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# Cold atmospheric plasmas as regulators of seed germination, stress adaptation, and microbial interactions during early plant development

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Thèse de doctorat de Biologie et de physique

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# Résumé substantiel

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Ce projet doctoral se situe à la croisée de deux disciplines : la physique du plasma froid et la biologie des semences. Les expérimentations ont été menées au sein de deux laboratoires de recherche : le laboratoire Développement, Adaptation et Vieillissement (Dev2A) de l'Institut de Biologie Paris-Seine (IBPS), et le Laboratoire de Physique des Plasmas (UMR 7658), tous les deux localisés sur le campus Pierre-et-Marie-Curie de Sorbonne Université, à Paris.

L'objectif principal de cette étude était d'évaluer les effets d'une exposition au plasma froid sur la germination, la croissance et la décontamination des semences. Deux modèles biologiques ont été utilisés : le tournesol (*Helianthus annuus*), pour étudier la vigueur germinative et la croissance des plantules sous stress hydrique ; et deux écotypes d'*Arabidopsis thaliana* (Landsberg et Columbia), pour explorer les effets sur la décontamination des graines et le microbiote associé aux plantules.

Ce manuscrit est structuré en quatre sections complémentaires. La première section est une introduction qui présente l'état actuel des connaissances sur les applications agricoles du plasma froid, le contexte scientifique et les objectifs de la thèse. La deuxième et la troisième section rassemblent les principaux résultats expérimentaux, présentés sous forme de deux articles scientifiques. Ces articles décrivent les effets du traitement au plasma sur la germination, la croissance des plantules et la décontamination des semences. La quatrième section propose une analyse mécanistique visant à dissocier les différentes composantes du plasma (espèces réactives, rayonnement, chaleur, champ électrique) pour mieux comprendre leurs contributions respectives aux effets observés. Elle s'achève par une discussion générale des résultats obtenus, identifiant les limites de l'approche et proposant des pistes d'amélioration pour renforcer la robustesse et la portée des futures recherches dans ce domaine.

Dans un contexte de croissance démographique continue, avec près de 9,7 milliards d'habitants attendus d'ici 2050, les enjeux liés à la sécurité alimentaire s'imposent comme une priorité pour la recherche agronomique. Parallèlement, le changement climatique accentue la pression sur les agrosystèmes, en rendant les conditions de production agricole plus instables. Les événements climatiques extrêmes ont ainsi augmenté de 85 % depuis les années 1990 [1]. Dans ce contexte, les semences, point de départ de toute production végétale, se retrouvent en première ligne face aux stress abiotiques. Sécheresses, inondations et températures extrêmes perturbent la germination et compromettent la levée des plantules. À ces contraintes s'ajoutent des stress biotiques, notamment les attaques fongiques, bactériennes ou d'insectes, qui altèrent la qualité sanitaire des semences, réduisent leur pouvoir germinatif et favorisent la transmission de pathogènes [2, 3].

Par ailleurs, la dégradation continue des sols aggrave la situation : appauvrissement en matière organique, compaction et perturbation de leur équilibre microbien, compromettent durablement la résilience des cultures. L'ensemble de ces facteurs menace non seulement les rendements agricoles, mais remet aussi en question l'efficacité des stratégies conventionnelles.

Pour faire face à ces défis, l'agriculture conventionnelle a longtemps reposé sur l'utilisation intensive de produits phytosanitaires. Cependant, de nombreuses études soulignent aujourd'hui leurs effets délétères sur la santé humaine et les écosystèmes. Des substances comme le glyphosate, longtemps considérées comme sûres, sont désormais reconnues comme cancérogènes probables par le CIRC (2015), et impliquées par plusieurs études pour son rôle dans le développement de lymphome non hodgkinien et de cancer du sein [4, 5].

Malgré ces risques, certaines molécules controversées ou interdites pour des raisons sanitaires continuent d'être utilisées, ou bénéficient de ré-autorisations provisoires.
Par exemple, en 2024, la France a prolongé l'autorisation d'usage du S-métolachlore, un an après le vote de son interdiction. Cet herbicide, détecté dans les nappes phréatiques, appartient à la famille des acétanilides et est suspecté d'être cancérogène ainsi que perturbateur endocrinien [6].
De même, la loi Duplomb, définitivement adoptée en juillet 2025, a temporairement réintroduit certains néonicotinoïdes pour la culture de betteraves sucrières, en dépit de leur impact avéré sur les pollinisateurs. Cette loi concerne notamment l'acétamipride et allège les contraintes encadrant son utilisation [7]. Ces décisions illustrent l'impasse dans laquelle se trouve l'agriculture conventionnelle, encore fortement dépendante des intrants chimiques, malgré des risques bien établis pour la santé humaine et l'environnement.

Face à cette situation, il devient prioritaire de renforcer la qualité et la résilience des semences sans recourir à ces substances. Cela encourage la recherche à explorer des stratégies alternatives, innovantes et plus respectueuses des écosystèmes. Parmi elles figurent les biostimulants, qui renforcent les défenses naturelles des plantes et améliorent leur vigueur. Il en existe de plusieurs types : acides humiques, extraits d'algues, microorganismes bénéfiques (comme *Trichoderma*), peptides végétaux, etc. [8]. Toutefois, cette diversité implique une variabilité des effets selon les espèces végétales et les conditions d'application.

Parmi ces approches, le traitement au plasma froid émerge également comme une alternative crédible. Grâce à ses propriétés physiques et chimiques, il peut agir sur plusieurs aspects fondamentaux de la vie des semences : stimulation de la germination, amélioration de la tolérance au stress, réduction de la charge microbienne… Autant de propriétés qui en font une piste de recherche sérieuse pour accompagner la transition vers des systèmes agricoles plus durables.

Le plasma est considéré comme le quatrième état de la matière. Il s'agit d'un gaz partiellement ionisé, contenant des ions, des électrons, des espèces réactives de l'oxygène et de l'azote (RONS), un rayonnement (UV, visible) et un champ électrique. Ils peuvent être distingués selon leur degré d'ionisation, les plasmas fortement ionisés sont considérés comme « chauds » et sont utilisés en industrie du fait de leur température extrêmement élevée (plusieurs milliers de kelvins) et les plasmas faiblement ionisés ou « froids », qui opèrent à température ambiante et dont l'utilisation est compatible avec les systèmes biologiques. Sur Terre, les manifestations du plasma sont plutôt rares, on retrouve par exemple la foudre, qui est un plasma chaud, fortement ionisé et les aurores boréales qui sont des plasmas froids faiblement ionisé. A l'inverse, dans l'univers, les plasmas représentent 99,9 % de la matière [9].

Les plasmas froids générés à pression atmosphérique (PAFs) du fait de leur faible température et de leur propriétés physiques et chimiques, sont utilisés dans de nombreux domaines. En médecine ils sont utilisés pour le traitement de la peau (cicatrisation, vieillissement), la stérilisation de surface et de matériel médical et plus récemment en oncologie pour leur action anticancéreuse [10]. Depuis une 20aine d'année, des applications agricoles notamment le traitement direct des semences ou l'application d'eau activée par plasma sont également largement étudiées.

Dans cette étude, le plasma d'air ambiant a été généré dans un dispositif à barrière diélectrique (DBD) composé de deux électrodes planes de 30 × 40 cm de surface, l'une connectée à la haute tension et l'autre à la masse. Les deux électrodes étaient séparées par un matériau diélectrique isolant de 2 mm d'épaisseur, afin d'empêcher toute transition streamer-arc et d'assurer ainsi une distribution homogène du champ électrique. Le gaz de fond utilisé était l'air ambiant, avec une humidité relative proche de 40 %.

Dans le chapitre 2, nous explorons les effets de l'exposition au plasma de 15 et 30 minutes sur la germination des semences et la croissance des plantules en situation de stress hydrique. Nous avons mené ces expériences sur deux variétés de tournesol, dont l'une présentait une germination très faible à 15 °C, révélant son caractère de dormant. Nous avons montré que l'exposition au plasma froid a permis d'augmenter significativement la germination des deux variétés en condition de stress hydrique (PEG). En absence de stress, seule la variété dormante a montré une amélioration de la germination qui a été multipliée par 3 après 15 minutes de traitement. Ces observations ont été confirmées en serre, où nous avons évalué la croissance des plantules issues de graines traitées, soumises à un arrosage normal ou à une restriction hydrique. Nous avons observé une augmentation de la croissance uniquement lorsque les plantules étaient exposées au stress hydrique. En condition optimale, la croissance des plantules issues de graines traitées était équivalente à celle des témoins.

Des résultats similaires ont été obtenus sur d'autres espèces d'intérêt agronomique, telles que le blé et le colza [11, 12]. Guo et al. ont montré que le traitement au plasma augmentait l'activité d'enzymes antioxydantes telles que la catalase (Cat) et la peroxydase (POD), contribuant à une meilleure tolérance au stress hydrique [Guo].

Nous avons également montré que la configuration du dispositif expérimental, et la présence ou l'absence de graines entre les électrodes modifiait la composition chimique du plasma. Par exemple, l'augmentation de l'espace inter-électrode réduisait la concentration relative d'ozone, tandis que l'introduction des graines dans le dispositif entraînait son augmentation. Les espèces réactives de l'oxygène (ROS) jouent un rôle ambivalent chez les organismes vivants. En excès, elles induisent un stress oxydatif, susceptible d'endommager les membranes cellulaires, les protéines et l'ADN, pouvant conduire à l'apoptose [13]. Toutefois, à des concentrations modérées et régulées, les ROS agissent comme des signaux moléculaires essentiels, activant les voies enzymatiques de détoxication et régulant des processus physiologiques clés [13]. Chez les plantes, elles participent notamment à la levée de dormance et au déclenchement de la germination [14], leur accumulation en post-maturation conduit à l'oxydation sélective des ARNm codant pour des inhibiteurs de germination, permettant ainsi la reprise du développement embryonnaire [15]. Cette dualité souligne l'importance d'un équilibre strict entre leurs accumulations et leur élimination. Pour faire face au stress oxydatif induit par les ROS, les plantes activent deux voies principales de détoxication : les voies enzymatiques et non enzymatiques. Les voies enzymatiques mobilisent des enzymes antioxydantes comme la catalase (CAT), la superoxyde dismutase (SOD) ou encore les peroxydases (POD), qui neutralisent les ROS en les transformant en composés moins toxiques. En parallèle, les voies non enzymatiques reposent sur des molécules antioxydantes telles que l'acide ascorbique (vitamine C), le glutathion ou les flavonoïdes, capables de piéger directement les ROS [13].

Dans le troisième volet, nous avons travaillé sur les effets du plasma sur la décontamination des graines d'*Arabidopsis thaliana* ainsi que sur l'effet de ce traitement sur le microbiote des plantules issues de graines traitées pendant 5 et 15 minutes. Nous avons également caractérisé le microbiote fongique et bactérien des plantules de deux écotypes : Columbia et Landsberg. Pour amplifier l'ADN microbien, nous avons ciblé deux régions de l'ADN ribosomique : la région ITS1 pour l'ADN fongique [16], et la région V4 pour le microbiote bactérien. Ces régions présentent une grande variabilité interspécifique, ce qui permet une attribution taxonomique fine des micro-organismes. Deux lots de graines présentant des taux de contamination différents ont été considérés : le lot A (faiblement contaminé) et le lot B (fortement contaminé). Nous avons évalué les effets de la décontamination des graines de manière directe et indirecte grâce à la néphélométrie. Ces deux méthodes nous ont confirmé que les

micro-organismes présents à la surface des graines n'étaient plus capables de se développer après 5 minutes d'exposition au plasma, quel que soit le niveau de contamination initial du lot et l'écotype.

À l'échelle des plantules, l'analyse du microbiote fongique a montré que le traitement induisait une baisse de la diversité globale, marquée par une diminution de l'abondance relative de taxons dominants tels que *Penicillium* et *Acremonium*, retrouvés en abondance sur les plantules Columbia et Landsberg témoins. Nous avons pu constater des différences de sensibilité entre les taxons : *Acremonium sclerotigenum* voyait son abondance réduite à moins de 1 % dans tous les échantillons traités, tandis qu'à l'inverse, l'abondance relative de *Cladosporium cladosporioides* augmentait dans certains échantillons traités. Certains genres n'étaient pas partagés entre les deux écotypes, c'est le cas du genre *Trichoderma*, retrouvé uniquement dans l'écotype Landsberg, dont l'abondance relative était plus importante dans les échantillons du lot B exposés au temps de traitement le plus court. Il est intéressant de noter que plusieurs espèces de ce genre ont attiré l'attention en raison de leurs effets positifs sur la croissance des plantes [17].

Dans les échantillons traités de l'écotype Columbia, la majorité de l'ADN amplifié ne pouvait pas être attribuée au-delà du phylum *Ascomycota*. Cet ADN, provenant des taxons dominants, était trop dégradé pour être attribué taxonomiquement. Nous avons également observé, pour l'écotype Landsberg, dont la diversité des plantules témoins était très limitée (indices de Shannon < 2 pour tous les échantillons), que l'ADN de *Penicillium* était majoritairement retrouvé dans l'ensemble des échantillons, bien qu'il ne se développe pas sur les graines traitées. À l'inverse de l'écotype Columbia, cet ADN ne semble pas avoir été dégradé.

L'analyse du microbiote bactérien nous a fourni peu d'informations sur les communautés hébergées par les plantules et leur dynamique. Aucune bactérie n'a été détectée sur les échantillons traités, et la diversité observée sur les plantules témoins était relativement faible. Le genre *Massilia* ainsi que les familles *Comamonadaceae* et *Burkholderiaceae* étaient néanmoins particulièrement représentés. La région V4 nous a fourni un grand nombre de lectures, mais la majorité d'entre elles n'ont pas pu être attribuées taxonomiquement, car elles correspondaient à de l'ADN de chloroplastes et de mitochondries. De plus, cette région ne permet une identification fiable qu'au niveau du genre, ce qui a limité la pertinence de l'analyse. À l'inverse des communautés fongiques, où un petit nombre de taxons très dominants étaient partagés entre tous les échantillons, les taxons bactériens variaient d'un échantillon témoin à l'autre, tant en composition qu'en abondance relative.

Cette analyse nous a montré que le traitement au plasma avait un impact sur le microbiote bactérien et fongique associé aux plantules. Il s'est montré efficace pour réduire l'abondance relative de *Penicillium* sur les plantules Columbia issues de graines traitées : après 15 minutes, son ADN

représentait moins de 1 % contre plus de 80 % dans les plantules témoins. Le traitement a également inactivé le développement de *Penicillium* sur les graines Columbia et Landsberg dès 5 minutes d'exposition.

Enfin, pour clore cette étude, nous avons, dans le chapitre 4, cherché à isoler les effets des propriétés physiques et chimiques du plasma afin de déterminer la contribution respective de chacune dans le processus de décontamination. Nous avons ainsi pu distinguer l'influence du champ électrique, de la température, du rayonnement et des espèces réactives de l'oxygène et de l'azote (RONS). Pour isoler le champ électrique, nous nous sommes positionnés à la tension maximale atteignable avant de dépasser le seuil de claquage au-delà duquel le plasma se forme, soit aux alentours de 7 kV. Les graines d'*Arabidopsis thaliana* ont été placées dans le dispositif pendant 5 et 15 minutes. Deux lots de graines Columbia, présentant des niveaux de contamination différents, ont été exposés : le lot A, moyennement contaminé, et le lot B, fortement contaminé.

Nous avons constaté, six jours après le dépôt sur milieu malt agar, que le champ électrique avait significativement réduit le nombre de graines contaminées dans le lot A. En revanche, pour le lot le plus contaminé (lot B), aucun effet du champ électrique sur le niveau de contamination des graines n'a été observé. Ensuite pour évaluer les effets potentiels de la température, nous avons mesuré celle du dispositif à l'aide d'une caméra thermique pendant 30 minutes avec le plasma allumé. Nous avons ensuite exposé nos graines à la température moyenne maximale enregistrée par la caméra (37 °C), pendant 5 et 15 minutes. Aucun effet de la température sur le niveau de contamination des graines n'a été observé.

Concernant le rayonnement, nous avons caractérisé le spectre du plasma froid, qui présentait des raies d'émission de faible intensité dans le domaine UVA. Les graines ont été placées sur la tranche du dispositif, afin d'être directement exposées au rayonnement pendant 5 et 15 minutes. Pour le lot A, une diminution du nombre de graines contaminées a été observée, mais celle-ci n'était pas significative. Pour le lot le plus contaminé, aucune différence n'a été constatée par rapport aux graines témoins. Ces résultats nous ont permis de conclure à la faible implication du rayonnement émis par le plasma dans la décontamination des graines.

Enfin, nous nous sommes intéressés aux espèces réactives de l'oxygène et de l'azote (RONS) présentes dans notre plasma. Grâce à des mesures de spectrométrie de masse réalisées en conditions plasma ON et plasma OFF, nous avons constaté une augmentation marquée de l'ozone ($O_3$) et du dioxyde d'azote ($NO_2$) à l'allumage du plasma. Pour exposer uniquement les graines aux RONS, nous les avons placées sous une plaque métallique reliée directement à la masse, de sorte que le plasma se formait entre l'électrode haute tension et cette plaque, sans contact direct avec les graines.

Nous avons constaté que la décontamination des graines des deux lots était totale dès 5 minutes d'exposition, un résultat similaire à celui obtenu lors d'un traitement direct au plasma froid. Ces observations suggèrent que les RONS constituent la composante majoritairement responsable de la décontamination des graines. Les autres composantes, telles que le rayonnement ou le champ électrique, pourraient jouer un rôle synergique, mais mineur.

Cette étude multidisciplinaire nous a permis de caractériser les effets biologiques du plasma sur les semences ainsi que les mécanismes clés impliqués, en particulier chez les semences exposées à des problématiques de stress hydrique et de contamination. Nous avons démontré que l'exposition au plasma froid pouvait améliorer significativement la germination et la croissance des plantules en condition de stress hydrique. L'analyse mécanistique menée en fin d'étude a permis de dissocier les composantes physico-chimiques du plasma, révélant que les espèces réactives de l'oxygène et de l'azote (RONS) sont les principaux agents responsables des effets observés, notamment en matière de décontamination. En comparaison, le rayonnement UV, le champ électrique et la température se sont révélés jouer un rôle mineur, voire négligeable dans les conditions expérimentales testées. Ces résultats renforcent l'hypothèse selon laquelle l'action du plasma repose principalement sur des mécanismes chimiques.

Dans la dernière partie de cette thèse, nous discutons des améliorations possibles ainsi que des ajustements à envisager afin de compléter cette étude et d'en renforcer la pertinence scientifique. Cela inclut notamment l'optimisation des paramètres d'exposition pour limiter les effets délétères potentiels, l'approfondissement des analyses sur le microbiote, et la transposition des résultats à d'autres espèces d'intérêt agronomique. Ces perspectives sont essentielles pour consolider le potentiel du plasma froid comme levier agroécologique au service d'une agriculture plus durable et respectueuse de la santé des écosystèmes.

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# Chapter 1: General introduction: State of the art

**1. Agriculture and food issues in the context of climate change: why seeds are important**

At the heart of contemporary agricultural challenges lie two major issues: climate change and the rapid growth of the global population. Global food security increasingly depends on our ability to produce high-quality seeds and maintain sustainable agricultural productivity in the face of mounting environmental and demographic pressures. By 2050, the world population is expected to reach approximately 9.7 billion people, significantly intensifying food demand and placing additional strain on agricultural systems [1].

In parallel, the SSP2-4.5 climate scenario projects a global average temperature increase of around 2 °C by 2050, should current greenhouse gas emission levels persist. This trajectory would lead to profound and lasting consequences: more frequent and intense extreme weather events (droughts, floods), shifts in precipitation patterns, and a gradual deterioration of optimal growing conditions. The early stages of plant development, particularly seed germination and seedling establishment are especially vulnerable to these environmental disruptions, making them key targets for agricultural adaptation strategies. Abiotic stress disrupts essential physiological processes in seeds and young plants, compromises germination success, and hinders growth (Fig. 1). Beyond the decline in productivity, it also affects the sexual reproductive cycle of seed-bearing plants, weakening the resilience of ecosystems. Seeds and seedlings are further exposed to pests and diseases, whose distribution ranges are expanding or shifting due to climate change [Singh]. Rising temperatures promote the survival, multiplication, and spread of numerous pathogens, insects, and parasites, thereby increasing the pressure on seeds and young plants (Fig.1).

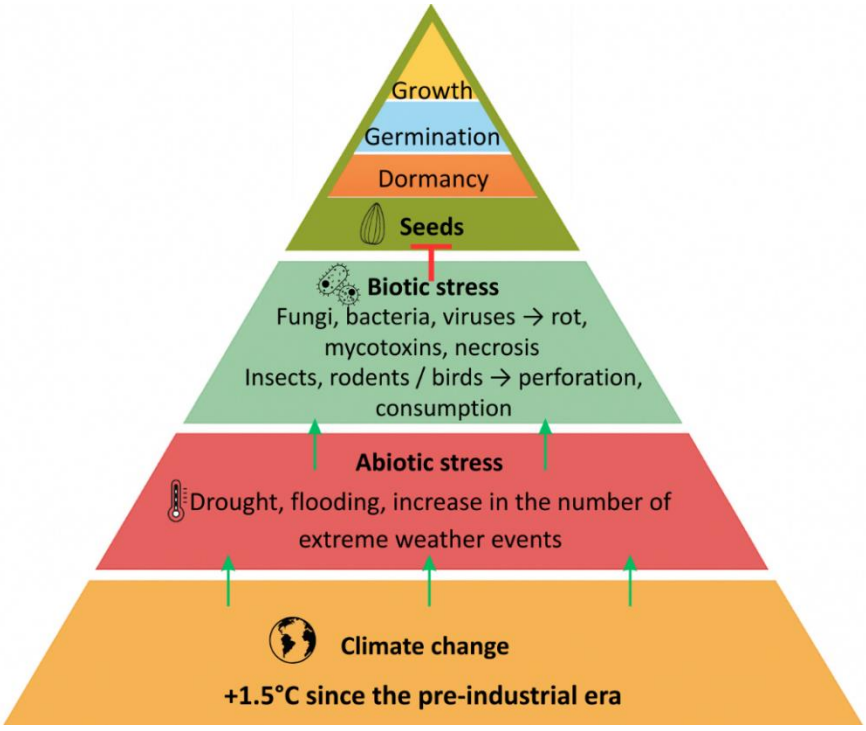


Fig. 1: Maslow's pyramid of stressors influencing seed germination, dormancy and seedling

Seeds form the foundation of the food chain for most living organisms. They ensure the reproduction and dispersal of plant species, thus contributing to genetic renewal and the stability of ecosystems. As producers of organic matter, plants through their seeds and biomass feed herbivores, which in turn support carnivorous diets, thereby sustaining entire trophic networks.

At the same time, seeds are central to both human and livestock nutrition. They provide staple crops such as rice, wheat, maize, millet, and sorghum, which account for 60% of the world's energy intake. More broadly, plants represent over 80% of human food and nutrition [2]. However, their survival whether in natural ecosystems or agroecosystems is now under threat from climate disturbances, overexploitation of natural resources, and the lack of adaptive strategies. These disruptions, often sudden and unpredictable, catch farmers off guard, with sowing and harvests compromised or even wiped out by biotic (pests, diseases) and abiotic (drought, heat, salinity, etc.) stresses. The effectiveness of control and adaptation strategies is also being undermined, limiting the resilience of agricultural systems.

The direct effects of these disruptions are already observable in several regions of the world, notably in China the world's leading producer of wheat, rice, and potatoes, and the second-largest producer of maize. Between 2000 and 2019, China recorded nearly 600 natural disasters, including floods, heatwaves, and storms [3, 4]. These events underscore the urgent need to rethink agricultural systems to make them more resilient in the face of future climate change.

### 1.1 Seed dormancy & germination: the two stages and their regulation

In the context of climate change, a thorough understanding of the biological mechanisms governing the early stages of plant development particularly dormancy, germination, and emergence is essential, as these processes largely determine the successful establishment of crops under field conditions.

In a plant's life cycle, the seed resulting from sexual reproduction protects the embryo and contains all the necessary structures to form a complete individual. Moreover, produced through fertilization and dispersed upon maturation, the seed contributes to genetic diversity and the colonization of the environment by a population [5]. Germination is the biological process by which a viable seed initiates growth to give rise to a new plant in response to favorable environmental cues. It involves the reactivation of metabolic activity, cell expansion, and the protrusion of the radicle through the seed coat. A seed is considered germinated as soon as the radicle breaks through the testa [6]. These events occur during imbibition a phase in which water absorption generates the turgor pressure necessary to mechanically rupture the seed coat. This process is controlled by both endogenous and exogenous

factors. Oxygen [7], temperature, and water availability are the three essential exogenous factors regulating germination (Fig.2).

During maturation, the seed acquires desiccation tolerance and undergoes significant dehydration. Water is essential for imbibition, which is characterized by the reactivation of seed metabolism, including ATP synthesis via glycolysis, the Krebs cycle, the respiratory chain, and mRNA translation [8].

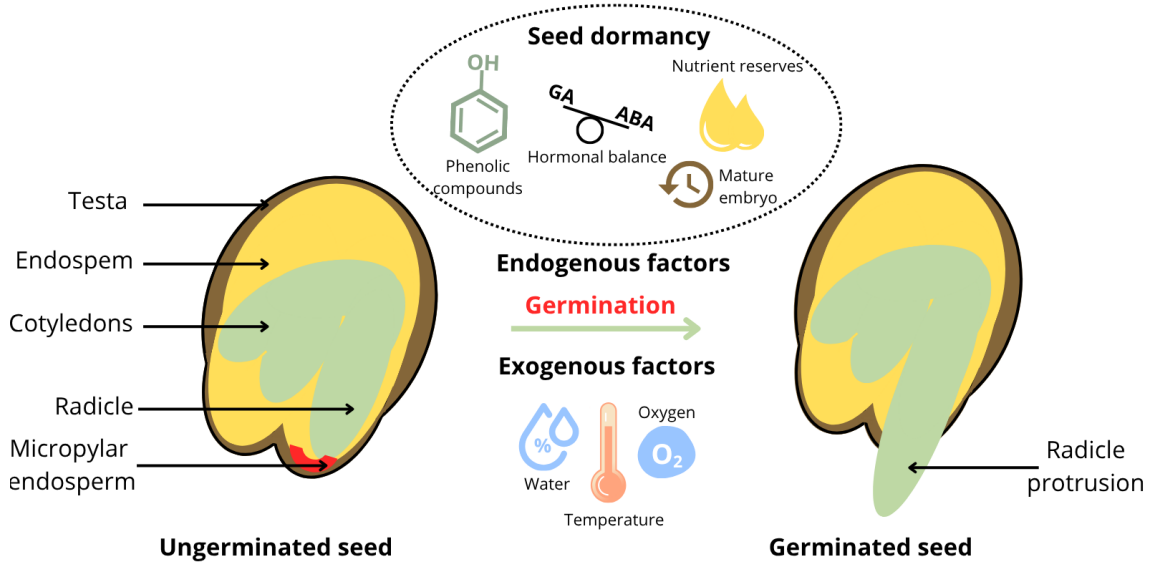


Fig. 2: Regulation of seed germination: endogenous and exogenous factors

Oxygen is crucial for cellular respiration, but its role in germination depends on both temperature and plant species. For instance, rice can tolerate hypoxic conditions through alcoholic fermentation, enabling it to mobilize its reserves [9], whereas other species such as carrot, celeriac, and parsley experience a significant drop in germination rates below 15% oxygen concentration [10].

Temperature is another critical factor: it affects the speed and rate of germination as well as water uptake. Suboptimal temperatures reduce enzymatic activity and slow the mobilization of seed reserves [8].

The inability of a seed to germinate despite favorable conditions may also result from internal factors; in this case, the seed is said to be dormant. Dormancy is a temporary physiological state that prevents germination even under favorable environmental conditions. It consists of three phases induction, maintenance, and release and plays a key evolutionary role by preventing premature germination [11]. There are several types of dormancy, notably primary and secondary dormancy, depending on when it is imposed during the seed's life cycle.

Primary dormancy is acquired during seed development on the mother plant. Considered innate, it is established during the final stages of maturation, marked by seed dehydration, acquisition of desiccation tolerance, and the activation of dormancy-related signaling pathways. It can also be morphological (immature embryo) or physical/mechanical (impermeable seed coat), depending on the structure responsible for inhibition [12]. The post-maturation phase, or after-ripening, which occurs during storage, allows for the progressive release of dormancy in relation to humidity and temperature conditions [13].

Dormancy regulation relies on a complex interaction between environmental signals and plant hormones. Abscisic acid (ABA) plays a central role in dormancy induction and maintenance. Before dormancy release, auxin (IAA) also contributes to its maintenance [14]. Conversely, gibberellins (GAs) promote dormancy release by stimulating the production of enzymes such as α-amylase, which mobilize seed reserves, weaken the seed coat (testa), and enhance water absorption (imbibition). They also enable the degradation of DELLA proteins, which inhibit embryonic growth. In parallel, ethylene increases after maturation and acts synergistically with GAs, particularly by inducing enzymes that degrade ABA (e.g., ABA 8'-hydroxylases) [15].

Additionally, reactive oxygen species (ROS) play a role in dormancy regulation. Their accumulation during after-ripening leads to selective oxidation of mRNAs encoding germination inhibitors, thereby enabling the resumption of embryonic development [16]. A certain ROS threshold referred to as the "oxidative window" is required to permit germination [17]. These oxidative signals interact with hormonal pathways (GA, ethylene, ABA), creating a favorable biochemical environment (Fig. 3).

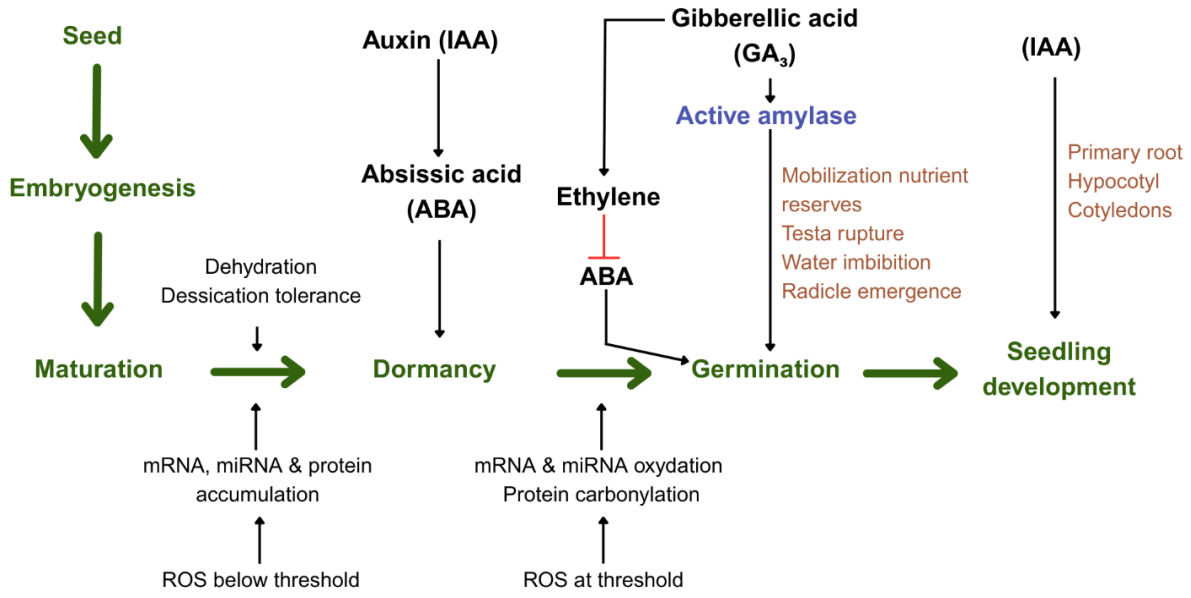


Fig. 3: Phytohormones and ROS signaling cascade in regulation of seed dormancy, germination and seedling development, adapted from [15]

In certain species such as pea (*Pisum sativum*) or soybean (*Glycine max*), dormancy is primarily mechanical, caused by a seed coat that is impermeable to water or oxygen [18]. Furthermore, if environmental conditions remain unfavorable after the release of primary dormancy, secondary dormancy may be induced by stress during imbibition. This induction is a natural process in which seeds that are competent for germination enter a reversible state of dormancy due to environmental stress conditions [19].

Following dormancy release, IAA promotes cell elongation in the primary root and hypocotyl. When the radicle emerges through the micropyle to form a root, the seedling is initially heterotrophic and depends on the reserves contained in the cotyledons, which may also be photosynthetic. These enable the development of a functional aerial system capable of utilizing water, mineral nutrients, and light energy, marking the gradual transition to autotrophy [20]. A rapid, uniform, and vigorous seedling establishment is therefore a key factor for ensuring high crop yields.

### 1.2 Abiotic stress and climate change: strategies of adaptation in plants and seeds

Climate change is driving rapid shifts in precipitation patterns, increasing the frequency of droughts, and amplifying thermal variability. Forecasts predict a global temperature increase of 2°C. This warming is disrupting the water cycle in a lasting way and altering rainfall patterns, which are becoming both less frequent and more intense, thereby increasing agricultural exposure to climate-related hazards. More broadly, major climate-related disasters have intensified globally over the past two decades: +28% for droughts, +46% for wildfires, +40% for storms, +232% for extreme temperature events, and +137% for floods [3] (Fig. 4). Overall, the frequency of these events has increased by 85% compared to the 1980–1999 period, illustrating the growing climate pressure on global agriculture.

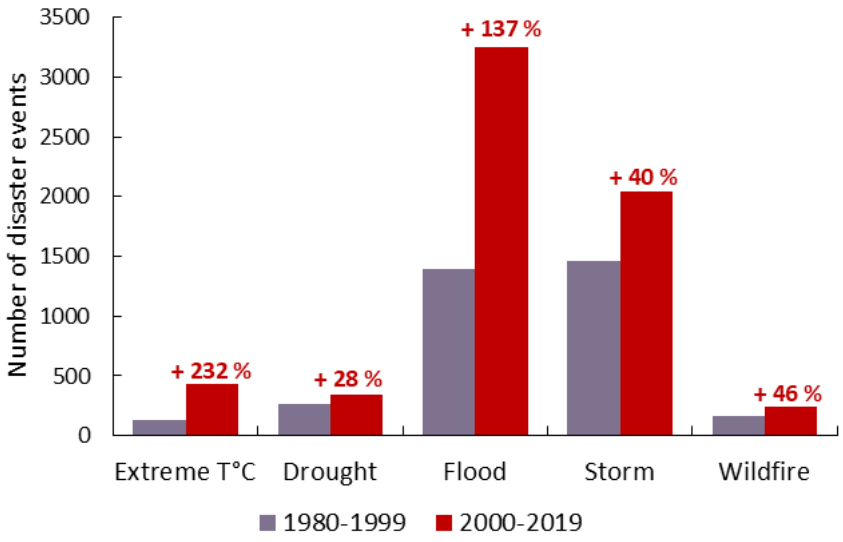


Fig. 4: Total disaster events by type: 1980-1999 vs. 2000-2019

Environmental changes have direct impacts on seed physiology and cropping cycles. As mentioned previously, germination and the early stages of plant development are particularly sensitive to water availability. Water stress and temperature variations, the main manifestations of climate change, disrupt the genetic and hormonal networks that regulate germination in species such as rice, wheat, or tomato [21]. Moreover, an unfavorable environment at the time of germination can induce secondary dormancy in mature seeds, thereby delaying dormancy release.
The effects of water stress on seeds are well documented. A meta-analysis by Daryanto et al. showed that drought causes an average yield loss of 20% in wheat and 40% in maize [22]. In rice, drought reduces the number of panicles and flowers per plant [23]. However, yield losses are rarely attributable to a single factor, as multiple abiotic stresses often act synergistically, thus amplifying their impact. In response to these constraints, seeds have developed adaptive strategies.

Naturally dehydrated, they can remain dormant in soil seed banks for years, even decades. They can also delay germination via secondary dormancy when environmental signals are unfavorable, acting as an evolutionary safeguard mechanism to optimize reproductive success. Water stress also induces excessive production of reactive oxygen species (ROS), leading to oxidative stress [24]. Prolonged accumulation of ROS causes membrane damage and oxidative alterations to DNA, proteins, and lipids, potentially leading to programmed cell death [25].

To protect themselves, seeds activate enzymatic antioxidant mechanisms such as superoxide dismutase (SOD), which converts superoxides into hydrogen peroxide, catalase (CAT), and peroxidases (APX, GPX), which convert hydrogen peroxide into water and oxygen, as well as glutathione reductase (GR), which regenerates reduced glutathione (GSH), essential for ROS neutralization. In addition, non-enzymatic compounds such as reduced glutathione, ascorbate (vitamin C), tocopherols (vitamin E), and carotenoids scavenge ROS and limit lipid oxidation, a major source of ROS [26]. Thioredoxin/glutathione systems also maintain protein reduction and protect against oxidative damage [27].

Also, as previously mentioned, seeds require oxygen ($O_2$) to germinate. Oxygen demand increases significantly during imbibition, as metabolic activity resumes. This process can be disrupted by flooding, leading to environmental hypoxia [28], which negatively affects germination. In a study involving six agriculturally important plant species (both monocotyledons and dicotyledons), Tang et al. demonstrated that suboptimal $O_2$ concentrations significantly reduced germination rates in all species tested, with dicotyledons being the most sensitive [29]. Under such hypoxic conditions, the aerobic mitochondrial pathway becomes insufficient. Seeds can then activate alternative metabolic

pathways, such as glycolysis and fermentation, which occur outside the mitochondria. These pathways play a crucial role in hypoxia acclimation.

They are particularly well developed in aquatic species tolerant to hypoxia [7], such as rice a semi-aquatic annual grass [30]. In maize (monocotyledon), Gayral et al. observed a spatial regulation of metabolic pathways within the seed:

- Glycolytic genes were upregulated in the seed periphery, a less hypoxic zone,
- Fermentation-related genes were activated in the central region of the seed, which is more hypoxic.

This spatial organization of metabolism enables targeted ATP production in the peripheral regions of the endosperm and embryo, where energy-intensive processes, such as protein and lipid synthesis, are concentrated [31].

In plants, beyond antioxidant mechanisms, abiotic stress such as drought or extreme temperatures also triggers physiological, biochemical, and molecular responses:

- Physical: Plants may, for example, reduce leaf area and close their stomata via ABA to limit evapotranspiration [32], and modulate root architecture by increasing the number of absorbing root hairs [33].
- Biochemical: They accumulate osmoprotectants such as proline or glycine betaine, which contribute to osmotic adjustment, ROS detoxification, membrane protection, and enzyme stabilization [34]. These compounds enhance tolerance to salt, water, or metal stress. Forlani et al. demonstrated a correlation between relative proline increase and salt stress tolerance in various rice genotypes [35]. Badawy et al. showed that in sugar beet, foliar application of glycine betaine could compensate for cadmium-induced growth delay, significantly increasing shoot length (+29.11%), root length (+24.66%), and leaf number (+16.67%) [36].
- Molecular**:** Exposure to stress also triggers the expression of response genes encoding, notably, heat shock proteins, ion channels, or transcription factors. These responses are largely regulated by phytohormones such as abscisic acid (ABA), ethylene, and cytokinins, which coordinate stress signaling and metabolic adjustments [37].

Under hypoxic conditions, plants also activate alternative metabolic pathways, including glycolysis and fermentation, to maintain a minimal production of ATP. This response is regulated at the molecular level by transcription factors triggered by mitochondrial respiration dysfunction, particularly those

from the ANAC and ERF-VII families [38]. In addition, morphological changes may occur. Pan et al. showed that excessive watering in cotton induces the formation of aerenchyma (a porous cortical tissue that facilitates gas exchange with the atmosphere) [39], a response regulated by ethylene [40].

These strategies represent a significant energetic cost for the plant and are often implemented at the expense of reproduction. Under unfavorable conditions, plants may abort their reproductive organs or develop abnormal structures that prevent fertilization [41]. Seed and fruit development is therefore particularly sensitive to abiotic stresses.

However, certain environmental stresses experienced by the mother plant can also trigger adaptive responses that are beneficial for the offspring. For example, in sunflower (*Helianthus annuus*), a moderate water stress applied to mother plants after flowering improves the germination of seeds derived from these plants, revealing a maternal effect [42]. This treatment also influences seed dormancy levels across two generations, suggesting the existence of a transgenerational stress memory mechanism. Transcriptomic analysis, coupled with methylome profiling, revealed an epigenetic component associated with this response [42]. Among the ten major global crops, six are primarily harvested for their fruits and seeds [4]. Therefore, maintaining reproductive success and seed vigor under stress conditions remains a major challenge for ensuring stable crop yields. In this context, understanding the epigenetic mechanisms involved in the transmission of stress tolerance is essential for developing effective crop adaptation and improvement strategies.

### 1.3. Challenges of response to biotic stresses: vectors and defense mechanisms in plants & seeds

#### 1.3.1. Vectors of biotic stress

In addition to their direct effects, abiotic stresses indirectly affect plant health by promoting the development and spread of pathogens, thereby increasing the risk of seed contamination. Global climate warming is altering and, in many cases, expanding the geographical distribution of numerous pathogens [43]. As a result, temperatures once typical of tropical regions are now recorded in temperate zones, enabling the northward migration of pests and pathogens that were previously absent. Crops in these newly exposed regions face unfamiliar combinations of abiotic and biotic stresses, whose synergistic interactions often exacerbate negative effects. Moreover, seeds and plants weakened by repeated or intense abiotic stress become more susceptible to pathogen attacks, increasing yield losses [44].

Biotic stress, defined as any constraint exerted by a living organism on a plant, includes a wide range of agents such as bacteria, fungi, viruses, insect pests, and weeds that compete for resources. These

pressures, exacerbated by environmental disturbances, represent a constant threat throughout the plant life cycle [45]. Seeds are particularly vulnerable to biotic stress: their rich nutritional content makes them a prime target for many animals and crop pests. They can be directly consumed by rodents (rats, mice, voles) or granivorous birds (pigeons, sparrows), causing significant losses even before germination [46]. In the soil or in storage, insects such as grain weevils (*Sitophilus spp*.) and seed beetles (*Callosobruchus spp*.) attack seed interiors, destroying embryos and preventing germination [47] (Tab. 1). Additionally, some plant-parasitic nematodes (e.g., *Meloidogyne* spp.) can damage embryonic or root tissues during the germination stage [48]. Pests not only consume seeds and plants, reducing crop productivity, but also act as vectors of plant diseases, transmitting bacteria, viruses, and fungi that can further compromise plant health [49].

The table below summarizes major seed and plant pests:

| Pest | Type | Target | Effects |
|---|---|---|---|
| Sitophilus spp. | Insect (Coleoptera) | Seed (during storage) | Infest stored grain, reducing quality & quantity. |
| Callosobruchus spp. | Insect (Coleoptera) | Seed (during storage) | Common in legumes larvae develop inside seeds. |
| Rodents | Mammal | Seed (in field and storage) | Consume seeds or seedlings, reducing plant establishment. |
| Granivorous birds | Bird | Seed (in field) | Feed on sown seeds. |
| Meloidogyne spp. | Nematode | Plant (roots) | Cause root galls and reduce plant growth. |
| Tribolium spp. | Insect (Coleoptera) | Seed (during storage) | Infest stored grain, reducing quality & quantity. |
| Pheidole spp. | Insect (Hymenoptera) | Seed (in field) | Infest grain, reducing quality & quantity. |
| Trogoderma granarium | Insect (Coleoptera) | Seed (during storage) | Infest stored grain, reducing quality & quantity. |
| Diabrotica spp. | Insect (Coleoptera) | Plant (roots) | Larvae feed on maize roots, affecting stability and growth. |
| Agriotes spp. | Insect (Coleoptera) | Plant (roots) | Larvae damage plant roots, impairing nutrient uptake. |

Table. 1: Major crop pest [50]

In ecosystems, seeds and plants also interact with a wide variety of microorganisms, both pathogenic and symbiotic [51]. They host a considerable diversity of microorganisms, especially fungi. Infections caused by pathogenic fungi are responsible for a large proportion of seed diseases. It is estimated that over 80% of seed and plant diseases are caused by fungi or related organisms such as oomycetes [52]. These fungi can produce visible molds on seeds, compromise seed quality, and cause major problems

in the field such as seedling damping-off. They may also affect human and animal health via mycotoxins. Transmission can occur through the mother plant, biotic vectors like insects or animals, or abiotic environmental factors.

A prominent example is *Ustilago nuda*, a phytopathogenic fungus affecting barley. Transmitted directly from the mother plant to the seed, it colonizes the embryo during seed formation and later destroys the crop by causing ear rot [53]. Other fungi, such as those from the genus *Fusarium*, can grow during storage and cause grain rot. These pathogens, sometimes already present on seeds, thrive in poorly adapted storage conditions (e.g., high humidity, unsuitable temperatures) [54].

Some fungi also produce harmful mycotoxins, such as *Aspergillus*, *Fusarium*, *Alternaria*, or *Penicillium*. These secondary metabolites pose significant health risks; the most concerning include aflatoxins, altersolanols, trichothecenes, fumonisins, zearalenone, ochratoxin A, and certain alkaloids [54]. Fungal infections can not only reduce the nutritional quality of seeds but also compromise their agricultural or food use. The grain may be completely destroyed or partially degraded, reducing its market and agronomic value.

Seeds are also a major vector of diseases affecting seedlings and mature plants [55]. A classic example is damping-off disease: this condition affects germinating seeds and seedlings and is caused by fungi such as *Rhizoctonia solani* or *Pythium* spp. These pathogens attack young tissues lacking secondary thickening, such as radicles or seedling collars, leading to wilting, blackening, and rapid death [56].

Beyond fungi, other pathogens such as bacteria can cause serious diseases. This is the case for several species of *Xanthomonas*, pathogens of over 400 plant species. Certain strains, such as *Xanthomonas campestris* or *Xanthomonas oryzae*, cause black rot in cabbage and bacterial blight in rice, respectively [57]. Soil-dwelling bacteria from the genus *Pseudomonas* also cause serious plant diseases. Over 50 pathovars of *Pseudomonas syringae* (Tab. 2) have been identified as pathogenic on various plants, causing cankers, bacterial blights, and leaf spots. These lead to substantial economic losses due to reduced yields and increased control costs [58, 59]. Plants are also targeted by viruses, particularly mosaic viruses such as Tobacco Mosaic Virus (TMV). This RNA virus has a broad host range, infecting more than 350 plant species. It causes leaf mosaic discoloration, wilting, reduced flowering, fruit deformation, and overall plant weakening, resulting in global economic losses estimated at $2.5 billion annually [60].

In total, infections can lead to the complete destruction of seeds, seedlings, or crops, or to a severe degradation in their quality (e.g., mycotoxin contamination, loss of nutritional value, reduced fruit and vegetable size, etc.). It is important to note that global annual crop yield losses caused by microbial

pathogens and pests are estimated at $220 billion, directly impacting food security, regional economies, and related socioeconomic factors [43].

The main pathogens and their effects are summarized in the table below:

| Pathogen | Type | Target | Effects |
|---|---|---|---|
| *Ustilago nuda, Tilletia caries* | Fungus | Wheat, barley, maize | Loose smut/common bunt |
| *Fusarium graminearum, F. verticillioides* | Fungus | Maize, wheat, soybean | Seed rot |
| *Alternaria alternata, A. brassicicola* | Fungus | Carrot, lettuce, tomato | Leaf spots and blights/silique rot |
| *Colletotrichum lindemuthianum* | Fungus | Bean, pea, soybean | Anthracnose |
| *Penicillium, Aspergillus, Rhizopus* | Fungus | Cereals, legumes | Storage mold |
| *Pythium spp.* | Oomycete | Seeds and seedlings | Damping-off |
| *Rhizoctonia solani* | Fungus | Seedling stem (collar region) | Damping-off |
| *Xanthomonas campestris* | Bacterium | Brassicas (cabbage, mustard) | Bacterial wilt |
| *Pseudomonas syringae* | Bacterium | Lettuce, bean, spinach | Bacterial soft rot / blight |
| *Tobacco mosaic virus (TMV)* | Virus | Tobacco, tomato | Mosaic virus diseases |
| *Tomato spotted wilt virus (TSWV)* | Virus | Tomato, pepper | Tomato spotted wilt |

Table. 2: Major crop pathogen [61, 62, 56, 63, 64]

### 1.3.2. Defenses mechanisms

To counter these threats, seeds have developed a range of adaptive defense strategies that help them resist biotic stresses, especially from pathogens and predators. These defense mechanisms include physical and chemical barriers.

Physically, the seed is protected by a hard, often impermeable seed coat, which serves as the first line of defense. This coat limits the penetration of water and pathogens, reducing infection risk during critical stages such as storage or germination. In some cases, the seed coat structure also prevents internal access by insects or other predators (Fig. 5).

Chemical defenses complement this physical barrier. Seeds accumulate various antimicrobial compounds in their tissues, particularly in the seed coat and endosperm [65]. These include phenolic compounds, tannins, and saponins that can inhibit pathogen colonization or repulse herbivores. Such

substances may suppress fungal and bacterial growth or make the seed less palatable or more toxic. For example, tannins precipitate proteins, disrupting digestion in granivores, while saponins have well-documented antifungal and antibacterial properties [66, 67]. Another well-known example is castor bean (*Ricinus communis*), whose seeds are enclosed in a protective coat containing the highly toxic protein ricin. Ricin is a potent toxin that inhibits protein synthesis in cells, making it lethal even at very low doses [68]. These strategies allow the seed to survive in a hostile environment and preserve its germination potential until conditions are favorable.

Some signaling pathways triggered in response to biotic stress in seeds are similar to those observed in mature plants, though adult plants possess a more developed immune system. As sessile organisms, plants cannot flee from threats and have therefore developed adaptation strategies to survive not only abiotic fluctuations [69] but also biotic pressures from phytopathogens and herbivores.

Like seeds, plants have a passive first line of defense made up of physical barriers such as the cuticle, epicuticular waxes, trichomes, and sometimes thorns (Fig. 5). These structures help limit attacks from pathogens, herbivores, and insects [70].

When an attack is detected, active defense mechanisms can be triggered, leading to local or systemic responses. The first level of recognition involves membrane-bound receptors called PRRs (Pattern Recognition Receptors), which detect pathogen-associated molecular patterns (PAMPs). This response, called PAMP-Triggered Immunity (PTI), involves the production of reactive oxygen species (ROS) and activation of defense genes. Similar signals are triggered by herbivore attacks, including herbivore-associated elicitors (HAEs), herbivore-associated molecular patterns (HAMPs), or herbivore effectors recognized by PRRs [70].

PTI may be followed by a stronger immune response known as Effector-Triggered Immunity (ETI). This is activated by intracellular resistance proteins, especially NLRs (Nucleotide-binding Leucine-rich Repeat receptors), which detect specific pathogen effectors (Avr proteins). This activation initiates signaling cascades via MAP kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), leading to intracellular calcium ($Ca^{2+}$) accumulation. This signal promotes callose and lignin deposition and strengthens cell walls at infection sites. Concurrently, antimicrobial compounds such as phytoalexins are locally produced. If the infection persists, the plant may trigger a hypersensitive response (HR), consisting of localized programmed cell death to prevent pathogen spread [71].

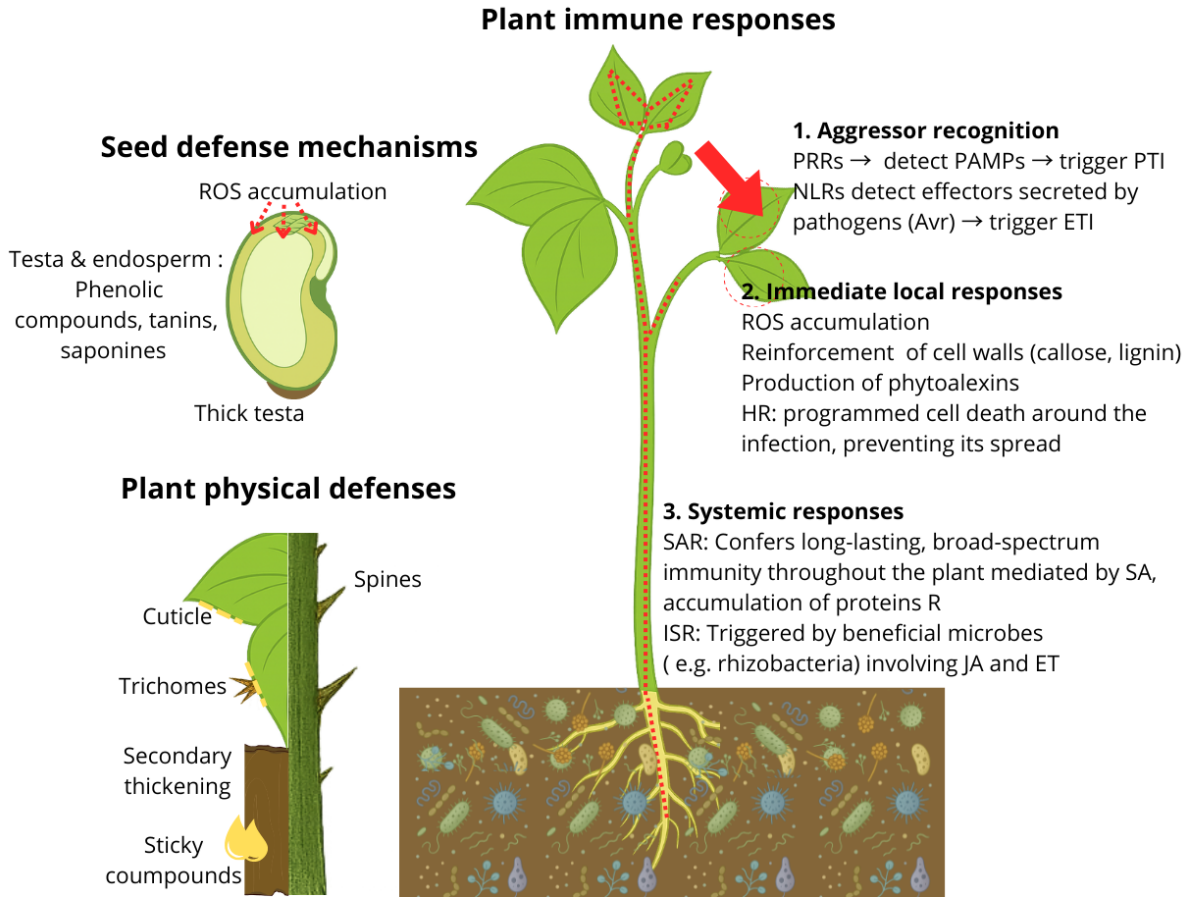


Fig. 5: Seed defense mechanisms and plant physical and immune responses to biotic stress

These local signals can also activate systemic responses, such as Systemic Acquired Resistance (SAR), mediated by salicylic acid (SA) and associated with R protein accumulation [71], extending immune responses beyond the infection site (Fig. 5). These defenses are energetically costly, often involving trade-offs with essential processes like growth, reproduction, and seed production, with significant ecological and economic consequences [72].

A critical yet often underestimated component of plant defense is the plant microbiota. A growing body of research highlights its importance, especially in seeds. The plant microbiota includes all microorganisms (bacteria, fungi, protozoa, etc.) that live on (epiphytes) or within (endophytes) plant tissues roots, stems, leaves, flowers, and seeds. These microbial communities play key roles in nutrient acquisition, plant growth, immune stimulation, and tolerance to abiotic and biotic stresses [73]. For example, plants may also activate another form of systemic defense: Induced Systemic Resistance (ISR). Unlike SAR, ISR is triggered by beneficial microorganisms, notably rhizobacteria such as *Pseudomonas fluorescens* or *Bacillus subtilis*, living in the rhizosphere. ISR is mainly mediated by

jasmonate (JA) and ethylene (ET) pathways and helps prevent future attacks without immediately activating costly immune responses (Fig. 5).

Microbial effects on plants may be beneficial (e.g., ISR), neutral, or harmful especially when promoting disease. These interactions depend heavily on plant genotype, microbial species characteristics, and environmental conditions [74]. Key microbiota functions include pathogen protection via antimicrobial production or ecological competition, plant growth promotion through hormone-like molecules (e.g., *Trichoderma harzianum*) [75], and improved nutrition via nitrogen fixation (*Rhizobium*), phosphorus solubilization, or mineral mobilization (e.g., *Pseudomonas*) [76].

However, today's environmental conditions are increasingly unstable due to climate change. These disruptions can unbalance host-pathogen dynamics, transform plant-microbe relationships (e.g., from commensalism to parasitism), or increase the virulence of opportunistic pathogens [43].

The seed and plant microbiota assemble via two complementary mechanisms: vertical transmission from the mother plant to the seed, and horizontal transmission directly influenced by the environment (soil, water, insects, etc.) (Fig.6).

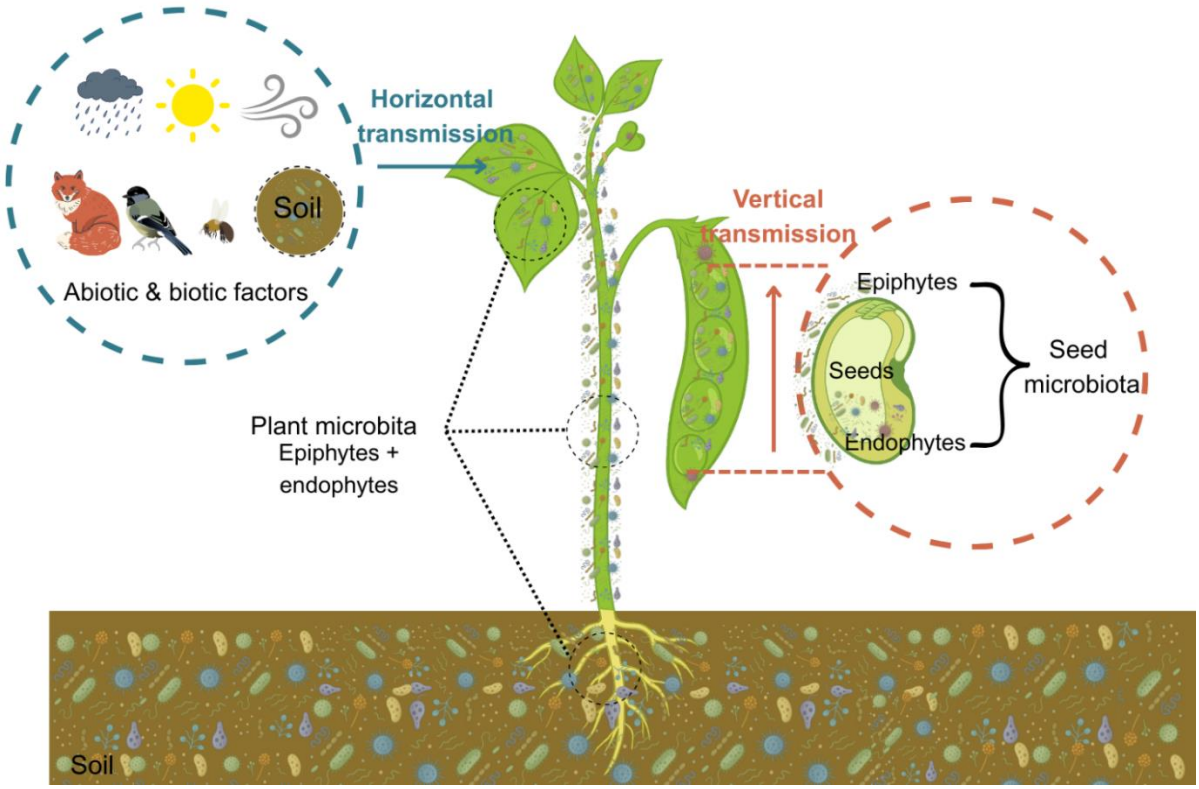


Fig. 6: Modes of plant and seed microbiota transmission: vertical and horizontal pathways and influencing factors

During germination, the transition from seed to seedling represents a major disturbance for the seed microbiome [77]. Furthermore, each seed has a unique microbial composition, making characterization difficult. Nonetheless, certain fungal and bacterial species are widespread. For instance, Simonin et al. (2022), in a meta-analysis using global seed data, identified 30 fungal and bacterial taxa found in many plant species. Major taxa included *Pantoea agglomerans*, *Pseudomonas viridiflava*, *P. fluorescens*, *Cladosporium perangustum*, and *Alternaria* sp., frequently found in seed microbiota [78].

Once the plant matures, microbiota composition depends on the colonized tissue (phyllosphere, rhizosphere, etc.). Although variation between individuals remains high, several taxa are consistently found, including bacterial genera such as *Pantoea* and *Pseudomonas*, and fungal genera like *Fusarium*, *Colletotrichum*, and *Cladosporium*. Due to the complexity of interactions and the many factors shaping these microbial communities, it remains extremely difficult to define a universal plant microbiota [78].

Despite these sophisticated defense mechanisms, seeds and plants face dual biotic and abiotic pressures that cause considerable yield losses and push agricultural stakeholders to rethink seed quality improvement strategies and plant resilience in the face of environmental change.

### 1.4. Current approaches towards resilient agricultural practices in a changing climate

#### 1.4.1. Crop breeding

Since Gregor Mendel's work on heredity, crop breeding has been established as a central lever of modern agriculture. This approach aims to select genotypes with superior agronomic traits, whether in terms of yield, disease resistance, or adaptation to environmental conditions. Since the Green Revolution of the 1960s, variety breeding programs, combined with a profound evolution of agricultural techniques, have enabled a significant increase in crop yields. In France, durum wheat breeding intensified in the 1970s with priorities such as reducing straw height to increase lodging resistance and better meet industrial requirements. The introduction of high-yielding semi-dwarf varieties, combined with agronomic advances, has led to a +232% increase in global yields since the 1960s [4] (Fig. 7).

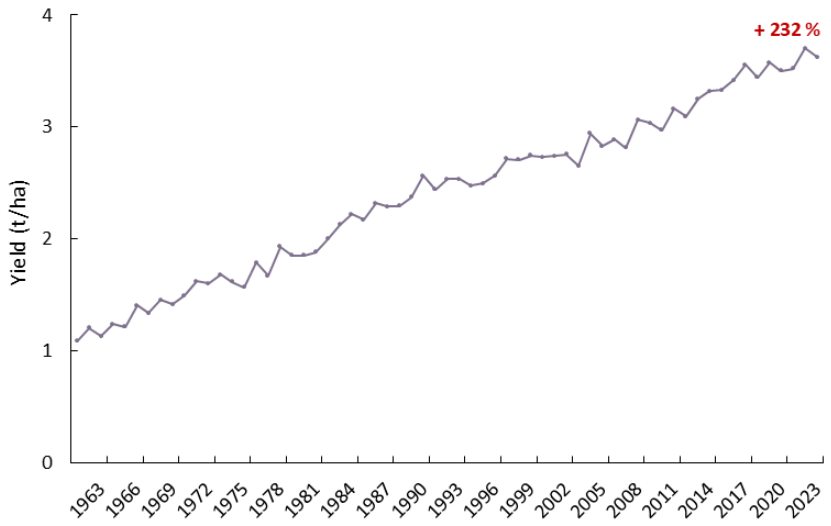


Fig. 7: Global trends in wheat yield improvement from 1961 to 2023, based on FAOSTAT data [4]

A landmark example of successful variety breeding is IR8 rice, developed in the 1960s by the International Rice Research Institute (IRRI). Resulting from a cross between a tall high-yield Indonesian variety (Peta) and a robust Chinese dwarf variety (Dee-geo-woo-gen), IR8 is considered the first high-yielding semi-dwarf rice variety. This variety doubled yields in many regions of Asia and played a major role in helping to reduce the risk of famine in several rapidly growing countries [79]

### 1.4.2. Genetic engineering

The discovery of the structure of DNA in the 1950s by Rosalind Franklin, Maurice Wilkins, Raymond Gosling, James Watson, and Francis Crick [80], along with the development of sequencing methods in the 1970s by Walter Gilbert and Frederick Sanger [81], paved the way for genetic engineering. This discipline has enabled the genetic improvement of plants, particularly through understanding the transferability of DNA and RNA [82]. A GMO (genetically modified organism) is defined as an organism (plant, animal, or microorganism) whose genetic material (DNA) has been altered in a way that does not occur naturally through mating or natural recombination [83]. One technique involves inserting a specific gene via a vector organism, such as *Agrobacterium tumefaciens*. This bacterium, used as a vector, transfers a fragment of its DNA (the T-DNA) into plant cells, allowing stable integration into the plant genome. These modifications aim to make plants more resistant to pests, diseases, or environmental stress.

The first GMO commercialized for food purposes was the Flavr Savr tomato, authorized in the United States in 1994 [84]. It was designed to better withstand transport and slow fruit softening. Subsequently, crops like Bt maize resistant to the European corn borer and Bt cotton were developed

to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis*, thereby reducing the need for chemical pesticides. Later, next-generation sequencing (NGS) revolutionized plant genetic engineering. The genetic modification of plants relies on a deep understanding of their genome. NGS technologies have enabled considerable progress in this field, particularly in identifying genes of interest. These methods now allow for the sequencing of millions of bases at once, at a much lower cost than traditional first-generation sequencing [85].

More recently, it has become possible to obtain a desired trait in food crops or animals without even needing to transfer genes from unrelated species. In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for their work on the CRISPR-Cas9 complex and the development of "molecular scissors." This discovery opened a new path for plant genetic engineering. The CRISPR-Cas9 complex, derived from a bacterial immune mechanism developed in response to viral infection, consists of a protein (the Cas9 endonuclease) coupled with a guide RNA. When the guide RNA recognizes a specific DNA sequence, the complex binds to it, and the Cas9 enzyme cuts the complementary DNA strand [86]. The cut can then be repaired by inserting a new fragment of target DNA. This technology, combined with NGS, enables the precise targeting of specific genetic sequences and the introduction of desirable traits into plant genomes. It is considered safer than techniques involving vector organisms, as it avoids transferring genetic material between unrelated species, which is often a source of uncertainty [82]. Moreover, one of the advantages of CRISPR technology is that it allows genes to be inserted or deleted more easily and precisely than conventional transgenic methods. It also makes it possible to bypass some of the strict regulations applied to transgenic GMOs, thereby facilitating its adoption in several countries [82].

### 1.4.3. Pesticides

To date, pest and weed control still relies largely on the use of pesticides. Nationally, France is the second-largest consumer of pesticides in the European Union, after Spain, with an average annual consumption of approximately 69,000 tonnes between 2011 and 2020 [87]. It also ranks third in the number of authorized active substances (291), behind Greece (298) and Spain (296), which is 32 % above the EU average [88].

Over 90 % of pesticides are used for agricultural purposes. In France, their distribution is as follows:

- Herbicides (44 %): for weed control,
- Fungicides and antimicrobial agents (41 %): against fungal and bacterial pathogens,

- Insecticides (10 %): against insect pests,
- Other products (5 %): including rodenticides, molluscicides, nematicides, and growth regulators [4].

Globally, the amount of pesticides used in agriculture increased by 104 % (Fig. 8) between 1990 and 2022 [4].

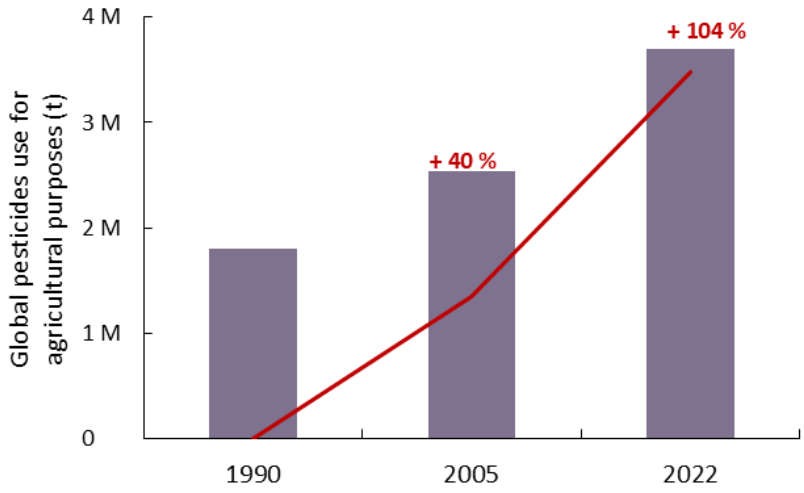


Fig. 8: Global agricultural pesticide use in 1990, 2005, and 2022, based on [4]

Pesticides can be applied directly to crops or used as seed coatings. Seeds are primarily treated with biocides to protect them against pests and pathogens, while plants are treated with both biocides and herbicides such as glyphosate to reduce weed competition [4]. Application methods vary depending on the type of pesticide, the crop, and the target pest. These methods include foliar spraying (either aerial or ground-based), soil incorporation, drip irrigation systems (chemigation), and seed treatments. Foliar spraying is one of the most common practices and can be carried out using tractors, drones, or aircraft in conventional agriculture. Soil application involves incorporating pesticides into the soil either before or after planting to target soil-dwelling pests. Chemigation enables precise delivery of pesticides through irrigation systems, thereby minimizing drift and exposure. Seed coating, in contrast, offers a targeted approach by embedding active ingredients directly onto the seed surface, which reduces the amount of pesticide required and limits environmental dispersion.

### 1.4.4. Seed priming

In addition to chemical inputs, various priming techniques have been developed to promote more uniform germination. Priming is a seed pre-hydration technique that induces a physiological state favorable to germination and improves seedling emergence [89].

There are several types of priming:

- Osmopriming: soaking in osmotic solutions (e.g., PEG, salts),
- Hydropriming: soaking in water,
- Nanopriming: soaking in a solution containing nanoparticles (often metallic: ZnO, Ag, Cu),
- Hormopriming: soaking in solutions of plant hormones,
- Biopriming: coating seeds with beneficial microorganisms, an emerging practice based on recent research on the seed microbiome.

1.4.5. Biostimulants

More recently, new molecules have been developed to enhance seed and plant vigor, by strengthening natural defenses and stimulating growth. These substances are grouped under the term biostimulants. Du Jardin (2015) describes them as follows: “A plant biostimulant is a substance or microorganism applied to plants with the aim of improving nutrient use efficiency, tolerance to abiotic stress and/or crop quality characteristics, regardless of its nutrient content.” [90]. By extension, this term also includes commercial products containing mixtures of these substances or microorganisms.

Biostimulants may be categorized according to the nature of the molecules used, as summarized in the table 3 below. They can be applied as priming or coating solutions on seeds, or by spraying or irrigation in the field.

| **Category** | **Examples** | **Main effects** |
|---|---|---|
| Humic and fulvic substances | Humic acid, fulvic acid | Improves nutrient uptake, root growth, and enzyme activity |
| Beneficial microorganisms (PGPR) | Rhizobium, Azospirillum, Bacillus, Trichoderma, Mycorrhizae | Promotes growth, nitrogen fixation, induces systemic resistance (ISR) |
| Seaweed extracts | Ascophyllum nodosum, Ecklonia maxima, Laminaria spp. | Enhances stress tolerance, metabolism, and photosynthesis |
| Amino acids and peptides | Hydrolyzed proteins, free amino acids | Supports plant under abiotic stress, stimulates enzymatic activity |
| Inorganic compounds | Al, Co, Na, Se, Si | Strengthens plant structure, improves resistance to stress |
| Biopolymers | Chitosan, glucans | Enhances plant defense, improves resistance to stress |

Table 3: Main categories of biostimulants [90]

### 1.4.6. Biological control

Lastly, biocontrol strategies are also emerging to combat pests and diseases. Agricultural biocontrol relies on the use of living organisms (predators, parasitoids, pathogens, or competitors) to reduce pest populations, ideally within an integrated pest management strategy [91].

Recent examples include:

- Use of *Trichoderma* fungal strains that exert mycoparasitic effects on pathogenic fungi and facilitate phosphate solubilization, benefiting plant growth [92, 75].
- In viticulture, *Bacillus subtilis* strains have demonstrated antifungal effects against fungi responsible for wood spot diseases [93].
- Insects are also commonly used, such as lady beetle larvae against aphids [94], or parasitoid hymenopterans against lepidopteran pests like the European corn borer (*Ostrinia nubilalis*) [Gagnon] or the tomato fruit-worm (*Helicoverpa armigera*) [95].

### 1.4.7 Limits and controversies

Pesticides

Since the 1960s, the massive use of pesticides has profoundly disrupted agroecosystems. These substances have depleted soils by destroying essential microorganisms, thereby increasing crops' dependence on external chemical inputs. They have also led to the long-term contamination of surface and groundwater, raising serious concerns for biodiversity and public health.

All application methods carry specific risks of runoff or non-target exposure, but spraying is particularly problematic. It significantly exposes farmers and nearby residents to pesticide drift, especially when used close to homes or under unfavorable weather conditions.

Certain pesticides, such as glyphosate and its degradation product AMPA, are suspected of increasing the risk of breast cancer (in vitro studies) [96] and non-Hodgkin lymphoma [97]. Others, like neonicotinoids mainly used against aphids in sugar beet cultivation are known neurotoxins for pollinators. While banned as early as the 2000s in countries like Slovenia due to their environmental impact [98], these substances remain highly controversial across the European Union. In France, the “Duplomb law,” definitively adopted by the National Assembly on July 8, 2025, reauthorized certain

previously suspended neonicotinoids, including acetamiprid, sulfoxaflor, and flupyradifurone [99], reigniting debates on the balance between agricultural productivity and ecological responsibility.

Crop breeding

Varietal selection is a long and costly, on average a conventional breeding program takes 8 to 10 years to develop a new stable and marketable variety, due to the multiple stages involved: crossing, generational selection, multi-site evaluation, and registration in national catalogs [100]. It also demands substantial human and technological resources and may reduce genetic diversity, making crops more vulnerable to emerging diseases or rapid environmental changes. Moreover, varieties developed under controlled conditions do not always perform as expected in field environments [101, 102, 100].

Genetic engineering

Today, GMOs are widely cultivated in certain countries, notably the United States, Brazil, and India, although they remain the subject of regulatory and ethical debates in other regions such as the European Union [103].

Despite their agronomic benefits (pest resistance, reduced pesticide use, etc.), GMOs raise several limitations and controversies:

- Environmental: the repeated use of Bt plants or herbicide-tolerant crops can promote the emergence of resistance in pests or weeds. This may reduce the effectiveness of the introduced traits and require the use of new chemical products or techniques.
- Economic and social: some GMO seeds are protected by patents, which limits their deployment and use by farmers. This can increase dependency on major seed companies, especially in developing countries [104].
- Health and societal: although scientific assessments conducted so far have not demonstrated any proven health risks, public perception often remains skeptical. In Europe, the precautionary principle is frequently applied, which slows the adoption of these technologies.

Furthermore, the development of a new variety whether through conventional breeding or genetic engineering can take between 10 and 15 years, depending on generation time and the field trials required before any greenhouse testing and marketing authorization. These steps aim to ensure safety for human and animal health as well as for the environment.

Priming

Priming can reduce seed storage duration, requires specific storage conditions, and involves high technical and economic costs. Misapplication can promote pathogens or cause premature germination. Its effectiveness remains variable depending on plant species and environmental conditions [105].

Biostimulants

Biostimulants have several drawbacks, including high variability in results. This is due to:

- The geographic origin of raw materials,
- Seasonal variations,
- Extraction and processing methods,
- Specific microbial strains used.

Each of these factors can significantly alter the composition and concentration of active compounds in the final product, making performance difficult to predict [106]. Furthermore, the regulatory framework is heterogeneous internationally. In Europe, they fall under EU Regulation 2019/1009 on fertilizers, which defines clear criteria, unlike in the United States, where the regulatory framework remains less defined [106]. These regulatory ambiguities may also hinder their commercial promotion, marketing, and use across countries.

Biocontrol

Biocontrol strategies require time to observe effects and numerous costly preliminary studies to precisely target pests. There is also risk to non-target species. For example, the introduction of the Asian lady beetle (*Harmonia axyridis*) in Europe caused a decline in native ladybird populations [107]. Finally, adopting biostimulants and biocontrol requires substantial R&D investment and specific training for farmers to encourage large-scale adoption.

## 2. Plasma

### 2.1. History, definition and properties

The discovery of plasma likely began with early cosmic observations and philosophical reflections on the states of matter, dating back to Ancient Greece. Greek philosophers were among the first to lay the conceptual foundations of matter and its various transformations.

As early as the 5th century BCE, within the framework of natural philosophy, thinkers such as Empedocles proposed that all matter was composed of four fundamental elements: earth, water, air and fire. Later, Aristotle expanded upon this model by attributing specific physical properties to each element. Unlike the atomistic theory of Democritus, Aristotle did not consider matter to be inert; rather, each element possessed intrinsic qualities: dry, hot, moist and cold. For instance, water was considered *cold and moist*, while air was *hot and moist*. Aristotle further developed these ideas in his works *Physics* and *Metaphysics*. His worldview and conceptualization of matter would remain dominant for centuries [108].

Attributing physical properties to natural elements marked an early attempt to categorize different states of matter, as we understand them today. In this framework, the immaterial and luminous nature of fire might be seen as an early analog of what we now identify as plasma. Although the Greeks lacked the scientific tools and theoretical models to identify an ionized state of matter, their reflections laid the groundwork for future exploration of matter's transformations, a subject that modern physics would later study in much greater depth.

In the modern history of plasma physics, particularly from the 19th to early 20th century, major contributors such as Michael Faraday and William Crookes made significant strides in understanding electrical phenomena in gases. Faraday's work in the 1830s particularly on electromagnetic induction and the continuity of electric current influenced Crookes, who in 1879 introduced the term "radiant matter" to describe a new gaseous state observed in vacuum tube discharges. This state was characterized by the presence of charged particles [109].

The term "plasma" was finally introduced in 1928 by Irving Langmuir and Levi Tonks, who used it to describe a quasi-neutral mixture of free electrons and ions that behaves collectively under the influence of electromagnetic fields. The term was inspired by an analogy with blood plasma the fluid that carries biological cells since this "physical plasma" similarly carries charged particles [110].

Since then, plasma physics has emerged as a distinct discipline, playing a critical role in diverse fields: from astrophysics (where an estimated 99% of the visible universe exists in the plasma state), to

nuclear fusion, material processing and display technologies. Today, plasma is widely recognized as the fourth state of matter, alongside the solid, liquid and gaseous states (Fig. 9).

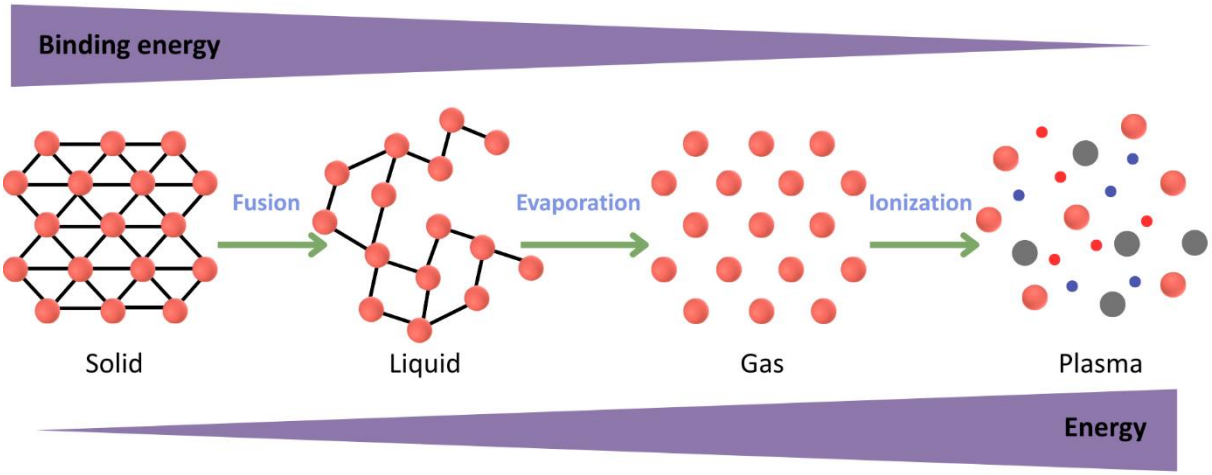


Fig. 9: States of matter: dependence on energy and binding energy

**2.2. Definition and ionization mechanisms**

Plasma is often defined as a quasi-neutral gas composed of charged particles (free electrons and ions) and neutral species (atoms or molecules). Despite being electrically neutral on macroscopic scales due to the near-equal densities of positive and negative charges, plasmas exhibit collective behavior governed by long-range Coulomb interactions [111]. These collective effects (eg, plasma oscillations, Debye shielding and self-generated electromagnetic fields) distinguish plasma fundamentally from ordinary neutral gases [112].

A gas enters the plasma state when sufficient energy (usually by heating, photoionization or electric discharge) is supplied to overcome the ionization energy of atoms or molecules, producing a population of free charges. One of the key parameters characterizing a plasma is the degree of ionization α, defined as follows:

$$\alpha = \frac{n_i}{n_i + n_n}$$

where $n_i$ is the ion density and $n_n$ is the density of neutral particles.

The value of α ranges from nearly zero (weakly ionized plasmas, ie non-thermal plasmas, ie cold plasmas) to approximately one (fully ionized plasmas, ie thermal plasmas). In weakly ionized plasmas, neutral species dominate and plasma behavior is significantly influenced by collisions between charged and neutral particles. In contrast, fully ionized plasmas, such as those found in stellar interiors or fusion

reactors, contain almost no neutral atoms and electromagnetic interactions dominate the dynamics [113].

Ionization is typically driven by inelastic collisions between energetic electrons and neutral atoms or molecules. These collisions can result in:

- Excitation, where an electron promotes an atom to a higher energy state, often followed by photon emission;
- Ionization, where an electron removes a bound electron from an atom, creating a positive ion and a secondary free electron.

The ionization rate is strongly dependent on the electron temperature $T_e$, which often differs significantly from the temperatures of ions ($T_i$) and neutral particles ($T_n$).

### 2.3. Classification by thermodynamic properties

#### 2.3.1. Thermal (equilibrium) plasmas

In thermal plasmas, all species (electrons, ions and neutrals) are in or near local thermodynamic equilibrium (LTE), meaning:

$$T_e \approx T_i \approx T_n$$

This condition is typically achieved at high particle densities and pressures, where frequent collisions allow for efficient energy exchange among all species. Thermal plasmas exhibit high ionization degrees ($\alpha \approx 1$), high energy density and strong thermal radiation.

Examples of thermal plasmas include the interior of the sun, where hydrogen and helium are almost completely ionized under extreme temperature and pressure conditions [114] and lightning, in which atmospheric gases are rapidly heated to temperatures in excess of 30,000 K, resulting in intense ionization [115]. In technological applications, thermal plasmas are at the heart of several industrial and experimental systems. Tokamaks aim to reproduce stellar fusion by using magnetic confinement to sustain high-temperature plasmas [116, 117]. Plasma torches are used for precision metal cutting and thermal spray deposition of ceramic coatings [118]. Thermal plasma reactors are also used for high-temperature processes such as the destruction of hazardous waste and the synthesis of advanced materials. In addition, the Variable Specific Impulse Magnetoplasma Rocket (VASIMR) uses radio-frequency heating to generate and accelerate plasma for efficient space propulsion [119, 120]. Although VASIMR does not operate under strict local thermodynamic equilibrium (LTE), it does operate at high energy densities characteristic of thermal plasma environments.

2.3.2. Non-thermal plasmas (or cold plasmas)

Non-thermal plasmas, also known as cold plasmas, are characterized by a significant deviation from thermodynamic equilibrium. In these plasmas, the various species (electrons, ions and neutral particles) do not share the same temperature. More precisely, and as depicted in Figure 10, the temperature of electrons ($T_e$) is significantly higher than the temperatures of heavier species, which generally satisfies the relationship:

$$T_e \gg T_i \approx T_n$$

While electrons in non-thermal plasmas can reach effective temperatures ranging from a few thousand to several 10 000 K (1-10 eV), ions and neutral gaseous components remain close to ambient temperature (typically 300-1000 K). This substantial thermal disparity is due to the much lower mass of electrons, which enables them to be preferentially accelerated by external electric fields while transferring only limited energy to heavier particles via collisions [121].

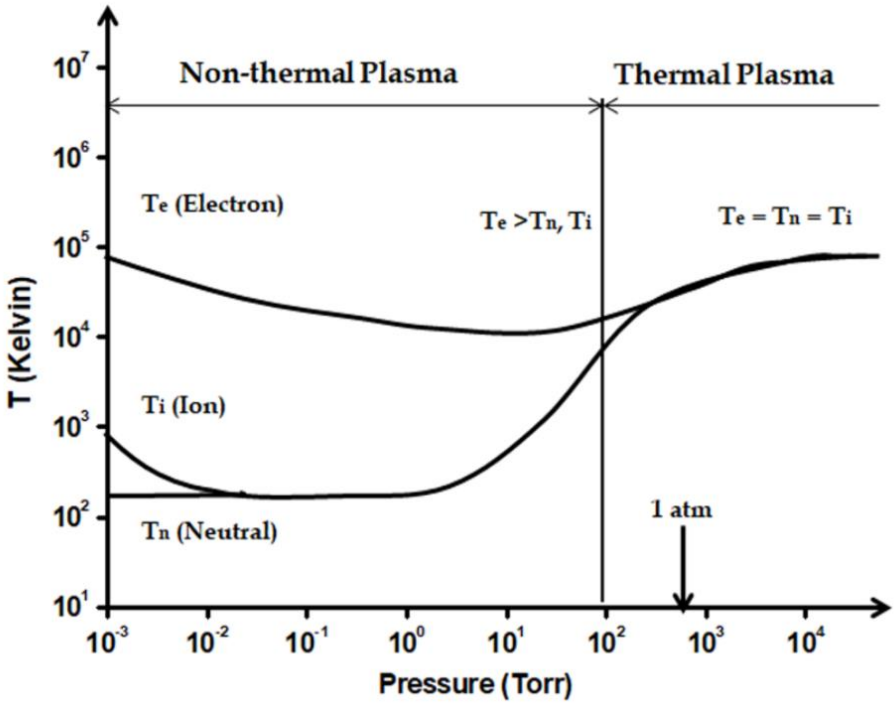


Fig. 10: Difference between thermal and non-thermal plasma. The notations used: Electron temperature (Te), ion temperature (Ti), and gas temperature (Tg) [122].

As a result, non-thermal plasmas enable the creation of highly reactive chemical environments while maintaining low bulk gas temperatures, making them particularly useful for processing heat-sensitive materials, including biological tissues and polymers [123, 124]. The energy of “hot” electrons is sufficient to cause electron impact excitation, ionization and dissociation of gas molecules, generating

a rich mixture of reactive species, including radicals (e.g. -OH, O-, N-), metastables, ions, UV photons and transient electric field. These active components are responsible for various physicochemical processes, despite the fact that the gas remains globally cold.

It is important to note that cold plasmas are defined not only by their temperature disparity, but also by a set of five interdependent physical characteristics that determine their application potential: (1) the electric field, which governs the transfer of energy to electrons; (2) the temperature of electrons and gas, which remains decoupled; (3) the reactive chemistry, driven by energetic electrons; (4) the dynamics of gas flow, which controls residence time and species transport; and (5) the emission of UV and visible radiation, which participates in surface activation and biological effects [Chatterjee]. These five components, represented in figure 11, act in synergy to produce complex, non-equilibrium plasma environments, adaptable to a wide range of fields, from materials processing to medicine.

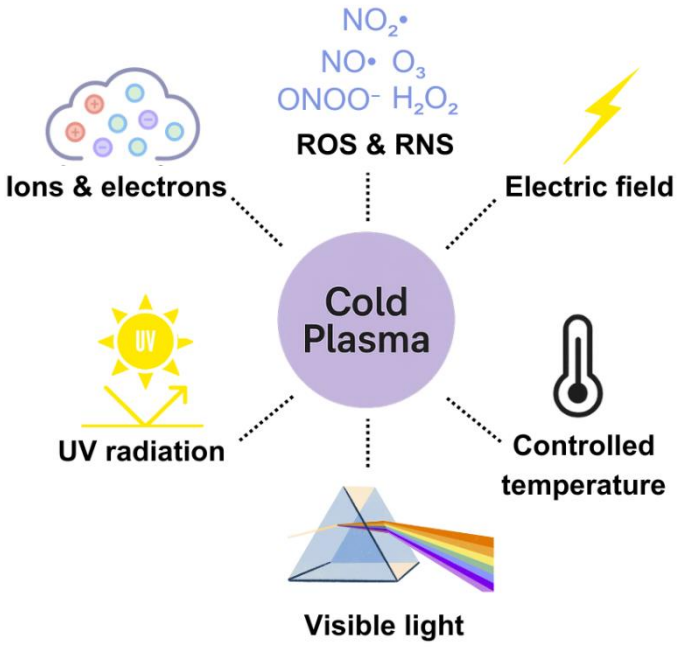


Fig. 11: Main properties of cold plasma.

The absence of thermal equilibrium also means that non-thermal plasmas cannot be described using a single Maxwell-Boltzmann distribution, as illustrated in Figure 12 [125]. Instead, the electron energy distribution function (EEDF) in these plasmas is often not Maxwellian and sometimes exhibits high-energy tails that enhance dissociation or ionization processes beyond what would be expected from a thermalized electron population [126].

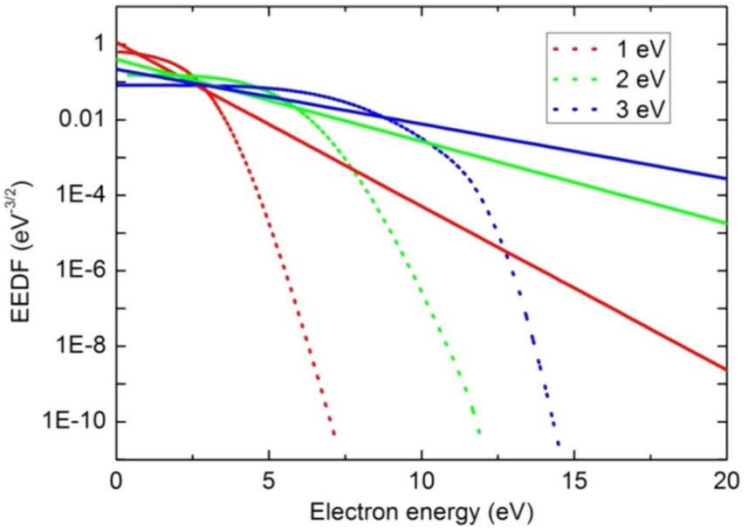


Fig. 12: Electron energy distribution functions (EEDFs) for Maxwellian (solid) and non-Maxwellian (dotted) cases at Tg=1600T_g = 1600Tg=1600 K. Non-Maxwellian curves are obtained with BOLSIG+ for mean electron energies of 1, 2, and 3 eV, matching Maxwellian values. The plot compares their shapes and highlights differences in the high-energy tails [127].

Plasma generation in the non-thermal regime is generally achieved by low-energy discharge sources such as dielectric barrier discharges (DBDs), corona discharges, plasma jets or microwave/RF plasmas, operating at or near atmospheric pressure. In many cases, pulsed or alternating electric fields are applied to prevent arcing and limit gas heating. Gas flow is often used to control residence times and stabilize plasma chemistry. From a kinetic point of view, the speed of electron-induced reactions is determined by the electron impact cross sections of the processes involved and by the shape of the EEDF [128, 129]. For example, dissociative excitation and ionization generally require electron energies above specific thresholds (e.g. 10-15 eV for oxygen or nitrogen molecules), which are readily available in the high-energy tail of the electron population in non-thermal plasmas.

As ionization levels remain low (typically $\alpha$ between $10^{-6}$ and $10^{-2}$), cold plasmas are generally weakly ionized. This implies that neutral-neutral and electron-neutral collisions dominate, with relatively fewer ion-ion or Coulomb interactions than in thermal plasmas. Despite this, cold plasmas still exhibit collective behavior, favoring phenomena such as sheath formation, electric double layers and self-organization under certain conditions [130, 131].

### 2.4. Classification following the temperature-density diagram

Plasmas occupy a vast range of physical conditions in terms of temperature and charge density, as illustrated in Figure 13 by their distribution across the temperature-density phase space. The plot

presents a classification of both natural and artificial plasmas according to these two fundamental parameters. On the horizontal axis, the charge density (typically representing the total number of charged particles, ie electrons and ions per $cm^3$) spans over 25 orders of magnitude, from the extremely dilute plasmas of interstellar space to the dense cores of stars. The vertical axis represents temperature in kelvins, ranging from hundreds of K to values exceeding one billion K, thus encompassing both cold, weakly ionized plasmas and the extreme conditions of fusion plasmas.

In the lower left corner of the diagram are low-temperature, low-density plasmas such as auroras and interstellar nebulae. These are naturally occurring, weakly ionized plasmas characterized by low particle interaction rates and long mean free paths [132]. Despite their low ionization levels, they exhibit typical plasma behaviors such as the generation of electromagnetic waves and collective field interactions [133].

Near the middle of the plot are atmospheric-pressure plasmas including flames and plasmas generated in liquids. These plasmas typically exhibit moderate temperatures (in the range of a few thousand K) and higher charge densities, especially in the case of plasmas in condensed media. Industrial plasmas, often used for surface modification, sterilization or material processing, appear in a similar region of the diagram [134]. These are often non-thermal plasmas, in which electrons are much hotter than the bulk gas, allowing for high reactivity without significant heating of the material being treated.

Higher up and to the right on the diagram are thermal plasmas such as lightning, which briefly reach extremely high temperatures (exceeding 30 000 K) and relatively high charge densities [135]. Also located in the high-temperature region are astrophysical and fusion-related plasmas. The solar corona, although tenuous, is extremely hot, while the solar core exhibits both high temperature and high density: conditions necessary for sustaining thermonuclear fusion [136]. Fusion reactors, such as tokamaks, aim to reproduce similar conditions artificially, though typically at lower densities to maintain magnetic confinement [137].

This classification highlights the extraordinary diversity of plasmas and the wide range of environments in which they occur. From the cold, rarefied plasmas of the upper atmosphere and interstellar medium to the dense, hot interiors of stars and man-made fusion devices, plasmas cover an expansive domain of thermodynamic states. Understanding their behavior across this spectrum is critical not only to astrophysics and fusion research but also to emerging technologies in materials science, medicine and agriculture.

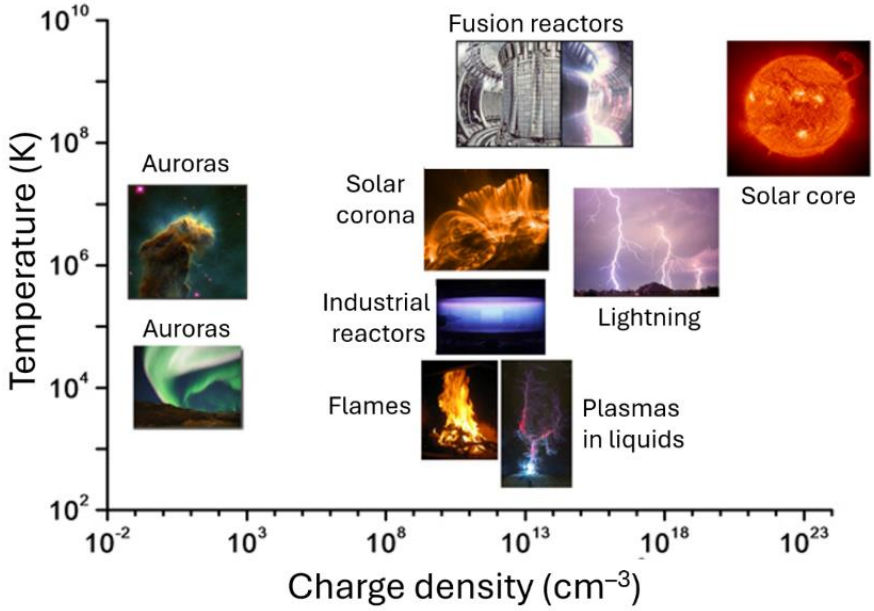


Fig. 13: Comparative overview of different plasma types positioned according to their characteristic temperature (K) and charge density ($cm^{-3}$). It includes natural plasmas (nebulae, auroras, lightning, …) and artificial plasmas generated in fusion reactors, industrial reactors, flames and liquid-phase discharges.

### 2.5. Cold plasma sources

The generation of cold (non-thermal) plasmas requires devices that selectively accelerate electrons while maintaining ions and neutrals at near-ambient temperatures. This is typically achieved by applying an electric field that accelerates the light electrons ($9.11\times10^{-31}$ kg) more effectively than the heavier species (ions, atoms), thus avoiding significant bulk heating. Several types of electrical discharge devices have been developed to create such plasmas under controlled conditions, particularly at atmospheric or low-pressure regimes. Each source operates under distinct physical principles and is optimized for specific applications.

### 2.5.1. Corona discharge

Corona discharge is one of the earliest known forms of electrical discharge in gases, with observations dating back to the 18th century. A systematic study was conducted by Michael Faraday in the 1830s and later, the phenomenon was extensively explored by physicists such as Lord Kelvin and John Townsend [138]. It became industrially significant in the early 20th century with its application in ozone generation and electrostatic precipitation [139]

A corona discharge occurs when a high electric potential is applied to an electrode with a highly curved geometry (typically a thin wire, needle or point) placed in proximity to a grounded or oppositely biased electrode [140]. The strong local electric field near the sharp tip exceeds the ionization threshold of the surrounding gas, initiating electron avalanches and partial breakdown of the gas. Unlike arc or glow discharges, corona is a non-uniform, self-sustained discharge that remains confined to the vicinity of the high-field electrode, forming a localized cold plasma region.

The typical structure of a corona discharge device includes a single high-voltage electrode (e.g., needle or wire) and a counter-electrode (plate or cylinder), separated by a small air gap. It can be operated in ambient air, with applied DC or pulsed voltages ranging from DC to tens of kV. The discharge is sustained by the continuous generation of primary and secondary electrons and stabilized by ion drift and space-charge effects [141].

Corona discharges are effective in generating a variety of reactive species, particularly in air or oxygen-containing gases. These include ozone ($O_3$), nitrogen oxides (NO, $NO_2$), superoxide ions ($O_2^-$) and hydroxyl radicals (•OH): species known for their strong oxidative properties [142, 143] Due to the low current and localized nature of the discharge, the gas temperature remains close to ambient, classifying it as a non-thermal plasma. The electron temperature may reach several eV, sufficient to induce dissociation and excitation processes [142].

Owing to their simple geometry, low gas consumption and ease of integration, corona discharge have led to wide adoption in air purification, electrostatic filtration, ozone generators and surface activation processes [144, 145, 146, 147]. In biology and more specifically in agriculture, they are widely used for decontamination of seeds such as rice and broccoli [148, 149] as well as the decontamination of leaves, e.g. tomato leaves to limit powdery mildrew [150] and localized sterilization, particularly where portability and minimal thermal impact are required.

However, corona discharges have several limitations. The treatment area is small, often limited to the vicinity of the high-field electrode, which restricts uniformity and scalability. Besides, the spatial inhomogeneity of the plasma can lead to non-uniform processing, especially on complex or large

surfaces. Finally, material erosion of the high-voltage electrode, especially under continuous operation, can also limit long-term device stability [151].

### 2.5.2. Atmospheric Pressure Plasma Jet

Atmospheric Pressure Plasma Jets (APPJs) are compact, non-thermal plasma sources developed in the early 2000s, with foundational work by researchers such as Mounir Laroussi, who pioneered their use in biomedical applications [152] APPJs evolved from dielectric barrier discharge (DBD) devices and are designed to generate plasma at atmospheric pressure without the need for vacuum infrastructure.

Regarding its configuration, an APPJ consists of a narrow dielectric tube which is commonly made of quartz, glass or ceramic and through which a noble or molecular gas such as helium, argon or an argon-air mixture flows continuously. One or more electrodes are placed around or inside the tube and an alternating or pulsed high-voltage signal, typically in the kHz or RF range (e.g., 13.56 MHz), is applied [153] When the applied electric field exceeds the gas's breakdown threshold, ionization occurs, forming a stable plasma that exits the tube as a visible and luminous jet (also called plume).

Its operating principle relies on the generation and maintenance of plasma through electron acceleration in the oscillating (or pulsed) electric field. These high-energy electrons initiate collisions with neutral gas molecules, leading to excitation, ionization and dissociation [Viegas]. While the electron temperature can reach several eV, the neutral gas temperature remains near room temperature due to the continuous inflow of cold gas, classifying APPJs as cold plasmas.

A defining feature of APPJs is their ability to project a plasma plume into open air, which corresponds to the propagation of ionization waves. These jets deliver reactive species such as O and OH to distant surfaces, even irregular or biologically sensitive ones [154, 155]. APPJs are highly effective for surface treatment, wound healing, sterilization and seed decontamination [156, 157].

However, APPJs face limitations including short lifetimes of reactive species, plume length constraints and the cost of noble gases. Controlling plasma-biological interactions and ensuring treatment uniformity remain key research challenges.

### 2.5.3. Microwave and radio-frequency (RF) discharges

Microwave and radio-frequency (RF) discharges transfer energy to electrons using oscillating electromagnetic fields, which explains why they are commonly considered as non-contact plasma

generation methods [157]. Microwave discharges typically operate at 2.45 GHz using magnetron generators, while RF discharges function between 0.1 and 100 MHz via RF amplifiers or function generators [158] The absence of direct electrode contact prevents erosion and contamination, making these discharges ideal for high-purity applications such as nanomaterial synthesis and plasma-enhanced chemical vapor deposition (PECVD) [159].

These plasmas are implemented in various configurations. Microwave discharges can be generated in resonant cavities, coaxial waveguides or torch designs, where electromagnetic energy is concentrated in a defined volume [160]. The working gas which is often argon, helium or air, is introduced into the source and when the electric field exceeds the breakdown voltage, a plasma forms and is sustained through continuous energy coupling. RF discharges are typically realized in either capacitively coupled plasma (CCP) sources, where electrodes are separated by a dielectric or inductively coupled plasma (ICP) sources, where an RF coil surrounds a dielectric tube and generates plasma through magnetic induction [161]

Plasma formation is governed by resonant energy absorption: electrons oscillate in the high-frequency field, gaining kinetic energy sufficient to ionize neutral gas molecules. This sustains a partially ionized plasma with electron temperatures between 2 and 10 eV and ionization degrees from $10^{-3}$ to $10^{-1}$ [161, 162]. Electron densities can reach $10^{11}$ to $10^{13}$ $cm^{-3}$, supporting the production of chemically reactive species [162].

Despite their advantages, these sources present challenges, including high equipment cost, complex impedance matching and limited plasma volume. At atmospheric pressure, additional stabilization techniques such as gas flow control or pre-ionization may be necessary. Localized heating, particularly in microwave sources, can also be problematic in thermally sensitive applications.

### 2.5.4. Gliding arc discharge

A gliding arc discharge is a transitional plasma source that combines features of both thermal and non-thermal discharges [163]. It typically consists of two diverging electrodes (usually metal rods or blades) arranged in a V-shaped configuration. A high voltage (DC or AC) is applied across the electrodes, initiating an arc at the narrowest point. A gas flow, commonly air, nitrogen or methane mixtures, is introduced at the base of the electrodes [164, 165]. The arc is carried upward by the gas, elongating along the diverging gap. As it stretches, its current density and temperature decrease, creating a plasma region where the electron temperature remains high (several eV), while the bulk gas temperature drops to a moderate range, typically higher than 500 K [166].

This dynamic behavior allows the arc to transition from a thermal to a non-equilibrium state. Near the base, the discharge behaves like a conventional arc, with high energy density and ionization. As it glides and expands, it enters a non-equilibrium regime that favors the generation of reactive species, including radicals, electronically excited molecules and metastables [167]. This unique property enables gliding arc plasmas to maintain high chemical reactivity without the excessive heat typical of thermal arcs.

Gliding arcs are effective in producing reactive oxygen and nitrogen species (ROS and RNS), such as ozone, nitric oxide and hydroxyl radicals, over large treatment volumes. They are widely used in environmental remediation (e.g., VOC destruction, NOx reduction), plasma-assisted fuel reforming and agricultural applications like seed decontamination and plasma-activated water (PAW) generation [168, 169, 170, 171]. However, limitations include elevated gas temperatures that may harm heat-sensitive materials, difficulty in controlling arc stability and uniformity and electrode erosion over prolonged use, requiring robust materials and regular maintenance.

### 2.5.5. Dielectric barrier discharges (DBD)

A typical DBD device consists of two electrodes separated by a dielectric barrier, such as glass, quartz or alumina. Depending on the application, the electrodes can be arranged in various configurations: planar, cylindrical or coaxial [172]. At least one electrode must be insulated by the dielectric material, which plays a critical role in regulating the discharge dynamics. The inter-electrode gap, generally between 0.1 mm and a few millimeters, is filled with a working gas (e.g., air, helium, argon, nitrogen). An alternating voltage (ranging from a few kV up to several tens of kV, typically in the frequency range of 50 Hz to several kHz) is applied across the electrodes [173]. This generates an oscillating electric field that accelerates free electrons, initiating gas breakdown.

As previously mentioned, a DBD operates by applying an alternating high voltage across two electrodes, at least one of which is coated with a dielectric material. As the electric field increases in the gas-filled gap during each voltage period (or cycle), it eventually exceeds the breakdown threshold of the gas, initiating a cascade of electron-impact ionizations. These cascades generate short-lived, localized plasma channels known as microdischarges, which typically last only a few nanoseconds [174, 173]. Unlike arc discharges, DBDs do not sustain continuous current flow. The dielectric barrier plays a crucial role by accumulating surface charges that locally reduce the electric field and quickly extinguish each microdischarge [175]. This self-limiting mechanism prevents transition to the arc regime *(i.e.*, thermal runaway) and ensures that the discharge remains a cold plasma, i.e. thermodynamically unbalanced. Rather than forming a continuous plasma, DBD consists of numerous transient micro-

discharges distributed over the electrode surface. In some cases (particularly with helium or rare gas mixtures) they may appear as a diffuse, homogeneous glow [176]. The electrons in the discharge acquire high energies (several eV), enabling gas molecules to be excited and dissociated, while heavy ions and neutral particles remain close to room temperature. Alternating voltage also facilitates periodic charge neutralization, allowing new micro-discharges to occur at different locations in successive cycles.

DBD plasmas present typical ionization degrees below $10^{-4}$, yet they are highly effective in generating significant concentrations of reactive species [177]. These include ROS such as O, $O_3$ and OH, as well as RNS like nitric oxide (NO), nitrogen dioxide ($NO_2$) and nitrous oxide ($N_2O$) [177]. In addition to these, DBD plasmas produce metastable atoms and molecules, ultraviolet (UV) photons and transient electric field, all of which contribute to their strong chemical reactivity. The electron temperature in a DBD typically ranges from 1 to 10 eV, while the temperature of the neutral gas can remain much lower, close to ambient air temperature up to a few 100 °C [178]. Spatially, the discharge can manifest in two main regimes: a filamentary mode, characterized by discrete, spatially confined microdischarges or a diffuse (or glow-like) mode, where the plasma appears homogeneous, often achieved when using helium or specific gas mixtures that suppress streamer formation [179, 180].

Despite their advantages, DBD sources may encounter some limitations. The non-uniformity of microdischarges in filamentary regimes can lead to inconsistent treatment across surfaces, especially for large or complex geometries. Additionally, control over plasma chemistry (especially in ambient air) is complex due to interactions with humidity and trace gases [181, 182].

### 2.6. Main application of cold plasmas

Cold plasmas, due to their highly reactive and non-equilibrium nature, have found diverse applications across multiple advanced technological domains. Their ability to deliver high electron energies (1-10 eV) while maintaining neutral gas temperatures near ambient conditions enables precise, energy-selective processes without inducing thermal degradation. This makes them particularly suitable for emerging applications in space propulsion, materials processing, gas conversion, medicine and agriculture.

In space propulsion, cold plasmas play a crucial role in electric thrusters such as Hall-effect thrusters and the Variable Specific Impulse Magnetoplasma Rocket (VASIMR) [183, 184]. These devices use electromagnetic fields to ionize and accelerate propellant gases, typically xenon or argon, to generate thrust. In Hall thrusters, electrons are trapped in a magnetic field, creating a cold, dense plasma that

ionizes the neutral gas, while an electric field accelerates the resulting ions [185]. In VASIMR, cold plasmas are first created by radiofrequency (RF) heating, then further accelerated by ion cyclotron resonance, enabling precise control over exhaust velocity and specific impulse [184]. The non-thermal nature of the initial plasma stages ensures efficient energy coupling and minimal heat loss, which improves propellant efficiency and reduces component erosion. These features make cold plasmas mandatory for long-duration, low-thrust missions.

In materials science, cold plasma techniques are essential for modifying surface properties without altering bulk material characteristics, especially for temperature-sensitive substrates like polymers (e.g., polyethylene, PTFE) [186, 187, 188]. Processes such as PECVD and plasma etching operate at low temperatures (~25-200 °C), enabling precise tuning of surface energy, roughness, wettability and chemical functionality [189]. These methods are widely used in semiconductor fabrication for depositing ultra-thin dielectric and conductive films, as well as in biomedical engineering to enhance the biocompatibility of implants by grafting functional groups or sterilizing surfaces. In polymer processing, cold plasmas improve adhesion and printability by breaking surface bonds and introducing polar functionalities (e.g., -OH, -COOH) [190, 191, 192]. Textile treatments use similar approaches to confer hydrophilic or antimicrobial properties. The high reactivity of cold plasma species (including radicals, ions and UV photons) allows these transformations to occur efficiently without damaging fragile materials, making cold plasma indispensable for multiple industries involved in precision surface engineering.

In gas valorization, cold plasmas enable the conversion of stable waste gases like $CO_2$ into valuable products through non-equilibrium chemistry. High-energy electrons generated in the plasma dissociate $CO_2$ into CO and O, which can recombine into CO and $O_2$ or further react with added $H_2$ to form syngas (CO + $H_2$): a key precursor for synthetic fuels and chemicals [193, 194]. These reactions occur at atmospheric pressure, often without catalysts and at significantly lower temperatures than conventional thermochemical methods. Such cold plasma reactors offer a promising route for carbon recycling, power-to-fuel systems and integration with renewable electricity, supporting sustainable, low-carbon process development in the circular economy [195].

In medicine, CAPs are emerging as innovative tools for wound healing, infection control and cancer therapy [196]. CAPs generate a complex mix of reactive oxygen and nitrogen species (OH, NO, $O_3$, …) combined with electric field capable of disrupting microbial membranes, denaturing proteins and damaging nucleic acids [197, 198]. This allows effective inactivation of a broad spectrum of pathogens, including antibiotic-resistant bacteria, while minimizing damage to surrounding healthy tissues. In chronic wounds, cold plasma treatment has been shown to stimulate angiogenesis, fibroblast

proliferation and collagen synthesis, accelerating tissue regeneration [199]. In oncology, cold plasmas exhibit selective cytotoxicity toward cancer cells by inducing oxidative stress, DNA damage and apoptosis, with minimal effects on non-malignant cells [200, 201]. This selectivity is attributed to the altered redox balance and membrane properties of cancer cells. Clinical trials have been carried out to evaluate CAP devices for applications such as chronic ulcers, implant sterilization and plasma-assisted surgery, establishing cold plasmas as a promising non-thermal therapeutic modality [202, 203].

In agriculture, cold plasmas have emerged as a sustainable and chemical-free technology for seed treatment, pathogen control and the generation of plasma-activated water (PAW) [204]. When seeds are exposed to cold plasma, reactive oxygen and nitrogen species (ROS/RNS), UV radiation and electric field interact with the seed surface, disrupting microbial contaminants and modifying the seed coat. This can enhance surface hydrophilicity, water uptake, break dormancy and increase germination rates and early seedling vigor, particularly in hard-coated or aged seeds [205, 206, 207, 208]. Plasma exposure also stimulates endogenous plant defense mechanisms by triggering redox-sensitive signaling pathways [209]. It is effective against a wide range of plant pathogens, including fungi (e.g., Fusarium spp.), bacteria (e.g., Pseudomonas, Xanthomonas) and even viral vectors [210, 211, 212]. It inactivates pathogens on seeds and leaf surfaces without the use of fungicides or antibiotics, reducing the risk of resistance development. PAW, produced by treating water with plasma, contains long-lived ROS/RNS such as hydrogen peroxide ($H_2O_2$), nitrates ($NO_3^-$), nitrites ($NO_2^-$) and peroxynitrite ($ONOO^-$). When applied to soil or foliage, PAW enhances nutrient uptake, stimulates root development and can improve tolerance to abiotic stresses such as drought or salinity [213]. It also exhibits antimicrobial properties, supporting plant health while reducing dependence on chemical pesticides and fertilizers.

## 3. Cold plasma technology in agriculture: an innovative approach to current challenges

### 3.1. Relevance of cold plasma sources in regard of their utilization for seeds and plants applications

In agricultural applications, the relevance of various cold plasma sources depends on their ability to generate reactive species, operate at atmospheric pressure and interact safely with biological materials. DBD sources are among the most widely used due to their scalability, stability in ambient air and ability to produce chemically rich plasmas at relatively low gas temperatures. As already discussed, they are particularly suited for treating seeds, enhancing germination and decontaminating surfaces. Corona discharges, though more localized and limited in plasma volume, are highly efficient and cost-effective for seed surface sterilization and microbial deactivation. Their simple design and low energy requirements make them attractive for small-scale or targeted agricultural treatments. APPJs are valuable for precise and localized treatments, such as sterilizing plant surfaces or stimulating seed

germination without damaging delicate tissues. Their use of noble gases allows for controlled plasma chemistry, although operational costs can be higher. Microwave and RF discharges, typically used in electrode-free configurations, are particularly relevant for the generation of PAW, offering a powerful means to deliver long-lived reactive species for irrigation, pathogen control or nutrient enhancement. Lastly, gliding arc discharges, while operating at higher gas temperatures, are capable of producing large volumes of RONS, making them ideal for PAW production, soil pathogen inactivation and even valorization of agricultural waste gases. Each plasma source offers specific benefits and limitations and the choice depends on factors such as treatment scale, biological sensitivity of the target and the desired plasma chemistry (Fig. 14).

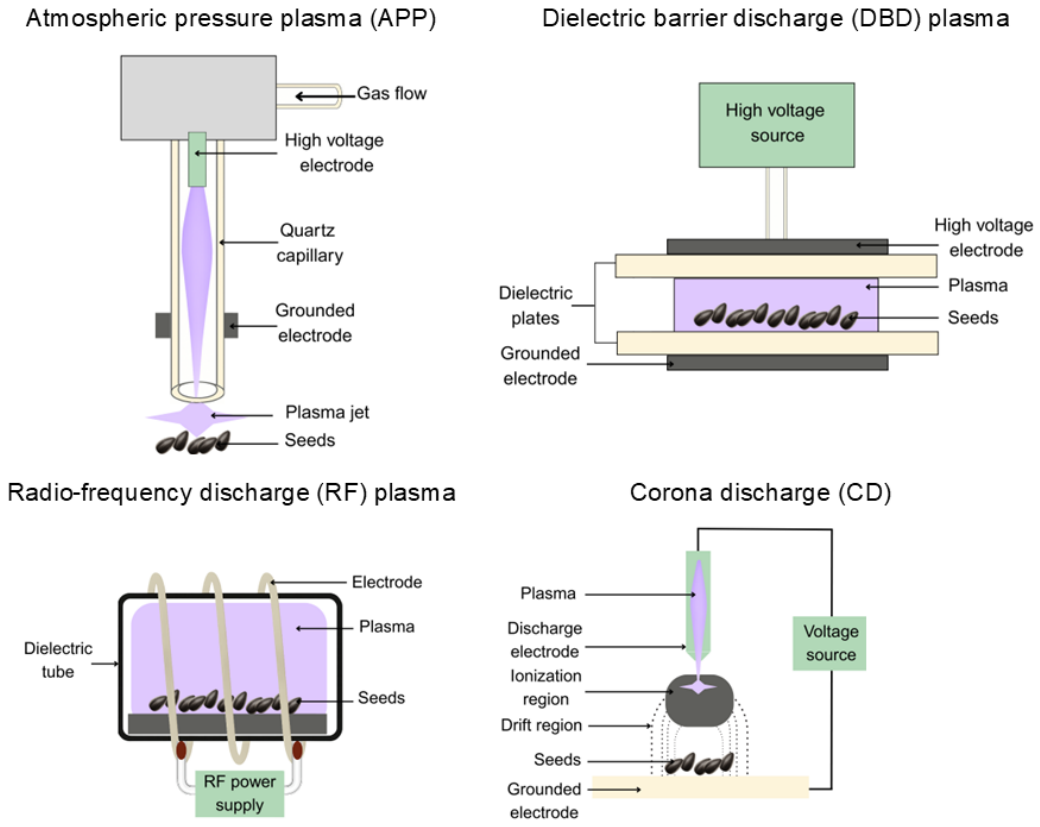


Fig. 14: Overview of plasma devices developed for direct seed treatment, adapted from [214]

### 3.2. Plasma treatment processes: direct and indirect approaches

The application of plasma in agriculture can follow two main strategies: direct exposure and indirect treatment, each offering distinct benefits depending on the biological target and treatment goals. In direct plasma treatment, seeds are placed in close contact with the active plasma region within the discharge device (Fig. 14). This approach ensures immediate interaction between the seed surface and

plasma-generated reactive agents, including ROS and RNS, UV photons and transient electric fields. Direct exposure is particularly effective for seed priming, as shown in the "pin-to-pin discharge" configuration in the figure 15, where an electrical discharge between two needle-like electrodes over a water-containing chamber creates a localized and non-thermal plasma environment. This direct contact promotes seed germination, reduces microbial load and modulates dormancy by altering seed surface properties and biochemical signaling pathways.

In contrast, indirect plasma treatment typically relies on the generation of plasma-activated water (PAW), where cold plasma is used to treat water which is subsequently utilized to irrigate seeds. The figure 15 illustrates this principle using different plasma sources, such as surface dielectric barrier discharge (DBD) and DBD plasma jets, where plasma is generated above or near a water surface. The treated water accumulates stable, bioactive species like $H_2O_2$, $NO_2^-$ and $NO_3^-$, which retain antimicrobial, antioxidant and growth-promoting properties. When used for plant watering, PAW can enhance plant growth, activate plant defense responses, reduce pathogen load in the rhizosphere and increase antioxidant compound synthesis. These benefits make PAW an effective, non-chemical strategy for boosting crop resilience and productivity.

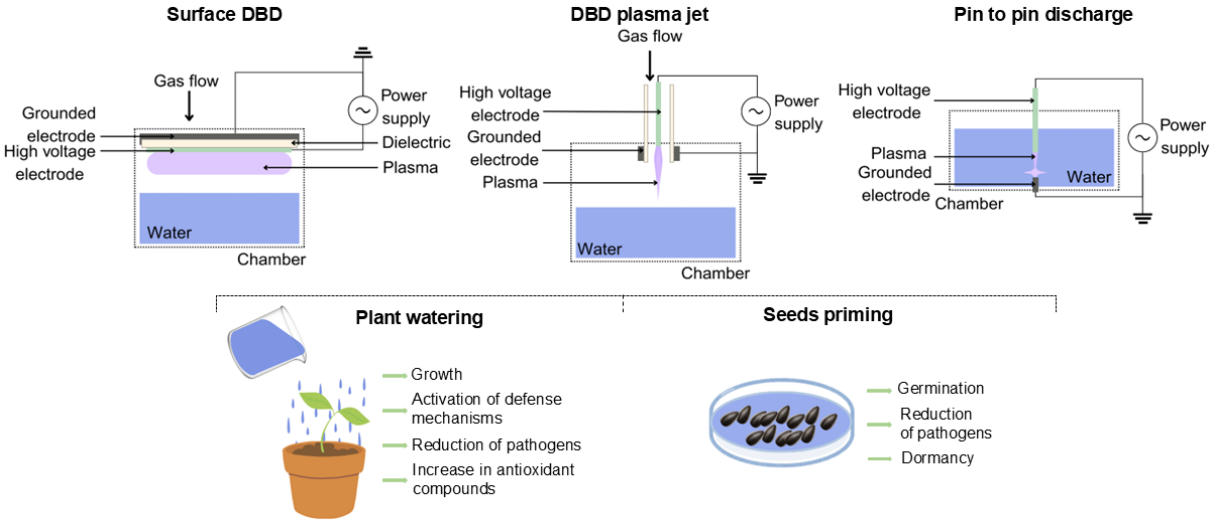


Fig. 15: Overview of plasma-activated water (PAW) generation systems & agricultural applications adapted from [215]

### 3.3. Effects and mechanisms of CAP on seeds and plants

ROS such as $O_3$, •OH, $H_2O_2$ and RNS, including NO, $NO_2$, $NO_3^-$, $NO_2^-$ and $ONOO^-$, are the primary biological mediators of atmospheric pressure plasma effects [216].

The lifetimes of these molecules vary. Depending on the pH, temperature and presence of metal ions or organic compounds in the water, reactive species such, as $O_3$ can present a lifespan of several tens of minutes while $H_2O_2$ can exist lasts over several hours, hence making PAW process particularly relevant to promote nitrogen fixation and stimulate plant growth. However, a major drawback of PAW lies in its limited stability: even long-lived species eventually degrade, which restricts its storage duration.

Conversely, highly reactive species (free radicals, NO, •OH) have extremely short lifetimes (on the order of microseconds and even shorter), which confines their activity to direct plasma treatments [216]. Direct treatment relies on immediate contact between the plasma and the surface of the biological sample. This configuration enables all active agents, including short-lived species, to interact with the sample's surface. Internal effects may also be observed, resulting from the diffusion of reactive species into plant tissues.

These properties justify their application in agriculture. The effectiveness of plasma exposure depends on the type of feed gas, the plant species being treated and the treatment modality (direct or indirect).

Jangra et al. demonstrated that air-DBD plasma treatment for 10 seconds significantly improved the physiological performance of wheat seeds: germination increased by 14% (98% in treated vs. 85.6% in control seeds), shoot length by 14%, and root length by 17% [217]. Moreover, wettability rose by 82% after 25 hours, indicating much faster water absorption in treated seeds compared to controls. Kamseu-Mogo et al. reported similar effects on maize germination following a few minutes of DBD treatment using an air-helium mixture [218]. They also highlighted a synergistic effect on seed germination and seedling growth when direct plasma treatment was combined with seed priming in PAW.

By altering the surface properties of seeds, cold plasma treatment improves their water absorption capacity, thereby reducing seed coat hardness associated with mechanical dormancy a phenomenon observed in several Fabaceae species such as lentil and soybean [219, 220]. The presence of long-lived ROS and RNS may also help overcome primary dormancy, as suggested by August et al. in their study on *Arabidopsis thaliana* [221].

Plasma exposure is also known to mitigate the effects of water stress on seed germination and plant growth. Guo et al. reported that dielectric barrier discharge (DBD) treatment alleviated the negative impacts of drought on wheat by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as increasing levels of proline and ROS [222]. The study also demonstrated a significant increase in proline content under water stress

conditions in the treated seeds. These physiological changes resulted in a 27% increase in germination compared to the control group.

In their study on sunflower, Tamošiūnė et al. (2020) reported that cold atmospheric plasma (CAP) treatment stimulated root and leaf growth by 9 to 14% compared to the untreated control [223]. Similarly, Guragain et al. demonstrated the synergistic effects of direct (CAP) and indirect (plasma-activated water, PAW) treatments in enhancing germination, growth, and vigor in agriculturally important species such as mustard and carrot [224].

Finally, a particularly promising application in the context of sustainable agriculture is the use of plasma for seed decontamination. Wannicke et al. showed that a short microwave plasma treatment effectively deactivated *Bacillus atrophaeus* spores on barley and rapeseed seeds [225]. Similarly, Liu et al. demonstrated that a 90 second treatment using gliding arc discharge significantly reduced *Fusarium oxysporum* contamination in seeds [226]. Silvetti et al. observed a reduction of approximately 1 log10 CFU/g in bacterial load on freshly cut lettuce leaves following a 10 second APPJ treatment [227].

In their study on the bacterial microbiota associated with the seeds and seedlings of *Arabidopsis thaliana*, Tamošiūnė et al. demonstrated that CAP treatment of seeds has a selective effect on the composition of this microbiota [228]. They showed that, beyond the direct antimicrobial effect on the seed microbiota, CAP treatment also induces long-term changes in the composition of the foliar microbiome in mature plants. For example, they reported a 3- to 4-fold increase in the relative abundance of endophytic bacteria belonging to the families *Sinobacteraceae* and *Nocardiaceae* compared to control plants [228].

Taken together, these findings highlight the potential of CAP as innovative tools to support sustainable agricultural practices. By enhancing stress tolerance, improving plant development, and reducing pathogen loads without chemical inputs, plasma treatments may offer a relevant approach to maintain crop productivity under increasingly variable and changing environmental conditions.

## 4. Thesis objectives

The main objective of this thesis is to characterize the effects of cold plasma treatment, generated using a DBD source operating in ambient air, on three key seed-related issues: germination, early seedling growth, and microbial decontamination.

This work fits into the broader context of agroecological transition in response to the challenges posed by climate change. As reducing the use of chemical inputs becomes increasingly necessary, cold plasma treatment emerges as a promising alternative to conventional agricultural methods. It offers an innovative response to several recurring agricultural issues, such as dormancy management, fungal and bacterial contamination control, and germination enhancement, while minimizing the environmental impact associated with synthetic chemical treatments, which tend to persist in ecosystems.

First, our study focused on the germination and early growth stages of an agronomically important species, *Helianthus annuus* (sunflower), under water stress conditions. The effects of cold plasma treatment were analyzed in relation to dormancy breaking, germination, and early seedling development. This model allowed us to explore the impact of plasma under realistic and challenging agricultural conditions.

To interpret these biological effects, the plasma phase was thoroughly characterized both chemically and physically using optical emission spectroscopy, mass spectrometry, infrared thermography, oscilloscope-based analysis, and other physical measurements. These analyses were conducted by modifying the configuration of the experimental setup to manipulate and better understand plasma properties.

We also investigated the decontaminating effect of cold plasma using a model species, *Arabidopsis thaliana*, with two levels of analysis: the seed surface and the microbiota of seedlings originating from treated seeds. This dual approach allowed for a better understanding of the treatment's impact on associated microbial communities, and more broadly, on the indirect effects of plasma exposure on plant-associated microbiota.

Lastly, an in-depth investigation was conducted to identify the plasma components responsible for pathogen inactivation. The respective contributions of reactive species (ROS/RNS), UV radiation, thermal effects, and the electric field were experimentally dissociated to better understand their roles.

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# Chapter 2: Treatment of sunflower seeds by cold atmospheric plasma enhances their tolerance to water stress during germination and early seedling development

# Treatment of sunflower seeds by cold atmospheric plasma enhances their tolerance to water stress during germination and early seedling development

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**Abstract**

The aim of this study was to investigate the impact of ambient air plasma treatment on both sunflower seed germination and early steps of seedling development under water stress. Dry seeds were exposed to a cold atmospheric plasma generated in a dielectric barrier device where excited molecular nitrogen and ozone were detected by optical emission spectroscopy and mass spectrometry respectively. Interestingly, we explain the crucial role of gap's accuracy when treating seeds with cold atmospheric plasma, especially to improve interaction between seeds and plasma and therefore ensure an efficient treatment. Cold atmospheric plasma significantly improved the germination rates of seeds of dormant and non-dormant sunflower genotypes under water stress, demonstrating its efficiency in alleviating seed dormancy and in improving germination under suboptimal conditions. Furthermore, greenhouse experiments demonstrated that plasma treatment also stimulated seedling development under water stress conditions. These findings highlight the potential of cold atmospheric plasma treatment as an effective approach to promote the whole process of emergence of crop species.

**Key words: Cold atmospheric plasma, germination, seed, sunflower, growth, water stress**

## 1. Introduction

The timing, success, and overall productivity of agricultural systems are significantly impacted by seed dormancy, a natural state that prevents a seed from germinating even under favorable conditions [1]. This survival strategy allows seeds to bypass unfavorable periods (like cold winters or dry seasons) and germinate when the chances of seedling survival are the highest. Dormancy can be due to several reasons like hard seed coats preventing water absorption, the presence of chemical inhibitors, or physiological blockage within the embryo [2]. In the case of sunflower seeds, two types of dormancy can be distinguished: (i) the integumentary dormancy where inhibitory mechanisms of the integument and pericarp prevent germination at temperatures above 25°C and (ii) embryo dormancy which inhibits germination at temperatures below 15°C [3]. In the case of non-dormant sunflower seeds, optimal germination is between 20 and 25 °C.

In addition to seed dormancy, the overall productivity of agrosystems is being significantly challenged due to the ever-increasing global population but also to the profound and enduring effects of climate change [4], [5], [6]. This dual pressure translates into considerable constraints on agricultural yields, compounded by exacerbating factors such as urbanization and loss of agricultural land, deforestation, increase in pests and diseases, sea-level rise and salinization, decreased genetic diversity and socioeconomic inequality [7], [8], [9].

Among these global factors, water pressure plays a pivotal role, taking the form of drought due to insufficient rainfall, high evaporation rates or inefficient soil moisture retention. In sunflower seeds, drought can lead to increased accumulation of linoleic acid and to significant decrease in oleic acid content accompanied with a reduction in oil yield [10], [11]. Conversely, water-saturated scenarios can lead to a shortage of oxygen, hampering seed germination and the early stages of growth [12]. In addition, irregular rainfall can create alternating periods of water abundance and deficiency, posing problems for young seedlings. On larger scales, climatic variations can amplify the frequency and intensity of droughts or floods, disrupting the regularity or volume of rainfall and increasing soil salinity due to rising sea levels or diminishing water supplies [13], [14], [15]. These water stress conditions can exacerbate the effects of dormancy on seed germination, as documented for several decades [16], [17].

The compounding effects of the aforementioned factors - whether seed-specific or global - highlight the pressing necessity for inventive and robust practices to enhance germination capacities and therefore securing global food supply in the years ahead. In response to these challenges and specifically drought issues, a range of seed treatment techniques are being employed today like

breeding, biostimulants, priming and coating. Although not resorting increased pesticide usage, these techniques suffer from other limitations:

- Breeding and varietal selection are particularly relevant for large-scale crop seeds such as wheat, sunflower or rice to obtain the varieties showing optimal stress tolerance index [18], [19], [20]. However, these approaches are time-intensive and expensive, involving careful breeding and testing over several generations of plants [21]. Besides, the outcomes of breeding programs can be unpredictable, with desirable traits sometimes linked to undesirable ones, making it challenging to produce an "ideal" variety [22].
- Biostimulants suffer from a lack of standardized regulations, leading to discrepancies in quality and efficacy among products [23]. The effectiveness of biostimulants can vary greatly depending on the environmental conditions and the specific crop, leading to inconsistent results [24]. Besides, combinations of biostimulants and times of application can have very different results as shown by de Morais *et al.* for soybean seeds in water deficit conditions [25]. Moreover, high-quality biostimulants can be expensive, which may limit their use, especially in regions where the economic return might not justify the investment.
- Seed priming can be employed to initiate the physiological processes leading to germination in water scarcity conditions. Hence, sodium hydrosulphide (NaHS) solutions can be used to prime sunflower seeds (*Helianthus annuus L.*) exposed to different drought levels although providing mitigate results [26]. Overall, hydropriming (simple rehydration), osmopriming (osmotic treatment) and hormopriming (hormonal treatment) can be effective techniques but operate over long durations, ranging from several hours to several days [27].
- Biopolymer-based seed coating can be developed to increase germination and water-stress tolerance in semi-arid and sandy soils [28]. However, these coatings may not always be biodegradable in the context of solving drought stress, especially when superabsorbent polymers (potassium polyacrylate, polyacrylamide-based polymer) [29]. Last and not least, pelleting and coating technologies can also be expensive [30].

To overcome these limitations and provide new and innovative solutions, alternative technologies are therefore expected such as cold atmospheric plasma (CAP) processes. CAPs are ionized gases that exist at or near room temperature, created by applying energy to a gas, causing the gas particles to become ionized. Plasma, which is often described as the fourth state of matter, can exist either as a fully ionized gas, termed 'hot plasma' where there is thermodynamic equilibrium, or as a partially ionized gas, known as 'cold plasma.' In the latter, the presence of neutral species is significantly higher compared to the charged ones, and the electron temperature is notably higher than both the ion and neutral temperatures [31]. Cold plasmas are a versatile approach widely used to meet challenges in life

sciences (medicine, environment, agriculture) especially thanks to their ability to generate short- and long-lived reactive species including O, OH, $NO/NO_2$, $O_2(a^1\Delta g)$, $O_3$, electronically excited $N_2$ ($N_2$*), and metastables, in addition to their radiative, electrical, thermal and fluid-mechanical properties [32] [33].

The use of cold plasmas has attracted increasing attention in agricultural circles due to its proven benefits in improving seed germination characteristics, including seed vigor, dormancy release and promotion of seedling growth. These beneficial effects have been successfully demonstrated on a range of economically important crops, including tomatoes [34] and soybeans [35]. Cold plasmas exert their beneficial influence by modifying the physico-chemical characteristics of the seed surface [36]. For example, they improve surface wettability, a vital characteristic for successful imbibition and initiation of germination [37]. More recent research highlights the effectiveness of cold plasmas in combating phytopathogenic attacks on crops such as maize [38], barley and wheat [39], as well as in the degradation of mycotoxins [40]. These observations establish cold plasma as a versatile and promising tool for seed treatment to improve or maintain germination capabilities.

Several research works have already employed cold plasma technology to improve the germinative properties of seeds in water stress conditions. Under drought stress, Ling et al. have demonstrated that cold plasma treatment could slightly improve the germination rate of drought-sensitive and drought-tolerant oilseed rape seeds [41]. Seedling growth parameters (shoot and root dry weights, shoot and root lengths) also significantly increased after plasma exposure. Feng et al observed similar effects on alfalfa seeds after exposure to helium-air plasma (DBD) [42]. Under increasing drought stress conditions, the treated seeds showed vigor indexes I increased compared to their respective controls. Phenotypic observations have also been performed by Adhikari et al. on tomato seedlings under drought stress [43]. They demonstrate that seedlings obtained from seeds treated 10 min by an air plasma jet had higher total chlorophyll content. While many studies propose to use CAP to improve germinative properties of seeds under drought conditions (eg in wheat and cotton [44], [45]) only few has been specifically focused on seed dormancy. In Arabidopsis, however, August et al. (2023) [46] demonstrated that a short CAP treatment could significantly release dormancy for seeds with higher water content (i.e. > $10\%_{DW}$).

The objective of the present study was to investigate the effect of ambient air plasma on dormancy breaking and early seedling development in sunflower. The potential of this plasma treatment was tested under conditions of increasing water stress. To this end, two distinct experimental procedures were formulated: one involved the use of polyethylene glycol (PEG) solutions for seeds sown on cotton substrates housed in Petri dishes, while the other managed the water supply for seeds and, subsequently, seedlings, rooted in soil substrates contained in pots.

## 2. Material and methods

### 2.1. Experimental plasma setup

Ambient air plasma was generated in a dielectric barrier device (DBD) consisting of two planar electrodes of 30 × 40 cm of area, one connected to the high voltage and the other grounded. As sketched in Figure 1, the two electrodes were separated by an insulating dielectric material (classical soda-lime glass) 2 mm thick to prevent any streamer-to-arc transition and therefore ensure a homogeneous distribution of plasma in the reactor volume where the seeds were exposed to ambient air plasma. Here, the background gas was the ambient air with a relative humidity close to 40%. The high voltage electrode was supplied with 9 kV in amplitude at a frequency of 140 Hz (sine form) using a high voltage generator composed of a function generator (ELC Annecy France, GF467AF) and a power amplifier (Crest Audio, 5500W, CC5500).

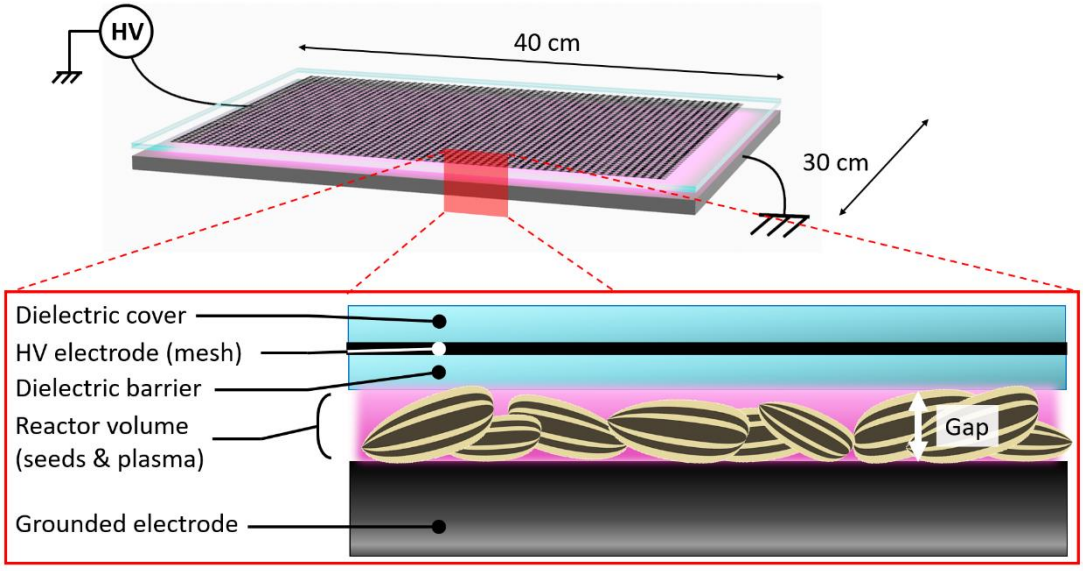


Fig. 1: Schematic of the dielectric barrier device operating in ambient air. Sunflower seeds are placed in the gap region 4 mm thick.

### 2.2. Plasma diagnostics

Measurements of electrical parameters were conducted using a Teledyne LeCroy Wavesurfer 3054 digital oscilloscope in conjunction with a high voltage probe (specifically, Tektronix P6015A 1000:1, Teledyne LeCroy PPE 20 kV 1000:1, Teledyne LeCroy PP020 10:1), as well as a Pearson 2877 current monitor. The distributions of current peaks were derived from original current signals, with a temporal precision of 500 ns, following a tri-step methodology: (i) the dielectric component was eliminated using the peak analyzer toolbox of the Origin software, specifically utilizing the asymmetric Least Squares Smoothing procedure, (ii) signal noise, which is indicative of peak magnitudes less than 2 mA, was isolated, (iii) the local peaks were organized in a sequence from highest to lowest.

Optical emission spectroscopy was utilized to identify the radiative species of the plasma phase. The spectrometer (SR-750-B1-R model from Andor company) was equipped with an ICCD camera (Istar model) which operates in the Czerny Turner configuration. Its focal length was 750 mm while diffraction was achieved with a 1200 grooves.mm$^{-1}$ grating blazed at 300 nm. Light was collected with an ANDOR SR-OPT-8014 single-fiber bundle (100 µm core, HOH UV/VIS silica, 2 m length) coupled to the spectrometer entrance slit. The fiber tip was positioned 1 cm from the discharge, at 90° to the electrode plane, viewing the center of the gap. The spectrometer slit width was 80 µm. The spectral resolution FWHM was 15 pm at 337.1 nm, determined with a Hg/Ar calibration lamp; wavelength accuracy was ±0.05 nm. The following parameters were selected for all experiments: exposure time = 0.1 s, number of accumulations = 20, readout rate = 3 MHz, gate mode = 'CW on', Gain level = 3000, insertion delay = 'normal'.

A quadrupole-based mass spectrometer (Model HPR-20 from Hiden Analytical Ltd) was utilized to identify the gaseous species considering their m/Z ratio. They were collected by a quartz capillary, 1 m in length, flexible, chemically inert and heated at 200 °C to prevent chemisorption. Then, a three-stage differentially pumped inlet system separated by aligned skimmer cones and turbo molecular pump, enabled a pressure gradient from $10^5$ bar to $10^{-7}$ bar at the entrance of the ionization chamber. There, ionization energy was set at 70 eV. The residual gas analyzer (RGA) mode was used for scanning masses from 1 to 50 amu, using a secondary electron multiplier (SEM) at 1600 V.

### 2.3. Plant material

This study was performed with seeds of two commercial sunflower (*Helianthus annuus* L.) hybrids, referred here as variety A and variety B, produced by NuSeed (USA) in 2020. Seeds were stored at −18 °C after harvest in order to maintain their initial physiological state [47]. At harvest, the two varieties exhibited distinct germination kinetics: variety A showed slower germination indicative of a

dormant-like behavior, while variety B germinated rapidly at 15°C. These contrasting germination profiles were used to investigate the effects of treatments under different physiological conditions.

**2.4. Germination assays**

Seed dormancy was assayed by distributing whole achenes into three separate replicates, each consisting of 25 seeds, and placing them onto a layer of cotton saturated with deionized water within 9 cm diameter glass Petri dishes at 15°C [47] Germination was scored daily for 15 days, a seed being considered as germinated when the radicle protruded through the pericarp. The results presented are means ± SEM of germination percentages obtained with 3 replicates. Results were also expressed as areas under the curve (AUC) which refers to the cumulative germination curve and which plots germination percentage (or number of seeds germinated) over time.

Area Under the Curve (AUC) was calculated using the trapezoidal rule as follows:

$$AUC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} . (x_{i+1} - x_i)$$

Where xi represents time (in days), and yi corresponds to the percentage of germination at time xi.

To decipher the effects of water stress on seed germination, achenes were germinated on a range of polyethylene glycol (PEG) solutions giving water potential values from −0.4 to −1.0 MPa [48]. Germination results in the presence of PEG were analysed using the hydrotime model [49] which allowed the calculation of $\Psi_{50}$, the water potential at which 50% of the seed population germinated, providing a comparative measure of drought sensitivity. Water uptake was calculated over a 23-hour period for seeds of variety B placed either on water or on a solution of PEG -0.8 MPa, using the following formula:

$$Water\ uptake\ (\%) = \frac{W_t - W_0}{W_0} . 100$$

Where $W_0$ is the initial dry weight of the seeds and $W_t$ is the seed weight after 23 hours of imbibition.

**2.5. Greenhouse experiments**

In greenhouse experiments with seedlings, an initial process consisted in dehydrating 100 g of Jiffy Substrates V1 potting soil at 105 °C until its relative humidity reached 0%. The soil's maximum retention capacity (100% humidity) was then determined by pouring a measured amount of water onto the dehydrated soil, which was placed in a filter within a funnel atop a graduated cylinder. The

difference between the initial volume of water and the volume recovered in the cylinder provided the maximum amount of water the soil could retain. This data served to calculate the precise amount of water required to achieve specific soil moisture percentages. This preliminary procedure ensured the possibility of maintaining controlled watering, thus preserving a steady and specific humidity percentage throughout greenhouse growth. The same treatment was applied to all potting soil used, enabling the preservation of initial watering conditions. Necessary adjustments were made based on daily weight assessments.

Three sets of seeds, later grown into seedlings, were used for greenhouse experiment: control seeds (C: unexposed to plasma) and seeds subjected to 15 (P1) and 30 min (P2) ambient air plasma exposure. In each case, 20 seeds were sown in pots at a density of 4 seeds per pot. Two distinct humidity conditions were adopted for growth: one ensured soil moisture content exceeding 70% within the pot (standard condition) and the other maintained 40% soil moisture content (stress condition). After initially fixing the soil's water saturation percentage, pots were weighted daily and watered with the appropriate water volume to maintain the initial soil moisture conditions. To mitigate potential biases such as border effects, seeds from various treatment groups were randomly allocated on the cultivation trays and repositioned daily. These trays were then housed in a greenhouse with a consistent temperature of 21°C. Following emergence from the soil, the length of the sunflower hypocotyls was recorded each day to monitor seedling growth progression.

In order to integrate both germination and early growth parameters, the vigor index I was calculated for each condition by multiplying the mean hypocotyl length (cm) of germinated seedlings by the corresponding germination percentage, and dividing the result by 100, according to the following formula:

$$Vigor\ index\ I = \frac{\text{Mean Hypocotyl Length (cm)} \times \text{Germination Percentage (\%)}}{100}$$

## 3. Results

### 3.1. Characterization of the gaseous plasma phase

The electrical characteristics of ambient air plasma were examined with a focus on two factors: first, the impact of the gap distance, and second, the presence or absence of seeds within the reactor volume. The determination of the optimal gap value is particularly significant as it results from a compromise between:

- Seed biology (the size of the seeds, and specifically the lowest dimension of the seeds which, in the case of sunflower, is estimated to $D_{low}$ = 3.8 mm)
- Plasma physics (the voltage breakdown is ruled by the Paschen law while operation voltage must ensure a continuous operation of ambient air plasma out of thermodynamic equilibrium).

Whatever the gap value (1, 4 or 7 mm) with/without seeds in the reactor volume, the Figure 2a indicates that the plasma voltage remained unchanged, with an amplitude as high as 9 kV and a frequency of 140 Hz. In that respect, two issues should be highlighted:

- Regarding the applied voltage, it is worth stressing that it was fixed by the high-voltage amplifier at 9 kV (peak) for all gaps. Therefore the applied voltage waveform was identical in Figure 2a. The gap primarily affected the discharge current pulse statistics and the plasma power, consistent with equivalent-circuit descriptions of DBDs [31,52]. Conversely, the time profile of the current peaks changed drastically considering different gap values, as shown in Figure 2b (without seeds). While 40 mA current peaks were obtained for a 1 mm gap, the current peak amplitude distribution was drastically reduced to values lower than 2 mA at 4 mm and 7 mm. Seeds could be introduced in the reactor volume only for gaps equal to at least 4 mm. As a result, the overall distribution of the current peak amplitude was significantly increased in Figure 2c compared with Figure 2b.
- Regarding the operating frequency, we selected the value of 140 Hz because it ensures efficient power transfer while maintaining a cold, spatially uniform discharge. In low-frequency operation, each half-cycle (about 3.6 ms at 140 Hz) allows surface charges on the dielectric to redistribute, so microdischarges ignite at different positions over the electrode area. This results in a diffuse glow, consistent with large-area homogeneous treatment. At higher frequencies (>300 Hz), current pulses overlap in time, charge relaxation is incomplete, and streamers tend to re-ignite at the same locations, producing bright filaments and non-uniformity [46]. In addition, the slower rise of the applied voltage at 140 Hz enables clear

resolution of individual current peaks, which is essential for the statistical analysis shown in Figure 3.

To more effectively evaluate the influence of the gap on the distribution of current peak amplitudes, each time-dependent signal was observed over the span of seven periods, utilizing three distinct triplicate sets. As outlined in the methodology (Section 2.2), the distribution of current peak amplitudes can be graphically represented on a log-log scale, as demonstrated in Figure 3a. For a gap distance of 1 mm (illustrated by the black curve), around 10 000 peaks were discerned, with a subset of 10 of these exhibiting magnitudes surpassing 30 mA, and a larger subset of approximately 9 000 streamers demonstrating amplitudes in the range of 2 to 7 mA. When the gap was extended to 1.5, 2, and 2.5 mm, there was a corresponding increase in the quantity of peaks possessing high magnitudes. Nonetheless, this tendency was inverted for larger gaps. The curve corresponding to a 3 mm gap presented an intriguing profile, notable for its high reproducibility, signifying a substantial decrease in low-amplitude peaks. This occurred because the gap enlarged to such an extent that it inhibited the corresponding micro-discharges from bridging the dielectric barrier with the counter-electrode, thereby limiting the operational discharges to a coarse estimate of a hundred (within the 10-400 mA range). As the gap continued to increase, a diminishing quantity of current peaks, typically less than 100, were still observed, presenting with relatively low current magnitudes (< 20 mA). This suggests isolated and stochastic electrical events that could no longer be associated with a homogeneous plasma discharge.

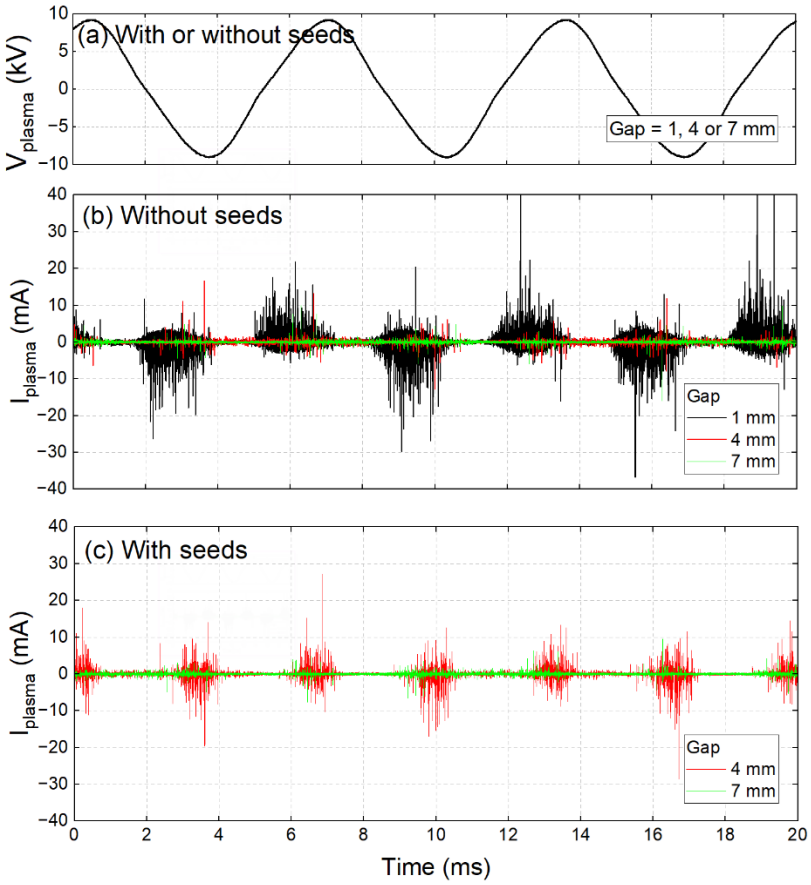


Fig. 2: (a) Time profile of the plasma voltage measured for gaps of 1, 4 and 7 mm without seeds and with sunflower seeds (20 g) in the reactor volume, (b) Time profile of the plasma current without seeds in the reactor volume for gaps of 1, 4 and 7 mm, (c) Time profile of the plasma current with seeds in the reactor volume for gaps of 4 and 7 mm.

In the seed-packed configuration, microdischarges occur within a noticeably narrower time window around each voltage crest (Figure 2c versus Figure 2b). Physically, the seeds act as a distributed dielectric 'bed': once ignition begins, many seed asperities experience field enhancement simultaneously. The ensuing streamers deposit surface charge over a very large effective area (seeds + barrier), which rapidly opposes the applied field so that the gas field $E_{gas}$ falls below breakdown soon after the crest. In parallel, hydrated pericarps are slightly conductive, shortening the effective RC time of the gap. Therefore, charges redistribute and neutralize more quickly than in the empty reactor. These two effects (phase-synchronous ignition at enhanced-field sites and fast charge accumulation/relaxation) both compress the active portion of each half-cycle. The result is a higher instantaneous pulse rate but a shorter overall 'on-time': pulses are more numerous, individually narrower, and strongly bunched near the crest, with little activity on the rising/falling shoulders. This temporal gating explains the tighter clusters seen in Figure 2c.

If the gap distance has an influence on the current peak amplitude distribution without seeds in the DBD, it is then important to study the same phenomenon when seeds are introduced in the reactor volume. For the sake of clarity, Figure 3b was only focused on minimum gap value at which seeds can be inserted in the volume reactor (gap = 4mm) and the gap value beyond which not even a micro-discharge can bridge the dielectric barrier with the counter-electrode (gap = 7 mm). At 4 mm, the introduction of sunflower seeds permitted a strengthening of the current peak amplitude distribution with a number of micro-discharges increasing from 200 (without seeds) to 2000 (with seeds) and maximum peak amplitudes increasing from 20 to 32 mA respectively. At this gap value, the micro-discharges could bridge the dielectric barrier with the counter electrode but also the dielectric barrier with the seed. For the larger gap at 7 mm, the same behavior was observed although the current peaks were so low in amplitude (< 10 mA) and so few in number (< 100) that they were unlikely to induce the expected biological effects.

While a gap of 4 mm seems appropriate according to the current peak amplitude distributions, another important parameter to assess is the electrical power deposited by the plasma in the reactor volume. This parameter was measured by the Lissajous method which required placing a measurement capacitor between the counter-electrode and the ground. The value of the capacitance (100 nF) was chosen at least 10 times higher than the one of the dielectric barrier (3.7 nF) to be non-intrusive [46]. Then, it was possible to plot the plasma voltage ($V_{plasma}$) as a function of the plasma charge ($Q_{plasma}$), as proposed in Figure 3c for a gap of 4 mm. Multiplying the area of the closed contour by the applied frequency gave the value of the plasma power, which was estimated to 430 mW and 2.5 W without seeds and with seeds in the reactor volume respectively.

A more general view of this discrepancy is reported in Figure 3d where the plasma power is plotted versus the gap, considering a reactor volume without seeds (black curve) and with seeds (red curve). In the first case, a slight increase in the power was obtained between 1 and 1.5 mm of gap, from 30.8 to 36.4 W before decreasing for larger gap values. Interestingly, a sharp fall of the plasma power (from 36.4 to 560 mW) was observed between 1.5 and 3.5 mm, i.e. on a very narrow gap range. Owing to the $D_{low}$ value of 3.8 mm, the sunflower seeds could only be introduced for a gap of at least 4 mm. In that case, the red curve indicates that the plasma power was significantly increased from 430 mW to approximately 2.8 W. For increasing gap values, the plasma power always decreased to lower values and remained higher when seeds were present in the reactor. These results indicate that an ambient air plasma generated in a dielectric barrier device may be a limitative option to treat seeds of larger dimensions and that the gap was a crucial parameter requiring a fine tuning.

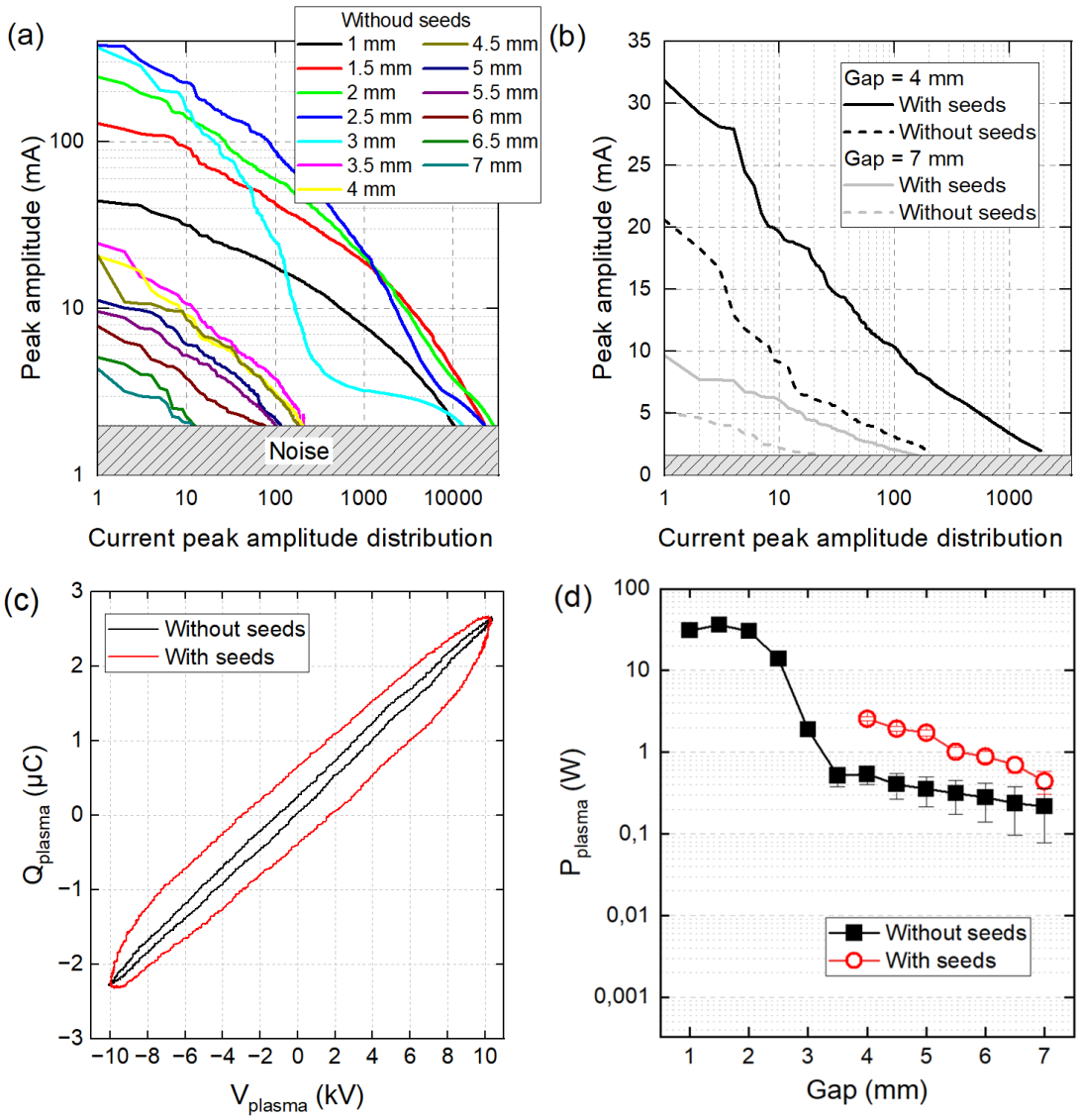


Fig. 3: (a) Current magnitude distribution of current peaks in ambient air plasma for different gaps without seeds, (b) Same distribution for gaps of 4 and 7 mm, with or without seeds, (c) Lissajous curves at 4 mm gap without and with seeds (d) Plasma power measured by Lissajous method as a function of the gap without/with seeds in the reactor volume of the DBD. Each measurement was performed in quadruplicate; error bars represent the standard error of the mean (SEM).

The importance of gap settings is underlined by the electrical characterization and can be complemented by the photographic report in Figure 4. The reactor volume in the DBD was captured from a side view, taking into account different scenarios: (i) plasma off versus plasma on, (ii) without seeds versus with seeds, (iii) gap distances ranging from 1 mm to 7 mm. Several observations are noteworthy :

- When the gap was only 1 mm, the ambient air plasma could uniformly occupy the entire reactor volume. Then, it became more filamentary at 2 mm, strongly heterogenous at 3 mm

and almost undetectable at 3.5 mm. Beyond 3.5 mm, it was almost imperceptible because the micro-discharges could occur randomly across the entire 30 × 40 $cm^2$ surface electrode, and did not necessarily appear in the area captured by the camera (limited to a side view of around 2 cm). In the absence of seeds and with larger gaps, no other micro-discharge was detectable.

- The inclusion of seeds in the reactor volume made the ambient air plasma more apparent when the gap was 4 mm. Interestingly, the plasma completely surrounded each seed, without forming a uniform layer filling the whole reactor volume. At 4.5 mm, the ambient air plasma still surrounded each seed although partially. At a gap of 5 mm, only one seed could interact with the plasma, and at larger gaps, no plasma was detectable.
- In the empty reactor, visible plasma emission is confined to gaps ≤3 mm and disappears beyond 3.5 mm. With seeds present, plasma reappears at the seed surfaces for gaps of 4-4.5 mm, confirming that field enhancement on the seed coat sustains localized micro-discharges. At 5 mm, emission becomes weak and patchy, and for ≥6 mm no discharge is detected. Thus, stable treatment is practically restricted to 4-4.5 mm, in agreement with the power trends of Figure 3d.

These results highlight that when treating seeds with ambient air plasma, an accurate adjusting of the gap value (precision of about 0.1 mm) is crucial to optimize the plasma-seed interaction. In particular, ambient air plasma might be a less recommended approach for treating seeds with excessively heterogeneous size distributions.

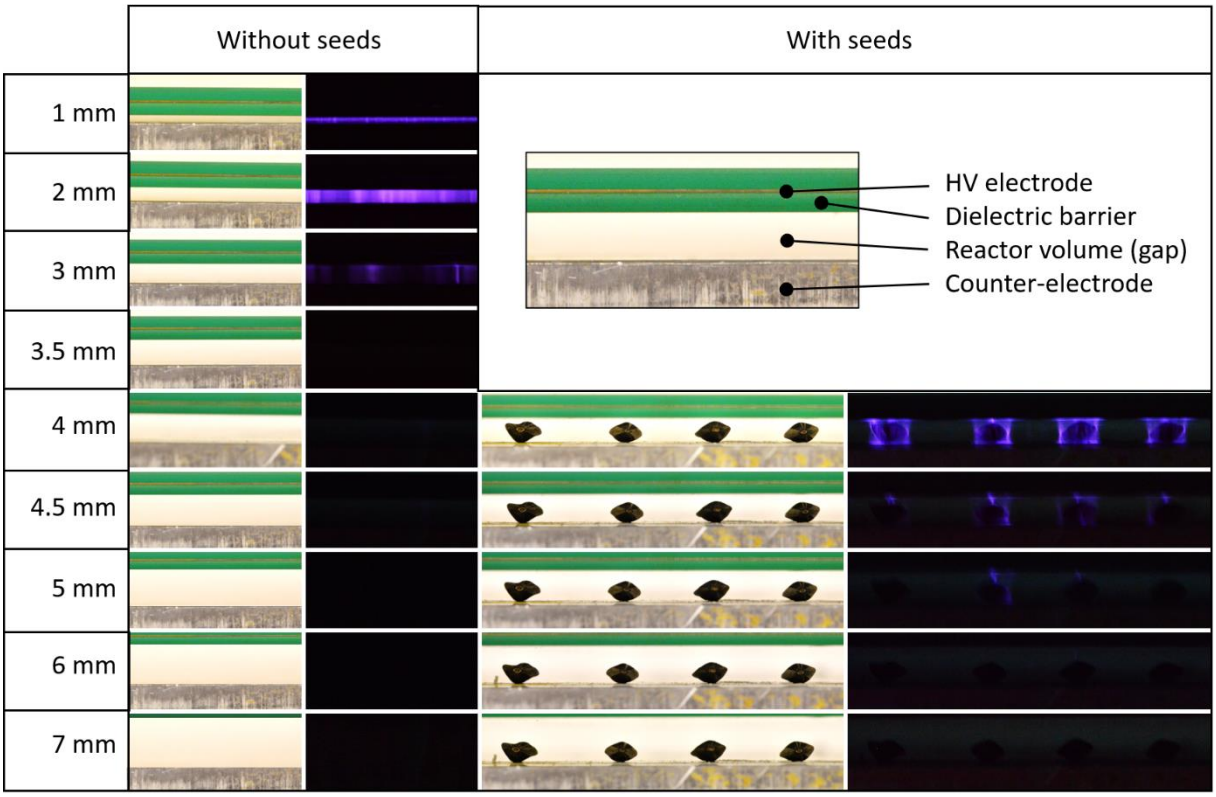


Fig. 4: Photographs of the DBD source viewed from the side, without seeds or with 4 seeds of sunflowers, without or with ambient air plasma (9 kV, f = 140 Hz), for various gap values. When room light is switched on, camera exposure time and aperture are 1/40 s and f/22 respectively. When room light is switched off, camera exposure time and aperture are 4 s and f/22 respectively. These later values are deliberately chosen to capture more detailed and clear images of the plasma. The case "with seeds" includes 4 aligned seeds for the sake of clarity.

Although camera imaging provides insights into how the ambient air plasma fills the reactor volume and how this filling depends on gap adjustments, the exact nature of the excited gaseous species remains unknown. Therefore, optical emission spectroscopy (OES) was carried out on the 250-800 nm range, considering 1-7 mm gaps. The OES spectrum in Figure 5a revealed that the ambient air plasma was composed solely of molecular nitrogen bands from the second positive system (SPS), characterized by the $C^3\Pi_u$-$B^3\Pi_g$ electronic transition states. This spectrum was derived without seeds and at a gap of 1 mm. Packing the DBD with seeds or extending this gap up to 7 mm neither added nor removed any lines or bands, but it did affect the overall emission of the SPS. This change was readily apparent in Figure 5b: without seeds, the optical emission of the band at 337.1 nm increased from $4.3 \times 10^5$ (at 1.0 mm) to $7.1 \times 10^5$ a.u. (at 1.5 mm) before decreasing sharply and vanishing completely at 3.5 mm. When sunflower seeds were placed in the interelectrode region, the minimum accessible gap was 4 mm. Consistent with the imaging results in Figure 4, the $N_2$ emission intensity at this gap reached $6.4 \times 10^5$ a.u., whereas no signal was detected at the same gap in the empty reactor. At larger gaps the emission again decayed rapidly, with no photons detected beyond 5.0 mm. As expected, these trends closely mirror the dependence of plasma power on gap distance in Figure 3d.

SPS was detected because the high-energy electrons in the plasma phase predominantly excited nitrogen molecules to the $C^3\Pi_u$ electronic state, as shown in equation {4}. These electrons can deliver sufficient energy to induce vibrational excitation of the excited nitrogen molecules ($N_2$*) within the $C^3\Pi_u$ state, allowing them to further relax back to a vibrational level of the lower $B^3\Pi_g$ electronic state through the emission of a photon, as depicted in equation {5}. Additionally, vibrational relaxation can proceed non-radiatively through collisions with other molecules. This mechanism, called quenching, is in competition with radiative de-excitation, as seen in equation {6}. In all three equations, v' and v'' represent the initial and final vibrational levels, j' and j'' stand for the initial and final rotational levels, and M symbolizes another molecule.

$$N_2 + e \rightarrow N_2^*(C^3\Pi_u, v', j') + e \quad \{4\}$$

$$N_2^*(C^3\Pi_u, v', j') \rightarrow N_2(B^3\Pi_g, v'', j'') + h\nu \quad \{5\}$$

$$N_2^*(C^3\Pi_u, v', j') + M \rightarrow N_2(v'', j'') + M \quad \{6\}$$

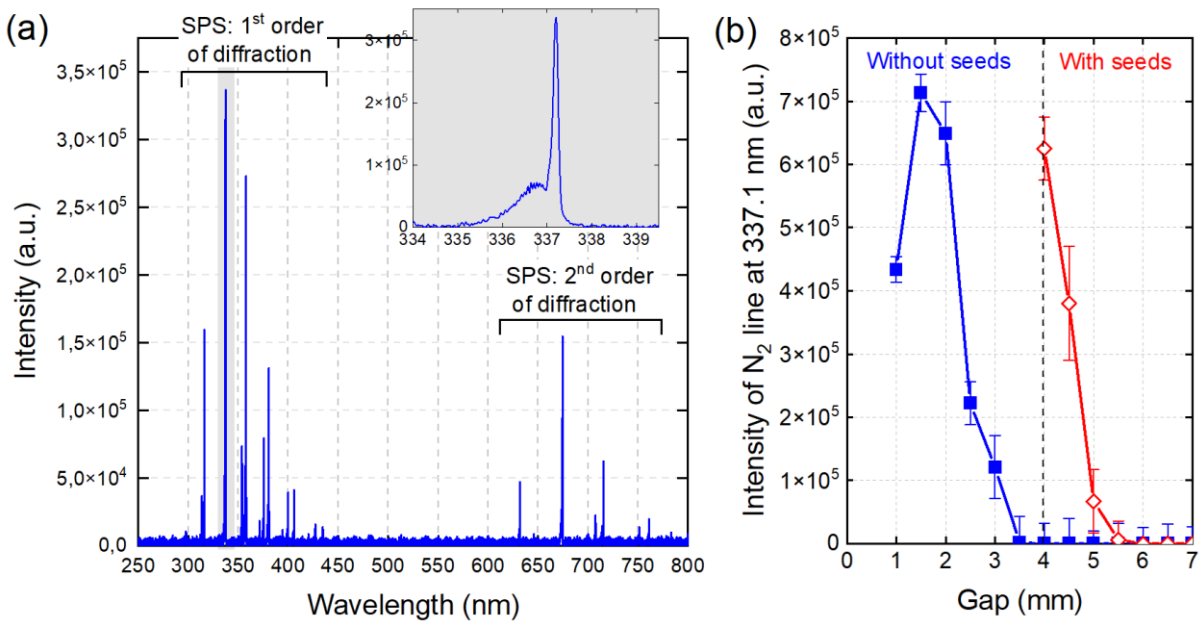


Fig. 5: (a) Optical emission spectrum of ambient air plasma measured in the reactor volume of the DBD, dominated by the second positive system (SPS) of molecular nitrogen (gap = 1 mm). Inset is a magnification view of the molecular nitrogen band headed at 337.1 nm. (b) Intensity of the $N_2$ band line at 337.1 nm as a function of gap. Each measurement was performed in quadruplicate; error bars represent the standard error of the mean (SEM).

While the vibrational structure of SPS is always clearly visible in the OES spectrum, it is surprising that no gaseous oxygenated species was detected. It is particularly relevant to remind the following points:

- O radicals are commonly observed through the green line at 557.7 nm and the infrared lines at 777.4 nm, 844.6 nm and 926.6 nm. However, no such line was detected in Figure 5a.
- The electronic transition ($A_2\Sigma^+$-$X_2\Pi$) of the hydroxyl radical (OH) is often used owing to its strong emission at around 306-309 nm. The upper excited state ($A_2\Sigma^+$) is commonly populated through the dissociation of water vapor or electron-impact excitation of OH molecules. Once in the $A_2\Sigma^+$ state, the OH radicals can decay to various vibrational levels of the $X_2\Pi$ ground state, emitting photons. Although this transition is spin-allowed and has a relatively high Einstein A coefficient, the OH band was not detected in Figure 5a.
- The lowest excited states of $O_2$ are triplets, which are forbidden to decay radiatively to the singlet ground state. This results in a low probability for emission (a low "Einstein A coefficient") and long lifetimes for these states. Thus, the emission from $O_2$ is generally much weaker than from $N_2$ and is more challenging to observe, especially in an air plasma dominated by molecular nitrogen.

The inability of OES to quantify these oxygenated species could indicate their absence from the plasma phase, or alternatively, their existence in a quenched state. To elucidate this ambiguity, we undertook

analytical investigations employing mass spectrometry. Figure 6a represents a mass spectrum (MS) of the ambient air plasma measured in a 1 mm gap, after air background correction (plasma off). Several noteworthy species could be identified considering their m/Z ratios expressed in $g.mol^{-1}$: N (14), O (16), OH (17), $H_2O$ (18), $N_2$ (28), NO (30), $O_2$ (32), $CO_2$ (44) and $O_3$ (48). After acquiring several spectra without/with seeds for gaps between 1 and 7 mm, the line intensities of the previous species were measured when plasma was off and on so, as to derive ratios plotted versus gap, as shown in Figure 6b (without seed in the reactor volume). It turns out that all the short lifetime species (O, N, OH and NO) presented ratios approximating 1, thereby suggesting they were not produced within the ambient air plasma. The same inference could be made for long lifespan species with the exception of ozone which exhibited a line intensity peaking for a 2 mm gap. This line intensity ratio dipped to a value of 3 for a 4 mm gap when the reactor volume was devoid of seeds. Interestingly, the introduction of sunflower seeds changed this MS profile. Figure 6c illustrates that for a 4 mm gap, this ratio escalated to 15; a value corresponding to a 1.5 mm gap in Figure 6b (without seeds), and equivalent to the average distance partitioning the lower dielectric barrier surface from the uneven upper surface of the sunflower seeds.

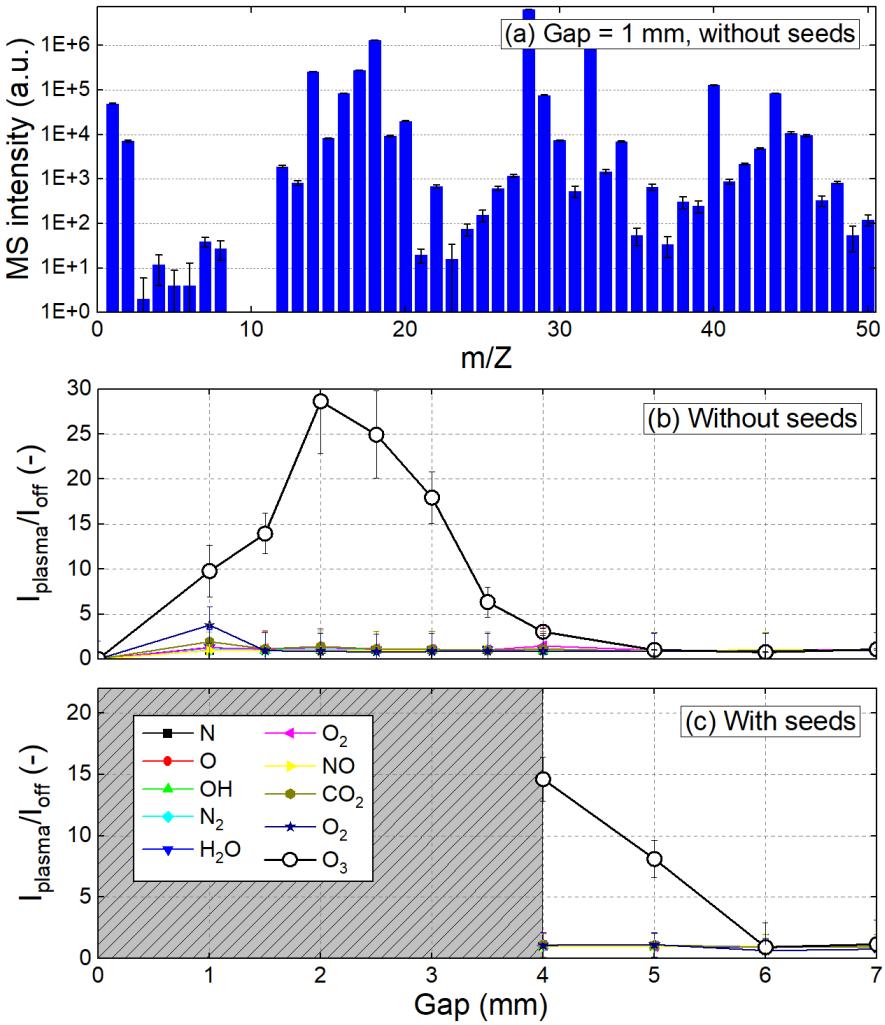


**Fig. 6:** (a) Mass spectrum of ambient air plasma generated in DBD for a gap of 1 mm, (b) Intensity ratio of the lines of each species detected by mass spectrometry as a function of the gap containing no seeds, (c) Same as (b) with seeds contained in the reactor volume. Each measurement was performed in quadruplicate; error bars represent the standard error of the mean (SEM).

The active species detected in the ambient air plasma were molecular nitrogen excited in the SPS and ozone molecules. Short lifespan reactive species (OH, NO, O) could not be detected by OES because of quenching or by MS because of potential recombination into more stable compounds during propagation within the spectrometer capillary. Still, the existence of O radicals was expected to explain the production of ozone. To effectively detect these species, techniques such as laser-induced fluorescence (LIF) or cavity ring-down spectroscopy (CRDS) would be required, but these were currently unavailable in our laboratory. No additional species were detected by OES or MS when changing the gap from 1 to 7 mm, without/with seeds in the reactor volume. For the rest of the article, all plasma treatments applied to treat the sunflower seeds were conducted with a 4 mm gap.

### 3.2. Effect of ambient air plasma on seed germination

Seeds of varieties A and B were subjected to a 15 min ambient air plasma treatment using the Dielectric Barrier Discharge (DBD) method, as illustrated in Figure 1, and their germination was assessed at 15°C on water and on PEG solutions.

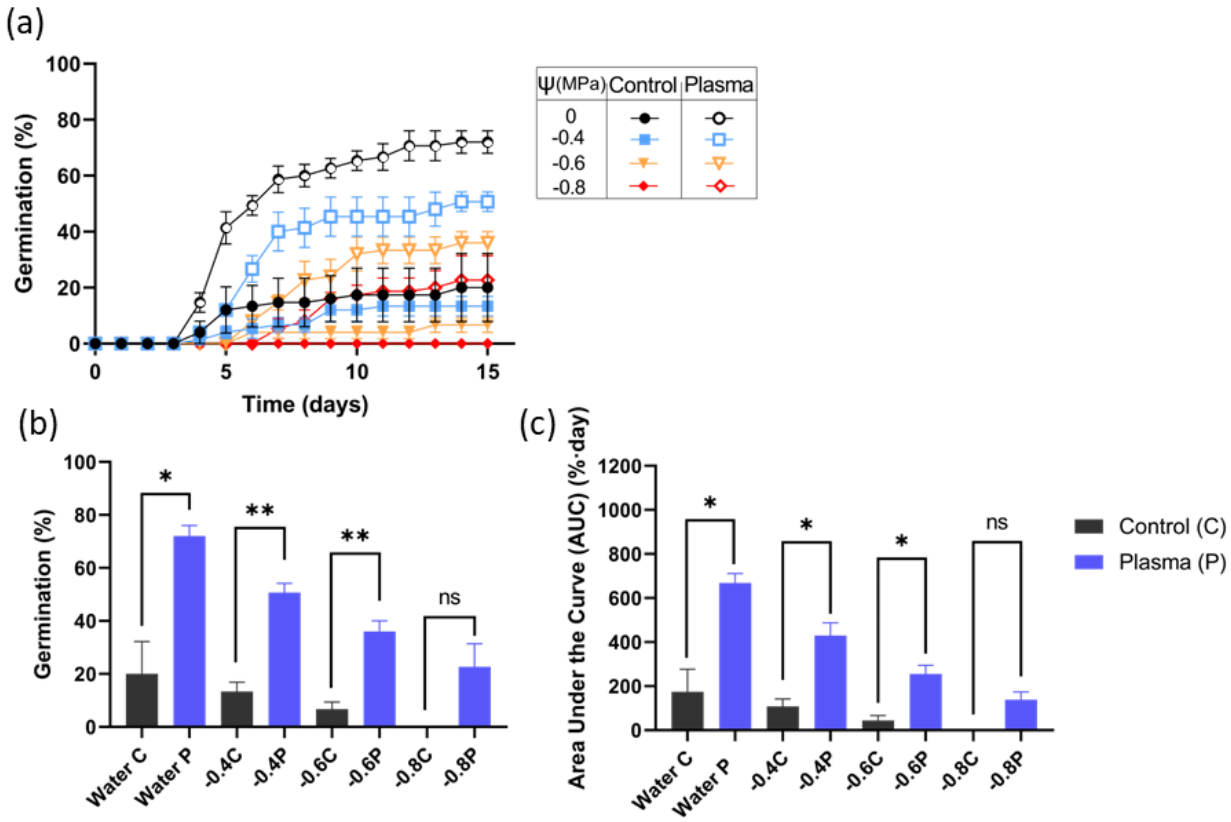


Fig. 7: Effect of plasma treatment on germination at 15 °C of sunflower seeds from variety A under PEG-simulated drought stress: Germination kinetics (a), final germination rate (b), and area under the curve (c). Each point or bar represents the mean of 3 biological replicates (25 seeds per replicate). Error bars indicate the standard error of the mean (SEM). Seeds were either untreated controls (C) or plasma-treated (P), and tested under no water stress (0) or increasing levels of PEG-induced water stress (−0.4, −0.6, and −0.8 MPa).

Germination of A seeds on water did not exceed 20 % after 15 d at 15°C, suggesting that they were dormant (Fig. 7a & 7b). In contrast, plasma-treated seeds exhibited a germination rate of about 70%, suggesting that the treatment partially released seed dormancy. As expected, B seeds fully germinated at 15°C on water within 4 days, but plasma treatment did not significantly improve their germination (Fig. 8a & 8b). Increasing PEG concentrations in the imbibition media progressively reduced germination kinetics and final percentage of A and B control seeds (Figs. 7 & 8). A seeds were very sensitive to water stress since their germination decreased at -0.4 MPa and was fully prevented at -0.8 MPa (Fig. 7a, b, c). In contrast seeds from the B batch were much less sensitive to water stress since they germinated to 80 % at -0.8 MPa (Fig. 8a, b, c). At -1.0 MPa however their germination did not

exceed 20 % (Fig. 8a). The positive effect of plasma on seed germination was clearly shown with dormant A seeds for which final germination percentage increased from 10 to 50 % at -0.4 MPa and from 5 to 35 % at -0.6 MPa (Fig. 7a, b). For non-dormant B seeds, the effect of CAP treatment was less pronounced and became clearly evident only at -1.0 MPa, where water stress significantly reduced germination (Fig. 8a, b). However, differences in the germination kinetics were already noticeable from -0.6 MPa. Figure 7c, where germination results are expressed as area under the curve, highlights the beneficial effect of CAP treatment on both final germination rate and mean germination time across all conditions, except under the most severe water stress. In contrast, in Figure 8c, the area under the curve of treated seeds is significantly higher only under the most severe water stress condition. Consistently, the decrease in $\Psi_{50}$ values following CAP treatment (Table 1) indicates that the treatment enhanced the ability of sunflower seeds to germinate under water stress conditions.

| **Batch** | **Control** | **Plasma (15 min)** |
|---|---|---|
| A seeds | ND | -0.57 MPa |
| B seeds | -1.15 MPa | -1.29 MPa |

Table 1. Water potential for 50% germination ($\Psi_{50}$) at 15 °C. ND, not determined (germination too low).

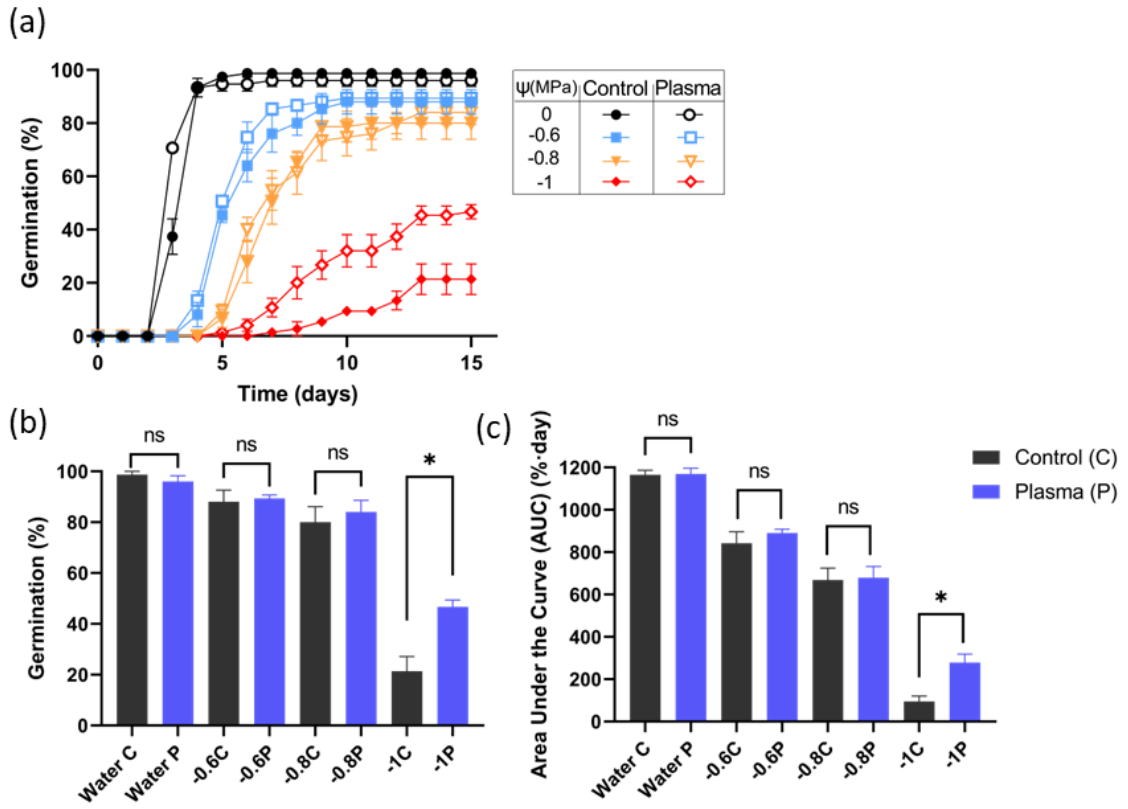


Fig. 8: Effect of plasma treatment on germination at 15 °C of sunflower seeds from variety B under PEG-simulated drought stress: Germination kinetics (a), final germination rate (b), and area under the curve (c). Each point or bar represents the mean of three biological replicates (25 seeds per replicate). Error bars indicate the standard error of the mean (SEM). Seeds were either untreated controls (C) or plasma-treated (P), and tested under no water stress (0) or increasing levels of PEG-induced water stress (−0.6, −0.8, and −1 MPa). Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

In order to determine whether the beneficial effect of CAP treatment on seed germination in water stress conditions could rely on an improved water uptake by the seeds, we investigated the changes of moisture content during imbibition on water and on a -0.8 MPa solution for seeds of variety B (Fig. 9). Water stress decreased markedly water uptake by whole achenes but it was not significantly stimulated by CAP treatment.

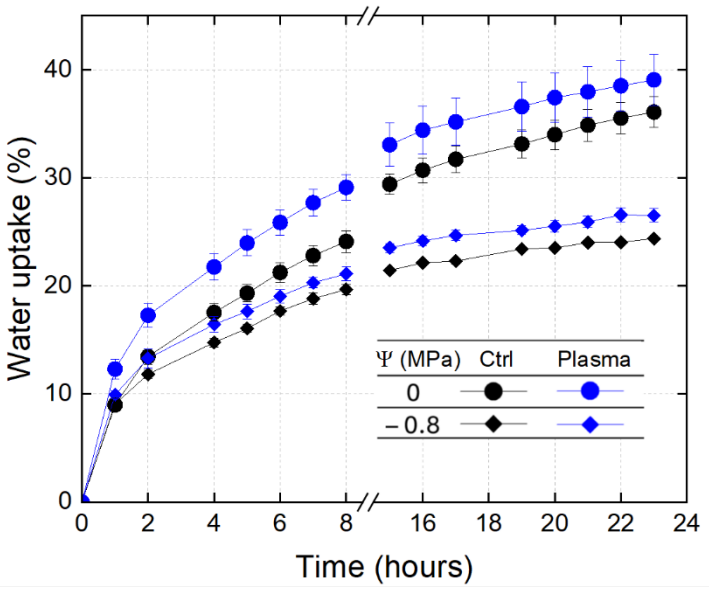


Fig. 9**:** Change in seed water content during imbibition of plasma-treated and control sunflower seeds from variety B under water and PEG -0.8 MPa conditions at 20 °C. Each point represents the mean water uptake calculated from 3 replicates of 20 seeds. Error bars indicate the standard error of the mean (SEM).

### 3.4. Post-germinative effects of ambient air plasma on seedling growth

Since we established that CAP treatment potentially increased the germination rate of B and, more significantly, of A seeds under water stress, we then investigated whether the treatment could have an effect beyond germination, during the early stages of seedling development.

Seedling development was followed under well-watered and water stress conditions in a greenhouse maintaining a constant temperature of 21°C, which also allowed A seeds to germinate. Seeds were treated for 15 or for 30 min before being sown directly in the pots. In the absence of water stress both A and B seeds almost fully germinated in the greenhouse condition (soil and 21°C) (Fig. 10, 11 a & e). As previously shown, water stress dramatically decreased germination of control A and B seeds (Fig. 10, 11 c & g), which reached 20 and 40 % only, respectively. In A seeds, increasing durations of CAP treatment from 15 to 30 min allowed seeds to germinate to more than 60 % (Fig. 11 c) whereas in B seeds 15 min CAP treatment were sufficient to trigger germination rate higher than 80 % (Fig. 11 g).

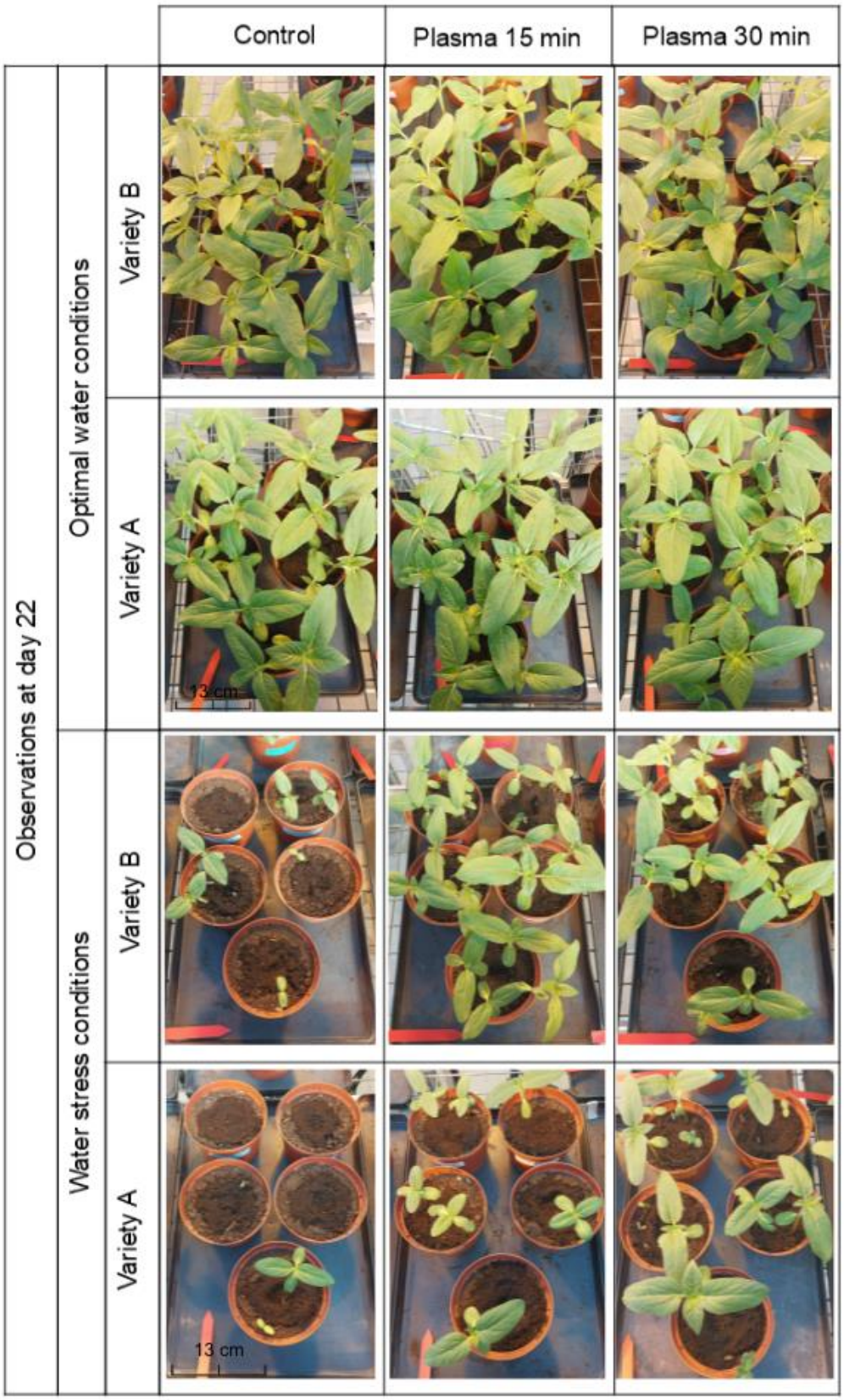


Fig. 10**:** Photographs of sunflower seedlings, taken 22 days after seed sowing in pots placed in a greenhouse. Seedlings from variety A and variety B issued from untreated seeds (control) and from seeds subjected to plasma treatment in ambient air for 15 and 30 min.

We then evaluated the effect of CAP treatment on seedling growth. Fig. 10 shows the general appearance of seedlings that grew-up for 22 days with/without stress conditions. Supplemental Annex 1 shows the appearance of the seedlings at an earlier time point, ie 14 days. From these pictures it appears clearly that a 15 min CAP treatment was sufficient to stimulate seedling emergence in water stress conditions for A and B seeds.

Measurement of hypocotyl length after 19 days confirmed these observations. Figs 11 b, d & f, h show the frequency distribution of hypocotyl lengths according to treatments. In unstressed A seeds, 30 min CAP treatment slightly stimulated hypocotyl growth since most of the hypocotyls ranged in the 8-9 cm class, versus 7-8 cm for control and 15 min treated seeds (Fig. 11 b). This effect was not observed for B seeds (Fig. 11 f). For both A and B seeds CAP treatment induced a shift of hypocotyl mean lengths toward higher values, which was more important for A seeds (Fig. 11 d) than for B seeds (Fig. 11 h).

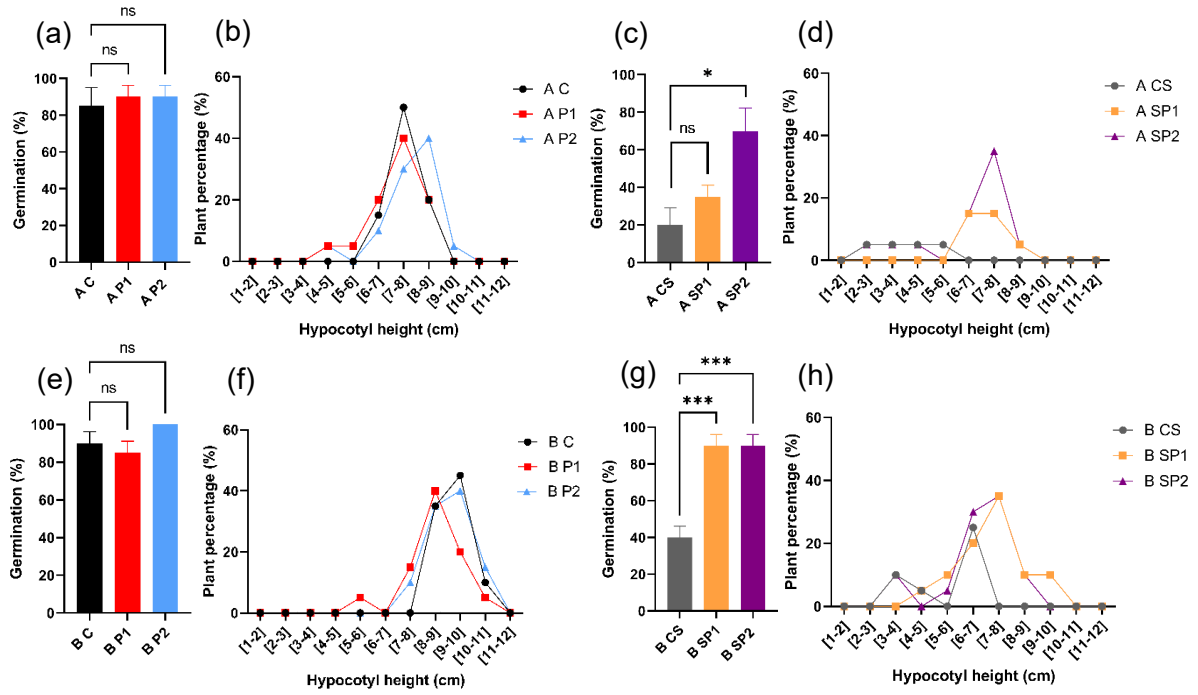


Fig. 11**:** Effect of plasma treatments on seed germination rate and hypocotyl growth for variety A (a, b, c, d) and variety B (e, f, g, h) under control (a, b, e, f) and water stress conditions (c, d, g, h) over a 19-day period. Hypocotyl length distributions (b, d, f, h) are shown as the percentage of seedlings falling within defined size intervals (e.g., [1–2] cm). C, untreated seeds, P1 and P2, seeds treated for 15 and 30 min by plasma, respectively. For histograms (a, c, e, g), each bar represents the mean percentage of germinated seeds per pot, calculated from five pots with four seeds each (20 seeds in total per condition). Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

Finally, the effects of CAP treatment on both seed germination and seedling growth can be appreciated using the vigor I index which was calculated as the product of the average hypocotyl length (at day 19), by the germination rate (at day 19), divided by 100. In the case of A seeds, the CAP treatment increased the vigor index I from 0.9 (C) to 2.5 (P1) or 4.4 (P2) (Fig. 12a). For the seeds in Batch B, this index increased from 2.3 (C) to 6.77 (P1) or 6.25 (P2) (Fig. 12b). On the other hand, Figure 12a, b shows that in the absence of water stress, cold plasma had no effect, even significant.

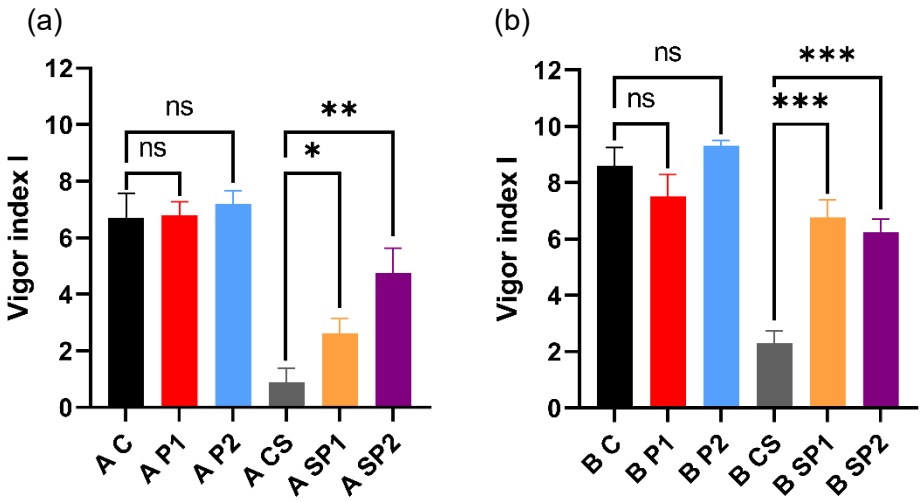


Fig. 12: Effect of plasma treatments on the vigor index I of sunflower seeds under control and water-stress conditions: (a) variety A, (b) variety B, over a 19-day period. The vigor index I corresponds to the product of the average germination rate and the average hypocotyl length, divided by 100. Each bar represents the mean vigor index I calculated for all pots per condition. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

## 4. Discussion

The characterization of ambient air plasma generated using a dielectric barrier discharge (DBD) reactor revealed a complex, interdependent relationship between discharge parameters and the generation of reactive species. Notably, the production of strong oxidants such as ozone ($O_3$) in Fig. 6 was found to be highly sensitive to the electrode gap, in agreement with the findings of Kumar Shah et al. [50]. In addition, the physical filling state of the reactor (whether empty or packed with seeds) significantly influenced the yield and composition of gaseous reactive species, as previously reported by Molina et al. [51] and Judée et al. [52].

OES indicated that the plasma emission was dominated by the second positive system (SPS) of molecular nitrogen (Fig. 5), which is characteristic of non-thermal atmospheric plasmas in air [46]. In

contrast, short-lived oxygenated species such as atomic oxygen (O), hydroxyl radicals (OH), and nitric oxide (NO) were not detected. This absence is most likely due to rapid collisional quenching and energy transfer processes occurring under the specific discharge conditions used in this study, including atmospheric pressure, limited residence time, and frequent surface interactions [53, 2016]. Under such conditions, these transient species are either converted into more stable molecules before they can emit detectable photons or quenched via vibrational and rotational energy relaxation, rendering their optical signatures too weak to be captured by time-averaged OES measurements [54] To overcome this limitation, advanced diagnostic techniques such as time-resolved OES, laser-induced fluorescence (LIF), or cavity ring-down spectroscopy (CRDS) could be employed to detect and quantify these highly reactive, short-lived species with greater sensitivity and temporal resolution [55].

Complementarily with OES, mass spectrometry enabled the detection of more stable species, particularly ozone, whose abundance significantly increased in the presence of seeds. Two factors could explain this trend: the dielectric nature of the seeds and their sharp geometrical structure.

Regarding seeds dielectric nature, it is worth reminding that seeds act as microscale capacitive elements within the discharge gap [46]. When densely packed, they modify the local electrical properties of the reactor by increasing the effective dielectric constant and contributing to heterogeneous charge storage [52]. This could result in altered electric field distributions and enhanced microdischarge activity, as observed in DBD systems packed with dielectric glass beads [56] and here in Fig. 4. Such capacitive behavior likely facilitates a more intense and spatially distributed generation of ozone, providing a plausible explanation for the elevated $O_3$ concentrations detected in the presence of seeds.

Seeds of sunflower often present sharp tips and ridges. This morphology can induce local electric field enhancement at the apexes of the seed coat, a phenomenon well-documented in patterned dielectric barrier discharges (p-DBDs). Recent work by Berger et al., (2024) [57] demonstrated that dielectric pellets with sharper apexes (such as conical or inverse hemispherical shapes) exhibit distinct streamer dynamics compared to flat or rounded pellets. Specifically, sharper features lead to stronger local polarization, increased electron impact excitation rates, and higher streamer velocities, due to enhanced electric fields at the apex. By analogy, the natural topography of sunflower seeds may act as field-enhancing microstructures under DBD treatment, concentrating electric field and intensifying microdischarges in their vicinity. This localized enhancement likely promotes the formation and stabilization of reactive species, including ozone, via more efficient electron-driven reactions.

These physico-chemical properties have direct implications for the biological efficiency of the plasma treatment. Reactive nitrogen species such as $N_2$* and long-lived oxidants like $O_3$ are known to influence seed coat permeability and surface chemistry. $O_3$ may indeed facilitate oxidative degradation of inhibitory compounds or modify hydrophobic barriers on the seed surface [58]. Although OH and O radicals were not detected via OES, their possible transient presence at the plasma-seed interface cannot be excluded and may contribute to the activation of reactive oxygen species (ROS)-mediated signaling pathways involved in dormancy alleviation and water uptake enhancement.

Plasma treatments have been demonstrated to stimulate seed germination of a wide range of species (see Waskow et al. 2021 for review [59]) including in sunflower, but in this case using argon plasma [60]; [61] or vacuum plasma [62]. In contrast to what is shown here, using a similar device (coaxial DBD reactor operated in air 10 min) but with different settings (sinusoidal voltage of 16 kV and amplitude at 50 Hz frequency) Florescu et al. (2023) [63] did not evidence any effect of plasma on germination of sunflower seeds, but they showed that the treatment could stimulate seedling growth. This demonstrates the importance of the plasma characteristics when designing a treatment to stimulate germination, as already suggested by Sarapirom and Yu (2021) [64]. Here we took advantage in phenotypic variability that exists between seeds of different hybrids of sunflower to explore the effect of plasma on both dormancy and germination under water stress. A short plasma treatment significantly stimulated germination of dormant seeds at 15°C (Fig. 7), a temperature which allows expression of embryo dormancy [65]; [3]. Alleviation of embryo dormancy naturally occurs during dry storage (dry after-ripening), but may take several weeks or months [65]. Therefore, the ability to alleviate sunflower seed dormancy within a few minutes constitutes a striking advancement, with potential practical implications for the evaluation of seed quality and the acceleration of sunflower breeding programs. Sunflower seed dormancy alleviation during dry storage has been associated with non-enzymatic ROS generation [66]. Interestingly, it is also well known that plasma generates ROS, which might explain their beneficial effect on seed dormancy alleviation. Surprisingly the effect of CAP treatment on release of physiological dormancy is poorly documented but it has been evidenced in species as radish [67] or Arabidopsis [46]. Our results highlight that CAP is a promising treatment for releasing the dormancy of agroeconomically important species.

As previously demonstrated, the germination of sunflower seeds can be dramatically altered by water stress, but it also strongly relies on genotype [68]. As expected, dormant seeds were very sensitive to water stress and their germination rapidly became impossible with increasing PEG concentrations (Fig. 7). However CAP treatment always stimulated germination of dormant seeds in suboptimal conditions of water availability, which resulted both from dormancy release, as previously shown, and from the

improvement of germination in the presence of PEG. This was demonstrated using non-dormant seeds, which germinated quite well till -0.8MPa, but whose germination dropped down to 20 % at -1.0 MPa. In this later case, CAP treatment stimulated significantly germination, suggesting that plasma also improved tolerance to water stress at the germination stage. Interestingly CAP treatment significantly decreased $\Psi_{50}$ in a range that was quite similar to what can be obtained using seed priming. This effect has been shown for oilseed rape [41], wheat [44] or barley [69], and in most cases it was attributed to an improved water uptake by the seeds. This was not the case here, since we did not evidence a clear difference of water uptake between control and treated seeds in water stress conditions (Fig. 9), which suggests that the better germination of CAP treated seeds relies on other mechanisms, that need to be explored.

Since stand establishment relies on both germination and seedling growth, we investigated the effects of CAP on the early stages of sunflower seedling development in the soil under water stress conditions. Besides stimulating seed germination under water constraints CAP also dramatically stimulated post-germinative seedling growth. This is clearly shown Fig. 11 where the length of the seedlings was generally 2-3 cm longer after 19 days of growth in a poorly watered soil. This demonstrates that an appropriate CAP treatment was highly beneficial for sunflower seedling emergence, since it stimulated both components of this process, ie the seed germination and the seedling growth, as demonstrated by the calculations of the vigor index I (Fig. 12). In contrast, a decoupling of the effects of CAPs on germination and growth has often been reported, ie a positive effect on seedling growth without changes to the germination [70]. This discrepancy between effects on those two distinct developmental processes is not surprising since germination consists in cell elongation only whereas seedling development also requires active cell division through meristematic activity. Nevertheless, it is worth noting that a plasma treatment performed on dry seeds can last over time and modify seedling tolerance to water stress. It would be interesting to determine whether this treatment has also an effect on the long term; i.e. seed yield, and to better understand the mechanisms involved in this long-lasting effect.

**5. Conclusion**

Altogether our results demonstrate that CAP treatment is a promising technology to enhance crop emergence and resilience in changing environmental conditions. A key finding of this work is the critical importance of accurately adjusting the gap distance between electrodes in the plasma reactor. This highlights the need for precise engineering control in the design of plasma-based seed treatment systems, particularly for seeds with non-uniform or large size distributions. Future studies will have to determine if tolerance to other stresses during stand establishment, such as hypoxia or extreme

temperatures, can also be improved by plasma. Further research should also focus on optimizing plasma treatment parameters, including power, duration, and gas composition, to maximize benefits for different crop species and environmental scenarios. Investigating the mechanisms of action of the plasma species, such as reactive oxygen and nitrogen species, in promoting seed germination, seedling growth, and stress tolerance is also crucial. Advancing our understanding of CAP technology should support sustainable crop production in the face of environmental challenges.

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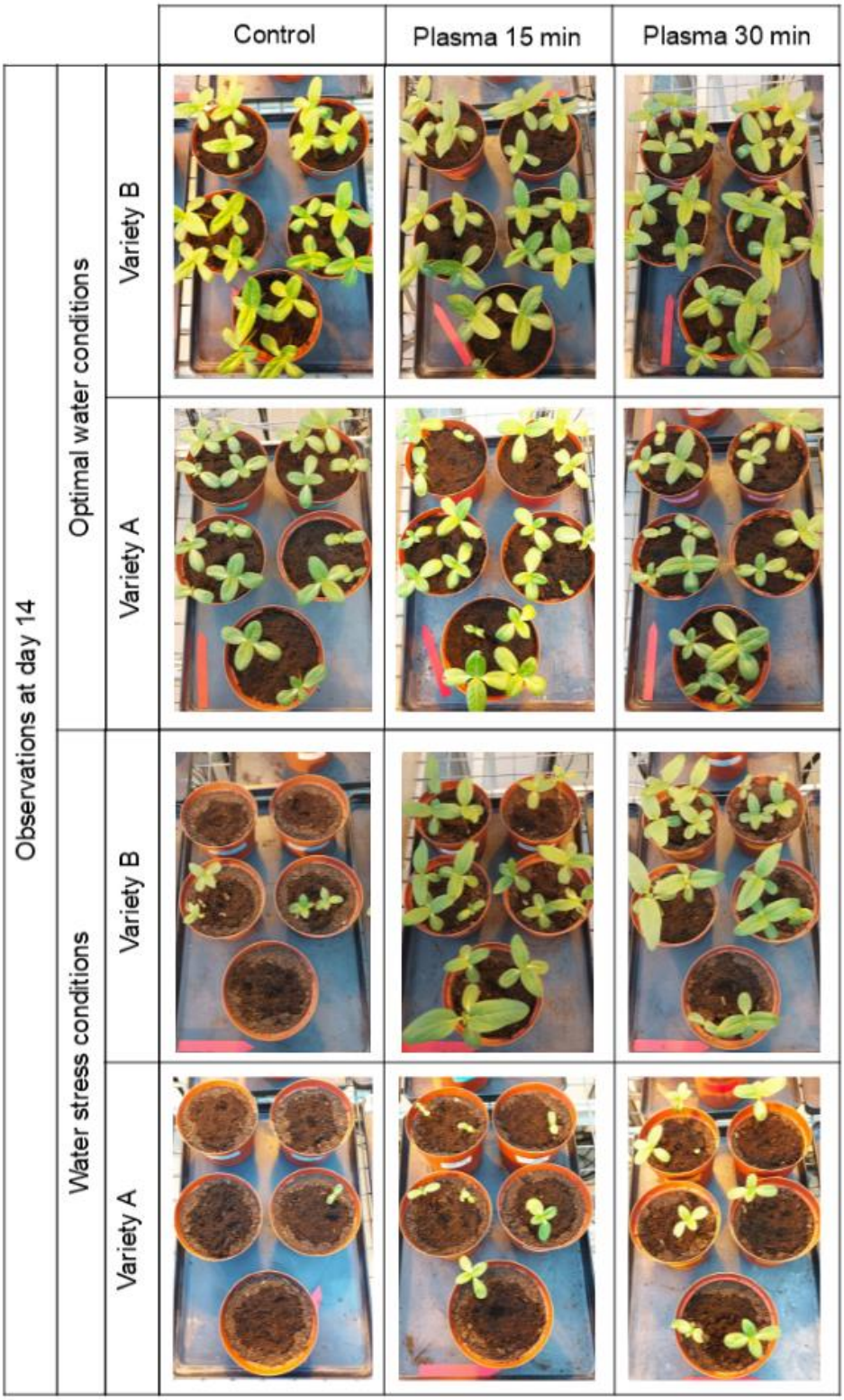
**8. Annex**




Photographs of sunflower seedlings, taken 14 days after seed sowing in pots placed in a greenhouse. Seedlings from variety A and variety B issued from untreated seeds (control) and from seeds subjected to plasma treatment in ambient air for 15 and 30 min.

# Chapter 3: Cold atmospheric plasma decontaminates Arabidopsis thaliana seeds and remodel seedling fungal and bacteria microbiota

# Cold atmospheric plasma decontaminates *Arabidopsis thaliana* seeds and remodel seedling fungal and bacterial microbiota (to be submitted)

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**Abstract**

This study evaluates the effect of cold plasma (CAP) exposure on the decontamination of *Arabidopsis thaliana* seeds from two ecotypes (Columbia and Landsberg) and two batches exhibiting varying levels of *Penicillium* contamination. A 5 min treatment effectively inhibited *Penicillium* growth on seeds placed on MEA medium. Concurrently, the fungal and bacterial microbiota of seedlings derived from untreated and treated seeds were analyzed by amplification of the ITS1 and V4 regions of ribosomal DNA. For the fungal microbiota, we show that the treatment significantly reduced the relative abundance of dominant taxa, notably *Penicillium* and *Acremonium*, in the Columbia ecotype. *Penicillium*, initially dominant at 80% relative abundance, was reduced to less than 1% after 15 minutes of treatment. Regarding the bacterial microbiota, CAP treatment resulted in complete elimination of bacteria in all treated samples. Unlike the fungal microbiota, the dominant bacterial taxa in controls varied in nature and abundance between samples, with notable presence of the genera *Massilia*, *Pseudomonas*, *Paenibacillus*, as well as the families *Comamonadaceae* and *Burkholderiaceae*. These results demonstrate the efficacy of CAP in seed decontamination and its differential effects on the fungal and bacterial microbiota structures of seedlings, opening avenues for alternative phytosanitary management strategies.

**Keywords: cold plasma, seedlings, microbiota, seeds, decontamination**

## 1. Introduction

The plant microbiota comprises microbial communities inhabiting seeds and plants, which can be classified as either endophytic (residing within the plant or seed) or epiphytic (living on external surfaces) [1]. This microbiota forms a dynamic set of communities shaped by both environmental factors and by the genotype of the host plant [2]. From seed to plant, different compartments and tissues create distinct ecological niches, making the microbiota an ecosystem in its own right, sensitive to environmental fluctuations.

Microbial communities can exert beneficial, harmful, or neutral effects on the host plant, depending on their composition and surrounding conditions [3]. For example, soil composition plays a crucial role in shaping microbial communities. Hiruma et al. (2016) [4] demonstrated a link between soil phosphate content and the role of plant-associated microbial communities, showing that *Colletotrichum tofieldiae* enhances the growth of *Arabidopsis thaliana* in phosphate-deficient environments. Conversely, Finkel et al. (2019) [5] reported that low phosphate levels can trigger a shift in *Burkholderia* spp. from commensalism to parasitism. Similarly, certain microbial species present on seeds before harvest can become opportunistic during storage, negatively affecting seed quality [6].

These findings underscore the extent to which environmental disturbances can alter microbial balance, potentially revealing the pathogenic capabilities of species that remain functionally neutral under stable conditions.

Plant diseases are a major cause of agricultural yield losses, driving the widespread use of phytosanitary products. Over the past two decades, the global use of antimicrobial agents and fungicides in agriculture has increased by more than 50% [7], with these chemicals accounting for 21% of pesticide applications in 2022. Many plant diseases are caused by microorganisms already present in the environment or introduced through agricultural practices. The post-harvest fate of seeds, particularly during storage, plays a crucial role in ensuring seed viability and, consequently, future crop yields. Under unfavorable conditions, such as increased moisture or prolonged storage duration, microorganisms already present on seeds can proliferate, leading to contamination, reduced germination rates, and the potential transmission of pathogens to emerging seedlings [6]. Therefore, proper management of storage conditions is essential to minimize post-harvest contamination that may threaten the health of future plant generations. The role of plant-associated microbiota has also been highlighted in numerous studies for its potential applications in sustainable agriculture It can act as a biological control agent against phytopathogens, promote plant growth, enhance resilience to environmental stresses, and contribute to the production of beneficial molecules such as antibiotics and facilitate access to nutrients [3]. For these reasons, certain microorganisms, such as mycorrhizal

and non-mycorrhizal fungi, bacterial endosymbionts (like *Rhizobium*), and rhizobacteria, are also central to the formulation of biostimulants [8]. As a result, research on microbiota is increasingly being prioritized to support ecological restoration of soils and to develop more sustainable agricultural practices [9].

Conventional agricultural methods for controlling phytopathogens can disrupt microbial communities associated with soils and plants, potentially eliminating beneficial microorganisms. Practices such as plowing and pesticide application degrade soil biological quality by harming resident microbial populations. The persistence of chemical inputs and their degradation products in the soil contributes to environmental pollution, affecting waterways and groundwater, which are crucial for food production and human health [10]. Moreover, the routine use of these chemicals fosters microbial resistance, thereby necessitating the continuous development of new molecules [11]. These challenges are directly linked to food security, with seeds playing a central role in ensuring agricultural sustainability. Managing pathogenic threats, enhancing agrosystem resilience to climate change, and improving soil health are key priorities for the future of agriculture. Current research focuses on developing innovative seed treatment methods that minimize environmental impact while maintaining productivity.

Among emerging technologies, cold atmospheric plasma (CAP) represents a compelling alternative to conventional decontamination methods. Often referred to as the fourth state of matter, cold plasma is an ionized gas composed of various charged and neutral species, including photons, electrons, and ions. The term "cold" or "non-thermal" indicates that the particles within the plasma are not in thermodynamic equilibrium; while electrons can reach temperatures of several thousand kelvins, the heavier species (ions and neutrals) remain near ambient temperature, making cold plasma particularly suitable for biological applications [12].

CAP, owing to their unique physico-chemical properties, are well recognized for their antimicrobial activity [13]. In the medical field, they are routinely employed for surface disinfection and for promoting the healing of chronic wounds [14], due to their ability to inactivate a broad spectrum of microorganisms. These antimicrobial effects are also being explored in agriculture, where numerous studies have demonstrated their efficacy in inactivating seed-borne pathogens [15, 16], thereby establishing plasma as a promising technology for plant disease management.

However, the specific effects of plasma treatment on fungal and bacterial communities associated with seeds and their resulting seedlings remain poorly understood. Recent investigations in *Arabidopsis* and sunflower have shown that plasma treatment can alter the structure of seedling-associated bacterial communities [17, 18]; nonetheless, data regarding the impact on fungal communities are still scarce.

The main objective of this study was to better understand the effects of CAP treatment on seed decontamination and seedling-associated fungal and bacterial communities in Arabidopsis thaliana. We investigated the decontaminating effects of CAP treatment using Arabidopsis thaliana seeds from the Landsberg and Columbia ecotypes as model organisms. By employing techniques such as nephelometry and microbial quantification before and after treatment, we directly assessed its impact on the microbial load associated with seeds. Furthermore, metabarcoding analyses were conducted to investigate how CAP exposure influences microbial communities in seedlings derived from treated versus untreated seeds. This study contributes to a better understanding of the potential of CAP as a sustainable seed treatment method and highlights its broader implications for shifts in the composition of microbial communities.

## 2. Material and methods

### 2.1. Plant material

This study was performed with two ecotypes of *Arabidopsis thaliana*: Columbia-0 (Col) and Landsberg-0 (Ler). The plants were cultivated in a climate-controlled chamber at 20–22°C with a long-day photoperiod (16 h light/8 h dark). Seeds were collected from mature siliques after 2 weeks drying at room temperature and subsequently stored at room temperature. Two batches of seeds (A and B) from two independent production cycles were used for each ecotype.

### 2.2. Plasma treatment

Dry seeds were treated with cold atmospheric plasma (CAP) using a dielectric barrier discharge (DBD) system. Air plasma was generated in a DBD device consisting of two flat electrodes, each measuring 30 × 40 $cm^2$, one connected to a high voltage source and the other grounded. The electrodes were separated by a 2 mm thick insulating dielectric material to prevent any transition from streamer to arc, ensuring uniform plasma distribution within the reactor where the seeds were exposed. The background gas used was ambient air, with a relative humidity of approximately 40%. The high-voltage electrode was powered by a 9 kV supply at a frequency of 140 Hz (sine wave), provided by a high-voltage generator comprising a function generator (ELC Annecy France, GF467AF) and a power amplifier (Crest Audio, 5500W, CC5500). Two treatment durations were tested: 5 min and 15 min. The inter-electrode gap was 1 mm.

### 2.3. Assessment of seed contamination on Malt Extract Agar medium

At least 40 non-disinfected seeds were placed under sterile conditions onto malt agar medium (30 g malt extract and 10 g agar per liter) for each untreated and plasma treated conditions (5 & 15 min of plasma treatment) and each ecotype (Columbia and Landsberg) in 2 replicates (each consisting of at least 40 seeds). Photos were taken daily to monitor contamination levels, and the percentage of contaminated seeds was calculated after 6 days at 21°C with a 16-hour light/8-hour dark photoperiod. Unpaired t-tests were performed using Prism 9 to compare results across conditions.

### 2.4. Nephelometry

Eighty mg of control and treated Col seeds were stirred for 1 h in sterile liquid malt medium (30 g malt extract per liter) to capture at least the epiphytic microorganisms. The incubation mixture was then filtered to retain the seeds and collect the medium containing the detached fungal spores. A volume of 200 µL per well were pipetted under sterile conditions into a 96-well plate. On the plate, 12 wells were filled per condition, changing rows every 4 wells to prevent cross-contamination between conditions. A negative control (sterile liquid malt medium) and a positive control, consisting of a spore suspension prepared in sterile liquid malt medium from a *Penicillium* culture isolated from control seeds, were included. To prepare the spore suspension, the spores were scraped off the mycelium grown on solid malt agar mixed with 100 µL of liquid malt medium and filtered using filter cloth **(**10 µm pores**)** and diluted in sterile liquid malt medium. The concentration of the 3 control suspensions was measured with a Malassez counting chamber and adjusted by adding liquid malt medium to reach final concentrations of $10^3$, $10^4$, and $10^5$ spores/mL**.** Nephelometric assays were conducted using a NEPHELOstar® Plus microplate reader (BMG LABTECH GmbH, Ortenberg, Germany), equipped with a 635 nm laser diode capable of detecting scattered light up to an 80° angle. Growth was automatically recorded for 72 h at 25 °C and measurements were done every 20 min with a laser intensity of 40%. The data is given in nephelometric turbidity units (NTU) and analyzed using MARS software.

### 2.5. Metabarcoding analysis

Seeds were sown under sterile conditions on moistened cotton pads with sterile water, in two replicates. Both water and cotton were sterilized. After 8 d of incubation at 21°C with a 16 h light / 8 h dark photoperiod, 0.1g of seedlings were collected per condition (control, 5 & 15 min) and per ecotype (Columbia and Landsberg) in triplicates. DNA extraction was performed using the Qiagen Plant Pro kit, following the manufacturer's protocol. The extracted DNA samples were sequenced by Novogene

using paired-end Illumina amplicon sequencing, targeting the V4 region of the 16S rRNA gene for bacteria and the ITS1 region of the internal transcribed spacer for fungi [19, 20]. Sequencing depths of 100k reads for the V4 region and 50k reads for the ITS1 region were applied.

The sterile conditions applied to both control and treated seeds, along with the two boxes sown per condition, ensured a robust experimental setup. This experiment focused on seedlings, as the transition from seed to seedling represents a critical phase of microbial community reorganization, driven by changing environmental conditions [21].

**2.6. Bioinformatic analyses**

Raw paired-end sequencing reads were first demultiplexed according to their unique barcodes, and barcode and primer sequences were removed. Paired-end reads were then merged using FLASH to generate contiguous sequences, referred to as raw tags. Quality filtering was subsequently performed following the QIIME pipeline to remove low-quality sequences and obtain high-quality clean tags. Chimeric sequences were identified by comparison with the Gold reference database using the UCHIME algorithm and removed, resulting in the final set of effective tags. All downstream analyses were conducted using the clean data.

The clean sequencing data for fungal ITS and bacterial V4 regions were analyzed using the QIIME2 (version 2024.5) bioinformatics platform, leveraging GitHub resources from Simonin et al. (2022) [16]. Taxonomic classification of each amplicon sequence variant (ASV) was performed using the naïve Bayes classifier implemented via the scikit-learn package in QIIME2, with the UNITE release 10 database used as reference for ITS sequences and SILVA release 138 for V4 sequences. Sequence data analyses were primarily conducted using QIIME2 and R packages. Alpha diversity indices at the ASV level, including the Chao1 richness estimator, Shannon index, and Pielou's evenness, were calculated in QIIME2. Principal coordinate analysis (PCoA) was performed in R to estimate and visualize beta diversity based on the Bray–Curtis distance matrix generated in QIIME2.

PERMANOVAs and pairwise PERMANOVAs were conducted in RStudio to assess the statistical significance of differences between groups, with Bonferroni correction applied for multiple comparisons. Differences in the number of contaminated seeds, diversity indices, and read counts prior to rarefaction were evaluated in Prism 9 using unpaired t-tests. Rarefaction thresholds were determined based on rarefaction curves to maximize diversity while retaining the highest number of samples in the analysis. Thresholds of 51,364 reads for fungal communities and 9,600 reads for

bacterial communities were applied. One of the Col replicates from batch A treated with CAP for 5 minutes was excluded from the fungal analysis after rarefaction.

## 3. Results

### 3.1. Effects of cold plasma treatment on seed contamination

We investigated the effect of CAP exposure on the contamination of *Arabidopsis thaliana* seeds using two batches from Col and Ler ecotypes. Without any treatment, seed contamination levels, evaluated after 6 days on MEA medium, ranged from 20 to 100 % in Col seeds (Fig.1 a, b) and from 80 to 100 % in Ler seeds, depending on the seed batch (Fig. 1 b, c). The morphology of the mycelium after 6 days

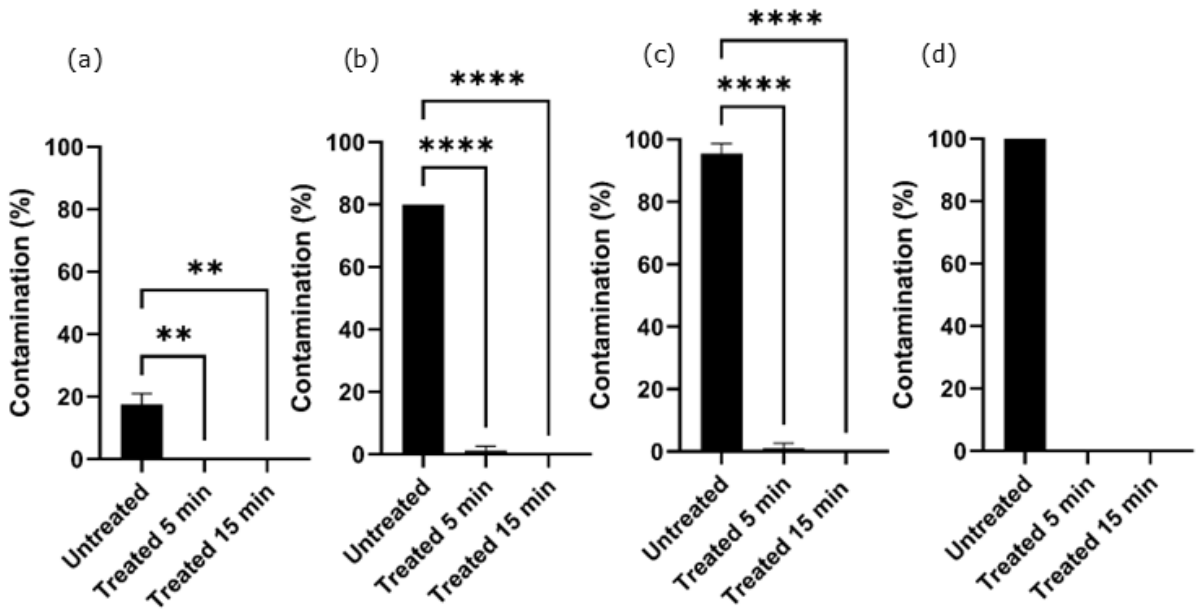



Fig. 1: Number of contaminated Columbia (Col) (a, b) and Landsberg (Ler) (c, d) seeds after 6 days on MEA medium. Two batches (A & B) were tested. The seeds were either untreated (C) or plasma-treated for 5 or 15 minutes. Each bar represents the average of two replicates of 45 seeds. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

To complete this experiment, we investigated the effect of CAP treatment on the development of fungal spores in suspension using nephelometry. This experiment was conducted with seeds from Col ecotype from the heavily contaminated batch (batch B, Fig. 1 b). Three hours after inoculation, Nephelometric Turbidity Unit (NTU) values were below 60,000 for the three tested conditions, indicating low initial turbidity in the wells (Fig. 2 a). However, after 48 h, turbidity significantly increased in the untreated condition and in the positive control, indicating fungal growth. In these two

conditions, NTU values were respectively 13 and 9 times higher than at the start of the experiment, reaching peak levels of 692,274 and 525,132. In contrast, in the wells corresponding to 15 min plasma exposure of seeds, turbidity did not increase between the 3 h and 48 h measurements and remained low (Fig. 2 b). These results, combined with the previous findings, indicate that a 15 min CAP treatment effectively prevents the development of fungal spores present on the seeds, thereby reducing the risk of contamination.

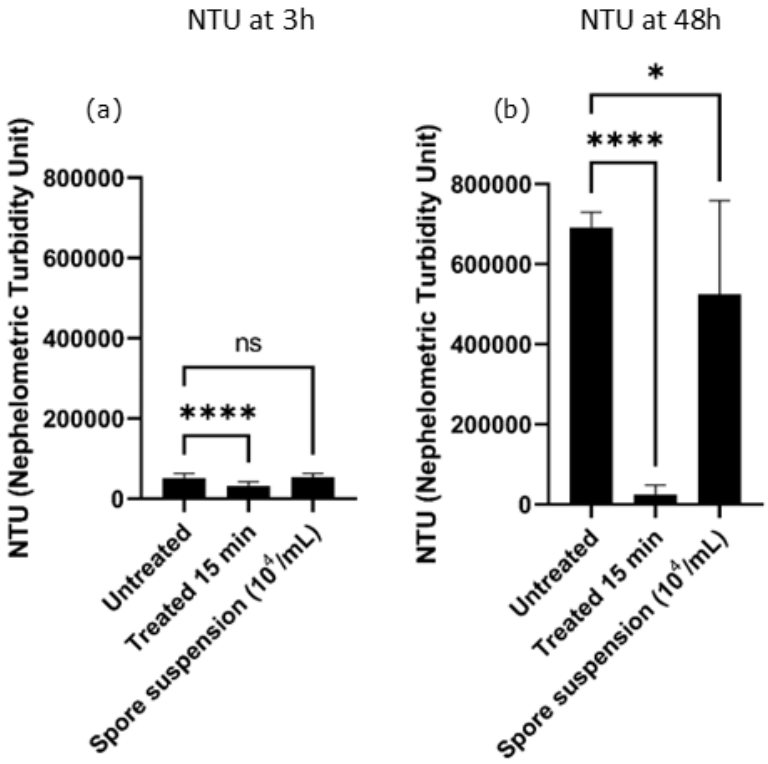



Fig. 2: Fungal growth (NTU) at 3h (a) and 48h (b) after nephelometry measurements. The tested liquid MEA suspensions containing spores were obtained from untreated and plasma-treated (15 min) Columbia seeds, as well as from a spore culture derived from Columbia seeds of the most contaminated batch (B). The results correspond to the average NTU values from 12 wells per condition, with blank correction applied. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

### 3.2. Deciphering the effects of plasma treatment on seed fungal communities

Amplicon library construction and sequencing were performed on seedlings derived from seeds germinated and grown under sterile conditions. Under these conditions, i.e. on cotton wool moistened with sterile water, no visible bacterial contamination was observed, and only a few cases of visible fungal contamination occurred, exclusively in the control plates, after 8 days. The fungal community associated with seeds contained a small number of dominant taxa common to both ecotypes and both

batches, notably *Penicillium olsonii*, *Acremonium sclerotigenum*, and *Cladosporium cladosporioides* (Fig. 3). Plasma exposure induced significant changes in the composition of fungal communities associated with Col seedlings compared to the untreated condition. In the less contaminated batch of Col seeds (batch A), 5 and 15 min CAP treatments induced major shifts in the microbiota. The dominant taxa in untreated seedlings, *Penicillium olsonii* and *Acremonium sclerotigenum*, became either undetectable or present at very low abundance (<1%), even after 5 min of CAP treatment. Furthermore, seeds treated with plasma for 5 and 15 min contained 90% and 70%, respectively, of fungal DNA sequences that could not be assigned to a genus or a species, with most of this DNA affiliated to the phylum *Ascomycota* (Fig. 3 a). Interestingly, after 15 min of plasma exposure, *Cladosporium cladosporioides*, which initially had an abundance of less than 1% in untreated seeds, accounted for 30% of the detected sequences (Fig. 3 a).

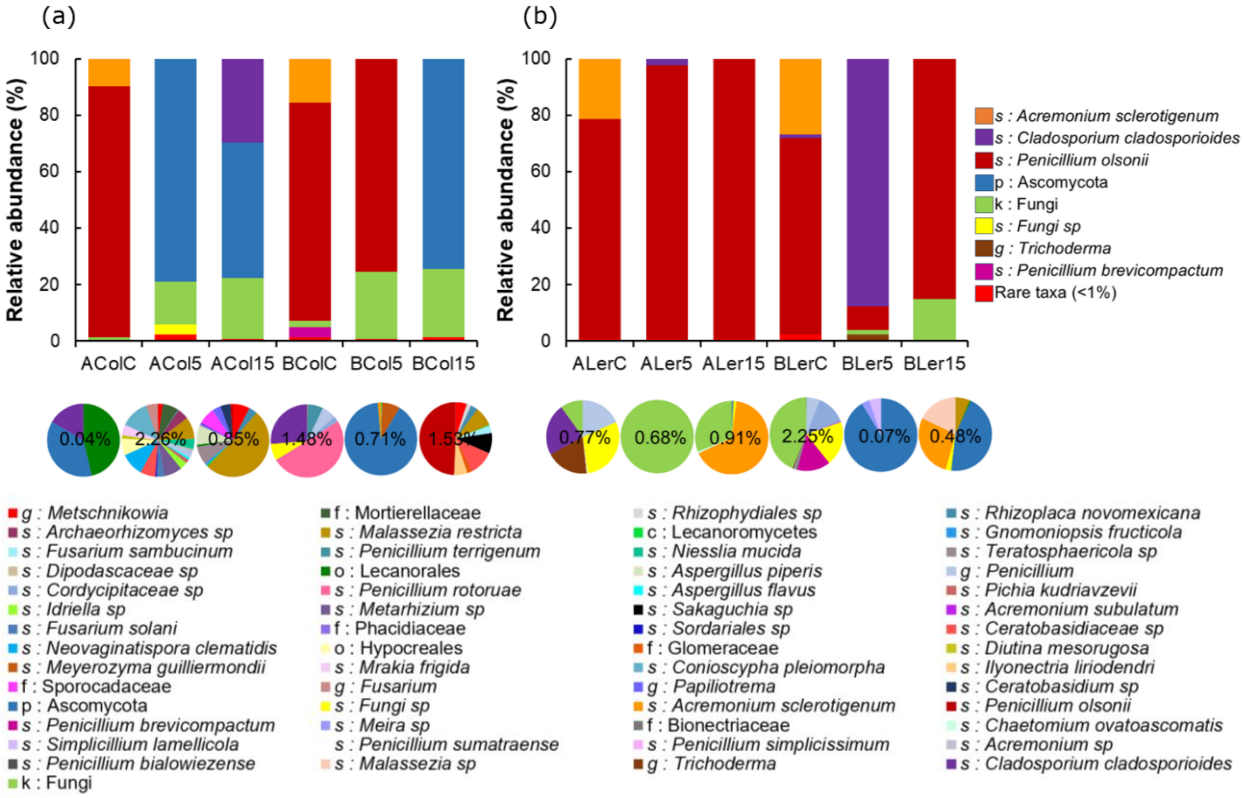



Fig. 3: Taxonomic profiles of fungal communities associated with *Arabidopsis* seedlings of Columbia (Col) (a) and Landsberg (Ler) (b) ecotypes. Fungal diversity associated at seedlings which provided of untreated (C) and plasma-treated for 5- or 15-minutes seeds from two batches (A & B). Each stacked bar represents the average relative abundance of taxa from three samples per condition. Taxa with an abundance greater than 1% are shown in the figure, while the others are grouped under "rare taxa," with details provided for each sample in the form of pie charts.

Alpha diversity analysis revealed an increase in both Shannon and Chao1 indices following plasma exposure, which tended to indicate a greater taxonomic diversity (Fig. 4 a, b). In untreated seeds, the fungal community was dominated by a few major taxa, with a low proportion of rare taxa (relative abundance <1%). After 5 min of treatment, these rare taxa accounted for 2.26% of the sequences, reflecting a rise in diversity. Although the differences were not statistically significant due to variability between replicates, the trend may reflect a reduction in dominant taxa, allowing rare taxa to emerge and contribute to the observed increase in Shannon and Chao1 indices (Fig 4 a, b).

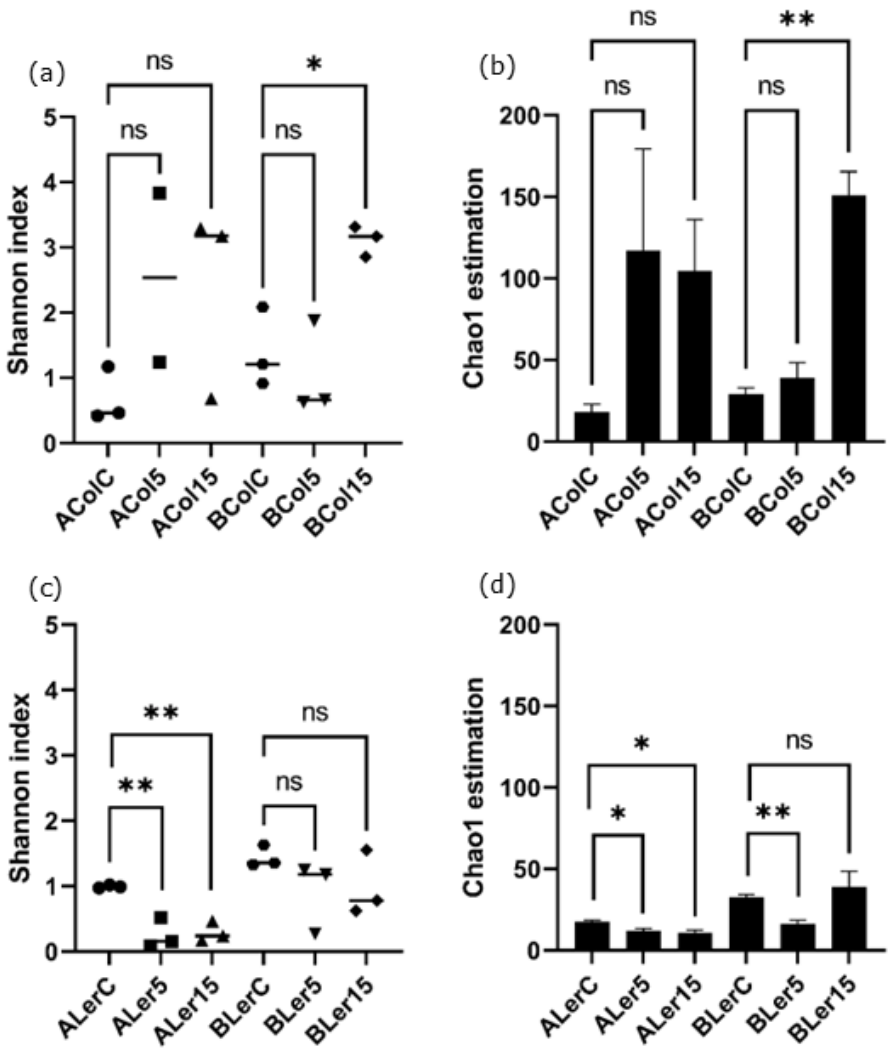



Fig 4. Alpha diversity metrics of the fungal microbiota (Shannon (a, c) and Chao1 (b, d)) for Columbia (Col) and Landsberg (Ler) across treatments C, 5, and 15 in lots A and B. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

In Col seeds with a higher level of contamination (batch B), *Penicillium olsonii* and *Acremonium sclerotigenum* were the dominant taxa in seedlings, similar to what was observed in seeds of batch A, with comparable relative abundances (Fig. 3 a). *Penicillium brevicompactum* was also detected,

accounting for 3.4% of the identified sequences. Additionally, a greater proportion of rare species was found, with eight taxa each having a relative abundance of less than 1%, collectively representing 1.48% of the total analyzed sequences. After 5 min of plasma exposure, *Penicillium olsonii* remained the dominant taxon, representing 75% of the sequences identified, compared to only 0.04% in batch A after the same treatment duration (Fig. 3 a).

Furthermore, 24% of the sequences could not be classified beyond the kingdom level. These unclassified sequences represent the majority of rare taxa, which were mostly affiliated with the phylum Ascomycota. After 15 min of exposure, 98% of the sequences remained unassigned beyond the phylum level, while the abundance of Penicillium olsonii dropped below 1%. Aside from these broadly unassigned sequences, no taxon exceeded a relative abundance of 1%. The number of rare taxa (relative abundance <1%) also increased following plasma exposure, with 11 taxa identified compared to 8 in untreated control seedlings. We did not observe any significant changes in the Shannon and Chao1 diversity indices between untreated and 5 min treated seeds. As expected, after 15 min plasma treatment, values of both indexes increased due to the increased number of rare taxa (<1%) (Fig. 4 a, b).

These observations were further supported by an analysis of the total number of reads before rarefaction, which varied across conditions. A significant decrease in the number of reads was observed in treated samples, particularly after 5 minutes for batch A and after 15 minutes for batch B (see supplemental data 2 a). The observed decrease in the total number of reads in CAP treated samples, particularly noticeable after 5 min for batch A and 15 min for batch B, may indicate a reduction in microbial load due to plasma treatment. This reduction in raw read counts may result from the elimination of abundant microorganisms, especially those located on the seed surface and thus directly exposed to plasma.

In Landsberg ecotype, the analysis of fungal communities revealed minor differences between untreated and treated conditions for both batches. Communities from all the samples were predominantly composed by *Penicillium olsonii* (≥70% of sequences), excepted for the 5 min treated seeds from batch B, which were dominated by *Cladosporium cladosporioides* (>87%) (Fig. 3 b). *Acremonium sclerotigenum* was more abundant (>20%) in untreated seeds but decreased (<1%) or disappeared in the plasma-treated seeds of both batches (Fig. 3 b).

In batch A, *Penicillium olsonii* remained the predominant taxon in both untreated and treated conditions. Unlike in the Columbia ecotype, its relative abundance increased following plasma exposure, reaching over 97% of the analyzed sequences (Fig. 3 b). *Cladosporium cladosporioides* was detected at an abundance greater than 1% in 5 min treated seeds and the total proportion of rare taxa

remained below 1% in this batch. This low diversity, both in dominant and rare taxa, was reflected by very low diversity indices, with Shannon <1 and Chao1 <20 that even decreased after plasma treatments (Fig. 4 c, d).

In the more contaminated batch of Ler seeds (batch B), *Penicillium olsonii* and *Acremonium sclerotigenum* represented 70% and 27% of the sequences, respectively, followed by *Cladosporium cladosporioides* (1.3%) (Fig. 3 b). *Cladosporium cladosporioides* became dominant after 5 min of plasma treatment (87%), while *Penicillium olsonii* dropped to 8% (Fig. 3 b). Species from the genus *Trichoderma*, detected only in the Ler ecotype, increased from less than 1% to 2.5% in the 5 min treated sample. After 15 min of plasma treatment, 85% of the sequences were assigned to *Penicillium olsonii* (Fig. 3 b). The proportion of sequences unassigned beyond the phylum level increased with exposure time (<1% in control, 1.3% after 5 min, 14% after 15 min), without significantly affecting Shannon and Chao1 indices which ranged from 0.89 to 1.4, and from 16 to 38 respectively (Fig. 4 c, d). Thus, diversity remained low in all samples, with few dominant and rare taxa.

Finally, no significant differences were observed in the number of reads before rarefaction between the different conditions and batches for the Landsberg ecotype (see supplemental data 2 b).

### 3.3. Fungal beta Diversity

Beta diversity was assessed using the Bray-Curtis distance. The Principal Coordinates Analysis (PCoA) revealed a clear separation of microbial communities between the different samples. For the Col ecotype, the first two axes explained 86% of the total variance (PC1 = 72.74%, PC2 = 14.15%) (Fig. 5 a), while for the Ler ecotype, they accounted for 91% (PC1 = 80.7%, PC2 = 10.31%) (Fig.5 b).

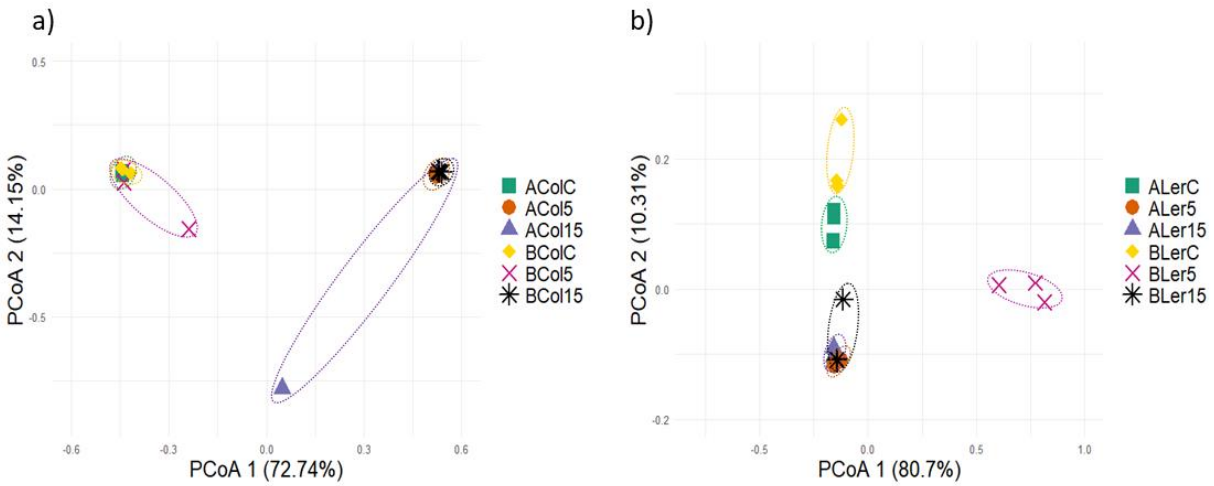



Fig. 5: Principal Coordinates Analysis (PCoA) of fungal communities associated with Columbia (Col) (a) and Landsberg (Ler) (b) seedlings, based on Bray curtis distance matrix analysis of triplicate DNA extractions. Each point represents a biological replicate, and the first two axes illustrate the explained variance in community composition.

PERMANOVA analysis confirmed a significant separation of microbial communities according to the group factor (which combines exposure time and seed batch) for both ecotypes. This factor had a major effect on microbial composition ($R^2$ = 77.5% for Columbia; $R^2$ = 91.5% for Landsberg; $p$ = 0.001 in both cases), indicating substantial differences between experimental conditions. In contrast, the batch effect (i.e., seed harvest) was not significant for Columbia ($R^2$ = 4.8%, $p$ = 0.431), but was statistically significant for Landsberg ($R^2$ = 19.5%, $p$ = 0.004), suggesting a secondary influence of seed production conditions on the initial microbiota (see supplemental data 4). This may reflect inter-batch variability in the vertical or environmental transmission of microorganisms. Pairwise PERMANOVA comparisons on group did not reach significance after multiple testing correction (adjusted $p > 0.05$), although many showed high $R^2$ values (e.g., ACol-treated5 vs ACol-untreated, $R^2$ = 0.99). This suggests strong structuring effects despite limited statistical power (see supplemental data 5 a & 6 a). Pairwise comparisons on treatment duration (time) revealed significant differences. In Col, 15 vs 0 min ($p$ = 0.003, $R^2$ = 0.94) and 5 vs 0 min ($p$ = 0.039, $R^2$ = 0.36) were both significant, but 15 vs 5 min was marginal

($p$ = 0.052). Ler showed similar results: 15 vs 0 min ($p$ = 0.012, $R^2$ = 0.70) and 5 vs 0 min ($p$ = 0.048, $R^2$ = 0.33) were significant; 15 vs 5 min was not ($p$ = 0.112) (see supplemental data 6 b).

Overall, the findings show that CAP treatment substantially shaped the β-diversity of seed-associated microbial communities, as evidenced by Bray-Curtis distances. Microbial shifts were detectable after just 5 min of exposure, with no major differences observed between 5 and 15 min. In Ler, a significant batch effect also contributed to β-diversity variation, suggesting an interaction between treatment and seed production conditions. Although some pairwise comparisons lacked statistical significance after correction, the high $R^2$ values highlighted a clear structuring effect of treatment and, to a lesser extent, batch.

### 3.4. Effects of plasma treatment on seed bacterial communities

Amplification of V4 region yielded between 95,052 and 210,968 reads, predominantly derived from plant chloroplast and mitochondrial DNA, due to their bacterial evolutionary origin. Consequently, these sequences did not represent the bacterial diversity associated with the seedlings (see supplemental data 3 a, b). After filtering and rarefaction to 9600 reads, only the untreated samples from Col seeds and batch A of Ler seeds were retained for further analysis because we were unable to detect any reads in the treated samples (see supplemental data 3 c, d). Even in these samples, taxonomic assignment was very limited, with only 9 genera and 4 families identified.

In Col seeds from batch A, the majority of bacteria belong to the *Comamonadaceae* family (55% of sequences) and to the genera *Massilia* (30%) and *Pseudomonas* (9%). Three other families (*Burkholderiaceae, Rhizobiaceae,* and *Oxalobacteraceae*) and 2 genera (*Bosea* and *Asticcacaulis*) were detected at relative abundances below 1%. Alpha diversity indices suggested moderate bacterial diversity in this sample, with Shannon values below 2 and Chao1 estimates below 30 (Fig. 6). In untreated Col seeds from batch B, the dominant bacteria belonged to the *Rhizobiaceae* family (68%) and the genera *Sphingomonas* (28%) and *Streptomyces* (1.2%) (Fig. 6 a).

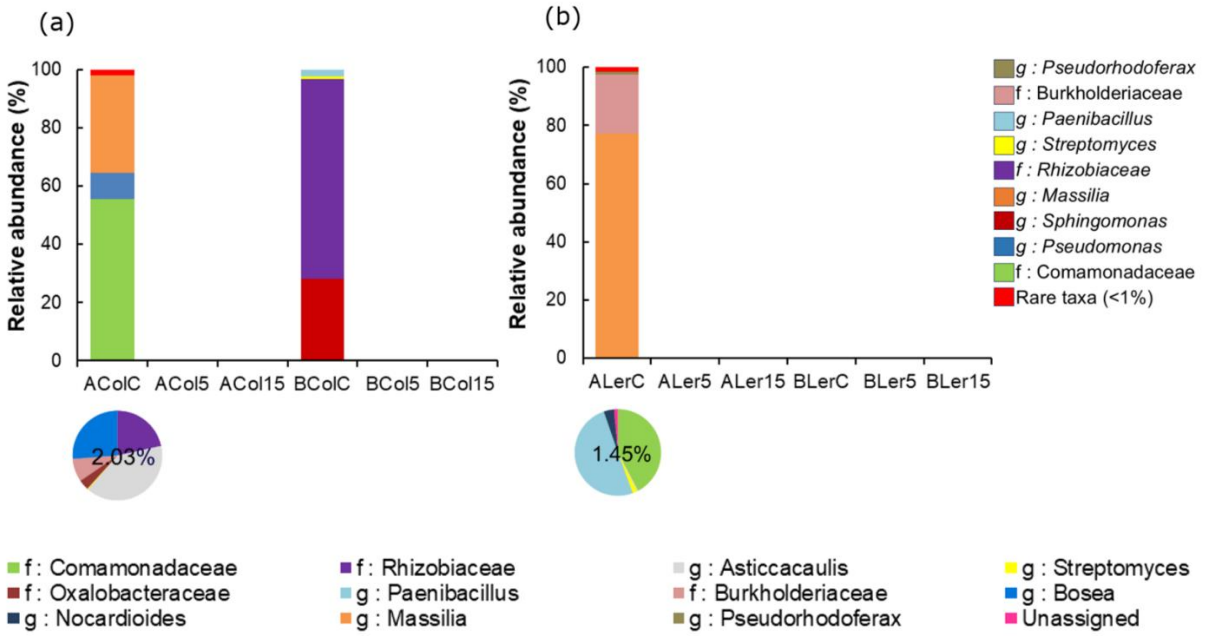



Fig. 6: Taxonomic profiles of bacterial communities associated with *Arabidopsis* seedlings of Columbia (Col) (a) and Landsberg (Ler) (b) ecotypes. Bacterial diversity associated at seedlings which provided of untreated (C) and plasma-treated for 5 or 15 min seeds from two batches (A & B). Each stacked bar represents the average relative abundance of taxa from three samples per condition. Taxa with an abundance greater than 1% are shown in the figure, while the others are grouped under "rare taxa," with details provided for each sample in the form of pie charts.

No taxa with a relative abundance below 1% were detected, which did not affect the Shannon index, which was similar to that of the untreated samples from batch A (Fig. 7 a). However, the Chao1 index was below 20, indicating a very moderate species diversity (Fig. 7 b).

In seeds from Ler ecotype, bacteria from the *Massilia* genus were the most abundant in samples from batch A, representing 77% of the taxonomically assigned sequences (Fig. 6 b). The remaining sequences were distributed as follows: 20% belonged to the *Burkholderiaceae* family and 1% to the *Pseudorhodoferax* genus. Additionally, three other genera (*Streptomyces, Paenibacillus,* and *Bosea*) and one family (*Comamonadaceae*) were detected at low relative abundances (<1%), together accounting for 1.45% of the assigned sequences (Fig. 6 b). The bacterial diversity in these samples was low, with a Shannon index below 2 and a Chao1 index below 20 (Fig. 7 a, b).

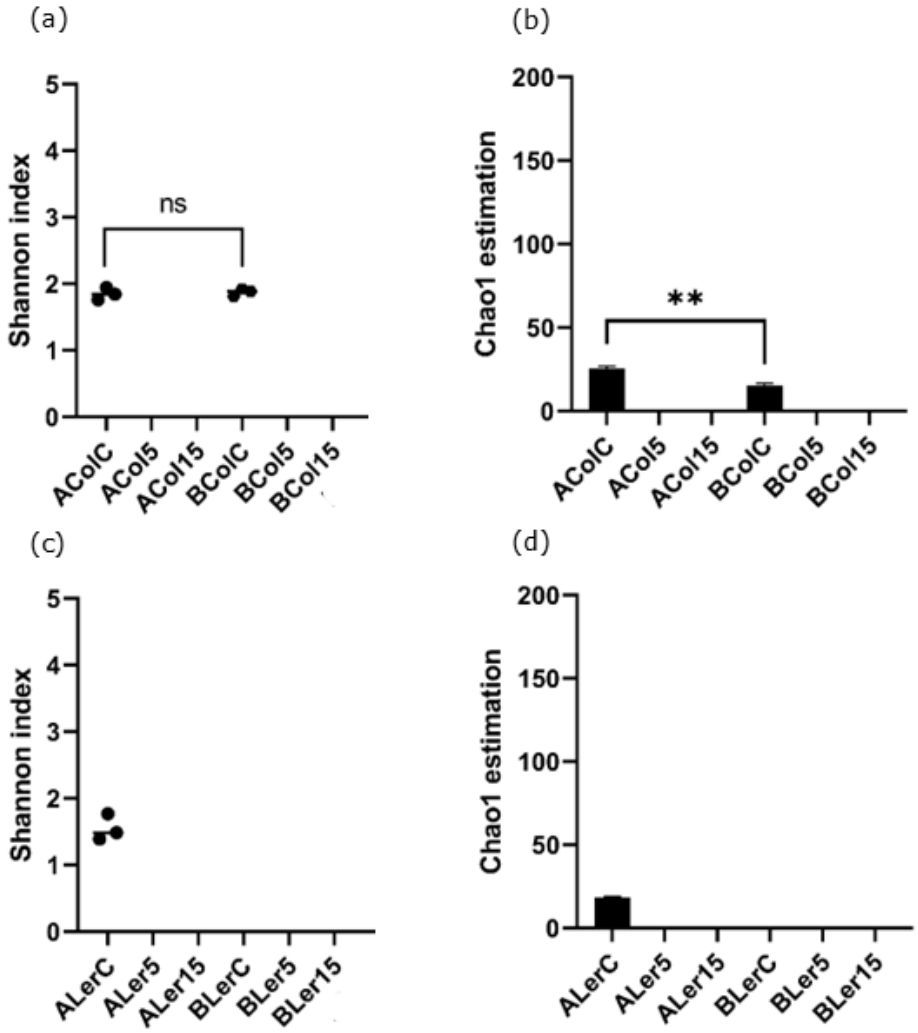



Fig. 7: Alpha diversity metrics of the bacterial microbiota (Shannon (a, c) and Chao1 (b, d)) for Columbia (Col) and Landsberg (Ler) across treatments C, 5, and 15 in lots A and B. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

For all samples, Good's coverage was calculated and consistently ranged between 0.99 and 1, indicating that the sequencing effort was sufficient to capture the vast majority of the microbial diversity present. This suggests that the probability of encountering new, unobserved taxa with additional sequencing is minimal, confirming the comprehensiveness of the sequencing depth (supplemental data 7).

**4. Discussion**

In this study, we show that short plasma treatments are highly effective in decontaminating Arabidopsis seeds, and we reveal their impact on the structure of seed-associated fungal and bacterial communities. The data obtained by assessing seed contamination levels either on MAE medium (Fig. 1) or by nephelometry (Fig. 2) demonstrate that 15 minutes of CAP treatment decreased contamination level to null for seeds of both ecotypes, regardless of their initial contamination level and thus even for the most contaminated batches. The effect of plasma treatments on seed contamination are well known and have been evidenced in a wide range of species for fungi and bacteria [21, 22]. Although not fully understood it has been proposed that this effect might mainly result from reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by the plasma which can in turn lead to various oxidative damage towards microorganisms [22].

Surprisingly, the Arabidopsis seed microbiota has been poorly studied until now, with Barret et al. [23] and Johnston-Monje et al. [24] among the few studies investigating fungal and bacterial communities in seeds of sf-2 and Col ecotypes, respectively. In their study, Johnston-Monje et al. [24] showed that the fungal microbiome of Col seeds was dominated by the phylum Ascomycota and bacteria were almost all proteobacteria. Here, as stated before, we have investigated the microbiota of 7 d old seedlings, issued from seeds that were treated or not by plasma. As shown by Barret et al. [23] the process of emergence significantly impacts the structure of microbiota, leading to a decrease in both bacterial and fungal diversity, thus we only provide a proxy of microbiota in seeds. Here *Penicillium olsonni* and *Acremonium scelortigenum* were the most represented fungi in all seedlings, independently of the ecotype and of the production (Fig. 3). In contrast the structure of bacterial community varied across ecotypes and productions. For example, in Col ecotype, bacteria from the family *Comamonadaceae* were predominant in seedlings from batch A whereas in batch B bacteria from the family *Rhizobiaceae* were the most represented, showing the strong effect of the environment (here the production cycle in the growth chamber) on the bacterial microbiota.

The effect of plasma on fungal communities greatly depended on the ecotype and the seed batch, as firstly shown by the number of reads in each sample; which decreased with plasma treatment in Col seedlings whereas it remained unaffected in Ler seedlings. The observed decrease in the total number of reads in Col plasma-treated samples, particularly noticeable after 5 min for batch A and 15 minutes for batch B, may indicate a reduction in microbial load due to plasma treatment. This reduction in raw read counts may result from the elimination of abundant microorganisms, especially those located on the seed surface and thus directly exposed to plasma. This suggests that the treatment may have led to a decrease in overall fungal abundance in the Col treated samples, but not in the Ler treated ones.

In all samples, plasma exposure resulted in the disappearance of *Acremonium sclerotigenum* DNA, while Penicillium olsonii DNA remained dominant in Ler seedlings, regardless of the treatment duration. (Fig. 3 b). However, since no fungal growth was detected on Col and Ler seeds exposed to plasma (Fig. 1), we hypothesize that the detection of *Penicillium olsonii* DNA in seedlings derived from plasma-treated seeds reflects the presence of DNA from non-viable fungi. In some cases (A Col5, A Col15, B Col15, and B Ler15; Fig. 3), we identified DNA from "ascomycetes" or even "fungi" as being dominant. These poorly affiliated sequences may originate from non-target DNA, including possible host-derived sequences from *Arabidopsis thaliana*, rather than representing true fungal taxa. In certain cases, plasma treatment was associated with the development of novel fungal communities, mostly rare species, in Col seedlings (Fig. 3 a), which was reflected by an increase in Shannon and Chao1 indexes (Fig. 4 a, b). We propose that the disappearance of the dominant and viable *Penicillium olsonii* allowed the colonization of seedling tissues by other fungi, notably endophytes present in low abundance, once *Penicillium olsonii* lost its competitive advantage. This was not the case for Ler seedlings, where diversity indices remained low before and after plasma treatment, with very few abundant or rare taxa. Only the relative abundance of the genus *Trichoderma*, found exclusively on Ler seedlings and initially below 1% in samples from batches A and B, was found at higher relative abundance in the samples exposed for 5 minutes from batch B. It's interesting to note that several endophytic fungi belonging to this genus are being studied for their potential to stimulate plant growth due to their ability to solubilize phosphorus present in the soil [25], as well as to enhance plant stress resistance, notably drought tolerance [26].

Our analysis identified exposure time as the primary driver of fungal community variation in both ecotypes. Interestingly, our findings indicate that fungal species exhibit different sensitivity, resilience and colonization potential thresholds to disturbance. Upon disturbance, *Cladosporium cladosporioides* appears more opportunistic and resistant, whereas *Acremonium sclerotigenum* systematically declines in relative abundance in exposed samples, even after just five minutes of treatment.

Bacterial community analysis provided limited insights, primarily due to the loss of most of the samples after rarefaction. The rarefaction threshold was relatively low because of the small number of remaining reads after filtering out chloroplastic and mitochondrial DNA. Arabidopsis seeds are known to harbor a core set of bacterial taxa, though the microbial community tends to be less diverse than that found in roots or leaves [27]. Common bacterial genera include *Pseudomonas*, *Sphingomonas* and *Burkholderia*, that were identified here in Col and Ler seedlings. However, bacterial diversity was inherently low in our study because no reads were detected in untreated Landsberg samples from batch B after filtration. Diversity associated in Ler was lower than the one estimated in Col seedlings as indicated by the diversity indexes (Fig.7). Unlike the fungal communities, the dominant bacterial

taxa vary in both genus and abundance between batches. *Massilia* bacteria are present on Columbia and Landsberg seedlings from batch A, but absent from Columbia seedlings in batch B. Several other taxa common in batch A seedlings are found in varying proportions, such as members of the *Comamonadaceae* and *Burkholderiaceae* families. In Columbia seedlings from batch B, bacteria from the genus *Sphingomonas* and the family *Rhizobiaceae* are dominant (Fig. 6). This variation could be due to environmental or genetic factors, both of which are known to strongly influence plant-associated microbial communities, but it is worth noting that seeds from various seed batches were produced in the same controlled growth chamber, thereby limiting the influence of the environment. No reads indicative of bacterial DNA were detected after a 5 min plasma treatment, suggesting that a short plasma exposure is sufficient to significantly reduce the number of viable bacteria. Despite a high number of reads obtained for all samples prior to filtering (see supplementary data 3), the majority of these reads were assigned to chloroplast and mitochondrial sequences, resulting in no taxonomic assignment. Furthermore, the use of the V4 marker does not allow identification beyond the genus level, which limits the relevance of the analysis, particularly for functional studies of the bacterial community associated with the seedlings. Therefore, it would be relevant to conduct a complementary study targeting the gyrB gene to overcome these limitations and achieve higher taxonomic resolution.

Numerous studies have demonstrated the antibacterial activity of CAP [13], in seeds, plasmas have been shown to control Xanthomonas campestris in cabbage [28], Ralstonia solanacearum in tomato [29], and E. coli in lentil [30], for example. The higher sensitivity of bacterial strains to plasma compared to fungi might be related to the absence of a thick layer of polysaccharides in the cell wall, which can protect cells from oxidative attacks [31]. In sunflower, a metagenomic analysis demonstrated that CAP treatment results in a reduction but not in an elimination of bacterial microbiota diversity in seedlings [17]. In Arabidopsis seeds a similar analysis also showed that CAP treatment reduced the abundance of bacteria of *Mycobacteriaceae* family, resulting in the domination of viable *Bacillaceae* in seedlings [18].

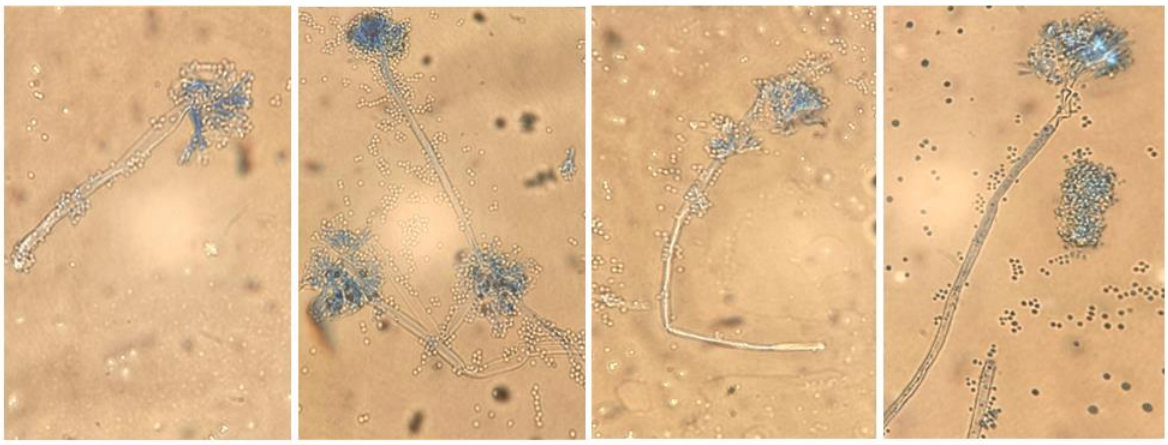
## 5. Conclusion

This work confirmed the effectiveness of a short plasma treatment in decontaminating *Arabidopsis thaliana* seeds. Therefore, in the context of reducing pesticide use for sustainable agriculture, plasmas appear as attractive and efficient methods to decontaminate seeds. Although this effect is already largely documented this study brings new findings that show that plasma can also modify the dynamics of microbiota populations, mostly fungi, during seedling emergence. The elimination of *Penicillium* from the seed surface by plasma treatment effectively freed up ecological niches that could be

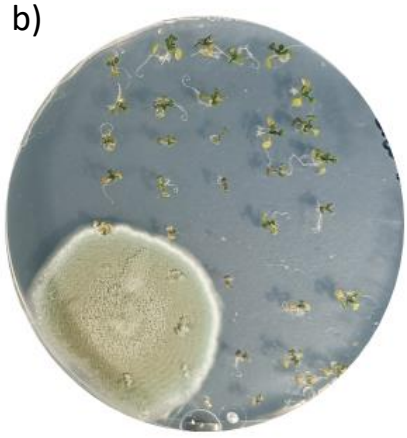
exploited by other fungal communities, particularly endophytes, and altered the contribution of other seed-borne taxa to the plant microbiome. Open questions nevertheless remain regarding the dynamics of microbial communities in seedlings following perturbation. Fungal diversity was differentially affected depending on the ecotype which questions the role of the genotype in response to plasma. Beyond pathogen control, plant-associated microorganisms can also have many beneficial effects such as to the suppression of disease development and the promotion plant growth, and research increasingly focuses on these microbial communities due to their potential applications in sustainable agriculture. Thus, appropriate plasma treatments should also be considered as useful tools to reshape microbial communities by eliminating seed epiphytes, thereby promoting the proliferation of endophytic microorganisms that could be beneficial for seedling growth. In their study on Arabidopsis seeds, Tamošiūnė et al. (2020) [18] used a short CAP treatment (3 times 1.5 min) that allowed bacterial microbiota to persist in developing seedlings. Thus, a deeper understanding of the impact of plasma treatments on microbial interactions is essential to design protocols that preserve beneficial microorganisms while effectively reducing surface pathogens. Future studies should explore the underlying mechanisms of microbial resistance to plasma exposure, especially in taxa that persist despite treatment, and assess the long-term consequences of plasma application on seedling development and microbiome recovery. It is also important to identify which physical or chemical components are involved in the decontamination observed in seeds. This knowledge will help refine biotechnological approaches aimed at improving plant health and agricultural resilience.

**6. Supplemental data:**

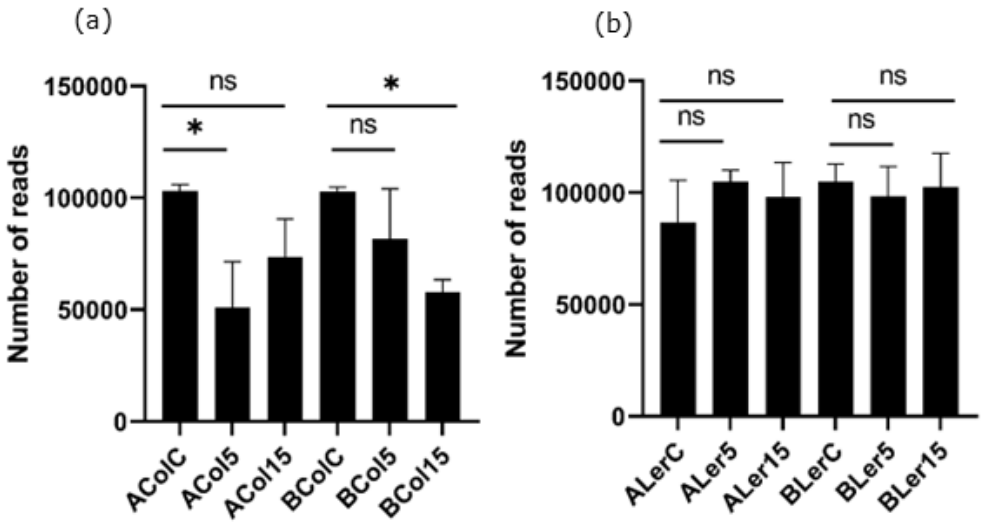
a)



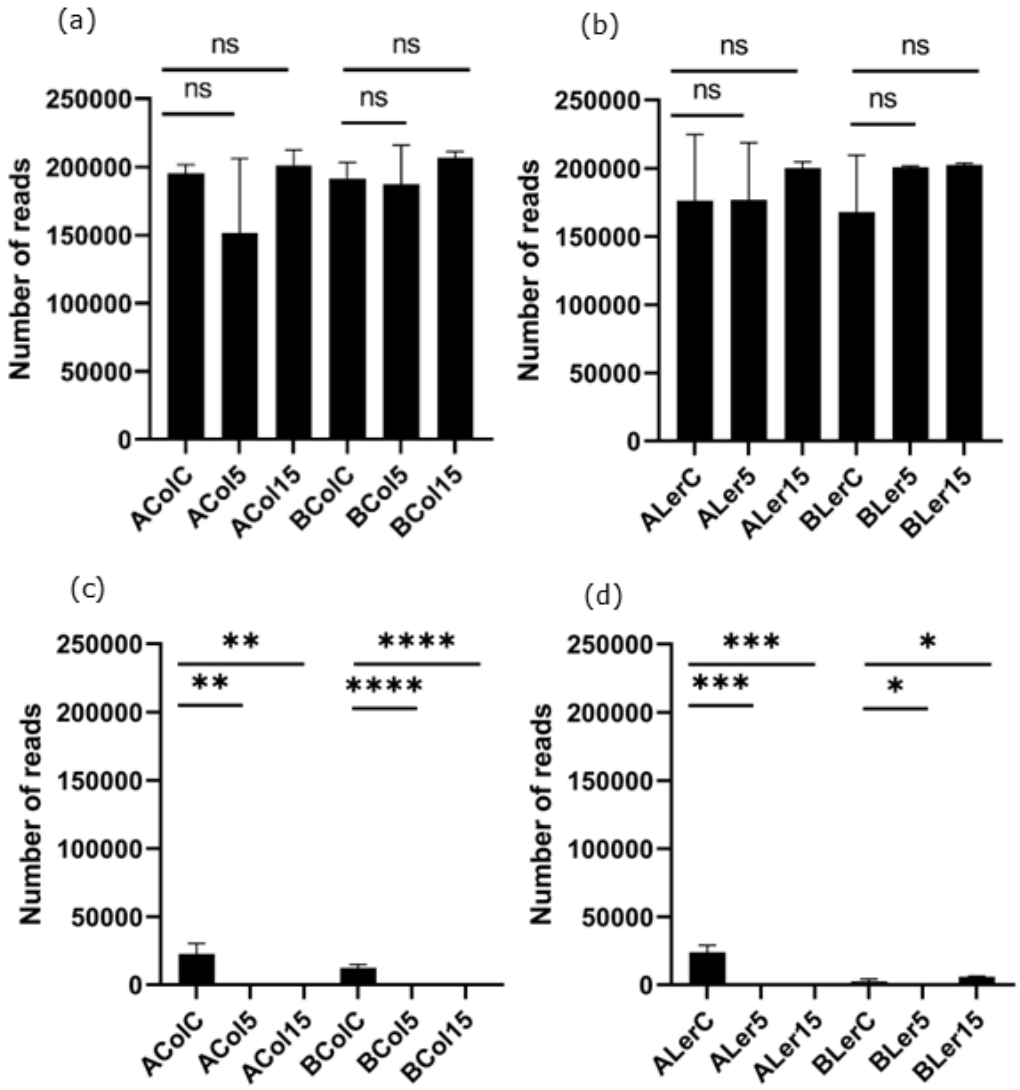
b)



Supplemental data 1 a: Microscopic observations (×64) of *Penicillium* structures isolated from *Arabidopsis* seeds after lactophenol blue staining, showing characteristic brush-like spore arrangements. b: Colony morphology of *Penicillium* on MS/2 Medium.




Supplementary data 2: ITS1 region read counts for Columbia (a) and Landsberg (b). Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).




Supplementary data 3: V4 region read counts for Columbia (a, c) and Landsberg (b, d), shown before (a, b) and after (c, d) filtering of chloroplast and mitochondrial DNA. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

| **Permanova** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ecotype** | **Facteur** | **Df** | **Sum of Squares** | **$R^2$ (%)** | **F-value** | **p-value** | **Significativity** |
| Columbia | Group | 5 | 3.7942 | 77.5 | 7.5741 | 0.001 | *** |
| Columbia | Batch | 1 | 0.2331 | 4.8 | 0.7497 | 0.431 | ns |
| Landsberg | Group | 5 | 2.20493 | 91.5 | 25.706 | 0.001 | *** |
| Landsberg | Batch | 1 | 0.47041 | 19.5 | 3.879 | 0.004 | ** |

Supplementary data 4: PERMANOVA results for group and batch factors in Columbia and Landsberg.

a)

| Group | Pairs | | Df | SumsOfSqs | F.Model | R2 | p.value | p.adjusted |
|---|---|---|---|---|---|---|---|---|
| 1 | ACol-treated15 vs | ACol-treated5 | 1 | 0.006162740 | 0.5123736 | 0.1458767 | 1.0 | 1.0000000 |
| 2 | ACol-treated15 vs | ACol-untreated | 1 | 0.370163822 | 39.5051072 | 0.9080568 | 0.1 | 0.1363636 |
| 3 | ACol-treated15 vs | BCol-treated15 | 1 | 0.013888544 | 1.5660550 | 0.2813582 | 0.1 | 0.1363636 |
| 4 | ACol-treated15 vs | BCol-treated5 | 1 | 0.272749075 | 16.9198347 | 0.8087939 | 0.1 | 0.1363636 |
| 5 | ACol-treated15 vs | BCol-untreated | 1 | 0.337688334 | 34.8861001 | 0.8971355 | 0.1 | 0.1363636 |
| 6 | ACol-treated5 vs | ACol-untreated | 1 | 0.387952957 | 328.1214557 | 0.9909399 | 0.1 | 0.1363636 |
| 7 | ACol-treated5 vs | BCol-treated15 | 1 | 0.002295132 | 4.4684221 | 0.5983087 | 0.1 | 0.1363636 |
| 8 | ACol-treated5 vs | BCol-treated5 | 1 | 0.294388327 | 28.9114344 | 0.9059898 | 0.1 | 0.1363636 |
| 9 | ACol-treated5 vs | BCol-untreated | 1 | 0.356102119 | 223.2198263 | 0.9867386 | 0.1 | 0.1363636 |
| 10 | ACol-untreated vs | BCol-treated15 | 1 | 0.551245430 | 750.6267627 | 0.9946994 | 0.1 | 0.1363636 |
| 11 | ACol-untreated vs | BCol-treated5 | 1 | 0.009148723 | 1.1455997 | 0.2226368 | 0.3 | 0.3461538 |
| 12 | ACol-untreated vs | BCol-untreated | 1 | 0.002139801 | 1.3844217 | 0.2571161 | 0.2 | 0.2500000 |
| 13 | BCol-treated15 vs | BCol-treated5 | 1 | 0.422011810 | 56.3852600 | 0.9337587 | 0.1 | 0.1363636 |
| 14 | BCol-treated15 vs | BCol-untreated | 1 | 0.506871213 | 485.4649180 | 0.9918278 | 0.1 | 0.1363636 |
| 15 | BCol-treated5 vs | BCol-untreated | 1 | 0.008338685 | 1.0051836 | 0.2008285 | 0.5 | 0.5357143 |

b)

| Time | Pairs | Df | SumsOfSqs | F.Model | R2 | p.value | p.adjusted | sig |
|---|---|---|---|---|---|---|---|---|
| 1 | 15 vs 5 | 1 | 0.2159165 | 5.191715 | 0.3658272 | 0.052 | 0.052 | . |
| 2 | 15 vs 0 | 1 | 0.8749702 | 151.681189 | 0.9381499 | 0.001 | 0.003 | ** |
| 3 | 5 vs 0 | 1 | 0.1861974 | 5.028466 | 0.3584473 | 0.026 | 0.039 | * |

Supplementary data 5: Pairwise PERMANOVA results for group a) and time b) factors in Columbia.

a)

| **Group** | Pairs | | Df | SumsOfSqs | F.Model | R2 | p.value | p.adjusted |
|---|---|---|---|---|---|---|---|---|
| 1 | ALer-treated15 vs | ALer-treated5 | 1 | 0.0006823338 | 1.203847 | 0.2313380 | 0.5 | 0.5000000 |
| 2 | ALer-treated15 vs | ALer-untreated | 1 | 0.0849479441 | 110.727552 | 0.9651348 | 0.1 | 0.1071429 |
| 3 | ALer-treated15 vs | BLer-treated15 | 1 | 0.0160558036 | 2.868672 | 0.4176458 | 0.1 | 0.1071429 |
| 4 | ALer-treated15 vs | BLer-treated5 | 1 | 0.6973550840 | 206.366012 | 0.9809855 | 0.1 | 0.1071429 |
| 5 | ALer-treated15 vs | BLer-untreated | 1 | 0.1167406590 | 45.995336 | 0.9199925 | 0.1 | 0.1071429 |
| 6 | ALer-treated5 vs | ALer-untreated | 1 | 0.0877770089 | 97.036564 | 0.9604104 | 0.1 | 0.1071429 |
| 7 | ALer-treated5 vs | BLer-treated15 | 1 | 0.0135712315 | 2.366658 | 0.3717269 | 0.1 | 0.1071429 |
| 8 | ALer-treated5 vs | BLer-treated5 | 1 | 0.6788136053 | 193.030587 | 0.9796986 | 0.1 | 0.1071429 |
| 9 | ALer-treated5 vs | BLer-untreated | 1 | 0.1184681461 | 44.278971 | 0.9171482 | 0.1 | 0.1071429 |
| 10 | ALer-untreated vs | BLer-treated15 | 1 | 0.0582138058 | 9.809008 | 0.7103340 | 0.1 | 0.1071429 |
| 11 | ALer-untreated vs | BLer-treated5 | 1 | 0.6624979056 | 178.234704 | 0.9780503 | 0.1 | 0.1071429 |
| 12 | ALer-untreated vs | BLer-untreated | 1 | 0.0136732853 | 4.754470 | 0.5430905 | 0.1 | 0.1071429 |
| 13 | BLer-treated15 vs | BLer-treated5 | 1 | 0.5667957709 | 66.317001 | 0.9431148 | 0.1 | 0.1071429 |
| 14 | BLer-treated15 vs | BLer-untreated | 1 | 0.0716811071 | 9.302412 | 0.6993027 | 0.1 | 0.1071429 |
| 15 | BLer-treated5 vs | BLer-untreated | 1 | 0.5383441707 | 98.096299 | 0.9608213 | 0.1 | 0.1071429 |

b)

| **Time** | Pairs | Df | SumsOfSqs | F.Model | R2 | p.value | p.adjusted | sig |
|---|---|---|---|---|---|---|---|---|
| 1 | 15 vs 5 | 1 | 0.2917675 | 3.989581 | 0.2851823 | 0.112 | 0.112 | ns |
| 2 | 15 vs 0 | 1 | 0.1509272 | 23.723086 | 0.7034672 | 0.004 | 0.012 | * |
| 3 | 5 vs 0 | 1 | 0.3573002 | 4.975931 | 0.3322619 | 0.032 | 0.048 | * |

Supplementary data 6: Pairwise PERMANOVA results for group a) and time b) factors in Landsberg.

a)

| Alpha diversity ITS | Shannon | Pielou | Chao1 | Observed features | Goods coverage |
|---|---|---|---|---|---|
| AColC | 0,68521836 | 0,16577108 | 18,3809524 | 18,3333333 | 0,99998053 |
| ACol5 | 2,53814939 | 0,36365919 | 117,166667 | 117 | 0,99996106 |
| ACol15 | 2,38571795 | 0,34703811 | 104,733333 | 104,333333 | 0,99995457 |
| BColC | 1,40402032 | 0,28718427 | 29 | 29 | 1 |
| BCol5 | 1,05548656 | 0,19452278 | 39,4166667 | 39,3333333 | 0,99998053 |
| BCol15 | 3,11323685 | 0,43121275 | 150,833333 | 150,333333 | 0,99997404 |
| ALerC | 0,99296607 | 0,23988716 | 17,6666667 | 17,6666667 | 0,99999351 |
| ALer5 | 0,25434314 | 0,06963088 | 12 | 12 | 0,99998702 |
| ALer15 | 0,29343045 | 0,09114556 | 10,6666667 | 10,6666667 | 0,99999351 |
| BLerC | 1,43811234 | 0,28656182 | 32,3333333 | 32,3333333 | 0,99999351 |
| BLer5 | 0,89870775 | 0,23608469 | 16 | 16 | 0,99998702 |
| BLer15 | 0,98531905 | 0,18454257 | 38,6666667 | 38,6666667 | 0,99999351 |

b)

| Alpha diversity V4 | Shannon | Pielou | Chao1 | Observed features | Goods coverage |
|---|---|---|---|---|---|
| AV4ColC | 1,84961586 | 0,39541987 | 25,6666667 | 25,6666667 | 0,9999653 |
| BV4ColC | 1,87230908 | 0,47784813 | 15,3333333 | 15,3333333 | 1 |
| AV4LerC | 1,54695337 | 0,36844473 | 18,3333333 | 18,3333333 | 1 |

Supplementary data 7: Alpha diversity index for Columbia and Landsberg based on ITS a) and V4 b) regions.

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# Chapter 4: Effects of individual properties of ambient air cold plasma on the decontamination of *Arabidopsis thaliana* seeds

# Effects of individual properties of ambient air cold plasma on the decontamination of *Arabidopsis thaliana* seeds

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**Key words: Cold atmospheric plasma, seeds, RONS, decontamination, characterization, electric field**

## 1. Introduction

Atmospheric cold plasmas (CAPs) are attracting increasing interest due to their physical and chemical properties, particularly in the fields of medicine and agriculture. These partially ionized gases, generated at atmospheric pressure, produce a complex mixture of reactive oxygen and nitrogen species (RONS), UV and visible radiation, as well as charged particles. This physico-chemical composition allows the inactivation of a wide range of microorganisms, including bacteria, fungi, and viruses [1], without causing thermal damage to sensitive surfaces such as biological tissues.

In the medical field, CAPs are primarily used for surface disinfection [2], wound treatment, and biofilm elimination [3]. They have been shown to effectively reduce bacterial load, including antibiotic-resistant strains, notably in *Escherichia coli* [4]. Their effectiveness has also been reported in wound healing [5], as well as in the sterilization of surgical instruments [6]. Moreover, CAPs have been shown to promote tissue regeneration and accelerate healing [7], opening promising prospects for the treatment of chronic wounds and various skin conditions. More recently, their use has extended to aesthetic medicine, particularly for skin rejuvenation, characterized by the stimulation of skin elasticity and thickness [8].

Finally, preclinical studies have shown that CAPs also exhibit inhibitory effects on the growth and migration of certain tumor cells [9]. These effects have been reported in studies focused on skin, breast, and lung cancers, for example [10].

In agriculture, in the context of climate change and the search for sustainable solutions to maintain seed vigor and crop yields, cold plasmas also emerge as an innovative technology thanks to their physico-chemical properties and non-thermal effects. Two main approaches are being explored: direct exposure and indirect exposure.

Indirect exposure involves the use of plasma-activated water (PAW), either for watering plants or as a priming solution for seeds. These two methods can also be used simultaneously. Kamseu-Mogo et al. showed synergistic effects of these two applications on maize growth and germination [11]. These biological effects are mainly explained by the presence of nitrates ($NO_3^-$) and nitrites ($NO_2^-$) in the PAW, which enhance nitrogen availability an essential nutrient for plant development [12], thus offering an alternative to fertilizers.

In direct exposure, CAPs are applied to seeds to stimulate germination [13], improve seedling vigor, especially under water stress conditions [14], and ensure decontamination [15]. Seeds naturally harbor microbial communities that may have beneficial, neutral, or harmful effects depending on their composition and environmental conditions [16]. Additionally, certain microbial species present on

seeds before harvest may become opportunistic after harvest, during storage periods, negatively affecting seed quality [17]. These microorganisms can proliferate, cause contamination, reduce germination rates, and promote pathogen transmission to seedlings. Seeds are thus a major vector in the transmission of plant diseases.

In this context, as reducing the use of chemical pesticides becomes increasingly necessary, cold plasma treatment appears as an interesting alternative to conventional agricultural methods.

In this chapter, we examine the specific contributions of the various components of cold plasma (electrical, thermal, radiative, and chemical) in the seed decontamination process of *Arabidopsis thaliana*. Understanding the relative influence of each of these factors is essential to optimize treatment protocols, ensuring microbial inactivation without affecting seed viability.

In Chapter 3, we demonstrate that a 5 min plasma treatment enables complete seed decontamination. However, in most studies, this decontamination is often attributed, albeit with some uncertainty, to reactive oxygen and nitrogen species (RONS) and UV radiation [18, 1], while the contribution of other parameters, such as electric fields or temperature, is rarely considered or measured.

We therefore aimed to separate the different components of cold plasma in order to better understand the specific role of each in the decontamination process. To address this question, we combined several diagnostic tools: electrical measurements, optical emission spectroscopy (OES), infrared thermal imaging, and mass spectrometry (MS), to systematically analyze the mechanisms involved. The approach begins with a thorough characterization of the electrical regime and then extends to evaluating thermal and radiative effects. Two seed batches of the *Arabidopsis thaliana* Col-0 genotype, with different initial contamination levels, were used: batch A, with approximately 60% contaminated seeds, and batch B, with a contamination rate between 90 and 100%.

## 2. Material and methods

### 2.1. Experimental plasma setup

A dielectric barrier device (DBD) in a plane-to-plane configuration with a gap of 2.5 mm was used in this study. The device was composed of a 2 mm thick dielectric barrier and two electrodes: one electrode biased to high voltage (stainless-steel mesh) and a grounded counter-electrode (bulk alumina plate). The area of the reactor was 30 × 40 $cm^2$. The mesh electrode was powered with a high-voltage generator composed of a function generator (ELC Annecy France, GF467AF) and a power amplifier (Crest Audio, 5500 W, CC5500).

### 2.2. Current and voltage

Voltage and current of the DBD were measured using a digital oscilloscope (WaveSurfer 3054 from Teledyne LeCroy), with a 500 MHz bandwidth and a 4 GS/s sampling rate. The voltage applied to the mesh electrode was measured using a high-voltage probe (Tektronix P6015A 1000:1 and Teledyne LeCroy PPE 20 kV 1000:1). The discharge current was monitored using a current transformer (Pearson, model 2877) placed between the counter-electrode and the ground. A measurement capacitor (80 nF) was inserted between the counter-electrode and the ground to plot Lissajous curves. The area enclosed by the Lissajous curves was calculated to determine the energy dissipated in the DBD. Multiplying this value by the operating frequency provided the electrical power injected into the DBD.

### 2.3. Thermographic infrared imaging

A thermographic camera (Infrared camera Jenoptik VarioCAM HD Head 900, InfraTec) was used to assess the temperature of the material surface in contact with the plasma, particularly the counter-electrode and the dielectric barrier. Temperature monitoring was carried out in real-time while the plasma operated for 30 min.

### 2.4. Optical emission spectroscopy (OES)

Optical emission spectroscopy was carried out to identify the radiative species emitted by the plasma. The spectrometer (Andor, SR-750-B1-R) was used in the Czerny–Turner configuration, with a focal length of 750 mm and a spectral range of 200–800 nm. The system included an optical fiber (Leoni

Fiber Optics SR-OPT-8014, 100 µm diameter), a diffraction grating (1200 grooves/$mm^{-1}$) blazed at 500 nm, and an ICCD camera (Andor iStar, 2048 × 512 imaging array with 13.5 µm × 13.5 µm pixels).

### 2.5. Mass spectrometry (MS)

Mass spectrometry was performed to analyze the gas phase and identify the relevant species generated by the plasma. The mass spectrometer used in this study was a quadrupole-based instrument (Model HPR-20 from Hiden Analytical Ltd). Air plasma chemical species were collected via a quartz capillary whose inlet is fixed onto a 2-axis stages plate placed in the middle-left of the plasma reactor. The 1 m long capillary is flexible, chemically inert, and heated to 200 °C to prevent chemisorption. A three-stage, differentially pumped inlet system separated by aligned skimmer cones and turbomolecular pump, enabled a pressure gradient from $10^5$ bar to $10^{-7}$ bar at the entrance of the 70 eV ionization chamber. The species detected by mass spectrometry were quantified by measuring their intensities in the ionized gas (plasma on) and subtracting the corresponding background intensities when the gas is non-ionized (plasma off). In both cases, measurements were performed in steady state, obtained after pressure stabilization following the ignition of the filament.

### 2.6. Plant material

This study was conducted using seeds of *Arabidopsis thaliana* ecotype Columbia-0 (Col-0). The plants were grown in a climate-controlled chamber at 20–22 °C under a long-day photoperiod (16 h light / 8 h dark). Seeds were harvested from mature siliques after two weeks of drying at room temperature and subsequently stored under the same conditions. In this study, two seed batches (A and B) obtained from two independent production cycles were used. Treatments of 5 and 15 min were performed, and seeds from both batches were distributed in duplicate Petri dishes containing 40 to 45 seeds each on malt agar medium. The plates were then incubated at 21 °C with a photoperiod of 16 h light / 8 h dark. Photographs of the plates were taken on days 1 and 6, and contamination rates were assessed on day 6, as illustrated in Fig. 1.

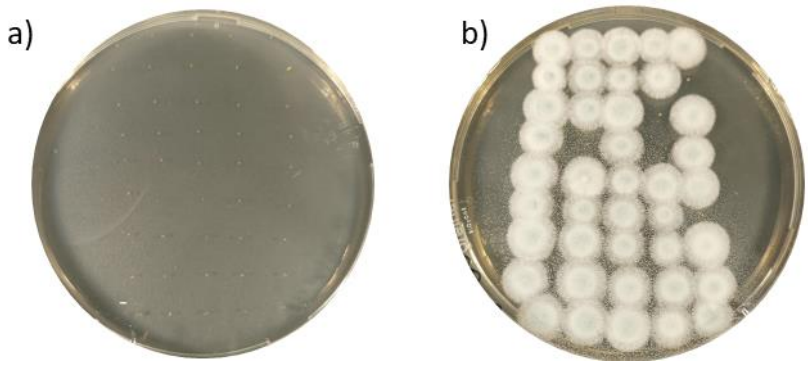


Fig. 1: Evolution of contamination on group B Arabidopsis control seeds from day 1 (a) to day 6 (b) on MEA.

## 3. Results/discussion

### 3.1. Influence of CAP current and voltage on seeds decontamination

The time-resolved voltage signals applied to the DBD are represented in Fig. 2 a for generator amplitudes ranging from 1 kV to 7 kV, at a frequency of 500 Hz. All voltage signals exhibit a clean sinusoidal shape, with no visible distortion or collapse, even at the highest amplitudes tested. This indicates strong signal stability and linearity of the high-voltage source throughout the operating range. The corresponding electric field, shown on the right axis and calculated as E=V/d with a fixed inter-electrode gap of 2.5 mm, increases proportionally with the applied voltage, reaching peak values up to 28 kV/cm at 7 kV. This linear scaling confirms that the system remains predominantly capacitive, ie no cold plasma is generated within the interelectrode gap because the supplied voltage is below the breakdown threshold.

The associated current waveforms measured across the DBD are represented in Fig. 2 b under the same voltage conditions. For all tested amplitudes, the current signals remain cleanly sinusoidal, with no significant distortion or high-frequency components. This behavior is consistent with a dielectric system exhibiting a purely displacement current response I=C.dV/dt, where C is the equivalent capacitance of the DBD assembly. The absence of visible current spikes or conductive bursts confirms that no plasma filaments or microdischarges are present. Occasional minor transients may appear but are sporadic and not correlated with the applied voltage phase, suggesting they are more likely due to measurement noise, external perturbations, or rare ionizing events from cosmic radiation.

Both voltage and current signals validate that the DBD remains in a stable, fully capacitive regime across the full voltage range explored here.

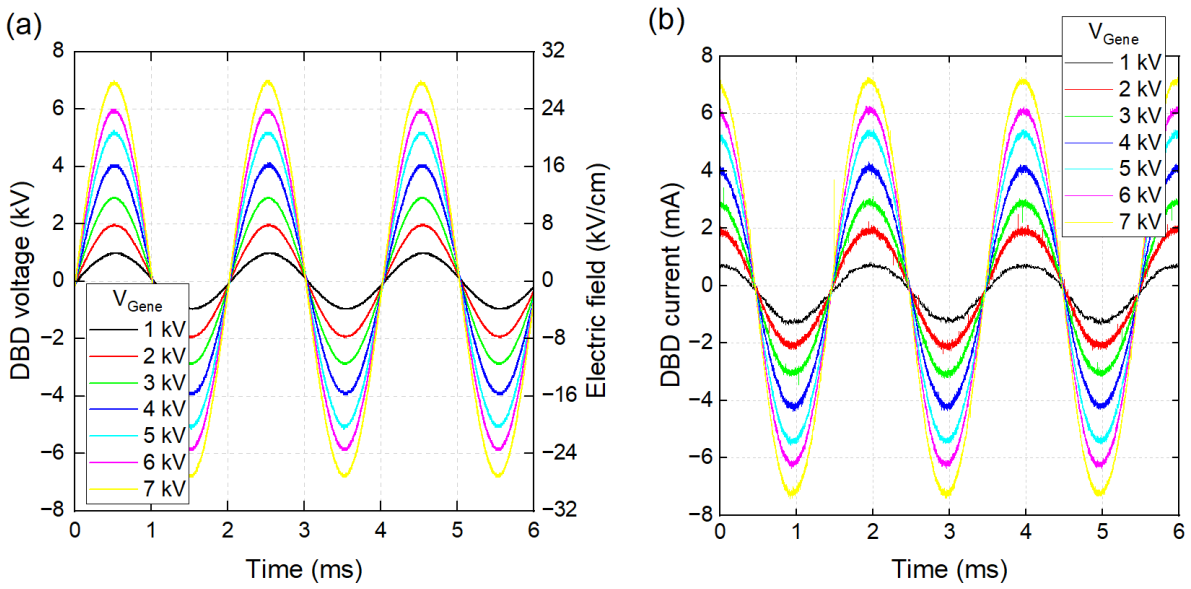


Fig. 2: (a) Time-resolved voltage of the dielectric barrier device ($V_{DBD}$) for generator voltages ranging from 1 to 7 kV at 500 Hz. Geometrical electric field is indicated on right-axis. (b) Measured current waveforms across the DBD for generator voltages ranging from 1 to 7 kV at 500 Hz. In both figures, all signals exhibit smooth sinusoidal shapes without spikes or distortion, confirming a displacement current typical of a purely capacitive regime without any formation of cold plasma.

The time-resolved measurements of the plasma voltage (black curve, left axis) and plasma current (red curve, right axis) are reported in Fig. 3 for the DBD supplied at a voltage as high as 8 kV in magnitude at 500 Hz. The applied voltage exhibits a clear sinusoidal waveform, reflecting the stable and linear response of the high-voltage power supply under these operating conditions. The current waveform presents a dual-component structure: a smooth sinusoidal baseline associated with the displacement current and a series of sharp, transient peaks that appear periodically around the voltage extrema.

The sinusoidal background corresponds to the capacitive component of the current, governed by the classical relation $I=C.dV/dt$ , where C is the effective capacitance of the dielectric barrier device. Superimposed on this baseline are narrow, high-amplitude current peaks, attributed to microdischarges that are stochastically triggered when the local electric field exceeds the breakdown threshold of air. These microdischarges are confined to brief time windows of a few microseconds (about 5 µs) and exhibit peak amplitudes surpassing ±20 mA. Their recurrent emergence near the voltage maxima and minima is indicative of a filamentary discharge regime, in which localized streamer discharges are periodically initiated and quenched within each half-cycle of the applied field.

A noticeable phase shift between the voltage and the sinusoidal current baseline is observed, characteristic of capacitive devices. The phase angle $\phi$ can be quantitatively estimated using the relation $\phi=\Delta t/T\times360°$ where $\Delta t$ represents the temporal delay between corresponding features (e.g.,

zero-crossings or peaks) of the voltage and current waveforms and T is the signal period. Here, a time shift of 0.49 ms over a 2 ms period yields a phase angle close to 90°, confirming that the capacitive behavior remains dominant.

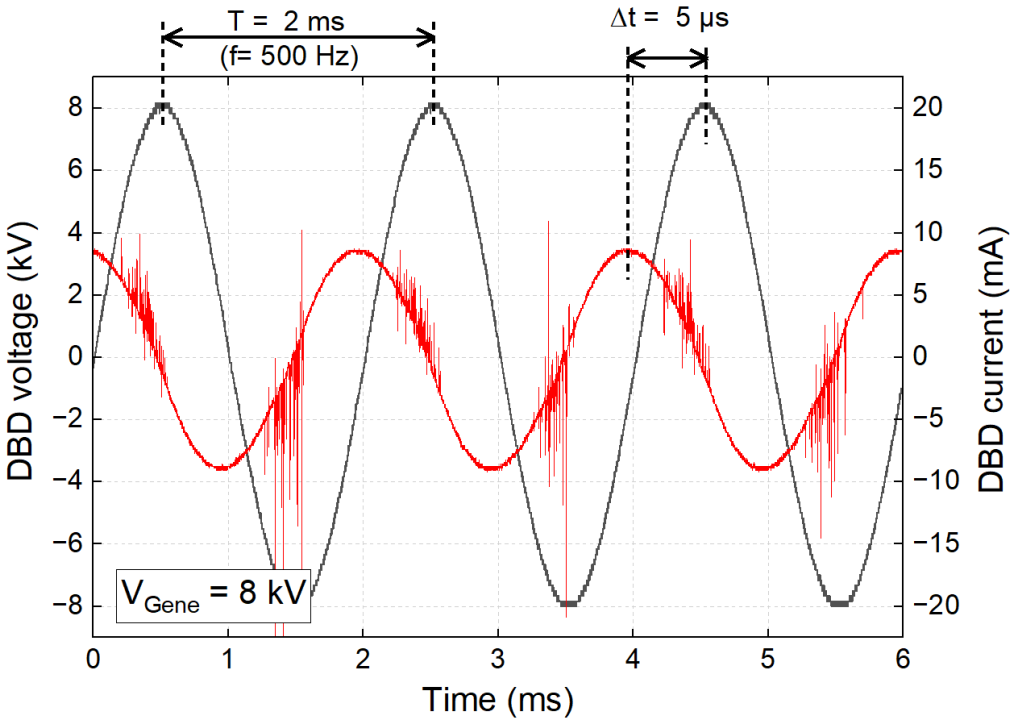


Fig. 3: Time-resolved voltage (black, left axis) and current (red, right axis) waveforms across the dielectric barrier device (DBD) operated at voltage generator $V_{Gene}$=8 kV at 500 Hz.

To further characterize the electrical behavior of the discharge, voltage-current (V-I) plots over a full AC cycle are examined. These Lissajous figures provide a complementary perspective, enabling clear identification of capacitive versus conductive regimes based on the shape and area enclosed by the V-I contours. Fig. 4 a presents the Lissajous plots measured over several full AC cycles for increasing DBD voltage amplitudes ranging from 1 kV to 8 kV. Each colored contour corresponds to a full period of sinusoidal excitation at a given voltage amplitude, reflecting the relationship between the applied voltage and the resulting current. For voltages from 1 to 7 kV, the curves exhibit clean elliptical shapes centered around the origin, which are characteristic of a linear, purely capacitive regime. As already mentioned, this regime is characterized by the fact that the current leads the voltage by approximately 90°, with minimal hysteresis and negligible conduction current, confirming that the system remains dominated by displacement current.

As the applied voltage increases, the area enclosed by each ellipse also increases, indicating a larger exchange of reactive power (Q) per cycle. This area is directly proportional to the reactive power $Q=1/T.\oint V(t).dI(t)$, a signature of energy storage and return in the dielectric barrier. While the overall capacitive behavior persists up to 7 kV, subtle distortions begin to emerge in the outermost contours, particularly at 6 and 7 kV, hinting at the onset of weak nonlinearity.

At 8 kV, the V-I contour visibly departs from ideal ellipticity and exhibits irregularities and superimposed features, such as sharp corners and kinks, indicative of microdischarge activity. These features signal the transition from a purely reactive to a mixed reactive/conductive regime. In this filamentary discharge regime, transient plasma channels contribute a resistive component to the current, causing deviations from the ideal capacitive loop. Thus, while the device is entirely non-dissipative and capacitive from 1 to 7 kV, the behavior at 8 kV clearly marks the emergence of conduction through microdischarges and the breakdown of the ideal dielectric response.

Fig. 4 b shows the corresponding evolution of the reactive power Q, plotted as a function of the DBD voltage amplitude. This quantity was derived from the area enclosed by each V-I loop in Fig. 4 a, following the standard method used in dielectric discharge diagnostics. As expected for a capacitive system under sinusoidal excitation, the reactive power increases nonlinearly with the applied voltage. The growth follows the relation $Q \propto V_{RMS}^2 \times f \times C$, where f is the frequency and C the effective capacitance of the DBD. Given that both frequency and capacitance remain constant, the observed increase in Q primarily reflects the quadratic dependence on the RMS voltage.

Quantitatively, the reactive power rises from approximately 20 VAR at 1 kV to over 550 VAR at 8 kV, confirming that a significant amount of energy is cyclically stored and returned to the generator without being dissipated. This behavior is characteristic of a system dominated by displacement current, where the phase angle ϕ remains close to 90° and the reactive power can also be expressed as $Q = V_{RMS} \times I_{RMS} \times sin\phi$. The smooth, monotonic increase up to 7 kV corresponds to a purely capacitive regime with minimal hysteresis. At 8 kV, however, the sharper rise in Q, in combination with distortions in the V-I loops, suggests that microdischarges begin to emerge. These introduce transient conduction channels that slightly perturb the reactive nature of the device, leading to a partial departure from ideal capacitive behavior. Nevertheless, the reactive contribution remains dominant in the power balance, underlying the efficient non-dissipative energy exchange which is inherent to atmospheric-pressure DBDs.

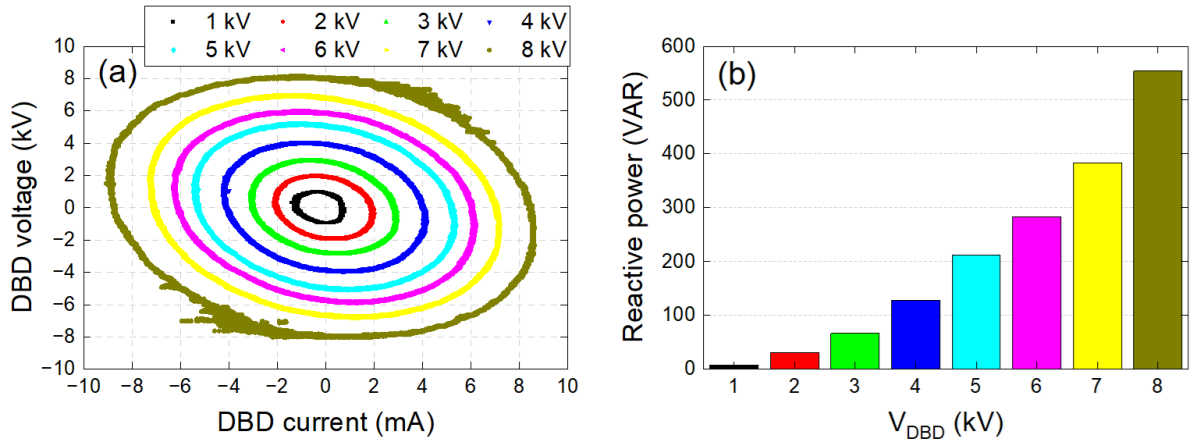


Fig. 4: (a) Lissajous plots (voltage-current characteristics) of the DBD over 10 full AC cycles for generator voltages ranging from 1 kV to 8 kV. At 8 kV, the loop deviates from ideal ellipticity and shows sharp features due to microdischarges, indicating the onset of a filamentary conductive regime. (b) Corresponding reactive power calculated from the area enclosed by each V-I loop in (a).

To complement the reactive power analysis and further investigate the onset of dissipative phenomena, we now examine the time-resolved behavior of instantaneous power. Fig. 5 a displays the time evolution of the instantaneous dissipated power in the DBD, calculated as p(t)=v(t).i(t), for voltages ranging from 1 kV to 8 kV. For voltages lower than 8 kV in amplitude, the power curves are smooth, symmetric and sinusoidal: a behavior which is typical of a purely reactive regime dominated by displacement currents. But at 8 kV, the power waveform clearly deviates from this ideal behavior where sharp and unidirectional peaks emerge; current peaks that are associated with the onset of filamentary microdischarges. The dissipated power reaches peak values exceeding 150 W during microdischarge bursts, although these events remain confined to microsecond timescales.

By subtracting the purely capacitive baseline from the total instantaneous power, it is possible to isolate the contribution arising from plasma conduction, as depicted in Fig. 5 b at 8 kV. The resulting waveform consists only of the sharp and narrow current peaks, ie the ignition of filamentary microdischarges, triggered when the electric field exceeds the breakdown threshold in atmospheric air. Each peak is highly localized in time, lasting only a few microseconds and reaches instantaneous power levels up to 180 W. Between these events, the power returns close to zero, confirming that conduction is absent outside the discharge windows and that the system otherwise behaves capacitively. These power bursts reflect real, resistive energy transfer associated with Joule heating, ion acceleration and electron-neutral collisions within the transient plasma filaments.

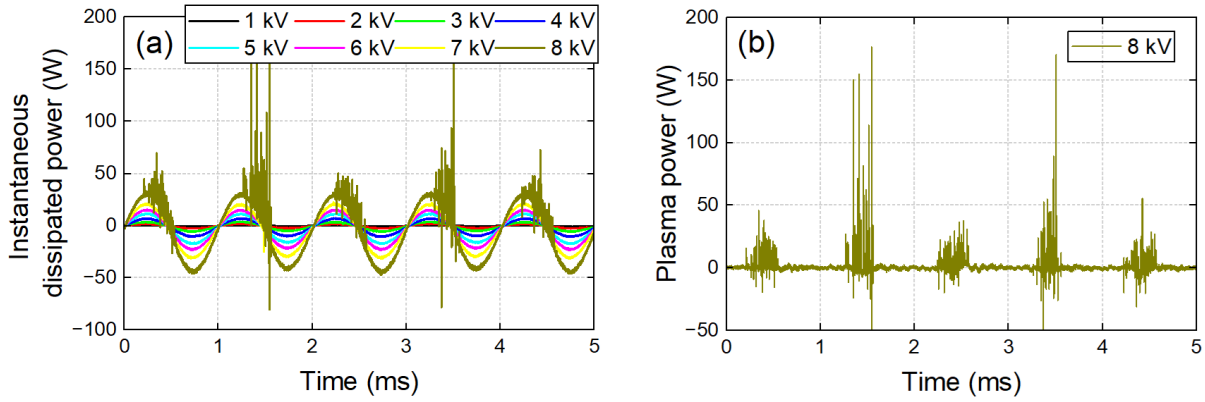


Fig. 5: (a) Instantaneous dissipated power p(t)=v(t)·i(t) as a function of time for applied voltages from 1 kV to 8 kV. From 1 to 7 kV, the signal remains sinusoidal and centered around zero, indicating a purely reactive regime with negligible real power loss. At 8 kV, however, sharp unipolar peaks indicate localized energy dissipation via transient microdischarges of cold plasma. (b) Extracted plasma power signal at 8 kV, obtained by isolating the conductive component of the dissipated power. The waveform reveals discrete, high-amplitude peaks that indicate real energy transfer to the plasma during streamer ignition.

To better quantify the real power deposition associated with these microdischarges, we now turn to averaged power metrics across voltage amplitudes. This allows us to distinguish the onset and growth of conduction-related losses and assess their relative importance in the overall energy balance of the discharge. The bar chart in Fig. 6 a displays the mean dissipated power $P_{diss} = \frac{1}{T}.\int_T p(t).dt$ over a full AC cycle as a function of the applied DBD voltage, ranging from 1 kV to 8 kV. $P_{diss}$ increases quasi-exponentially with the supplied voltage, from 80 mW at 1 kV to 2.2 W at 7 kV and 2.4 W. Notably, the bar corresponding to 8 kV is decomposed into two components: a dominant capacitive contribution and an additional conductive component (hatched), which reflects real energy dissipation through transient plasma filaments. These contributions arise from Joule heating and other irreversible processes (e.g., ionization, excitation). Despite the relatively low absolute values compared to the reactive power, this dissipated energy is critical for enabling plasma-induced chemical reactivity, seed surface modification and gas heating. Here again, the emergence of this conductive contribution at 8 kV clearly marks the transition from a non-dissipative capacitive regime to a mixed capacitive-conductive discharge regime.

Defined as the ratio of average dissipated power $P_{diss}$ to reactive power Q, the dielectric loss tangent $\tan \delta = P_{diss}/Q$ is reported as a function of $V_{DBD}$ in Fig. 5 b. Tan δ quantifies the extent of real energy losses relative to the energy cyclically stored and released in the DBD. Throughout the entire voltage range from 1 kV to 8 kV, tan δ remains extremely low, on the order of $10^{-6}$, indicating that the DBD remains highly capacitive with minimal net energy dissipation. From 1 kV to 7 kV, the value remains

nearly constant around $6\times10^{-6}$, reflecting a stable low-loss regime dominated by displacement currents. Interestingly, at 8 kV, tan δ slightly decreases, despite the onset of filamentary discharges and a rise in absolute dissipated power. This counterintuitive trend is explained by the even steeper growth in reactive power at higher voltages, which causes the proportion of dissipated energy relative to the total exchanged energy to diminish. Thus, even in the presence of microdischarges, the discharge remains predominantly governed by capacitive dynamics and the system continues to operate with exceptionally low dielectric losses.

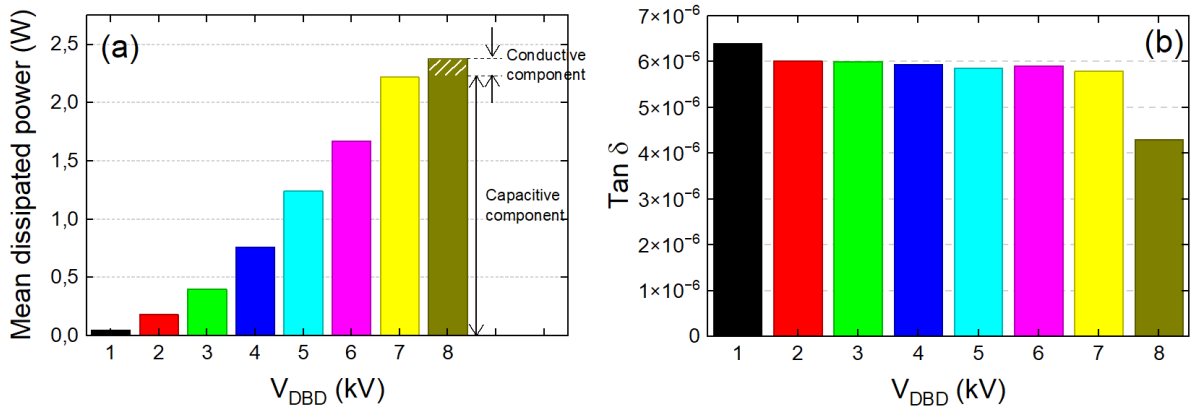


Fig. 6: (a) Mean dissipated power as a function of DBD voltage amplitude, highlighting the transition from a purely capacitive regime (1-7 kV) to a mixed capacitive-conductive regime at 8 kV. At 8 kV, the total power is decomposed into a dominant capacitive contribution and an additional conductive component (hatched), arising from transient microdischarges during filamentary plasma operation. (b) Evolution of the dielectric loss tangent tan δ = P/Q. Across the voltage range, tan δ remains extremely low, indicating a highly reactive system with minimal real power loss.

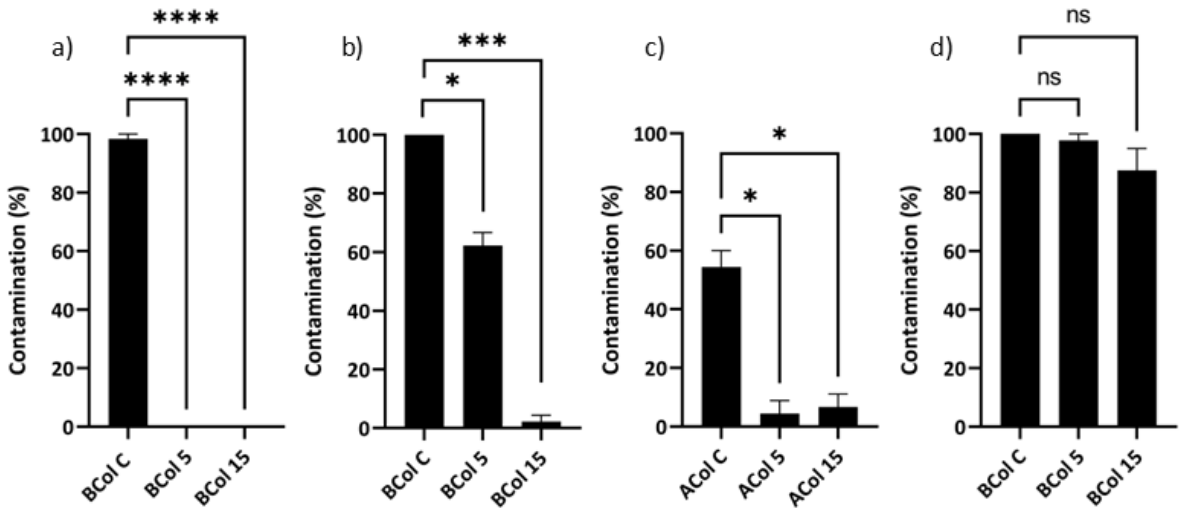


Fig. 7: Assessment of the effect of the electric field on the contamination level of *Arabidopsis thaliana* seeds from lots A and B at the frequency of 500 Hz (c, d) and at 140 Hz (lot B) (b) for 5 and 15 min, followed by the effect of plasma treatment on the decontamination of lot B seeds (a). Each bar represents the mean percentage of contaminated seeds across duplicates, with each replicate containing 40 to 45 seeds. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

In this experiment, we aimed to isolate the effect of the electric field from other plasma components. We first showed that at a frequency of 500 Hz and with a 2.5 mm gap, no plasma formation was observed up to a voltage of 7 kV (Fig. 6 a). Indeed, the applied voltage remained below the breakdown threshold, estimated between 7.5 and 8 kV. This configuration allowed us to expose the seeds solely to the electric field, without plasma generation, for durations of 5 and 15 min (Fig. 7b, c, d).

To provide a positive control and confirm the effectiveness of plasma treatment under these experimental conditions, we also conducted a “plasma ON” treatment at a voltage of 8 kV (above the breakdown threshold) on the most contaminated seed lot (Fig. 7 a).

An additional test was carried out at a much lower frequency (140 Hz) on the same lot, in order to assess the influence of frequency on treatment efficiency (Fig. 7 b). The results show that at 500 Hz (Fig. 7 c, d), a significant decontaminating effect was observed after just 5 min of exposure, with the contamination rate dropping from over 50% to less than 10% in the less contaminated lot (Fig. 7 c). In contrast, no notable effect was observed for lot B, which was more heavily contaminated (Fig. 7 d). These findings suggest a correlation between the initial contamination level of the seed lots and the effectiveness of the electric field. At 140 Hz, a stronger decontaminating effect was observed, particularly after 15 min of treatment (Fig. 7 b), with the percentage of contaminated seeds decreasing

from nearly 100% in the control condition to less than 5%. However, at this frequency, the seeds were subjected to electrostatic forces that gradually ejected them from the device. In contrast, at 500 Hz, the seeds tended to agglomerate into clusters, which kept them in place within the setup. We hypothesize that the differences in treatment efficiency observed between the two frequencies are related to the physical dynamics of the seeds within the system. Seed agglomeration at 500 Hz may limit the uniformity of the treatment across the entire surface of the seeds, thereby reducing the overall efficiency of the process.

### 3.2. Influence of temperature on seeds decontamination

In the context of seed decontamination, particularly from microbial pathogens, moderate temperature increases during treatment (whether plasma-based or not) can contribute to microbial inactivation by weakening cell membranes, denaturing proteins, or disrupting the metabolic functions of contaminating organisms [19]. Thermal treatments are already commonly used methods for disinfection or for reducing microbial loads of fungal and bacterial pathogens, without relying on chemical agents. However, if heating becomes excessive or prolonged, especially in the case of localized filamentary discharges it may adversely affect the seeds themselves, leading to structural damage, partial desiccation, or reduced viability [20]. Although DBDs are typically classified as “cold plasmas” due to their low average operating temperatures, the presence of transient microdischarges and limited heat dissipation can generate hot spots that introduce heterogeneity in the treatment.

As Joule heating was identified in the previous section as a potential byproduct of plasma exposure, it is important to determine whether such thermal effects occur in our specific dielectric barrier configuration and whether they influence decontamination efficiency on *Arabidopsis thaliana* seeds.

Gas temperature measurements derived from OES have not been achieved owing to the incertitudes of this approach, typically +/- 20 °C. As an alternative, IR thermographic imaging has been carried out on upper dielectric barrier of the DBD, which provides a reasonable estimation of seeds temperature upon their plasma exposure.

The surface temperature of the DBD HV electrode has been viewed and measured in the XY plane over a continuous 30-min plasma operation. The corresponding chronological sequence of infrared thermal images is detailed in Fig. 7. Each frame corresponds to a specific time point, from 1 min to 30 min, with a spatial temperature distribution captured and color-coded according to the scale on the right, ranging from 28 °C (dark blue) to 37 °C (magenta).

In the early phase (1-6 min), the electrode appears uniformly cool, with temperatures predominantly below 30 °C, suggesting minimal heating and nearly uniform thermal distribution. Starting from around 7 min, small localized regions begin to warm up, as indicated by the appearance of light green areas (30-31 °C), marking the onset of localized energy deposition likely due to the electrical wire which supplies the whole HV electrode.

Between 10 and 18 min, these warmer regions become more distinct and slightly expand, with yellow (32-33 °C) and orange (34 °C) zones emerging intermittently. This indicates a gradual accumulation of heat in specific areas, where plasma activity may be more intense or more frequent.

From 19 min onward, the temperature pattern becomes more heterogeneous and pronounced. Several stable hot spots appear in the central and peripheral regions of the electrode, some reaching red and even magenta levels (>35-37 °C). These hot spots coincide with localized zones of enhanced plasma-surface interaction, suggesting persistent microdischarges in those areas. The spatial pattern stabilizes by the final minutes (25-30 min), with a clear thermal footprint composed of multiple concentrated regions of elevated temperature (up to 36 °C) surrounded by cooler zones (32 °C).

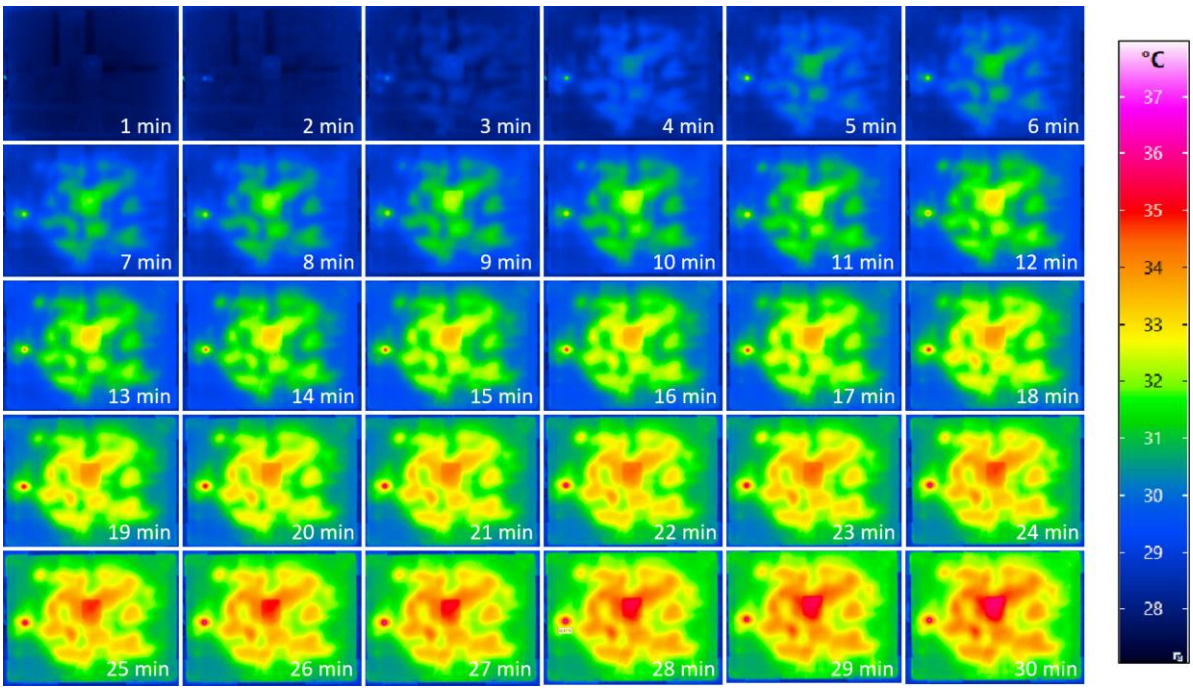


Fig. 8: Time-resolved infrared thermographic images of *Arabidopsis thaliana* seeds exposed to DBD plasma treatment over a 30-minute period. Each frame represents the thermal distribution at 1-minute intervals. The temperature scale bar (right) highlights spatial variations and the emergence of thermal heterogeneities linked to Joule heating and transient microdischarges.

These raw data have also been represented in Fig. 9 for a treatment time of 30 min. In this figure, the black curve represents the average temperature of the 26*34 $cm^2$ area of the HV electrode while the upper red and lower blue curves correspond respectively to the camera pixels with the highest and lowest temperature at distinct given times.

At the beginning of the treatment (t = 0), the electrode temperature is close to room temperature, around 27 °C. As the DBD operates continuously, the electrode gradually heats up, reaching an average temperature of approximately 31.5 °C after 30 min. The upper envelope, corresponding to local hot spots, rises nearly 36.5 °C, whereas the coolest regions of the electrode remain around 30.5 °C, as shown by the lower envelope. This thermal spread reflects spatial inhomogeneity in energy deposition with a value of approximately 6 °C for a treatment duration of 30 min. However, for 5 and 15 min (which correspond to the treatment times for seeds decontamination), remain close to 4 °C and 5 °C respectively.

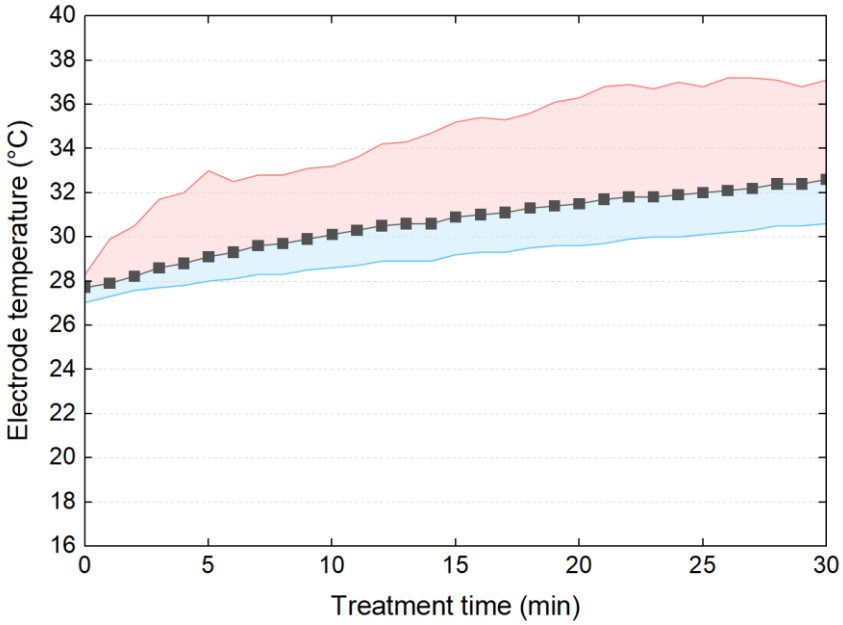


Fig. 9: Evolution of the electrode temperature during 30 min of DBD plasma treatment. The black squares represent the mean temperature measured on the electrode surface at each time point, while the red and blue shaded areas indicate the upper and lower bounds of the temperature range, respectively. To verify if the processing temperature alone can alter seeds decontamination, several samples of A. Thaliana seeds have been placed on a hot plate during 5 and 15 min, at 37 °C which corresponds to the most penalizing scenario.

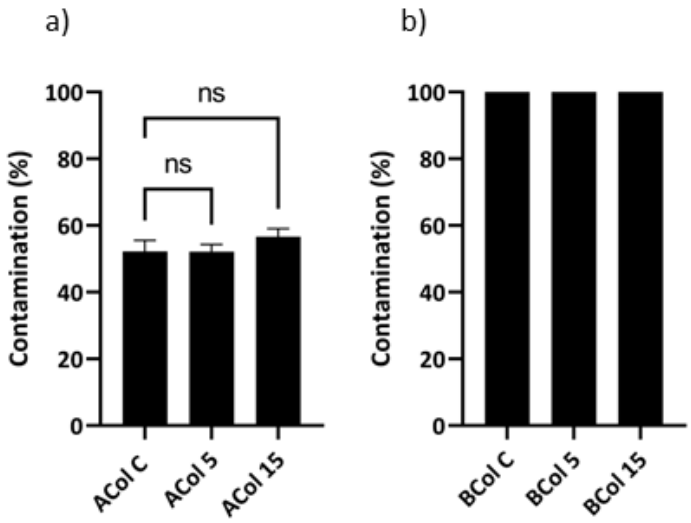


Fig. 10: Assessment of the effect of temperature (37°C) on the contamination level of *Arabidopsis thaliana* seeds from lots A (a) and B (b). Seeds were either not exposed (C) or exposed for 5 and 15 min. Each bar represents the mean percentage of contaminated seeds across duplicates, with each replicate containing 40 to 45 seeds. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

These results (Fig. 10) confirm that temperature does not play a significant role in the decontamination process. Even under the most extreme thermal conditions observed during our experiments i.e., after 30 min of continuous plasma operation (Fig. 9), when temperature levels were at their highest no notable decontaminating effect was observed. It is important to emphasize that our actual plasma treatments never exceeded 15 min, and therefore, the thermal exposure experienced by the seeds during standard treatment durations was even lower (approximately 32°C) (Fig. 9). This strongly suggests that the temperature rise induced by the plasma device is insufficient to contribute meaningfully to microbial inactivation, and that the decontamination effect observed in our study originates from other plasma-related mechanisms.

### 3.3. Influence of CAP radiation on seeds decontamination

While CAP is well-established for its antimicrobial efficacy, the extent to which its radiative output contributes to seed surface decontamination remains unclear, especially in the ultraviolet (UV) range. This section examines the optical emission spectrum of our air-based CAP DBD and compares it to two radiative benchmarks: ambient daylight under overcast conditions and a low-pressure mercury germicidal UV lamp. By analyzing spectral intensity and distribution particularly within the germicidal

UVC (100–280 nm) and UVB (280–315 nm) ranges, as well as the UVA range (315–400 nm) [21] we aim to determine whether CAP-generated photons alone can meaningfully contribute to microbial inactivation on the surface of *Arabidopsis thaliana* seeds, independently of reactive chemical species. The comparative analysis of the optical emission spectra for the three different light sources is presented in Fig. 11, including ambient daylight under a cloudy sky, a germicidal UV lamp and ambient air cold plasma generated in the dielectric barrier device. The spectra are plotted as intensity (in arbitrary units) versus wavelength, covering the UV-visible range from 200 to 800 nm. Each source is associated with a very specific spectrum, revealing its radiative characteristics and underlying physical processes.

The spectrum of the cloudy sky appears as a smooth, continuous curve (shown in rainbow colors), typical of natural sunlight diffused by atmospheric scattering. This broadband emission spans the entire visible range, peaking around 450-550 nm and tapering off into both the UV and infrared. This continuous distribution indicates blackbody-like emission dominated by the Sun, with minimal line features.

The germicidal lamp spectrum, shown in hatched purple, exhibits sharp and intense peaks, especially in the UVC range (200-300 nm), where low-pressure mercury lamps typically emit. These spectral lines correspond to atomic mercury transitions, especially the strong line near 254 nm, which is highly effective for microbial inactivation. The intensity of these lines is orders of magnitude higher than those of the CAP, as indicated by the y-axis scaling, with values as high as 22 000 a.u.

The CAP/DBD emission, plotted in white, displays a structured but much lower-intensity spectrum, composed of discrete peaks and molecular bands rather than a continuous distribution. For the sake of clarity, the same spectrum is reported in Fig. 12 in order to highlight the dominant radiative transitions. While the emission intensity in the deep UVC and UVB is nearly negligible, several strong emission peaks appear in the UVA region, with intensities exceeding 1000 a.u. for certain transitions. Specifically, distinct nitrogen emission lines are labeled: (i) $N_2$ 315.9 nm, marking the transition at the UVB/UVA boundary, (ii) $N_2$ 337.1 nm, the most intense feature, commonly regarded as the reference band of the second positive system (SPS) of molecular nitrogen ($N_2$), corresponding to the $C^3\Pi_u \rightarrow B^3\Pi_g$ transition, (iii) Additional prominent peaks include 353.7, 357.7, 371.0, 375.5, 380.5, 399.8 and 405.9 nm. These emissions correspond to radiative decays of electronically excited nitrogen molecules ($N_2$*) in ambient air. The dominance of the SPS transitions indicates that the plasma is largely non-thermal, with electron energies sufficient to excite $N_2$ to higher electronic states but without substantial dissociation or ionization that would lead to atomic lines or higher-energy UV radiation. Notably, the absence of significant intensity in the UVC range (<280 nm) reinforces that the plasma emits minimal

germicidal radiation, unlike low-pressure mercury lamps. This suggests that any biocidal effects from the CAP are unlikely to be photonic (UV-driven) and are instead attributed to reactive species such as $O_3$, OH or $NO_X$.

From a biological standpoint, it is important to note that UV-C and UV-B rays constitute the most energetic portions of the ultraviolet spectrum and are therefore the most concerning due to their mutagenic effects. The use of UV-based technologies for seed decontamination thus raises concerns about potential damage to seed DNA [22]. In contrast, UV-A rays represent the least energetic part of the UV spectrum (see Fig. 12) and are generally considered less harmful to biological structures. While UV radiation is effective for microbial inactivation, selecting the appropriate wavelength is crucial to minimize adverse effects on the genetic integrity of seeds.

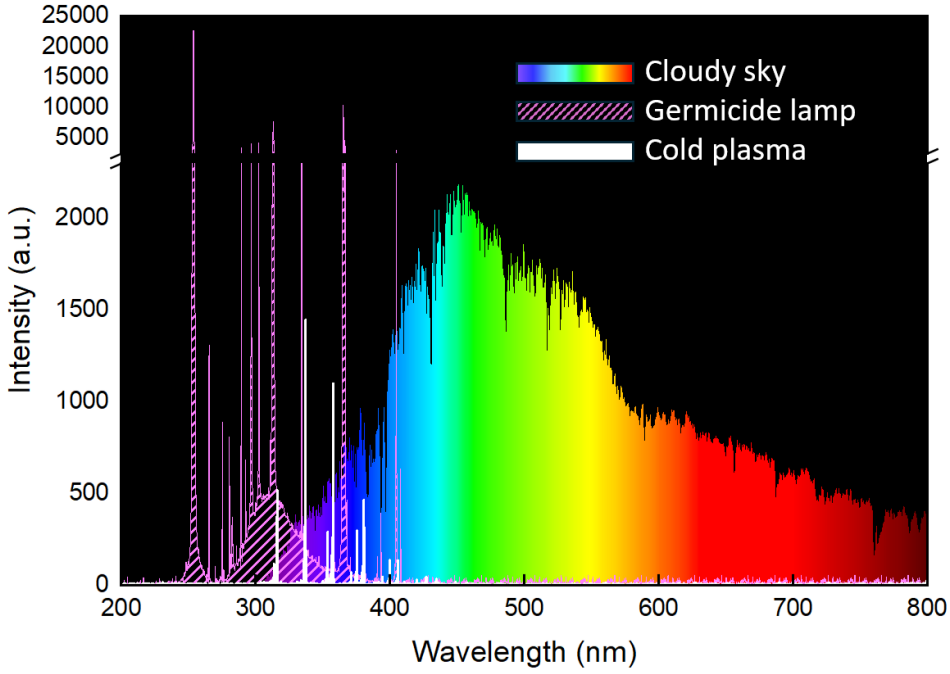


Fig. 11: Comparative optical emission spectra of 3 light sources relevant to seed surface treatment: cloudy daylight (rainbow-filled spectrum), a germicidal UV lamp (hatched purple) and an ambient air cold atmospheric plasma (CAP, white spectrum).

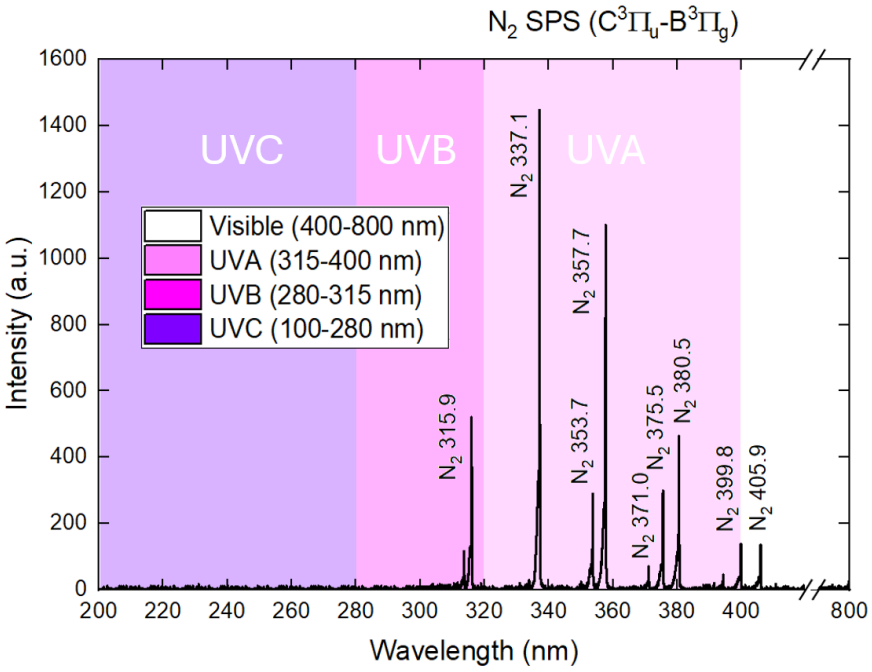


Fig. 12: Optical emission spectrum of ambient air CAP generated in the dielectric barrier device (DBD). The background shading delineates standard UV subranges: UVC (100-280 nm), UVB (280-315 nm), UVA (315-400 nm) and visible light (400-800 nm).

Fig. 11 and Fig. 12 highlight that while CAP emits a rich but weak spectrum composed of discrete molecular and atomic transitions, it lacks both the intensity and broadband coverage of sunlight or the strong germicidal UV peaks of mercury lamps. This underlines that CAP radiation is not the dominant mechanism for biological effects such as sterilization and that its action likely relies more on reactive species than on radiative power.

To verify if the processing temperature alone can alter seeds decontamination, several samples of *Arabidopsis thaliana* seeds have been placed in the very close vicinity of the DBD, ie at 2 cm so as to be lighted by the plasma radiation, without any possible influence of the other plasma properties, whether electrical, chemical or thermal. As a reference, we also exposed our seeds for 5 and 15 min under agitation to the radiation of the lamp, the spectrum of which is characterized in Fig. 11.

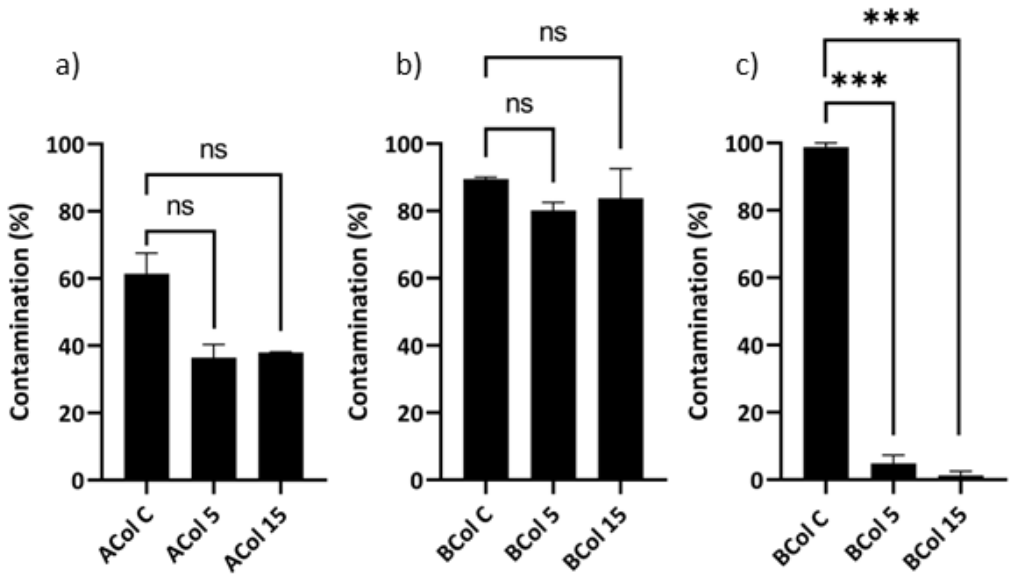


Fig. 13: Assessment of the effect of CAP radiation (a, b) and UVB-C lamp radiation (c) on the contamination level of *Arabidopsis thaliana* seeds from lots A (a) and B (b). Seeds were either not exposed (C) or exposed for 5 and 15 min. Each bar represents the mean percentage of contaminated seeds from two replicates, each containing 40 to 45 seeds. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

To establish a positive control and demonstrate the germicidal potential of UV-B and UV-C radiation, the most heavily contaminated seed lots were also exposed to UV-B and UV-C irradiation emitted by a lamp, whose spectrum is shown in Fig. 11, for 5 and 15 min. Regarding the results obtained with the CAP radiation, a reduction in the percentage of contaminated seeds was observed after just 5 min of exposure for lot A (Fig. 13 a). However, this reduction was not statistically significant, suggesting a limited effect under the tested conditions.

For lot B, which was more heavily contaminated, no significant differences were observed between the plasma-treated seeds and the controls, regardless of exposure duration. These results indicate a limited contribution of CAP radiation to the decontamination process under the tested conditions (Fig. 13 b). In contrast, direct exposure to UVB-C radiation from the lamp (Fig. 13 c) resulted in a significant reduction in seed contamination, with the contamination rate dropping from over 95% to below 10% after 5 min, and falling to less than 5% after 15 min. These findings confirm the strong germicidal efficacy of this type of radiation.

In conclusion, UV radiation generated by the plasma appears to have a very limited effect on seed decontamination. Furthermore, the overall UV spectrum emitted by the plasma consists mainly of low-intensity emission lines, predominantly located in the UV-A region (Fig. 12 & 13), which represents the

least energetic portion of the ultraviolet spectrum. These observations suggest that UV radiation plays only a minor role in the overall decontamination efficiency of plasma treatment.

### 3.4. Influence of CAP reactive species on seeds decontamination

Since the preceding sections have shown that CAP electrical, thermal and radiative effects contribute only marginally to the decontamination of *Arabidopsis thaliana* seeds, the focus is now shifted to the chemical reactivity of the plasma, specifically, the role of reactive oxygen and nitrogen species (RONS). To probe this, we first characterize the reactive chemistry in the DBD where cold plasma of ambient air is generated. Two complementary diagnostics are utilized: optical emission spectroscopy (OES), which provides insight into excited species within the active discharge zone and mass spectrometry (MS) which detects non-emissive neutral products in the effluent. This combined approach allows us to correlate the presence of RONS with observed microbial inactivation.

#### 3.4.1. Experimental condition where cold plasma is generated in ambient air

Characterization by optical emission spectroscopy

The optical emission spectrum of the DBD operating in ambient air is presented in Fig. 12: It reveals exclusively the presence of excited molecular nitrogen species, specifically from the second positive system ($C^3\Pi_u \rightarrow B^3\Pi g$). Notably, no emission features associated with reactive oxygen or nitrogen species such as atomic oxygen (O), hydroxyl radicals (OH), or nitric oxide (NO) are observed. This absence is particularly surprising given that these species are commonly cited as key actors in plasma-driven microbial inactivation. Understanding why they are not optically detected under these conditions is critical for clarifying the limits of OES and accurately interpreting the chemical composition of the discharge. Several plasma-physical and diagnostic factors contribute to this outcome and are discussed in the following paragraphs.

First, the optical emission spectrum of DBD operating in ambient air is typically dominated by molecular nitrogen ($N_2$) transitions from SPS corresponding to the $C^3\Pi_u \rightarrow B^3\Pi g$ transition. This prominence arises from the high abundance of $N_2$ in air (about 78%) and its favorable excitation cross-sections under the reduced electric field conditions characteristic of non-thermal plasmas. As a result, excited $N_2$ species are generated efficiently and radiate strongly in the 300-400 nm range, leading to bright, structured spectral features that often overshadow emissions from other, less abundant species.

In contrast, species such as OH, NO and O are formed through slower chemical kinetics and secondary reaction pathways that are not directly driven by electron impact excitation. Their excited states are either less populated or not as efficiently produced under the same plasma conditions. Consequently, their radiative transitions occur less frequently and the resulting optical emissions are intrinsically weaker. These species may still play important roles in plasma chemistry and surface interactions, but their low radiative efficiency means they often remain undetected in conventional OES.

Another important factor is the collisional environment of atmospheric-pressure plasmas. Excited states of OH (such as $A^2\Sigma^+$), NO ($A^2\Sigma^+$) and atomic O are particularly short-lived and highly susceptible to collisional quenching by background gas molecules, especially $N_2$ and $O_2$. Under high-pressure conditions, the probability of non-radiative de-excitation increases significantly, effectively suppressing the observable emission even if these species are present. Thus, the absence of OH or NO bands in OES does not necessarily imply their chemical absence but may instead reflect rapid quenching before photon emission can occur.

Finally, the nature of the discharge regime and the associated plasma conditions strongly determine which species are excited and how efficiently. DBDs operate in a pulsed microdischarge mode with low average power and weak electron temperatures, typically yielding limited thermal excitation and minimal dissociation of $O_2$ and $H_2O$ molecules. As a result, the formation of OH and NO radicals tends to be more pronounced in downstream or afterglow regions rather than in the active discharge zone itself. These considerations explain why nitrogen emissions dominate the OES signal in ambient air DBDs, while RONS may be better detected by complementary diagnostics such as laser-induced fluorescence, absorption spectroscopy or mass spectrometry, this later diagnostic being proposed in the next section.

Characterization by mass spectrometry

A time-resolved analysis of gas-phase species concentrations has been measured by mass spectrometry, as illustrated in Fig. 14 before and during the generation of cold plasma of ambient air. The discharge is switched on at t = 0 min and species concentrations are plotted on a logarithmic scale in ppm over a 30-minute window (-10 to +20 min). The system initially exhibits a stable baseline for all measured gases in the plasma-off region, dominated by $N_2$ (about $10^6$ ppm), $O_2$ (about $2.1 \times 10^5$ ppm), $H_2O$ (about $10^4$ ppm) and Ar (about $10^4$ ppm). Upon plasma ignition, several RONS exhibit marked changes in concentration. However, great care must be brought to their interpretations because the gas sample (issued from plasma) travels through a long quartz capillary before reaching the ionization chamber of the mass spectrometer, where high-energy electron impacts can induce secondary dissociation. Consequently, species such as atomic oxygen and OH may not reflect their actual

presence in the plasma effluent but rather result from *in situ* fragmentation of stable precursors like $O_2$, $O_3$, or $H_2O$. These artifacts can lead to overestimation of radical concentrations. In contrast, more stable species (eg $NO_2$, $O_3$, $H_2O_2$, $N_2O$) are more reliably measured, as they survive transit and ionization without major distortion. This limitation can be overcome considering the relative variations in concentration of all species before/during plasma.

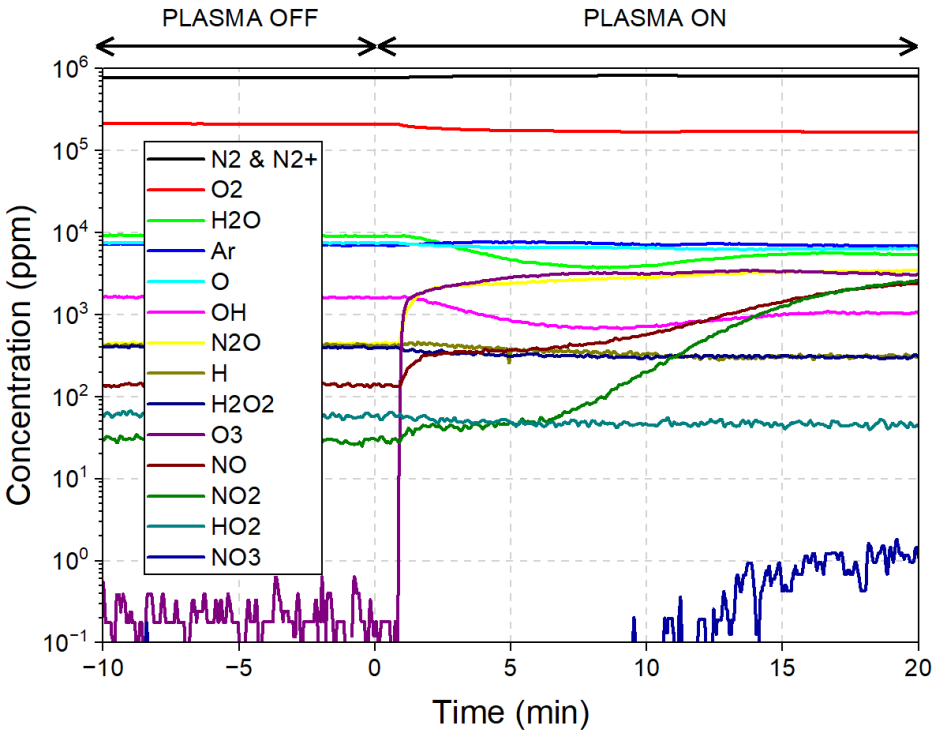


Fig. 14: Time-resolved gas-phase composition measured by mass spectrometry during DBD operation in ambient air.

The relative variation of key long-lived RONS before/during cold plasma treatment is plotted in Fig. 15. The y-axis shows the normalized increase in concentration, defined as $\left(\frac{C_{pl}^{on}-C_{pl}^{off}}{C_{pl}^{off}}\right)$, which expresses the percentage increase relative to the baseline (pre-discharge) concentrations. The species tracked include ozone ($O_3$, black), nitrogen dioxide ($NO_2$, red), nitric oxide (NO, green) and nitrous oxide ($N_2O$, blue).

At t=0 min, the plasma is turned on. $O_3$ shows the most immediate and dramatic increase, reaching a normalized ratio above $10^4$, indicating extremely efficient generation relative to its initially low background level. This rapid accumulation reflects the highly effective production of $O_3$ via oxygen dissociation and subsequent three-body recombination in the presence of $O_2$.

$NO_2$ also increases substantially, though more gradually than $O_3$. Its curve suggests formation via secondary gas-phase reactions, notably the oxidation of NO by $O_3$ or O atoms. The delayed and exponential-like profile of $NO_2$ implies it is not directly formed by the plasma but accumulates through post-discharge chemistry.

NO shows a modest but steady increase, reaching about 8-9 times its initial level after 20 min. Its slower evolution compared to $O_3$ and $NO_2$ reflects its role as both a primary plasma product and a reactive intermediate, partially consumed in the formation of $NO_2$ and other nitrogen oxides.

Finally, $N_2O$ exhibits the smallest relative increase, stabilizing quickly after ignition. This plateau suggests that $N_2O$ is likely produced in a short time window following plasma onset, probably via electron-driven recombination of N and NO species, but its formation is limited by slower kinetics or lower reaction efficiency.

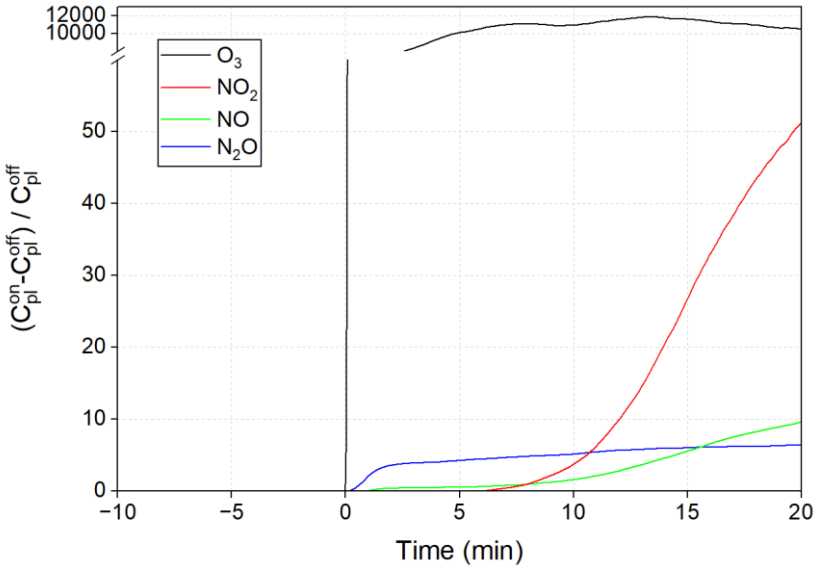


Fig. 15: Relative variation in the concentrations of key long-lived reactive species (ozone ($O_3$), nitrogen dioxide ($NO_2$), nitric oxide (NO) and nitrous oxide ($N_2O$), as a function of time, during DBD plasma ignition in ambient air.

Influence on seed decontamination

To determine whether plasma-derived RONS alone are sufficient to induce seed decontamination, *A. thaliana* seeds were placed in the interelectrode gap of the DBD device but upper-shielded by a grounded aluminum foil. This configuration effectively blocks direct exposure to both the electric field and plasma-generated UV radiation. Although seeds may still experience a mild temperature rise during treatment, previous results have shown that heating up to 37 °C has no measurable antimicrobial effect, thereby eliminating thermal influence as a perturbative factor. Under these conditions, seeds are exposed exclusively to RONS that diffuse laterally beneath the foil, from the plasma zone toward the seed surface. This setup isolates the chemical component of the plasma, allowing us to directly assess the contribution of neutral reactive species to microbial inactivation.

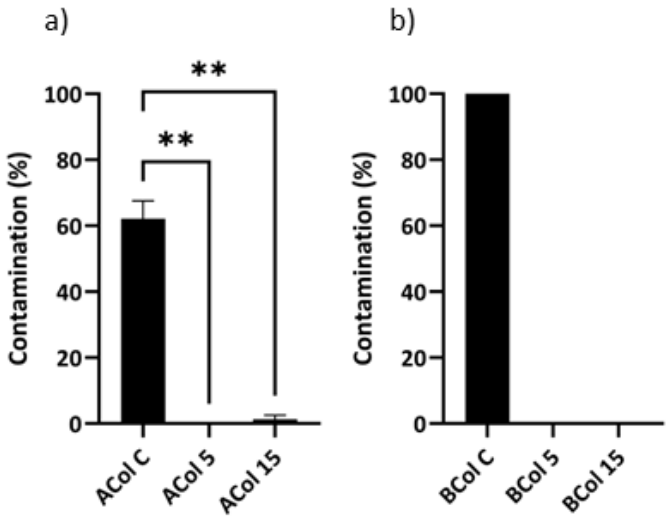


Fig. 16: Assessment of the effect of plasma-derived RONS on the contamination level of *Arabidopsis thaliana* seeds from lots A (a) and B (b). Seeds were either not exposed (C) or exposed for 5 and 15 min. Each bar represents the mean percentage of contaminated seeds from two replicates, each containing 40 to 45 seeds. Statistical significance was assessed using an unpaired t-test. Significance levels are indicated as follows: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

An almost complete decontamination by reactive oxygen and nitrogen species (RONS) was observed after just 5 min of treatment. For seeds from lot B, the decontamination was total, while for lot A it was nearly complete. Initial contamination levels were approximately 100% for lot B and 60% for lot A, and dropped to below 2% for both lots after only 5 min of exposure (Fig. 16). This decontamination efficiency is comparable to that obtained with plasma treatment shown in Fig. 6(a) under equivalent exposure times.

Among the various chemical species generated, ozone ($O_3$) and nitrogen dioxide ($NO_2$) exhibited the most significant relative increases in concentration following plasma ignition (Fig. 14 & 15). Among the parameters tested, these two compounds appear to be directly involved in the decontamination process, playing a more significant role than other components such as radiation or the electric field, which seem to have only a minor effect but may potentially act in a synergistic manner.

**5. Conclusion**

In this study, we demonstrated the major role played by reactive oxygen and nitrogen species (RONS) in the decontamination of *Arabidopsis thaliana* seeds. Our results confirm that these reactive species are primarily responsible for the microbial inactivation. However, their high reactivity also presents a potential downside: ROS, in particular, can induce oxidative stress, which may negatively impact seed viability or germination [23]. To address this limitation, it would be relevant to investigate whether shorter plasma exposure times could still ensure effective decontamination while minimizing oxidative exposure.

Additionally, our experiments revealed interesting effects associated with the electric field component of the plasma, particularly at a frequency of 140 Hz. These effects were observed in the absence of RONS generation, suggesting that the electric field alone may contribute to microbial inactivation under certain conditions. Future work should therefore focus on optimizing the experimental device to enable stable treatment at 140 Hz while eliminating electrostatic issues affecting the seeds, to ensure they remain within the device during treatment.

In summary, our findings highlight both the central role of RONS in plasma seed decontamination and the potential of alternative mechanisms, such as electric field effects, as complementary or substitute strategies.

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# General conclusion

# & perspectives

# General conclusion & perspectives

This work has provided new insights into the use of cold plasma as a sustainable solution to address biotic and abiotic challenges affecting seeds in agriculture. The main objective of this thesis was to characterize the effects of a cold plasma treatment generated by a dielectric barrier discharge (DBD) on three key aspects related to seeds: germination, early seedling growth, and microbial decontamination.

We have demonstrated that short plasma exposure improved seed vigour and growth of sunflower seedlings subjected to significant water stress. Similar studies conducted on other agronomically important crops such as wheat and rapeseed confirm these findings [1, 2]. Guo et al., in their study on wheat, showed that plasma treatment increased the concentrations of certain antioxidant enzymes (CAT, POD), enabling plants to better tolerate drought stress [3]. In addition to drought tolerance, future research could also explore the effects of plasma treatment on plant responses to other abiotic stresses, such as hypoxic stress. Hypoxia, which often occurs in waterlogged or compacted soils, can severely impair seed germination, root respiration, and early seedling development. Investigating whether plasma pre-treatment can enhance seed resilience under such conditions would be highly valuable, particularly in the context of increasingly frequent extreme weather events. These experiments should also be conducted on other plant models of agronomic interest, in order to assess relevance of the observed effects.

We have also showed that the physico-chemical properties of plasma are directly influenced by the experimental setup. Changes in the inter-electrode distance or the introduction of seeds into the reactor not only affected plasma formation but also altered its chemical composition. More precisely, we observed a relative decrease in ozone concentration when the inter-electrode distance increased, but an increase when seeds were present in the device. This suggests that the GAP setting is an important parameter that could impact both plasma composition and, consequently, treatment efficiency. However, it may also represent a limitation for the treatment of large seeds, as a small inter-electrode distance restricts the available volume of the device, thereby making the treatment of larger seeds physically impossible. Conversely, increasing the gap may negatively affect plasma formation and reduce its homogeneity. Future work could therefore focus on optimizing the DBD reactor design to enable effective treatment of larger seeds while preserving the desired plasma characteristics.

As discussed in Chapter 4, ozone plays a central role in decontamination processes, suggesting that parameters such as seed size and shape must be considered to optimize treatment efficiency. These compositional variations can also lead to inconsistent results, posing a challenge for the

standardization and broader implementation of this technology. Our results indicate that reactive oxygen and nitrogen species (RONS) are a common and central factor involved in both germination and decontamination processes. Living organisms must maintain a delicate balance between physiological ROS requirements and their accumulation, which can lead to oxidative stress. The latter may result in loss of membrane integrity and trigger cellular apoptosis [4]. It is therefore particularly noteworthy that brief exposure (5 minutes) is sufficient to significantly reduce the pathogenic load of seeds, even those heavily contaminated, as demonstrated in Chapters 3 and 4. On the other hand, prolonged exposure could have detrimental effects on seed germination and nutritional quality [5]. It would thus be relevant to investigate the effectiveness of exposure times shorter than 5 minutes to minimize the seeds exposure to RONS present in the plasma. The dual role of ROS was discussed in Chapter 1, in which we detailed the physical, molecular, and biochemical defense mechanisms implemented by seeds to cope with stress. ROS also serve as early signals for the activation of enzymatic pathways following an attack. Furthermore, we recalled that dormancy release is associated with a controlled increase in ROS, which must remain confined within a specific "oxidative window" [6].

We explored the decontaminating properties of plasma on seeds and the induced changes in the seedling microbiota. Until now, few studies have examined the effect of plasma on fungal microbiota even though many fungi present on the seed surface can develop opportunistically under poor storage conditions, thereby compromising germination potential or nutritional quality (e.g., rots, mycotoxins) [7]. The elimination of these epiphytic populations through a brief treatment, without the use of biocides, presents a promising approach toward more sustainable agricultural practices that safeguard both human health and the environment. Our results indicate that plasma treatment of seeds induces a disruption in the seedling microbiota. We observed a reduction in overall diversity and a decrease in the relative abundance of certain dominant taxa, such as *Penicillium* and *Acremonium*, in seedlings from treated seeds. Furthermore, differences in sensitivity towards plasma were observed among taxa, with *Cladosporium cladosporioides* showing higher resistance and *Acremonium sclerotigenum* displaying high sensitivity. This reduction in dominant taxa was sometimes accompanied, especially with shorter treatments, by an increase in the relative abundance of initially underrepresented taxa. For example, in Landsberg seedlings from seeds treated for 5 minutes, we observed an increase in the relative abundance of the genus *Trichoderma*. We hypothesize that these variations result from the decrease in dominant taxa, which frees up ecological niches and thereby reduces competitive pressure for microorganisms, particularly endophytes. It is interesting to note that several species belonging to this genus are known for their ability to solubilize soil phosphorus and produce antimicrobial compounds. This implies that *Trichoderma* is now at the heart of research programs focused on the

formulation of microbial biostimulants [8]. Thus, the seedling microbiota is now considered a key ally in plant health, playing a central role in plant adaptation to stress. It provides a solid foundation for developing new adaptive agroecological strategies [9].

The study of the bacterial microbiota yielded limited information. The bacterial diversity of our samples was initially very low, and the treatment eliminated residual bacteria. Moreover, we chose to amplify the V4 region of ribosomal DNA; this region generates a large number of reads, most of which are associated with chloroplasts and mitochondria, and do not result in taxonomic assignments. Furthermore, this region does not allow identification beyond the genus level, resulting in a lack of precision in characterizing microbial communities and complicating the interpretation of results, especially for functional microbiota analyses. It would be worthwhile to repeat this analysis using the *gyrB* marker, which is more relevant for these reasons.

Recent work by Tamošiūnė et al. on *Arabidopsis thaliana* showed that plasma seed treatment could induce a reduction in initial bacterial diversity on seeds, followed by an increase in certain endophytic bacteria present in the phyllosphere of mature plants [10]. These observations encourage us to extend our investigations by analyzing microbial communities at different stages of plant development and considering the various microbial compartments (rhizosphere, phyllosphere). This research could also be conducted on species of agronomic interest, to better understand the mechanisms of microbiota reorganization following a disturbance in the context of potential agricultural applications.

Finally, it is essential to emphasize that our study was conducted under highly controlled conditions, which do not reflect the complexity of microbial community interactions associated with plants in natural environments. We studied the microbiota of seedlings that developed in a sterile environment. The environment, especially soil, plays a central role in the assembly of plant-associated microbial communities [11]. The low bacterial diversity observed in our study could also be linked to the absence of these compartments. It is therefore crucial to continue this research under more representative conditions, for example by conducting a greenhouse experiment with potted mature plants, and performing a targeted analysis using gyrB amplification in the different plant tissues. In conclusion, we reported that short plasma exposure had beneficial effects on seed vigor, growth, and seed decontamination. These three aspects, as outlined in Chapter 1, are fundamental to plant health. Our results support that cold plasma represents an innovative and versatile solution that paves the way for more sustainable agricultural practices.

By enhancing germination performance, improving stress tolerance, and reducing microbial contamination without relying on chemical treatments, plasma technology holds strong potential for

addressing key challenges in modern agriculture. Further studies on a wider range of species, stresses, and plasma configurations will be essential to fully unlock its practical applications.

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**Les plasmas froids d'air ambiant : régulateurs de la germination des semences, de l'adaptation au stress et des interactions microbiennes au cours du développement initial des plantes.**

Les semences, piliers de la sécurité alimentaire, assurent 60 % de l'apport énergétique mondial via des cultures majeures telles que le riz, le blé ou le maïs. Ces cultures subissent aujourd'hui une double pression : d'une part, les stress abiotiques liés aux dérèglements climatiques (sécheresse, inondations, appauvrissement des sols), et d'autre part, une intensification des stress biotiques due à la prolifération et à l'expansion géographique des ravageurs et pathogènes. Cette conjonction fragilise l'efficacité des approches conventionnelles et interroge leur utilisation au regard des exigences écologiques actuelles. Dans ce contexte, la recherche de nouvelles stratégies pour améliorer la vigueur des semences est essentielle. Le plasma froid est considéré comme une alternative crédible face aux limites des méthodes traditionnelles. Il s'agit d'un gaz faiblement ionisé aux propriétés uniques, mêlant effets physiques (champs électriques, électrons, température, rayonnement UV/visible) et chimiques (RONS, ions). Cette étude explore son potentiel pour renforcer la vigueur des semences et stimuler la croissance des plantules chez le tournesol (*Helianthus annuus*). Par ailleurs, son efficacité dans la décontamination des semences d'*Arabidopsis thaliana* a été évaluée, accompagnée d'une analyse de l'impact du traitement sur les communautés microbiennes associées aux plantules. Enfin, une étude mécanistique complémentaire a permis d'identifier les composantes actives impliquées dans la décontamination. Les résultats obtenus ouvrent de nouvelles perspectives pour améliorer les traitements et favoriser une agriculture plus résiliente et durable.

**Mots clés : plasma froid, semences, stress, décontamination, plantules, microbiote**

**Cold atmospheric plasmas as regulators of seed germination, stress adaptation, and microbial interactions during early plant development.**

Seeds, as pillars of food security, provide 60% of the world's energy intake through major crops such as rice, wheat, and maize. Today, these crops are under dual pressure: on the one hand, abiotic stresses linked to climate change (drought, flooding, soil degradation), and on the other hand, an intensification of biotic stresses due to the proliferation and geographic expansion of pests and pathogens. This combination undermines the effectiveness of conventional approaches and calls into question their continued use in light of current ecological requirements. In this context, exploring new strategies to enhance seed vigor is essential. Cold plasma is considered a credible alternative in response to the limitations of traditional methods. It is a weakly ionized gas with unique properties, combining physical effects (electric fields, electrons, temperature, UV/visible radiation) and chemical effects (RONS, ions). This study explores its potential to strengthen seed vigor and stimulate seedling growth in sunflower (*Helianthus annuus*). Additionally, its effectiveness in the decontamination of *Arabidopsis thaliana* seeds was evaluated, along with an analysis of the treatment's impact on microbial communities associated with the seedlings. Finally, a complementary mechanistic study identified the active components involved in the decontamination process. The results open up new perspectives for improving treatments and promoting more resilient and sustainable agriculture.

**Keywords: cold plasma, seeds, stress, decontamination, seedlings, microbiota**