl-polypyrrolidone (PVPP) per gram fresh weight to M1; M3 was prepared by further addition to M2 of 4 % (w/v) choline-Cl, 4 % (v/v) ethanolamine and 10 mM glycerol-1-P, as described by SCHERER and MORRÉ (1978), lowering pH to 7.2 with HCl. Root homogenates were squeezed through

Table 1

Microsomal protein and ATPase activity from grapevine roots in representative experiments carried out using extraction media containing different sets of additives · Additions in M1: EDTA, DTT; in M2: EDTA, DTT, PVPP and BSA; in M3: EDTA, DTT, PVPP, BSA, choline-Cl, ethanolamine and glycerol-1-P · Reaction mixture for enzyme assay contained 33 mM Tris-MES pH 6.5, 3 mM ATP-Tris and, when added, 3 mM $MgSO_4$ and 50 mM KCl · Reaction time was 30 min, temperature 38 °C

Protéine microsomale et activité ATPasique des racines de vigne dans des expériences représentatives effectuées en utilisant des milieux d'extraction qui contenaient différents agents protectifs · Les additions dans le M1 étaient: EDTA, DTT; dans le M2: EDTA, DTT, PVPP, BSA; dans le M3: EDTA, DTT, PVPP, BSA, choline-Cl, éthanolamine, glycerol-1-P · Milieu d'incubation: tampon Tris-MES 33 mM pH 6,5; ATP-Tris 3 mM et, lorsque ajoutés, $MgSO_4$ 3 mM et KCl 50 mM · Incubation de 30 min à une température de 38 °C

| Homogenizing media | Microsomal protein (μg/g f.w.) | ATPase specific activity of microsomes (μmol Pi/mg protein · h) in the presence of | | |
|---|---|---|---|---|
| | | — | 3 mM $Mg^{2+}$ | 3 mM $Mg^{2+}$ + 50 mM $K^{+}$ |
| M1 | 57.1 | 3.0 | 2.6 | 2.8 |
| M2 | 73.8 | 3.6 | 7.6 | 11.8 |
| M3 | 91.9 | 4.7 | 11.3 | 13.3 |

four layers of cheesecloth and centrifuged for 5 min at 1,000 *g*. The supernatant was subsequently treated according to procedure outlined by HODGES and LEONARD (1974) to obtain the microsomal fraction as a pellet at 82,500 *g* which was resuspended with 18 % (w/v) sucrose and used for enzyme assays.

### Enzyme determinations

Assays were run by adding 0.1 ml of microsomal suspension (25—50 µg protein) to a mixture containing 3 mM ATP (Tris-salt), or other substrates when specified, at the desired pH, 33 mM buffer solution (Tris-MES at pH 5.0—8.5, Tris-HEPES at pH 9.0), 3 mM $MgSO_4$ when required, and 50 mM KCl unless differently specified, in a final volume of 1 ml. Reaction time was 30 min at 38 °C, the reaction being stopped by adding 2 ml of 1 % ammonium molybdate in 2 N sulfuric acid. Inorganic phosphate (Pi) was determined after addition of 0.4 ml of reducing solution according to FISKE and SUBBAROW (1925). Trichloroacetic acid precipitable protein was determined by the method of LOWRY *et al.* (1951) using BSA as a standard.

## Results

### Isolation procedures and ATPase activity

ATPase activity of microsomal fraction extracted from grape roots according to the unmodified procedure of HODGES and LEONARD (1974) (medium M1) was, in different experiments, 3—4 µmol Pi/mg protein · h. It was inhibited about 10—25 % by $Mg^{2+}$ and addition of 50 mM $K^+$ unevenly and partially relieved the inhibition. Results of representative experiments are summarized in Table 1.

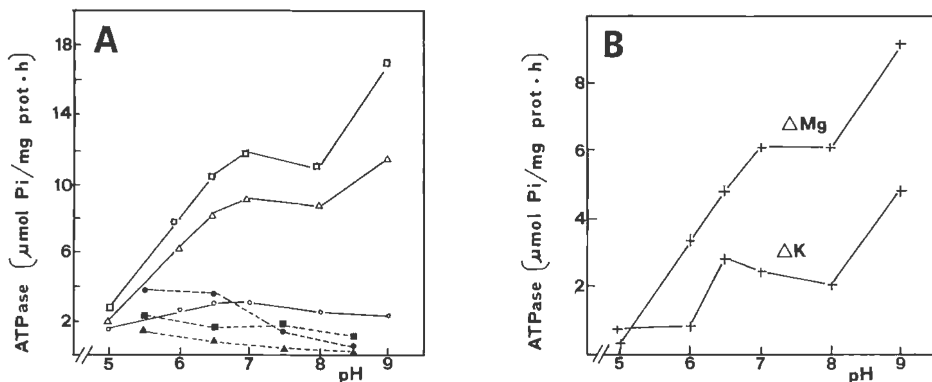


Fig. 1: A) Effect of assay pH on ATPase activity of grapevine microsomes. Microsomes extracted with M1 (full symbols) and with M3 (open symbols) were assayed in the absence of cations (● and ○), with 3 mM $Mg^{2+}$ (▲ and △) and with 3 mM $Mg^{2+}$ plus 50 mM $K^+$ (■ and □). For details see 'Materials and methods'. — B) Effect of assay pH on $Mg^{2+}$-dependent (Δ Mg) and $K^+$-stimulated (Δ K) ATPase activities extracted with M3 medium.

A) Effet du pH sur l'activité ATPasique de microsomes de vigne. Les microsomes extraits avec le M1 (symboles pleins) et M3 (symboles ouverts) étaient éssayés sans cations (● et ○), avec $Mg^{2+}$ 3 mM (▲ et △) et avec $Mg^{2+}$ 3 mM plus $K^+$ 50 mM (■ et □). Pour les autres détails voir «Matériel et méthodes». — B) Effet du pH sur les activités ATPasique dépendant du $Mg^{2+}$ (Δ Mg) et stimulées par $K^+$ (Δ K) extraites avec le milieu M3.

When BSA and PVPP were present in the homogenizing medium (M2), basal (without added cations) activity was about the same as in previous conditions. However, $Mg^{2+}$ caused a strong increase, approximately doubling specific activity, and 50 mM KCl caused further stimulation so that enzyme activity reached figures of about 11 μmol Pi/mg protein · h.

Furthermore, when choline-Cl, ethanolamine and glycerol-1-P were added (medium M3), the positive effects of $Mg^{2+}$ and $K^+$ were fully confirmed and higher values of specific activity could be reached. The protein content of microsomal fractions extracted by this method was higher in comparison with both previous procedures. Therefore, total ($Mg^{2+}+K^+$)-ATPase activity extracted per gram of root tissue was markedly higher since both protein content and specific activity increased.

To better clarify the different properties of enzyme preparations extracted in the absence (M1 medium) and in the presence (M3 medium) of preservative substances in the homogenizing medium, the two preparations were further studied with respect to pH effect and substrate specificity.

### Effects of pH

Fig. 1 A shows changes as a function of pH of enzyme activities isolated with media M1 and M3. In the first case and in the absence of cations, higher activity levels were reached at pH 5.5. However, activity peaks were absent. In the presence of $Mg^{2+}$ the curve was similar, though at a lower level. $K^+$ did not modify this pattern, only partially relieving the inhibition. When activity was assayed on the microsomal fraction isolated with medium M3, a slightly higher activity zone could be detected at pH 6.5—7.0 in the absence of cations, at a level which is similar to that of the previous preparate. In the presence of $Mg^{2+}$ and $Mg^{2+}$ plus $K^+$ a peak of activity at pH 7.0 became evident together with another raise of activity at higher pH. It is noteworthy that the effect caused by $K^+$ was also pH-dependent (Fig. 1 B), with maximum stimulation at pH 6.5 and a further increase at pH 9.0.

Table 2

Comparison of total (inorganic phosphate released per gram f.w. of root tissue) microsomal phosphohydrolase activities at pH 6.5 on different substrates for microsomal preparations extracted by homogenizing in the presence of EDTA and DTT (M1) or with further addition of PVPP, BSA, choline-Cl, ethanolamine and glycerol-1-P (M3) · Data shown as % of total ($Mg^{2+}$ + $K^+$)-ATPase activity extracted with M3

Comparaison des activités phosphohydrolasiques microsomales totales (phosphate inorganique délivré par gramme p.f. de racine) à pH 6,5 en présence des différents substrats · Les microsomes étaient extraits en homogénisant en présence d'EDTA et DTT seulement (M1), ou avec l'addition de PVPP, BSA, choline-Cl, éthanolamine et glycérol-1-P (M3) · Les résultats sont exprimés en % de l'activité ($Mg^{2+}$ + $K^+$)-ATPasique extraite avec M3

| Homogenizing media | Additions | Substrates | | | |
|---|---|---|---|---|---|
| | | ATP | ADP | AMP | PNP |
| M1 | $Mg^{2+}$ | 29 | 34 | 22 | 83 |
| | $Mg^{2+}$ + $K^+$ | 29 | 37 | 22 | 91 |
| M3 | $Mg^{2+}$ | 76 | 47 | 42 | 61 |
| | $Mg^{2+}$ + $K^+$ | 100 | 47 | 44 | 61 |

Table 3

Relative ability of 3 mM divalent cations to substitute for $Mg^{2+}$ as ATPase cofactor and relative capacity of 50 mM $K^+$ to stimulate ATPase activity over the level reached with the divalent cation · Data are expressed as % of controls with $Mg^{2+}$ and $K^+$, respectively

Capacité relative des cations divalents (3 mM) à remplacer le $Mg^{2+}$ comme cofacteur de l'ATPase et du $K^+$ (50 mM) à stimuler l'activité ATPasique au-dessus du niveau atteint avec le cation divalent · Les résultats sont indiqués en % des témoins en présence de $Mg^{2+}$ et de $K^+$, respectivement

| | Relative ATPase activity | |
|---|---|---|
| | Divalent ion dependent | KCl stimulated |
| Mg | 100 | 100 |
| Mn | 71 | 84 |
| Ca | 11 | 16 |
| Zn | 0 | 18 |

### Substrate specificity

Enzyme isolated by the two procedures was studied by comparing reaction rates with adenosine mono-, di- and triphosphate as substrates to assess the specificity as a triphosphatase, and with p-nitrophenyl phosphate (PNP) at pH 6.5 to ascertain the level of phosphatase activity. Table 2 shows that with membrane preparations obtained through homogenization without preservative compounds the preferred substrate was PNP followed by ADP and then by ATP, AMP being the last one in the order of preference. Activity with ATP in the presence of $Mg^{2+}$ and $K^+$ represented only 32 % of the level obtained with PNP. When microsomes were isolated with added preservatives, the preferred substrate was ATP both in the presence and in the absence of $K^+$, followed by PNP at a level representing 61 % of activity with ATP, and then by ADP and AMP. It is important to note that with this preparation $K^+$ caused stimulation of enzyme activity only when ATP was used as a substrate. This shows a sharp specificity as a triphosphatase of that part of activity which is $K^+$-stimulated.

Table 4

Relative efficiency of monovalent ions to stimulate ATPase activity over the level reached in the presence of $Mg^{2+}$

Capacité relative des ions monovalents de stimuler l'activité ATPasique au-dessus du niveau en présence de $Mg^{2+}$

| Monovalent ion 50 mM | Ion stimulated ATPase % |
|---|---|
| K | 100 |
| $NH_4$ | 111 |
| Na | 85 |
| Rb | 80 |
| Li | 59 |
| Cs | 49 |

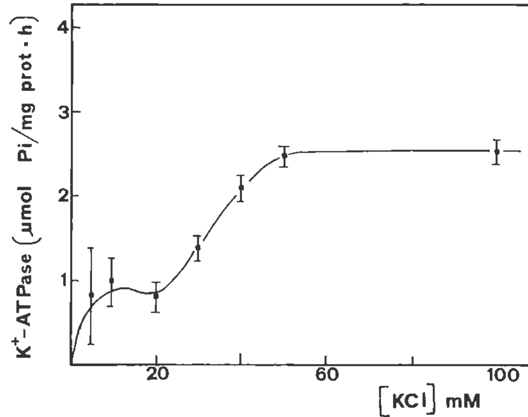


Fig. 2: Effect of KCl concentration on $K^+$-stimulated ATPase activity of grapevine microsomes. Standard errors of the means of two experiments, each run in triplicate, are indicated as vertical bars.

Effet de la concentration du KCl sur l'activité ATPasique stimulée par $K^+$ des microsomes de la vigne. Les barres verticales représentent l'erreur standard de la moyenne de deux expériences, chacune avec trois répliques.

### Effect of cations

Ion requirements of ATPase activity extracted with all preservative compounds present in the homogenizing medium were further studied by assaying the effects of different cations both divalent added as sulfates and monovalent given as chlorides. Table 3 shows that $Mg^{2+}$ had the highest capacity of stimulating ATPase activity over the basal level, followed by $Mn^{2+}$. In the presence of $Ca^{2+}$, stimulation was very low and $Zn^{2+}$ caused no stimulation over basal activity. Strictly and positively correlated with the effect of divalent cations was the capacity of $K^+$ to stimulate further activity.

All monovalent cations tested in the presence of $Mg^{2+}$ showed stimulative capacity (Table 4). $NH_4^+$, $Na^+$ and $Rb^+$ cause stimulation at levels similar to $K^+$, while $Li^+$ and $Cs^+$ ions were less effective.

### Kinetics of $K^+$ stimulation

The capacity of $K^+$ to stimulate ATPase activity over the level reached in the presence of $Mg^{2+}$ was tested at $K^+$ concentration ranging from 5 to 100 mM. Results in Fig. 2 show that a biphasic pattern described the effect of $K^+$ on the enzyme with a first step reaching its maximum at 10—20 mM $K^+$ and a second phase of stimulation with a maximum effect at 50 mM $K^+$, without further increase at higher concentrations.

## Discussion

Alterations of membranes caused by hydrolytic and oxidative enzymes during isolation were prevented in most cases by using chelating (EDTA and EGTA) and antioxidative (DTT) agents (LEONARD and HODGES 1980) and plasma membranes with high levels of $Mg^{2+}$-dependent, $K^+$-stimulated ATPase activity were isolated from roots of

oat (LEONARD and HODGES 1973), barley (NAGAHASHI *et al.* 1978), corn (LEONARD and HOTCHKISS 1976), soybean (HENDRIX and KENNEDY 1977), and from germinating seeds of radish (COCUCCI and BALLARIN-DENTI 1981). However, the same procedure applied to strawberry (BEN-ARIE and FAUST 1980) and apple (LURIE and BEN-ARIE 1983) resulted in membrane ATPases inhibited by divalent cations. Our results with grapevine roots from woody cuttings show that the isolation of microsomal $Mg^{2+}$-dependent, $K^+$-stimulated ATPase activity becomes possible by homogenizing not only in the presence of EDTA plus DTT but also of PVPP, BSA, choline-Cl, ethanolamine, glycerol-1-P.

The main function of PVPP and BSA is to protect enzymes and membrane integrity against phenolic compounds and free fatty acids of hydrolytic origin, respectively (LOOMIS and BATTAILE 1966; GALLIARD 1974). Other additives used here should prevent hydrolases from using membrane phospholipids as substrates (SCHERER and MORRÉ 1978). Therefore, our results indicate that the extraction of active membrane-bound cation-stimulated ATPase can be achieved by protecting membranes firstly from phenolics and free fatty acids. In addition, the inhibition of hydrolytic activities allowed to increase yields of both microsomal protein and total membrane-bound enzyme activity. Additional results, here not reported, suggest that PVPP more than BSA is important in preventing negative effects on membrane proteins. This agrees with observation by electron microscopy (N. RASCIO, personal communication) that phenolic cells are present both in endodermis and parenchyma of grapevine roots from woody cuttings.

As to the properties of microsomes extracted with M1 medium (without preservative compounds), the effect of pH and the preferred substrates indicate that the enzyme system behaves mainly as an acid phosphatase rather than as an ATPase. The inhibition observed with $Mg^{2+}$-ATP as a substrate confirms this inference. In fact, similar inhibitions were observed with microsomes obtained from other plant tissues and were shown to be due to the presence of acidic phosphatases (RUNGIE and WISKICH 1973; D'AUZAC 1975). Therefore, the lack of membrane protection during isolation may cause changes or disappearance of membrane-bound enzyme activities. In the case of ATPases presumed to be involved in ion transport, this could lead to the conclusion that different mechanisms work in tissues of different plants.

The characteristics of ATPase activity associated with microsomes extracted from grapevine roots, supposed to be representative of plasmalemma ATPase, are similar to those shown by both monocotyledon and dicotyledon annual plants (LEONARD and HODGES 1973; LEONARD and HOTCHKISS 1976; HENDRIX and KENNEDY 1977; NAGAHASHI *et al.* 1978; COCUCCI and BALLARIN-DENTI 1981; BRISKIN and POOLE 1983). In fact, enzyme activity requires $Mg^{2+}$ rather than other divalent cations, is stimulated by $K^+$ and is specific as a triphosphatase. In addition, it shows optimal activity at neutral pH, and $K^+$ stimulation, which is shown only when ATP is used as a substrate, peaks at pH 7. The increase in activity recorded at alkaline pH can be attributed to the presence of other types of membrane in the microsomal fraction, particularly from mitochondria which are known to possess ATPase activity with higher optimum pH (HODGES and LEONARD 1974) and, possibly, from tonoplast (LEIGH and WALKER 1980). To clarify also these aspects researches are in progress to purify plasma membrane and characterize it by using ATPase selective inhibitors.

LEONARD and HODGES (1973) showed that both $K^+$ uptake and $K^+$ stimulation of plasma membrane ATPase were affected by $K^+$ concentration in a way fitting in with the negative cooperativity model of oligomeric enzyme proteins (KOSHLAND 1970). Results obtained by HÅVARSTEIN and NISSEN (1981) showed that multiphasic patterns of $K^+$ stimulation of plasma membrane ATPase paralleled multiphasic kinetics of $K^+$ uptake by roots. Our preparations show a biphasic pattern in the 5—100 mM concen-

tration range. Therefore this result is in accordance more with the second model than with the first one. The jump recorded close to 20 mM $K^+$ occurred at about the same concentration where HÅVARSTEIN and NISSEN (1981) also recorded phase transition. Our kinetics of enzyme stimulation by $K^+$ could not compare with $K^+$ uptake by roots because of the already reported (VARANINI and MAGGIONI 1982; MAGGIONI and VARANINI 1983) unsatisfactory linearity of $K^+$ uptake, which did not allow to obtain surely defined uptake kinetics.

In conclusion, $(Mg^{2+} + K^+)$-ATPase of grapevine root microsomes can be considered an indication of the presence of plasma membrane-bound ATPase accomplishing energy transduction for ion transport, associated with, or being itself, the carrier for ion uptake. The properties of this enzyme associate grapes with the annual plants known for using this molecular device for membrane transport. Finally, our results show that care must be taken in order to prevent changes of membrane integrity. These can lead to the appearance of different enzyme properties, capable of inducing misleading conclusion on mechanism carrying out ion transport.

## Summary

The microsomal fraction extracted from grapevine roots obtained from woody cuttings possesses $Mg^{2+}$-dependent, monovalent cation-stimulated ATPase activity. Addition of BSA, PVPP, choline-Cl, ethanolamine, glycerol-1-P besides EDTA and DTT to the homogenizing medium as preservative compounds was required in order to achieve a successful isolation.

When microsomes were extracted according to HODGES and LEONARD (1974) by homogenizing root tissue in the presence of EDTA and DTT, the enzyme activity exhibited inhibition by $Mg^{2+}$ only partially relieved by $K^+$, and properties of acid phosphatase rather than ATPase.

Cation-stimulated ATPase of microsomes protected during homogenization was further characterized and it showed optimum activity at pH 7.0 in the presence of $Mg^{2+}$ plus $K^+$. It was specific as a triphosphatase and $Mg^{2+}$, rather than other divalent cations, was preferred as a cofactor. As monovalent stimulating cation, only $NH_4^+$ could substitute for $K^+$ with equivalent efficiency. The kinetics of $K^+$ stimulation showed a biphasic pattern.

Biochemical bases of ion uptake by grapevine seem to be similar to those clarified for annual plants.

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## Literature cited

BEN-ARIE, R.; FAUST, M.; 1980: ATPase in ripening strawberries. Phytochemistry **19**, 1631—1636.

BRISKIN, D. P.; POOLE, R. J.; 1983: Characterization of a $K^+$-stimulated adenosine triphosphatase associated with the plasma membrane of red beet. Plant Physiol. **71**, 350—355.

BOULTON, R.; 1980: A hypothesis for the presence, activity and role of potassium/hydrogen, adenosine triphosphatases in grapevines. Amer. J. Enol. Viticult. **31**, 283—287.

COCUCCI, M.; BALLARIN-DENTI, A.; 1981: Effect of polar lipids on ATPase activity of membrane preparations from germinating radish seeds. Plant Physiol. **68**, 377—381.

— — ; — —; MARRE, M. T.; 1980: Effects of orthovanadate on $H^+$ secretion, $K^+$ uptake, electric potential difference and membrane ATPase activities of higher plant tissues. Plant Sci. Lett. **17**, 391—400.

D'AUZAC, J.; 1975: Caractérisation d'une ATPase membranaire en présence d'une phosphatase acide dans les lutoïdes du latex d'*Hevea brasiliensis.* Phytochemistry **14**, 671—675.

DOUGLAS, T. J.; WALKER, R. R.; 1984: Phospholipids, free sterols and adenosine triphosphatase of plasma membrane-enriched preparations from roots of citrus genotypes differing in chloride exclusion ability. Physiol. Plant. **62**, 51—58.

FISKE, C. H.; SUBBAROW, Y.; 1925: The colorimetric determination of phosphorus. J. Biol. Chem. **66**, 375—400.

GALLIARD, T.; 1974: Techniques for overcoming problems of lipolytic enzymes and lipoxygenases in the preparation of plant organelles. Methods Enzymol. **31**, 520—528.

HÅVARSTEIN, L. S.; NISSEN, P.; 1981: Stimulation of plasmalemma adenosine triphosphatase from oat roots by inorganic and organic cations: concentration-dependence, selectivity and sites. Plant Physiol. **68**, 597—604.

HENDRIX, D. L.; KENNEDY, R. M.; 1977: Adenosine triphosphatase from soybean callus and root cells. Plant Physiol. **59**, 264—267.

HODGES, T. K.; LEONARD, R. T.; 1974: Purification of a plasma membrane-bound adenosine triphosphatase from plant roots. Methods Enzymol. **32**, 392—406.

KOSHLAND, D. E. JR.; 1970: The molecular basis for enzyme regulation. In: BOYER, P. D. (Ed.): The Enzymes, Vol. **1**, 3rd Ed., 342—396. Academic Press, New York.

LEIGH, P. A.; WALKER, R. R.; 1980: ATPase and acid phosphatase activities associated with vacuoles isolated from storage roots of red beet (*Beta vulgaris* L.). Planta **150**, 222—229.

LEONARD, R. T.; HODGES, T. K.; 1973: Characterization of plasma membrane-associated adenosine triphosphatase activity of oat roots. Plant Physiol. **52**, 6—12.

— — ; — —; 1980: The Plasma Membrane. In: STUMPF, P. K.; CONN, E. E. (Eds.): The Biochemistry of Plants. A Comprehensive Treatise. Vol. **1**, 163—182. Academic Press, New York.

— — ; HOTCHKISS, C. W.; 1976: Cation-stimulated adenosine triphosphatase activity and cation transport in corn roots. Plant Physiol. **58**, 331—335.

LOOMIS, W. D.; BATTAILE, J.; 1966: Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry **5**, 423—438.

LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L.; RANDALL, R. J.; 1951: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265—275.

LURIE, S.; BEN-ARIE, R.; 1983: Characterization of plasmalemma ATPase from apple fruit. Phytochemistry **22**, 49—52.

MAGGIONI, A.; 1980: Iron absorption by excised grapevine roots. Vitis **19**, 105—112.

— — ; VARANINI, Z.; 1983: Free space binding and uptake of ions by excised roots of grapevine. In: SARIC, M. R.; LOUGHMAN, B. C. (Eds.): Genetic Aspects of Plant Nutrition, 133—137. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Boston, Lancaster.

MENGEL, K.; MALISSIOVAS, N.; 1982: Light dependent proton excretion by roots of entire vine plants (*Vitis vinifera* L.). Z. Pflanzenernähr. Bodenk. **145**, 261—267.

NAGAHASHI, G.; LEONARD, R. T.; THOMSON, W. W.; 1978: Purification of plasma membranes from roots of barley. Plant Physiol. **61**, 993—999.

RUNGIE, J. M.; WISKICH, J. T.; 1973: Salt-stimulated adenosine triphosphatase from smooth microsomes of turnip. Plant Physiol. **51**, 1064—1068.

SCHERER, G. F. E.; MORRÉ, D. J.; 1978: Action and inhibition of endogenous phospholipases during isolation of plant membranes. Plant Physiol. **62**, 933—937.

VARANINI, Z.; MAGGIONI, A.; 1982: Iron reduction and uptake by grapevine roots. J. Plant Nutr. **5**, 521—529.

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Z. VARANINI
A. MAGGIONI
Istituto di Chimica Agraria
Università di Padova
Via Gradenigo, 6
35100 Padova
Italia