Review

Adaptation of Legume Seeds to Waterlogging at Germination

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Abstract: Legume seeds are often sown on standing rice crops a few weeks before rice harvest (relay cropping). Seeds cannot germinate in waterlogged soil under relay sowing as oxygen is depleted. However, seeds may survive under soil waterlogging if the seeds can initiate anaerobic respiration, have a large seed reserve such as carbohydrates, perform a slow water uptake during imbibition and are small in size. An example of a seed crop that can initiate anaerobic respiration is rice. The seed embryo of rice can use an alcoholic fermentation pathway from carbohydrates to produce enough energy to germinate. In legumes, seeds with a slow imbibition rate were more waterlogging tolerant than seeds with a rapid rate. This is likely due to seeds with low imbibition rates having less electrolyte leakage than seeds with a rapid imbibition rate during germination under waterlogging. A small amount of oxygen may remain on the surface of waterlogged soil. Small seeds can use the small amount of oxygen on the surface of waterlogged soil to germinate. However, large seeds often fail to use the oxygen on the surface of waterlogged soil to germinate because only a small part of large seeds remain on the surface of waterlogged soil. Therefore, small seeds are more adapted to soil waterlogging than large seeds under relay cropping. This review is focused on the physiological adaptation of legume seeds under low oxygen concentration during soil waterlogging.

Keywords: seeds; waterlogging; oxygen; germination; legumes

1. Introduction

Legume seeds are often sown two weeks before rice harvest (relay cropping) [1–4]. This relay cropping can increase legume production by using the soil moisture from the rice, while maintaining rice productivity [3–5]. However, legume seeds may fail to germinate under this relay cropping due to seed submersion at germination [4].

Oxygen is one of the main factors for seeds to germinate [6–8]. Oxygen dissolved in water enters the seed embryo through micropyle and/or seed coat [8–10]. Oxygen initiates aerobic respiration in the seed embryo and promotes germination [11]. Rate of oxygen absorption in the seed embryo is low at early germination stage (imbibition stage), but the rate slightly increases during mRNA transcription and is high toward the end of the germination stage [11]. The germination finishes when the radicle elongates and penetrates the seed coat [11]. However, seeds may fail to germinate when there is an absent or small amount of oxygen entering the seed embryo through the micropyle under anoxia and/or hypoxia [12]. Anoxia is a condition when oxygen is unavailable (0 kPa oxygen) and hypoxia is a condition when oxygen is limited (<20.6 kPa oxygen) for seeds to germinate [12,13].

Seeds of some species or genotypes may germinate and survive under soil waterlogging, anoxia and/or hypoxia [12,14,15]. In rice, for example, during seed submergence, rice seeds germinate and coleoptiles elongate to the water’s surface to supply oxygen to the seed embryo [15]. This long coleoptile develops an air canal, like a snorkel (aerenchyma tissue), to connect between air and the embryo, so the seed embryo of rice could survive, grow and become seedlings [15]. In legumes, seeds of soybean remained quiescent under low oxygen concentrations and germinated during drainage [13]. In comparison among grass pea genotypes, seeds of waterlogging-tolerant and waterlogging-sensitive grass pea germinate...
under hypoxia, but only seeds of waterlogging-tolerant grass pea survive during draining after hypoxia [16]. In addition, small seeds can be more tolerant to soil waterlogging than large seeds, probably because the small seeds are exposed to higher oxygen concentrations than large seeds during waterlogging under relay cropping [12,16].

In this review, aerobic respiration, seed imbibition, germination and soil condition under waterlogging will be explained. Morphological and physiological adaptation of seeds to survive under waterlogging will also be presented. This literature review aims to understand seed adaptation under waterlogging at germination so as to help farmers to identify the seed characteristics of waterlogging-tolerant genotypes and/or species at germination.

2. Aerobic Respiration

Aerobic respiration is one of the key processes during germination [17]. Aerobic respiration occurs when there is an interaction between glucose and oxygen [17,18]. The main products of aerobic respiration are energy (ATP), carbon dioxide (CO$_2$) and water (H$_2$O) [17,18].

There are three stages during aerobic respiration (Figure 1). First stage (glycolysis) occurs in cytosol and second stage (TCA cycle) occurs in mitochondria and both stages do not require oxygen, while last stage (electron transport chain) requires oxygen and happens in mitochondria [17]. Figure 1 shows that glucose is converted into pyruvate and energy in the form of ATP and NADH through glycolysis [19]. The pyruvate is then transferred to mitochondria and TCA cycle starts (Figure 1). The products of TCA cycle are carbon dioxide (CO$_2$) and energy in the form of ATP, and ‘reducing power’ in the form of NADH and FADH2. FADH2 from the TCA cycle and NADH from the glycolysis and the TCA cycle are converted into energy (34–36 ATP) during the last stage of aerobic respiration (Figure 1). A terminal electron acceptor in the last stage of aerobic respiration is used for receiving oxygen [17].

![Figure 1. Metabolic pathway of aerobic respiration. There are three main stages of aerobic respiration (glycolysis, TCA cycle and electron transport chain) which can produce energy (ATP). Oxygen is required in the last stage of electron transport chain, which produces the highest amount of energy (34 ATP). Glycolysis and TCA cycle do not require oxygen and produce less energy (4 ATP) than the electron transport chain. Glycolysis occurs in cytosol, while TCA cycle and electron transport chain happen in mitochondria [Adapted with permission from Ref. [18]. 2014, Knox et al.].](image-url)
Aerobic respiration produces optimum energy (38 ATP) for seeds to germinate [18]. However, the amount of energy in the form of ATP is small under low oxygen concentrations [17]. The low oxygen concentration can result in low germination percentages and seeds may even fail to germinate [12,20]. This germination failure is mainly caused by sub-optimal respiration (e.g., under hypoxia) that produces less than 20% of the energy than seeds under aerobic conditions [12,20,21].

3. Seed Imbibition

Imbibition can occur when dry mature seeds are exposed to water such as submergence [13]. Imbibition rate is rapid in the first 10 min of seed submersion [22]. The imbibition rate then slows and imbibition is complete after 5–10 h of seed submersion [22,23]. Seed coat plays a key role in the rate of imbibition and seedling growth development [24,25]. For example, in field pea, imbibition was completed in 8 h for seeds without a seed coat but when the seed coat was intact, imbibition took up to 12 h [24]. Moreover, seedling growth of seeds without a seed coat were less developed than seeds with a seed coat, which was probably due to an imbibition injury during early soaking [24].

The seed embryo needs oxygen to respire (aerobic respiration) [25–27]. Oxygen dissolved in water enters the seed embryo through the cell wall of the seed coat, micropyle and hilum during imbibition [28,29]. However, when the seed embryo receives a small amount of oxygen, respiration can be sub-optimal and the seed embryo may suffer [17]. The embryo may even die if there is too little oxygen (severe hypoxia) [25,26].

4. Germination

Germination occurs when mature seeds are exposed to sufficient oxygen and water [30,31]. After receiving adequate moisture and oxygen, mature seeds can start to respire and produce energy [30]. The energy, which is produced from carbohydrate degradation in the mature seeds, is used for radicle elongation [30,31]. Radicle that grows for more than 50 mm is then categorized as germinated seeds [12,16].

Germination can be divided into three stages (Phase I, II and III) according to the rate of water imbibition and oxygen needed in these three stages [11]. In Phase I, water and oxygen are absorbed rapidly into dry mature seeds during early imbibition (Figure 2). This rapid absorption causes a rapid outflow of solutes (e.g., N-acetyl phosphatidylethanolamine) from the seeds to the surrounding solution. The rate of oxygen consumption also increases to produce enough energy for germination in Phase I. In Phase II, the rate of oxygen consumption continues to increase, but the rate of water absorption is slow and new mRNAs and protein synthases such as germin are transcribed in the seed embryo as germination begins [11,32,33]. In the last phase, the radicle becomes visible and water and oxygen consumption rates increase [11,34].

The three phases during seed germination require oxygen and seeds may fail to germinate under low oxygen concentrations as protein synthase in the seed embryo is prevented [11,21]. Seeds of soybean and field pea, for example, fail to germinate under low oxygen concentrations, often due to the limitation of protein synthase during imbibition [21,26]. However, germination waterlogging-tolerant species such as rice are not inhibited by low oxygen concentrations [15]. The rice coleoptile can even grow up to the water surface and supply oxygen to the embryo of germinated seeds under submersion [15,35]. Therefore, oxygen is an essential factor for seeds of most crops to germinate but not for hypoxia and/or anoxia-adapted crops such as rice.
Figure 2. Oxygen consumption and water imbibition in the three phases of seed germination. The dashed line indicated the rate of water imbibition and the solid line showed the rate of oxygen consumption in aerobic conditions. The time on the X axis can be varied between hours and days [Adapted with permission from Ref. [11]. 1997, Bewley].

5. Soil Waterlogging

Waterlogged soil is often in hypoxic and/or anoxic conditions because of the rapid oxygen consumption by soil microorganisms and a slow oxygen diffusion from air to the soil surface in waterlogged soil [36–38]. Soil nutrient availability is also influenced by soil waterlogging [39–41]. The concentrations of soluble Fe, for example, increased by 30 times after 7 days of waterlogging but the concentration of Fe remained stable during 7 days of incubation in aerated conditions [39]. The low oxygen concentration and changes in aerated conditions in nutrient availability can cause germination failure, disturbance in plant growth and dysfunction of the root system [21,37,42]. In legumes, more than 70% of seeds failed to germinate after being exposed to soil waterlogging for 6 days [4,43].

Some oxygen may remain in a thin surface layer of waterlogged soil [12,44,45], which is caused by algae photosynthesis during illumination [46]. However, the thin layer of oxygen in waterlogged soils can soon be unavailable because microorganisms continue to consume oxygen in the dark, while algae stop photosynthesis [44,46,47]. Therefore, the remaining oxygen in the thin surface layer of waterlogged soil may not support seed germination under relay cropping due to oxygen unavailability during the dark condition (night).

Oxygen concentrations decrease with increased durations of soil waterlogging [12,44]. In a laboratory experiment, oxygen concentration was low at 2.3 kPa at a depth of 6 mm after 2 days of soil waterlogging and the oxygen became unavailable at the same depth after 4 days of waterlogging (Figure 3) [12]. In a glasshouse experiment on seedlings in a rice paddy, oxygen concentration was not available at 3.5 mm below the soil surface after 1 day of waterlogging [44]. The rapid reduction of oxygen concentration in the glasshouse experiment is caused by aerobic respiration of cyanobacteria and algae on the soil surface of rice paddy seedlings [44,46,47].
6. Morphological and Physiological Adaptation of Seeds under Low Oxygen Concentrations

Soil waterlogging or submergence causes hypoxia and/or anoxia [12,44]. Seeds often fail to germinate under submergence due to a lack of oxygen availability [12] (Figure 1; Table 1). However, some seeds may adapt to soil waterlogging and seeds may even germinate and survive after being exposed to anoxia [12–14]. There are morphological and physiological adaptations of seeds to germinate and survive under soil waterlogging, such as an initiation of anaerobic respiration and slow water uptake during imbibition.

Table 1. Germination percentage of different legume crops after being exposed to submergence or waterlogging in different durations.

| Grain Crops | Temperature (°C) | Duration of Submergence or Waterlogging (d) | Germination Percentage (Relative to Total Seed Sown) | Reference |
|-------------|------------------|--------------------------------------------|-----------------------------------------------------|-----------|
| Faba bean   | 25               | 4                                          | 38                                                  | [14]      |
| Field pea   | 25               | 8                                          | 54                                                  | [4]       |
| Grass pea   | 25               | 6                                          | 50                                                  | [16]      |
| Soybean     | 25               | 3                                          | 38                                                  | [13]      |

6.1. Seed Reserve Metabolism

Seed reserves, such as carbohydrate (starch) in the seed endosperm, are critical for seeds to germinate and survive under any unfavorable conditions such as waterlogging [15,48,49]. Rice seeds with high starch content in the seed endosperm, for example, were more adapted to low oxygen concentrations than seeds with low starch content [48].

The degradation of carbohydrates in the seed endosperm into glucose and glycolysis provides substrate and energy for respiration and germination [21,50]. Waterlogging-sensitive species such as soybean cannot degrade glucose under anoxia, so the seeds fail to germinate [20,50,51]. However, waterlogging-tolerant species such as rice can degrade glucose into energy without oxygen via rapid alcoholic fermentation (anaerobic respiration) [21,50]. Therefore, waterlogging-tolerant species can germinate under severe hypoxia and/or anoxia.
6.2. Anaerobic Respiration

Aerobic respiration is inhibited under anoxia [17]. However, waterlogging-tolerant seeds can carry out anaerobic respiration and produce enough energy to germinate under anoxia [15,52]. An embryo of rice seeds, for example, uses the enzyme pyruvate decarboxylase (PDC) to convert pyruvate into ethanol and acetate, which can produce enough energy in the form of NAD+ and NADH, to germinate under anoxia [50] (Figure 4). The conversion of pyruvate into ethanol is more common than conversion into acetate as the conversion of pyruvate into ethanol does not decrease pH in the cell of the seed embryo during anaerobic respiration [53]. Moreover, proteolytic enzymes, which support seeds to germinate, are inhibited at low pH, so seeds fail to germinate if the cell pH is not constant [54].

![Figure 4. Anaerobic respiration of rice under anoxia. The enzyme abbreviation of PDC, ADH and mALDH are pyruvate decarboxylase, alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase, respectively [Adapted with permission from Ref. [50]. 2013, Miro and Ismail].](image)

6.3. Water Uptake

Water uptake differences are found between seeds that tolerate waterlogging and seeds that are sensitive to waterlogging [55,56]. In soybean, water uptake of waterlogging-sensitive genotypes was three times faster than of waterlogging-tolerant genotypes in the first hour of seed soaking [55].

The slow rate of water uptake in waterlogging-tolerant genotypes can be associated with seed color [56,57]. The dark seed coat of waterlogging-tolerant genotypes often contains a high concentration of phenolic compounds (e.g., melanin) [57,58]. Moreover, the seed coat compounds reduce the rate of water absorption and imbibition injury during early soaking [58]. Therefore, the concentration of phenolic compounds in the dark seed coat can be used to identify seeds of waterlogging-tolerant genotypes [56–58].

The rate of water uptake during seed soaking can relate to the thickness of aleurone layer under the seed coat [55]. Waterlogging-tolerant genotypes often have a thicker aleurone layer than waterlogging-sensitive genotypes [55,59]. The thick aleurone layer in waterlogging-tolerant genotypes can protect seeds from high solute leakage, which can result in imbibition injury during early seed soaking [55,59]. Moreover, seeds with a thick aleurone layer can reduce the rate of oxygen diffusion into the embryo of the seed, which can result in the delay of seed germination of waterlogging-tolerant soybean [55,59].
6.4. Seed Size (Relation between Seed Size and Oxygen Availability to Support Aerobic Germination)

Small seeds were recorded to be more adapted to soil waterlogging than large seeds [4,16,60] (Table 2). Oxygen concentration in waterlogged soil decreases with the rise of soil depth (Figure 3). Small seeds are exposed to high oxygen concentrations at shallow soil depths, while large seeds are exposed to low oxygen concentrations at deeper soil depths [12,44]. Moreover, small seeds have a larger seed surface area to volume ratio and a shorter distance of oxygen and water diffusion from seed coat to seed embryo than large seeds [61]. The large surface area to volume ratio and a short distance of oxygen and water diffusion in small seeds can result in a high oxygen concentration in the embryo of small seeds, which can support aerobic respiration to produce enough energy for seeds to germinate [10,61]. Therefore, small seeds are more tolerant to soil waterlogging than large seeds under relay cropping [4].

Table 2. The relation between waterlogging tolerance and seed size (100 seed weight) of different crops in various durations of waterlogging.

| Crop Species | Waterlogging (d) | Seed Size (100 Seed Weight g) | Germination or Emergence (%) | Treatment of Soil Waterlogging | Reference |
|--------------|------------------|-------------------------------|-------------------------------|-------------------------------|-----------|
| Field pea    | 14               | 11.2                          | 50                            | Relay cropping                | [4]       |
|              |                  | 24.2                          | 10                            |                               |           |
| Grass pea    | 6                | 8.9                           | 60                            | Water level maintained above the soil surface at 9 mm | [16] |
|              |                  | 15                            | 14                            |                               |           |
| Lentil       | 6                | 1.8                           | 30                            | Water level maintained above the soil surface at 10 mm | [43] |
|              |                  | 4.6                           | 0                             |                               |           |
| Soybean      | 10               | 6.2                           | 14                            | Water level maintained above the soil surface at 20 mm | [60] |
|              |                  | 15.7                          | 5                             |                               |           |

Note: Percent germination was shown after the correction from the number of germinated seeds under control condition for field pea, grass pea and lentil, while germination percentage of soybean was relative to total seed sown.

7. Conclusions

Legume seeds may fail to germinate under relay cropping. However, seeds of some genotypes or species can have particular morphological and physiological characteristics to adapt to sub-optimal conditions such as waterlogging. Seeds, which are able to carry out anaerobic respiration, can produce enough energy to germinate. Seeds with a large portion of carbohydrate are more waterlogging-tolerant than seeds with a small portion of carbohydrate because carbohydrate in the seed is used as a source to produce energy to germinate by converting the carbohydrate into glucose, pyruvate acetaldehyde and acetate. Slow water uptake in the early seed submerge can also identify seeds that tolerate waterlogging. A slow rate of water uptake reduces the amount of solute leakage during imbibition. Under relay cropping, where seeds are exposed to low oxygen concentration, small seeds are more tolerant to waterlogging than large seeds because the small seeds have more access to use remaining oxygen on the soil surface of waterlogged soil than large seeds.

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