

Hatching Grass Seeds in Water with Elevated Levels of Dissolved Oxygen

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Abstract

Background: Food security in sustainable food production aims to ensure that all people have access to sufficient, safe, and nutritious food and maintain the balance of the ecosystem and the efficiency in agriculture. For instance, globally, approximately 1.3 billion tons of food are lost or wasted each year that not only leads to substantial economic losses but also contributes to approximately 8% of global greenhouse gas emissions, resulting in serious environmental harm. Therefore, efficiency in agriculture must be continuously modernized to optimize resources and get closer to the goals of producing more food by reducing significantly the consumption of energy, water, and agrochemicals. One of the areas where we must move forward is in the mysteries of seed germination. Seed germination that begins with water imbibition and ends with radicle emergence is the first step for plant growth. Successful germination is not only crucial for seedling establishment but also important for crop yield. After being dispersed from mother plant, seed undergoes continuous desiccation in ecosystem and selects proper environment to trigger germination. The hatching of seeds has been extensively studied, but despite the remarkable efforts of researchers, the role of pigments in the logic of the chemical sequence of the seed has been overlooked. The purpose of this work is to demonstrate the omnipresence of pigments throughout the process, and to concatenate the hitherto unsuspected role of pigments in the initial phases of seed hatching. **Methods:** Grass seeds were placed in water with minimum dissolved oxygen levels of 6.5 mg/L. and the changes in subsequent hours were photographically recorded. **Results:** The first phase of seed hatching occurred as expected according to our working hypothesis. **Conclusion:** The already well described germination-changes including morphological changes, cell and its related structure recovery, metabolic activation, hormone behavior, and transcription and translation activation, inevitably require water imbibition as very first step. But the water that soaks the seed should ideally contain high levels of dissolved oxygen (greater than 6.5 mg/L) for germinative changes

in the seeds to occur optimally.

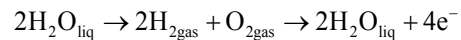
Keywords

Dissociation, Gas, Hydrogen, Imbibition, Oxygen, Water

1. Introduction

The unsuspected presence of molecules in human cells capable of transforming the power of sunlight into chemical energy that can be used by living beings, through the dissociation of water molecules present inside cells [1], constitutes a disruptive discovery that represents a before and after in biology and related sciences [2]. And agriculture and interrelated areas are no exception.

Briefly, during an analytical observational study, about the possible correlation between the tiny vessels that enter and exit the eyeball through the optic nerve and the three leading causes of blindness in the world: macular degeneration, diabetic retinopathy, and glaucoma; We identified a previously unknown chemical reaction in human cells: the unsuspected ability to dissociate and re-associate water molecules located inside cells. Such a reaction can be described as follows:



Our study began in 1990 and ended in 2002 and included the ophthalmological records of 6000 patients.

Until our discovery, only the chlorophyll molecule was considered capable of dissociating the molecule from water, and this irreversibly. But now, things change radically because not only are plants able to generate their own oxygen without having to take it from the environment that surrounds them, whether it is air, water, or subsoil; but also, humans and living beings in general, also produce their own oxygen.

Our results overturn the basic concepts of biology, medicine, and in general in all life sciences, such as agriculture. And in relation to this article, with which we want to demonstrate the powerful reason why Nature insists on placing pigmented molecules in all seeds, it is extremely important, because from the interaction between these pigment molecules, water and sunlight, the process of seed germination begins, which has not been considered to date by the scholars of these phenomena, and proof of this are the following fragments taken from various sources on the subject:

2. Background on Dormancy and Germination of Seeds in Current Literature

In the current literature there is no support (either for or against) about the role of biological pigments in the dissociation of water molecules, except for those works written in relation to chlorophyll.

The morphology of seeds involves a careful design structured from millions of years of evolution (**Figure 1**) [3]. Plants have evolved complex molecular mechanisms to regulate seed germination to ensure successful seedling establishment under favorable conditions and seed dormancy to allow for seed dispersal in space and time in the natural environment [4].

Seed germination occurs in stages that include imbibition, hydration, enzyme activation, cell division, and enlargement. The embryo also breaks through the seed coat and develops into a seed [5].

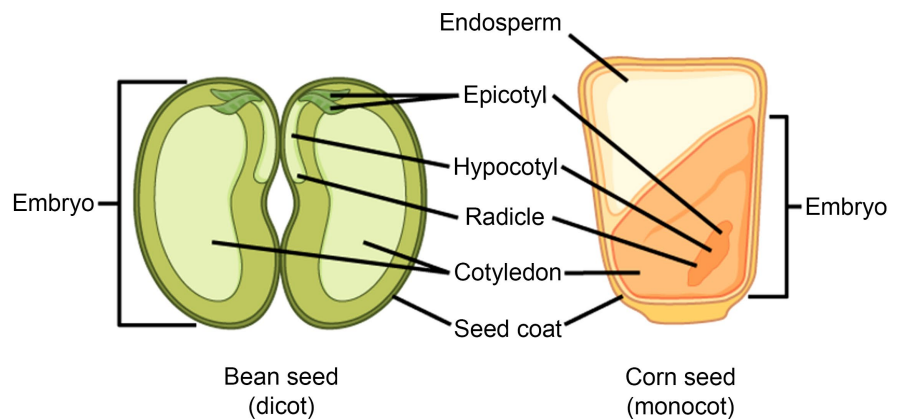


Figure 1. In general, the morphology of seeds is similar in terms of the elements that make up, including the fundamental pigment layer. Photographs by unknown author. Under license from CC BY.

But water imbibition, this is: deep hydration; it is essential for the hatching of the seed and its consequent germination. It is a first step that must happen so that the rest of the mechanisms involved can happen in a careful order established thanks to millions of years of evolution.

Each stage of germination and dormancy is regulated by a precise molecular mechanism that is not well understood [6]. In addition to the indispensable hydration of the Seed, light is required to initiate germination in the photo-dormant seed.

Both lighting and hydration are indispensable factors, but they must happen simultaneously. When they are in isolation from each other, the results are not as expected.

There is a third element that has a decisive influence on the emergence of the seed and that has gone relatively unnoticed: pigmentation. Until now, no article published on the subject gave any importance to the omnipresent presence of pigments in seeds.

3. The Function of Pigments in Seed and Water Dissociation [7]

A pigment is defined as any substance capable of absorbing light [8], so it could be considered pigments almost all substances. Usually, substances absorb very

specific wavelengths and emit a characteristic electromagnetic signal that allows us to identify them. Color is the property of electromagnetic radiation with a wavelength between 300 - 400 and 700 - 800 nm. The color of individual chemical substances derived from their interaction with white light. If the total radiation falling on a chemical compound passes through or is reflected from it, the substance has a white color. If white light on contact with a substance is completely absorbed, it appears dark. Most often chemical compounds selectively absorb within certain radiation ranges, and the color of a given compound is complementary to the absorbed radiation.

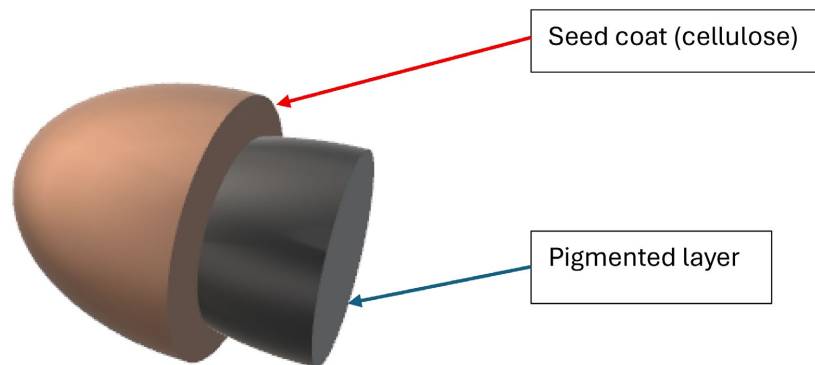


Figure 2. Diagram showing the rigid outer layer of the seed (brown), formed by cellulose compounds, and the surface inner layer (dark) which is the main location of the pigments.

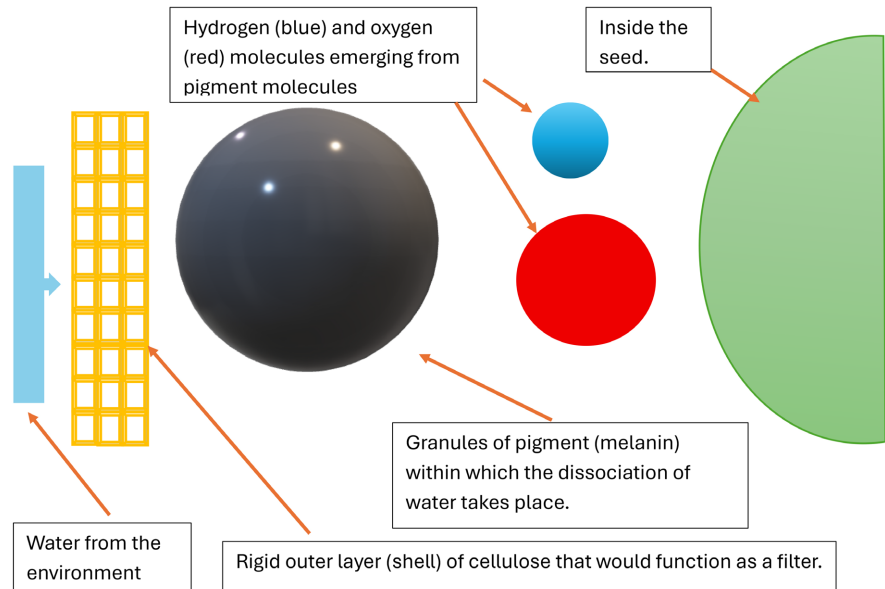


Figure 3. The diagram represents the path of liquid water from the immediate environment of the seed, through the different structures of the seed, where it ends up being transformed into oxygen and hydrogen molecules, both gaseous, and which are pushed to the internal parts of the seed by the new gas molecules that are continuously formed, since the dissociation of water inside the melanin molecules is constant. When the water from the outside reaches the pigmented layers, it can be said that it is filtered water, and when it is dissociated and reaches the inner portions of the seed, it is something like “distilled water”.

The pigment is usually relatively isolated from the environment by a rigid layer of cellulose (**Figure 2**), and when the outer layer is not imbibed with water, the pigment is also isolated from the environment. Once the Seed, both the outer (rigid) and pigmented layer, is soaked in water, then the dissociation of the water molecules that happens strictly inside the pigment molecules, is created a positive pressure that breaks down the cellulose shell allowing the internal components of the seed to meet the outside and therefore hatch easily, due to the greater contact with water and the greater availability of space for the germinative elements of the Seed that gain size once they are activated. The outer layer of the seed, composed mainly of cellulose and derivatives, is not completely impermeable to water, so it would also function as a kind of filter that would protect the pigment molecules inside the seeds to some extent of contact with contaminated water (**Figure 3**).

4. Oxidative Signaling in Seed Germination and Dormancy

According to the literature on the role of oxygen during germination and dormancy, we found the following:

Reactive Oxygen Species (ROS) play a key role in various events of seed life. In orthodox seeds, ROS are produced from embryogenesis to germination, *i.e.*, in metabolically active cells, but also in quiescent dry tissues during after ripening and storage, owing various mechanisms depending on the seed moisture content. Although ROS have been up to now widely considered as detrimental to seeds, recent advances in plant physiology signaling pathways have led to reconsider their role. ROS accumulation can therefore be also beneficial for seed germination and seedling growth by regulating cellular growth, ensuring protection against pathogens or controlling the cell redox status. ROS probably also act as a positive signal in seed dormancy release [9].

It is interesting that the fundamental role of oxygen during the life of the seed is mentioned. They even report that the seed produces it, but not as molecular oxygen (O_2), but as Reactive Oxygen Species (ROS). Likewise, they stop considering the ROS as detrimental to seeds and give them a different and important role in plant physiology signaling pathways.

So far, Reactive oxygen species (ROS) derivate from the reduction of oxygen which gives rise to superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO.) and singlet oxygen (1O_2).

One of the most remarkable features of orthodox seeds is that their moisture content and metabolism vary dramatically from development to the completion of germination. Seed moisture content (MC) is high at the early stages of seed development, *i.e.*, during embryogenesis, a developmental stage which is accompanied by an intense metabolic activity resulting in cell division. This is consistent with our finding about the dissociation of water, since the greater the amount of water, the greater the dissociation of the water molecules, which is consistent with the greater need for hydrogen (energy) and oxygen, by cells that are rapidly developing.

At shedding, orthodox seeds are therefore fully desiccated (seed moisture content is generally below 0.10 g H₂O g dry weight (DW)) and quiescent, with a metabolic activity almost at a standstill [10] as in hibernation in vertebrates. This was to be expected, since all living beings produce their own oxygen through a universal mechanism that is the dissociation of water molecules, which prokaryotic or eukaryotic cells contain inside.

Thereby, the hydration of the seed's tissues has no direct consequences in terms of ROS production. Instead, in developing or germinating seeds, the active mitochondria is probably one of the major sources of ROS, generating superoxide, and subsequently H₂O₂ [11] but once enough molecular oxygen and hydrogen are generated through water dissociation, and in turn they activate or re-activate all the biochemical processes inside the seed, including the mitochondria.

Chloroplasts can also generate ROS in the beginning of seed development, but they rapidly become nonfunctional. The reason is that chlorophyll does not tolerate oxygen toxicity. ROS production in dry seeds would probably result mainly from a slight dissociation of water by the pigmented layers of the dry seed, by virtue of the scarce water available, and not so by non-enzymatic mechanisms, such as those of Amadori and Maillard [12] and lipid peroxidation [13]. Lipid peroxidation is even favored at very low moisture content (MC) [14]. This can be explained by the generation of hydrogen from the dissociation of water. This largely prevents seed corruption, since hydrogen is the best antioxidant known, and the corruption of molecules can be defined as disordered oxidation.

The ROS signaling transduction pathway in plants, and therefore in seeds, from sensing to changes in gene expression, is not fully understood yet. Because the generation of ROS, as well as its activity and impact on the hatching of the seed, is largely random, and instead, the generation of hydrogen and oxygen molecules from the dissociation of water is an amazingly exact and therefore very orderly process.

The changes in cell water content during seed life suggest that seeds have sensing mechanisms. And melanin would explain it, as it has been found that melanin seems to take information from the immediate environment. [15]

In dry quiescent seeds, the scarcity of free water and an elevated cytoplasm viscosity which traps better the few gas molecules of H₂ and O₂ that are formed when water dissociates, it also limits the generation and diffusion of ROS.

5. Seed Germination

Germination is the process which leads to the elongation of the embryonic axis from a seed, allowing subsequent seedling emergence [16]. It consists in hydration of the quiescent seed (imbibition, phase I of the full process), and in the achievement of many metabolic and molecular events during the so called germination *sensu stricto* phase which occurs at a constant seed MC (phase II). Completion of the germination *sensu stricto* is the critical step of germination because it requires the activation of a complex regulatory system which is controlled by intrinsic (*i.e.*,

dormancy) and extrinsic (*i.e.*, environmental conditions, such as light, temperature, hydrogen, oxygen and water availability) factors. Numerous reports have shown that the transition from a quiescent seed to a metabolically active organism is associated with ROS generation, suggesting that it is a widespread phenomenon. But where does the substrate (oxygen) that gives rise to the ROS come from, and even more, where does the activation energy (Hydrogen) that leads to the reactivation or intensification of metabolism come from, given that in reality, metabolism is always active in the seed, because otherwise the conservation and therefore minimum maintenance of the molecules that intervene in the germination process could not be explained, and even less over decades, lustrous, hundreds or thousands of years.

Production of hydrogen peroxide has been demonstrated at the early imbibition period of seeds of soybean [17], radish [18], maize [19], sunflower [20], wheat [21], pea [22], and tomato [23] seeds. Here we would have two questions: where does the water needed as a substrate to form H_2O_2 come from, and where does the oxygen atom also necessary for it come from.

Also, the intracellular sources of ROS production are poorly documented. Presumably, most of the ROS produced should originate from mitochondria, since resumption of respiration in imbibed seeds might lead to electron leakage and increased production of ROS.

If for a moment we accept, without panicking, that our observation about the unsuspected intrinsic property of melanin to transform the power of sunlight into chemical energy, by means of the dissociation of the water molecules contained within the cell itself, then the explanation about the origin of the aforementioned elements, that they must necessarily be increased so that the seed passes from the state of dormancy to germination, is within reach, and the mechanism is so universal that it occurs in all seeds, in addition to those referred to above.

Since the purpose of this article is to draw attention to a so far unknown mechanism that is basic both for the seed to preserve the chemical attributes essential to remain viable, which is called latency; and once the same process is naturally increased under appropriate environmental conditions, such as light, temperature, hydrogen, oxygen and water availability, And this is our brief review of the relevant literature and now we move on to the main topic that motivated us to make the demonstrative and relatively simple experiments, the results of which induced us to communicate them through this article.

6. The Subtle but Fundamental Role of Oxygen (O_2) and Hydrogen (H_2) in the Hatching of Seeds

The hydrogen (H_2) and oxygen (O_2) molecules that are generated, in addition to playing an important role in the release of the internal parts of the seed into the close environment through a mini-explosion, also play a fundamental role in the almost homogeneously activation of the all biochemical pathways of the seed, which finally leads to the emergence of the seed, because all the chemical processes

involved in the hatching of the Seed require activation Energy. The hydrogen atoms, being the smallest, permeate with some ease through the internal structures of the seed, with their valuable energetic charge, which provides the indispensable activation energy for every one of the chemical reactions that are required to bring the seed out of the dormancy state. followed by the growth of biomass, for which oxygen besides hydrogen is also indispensable, since it is part of more than 90% of the present and future stuff. But the size of the oxygen molecule requires 16 times more space than hydrogen, so it takes a little longer to penetrate the interior of the seed to carry out the task entrusted to it by nature, and that is in relation to the complex growth of the germinative biomass of the seed.

Therefore, pigments have a double initial function which is both mechanical and chemical and are as follows: 1) to break the envelope through the positive pressure (mechanical) generated by the continuous dissociation of water (H_2 and O_2), and 2) biochemical, since both gaseous elements have different but indispensable effects both on the rigid shell, which finally weakens it, and on the germinative mass of the Seed, to which it provides the two most important initial elements of the germination process. And at the same time water dissociation can provide these fundamental gases to the internal structures (and immediate surroundings) of the seed so that all the biochemical processes that lead to germination and that have been rigorously and carefully structured over millions of years of evolution, begin simultaneously, both in time, form, space, location, composition, etc., so that they happen in a harmonious, congruent, and coherent way; as required by the astonishingly exact processes that give rise to what we call life, which is achieved through H_2 , which transports energy and therefore provides providing the indispensable activation energy. Let us remember that hydrogen (H_2) is the carrier of Energy that nature uses at most in the entire universe [24].

It is no coincidence that sucrose enzymes, so-called synthases, are because they do not use ATP as an energy source, and then molecular hydrogen becomes the ideal candidate. By the way, Enzymes that use ATP as an energy source are called synthetases, and characteristically they appear later in evolution.

The blue-green spheres (**Figure 4**) represent the hydrogen molecules that are directed in and out of the seed, and their valuable energy charge provides the activation energy of all the molecules and structures that make up the biological germination machinery of the seed, and this happens almost simultaneously, as if we activated all the germinal internal parts of the seed at the same time. which explains the pH changes inside the seed (and close surroundings) during germination [25]. Although it can be thought that the parts adjacent to the pigmented layer are the first to be saturated with the molecular hydrogen (H_2) that emanates from the pigmented layer, and later the subsequent or deeper ones are saturated (and activated).

The oxygen molecules (O_2), also in **Figure 4**, represented by the orange circles, have more difficulty moving to the inner layers of the seed, unlike H_2 which penetrates quickly due to its size. But oxygen (O_2) is also important since it becomes

part of 99% of the molecules that form and make up the total seed's biomass when germinating. The carbon atoms necessary for the formation of organic molecules necessary for germination are taken by the plant from CO_2 from the atmosphere mainly (Figure 4).

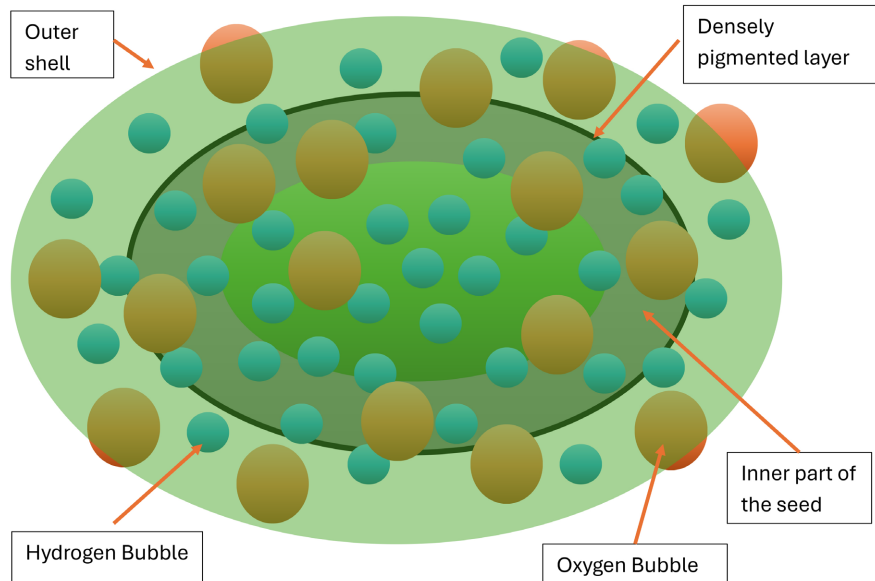


Figure 4. The dissociation of water molecules, a non-compressible liquid, creates a negative pressure zone, *i.e.* a vacuum, which in turn attracts more water molecules into the seed. The light green oval represents the outermost covering of the seed, the dark green oval represents the pigmented layer of the seed, which is where the dissociation of water takes place, strictly inside the pigment molecules; and the inner oval of flag green, represents the internal parts of the seed. The brown-orange spheres represent oxygen molecules, and the smaller blue spheres represent molecular hydrogen, both molecules coming from the dissociation of the molecule from water that occurs strictly inside melanin granules or molecules, called melanosomes in mammals, and melanophores in dermis of cold-blooded vertebrates.

From the CO_2 that the plant takes from the atmosphere, and thanks to the high levels of oxygen and hydrogen that the plant's cells obtain through the dissociation of water, CO_2 is hydrated to form carbohydrates. Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) is the end-product of photosynthesis and the primary sugar transported in the phloem of most plants [26].

Recall that Photosynthesis carried out by plants, algae and cyanobacteria is the major source of fixed carbon for all life on earth (Figure 5). Therefore, its requirements for oxygen and hydrogen (from the dissociation of water, not from the atmosphere), as well as atmospheric CO_2 are particularly significant.

Oxygen deficiency (hypoxia) and a complete absence of oxygen (anoxia) are forms of serious abiotic stress that often cause reduced plant growth and productivity. Low-oxygen stress in plants is often caused by flooding, but may also occur naturally in dense, bulky and inner organs and tissues or in very rapidly growing tissues in which oxygen consumption is high. So far, Oxygen is considered as final

acceptor in the mitochondrial electron transport chain and the absence of oxygen blocks electron transfer and cellular ATP production. Although these concepts are mostly theoretical [27]. Some of the plants' responses to oxygen deficiency can occur very rapidly and may involve changes in the transcription and activity of metabolic enzymes [28].

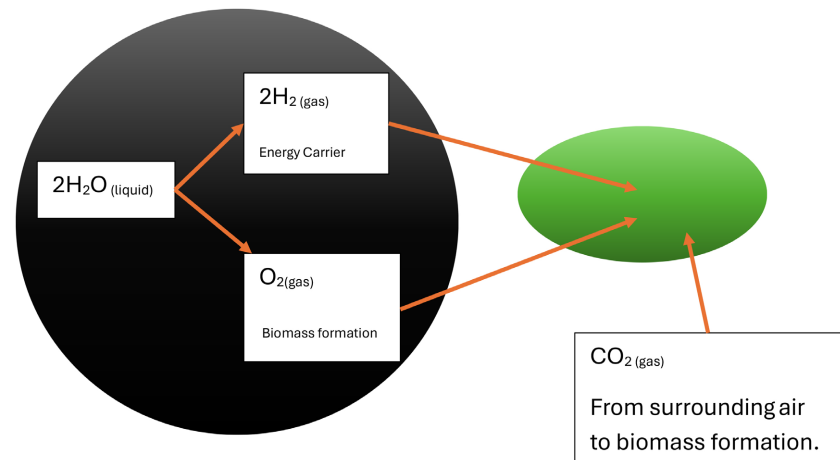


Figure 5. The dissociation of water happens strictly inside the pigment molecules, hence the gases diffuse in all directions, both inside and outside the seed, so in addition to the effect on the internal structures of the seed, they also have effects on the immediate environment of the seed, promoting its hatching.

The oxygen needs of both the plant and the seed are indisputable, and also intense and constant, therefore the high amounts of oxygen that are required for living beings, in this case the seeds, could not come from the atmosphere, because among other things, oxygen tends to rise, and on the other hand the proportion of oxygen in the atmosphere is much lower than the needs of the plant and of the seeds. That is why the dissociation of the water molecule is a fundamental process for life, and it needs to be carried out inside each cell, whether it is a prokaryotic or eukaryote.

We can think that plants, algae and cyanobacteria, handle CO_2 differently from vertebrates, because they do not emit CO_2 but hydrate it inside each cell forming sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$), and vertebrates emit significant amounts of CO_2 , since the theoretical formation of sucrose inside them is remotely possible and if so, it would be in very small amounts. For instance, when the Krebs cycle goes into reverse.

7. Seeds Dormancy

Plants rely on seeds for reproduction, genetic continuity, and colonization [29]. However, the successful establishment of seedlings depends on the timing of seed germination; consequently, seeds must possess unknown strategies to perceive their surrounding environment and trigger germination [30]. Seed dormancy is a plant strategy that optimizes germination, seedling emergence, and establishment

so that these processes occur when environmental conditions are optimal for plant survival and growth [31]. It refers to inhibited germination in mature seeds even under favorable environmental conditions for germination [32]. Seed dormancy can be particularly important for plants in harsh and highly unpredictable environments (e.g., in arid ecosystems), where windows for recruitment are few and sparse; hence, it is more prevalent in these environments than in those with more benign conditions [33].

Most families of plants have seeds with physiological dormancy (PD) [34], where a physiological inhibiting mechanism (low levels of water dissociation) in the embryo results in low growth potential, which prevents the emergence of the radicle through covering layers, hence seeds require a specific set of conditions (*i.e.*, often a combination of temperature, moisture, pigmentation, and light) to initiate germination; and depending on the strength of the physiological inhibitory mechanism. Here we could add levels of dissolved oxygen (DOL) in the water, since water with DOL levels above 6.5 mg/L is when the biological pigments act best to dissociate the water molecules [35] (Figure 6).

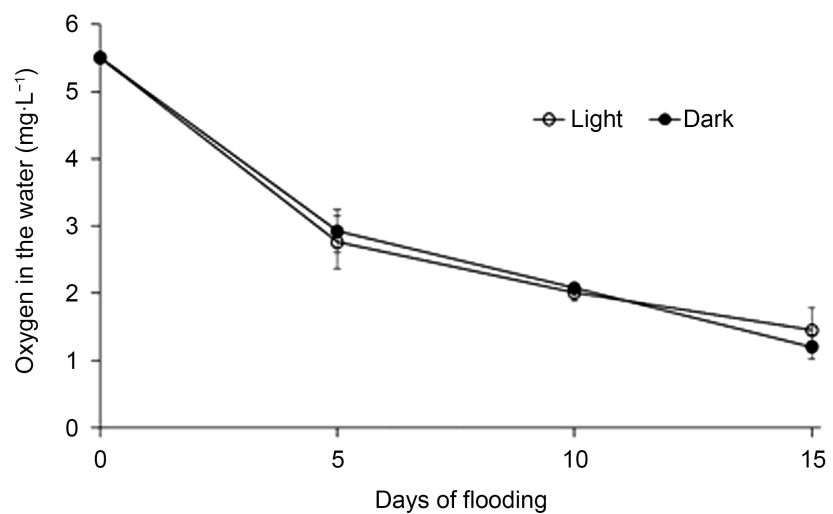


Figure 6. Dissolved oxygen concentration ($T = 27^{\circ}\text{C}$) at 5-cm depth for seed germination in water experiment. Seeds were immersed in water in sealed transparent plastic pots (300 mL) and kept in the dark or in the light during a 15-day period. Measurements were taken at 5-day intervals for 15 days in light and in dark. Data expressed as mean \pm standard deviation ($n = 4$) [35].

Flooding is one of the major abiotic stresses that threatens the growth and survival of plants in both natural and agricultural ecosystems. Flooded soils are hypoxic or even anoxic [36] because floods also deliver oxygen-consuming pollutants that impact that have had impoverishing respiratory oxygen consumption and the sharp decrease in replenishment resulting from the oxygen diffusion rate in the water being 10^4 times slower than in air. An adequate supply of oxygen is essential for successful seed germination of most terrestrial plants. But living beings with chlorophyll produce their own oxygen, dissociating the water molecules

that the plant or seed contains inside each cell, so they do not take it from the environment that surrounds them, whether it is water, air, or subsoil. The levels of dissolved oxygen refer more to the quality of the water than to the oxygen available for the plants or seeds to supposedly absorb it, which does not happen, because they do not need to do so, since thanks to the process called photosynthesis, through which they separate the water molecules into H_2 and O_2 , the plant entities are able to produce the oxygen they require by their own means. and this, from the water they contain inside each cell.

8. No Living Entity Takes Oxygen from the Environment That Surrounds It, Whether It Is Water, Air, Or Soil. Seeds Are No Exception

Oxygen plays a fundamental role in ocean chemistry and photosynthesis by microscopic plants—a vital part of the marine food chain. In oxygen-depleted waters that means low level water dissociation; then microbial processes can produce hydrogen sulfide, which is toxic to other organisms, and methane, a potent greenhouse gas. That is, microbes that also produce their own oxygen cannot generate enough hydrogen and oxygen that their own metabolism requires, and then abnormal products of metabolism appear.

Starting from the fact that all living beings (including seeds) produce oxygen and hydrogen by dissociating the water they contain inside, and mainly for their own consumption, expelling a minimum part of it into the immediate environment, so that hypoxia or anoxia in the water or in the subsoil, is a mere reflection of the damage caused by air pollution, of water or subsoil. And in the case of water, the gradual loss of marine life, both animal and vegetable, is observed; and in the case of the subsoil, it is the low yield of the crops. And as environmental pollution increases, the capacity of water, air, and subsoil to sustain life inevitably decreases (Figure 7).

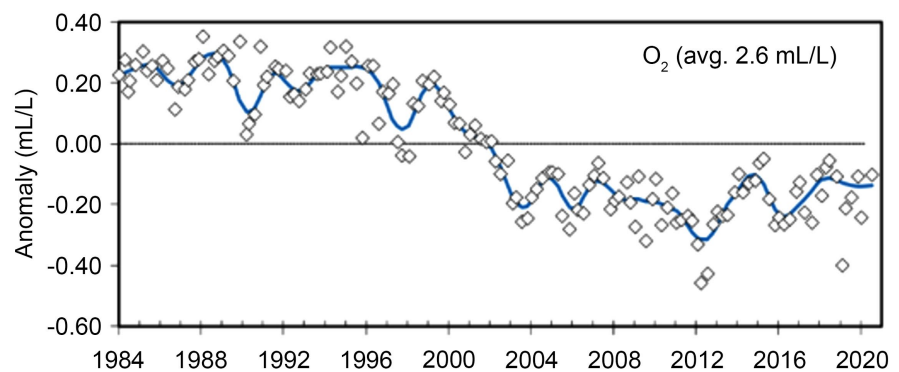


Figure 7. Dissolved oxygen concentrations across 66 monitoring stations in Southern California waters (1984-2020) [37].

Specifically to the topic of this article, referring to the fact that if the water with which the seed is moistened, has low levels of dissolved oxygen, then the seeds

cannot dissociate the available water molecules with sufficient speed and quantity, so from the very first step of the seed hatching, the process is impoverished, which results in a lower yield in the final harvest.

9. The Unsuspected Role of Pigments in Seed Biology

Pigments always have water, so the dissociation of these molecules is incessant, but differs in the amount of hydrogen and oxygen available, and the molecules of H_2 and O_2 also play a very important role in the preservation of seeds during surprising long periods of time, without being corrupted and preserving its form, structure, and therefore germination capacity, due to the finely directed (by Nature) effect of O_2 and H_2 , on the Seed (**Figure 8**) as a whole, during the latent phase of it. When the dissociation of water molecules is limited to the water present inside the seeds and inside the pigment molecules, what we could call basal dissociation, then the hydrogen and oxygen that is generated is only enough for the seed and its content of organic molecules to be preserved, but only viability is maintained. but the seed will not be able to germinate until the amount of water available, and therefore of H_2 and O_2 , is sufficient to impregnate and therefore drive at the same time the complex machinery of seed hatching.



Figure 8. Macadamia tetraphylla seeds. The pigments present in the different seeds provide the hydrogen and oxygen necessary for the seed to remain viable, in dormancy, or, if the conditions of water, light, pigment, etc., allow it, then it will hatch. This depends mainly on the amount of oxygen and hydrogen available, both of which come from the dissociation of water molecules.

Biological pigments best play their role in transforming the power of light into chemical energy by dissociating the water molecules they contain within them, when the levels of dissolved oxygen in the water that hydrates them contain a minimum of 6.5 mg/L of dissolved oxygen, so the following images (**Figure 9** to **Figure 23**) were taken from an experiment in which the dissolved oxygen content in water was raised with our novel material (QBLOCK®), developed in basis of the biology of the human eye; to consequently place grass seeds in it and demonstrate how the rigid seed cover is broken in few hours.



Figure 9. When grass seeds are placed in water, they tend to float, at the bottom of the blue plastic container, the dark gray QBLOCKS® can be seen.



Figure 10. Magnification allows us to observe the integrity of the seed structure. The cubic blocks at the bottom of the blue container are the QBLOCKS®, so that they raise the levels of dissolved oxygen in the tap water that was used in the experiment.



Figure 11. The photograph allows us to observe the distribution of the QBLOCKS® at the bottom of the container, as well as the distribution and buoyancy of the grass seeds. Note the pigmented parts of the Seed that begin to appear in a matter of few hours.



Figure 12. The magnification shows the integrity of the rigid envelope of the seed, at the beginning of the experiment, characterized by its brownish-yellow color and buoyancy. As the hours go by, the pigmented parts of the seeds seem to multiply quickly.



Figure 13. In this enlargement, the loss of integrity of the external rigid envelope, which normally surrounds the seeds, is appreciated. Note the QBLOCK® at the bottom of the container. The pigmented inner portions of the seeds are becoming increasingly numerous as the hours go by, which tend to submerge due to the greater weight of the structures imbibed in water.

QBLOCK® is a material developed in our laboratory, based on the biology of the human eye, and is characterized by raising the levels of dissolved oxygen in water, when immersed in an aqueous medium, without requiring electricity or added chemicals [38]. There are several ways to keep dissolved oxygen levels in water elevated, whether fresh, salty, waste, etc., but to be effective, they require continuous medical-grade O₂ injection into the body of water, which significantly raises both costs and technical difficulties in this regard, making them impractical. Injecting atmospheric air continuously into the water does not effectively solve the serious and growing problem of low levels of dissolved oxygen, given the low proportion of oxygen in atmospheric air, which ranges from 18% to 21%, with 78% being nitrogen. Not to mention, significant amounts of electricity are required to be spent continuously, which involves emitting greenhouse gases in significant quantities. And to top it off, the results of such practices that are unfortunately very common, are less than mediocre.

That is why we use QBLOCK®, both for experimentation and for larger-scale applications. since it is surprisingly efficient in continuously raising the levels of dissolved oxygen in both water, air, and even in the subsoil. The QBLOCK® was developed in our Human® Photosynthesis Research Center, based on the biology

of the human eye. QBLOCK® does not require electricity or added chemicals, and due to its form of action it reduces the formation of toxic sludge by more than 90%. The average lifespan of QBLOCK® is 25 years minimum. They can be manufactured in almost any shape, size, and weight.



Figure 14. Closer view, As the outer rigid layer is uncovered, the inner pigmented parts of the seed tend to escape into the water. Note the QBLOCK®, in the form of cubes of 1 cm X 1 cm X 1 cm, at the bottom of the container.

In a matter of 2 or 3 hours, Once the seeds are deposited on the water surface of the container, the pigmented structures of the seed begin to appear. The pigmented parts of the Seed are visible to the naked eye, given the dark color of the pigment.

It is possible to observe the residual pigment inside the external part of seeds. Most seeds tend to sink due to the increased weight of the seed once the seed becomes increasingly hydrated.

As the hours pass, more seeds turn dark as the rigid layer of cellulose in the seed cracks, and the dark interior of the seed is usually expelled or released. The inner portions of the seeds, characteristically covered with pigment, tend to sink, since this part of the seeds, once hydrated, has a specific weight greater than that of water, and ends up sinking.

As the minutes go by, more seeds expel the pigmented content. The remains of the rigid layer of the seeds, composed mainly of cellulose, continue to float on the surface of the container.



Figure 15. When most of the water is removed from the container, the large number of pigmented (dark) portions inside the seeds are observed, once the outer rigid envelope breaks due to the positive pressure created by the gases (O_2 , H_2), coming from the dissociation of the water molecules. The visible cubes are our QBLOCKS®.

The QBLOCKS® at the bottom of the container, and the seeds tend to get close to them.

In the close-up, we can observe in greater detail the internal structures, dark in color (due to their abundant pigmentation), which were expelled from the interior of the seeds. Once the outer cellulose shell is cracked by the pressure of gases (H_2 and O_2) from the dissociation of water that happens inside each pigment molecule.

For the inner portions of the seeds to continue to hatch, they are released from the rigid seed coat, formed mainly by cellulose; because once released into the environment, the pigmented portions will have more room to grow; as well as greater accessibility to ambient light, water and CO_2 from the atmosphere.

CO_2 gas, from the atmosphere is captured by the seed and introduced into the seed, where it is reduced to sucrose. This is how nature recycles toxic gas (CO_2), this is by hydrating it and therefore forming carbohydrates. And this has been since the beginning of time. The affinity of the seed for QBLOCK® is remarkable. In the macroscopic views, it is possible to identify the empty shells of the seeds, as well as the pigmented interior that constitutes the germinative part of the seeds.

At the beginning of the experiments carried out, in a blue plastic container, grass seeds were placed in running water, to which some blocks of QBLOCK were previously placed®.



Figure 16. The empty rigid shells of the seeds resemble small canoes seen from above. Most seeds expel a dark brown content, one or another is greenish in color. The seeds “expelled” dark in color, and the “canoes” are easily visible.



Figure 17. In the photograph composed of several shots, we can see the changes that the seeds normally present when the levels of dissolved oxygen in the water are above 6.5 mg/L. The use of QBLOCKS® in the experiment is to ensure that these levels of dissolved oxygen were adequate for the hatching of the seeds. Seeds have a significant affinity for QBLOCKS.®

Water with dissolved oxygen (DOL) levels greater than 6.5 mg/L is when pigments work best in their unsuspected function of dissociating water molecules. This is why, ocean warming makes warmer ocean waters more buoyant, holding less O₂ and reducing the mixing between surface O₂-rich and deep O₂-poor water masses. It also raises the O₂ demand while reducing the available amount for marine organisms to respire. Ocean warming also contributes to the O₂-depletion of ocean dead zones in coastal and open ocean areas, rendering them largely uninhabitable by marine life. As ocean temperature increases at nutrient-poor subtropical ocean gyres, iron availability constrain marine nitrogen fixation in those regions [39]. These ocean warming-caused changes in the ocean's physics and chemistry are triggering variations in productivity [40] and shifts in marine ecosystem composition, functionality, and biodiversity, with adverse effects. In some photographs, the number of "canoes" is remarkable.

10. Part II

The use of surfactants in households and industries is inevitable and so is their discharge into the environment, especially into the water bodies as effluents. Being surface-active agents, their utilization is mostly seen in soaps, detergents, personal care products, emulsifiers, wetting agents, etc. Anionic surfactants are the most used class [40]. These surfactants are responsible for the foam and froth in the water bodies and cause potential adverse effects to both biotic and abiotic components of the eco-system. Surfactants can penetrate the cell membrane and thus cause toxicity to living organisms.



Figure 18. To contrast the impact of water pollution, in this case detergents (see foam), the behavior of the seeds was different, despite having QBLOCKS®.

Considered one of the major pollutants in the aquatic and terrestrial environment [41], surfactants are amphipathic compounds having both hydrophobic and hydrophilic groups which enable the property of reduction of surface tension, and thus, their use in the laundry and cleaning industries. Surfactants are used in households and industries every day, and most of them end up being discharged into the environment.

To clarify some doubts of the reviewers and readers, we did a second experiment in which in a plastic container, we placed the QBLOCKS® and the seeds in soapy water, with 2 grams of a common detergent for washing dishes.

Once the QBLOCKS® were placed in soapy water, the seeds were placed in a similar quantity and arrangement to the previous experiment.



Figure 19. In the photograph you can see the grass seeds freshly placed in the soapy water.

The macroscopic changes in the seeds were recorded by photographic control every 3 or 4 hours, in the same way as the previous experiment, which was carried out with running water.

Almost anything that gets into the water decreases dissolved oxygen levels, and detergents have a very marked effect [42].



Figure 20. Despite the passage of several hours, the appearance and color of the seed have significant differences from seeds placed in non-soapy water.



Figure 21. After almost 6 hours, the presence of dark-colored seeds is significantly less than when the seeds were placed in non-soapy water.



Figure 22. After almost 9 hours, the soap bubbles disappeared, leaving a brownish-dirty water appearance, and the hatching of the seeds is significantly less and different than seeds placed in non-soapy waters.



Figure 23. An approach that allows us to appreciate the brown water and the dark pigments, observed in the previous experiment, is different in this case, since it seems that few green pigments predominate. The absence of dark color pigments is perceptible to the naked eye. The brownish coloration of the water is different. The shape and color of the seeds placed in soapy water with QBLOCKS® is different enough in texture, color, and shape to conclude that there are significant differences between both experiments.

At greater magnification, it is possible to discern the presence of some dark-colored seeds, with green color seeds predominating (**Figure 24**).



Figure 24. The seeds were placed in soapy water, 24 hours after the experiment began, showing significant differences with the seeds of the previous experiment. Some magnification pictures, shows stem-like structures in seeds placed in soapy water.

When removing the surface layer of seeds, a large proportion of submerged seeds can be seen, but unlike the experiment with clean water, brown seeds predominate (**Figure 25**).



Figure 25. When the QBLOCKS® were removed 28 hours after the start of the experiment with soapy water, the affinity of the seeds to the QBLOCKS® was not observed, as in the previous experiment, carried out with plain water.

The approach of the seeds placed in soapy water, almost 28 hours after the experiment began, allows us to observe the scarcity of dark-colored seeds, in contrast to the previous experiment, in which they were significantly more abundant.

11. Comment

Quantitative structure-activity relationship (QSAR) studies have suggested that the toxicity of surfactants increases with the length of the alkyl chain within a surfactant class [43] (Table 1). Several negative effects on aquatic organisms have been described, given the importance of their biodiversity, and when it affects it, the adverse effects are significant, as they even lead to the disappearance of life in the so-called dead zones of the seas, oceans, rivers and lakes.

In this relatively simple experiment, we show that by affecting the intrinsic property of the different pigments found in the seeds, the very first step of germination is affected, which is that the rigid envelope is destroyed enough so that there is a better interaction between the seed and the environment, while creating enough space that allows the elements that make it up to grow properly as they hatch.

Table 1. Adverse impacts of surfactants on aquatic flora and fauna.

Flora & Fauna	Surfactant	Species	Adverse impacts	References
Planktons	LAS	Cryophytes	Cessation of photosynthetic activity	[44]
	SDS	Dunaliella salina	Reduced chlorophyll-a and carotenoid content	[45]
	Personal care products	Daphnia magna	100% mortality of female organisms	[46]
Aquatic Plants	SLS	Azolla pinnata	Reduced chlorophyll content	[47]
	SLS	Hydrilla verticillata	Leaves turned brown	[48]
	SDBS	Azolla pinnata	Reduced chlorophyll content	[47]
	SDBS	Hydrilla verticillata	Reduced chlorophyll content	[47]
	LAS	Elodea canadensis	50% reduction in photosynthesis	[49]
	LAS	Myriophyllum spicatum	50% reduction in photosynthesis	[49]
Fishes	LAS	Lates calcarifer Bloch	Sight disruption, gill damage	[50]
	Detergents breathing	Anabas testudineu	difficulties, signs of distress	[47]
	Anionic surfactants	Danio rerio	larval abnormalities, higher cortisol levels	[51]
	LAS	Clarias gariepinus	Histopathological damages to liver cells, gills, intestinal walls and mucosa	[52]
	LAS	Clarias gariepinus	Disturbed haematological parameters.	[53]

Surfactants entering the environment mostly through the discharge of sewage effluents into surface waters and sewage sludge on land have potential negative impacts on the ecosystem. As a component of grey water, surfactants have been identified to cause water repellence and a decrease in hydraulic conductivity of soil [54]. Increased foaming of the water bodies and changes in several physico-chemical properties of soil are two major effects of the surface-active agents on the abiotic environment.

Given the diversity of effects that have been reported, possible mechanisms have been sought in both abiotic and biotic systems. But our discovery of the unsuspected ability of living beings to produce their own oxygen (and hydrogen) through the dissociation of water, opens a light at the end of the tunnel, as we now have the possibility of modulating the levels of dissolved oxygen in the water that is used both for irrigation and for fish farming.

The hitherto unknown role of the various biological pigments in transforming the power of light into chemical energy by dissociating water molecules, mainly inside the seeds and inside the pigment molecules present in them, marks a before and after in the study of seed germination, which until now was poorly understood.

The following figure (Figure 26) explains the formation of H_2 and O_2 bubbles from the dissociation of water molecules that occur strictly inside each pigment molecule.

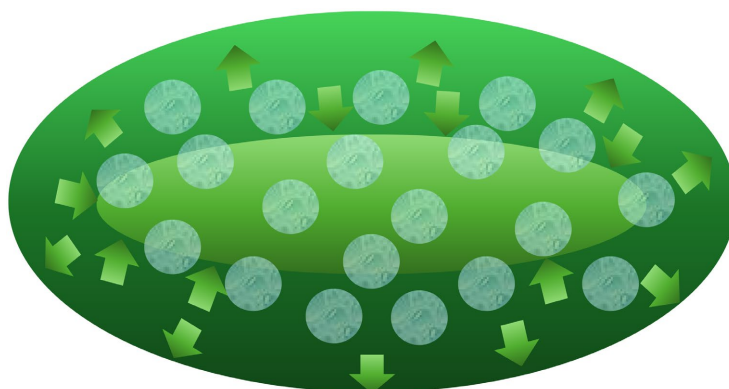


Figure 26. The molecules of hydrogen (H_2) and oxygen (O_2) are generated symmetrically, in all directions, coming from water dissociation, both towards outside of the pigmented part and inside of it, simultaneously fulfilling mechanical functions (thrust) and nutrition at the same time.

Hydrogen and oxygen bubbles play a fundamental role in both the preservation and hatching of seeds. Because when water is scarce, the bubbles are few, so they are only enough to maintain the shape and function of the whole structure, in terms of its rigidity and shape; and also for the structures that the pigment envelops, and which are also reached by H_2 and O_2 , they contribute to maintaining the biological molecules functional through dormant stage that make it up. Let's remember that hydrogen is the best antioxidant known, so its constant supply to the

interior of the cell, from the pigment molecules inside it, preserves both form and function, as well as its ability to germinate for periods of time that seem incredible, for example: thousands of years. But such an amazing capacity for germination is since melanin is the most stable substance known, demonstrated 162 million years ago [55].

12. Conclusions

The unsuspected ability of pigments to transform the power of sunlight into chemical energy through the dissociation of water molecules opens a new era in the study of seeds, since now the need for water for seed germination is explained in a more congruent way, since in addition to the “mechanical” explanation of the necessary rupture of the rigid outer shell, both hydrogen and oxygen play a crucial role in the chemical logic of the hatching of the internal structures of seeds, by providing the much-needed activation energy, taking into account that molecular hydrogen is the most used energy carrier by nature in the entire universe, and seeds and living beings in general cannot be different.

And the oxygen that comes from the dissociation of the molecule from water also has a relevant role in the biology of seed germination, as it is part of 99% of the organic molecules of living beings, and oxygen does not come from the air, since all living beings can produce their own oxygen [56].

And finally, the role of melanin is very relevant not only in the hatching of the seed, because in addition to providing hydrogen with its valuable energy charge, and the necessary oxygen to make up the biomass, melanin also produces a slight, although very continuous voltage of about a little less than a volt and between 10 and 12 microamperes [57], which presupposes that melanin provides energy of two different types: that which is carried by hydrogen, which is positively charged, and that which is carried by electrons, (when water molecules is re-formed) that is, negatively charged, and both are available inside seeds as well as prokaryotic and eukaryotic cells, and as a whole, they effectively lead to what we call life, and this since the beginning of time.

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Conflicts of Interest

The development of the QBLOCK,® to raise the levels of dissolved oxygen inside the water, was developed in our research center, based on the biology of the human eye.

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