Mechanisms of plasma-seed treatments as a potential seed processing technology

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Abstract
Plasma treatments are currently being assessed as a seed processing technology for agricultural purposes where seeds are typically subjected to pre-sowing treatments to improve the likelihood of timely and uniform germination. The aim of this review is to summarize the hypotheses and present the evidence to date of how plasma treatments affect seeds, considering that there is difficulty in standardizing the methodology in this interdisciplinary field given the plethora of variables in the experimental setup of the plasma device and handling of biological samples. The ever increasing interest for plasma agriculture drives the need for a review dedicated to seeds, which is understandable to an interdisciplinary audience of biologists and plasma physicists. Seeds are the first step of the agricultural cycle and at this stage, the plant can be given the highest probability of establishment, despite environmental conditions, to exploit the genetic potential of the seed. Furthermore, seedlings seem to be too sensitive to the oxidation of plasma and therefore, seeds seem to be the ideal target. This review intentionally does not include seed disinfection and sterilization due to already existing reviews. Instead, a summary of the mechanisms of how plasma
may be affecting the seed and its germination and developmental properties will be provided and discussed.

Introduction

The motivation driving plasma-seed treatment research is the importance of food. The world population is projected to increase to 10 billion by 2050 and even without increasing the food supply, it is necessary to maintain the current food production and quality (FAO, 2017). Most food begins with planting seeds.

The aim of this review is to centralize the hypotheses and present the evidence to date of how plasma treatments affect seeds, considering that there is difficulty in standardizing the methodology in this interdisciplinary field given the plethora of variables in the experimental setup of the plasma device and handling of biological samples. Considering that plasma agriculture has been gaining more attention recently, it is therefore useful to have a review dedicated to seeds, which is understandable to an interdisciplinary audience of biologists and plasma physicists.

The review is organized as follows. Seed structure and development will be presented in section 1 to establish a common ground, followed by a summary of current techniques used to improve seed survival in section 2. In section 3, plasma will be introduced and the motivation for its application on seeds followed by a corroboration of recent research to provide an overview of physical, chemical and biological mechanisms that can be triggered by plasma components in section 4.

1. Seed structure and development

Although very diverse, all seeds have generally evolved to contain all their needs to develop into plantlets once the environmental conditions are perceived as appropriate. The living tissues of seeds are protected by the seed coat (testa), which
can vary between species and cultivars, or depend on the plants being fertile or clones unable to produce the next generation. Inside the dry mature seed, the embryo is in a partially desiccated, quiescent state, poised to germinate upon the addition of water or in other words, imbibition. It is then provided with stored foods through the endosperm during germination, a process which is the transition from an inactive to active seed that grows, ruptures the seed coat and develops from a seedling first into a plantlet, which is generally still frail and particularly sensitive to external stresses, to then become a more stress-resistant autotrophic plant.

The trigger for germination requires a number of external parameters to be met, which will vary from seed to seed, but are generally a combination of water, temperature and light. Additionally, each seed has different requirements due to structural differences, particularly in the seed coat. Imbibition is the first step where the seed undergoes three stages with initially rapid, then slow and finally rapid water uptake. Water reactivates the enzymes that can repair DNA and membrane damages by using pre-existing RNA transcripts produced during seed maturation, and activates enzymes involved in beta-oxidation and amylases to break down stored oil and starch into sugars for energy and cell wall production. Proteases break down storage proteins into amino acids for protein synthesis.

After the third water uptake stage, the seed is swollen since the tissues have expanded and the embryo grows. The first visible sign of germination is the protrusion of the radicle, which later becomes the root. From there, the hypocotyl, which connects the root and shoot, hooks out and brings out the shoot with the cotyledon/s, then the true leaves. Once photosynthesis starts, the plantlet can grow independently from prior storages of organic matter and will only take nutrients and water from the soil and the surrounding media.
Prior to germination, there are hormones and inhibitors that prevent the process, to ensure the right environment and maximize the probability of the germinated seed to survive and thrive. The hormone abscisic acid (ABA) is known for its role in maintaining dormancy and for inhibiting germination. When this is removed by a lengthy water imbibition, another hormone gibberellic acid (GA) is produced and germination begins. As the embryo grows, both auxin and cytokinin are involved in cell expansion and cell division, respectively, whereas later during the stressful life of the plant, hormones such as salicylic acid, jasmonic acid and ethylene will play a role in plant defense to protect it against abiotic stresses such as cold, heat, dehydration, and biotic stresses, such as herbivores, viral, fungal and bacterial pathogens. More information about plant defense can be found in (Andersen et al., 2018).

2. Seed performance

The best chance for survival is to provide the seed with an opportunity to germinate from the very beginning, assuming they are not dead, and for food production, there are additional criteria beyond successful germination which are germination uniformity and rate for a single harvest when done on an industrial scale. For this reason, many centuries ago, a method called priming was developed to ensure a more uniform and faster germination.

Seed priming is a method which can increase plant growth parameters, such as germination rate and uniformity and contribute to higher yields and greater plant resistance. As reviewed by Pawar and Laware (2018) and Lutts et al. (2016), the concept of priming is to provide water and activate the metabolism of the seed to repair damage before continuing the embryo development and the emergence of the root.
Priming is frequently applied using water (hydro-priming), which requires soaking the seeds for a given timeframe. This water treatment can be modified to ameliorate germination rate, efficiency and uniformity with the addition of salts (halo-priming), solutes to change the osmotic pressure (osmo-priming), micronutrients like boron and iron (nutri-priming), hormones like gibberellic acid (hormonal priming) or beneficial microorganisms, such as *Pseudomonas* species (bio-priming), and metallic nanoparticles like iron and silver (nano-priming). Priming can also be done without water by using a solid and non-soluble material such as sand or clay called matrix priming (Pawar and Laware, 2018).

Depending on the seed and its structure, it can be primed with wet treatments by soaking in cold, warm, boiling water or dry treatments using dry heat or microwaves. Seeds can also be primed using acid scarification and physical scarification. Seeds, specifically their seed coats, can also be modified with compounds such as selenium or salicylic acid (Wang et al., 2016) or agents that are protectants, nutrients, symbionts, soil adjuvants (hydrogels) and colorants (Pedrini et al., 2017). Protectants, such as pesticides, and colorants make up the bulk of coatings and are applied mostly to crops and vegetables to mainly deter insects (44%), weeds and fungi (Aktar et al., 2009).

Pesticides in the seed coatings can transfer into the soil through rain and enter the groundwater and wastewater treatment plants. Since these compounds are highly toxic, persist for a long time and become more toxic with time when held in storage, remediation methods, used to remove toxic chemical compounds, can be done using with microorganisms, clay, polymeric materials or UV-\(\text{H}_2\text{O}_2\) and UV- ozone, and hydroxyl radicals and at times, even require additional water resources (Marican and Duran-Lara et al., 2018).
Although protective coatings are important and occupy a multi-billion-dollar market, the amount of investment in remediation techniques highlights that it would be beneficial to consider alternatives to minimize pesticide use. For this reason, alternatives have been considered such as biocontrol using fungi or bacteria, biopesticides derived from natural compounds such as grapefruit seed extract (Choi, 2017), physical methods such as ultrasound (Tito, 2017; Guo et al., 2018) as well as genetic engineering (Paoletti and Pimentel, 2000). It would also be ideal to find a method with minimal energy consumption in view of energy savings considering that energy input is five to ten times greater than the output in the form of food in North America (Lockeretz, 2012).

3. Non-thermal plasma (NTP) as a seed processing technology

Ideally, more effective solutions for seed treatment to ensure rapid and uniform germination should not include toxic residues, consume little energy, have low penetration depth to avoid injuring cells, and favor long storage time (Hussain et al., 2015), while subsequently supporting optimal seed development. These criteria can possibly be met with non-thermal plasma (NTP) treatments.

Plasma is an ionized gas that can be ignited under low or atmosphere pressure conditions. The plasma composition depends on the operating parameters such as voltage, frequency, humidity, flow rate and gas mixture. Gases such as argon, oxygen, nitrogen, helium, and/or air can be ionized by electric fields to form electrons, ions, UV, thermal radiation and reactive species. Specifically, air plasmas contain reactive oxygen species (ROS) such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^-$), singlet oxygen ($^1$O$_2$) and ozone (O$_3$) and reactive nitrogen species (RNS) such as nitric oxide (NO$^-$), peroxynitrite and nitrogen dioxide radical (NO$_2^-$) (Li et al.,
Han et al. (2019) and Laroussi et al. (2020) describe the types of plasma treatments in detail. The seeds and seedlings can be treated directly with the plasma or indirectly at a distance away from the plasma. They can also be soaked or watered using liquids exposed to plasma. These liquids are considered as plasma-activated media (PAM) and if water is used, it is called plasma-activated water (PAW). Comparing the gaseous and aqueous treatments, similar effects on macroscopic plant properties have been reported (Sivachandiran et al., 2017).

Plasma treatment may enhance seed survival without toxic residues since all the constituents in plasmas may be found in nature and recombine shortly afterwards. Moreover, components in plasma have approximately 10 nm deep penetration, limiting it to surface functionalization (Guo et al., 2018). Importantly, plasma treatments are considered to be low maintenance with low energy costs (Randeniya and de Groot, 2015). Therefore, many studies have been performed involving plasma treatments of agronomic interest, such as quinoa, basil, tomato, wheat, radish, soybean, mung bean, rice, Ajwain and Umbu and seeds deemed important for the landscape like Norway spruce (Pauzaite et al., 2018).

Randeniya de Groot (2015) and Puac et al. (2018) reviewed the observed effects on germination and subsequent plant growth. The extensive list of these effects in this review has been summarized in Figure 1. It can increase germination probability and biomass (Măgureanu et al., 2018) or increase disease resistance or stress resistance (Jinkui et al., 2018; Wu et al., 2007). Additionally, it can accelerate or delay germination and subsequent development (Volin et al., 2000), decrease water consumption (Bormashenko et al., 2012), decrease levels of microbial pathogens or insects (Shintani et al., 2007; Shintani et al., 2010), without detectable toxic residues (Sivachandiran et al., 2017). Molina et al. (2018) proposed to make a hydro-absorbant
polymer coating with short plasma treatments on specific seed types, and Kopacki et al. (2017) suggested to use plasma for seed coatings against fungal pathogens.

Other than treating the seed or plant directly, plasma treatment can also be used on the plant’s surroundings by degrading volatile organic compounds in the soil to improve soil health (Chen et al., 2009; Stryczewska et al., 2005). It may also have potential as an alternative to fungicide (Pérez-Pizá et al., 2019) and be implemented in industry as field studies have been shown (B. Zhang et al., 2018; Li et al., 2016).

Table 1 shows the legend describing the categories of Table 2, which is a compilation of papers described in four categories: seed coat modification, growth parameters, metabolism and disease or stress resistance and further divided by the scale of information i.e. macroscopic, microscopic or molecular properties. Seed disinfection and sterilization were intentionally left aside and can instead be found in more detail in the food processing field (Butscher et al., 2020).

4. Mechanisms of how non-thermal plasma affects seeds and their subsequent development

Quite consistently, most reports refer to optimized plasma setups that can significantly change germination and plant growth parameters. For example, germination rate may be accelerated, shoot and root lengths may be longer (Jiang et al., 2018) and more branching of the roots (Măgureanu et al., 2018) or stronger root system (Mildažienė et al., 2017) were reported although it is difficult to know the causative agent behind these effects due to varying plasma-seed treatment methodologies.

Not all observe the same effects and instead, plant growth can be improved without changing germination rate (Sidik et al., 2019) or scientists have strong variation in their experiments (Hosseini et al., 2018; Mildažienė et al., 2019a). This could mean
that further optimization is required, which is time-consuming considering the number of variables in the experimental design. It is not yet clear by which mechanism(s) these effects arise but understanding this will simplify the experimental design and potential scale up for the future so that the results are reproducible. Here, we take a closer look at the physical, chemical, and biochemical factors derived from plasma treatments to have a more detailed understanding of what is happening to the seed. A summary of the plasma-seed interactions at the seed surface and at a molecular level are given in Figure 2 and Figure 3, respectively.

4.1 Physical factors

The physical factors such as heat, ultraviolet and electromagnetic fields and mechanical scarification are the first contact point with the seed coat and may then trigger downstream consequences from this initial interaction. In the following section, each physical factor and its effect will be presented individually.

4.1.1 Heat

The temperature of plasma treatments can range from room temperature up to 90°C, so in principle it is possible to have an effect depending on the temperature and treatment time although not many authors think that increased temperature is responsible for the changes in plants. Temperature is monitored in a few studies by measuring the electrode temperature, calculating the gas temperature from spectra or measuring the temperature of the seed directly using an infrared camera or thermocouple (Lotfy, 2017; Kobayashi et al., 2020) but it has not yet been done on a molecular level by assessing the seed or plant response. Kitazaki et al. (2014) compared plasma and heat treatments by heating the seeds on a hot plate but did not see the same effect on the plant development.
Others have tried to look into heat shock proteins (HSPs), which are induced with temperatures ranging from 31 to 37°C, but HSPs are often not exclusively induced by heat. They may also accumulate under oxidative stress, high intensity irradiation, and desiccation (Al-Whaibi, 2011). Iranbakhsh et al. (2018) treated wheat seedlings with plasma and observed increased expression in heat shock factor A4A in both the root and shoot system in correlation with an increase of growth parameters and a mitigating negative effect of salinity stress. They only reached a maximum of 29°C in a 2-minute plasma treatment, suggesting that heat alone is not responsible for the observed effects in this particular study but the role of HSPs during plasma treatment remains unclear since it was shown not to change in the study by Mildažienė et al. (2019).

4.1.2 Ultraviolet light (UV) and electromagnetic fields (EMF)

The role of high-energy photons in plasma-seed treatments has been controversial since UV has had a negligible effect so far but the effects of UV on seeds and plants could theoretically contribute to the wettability or growth enhancement effects indirectly; for example, by producing radicals or reactive oxygen species (ROS) such as ozone (Lotfy et al., 2019). Gao et al. (2019) checked the effects of UV separately from plasma and observed that UV had only a minor contribution towards the seed wettability. Sarinont et al. (2016) also reported that there was no effect from UV when they saw the lack of growth enhancement effect after they blocked UV with a quartz plate.

It is known that just UV-B can accelerate germination of safflower seeds but then negatively affects growth (Farokh et al., 2010). Noble (2002) made the same conclusions with kale, cabbage, radish and agave seeds. Likewise, Sadeghianfar et
al. (2019) showed accelerated germination of maize and sugar with UV-C treatment but instead, saw an increase in plant growth parameters and suspected this may be due to breaking down the seed coat and increase in temperature.

There may be differences in effects depending on the wavelength since it has been shown that UV-A had a more pronounced effect than UV-C but both were able to accelerate the germination rate and improve growth parameters (Ibrahim et al., 2012). On the one hand, UV is often associated with inducing DNA damage. Prakrajang et al. (2020) compared gamma radiation with plasma and saw only with gamma irradiation that the plants did not grow well and thus suspected it was induced DNA damage. On the other hand, plants treated with UV were able to better cope with drought stress, possibly by activating DNA repair mechanisms (Caldwell et al., 1998). This may be due to an increase in phenolic compounds, which often have a role in disease or stress resistance and this response can be triggered by intense UV light which is accompanied by high temperature and photo-oxidative damage in nature (Veberic, 2016).

Babajani et al. (2019) and Iranbakhsh et al. (2017) both considered that plasma-derived UV may be detected by photoreceptors which then might affect secondary metabolism and trigger stress responses if the treatment is done briefly. UV is also linked with photomorphogenesis and cell elongation, division and differentiation (Huché-Thélier et al., 2016). Iqbal et al. (2019) compared laser and plasma treatment separately on seeds and saw similar types of effects: damage to the seed coat, an increase in water uptake and protein content.

Pauzaite et al. (2018) and Mildažienė et al. (2019) also attributed the changes in growth parameters to radiation since Mildažienė et al. (2019) saw similar protein
expression profiles when comparing seeds treated with plasma or electromagnetic fields (EMF). This is not entirely surprising since pulsed electric fields also affect seed germination (Su et al., 2015; Dymek et al., 2012). It needs to be kept in mind though that electric field treatment is also accompanied by oxidative stress and ozone (Teissie et al., 2005) where ozone has been suggested by Patwardhan et al. (2013) to be the main effective parameter in the treatment.

Static or alternating magnetic fields can also change germination probability, growth rates, increase root and shoot length, change redox status of plants possibly by increasing hydrogen peroxide ($H_2O_2$), altering photosynthesis, alleviating drought stress or increasing mineral content (Maffei et al., 2014; Bilalis et al., 2012). Arguably, UV may have a role but a minor contribution in terms of direct effect considering that the above studies used several hours long UV treatments to have an effect whereas most plasma treatment are in the seconds or minutes range. Nevertheless, photons and electromagnetic fields can still possibly contribute indirectly through the production of RONS.

4.1.3 Mechanical scarification and erosion

In terms of mechanical effects, it has been suggested that altering the seed coat may play a role in modifying germination rate. Typically, a seed has four layers: the cuticle, epidermis, hypodermis, and parenchyma. The differences in the microstructures and chemistry of these layers determine differences between species and even cultivars. The seed coat role is like a water modulator; it controls the entry of water so it can be absorbed slowly by the cotelydons to minimize or avoid imbibition damage (Souza and Marcos Filho, 2001).
It has been suggested that the removal of the lipid layer allows for better access to water, a requirement for triggering germination (Sehrawat et al., 2017). Bafoil et al. (2019) showed the importance of the seed coat by using mutants of *Arabidopsis* plant model Ler and Col-0 ecotypes and showed the rearrangement of lipid components, changes in lignin and seed coat erosion. Many, although not all authors have observed with scanning electron microscopy (SEM) that seed surfaces treated with plasma have an eroded appearance (Y. Li et al., 2017; Junior et al., 2016; Stolárik et al., 2015; Gao et al., 2019; Wang et al., 2017; da Silva et al., 2017), where Mildažienė et al. (2016) only saw etching on the seed surface facing plasma treatment and others did not see any changes (Sera et al., 2010; Bormashenko et al., 2012; Kitazaki et al., 2014).

A majority of these authors have been able to correlate these changes with increased water uptake. For example, Pawlat et al. (2018) observed that plasma treatment changed the seed structure by removing the upper cuticle layers covered with wax, a polymer present to prevent water loss under heat stress, and may form micro-pores to aid water absorption. Bafoil et al. (2019) also worked with an *Arabidopsis* mutant *gtap5*, which is not able to make suberin or cutin and saw that plasma was not able to improve the germination without these polymers in the seed coat, suggesting their importance. This waxy layer was even considered as a parameter in the experimental design of a study done by Park et al. (2018). They used two seed types, one with and the other without a waxy layer, and their results indirectly implied that this waxy layer plays a role in the effect that plasma treatment will have on the seeds, suggesting plasma indeed may be improving the seed coat permeability, considering wax limits water loss and controls gas exchange.

Billah et al. (2020) pointed out that there may be exothermic reactions from the plasma that release heat and may melt the wax due to its low evaporation temperature.
of 37°C or eroded by reactive species. This was also pointed out by Holc et al. (2019). Wang et al. (2017) also suggested that through surface modification via etching, the seed is able to absorb water through increased hydrophilicity. This enhanced water absorption by modifying the seed structure is further supported by others who observed the degradation of cellulose on seed surfaces (Yamauchi et al., 2012). Currently, the analysis of components with FTIR-ATR is limited to cellulose as Pawlat et al. (2018) expressed the difficulty in differentiating between different organic plant components like cellulose, hemicellulose, pectins, etc. Wang et al. (2017) used both FTIR-ATR to measure seed surface changes as well as FTIR to analyze the gas exhaust from the plasma treatment. They too needed to omit wavenumbers i.e. peaks below 1500 cm\(^{-1}\) that were difficult to assign to specific functional groups during analysis but also suggested that the spectral bands are mainly attributed to cellulose rather than wax.

Junior et al. (2016) showed that water may be guided differently after plasma treatment due to surface modifications. They observed that the hilum increased the amount of water absorption, the micropyle had a more open configuration and in particular, the water absorption was improved mainly through the hilum rather than micropyle. In any case, by thinning the seed coat, it is logical to assume that water will be more readily absorbed.

Interestingly, the water absorption can be controlled by the plasma treatment using different gases and coating thickness as mentioned by Volin et al. (2000). Depending on the working gas, 0.5 - 2 \(\mu\)m thick coatings were applied and their thickness modified imbibition. This suggests that the seed coat thickness can be mechanically modified by etching or by changing the chemical properties.
Despite adding to the thickness with an additional layer of coating, germination was improved in a few instances, which highlights the importance of chemistry, but it is difficult to separate the etching effect from the chemistry in this study. As it still remains, it is not known yet whether this increased permeability is principally due to mechanical mechanisms such as etching as Pawlat et al. (2018) suggested, a combination of both mechanical and chemical as mentioned by Gómez-Ramírez et al. (2017), Tounekti et al. (2018) and Park et al. (2018) or solely due to chemistry.

SEM is an insightful tool, which can provide quick qualitative results of the plasma-seed treatment. Nevertheless, caution should be exercised when interpreting SEM images because plant genetics influence the seed coat pattern. Otherwise, observed changes might already be pre-existing and should instead be attributed to biological variation rather than plasma treatment and therefore, using the same seed before and after treatment is recommended.

Pawlat et al. (2018) noticed that the seed shape influences the type of change affecting the seed and showed in their SEM images that the seed edge was torn off whereas the middle grew in sharpness. This brings attention to the fact that this process needs to be delicately handled. For example, Cui et al. (2019) used tape to prevent the movement of seeds during the plasma treatment but this may affect the seed coat especially if it is done in the presence of moisture. As a result, it seems that there is a limit to how much information can be extracted from surface analysis using microscopy. Instead, it would be useful to further explore the chemical modifications since this is currently limited at the moment in the literature.

It may very well be that the chemistry is sufficient to affect downstream processes considering that an effect in growth parameters without visible seed coat
modifications have been observed (Los et al., 2019). Mildąžienė et al. (2017) also observed positive growth effects using vacuum and EMF without visibly changing the seed coat structure using SEM. It may also be the case that the results will depend partly on whether there is physical or physiological dormancy meaning whether dormancy is due to the seed coat or embryo.

4.2 Chemical factors

On the one hand, etching may play more of a role in certain seed types with impermeable seed coats but, on the other hand, it may be that a specific concentration and/or mixture of reactive species from a higher power plasma is required to observe an effect. In the latter, mechanical damage might merely be a side effect, which too can contribute to enhanced water absorption but is not the primary factor. For this reason, chemical factors will now be discussed in the next section.

4.2.1 Nutrient absorption

In addition to analyzing mechanical damage, SEM can be coupled with energy dispersive X-rays (EDX) to look at the composition of seed surface. Other methods such as X-ray Photoelectron Spectroscopy (XPS) and micro X-ray fluorescence spectroscopy (μ-XRF) can also be used to acquire information about the elemental distribution. It has been observed by several authors like Pérez-Pizá et al. (2019) and da Silva et al. (2017) that lipid layers undergo chemical oxidation, which can therefore improve interaction with water.

Shapira et al. (2018) found that irreversible wettability is not due to electric charging, which could indirectly imply that it may be done chemically. Pérez-Pizá et al. (2019) also correlated the increased hydrophilicity with oxidized seed using nitrogen and oxygen plasmas. This perhaps suggests that hydrophilicity again is done through
chemical rather than mechanical changes. This may not be entirely dependent on the
gas type but rather about producing an appropriate profile of reactive species at
sufficient concentrations that will oxidize the outermost lipids. It has been shown by
Gómez-Ramírez et al. (2017) using XPS and EDX that plasma treatment can
oxygenate carbon and deposit nitrogen groups on the seed surface. They
hypothesised that these elements, as well potassium, are later absorbed by the seed
in the presence of water (Cakmak, 2005).

Ambrico et al. (2019) instead used μ-XRF and showed the concentration and
redistribution of macro- and micronutrients such as potassium after plasma treatment.
As an example, on the one hand, the diffusion of potassium into the seed interior may
improve the germination through enzyme activation, help with water retention, or
detoxify ROS (Wang et al., 2013). On the other hand, potassium at the surface can be
interpreted as seed damage since it may be used as an indicator of cell membrane
integrity (Miguel and Marcos Filho et al., 2002). Interestingly, Zhou et al. (2016)
exposed seeds to plasma-treated water and they had the lowest leakage rate by
measuring electrical conductivities, which is used to measure the cell membrane
integrity. This mobilization of nutrients perhaps is not lost to the environment but is
loosened and absorbed immediately by the seed (Carvalho et al., 2009).

4.2.2 Gas exchange

When considering gases, it could be that oxygen on the surface is responsible
for not only increased wettability but may also assist in seed respiration. Considering
that oxygen is another requirement for germination, Sarinont et al. (2016) observed
oxygen, NO and nitrogen gas to be the most effective for plant growth but fumigation
with oxygen gas already had an effect on growth parameters, albeit mild compared to
plasma. This fumigation might be sufficient to improve plant growth like in the case of nanobubbles (Ahmed et al., 2018). Although fumigation had a positive effect on plant growth, plasma application had a stronger effect which may be due to the transport process being more efficient i.e. speed up the process by not relying on diffusion or provide more directionality. This highlights the presumably overlooked importance of oxygen especially considering that Rahman et al. (2018) saw also more obvious changes in growth parameters when using an oxygen admixture instead of air.

This principle could likewise work for nitrogen gas but instead of modifying respiration, it may be directed towards other cellular processes such as photosynthesis since it is a major component of chlorophyll or protein synthesis (Leghari et al., 2016). As mentioned in the paper of Gao et al. (2019), the presence of CO and O$_2^+$ signals confirmed that chemical etching of the seed surface by plasma played an important role in stimulation of seed germination (Filatova et al., 2011). These chemical changes may then be responsible for changing the biochemistry and molecular events in the plant and therefore, these will be discussed in the next section.

4.3 Biochemical and molecular factors

4.3.1 Chemical species

4.3.1.1 RONS and seed coat interactions

Regardless of whether the changes to the seed coat surface might loosen an elicitor such as oligosaccharins, as mentioned by Iranbakhsh, Ardebili et al. (2018), many authors are in agreement and speculate that it is principally reactive oxygen and nitrogen species (RONS) that trigger biological processes.

Oxidation of the seed coat is very often observed but this can be propagated internally since there can be an increase in maldonaldehyde (MDA), a product of lipid
peroxidation, after plasma treatment as seen by both Los et al. (2019) and Cui et al. (2019). This may be among the first steps in the signal transduction considering that lipid peroxidation does not rely on enzymatic activity, which is very limited in dry seeds (El-Maarouf-Bouteau and Bailly, 2008).

Mujahid et al. (2020) mentioned that it may be instead the hydroxyl radicals which are responsible for the cell wall loosening. Additionally, Bafoil et al. (2019) calculated an increased expression of 23 genes for class III peroxidases after plasma treatment, which are proteins localized in the seed coat which regulate concentrations of hydrogen peroxide and precede the rupture for germination. Pauzaitė et al. (2018) also suggested that ROS may alter the seed coat pigmentation, which is known to be linked to germination. The flavonoid biosynthetic pathways and abscisic acid, a hormone for dormancy, are regulated by the same gene locus. Although they had changes in the seed coat flavonoids, they could not find any clear connection in their study between flavonoids and seed germination parameters.

Others have seen that the seed coat pigmentation does in fact influence seed permeability and the rate of imbibition where brown seeds had faster water uptake, reached a germination optimum sooner but were more susceptible to imbibition damage than black seeds (Siddiqui and Khan, 2010). Liu et al. (2019) also attempted to decipher between seed types using PAW and brought up in their discussion that seed storage proteins are typically oxidized during germination, which may facilitate the mobilization of the storage reserve. Therefore, it may be possible that plasma-derived ROS may be affecting the seed pigmentation first and then downstream the germination rate. Additionally, considering that Mueller et al. (2009) suggested that ROS play a role in the cleavage of cell wall polymers, it may be that changes inside the seed in turn modify the outer layers. The mechanical pressure on the endosperm
needs to be relieved for the radical protrusion but this is based on cell wall loosening, which is linked to the action of ROS (El-Maarouf-Bouteau and Bailly, 2008) and this, again, may be another argument that external seed coat erosion might not be necessary for modifying germination.

Alternatively, it could also be that chemical modifications to the seed coat, for example through lipid oxidation, may lead to the carbonylation of proteins, rendering them more susceptible to cleavage and leading to the breakdown of aleurone layer (Weber et al., 2015; El-Maarouf-Bouteau and Bailly, 2008). Therefore, post-translational modifications, such as carbonylation and sulphydryl group’s oxidation, are also another mechanism by which ROS, specifically H$_2$O$_2$, can shift a seed from a dormant to nondormant state as shown by Oracz et al. (2007) and Valderrama et al. (2019).

4.3.1.2 ROS entry and involvement in seed development

Generally, it is not fully understood how external ROS are detected and how these signals are transduced in seed cells but this process may partly depend on the presence of water. Water is needed to trigger germination and increase respiration, a reaction which oxidizes sugars to release energy in the form of ATP. During respiration, ROS are produced as by-products and thus, ROS generation is the hallmark from dormant to metabolically active seeds (El-Maarouf-Bouteau and Bailly, 2008). Where the moisture content is low, there is very little, if any, enzymatic activity but there may be hydrated pockets within the seed, permitting limited metabolic activity. It could be that this pocket of water may assist the diffusion and retention of ROS in the seed and, therefore it may be that plasma-derived ROS accumulate in these pockets and trigger signaling for intracellular programs. This process may be done more efficiently with hydrated instead of desiccated seeds due to the higher water content. Nevertheless,
in dry conditions, ROS may have an effect that is simply paused until imbibition (El-Maarouf-Bouteau and Bailly, 2008) and this may be why there are long term effects (Pauzaite et al., 2018) or why long-term storage of plasma-treated seeds can still have a positive effect on growth parameters relative to the untreated seeds (Sarinont et al., 2016). On the other hand, the effects of the plasma treatment may be continuously ongoing but are not morphologically obvious until imbibition and subsequent growth. Mildažienė et al. (2016) pointed out that biochemical changes continue to occur in seeds at various levels, including hormonal balance, gene expression, oxidative processes, mRNA content, and protein translation during storage and these changes may occur across all of these levels after plasma treatment.

Although it is not known which ROS is/are responsible for the effect and how it/they enter, it is known that seeds have pores whose size is genetically regulated and this also may assist the diffusion of ROS into the seed (Souza and Marcos-Filho, 2001). H₂O₂ can interact with the surface and diffuse through the membrane but charged species such as superoxide are not able to bypass the membrane and are dependent on voltage-dependent anion channels called porins, which are only found in the mitochondria (Parvaiz Ahmad, 2014). Despite its charge, superoxide can break down into hydroxyl and singlet oxygen and these may be able to bypass more easily (Grene, 2002). In some cases, RONS bypass the membrane using aquaporins, protein channels used for water transport (Yusupov et al., 2019). Transport also depends on the life stage of the plant since ozone can be taken up through the stomata in the leaves. Seol et al. (2017) observed an accumulation of ROS in chloroplasts, and suggested that through the micropores, ROS can travel down further into the plant tissue, from the epidermis into the mesophyll but if too potent, chloroplast degradation occurs.
Considering the lifetime and complexity of the reactive species reactions, there is a bias for researchers to measure longer-lived species like H$_2$O$_2$, O$_3$, and NO and therefore, most of the focus for this section will be dedicated to these species herein (Mhamdi and Van Breusegem, 2018).

4.3.1.3 Ozone

Surprisingly, little has been done to monitor and measure ozone during plasma treatments and it seems that many overlook the effect of ozone on seed germination despite it being possible to enhance germination with optimized treatment parameters. On the one hand, ozone can impair plant growth by replacing CO$_2$ and reducing photosynthesis. This is done by inhibiting the opening of the stomata due to the reduced flow of potassium ions (Torsethaugen et al., 1999). In some cases, plants like mung beans are not able to overcome ozone stress with their antioxidant defenses (Chaudhary et al., 2015). On the other hand, ozone has been used to enhance seed germination (Avdeeva et al., 2018) or improve fruit quality (Rodoni et al., 2009). Ozone seemingly has an ambivalent role that is dependent on the concentration and length of treatment (Abeli et al., 2017) as it is the case for many other treatments i.e. hormone, nanoparticles, heat. The authors mentioned that there is a variable response to the same ozone treatment based on the species and it is weak and transient. By using electron paramagnetic resonance (EPR), they showed that O$_3$ increased the concentration of radicals (carbon and oxygen species) in all tested species except one.

Pawlat et al. (2018b) measured 0.01 ppm of ozone produced during plasma treatment and had an effect on the growth parameters but it is not clear whether this is due to ozone or other treatment parameters, such as the short heat treatment at 40ºC. Perhaps this ozone treatment might not have been sufficient, in terms of concentration and exposure time but is a good example to point out that it would be helpful to include
as a control in studies to clarify whether the effect is due to ozone, other reactive species or other parameters.

It is especially interesting to differentiate this because ozone can trigger the production of ethylene, which then breaks down abscisic acid, the seed dormancy hormone (Emerson et al., 2018). Alternatively, it could be that ozone generates short-lived species inside the seed, which may be recognized as a signal for germination since the accumulation of ROS and peroxidation products is linked with seed dormancy alleviation (Oracz et al., 2007). As Sudhakar et al. (2011) pointed out, the production of hydrogen peroxide is observed in the early imbibition period of tomato seeds and nitric oxide, hydroxyl radicals and superoxide radicals accumulate during seed germination in different species. For this reason, many scientists speculate that H$_2$O$_2$ and NO are the reason for the observed effects using plasma treatments, whether or not it is the short-lived species such as superoxide, hydroxyl and NO that are eventually converted into H$_2$O$_2$ (Cui et al., 2019).

Wang et al. (2017) used a nitrogen plasma in open air and suggested that nitrogen oxides, which are known to have a role in dormancy and germination signaling, are present after plasma treatment and may initiate these biological processes (Giba et al., 2003). Instead, Puac et al. (2014) looked at meristematic cells of carrots treated with a RF plasma for less than 2 minutes and suggested that at least H$_2$O$_2$ and superoxide can pass through the cell membrane and reactive species may be one mechanism since they observed fluctuations in levels of redox quenching antioxidant enzymes such superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), which control and limit damage from excessive levels of ROS. This was also observed by Henselova et al. (2012) in maize using a DSCBD for less than 2 minutes. Simultaneously, these antioxidative enzymes may behave like sensors to
detect ROS availability and redox perturbations so that an organism can respond appropriately (Noctor et al., 2017; Valderrama et al., 2019). There is also an overlapping relationship between these enzymes, NO and H$_2$O$_2$ where NO positively regulates APX1 through a post-translation modification, S-nitrosylation, which then enhances resistance to oxidative stress and improves immune responses (Yang et al. 2015) and NO regulates itself as well as ROS (Romero-Puertas et al. 2016; Valderrama et al., 2019).

4.3.1.4 NO and H$_2$O$_2$

Rahman et al. (2018) suggested that plasma induces H$_2$O$_2$ formation, which is found in hydrated seeds and is involved in imbibition and early germination. They compared Ar/O$_2$ to Ar/air admixtures and observed higher concentrations of H$_2$O$_2$ with Ar/O$_2$ without inducing scavengers that counter H$_2$O$_2$ production. They concluded that plants treated with this admixture had better growth parameters due to the increased H$_2$O$_2$ concentration and did not see any changes in NO. Likewise, after plasma treatment, an increase in H$_2$O$_2$ concentration was correlated with positive effects on germination, whereas a decrease was correlated with negative effects on germination (Pauzaite et al., 2018). The same authors also showed for the first time the oscillatory dynamics of H$_2$O$_2$, which occurs in Arabidopsis and conifers but it is not characteristic for other seed types such as radishes and sunflowers. This already suggests that there can be differences between species including on a molecular level.

Kang et al. (2020) interestingly did not see an effect and observed high variation in rice germination. Los et al. (2019) also saw no changes in H$_2$O$_2$ levels in wheat but still had growth enhancement. This suggests that there are either differences in the plasma that do not necessarily lead to changes in H$_2$O$_2$ and requires optimization, or
that seeds respond differently, and it is not exclusively due to H$_2$O$_2$ but instead to nitrites and nitrates.

This latter point was heavily emphasized by Billah et al. (2020) who observed an increase in H$_2$O$_2$ but think that nitrogen is the main contributor for enhanced growth in gram seeds. In contrast, Liu et al. (2019) compared different gases using direct and indirect plasma treatments to produce PAW and soaked a variety of seeds. They were tempted to believe that effects are more likely due to oxygen-derived species. Interestingly, NO can downregulate the signal for H$_2$O$_2$ and will activate genes for antioxidant enzymes as pointed out by Iranbakhsh et al. (2017) so once again, it is difficult to separate the effects of both H$_2$O$_2$ and NO.

In the case of H$_2$O$_2$, it is considered a long-distance signaling molecule, which is highly interconnected with hormones, metabolism and gene transcription. The signalling likely includes MAPK cascades as pointed out also by Babajani et al. (2019). ROS mediated signalling includes calcium signalling, protein phosphorylation and gene transcription which are redox sensitive. The relationship between ROS and MAPK is not fully elucidated and both are able to regulate each other. These complexes phosphorylate transcription factors, kinases, phosphatases or other proteins, which can then change enzyme activity or gene expression (Jalmi and Sinha, 2015).

Additionally, ROS may produce changes in calcium signaling and use different signatures in terms of duration and amplitude depending on the species and this will dictate what happens downstream, for example root elongation (Wilkins et al., 2016). It was shown by Cui et al. (2019) that there was increased calcium in the roots of 4-day-old Arabidopsis seedlings which were previously plasma-treated as seeds and
they mentioned this may eventually lead to plant growth if maintained at a low level. Since there is little evidence about signalling due to plasma treatments, the focus now will shift from signal transduction to how hormones, metabolism and gene expression are modified with plasma, starting first with hormones.

4.3.2 Hormones

It is difficult to differentiate between the action of ROS and hormones because ROS are highly interconnected with hormones like salicylic acid (SA) and jasmonic acid (JA), which are widely reported. Information remains scarce with auxin and cytokinins, which are hormones that affect germination properties (Mhamdi and Van Breusegem, 2018).

Nevertheless, “hormones modulate the effects of ABA/GA balance: auxin IAA (indole-3-acetic acid) is a negative regulator of germination; ethylene, cytokinins, brassinosteroids, and strigolactones can stimulate germination; SA and jasmonate (stress hormones) may affect germination positively or negatively depending on the situation” (Mildažienė et al., 2019).

There are authors like Kitazaki et al. (2014), who agree on the importance of reactive species. Specifically, they think it is the relationship between ROS and the hormones, which stimulates plant growth. It is true that the importance of hormones cannot be overlooked considering that the seed coat is a source of hormones for the developing seed (Smýkal et al., 2014; Sabelli and Larkins, 2015). Mildažienė et al. (2017) also demonstrated how a dry seed undergoes subtle metabolic modulation by using proteomics. They showed that vacuum affected the auxin/cytokine balance, cold plasma increased GA, and EMF decreased the amount of ABA and increased IAA and SA without changing GA despite not being able to make a clear connection between phytohormones and germination kinetics. Ji et al. (2016) also observed an increase in
GA in spinach seeds but did not measure any other hormones. The increase in GA may be the result of plasma-derived ozone (Sudhakar et al., 2011) although it is often speculated that it is specifically auxin and cytokinins that are affected, which are hormones that increase and stimulate cell division, proliferation and elongation. Pérez-Pizá et al. (2018) showed that with plasma treatment, there was a decrease in ABA and increase IAA, a hormone which increases growth by regulating enzyme activity. They also detected H$_2$O$_2$, which coincided with an increase in ethylene after 24 hours of imbibition.

It may be that plasma-derived ROS regulate hormone production while for example increased auxin levels may be the reason for increased lateral root growth as pointed out by Wang et al. (2018) where the authors correlated increased levels of auxin in tomato with lateral root growth under drought stress. Stolárik et al. (2015) found changes in auxin and cytokinins with a 2-minute plasma treatment in peas. They found an increase in IAA and oxIAA as well as zeatin, the most common cytokinin, and correlated this with increased growth parameters. This is logical since these two hormones work together; auxin is responsible for DNA replication and initiating the cell cycle whereas cytokinins trigger cell division and mitosis (Heldt and Piechulla, 2010).

It seems that plasmas may somehow modulate concentrations of hormones and there may be a link with auxin especially considering that this hormone also affects xylem differentiation (Fabregas et al. 2015). It has been observed that plasma treatment modified the diameters of root and stems as well as the differentiation of tissues such as xylem and phloem (Safari et al., 2017; Moghanloo et al., 2019; Gao et al., 2019; Seddighinia et al., 2019). These changes in root morphology may then enhance nutrient exchange (Jiang et al., 2018) which was also proposed by
Moreover, Babajani et al. (2019) pointed out that auxin transport may also be affected by NO, which is often produced in plasmas. Additionally, it could very well be the case that auxin biosynthesis is upregulated because it is known that abiotic or biotic stresses increase the shikimate pathway which produces the precursor for IAA.

4.3.3 Metabolism

As stated previously, there is a link between hormones and metabolism and both primary and secondary metabolism, which are involved in growth and defense respectively, are altered after plasma treatment (Ljung, 2013).

Regarding primary metabolism, increased ATP levels along with ethanol were observed, suggesting increased anaerobic respiration (J. J. Zhang et al., 2017). Moreover, higher concentrations of protein and sugar were observed by Islam et al. (2019) and Billah et al. (2020) due to an increase in reserve utilization enzymes. Billah et al. (2020) explained that this could be because H$_2$O$_2$ transduces the signal for soluble sugar synthesis and therefore, more soluble sugar and protein are seen after plasma treatment (Singh et al., 2019). Moreover, exogenous treatment with H$_2$O$_2$ can stimulate germination by breaking dormancy through the oxidative pentose phosphate pathway, which provides reducing power and carbon for the new growth (Tian et al., 1998). This pathway also links primary and secondary metabolism because it provides the precursor for the shikimate pathway, which is important for plant defense.

Regarding secondary metabolism, an increase in enzymatic antioxidants like catalase (Moghanloo et al., 2019), superoxide dismutase (Alireza Iranbakhsh, Ardebili, et al., 2018), phenylalanine ammonia lyase (PAL) and peroxidases (Babajani et al.,
2019) as well as non-enzymatic antioxidants like total soluble sugar and proline to better tolerate stress have been observed, likely due to the increased concentration of ROS. Furthermore, Ghasempour et al. (2020) showed an increase in phenols, chlorophyll, flavonoids and alkaloids with plasma treatment. Pauzaite et al. (2018) has seen changes in flavonoids after plasma treatment while others have observed increased phenolic compounds (Scholtz et al., 2015; Ji et al., 2016; Filatova et al., 2020).

4.3.4 Defense

ROS may also trigger defense compounds which can originate from the shikimate pathway and phenylpropanoid pathway to induce the production of precursors. For example, H$_2$O$_2$ can activate the shikimate pathway (Moon and Mitra, 2016) (Noctor et al., 2015). ROS can also make the plant readily available in a redox state to preserve as stated by Filatova et al. (2020). Iranbakhsh et al. (2017) suggests that the defense response, triggered by ROS and/or UV, modulates the hormone balance since they saw an increase in PAL, a key enzyme in the phenylpropanoid pathway. This pathway also produces protective proteins called pathogenesis-related proteins and depending on whether they are acidic or basic, they can be upregulated by salicylic acid and ROS or methyl jasmonate and ethylene, respectively (Jain and Khurana, 2016).

Although Perez et al. (2019) used PAW, they looked at several genes involved in plant defense in an infected tomato plant and saw an increase in the gene expression of pal (phenylalanine ammonia lyase) but not prf1 (pathogenesis related protein) or erf1 (ethylene response factor). The gene pal is involved in the phenylpropanoid pathway and makes defense compounds like phytoalexins and phenolic compounds
and therefore, this information complements the increase in phenolic compounds seen experimentally by others previously mentioned in the metabolism section 4.3.3.

4.3.5 Gene expression

ROS can also affect gene transcription either directly or indirectly through hormone conjugates as mentioned by Stolárik et al. (2015). Redox sensitive transcription factors are widespread among animal, bacteria and plants (Grene, 2002) and these factors can be modified through the formation of disulfides upon sensing ROS (El-Maarouf-Bouteau and Bailly, 2008).

Unsurprisingly, there are others who think plasma also modifies gene expression although molecular information concerning plasma-seed treatments is very limited. Hayashi et al. (2011) suggested that ROS generated in plasma with water vapor may be a method to control the redox state of the plant by changing the thiol quantity (oxidizing cysteine to cysteine with OH radicals). This has an important role in gene transcription and therefore can change the plant response.

Iranbakhsh et al. (2020) observed an increase in the transcription factor WRKY1 and other enzymes involved in secondary metabolism in plasma-treated hemp seeds. WRKY is of particular interest since it is a family of transcription factors involved in many biotic and abiotic stress responses such as fungus, cold stress, salt stress, and drought tolerance. Furthermore, they are known for regulating phenolic compounds and there are specific factors such as AtWRKY23 that regulate auxin. Moreover, there are also interactions between MAPK cascades and this WRKY family and they can also activate PR proteins (Phukan et al., 2016).

The other genes that have been studied were by Guo et al. (2017) who observed an increase in LEA chaperone during stress, Ji et al. (2016) who saw increased
expression of a hydrolytic enzyme called pullulanase in spinach seeds, Ghasempour et al. (2020) who saw an increase in gene expression for DAT, an enzyme in the biosynthesis of vinblastine and vincristine, alkaloids with anti-cancerous properties and Islam et al. (2019) who showed changes in gene expression of ascorbate peroxidase and catalase but not superoxide dismutase with an air plasma in rapeseed.

These few preliminary studies demonstrate that plasma treatment can change gene expression for both primary and secondary metabolism, for growth and defense, respectively.

4.3.6 Epigenetics and genetics

These changes by the plasma treatment in the short-term may be due to epigenetics such as regulating DNA cytosine methylation, which inactivates gene expression (Hayashi et al., 2016; Mira et al., 2020).

J.J. Zhang et al. (2017) checked the methylation of genes involved in ATP synthase, an enzyme needed to produce energy for the cell, and TOR kinase, an enzyme which may increase metabolism and biosynthesis for energy and biomass production, and saw decreased methylation, meaning that the expression of these genes increased. Nevertheless, it cannot be ruled out yet that there may be changes to the DNA considering that genotoxic effects from plasma treatment have been observed (Kyzek et al., 2017).

Although it was not verified, Mildąžienė et al. (2017) found many similarities between cold plasma and electromagnetic treatment in protein expression but this may also be done epigenetically or through post-translational modifications. Despite the authors attributing the changes to radiation, it may be the action of hydroxyl radicals or other ROS since they too are produced from high energy radiation (Tuteja et al., 2001).
Additionally, there also remains the possibility that instead the DNA repair process is triggered by for example ozone and this may be responsible for the improvement in germination kinetics (Kurek et al., 2019; Pandiselvam et al., 2019).

Lastly, the plant genome itself may play a role in sensitivity to plasma treatment. Kobayashi et al. (2020) treated *Arabidopsis* seedlings and saw that the ecotypes Col and Ler responded differently to the same plasma treatment although the results were not statistically significant. Lo Porto et al. (2019) also pointed out that asparagus germination is strongly influenced by ecotype although they did not include this in their study. Therefore, the genetic component should not be overlooked when considering plasma-seed treatments and studies should be done on multiple generations since plasma treatment can have long-lasting effects in the same generation as observed by Sarinont et al. (2016) where the growth enhancing effects remained even after 17 months of storage of plasma-treated radish seeds.

In summary, it remains difficult to infer whether these effects are primarily attributed to the action of a single mechanism like mechanical scarification, chemical modifications or changes in biochemistry through ROS; even within ROS, each species behaves and functions differently. Additionally, variables such as the state of dehydration or hydration of the seed may influence the interaction and retention of ROS.

Ozone specifically in the presence of UV radiation may undergo reactions to eventually generate superoxide and hydroxyl radicals in the seed coat or these short-lived species may directly interact with the seed coat and this could be the critical point that determines how the external physical and chemical stimuli are transformed into internal biological stimuli.
The seed coat pigments can be altered and seed coat embedded enzymes like superoxide dismutase, NADPH oxidases or peroxidases can transform these species into signalling molecules like H$_2$O$_2$, which can more easily diffuse through the membrane or the apoplastic space, and then transduce this signal either through secondary messengers like calcium and MAPK cascade or be transmitted without the assistance of enzymes. Once the signal is sent, the hormonal balance may be modulated between GA and ABA to break dormancy, to increase auxin and cytokinin to accelerate the germination process, and increase ethylene, SA and JA for stress or disease resistance.

In parallel, metabolism may be modulated through the addition of water and enhanced gas exchange to modify enzyme activity to break down food reserves but at the same time, increase enzymatic and non-enzymatic antioxidants to shield against this sudden burst of ROS either externally derived or from metabolism. As a result of this stressful stimulus, metabolism in other defense pathways like shikimate and phenylpropanoid may be then activated to produce precursors in anticipation of future stressful events such as hormones or protective proteins.
Conclusion and future outlook

What has been shown to date is a combination of plasma device geometries, treatment methods and seeds that are able to alter the plant parameters. On the one hand, this diversity emerges from individual researchers considering what is relevant for their society and local economy, but on the other hand, this also makes it difficult to standardize the current research. We seem to have reached a point where there is potential in this technology as a proof-of-concept although there may be an inherent bias by publishing solely positive results, giving the impression that finding these setups is simple and it only takes trial-and-error to optimize the treatment conditions. Therefore, it would be helpful for the readers to also publish negative results to know what is not working when designing these treatments to move towards standardization and for our fundamental understanding.

As a next step, it would be useful to understand how each parameter in the plasma treatment affects the outcome so it is more predictable to be able to control the output. To accomplish this, it will require the continued collaborative efforts of biologists, chemists and physicists and these standardized protocols will likely need to be tailored to the seed type due to the diversity in the seed coat and build on the information from industry or associations such as AOSA (Association of Official Seed Analysts) where plasma is an added parameter.

Much of the outcome of the plasma-seed treatment seems to depend on both the plasma setup and seed features. If the seed has many layers that need to be scarified, plasma may help with mechanical erosion through etching or by melting the wax with the heat produced as a by-product of plasma generation. If the seed is rather permeable, it may functionalize the surface through the addition of chemical groups on
the surface to become more hydrophilic and enhance gas exchange to then affect the
seed biochemistry. Therefore, it may be that there are several modes of plasma
treatment that can be selected i.e. if you need mechanical scarification and erosion,
then use high power AC and argon for ion bombardment, if you need a gentle dose
with ROS for chemical modification of seed coat, use nanopulse power for better ROS
generation and lower temperature, or if you want to generate NO or H₂O₂ or if you want
to delay or accelerate germination for storage or sowing respectively, choose the
appropriate gas type.

Very little has been done in terms of economic analysis other than by Niemira et al.
(2012) but it would be useful to mention explicitly the power density of the device, the
maximum number of seeds that can be treated to have an effect and potentially
disclose the cost of manufacturing the plasma device to then see which designs would
be easiest to implement and scale-up. This does not mean one plasma device is used
universally as one of the advantages is the flexibility in design so people can adapt
their treatment to their surroundings (seeds may vary in their value or importance
depending on the country).

By understanding the plasma-seed interactions and mechanisms, this will also help
with setting up regulations around this technology since it is still ill-defined. Provided
that scientists now focus on the molecular effects of plasmas, with time, we might
understand in detail how plasma-seed treatments work to develop this into a viable
seed processing technology.
Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

A.W. for conceptualization of review and figures, literature search, designing the figures, and writing the manuscript. I.F and A.H. for reading and approving the manuscript.

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Figure 1: Effects of plasma treatment on seeds which includes surface modifications, changing growth parameters, modulating disease and stress resistance through metabolism.
Figure 2: Summary of the possible mechanical, chemical and biochemical interactions of plasma components with the seed surface
Figure 3: Summary of hypotheses and current evidence of plasma-seed treatments on a molecular scale
Table 1. Legend describing each category of Table 2 for the type of plasma and seed used in the study as well as the main findings (see Table 1 for a description of the categories)

<table>
<thead>
<tr>
<th>Plasma</th>
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<tbody>
<tr>
<td>geometry</td>
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<td>pressure</td>
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<td>gas type</td>
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<table>
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<tr>
<th>Seed type</th>
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<tr>
<td>radish, wheat, rice etc.</td>
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<table>
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<tr>
<th>Micro(organism)</th>
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<tbody>
<tr>
<td>bacteria or fungi or insect</td>
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<td>native or artificial contamination of a microorganism or organism (pest)</td>
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<table>
<thead>
<tr>
<th>Main findings</th>
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</thead>
<tbody>
<tr>
<td>seed coat modification</td>
</tr>
<tr>
<td>growth parameters</td>
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<tr>
<td>metabolism</td>
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<td>disease or stress resistance</td>
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<tr>
<th>Scale</th>
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<tr>
<td>macroscopic</td>
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<tr>
<td>microscopic</td>
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<tr>
<td>molecular</td>
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</tbody>
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Table 2. Collection of plasma-seed treatment papers

<table>
<thead>
<tr>
<th>Citation</th>
<th>Plasma</th>
<th>Seed type</th>
<th>Microbe</th>
<th>Main findings</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iranbakhsh et al., 2020</td>
<td>DBD; atm pressure; Ar</td>
<td>Hemp</td>
<td></td>
<td>growth parameters, metabolism</td>
<td>macroscopic and molecular</td>
</tr>
<tr>
<td>Kang et al., 2020</td>
<td>1) Arc discharge; low or atm pressure; underwater 2) DBD; low and atm pressure; (0.6-1 atm); not clear</td>
<td>Rice</td>
<td>Fusarium fujikuroi</td>
<td>seed coat modification, growth parameters, disinfection/disease resistance</td>
<td>macroscopic</td>
</tr>
<tr>
<td>Billah et al., 2020</td>
<td>DBD; low pressure (400 torr); air</td>
<td>Black gram</td>
<td></td>
<td>seed coat modification, growth parameters, metabolism</td>
<td>macroscopic and molecular</td>
</tr>
<tr>
<td>Rezaei et al., 2020</td>
<td>not clear; atm pressure; air</td>
<td>Hyssop</td>
<td></td>
<td>tissue modification (dried leaves)</td>
<td>macroscopic</td>
</tr>
<tr>
<td>Koga et al., 2020</td>
<td>DBD; atm pressure; humid air</td>
<td>Radish</td>
<td></td>
<td>seed coat modification (colour)</td>
<td>macroscopic</td>
</tr>
<tr>
<td>Ghasempour et al., 2020</td>
<td>DBD; atm pressure; Ar</td>
<td>Catharanthus roseus</td>
<td></td>
<td>growth parameters, metabolism</td>
<td>macroscopic and molecular</td>
</tr>
<tr>
<td>Mujahid et al., 2020</td>
<td>DBD; atm pressure; He and O₂</td>
<td>grape cultivar Muscat of Alexandria</td>
<td></td>
<td>growth parameters, metabolism</td>
<td>macroscopic and molecular</td>
</tr>
<tr>
<td>Filatova et al., 2020</td>
<td>CCP RF; low pressure (200 Pa); air</td>
<td>Maize, wheat, lupine</td>
<td>Native fungi, Fusarium culmorum</td>
<td>growth parameters, metabolism, disinfection/disease resistance</td>
<td>macroscopic and molecular</td>
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<tr>
<td>Prakrajang et al., 2020</td>
<td>not given; not given; Ar</td>
<td>Chili pepper</td>
<td></td>
<td>growth parameters</td>
<td>macroscopic</td>
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<tr>
<td>Kobayashi et al., 2020</td>
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<td>Arabidopsis (seedlings)</td>
<td></td>
<td>growth parameters</td>
<td>macroscopic</td>
</tr>
<tr>
<td>Dawood, 2020</td>
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<td>Moringa</td>
<td></td>
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<td>macroscopic and microscopic</td>
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