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Plasma Membrane H$^+$-ATPase during Embryo Dormancy and Dormancy Release in Horse Chestnut Seeds

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Abstract

Horse chestnut seeds harvested in October were exposed to wet cold stratification for 14 weeks, thus providing a way for seeds to overcome the state of deep physiological dormancy and acquire the ability to germinate. During first 5-6 weeks, a small portion of seeds germinated hardly and slowly while later on seeds germinated more uniformly and rapidly. The experiments with facilitated water supply have shown that 5-6 weeks is the state of embryo dormancy, which did not depend on water inflow, whereas during 6-14 weeks these embryos became capable to germinate, but this capacity was limited by water penetration through seed coat. Two distinct periods were identified in deep dormancy, namely embryo dormancy and coat-imposed dormancy (dormancy release). They differ in plasma membrane H$^+$-ATPase behavior. During embryo dormancy, the the transformation occurred from autoinhibited form preformed during seed maturation to an active form, whereas during dormancy release the activity of enzyme increased up to the levels typical for germinating seeds.

Keywords: Seed; Embryo dormancy; Dormancy release; Seed germination; Plasma membrane H$^+$-ATPase

Introduction

Plasma membrane H$^+$-ATPase is a unique membrane enzyme capable to transport H$^+$ ions from cytoplasm to cell walls by exchanging H$^+$ ions for K$^+$ ions. It can fulfill a key role in seed germination, particularly in the beginning of cell elongation in the axial organs of seed embryo [1]. The mechanism of its action is the following: H$^+$ ions in cell walls cause acidification favoring the cell wall loosening resulting in their high extensibility. Under the pressure of entering water, these cell walls extend that vafors the beginning of cell elongation. This is the way how plasma membrane H$^+$-ATPase participates in seed germination providing H$^+$ ions extrusion prior to seed germination [2].

Our experiments are usually performed with the seeds of horse chestnut *Aesculus hippocastanum* distinguished by their germination occurring only by cell elongation; cell division starts in their roots at the length of 3cm [3]. These seeds easily germinate in warm countries with humid climate [4]. However, in Russia with its cold winter, these seeds after shedding enter deep dormancy to germinate in spring. In this paper, the behavior of plasma membrane H$^+$-ATPase was studied in such dormant and starting germination horse chestnut seeds.

Materials and Methods

Horse chestnut seeds were collected in the Main Botanical Garden of Russian Academy of sciences, in Moscow, Russia. After seed extraction from fruit covers, they were uniformly distributed in the boxes filled with wet sand and placed into cold room at 4 ^{0}C. Such treatment called "cold wet stratification" imitates the natural conditions for shed seeds inside the litter, but controls the environmental conditions.

The seeds were regularly taken from the boxes, washed and used for analyses. Some seeds were incubated in water at 27 ºC being intact, while in another portion of seeds the "windows" in seed coat were made, just above the embryo axis. This treatment facilitated water penetration to the embryo. Such seeds with "windows" were also incubated in water under the same conditions. In both seed portions, intact and with "windows", the rate of radical protrusion (growth initiation) was regularly recorded.

In another series of experiments, every three weeks the axial organs of the embryos (embryo axis) were isolated, weighed and transferred to Petri dishes with distilled water or 10^{-6}M fusicoccin, an activator and stabilizer of plasma membrane H$^+$-ATPase [5]. After incubation at 27 ^{0}C, axial organs were weighed and used for acidification measurements. Twenty axes were preliminary incubated for some minutes in10^{-3}M KCl and transferred to pH-meter cell filled with 10^{-4}M KCl. H$^+$ ions transferred by the enzyme from axial organs into cell walls (apoplast) were washed out into the ambient solution, their accumulation was recorded

by pH shift from pH 6.2.-6.5 (optimum pH for this enzyme) to acid values during 10 minutes.

Results

Horse chestnut seeds enter in autumn the deep physiological dormancy for 14 weeks in average. During first 5-6 weeks, seeds germinated very slowly and percent of germinating seeds was low (Figure 1). Later on percent of germinating seeds increased and rate of germination increased too. By week 14, practically all seeds were capable to germinate and germination time was 1-2 days in average. The seeds with the "windows" over axial organs of embryo were under more favorable conditions, they absorbed water through these "windows" at higher rate as compared to intact seeds. For this reason, the seeds with "windows" germinated earlier (Figure 2, curve 2) than intact seeds (Figure 2, curve 1).The germination rate in hours was calculated for each point on both curves, and used as the ordinate axis. The area plot between curves 1 and 2 indicates the dependence of germination on water supply, whereas the area plot between curve 2 and curve 3 (germination rate of non-dormant seeds) indicates the germination dependence on the dormancy itself. These observations permitted us to distinguish the dormancy of embryo (first 5-6 weeks) from the coat-imposed dormancy manifesting itself during 6-14 weeks and depending on water penetration through seed coat.

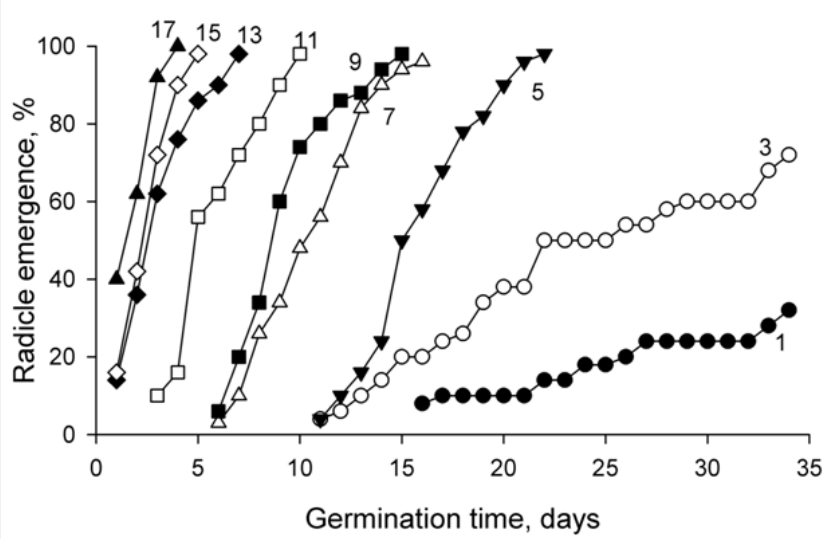

Figure 1: Dormancy initiation and release up to germination in intact horse chestnut seeds during cold wet stratification at 4 ºC. Seeds germinated in water at 27 ºC in darkness. Weeks of stratification are shown on curves.

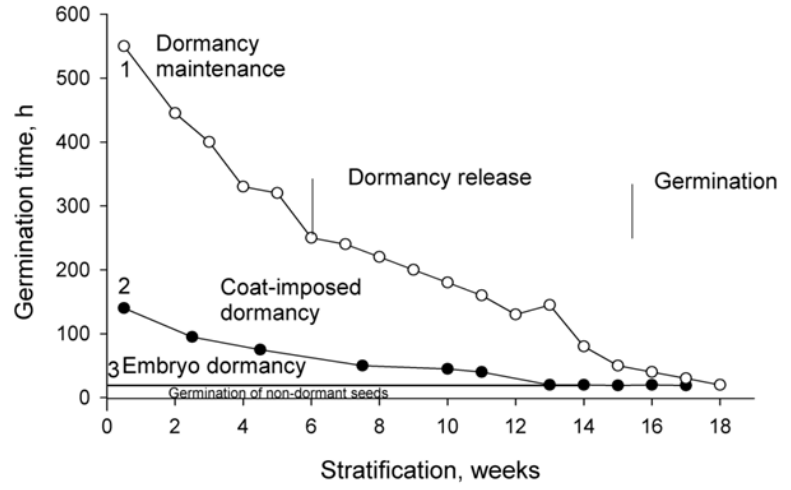

Figure 2: Scheme of embryo dormancy and coat-imposed dormancy during stratification.
1 – Germination of intact seeds.
2 – Germination of seeds with "windows".
3 – Germination of non-dormant seeds.

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The next aim of the work was the measurement of axial organ capacity to acidify the ambient solution and to verify whether the increase in acidification is sensitive to fusicoccin. The acidification of solution and effect of fusicoccin were measured every 3 weeks of stratification in axial organs from non-emerged seeds in wet sand, and then in axial organs of seeds gradually stratified in wet sand up to radicle protrusion (Figure 3).

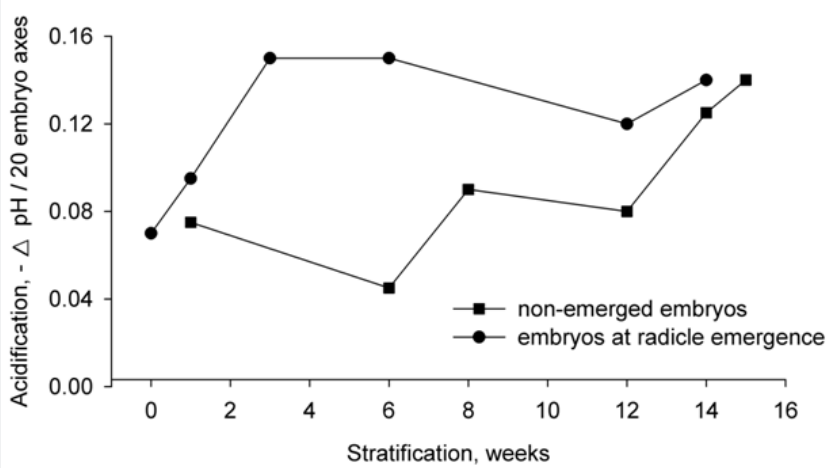

Figure 3: Acidification of ambient solution by non-emerged and emerged axial organs of horse chestnut seeds.

Another series of measurements was carried out with seeds achieving the radicle emergence (growth initiation) state. For the period of embryo dormancy (up to 6 weeks) very weak acidification was recorded, but later on, during the period of coat-imposed dormancy, rate of acidification increased (lower curve) up to the level typical of radicle protrusion. This level corresponds to axial organs at radicle emergence (upper curve). Therefore, the activation of acidification occurred in seeds during dormancy release. However, the acidification during embryo dormancy did not respond to the incubation in fusicoccin (Figure 4), whereas the activation of acidification during dormancy release responded by stimulation of acidification. This fact clearly indicates that activation of acidification is due to the activation of plasma membrane H^+-ATPase.

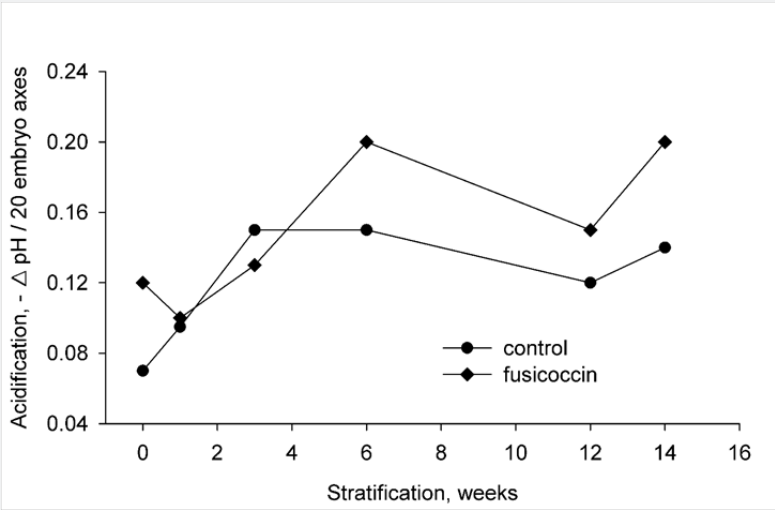

Figure 4: Effect of fusicoccin on acidification of ambient solution by emerged axial organs of horse chestnut seeds.

Discussion

The above data demonstrate the following results:

1. Embryo dormancy is characterized by low acidification, not stimulated by fusicoccin,

2. Dormancy release is the period of plasma membrane H^+-ATPase activation, as follows from additional activation of the enzyme by fusicoccin.

The first observation can be explained by the transformation of enzyme molecule from autoinhibited to active state.

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autoinhibitory domain is located in C terminus of the regulatory domain [6]. Phosphorylation of the penultimate threonine residue induces the binding of regulatory 14-3-3 proteins to the C terminus. This interaction is believed to cause a detachment of the autoinhibitory domain. This release of the regulatory domain goes along with a transformation of the enzyme from the low activity state to the high activity state [7]. According to recent observations, N terminus of the enzyme also participates in activation of regulatory domain; the N and C termini are constituting together the autoinhibitory regulatory module. It is tempting to suppose that by its nature embryo dormancy is an autoinhibitory state of the plasma membrane H^+-ATPase and that dormancy release of seed embryo is due to the enzyme transition from autoinhibited state to active state.

As to further activation of enzyme activity during dormancy release, we were unsuccessful to detect expected enhancement of phosphorylation of penultimate threonine in axial organs of horse chestnut seeds. It is quite possible that phosphorylation-independent interaction takes place between 14-3-3 protein and the plasma membrane H^+-ATPase [8]. It is supposed that 14-3-3 proteins react with phosphothreonine^{946} at the very end of C domain. Phosphorylation-independent binding of 14-3-3 protein with this motive is induced by fusicoccin, which binds to the (14-3-3 + H^+--ATPase) receptor. The presence of 14-3-3 protein side by side with H^+-ATPase and endogenous fusicoccin legends was demonstrated earlier [1].

Conclusion

Deep physiological dormancy can be subdivided in horse chestnut seeds to the following two periods:

1. initial embryo dormancy characterized by low germination capacity, weak dependence on water inflow and transition of plasma membrane H^+-ATPase from autoinhibited to active state;

2. coat-imposed dormancy, during which the embryo acquires the ability to germinate (dormancy release); its specific features are acceleration of germination rate, dependence of water inflow and activation of plasma membrane H^+-ATPase to the level typical for germinating seeds.

Acknowledgement

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