**Chapter**

# Artificial Intelligence Tools to Better Understand Seed Dormancy and Germination

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## Abstract

Despite a large number of publications available, the control mechanisms of seed dormancy and germination are far to be fully understood. Seed dormancy and germination are very complex biological processes and because they involve multiple factors (physiological, mechanical, and environmental) and their nonlinear interactions. This explains why extremely little variations on some of those factors and in the way they interact caused enormous variation in the obtained results. Multifactorial process like these can be modeled using computer-based tools to predict better results. In this chapter, some basic concepts relative to seed dormancy and germination and the main factors (physiological, involved in seed dormancy, particularly dormancy-inducers and dormancy-breakers, and seed germination) are reviewed. In the next two, we describe the use of artificial intelligence computer-based models to better understand the physiological mechanisms of seed dormancy (how dormancy is controlled and how can be released) and seed germination. Finally, some applications of artificial neural networks, fuzzy logic, and genetic algorithms to elucidate critical factors and predict optimal condition for seed dormancy-breaking and germination are given as examples of the utility of this powerful computer-based tools.

**Keywords:** primary dormancy, secondary dormancy, dormancy maintenance, dormancy release, germination factors, temperature fluctuations, light exposure, moisture, day length, after-ripening, stratification

## 1. Introduction

The importance of the seeds began with the dawn of agriculture, around 12,000 years ago, although seeds have been collected and eaten for many thousands of years before crop domestication (20,000–100,000 years). This domestication involved the selection of the desirable traits, as a high yield, appropriated seed size and good resistance/tolerance to biotic and abiotic stress, avoiding undesirable ones (mechanism of dispersion and seed latency).

The knowledge about the storage, distribution, germination, sowing, and harvest of seeds improved for the following centuries. The first written references on the germination of seeds can be found in religious texts or in the "naturalist-texts of Greeks and Romans." In those documents, Theophrastus and Pliny, the elder

explain various germination concerns, as the need of drying the seeds for storage or soaking them in water or in milk to stimulate their germination [1].

Germination is a complex physiologic process, beginning with water imbibition by the seeds and ending with the emergence of one part of the embryonic organ, the radicle. Harvested mature seeds are usually quiescent, meaning that they may survive many years with a standstill metabolism and low water content (<15%). Quiescent seeds must be imbibed for being able to activate its metabolism and germinate under suitable environmental conditions [2]. During seed imbibition, water uptake triggers the resumption of seed normal metabolic levels, and promote the damage repair occurred during drying. Once the seeds return to their normal metabolic state, an expansion of embryonic cells causes the embryo emergence and marks the end of germination. However, some imbibed and metabolically active seeds cannot germinate under a wide range of normal environmental factors and hence, they are considered dormant [3].

Seed dormancy plays a key role in the regulation of germination [4, 5]. Dormancy induction, maintenance, and release are determined by physiological and morphological seed characteristics and their control are governed by many genetic and environmental factors. Dormancy is then, a very complex biological process that involves multiple interactive factors (physiological, mechanical, and environmental), making it difficult to fully understand its performance despite the large number of publications available. Therefore, understanding how dormancy can be controlled and/or released should ensure the success of germination in desirable species with very interesting consequences in the socio-economic and research fields [4].

The factors that affect dormancy release and germination are generally studied independently, although they are obviously interconnected: a) no germination is being possible without dormancy-breaking, b) it is almost impossible to define the beginning and the end of each process and, c) many factors may interact or counteract in both processes.

Traditionally, data from dormancy or germination studies were analyzed using traditional statistical methods, nevertheless, complex biological process such as germination and seed dormancy, cannot be fully understand by simple comparison of means among treatments, analysis of variance, regression models or simple algorithms, with those approaches being necessary to integrate multidimensional data to describe complex biological interactions [6, 7].

Artificial intelligence (AI) tools have been shown as useful techniques for establishing relationships between multiple variables (factors and parameters) [8–10]. In addition, several studies have shown the effectiveness of those AI tools, such as artificial neural networks (ANNs) combined with fuzzy logic or genetic algorithms for modeling and optimizing complex biological processes [11, 12].

In this chapter, we describe how AI models can be used for a better understanding and selection of the critical factors that stimulate the physiological mechanism of dormancy-breaking and germination in seeds.

## 2. Seed dormancy and germination: some basic concepts

Dormancy is an evolutionary characteristic that has increased the survival of plant species, through the inhibition of seed germination in adverse conditions [13]. Seed dormancy could be considered a germination absence under suitable environmental conditions, in an intact viable seed. This germination lack has evolved differently across the species and hence, several dormancy mechanisms have been developed according to the diversity of climates and habitats [5].

Nikolaeva developed the first dormancy classification scheme including just two kinds of dormancy: endogenous and exogenous [14]. In the first one, embryo prevents germination, while in the second one, some seed structures or chemicals are responsible for germination inhibition [14]. Later, Bewley and collaborators described the mechanism involved in these two dormancies [2, 15]. According to these authors, the endogenous dormancy, re-named as embryo dormancy, can be induced by undifferentiated embryos, immature embryos, chemical inhibitors (present in seeds) and physiological constraints. On the other side, exogenous dormancy, re-named by these authors as coat-imposed dormancy, is caused by several covering tissues that interfered with or suppress seed germination. These tissues inhibit water uptake, gas exchange, chemical inhibitors release or cause mechanical restraint. Plants can exhibit one or both types of dormancy, acting simultaneously or successively.

These different terms and definitions of seed dormancy have caused confusion within the scientific community because they include both morphological and physiological properties of the seeds. In the earliest years of this century, Baskin and Baskin [3] proposed a comprehensive classification, which accurately reflects all the points of view mentioned above, which comprise five main classes of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY) and combinational (PY + PD).

Briefly, in PD class, seeds are water-permeable but present a physiological mechanism in the embryo that inhibits seed germination. Seeds with PD are affected by the phytohormone abscisic acid (ABA) and their physiological-inhibition grade (deep, intermediate and nondeep) varies according to their response to other phytohormone gibberellic acid (GA) and the breaking-dormancy requirements. Seeds showing MD dormancy have a small immature (underdeveloped, but differentiated) embryo and therefore, just needing an extra incubation time and suitable conditions for normal embryo development and following germination. However, in the case of seeds belonging to MPD dormancy, in addition to presenting an underdeveloped embryo, they also show PD that should be first broken to allow full embryo development, through warm/cold stratification and /or GA treatments. Concerning PY, it is caused by one or several cell layers that avoid the entry of water in the seed and can be broken under natural (high and/or fluctuating temperatures, fire, drying, digestive animal tract transit, and so on) or artificial (chemical or mechanical scarification or abrasion) conditions. By the formation of a gap between these waterproof coats, letting the water available for the embryo, germination can be restored. Finally, the combination of PY and PD, make the breaking the waterproof layers and the embryo physiological dormancy, necessary for achieving germination.

Despite all the above, it is important to emphasize that PD is the widest widespread, prevalent and abundant dormancy class for seeds from gymnosperms and angiosperms [3–5]. The wide distribution of plants with PD seeds have triggered in the appearance of different physiological mechanisms of induction or maintenance of dormancy. Moreover, these mechanisms are related to the environmental characteristics where the mother plant has grown [4, 5]. Many species with PD seeds show a cyclic change in dormancy states (primary and secondary), governed by several factors (**Figure 1**).

Induction of primary dormancy may impose during seed development by endogenous factors and its function is to prevent precocious germination, while seeds are being developed in the mother plant or immediately after their dispersal [2]. The plant hormone ABA is the main endogenous factor involved in the primary dormancy induction; however, exogenous factors such as environmental factors have also a high influence on this induction. In this sense, under optimal or at least
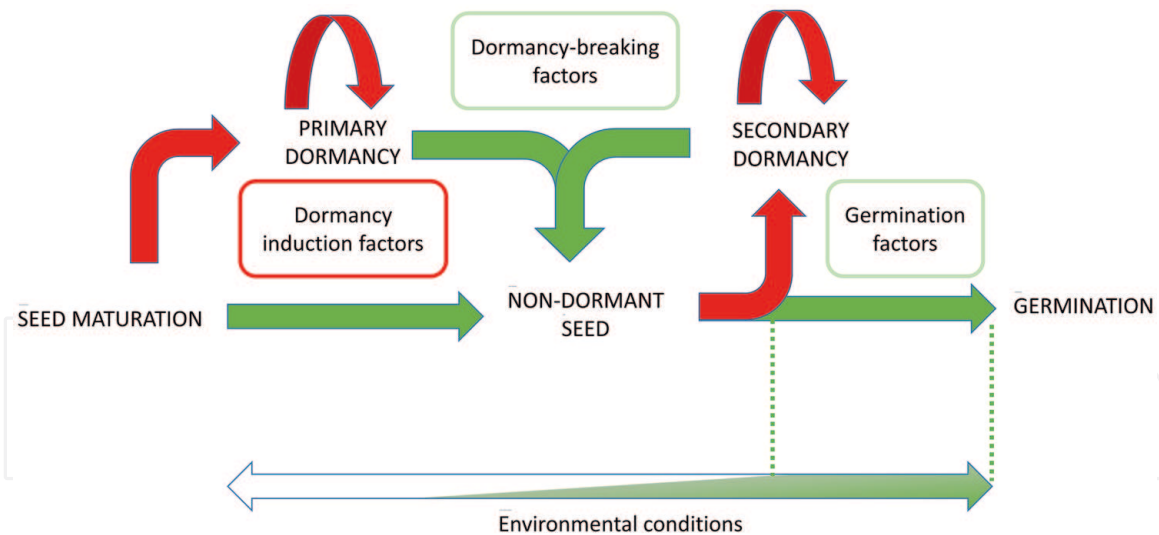
**Figure 1.**
*Scheme of seed dormancy and germination control and regulation in response to environmental conditions. Several factors are involved on dormancy induction (red) and release (green arrows). Germination process can only be fully achieved if seed germination requirements (thresholds or sensitivity) overlap with adequate environmental conditions (green dotted area).*

favorable conditions, the seeds do not suffer any germination block, moving to a nondormant state (**Figure 1**). Under favorable environmental conditions, germination starts with water uptake, followed by embryo expansion (embryo leave the dormant state, mobilizes stored nutrients, full elongate) and finish when breaks the covering coats and radicle protrusion occurs [4, 5, 16]. In any other circumstance, a blockage will happen at any time and the seeds will return to the dormant state.

Dormancy-breaking factors promote changes in dormant seeds (they cannot germinate in any condition) increasing their sensibility and allowing their germination under adequate environmental conditions [4, 5]. In addition, between dormancy and nondormant status, seeds are in a transitional state called conditional dormancy (CD), in which seeds are able to germinate but only in a small narrow of environmental conditions (**Figure 1**). In some species, under unfavorable conditions for germination, nondormant seeds may enter in a secondary dormancy status before germination [4, 17]. These seeds may continue in the transition between nondormant and dormant stage, which drives to a seasonal dormancy cycling (**Figure 1**). The cycle may continue for several years and is related to the maintenance of the soil seed bank, essential for the survival of plants communities.

The presence of nondormant and dormant seeds in a population depends on the effect of several induction factors during their development. These factors represent the main checkpoints in the control of germination and are summarized below.

## 2.1 Dormancy induction and maintenance factors

Seeds dormancy induction is highly correlated to the ABA content. This induction begins during seed development in the mother plant [18, 19]. In the initial stage, mother plant supplies ABA to seeds to prevent the premature germination [20–22]. In fact, Arabidopsis and maize mutants with reduced input of maternal ABA conduce to seeds germination in the mother plant [23, 24]. During the development, seeds begin to produce ABA by themselves and several scientific evidences demonstrated that this is the main factor required for inducing primary dormancy [5, 25, 26]. In fact, enhanced dormancy is evident in *Arabidopsis* mutants with overexpression of ABA biosynthesis genes, while ABA deficiency during seed development fails to induce primary dormancy. Therefore, it is widely accepted that the

ABA synthesized in the embryo and endosperm is the most critical factor inducing seed dormancy [16, 26].

ABA has also been proposed as the main factor involved in the dormancy maintenance of seeds, which still are dormant after their dispersion. In fact, *de novo* ABA biosynthesis has been associated to the maintenance of dormant state in several species (*Hordeum vulgare, Helianthus annuus*, and *Nicotiana plumbaginifolia*) [27–29]. In addition, *Arabidopsis thaliana* ecotype Cape Verde Island, imbibed seeds presented high ABA content and strong dormancy [17].

Recently, another phytohormone, the auxin indolacetic acid (IAA), has been found to regulate seed dormancy. Auxins have a similar effect than ABA, and transgenic seeds that overproduce auxin show a strong seed dormancy compared with wild-type seeds [30]. However, while the dormancy control of ABA by environmental signals is well-studied, the control of auxins in dormancy is not well-known yet [31].

The environment has a strong influence on the induction of dormancy during seed development (**Figure 1**). The main environmental factors affecting ABA content are temperature and light. They promote genetic expression changes in the mother plant during the seed development, which modify the ABA concentration and seed sensitivity to this phytohormone [2, 19]. Generally, low temperatures increase the dormancy induction during seed development in the mother plant, by increasing the expression of genes related to ABA biosynthesis, while high temperatures rise expression of ABA catabolism expression [2].

Dormancy induction is also influenced by natural light quantity (daily distribution), and quality (spectrum). Light daily distribution is controlled by the photoperiodic cycle: long days promote dormant seeds, while short days the opposite; the light quality is regulated via plant phytochrome (Prf): red wavelengths light (as white fluorescent) promote dormancy release, whereas far-red wavelengths (incandescent lights) promote dormancy induction and maintenance [32].

There are other factors, such as soil characteristics, that may also affect dormancy induction. Mineral nutrients such as nitrates, phosphate, sodium, potassium, zinc, iron, cooper taken up by the mother plant and translocated to the seeds has been included as dormancy inductors [2].

Finally, other mother plant physiological characteristics such as age, seed maturation timing, and seed position, which also can alter the dormancy induction [18].

## 2.2 Dormancy-breaking factors

The effect of ABA on dormancy induction and maintenance is counteracted by gibberellic acid (GA) since dormancy release depends mostly on ABA:GA balance (biosynthesis and catabolism) in seeds [33, 34]. This effect was demonstrated using plant deficient mutants. As example, Arabidopsis GA-deficient mutants present a strong seed dormancy and need exogenous application of GA for dormancy breaking [35], whereas mutants for genes involved in the negative regulators of GA biosynthesis pathway decreased the seed dormancy [36, 37]. Therefore, the release of latency depends on the concentration and sensitivity of the seeds to both phytohormones. Once seeds are imbibed, an increase in sensitivity and concentration of GA is necessary for dormancy release and some signals should trigger it. Nitric oxide (NO) has been proposed as a release dormancy signal since is related to the decrease of ABA sensibility and the increase of GA biosynthesis pathway in seeds of many species [38, 39]. Therefore, the dormancy release is established by the concentration and sensibility of both phytohormones in seeds.

Once seeds are released from the mother plant to the soil seed bank, they begin to behave as *sensors* which may detect environmental factors (signals) and change

their dormancy status, affecting the expression of genes related to phytohormone metabolism [40]. The combination of environmental signals and phytohormonal metabolism provides a complex network that allows controlling the germination according to ecological opportunities [5, 26].

The dormancy-breaking mechanisms have been modified by evolution processes, according to the environmental signals in which the species lives. This facilitated that plant species with PD were dispersed and adapted to different habitats. Soil temperature and moisture are the major factors that indicate the seasonal changes. Both factors trigger the main modifications in the depth of dormancy, by changing the seeds sensibility to other environmental factors such as, light or nitrate among others [41].

Concerning those main factors, two main dormancy patterns have been found in the field. For species autumn-germinating, the dormancy is release during summer under warm and dry conditions, while for spring-germinating species the pattern shows a release of dormancy during winter under cold and wet conditions [42]. The evolutionary usefulness of dormancy is that the seeds need going through adverse germination conditions as a requirement to be able to break it and germinate conditions become appropriate. As example of this, has been described for seeds autumn-germinating that extend periods of warm temperature and dryness allowed release primary dormancy. This dormancy-breaking process is termed as after-ripening, promoting a decrease in ABA concentration in seeds, an increase GA sensitivity and widespread the range of other environmental requirements for germination. Ecologically, this requirement prevents germination during the hottest period of summer, being necessary for breaking dormancy and allowing the following germination in autumn.

The time required of after-ripening for release dormancy is highly genotype-dependent. Moreover, using different temperatures and moisture content the after-ripening may be accelerated [43]. In fact, the after-ripening improved the germination of three autumn-germinating species (*Anthocercis littorea*, *Dioscorea hastifolia*, and *Z. fruticulosum*) when the temperature and moist conditions were modified from their normal summer conditions [44].

As described above, seeds spring-germinating need periods of cold temperatures under moist conditions. This process is usually known as cold stratification or chilling. Seed stratification, promote the expression of genes related to GA biosynthesis and also decline the activity of some GA catabolic genes [45]. The stratification is required for majority nontropical species, which are spring germinating. Ecologically, this requirement prevents germination during their unfavorable season (winter) and allows their germination during spring, where suitable environmental conditions for seedling growth are settled (**Figure 1**). However, some species have a long period of cold stratification as requirement for break dormancy. In this case, a combination of after-ripening and cold stratification can be required in order to release dormancy [3].

Once the temperature and soil moisture have modified seed dormancy depth, seeds increase their sensibility to other breaking dormancy and germination factors. For example, light is considered an important environmental factor for releasing dormancy. Many species only break their dormancy by exposure to white light (i.e., sunlight), while other seeds only release dormancy by a change in photoperiod (i.e., length of the day).

Oxygen or carbon dioxide (soil gases) incorporated into the pores soil or dissolved in soil solution may affect the dormancy of the seeds. The seed responses to soil gases are highly variable and are dependent of the other environmental factors [2].

### 2.3 Germination factors

After dormancy release, nondormant seeds increase their sensitivity to the environmental factors favoring germination. To that end, GA stimulates germination in nondormant seeds by induction of hydrolytic enzymes, which stimulate the embryo growth, mobilization of endosperm storage reserves and weakening of tissues that are recovering the embryo [13]. Other phytohormones, such as ethylene and brassinosteroids, seems to be involved in some extension and limited impact on dormancy and germination, by reducing the influence of ABA effects in seeds [26, 46]. Indeed, exogenous applications of phytohormones as GA, cytokinins, or ethylene promoted germination in some species [47, 48].

Once phytohormones have induced seed sensibility, several environmental factors are involved on seed germination.

Temperature is a good seasonal indicator for seeds germination capacity and rate, although may induce secondary dormancy too. Usually, the temperature ranges for germinate are opposite to the ranges for release dormancy, as we described above. The range of temperature, in which seeds are able to germinate, falls into the next three categories: minimum, optimum and maximum. These ranges are related with the adaption of each species to their habitat and the favorable conditions for later seedling growth. As example, *Carex* sp. evidenced different temperature requirements for seed germination since they need a cold stratification for break dormancy but they are not able to germinate at low temperature. In fact, the best germination temperature was determined around 25°C [49]. Contrary, a study with 50 autumn-germinating species with after-ripening requirements, dormant or conditional dormant, demonstrated that they germinated only at low temperatures [50].

Water, particularly soil moisture, is an essential factor for seed germination. Water availability affects to the rate and speed of germination. The imbibition process, explained previously, allows the normal metabolic process resumption in nondormant seeds. In addition, it allows the radicle growth and elongation for break the seed coat [18].

Light is well known for stimulating germination in several species, since some nondormant hydrated seeds acquire high sensitivity to this factor after releasing their dormancy by after-ripening or chilling. The light requirement for germination prevents this process in unfavorable time or places for the seedling growth. Natural (fire) or cultivation (agricultural management) events caused soil disturbances, letting soil seed bank to be exposed to sunlight and favoring their germination [2].

Nitrate, nitric oxide and nitrites may stimulate the germination of many species. The ecological significance is that the seedling requires large amounts of nitrogen for optimal development. However, other germination factor/s may change the seed responsiveness to nitrate and their interaction regulates the germination response [51].

In conclusion, dormancy release and germination are sequential processes and it is too difficult to distinguish the end and the beginning of each one. It seems that seeds need opposite environmental conditions to germinate that those for release dormancy (i.e., temperature as explained above). In addition, the environmental requirements for germination are species-dependent. Therefore, the combinations of multiple factors (endogenous and environmental conditions) regulating seed dormancy and germination, makes so difficult to understand and predict the best germination conditions [2, 18, 37, 41].

## 3. Integrating information to understand dormancy and germination

Seed dormancy and germination are complex biological processes, as described previously. Understanding these processes and, subsequently predict and optimize them is a quite difficult task due to the high number of variables (factors) involved in dormancy and germination [7, 11]. In fact, using traditional methods, only a few number of factors can be studied simultaneously, so the fully understanding of dormancy and germination still remain a challenge.

Other limitation derived on the different kind of data generated by biological processes. Then the importance of each factor needs to be determined by diverse statistical analyses depending on data nature (binary, discrete, continuous, and so on). In addition, the traditional statistical analysis is quite limited to the possible interaction among factors and does not allow predicting their best factor conditions to optimize both processes. Therefore, for these kinds of processes, simple stepwise algorithms are useless and therefore, more complex analytical tools are required; such as multivariable approaches (networks) using computational models [52].

To develop a model some crucial steps are needed to be followed [7]:

1. Identify clearly the whole procedure (all steps) before built model.

2. Define and select the variables (factors and parameters) to study in the model.

3. Create a database with accurate data and select the kind of model to be built.

4. Validate the model to assure not significant differences among observed (experimental) and predicted (by the model) data.

Ishikawa diagram (**Figure 2**) is a useful chart to identify the causes of a specific event in order to fix some factors as controlled variables and select some as independent variables to be test experimentally [52]. This diagram shows the high
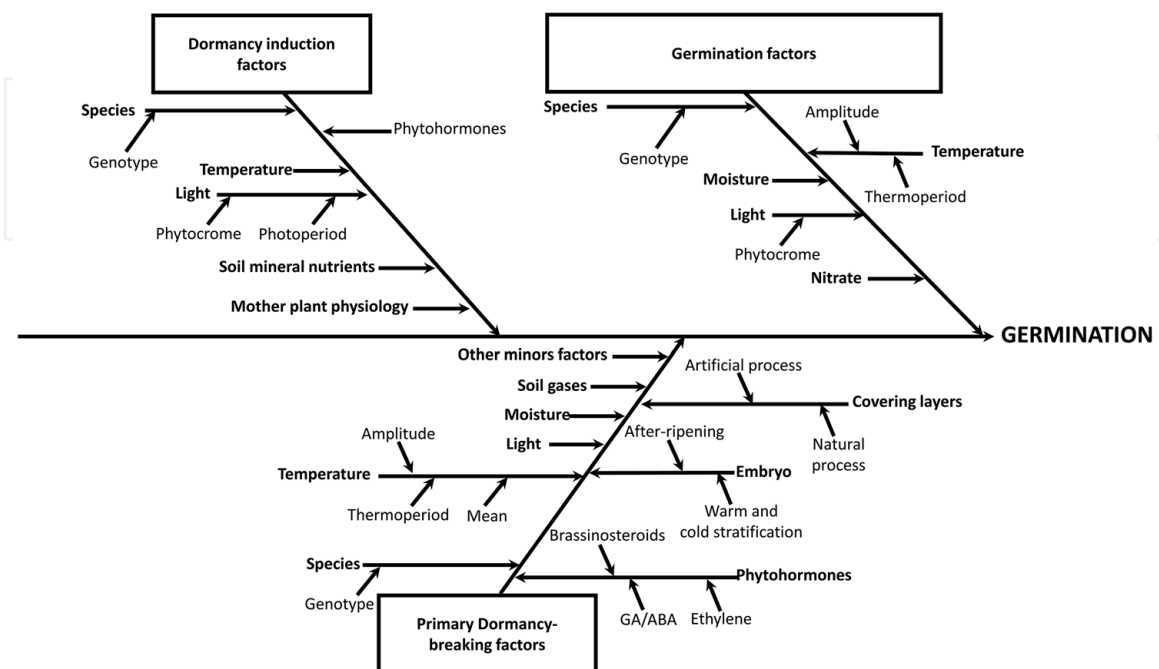


**Figure 2.**
*Ishikawa diagram showing the large amount of factors (endogenous and exogenous) involved in seed dormancy (induction and breaking) and germination.*

amount of factors and variables involved in the germination process and helps to identify some relationships among these factors overall process. After detecting the key variables (inputs) for germination, it is necessary to define the parameters for weight their effects (outputs) and an appropriate model. Although several models have been used to integrate data from complex biological processes, recent studies have shown the effectiveness of artificial intelligence tools as artificial neural networks (ANNs), genetic algorithms and neurofuzzy logic for modeling and optimize them [7, 12, 53, 54].

## 4. Artificial intelligence tools: from data to knowledge

Artificial Intelligence (AI) tools are problem-solving algorithms used when the number of solutions of one or several problems is huge, since they are capable of dealing with complex data in a versatile and powerful way [10]. This technology has been applied successfully to biomedical, pharmaceutical, and chemical applications for commercial and industrial purposes [8, 10, 55]. In addition, AI tools were used in basic and applied science research including ecology, environmental sciences, agriculture, food science, plant biology and biotechnology [9, 56, 57]. In this section, we summarized the most basic and important characteristics of the AI tools employed in plant biology [7].

### 4.1 Artificial neural networks (ANNs)

ANNs are AI tools that allow discovering nonlinear relationships among factors (inputs) and parameters (outputs) data. ANNs are one of the most effective method for revealing links among variables particularly if database are large and it is difficult to find direct relationships. In the case of germination (**Figure 2**), links between all factors and their effects on dormancy induction, dormancy-breaking and germination seems to be very complex, and difficult to be found using traditional statistical methodologies, but appropriate for ANNs. However, this technology has some limitations, related with the difficulties for results interpretation. In order to avoid this problem, ANNs are usually combined with other AI tools such as genetic algorithms or fuzzy logic (described below), which make easier the result interpretation [55, 58].

### 4.2 Genetic algorithms

Genetic algorithms (GA) are a heuristic algorithms based on genetic and natural selection. They are considered heuristic because generate useful solutions against problems. Concerning to plant biology, these algorithms have been used for optimization bioprocess [59]. Hybrids models between ANNs and GA have been performed allowing developing more accurate models for predict, optimize and control some biological process. Models achieved with this AI tools allowed determining the combinations of variables (factors or inputs) that provide the best results [7].

### 4.3 Fuzzy logic

Fuzzy logic tool is a computational tool, which allows analyzing and making deductions from uncertain or fuzzy data. Fuzzy logic assigns qualitative values using linguistic terms and degrees of membership called membership functions [7]. Interaction between linguistic terms (as low, medium or high) and membership functions allow the computer making meaning values to study [59]. Moreover, this

tool explains the behavior of a complex system using an easy language and improves description about any complex task or process. Knowledge acquired after modeling with a fuzzy logic-based system, is expressed by IF-THEN rules. These rules explain antecedent conditions of inputs variables (factors studied) and their consequent effect on the output variables (parameters measured) [7]. In conclusion, fuzzy logic tools have the ability for find consistent patterns or relationship between factors of complex process and generate understandable knowledge in an explicitly format (linguistic rules) [60, 61].

Fuzzy logic can be combined with ANNs forming neurofuzzy logic. This is a hybrid system that combines the adaptive learning capabilities from ANNs with the flexibility of representation of fuzzy logic [55].

## 5. Applications of artificial intelligence tools in seed germination and dormancy

AI is a novel technology in plant biology, and only a very scarce literature has published applying AI tools to germination and dormancy. However, these works are very interesting since they suggest that AI tools may predict and optimize germination and dormancy processes.

As previously discussed, a representative study of seed germination requires large experimental designs to include the effect of multiple factors (**Figure 2**). Therefore, the experimental design implies many treatments, replicates and, a huge number of seeds. However, not always is available a suitable size sample of seeds to draw clear conclusions. Under those circumstances, AI tools are an excellent alternative to conventional statistical methods. Advantageously, neurofuzzy logic technology allows working with not well-defined design spaces (reduced number of treatments) and different kind of data at the same time [62, 63].

The first papers published using AI in germination [64, 65], were devoted to predict field seed weed emergence, since is essential for minimizing economic losses and improve crop yield and management. Then, they really focus more in weed management and control than in elucidate the factors involved in seed weed germination. In those works, germination of the weed *Avena fatua*, was predicted with more accuracy with ANN models than with nonlinear regression analysis [64, 65]. In addition, those models were improved including some dormancy parameters such as after-ripening and implemented a genetic algorithm to optimize them. In this optimization process the mean square error between their experimental and training data were minimized. Therefore, they allow to obtain more parsimonious models and with better predictive capacity [66, 67].

Most germination research was carried out on seeds with commercial interest, however, many wild species, that never have been cultivated by humans, have deep dormancy and present seeds with underdeveloped embryos and with physiological dormancy. Moreover, some of those plants with poor germination are classified as vulnerable and endangered plants [68]. This is the case of *Eryngium viviparum* a threatened plant belonging to Apiaceae. This family has many species are well-known by a nonuniformity germination due to MD and MPD seeds. Recently [68], the hybrid neurofuzzy logic tool was used to decipher the relationship among several dormancy-breaking and germination factors (inputs) and several parameters (outputs), such as germination rate and embryo growth (E:S ratio). Neurofuzzy models allowed to found the most critical factors involved in the seed responses. In addition, IF-THEN rules pointed out the interaction of those factors to increase or promote the germination rate and the E:S ratio. The model revealed that the best germination rates were obtained with the combination of

1 mg L$^{-1}$ GA$_3$ (gibberellic acid) and high (24°C) incubation temperature and the combination of long incubation (20 weeks) and short warm (25°C) stratification periods (4 weeks) [68].

More recently, also neurofuzzy logic was used successfully in order to discover the critical factors that break dormancy and increase the germination rate in several kiwifruit cultivars, then allow to describe the best conditions for kiwifruit seeds germination [69]. The next factors were investigated: a) the effect of stratification time and type on dormancy-breaking and the effect of thermophotoperiod on germination. The results obtained demonstrated that neurofuzzy logic models greatly facilitate the data analysis and pointed out the critical role of cold-stratification time (long periods at 4°C) and stratification treatment (using gibberellic acid) on kiwifruit seed germination. In conclusion, neurofuzzy was able to model with high accuracy and predictability, to obtain a set of rules very useful for understand the cause-effect among the studied factors and dormancy-breaking and germination [68, 69].

## 6. Conclusions and future perspectives

Seed germination is a very complex bioprocess, dependent on many interacting factors. This kind of processes are not fully understood due to experimental limitations (low number of factors studied simultaneously or poorly designed experiments), which do not allow to study simultaneously all interactions among the factors involved. Currently, due to the emergence of computer-based technologies such as AI tools, those bottlenecks can be avoided. Artificial intelligence tools provide useful algorithms for studying complex processes, big datasets, being a quite novel technology in seed science.

In recent papers, ANNs combined with fuzzy logic had allowed to predict the germination of weed species, in a much easier way than traditional methods as statistical regressions. In addition, the use of ANNs combined with genetic algorithms allows to build up computer models to optimize, with high accuracy, the germination of these weeds, and hence, decreasing the economic losses in crop production. Moreover, the hybrid AI tool, neurofuzzy, demonstrated to be a successful technology to decipher the most critical factors and their interactions for increased germination and reduced the dormancy impact in some species.

In a near future, it seems that AI tools would be essential and very useful tools in germination studies, for the selection of most critical factors, with good accurateness in decipher the interaction between environment and physiological factors on dormancy and germination.

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## Conflict of interest

The authors declare no conflict of interest.

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