((please add journal code and manuscript number, e.g., DOI: 10.1002/ppap.201100001))
Article type: Full paper

Physicochemical surface analysis and germination at different irrigation conditions of DBD plasma-treated wheat seeds

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Abstract

Plasma treatment is increasingly being explored as an effective pre-sowing treatment improving seed germination. This paper studies the synergetic effect of the irrigation condition and the physicochemical surface properties of wheat seeds subjected to atmospheric dielectric barrier discharge (DBD) plasma activation on their water uptake and germination. An extensive surface analysis revealed a remarkably enhanced wettability of plasma-treated seeds due to the insertion of oxygen-containing functionalities on their surface. However, long plasma exposure damaged the outermost layers of the pericarp because of a pronounced oxidative etching effect. Although the seed germination capacity was not affected by the plasma treatments, short plasma exposures were shown to enhance water uptake and accelerate the seed germination especially under water scarcity conditions.

Keywords: Atmospheric plasma, dielectric barrier discharge (DBD), plasma agriculture, germination, wheat seed, irrigation, surface analysis.

1 INTRODUCTION

Nowadays, non-thermal plasma surface activation of seeds is increasingly being explored in the agricultural field as an effective pre-sowing treatment modulating seed germination and providing a certain degree of sterilization against endospores or fungi. [1–11] Plasma is in fact considered an ecofriendly alternative to the conventional chemical treatments used to surmount the tegumentary seed dormancy such as the use of sulfuric acid, dry heat, hot water or NaOCl treatments.[12–15] The other traditional widely used mechanical scarification methods, which are based on scratching the seed coat, also suffer from many disadvantages that are absent in plasma treatments. These disadvantages include the increased probability of microorganism infection, the decreased vigor, the destruction of some seeds, the non-homogenous treatment and the higher risk of abnormalities in seedlings and biomass production. [1–11] To overcome these issues, several studies have investigated the fast, non-destructive and uniform plasma activation of different seeds such as wheat, quinoa, cotton and many other plants.[3,5,6,10,16–22] This modern practice has been demonstrated efficient for wheat seeds subjected to oxygen or nitrogen-containing plasmas in terms of enhanced wettability and increased germination capacity up to an optimal treatment time, beyond which germination impairs.[10,19,23] Hence, plasma process parameters should be highly fine-tuned to generate the desirable chemical and morphological seed characteristics triggering a bio-stimulation. In fact, next to the complexity of plasma/living cell interactions, seed germination is an intricate physiological process and the seed itself is a histologically complex system. In particular, a wheat seed integrates multiple tissue types and chemical compositions in the seed coat, the endosperm and the embryo.[24–27] The outermost part of a wheat seed, so-called pericarp, defines a first interface layer controlling water diffusion into the seed which is a required step to initiate germination. The pericarp, consisting of an external (15-30 µm) and an internal (5-10 µm) part, is mostly composed of hemicellulose (60-65%), cellulose (25-30%), lignin (10-15%) and, to a lesser extent, other compounds such as arabinoxylans and phenolic compounds. Beneath the pericarp are located the testa (5-8 µm) and the hyaline layer (2-3 µm) which are rich in cellulose, hemicellulose and resistant starches. Further inside, the aleurone layer (30-65 µm) rich in cellulose, hemicellulose and β-glucan and the endosperm that occupies almost the entire volume of wheat seed are found.

Seed germination is initiated by water uptake or imbibition that leads to an ultimate radicle emergence. The minimal water content required for wheat grain germination is around 35 to 45 % of its weight.[28] Different authors have pointed out that plasma treatments enhance the wheat seed hydrophilicity and the water uptake velocity thus reaching the critical water content required to trigger a germination faster.[3,5,10,18–20] Despite being recently investigated, the plasma-induced mechanisms underlying wheat germination enhancement are still vague since no in-depth studies have been so far conducted on the specific chemical changes engendered on the outermost part of wheat seeds. Moreover, in addition to the seed surface properties, the environmental conditions surrounding the seed, of which the critical irrigation state, also play an influential role in the water uptake amount and kinetics.[26] Taken together, in the present work, the synergistic effect of the plasma-induced surface chemistry and topography and the irrigation conditions on wheat seed germination has been extensively studied. First, the surface modifications affecting the pericarp of the seeds subjected to an atmospheric pressure dielectric barrier discharge (DBD) plasma have been characterized by means of a variety of surface spectroscopy, goniometry and microscopy techniques. A correlation between this surface analysis and the water uptake properties of plasma-treated seeds has been unraveled at different irrigation conditions simulating dry, medium or wet environments in terms of water availability. The results of this investigation revealed that plasma treatments increase the water uptake velocity of wheat seeds resulting in a faster attainment of the water content threshold required to initiate germination.

2 EXPERIMENTAL SECTION

2.1 Atmospheric pressure plasma treatment

Ecological wheat seeds (Triticum aestivum L.) with a certificate of origin of NL-BIO-01 were obtained from Mapryser S.L. (Barcelona, Spain). The seeds were subjected to an atmospheric pressure plasma generated in a DBD made of very simple borosilicate glass containers and spacers (Figure 1).[7] In short, the reactor consists of two parallel metal electrodes (45 mm in diameter) separated by a gap of 12 mm and both covered by glass acting as dielectric plates. To avoid the arching or the microdischarges that might damage the seed coat, helium was used as primary plasma gas. Its flow (5 L_n min^{-1}) was controlled using a mass flow meter and controller (Bronkhorst, Ruurlo, Netherlands). Since the reactor has no tight closures, air impurities are mixed with helium thus leading to the generation of oxygen and nitrogen active species in the plasma which may directly interact with the seeds.[29] A 16 kHz signal was generated making use of a GF-855 function generator (Promax, L'Hospitalet de Llobregat, Spain) connected to a linear amplifier AG-1012 (T&C Power Conversion, Inc., Rochester, NY, USA). A matching network and two transformers (HR-Diemen S.A., Sant Hipòlit de Voltregà, Spain) were connected to the amplifier output in order to increase the voltage up to 20 kV (see the scheme of Figure 1). Wheat seeds (~ 2.5 gr total weight of seeds) with no apparent damage were placed onto the bottom glass piece covering the electrode. The incident power was adjusted to 30 W and the exposure time was varied between 10 s and 900 s.

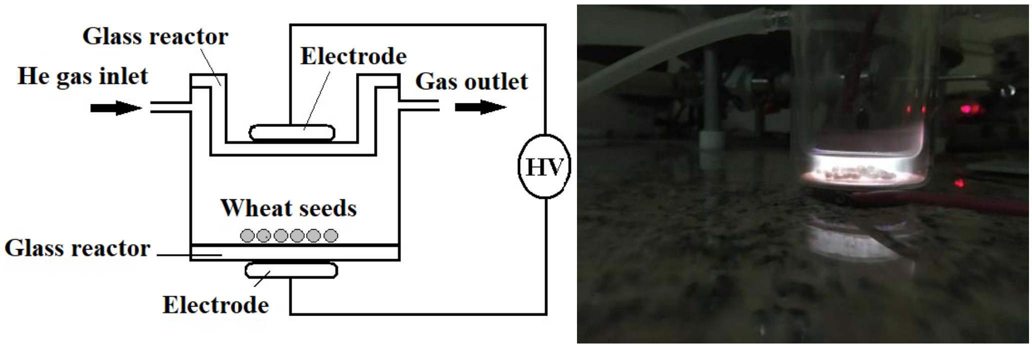

FIGURE 1. *Scheme of the experimental set-up used for the atmospheric plasma treatment of wheat seeds (Left) and photograph of the reactor under operation conditions (Right).*

2.2 Optical emission spectroscopy analysis

Optical emission spectroscopy (OES) measurements were carried out in order to determine the different active species in the He discharge in the presence and in the absence of wheat seeds inside the reactor. To perform this plasma diagnosis step, a quartz optic fiber was connected to a Black Comet spectrometer (Stellarnet, USA) with concave gratings. OES spectra were recorded in the UV–Vis wavelength range of 190–

850 nm with a spectral resolution of 0.5 nm, a solid angle of approximately 0.9 steradians and an integration time of 4 s. It is worth mentioning that no UV emission could be measured below a wavelength of 280 nm because of the DBD reactor being made of borosilicate glass.

2.3 Surface characterization of untreated and plasma-treated seeds

Initial moisture content of wheat seeds was determined gravimetrically according to the standard ASAE S352.2 (ASAE Physical Properties of Agricultural Products Committee). Three replicas of 10 g of wheat seeds were placed in an oven at 130 ºC for 19 h. Weight loss (W_{loss}) induced by plasma treatment was determined by weighing the seeds before (W_i) and just after each plasma treatment (W_f). An averaged weight loss was obtained from 6 replicas of 55 seeds per plasma treatment according to the following equation:

$$Wloss\ (\%) = 100 \times \frac{Wi - Wf}{Wi}$$

The morphology of untreated and plasma-treated seeds was assessed by scanning electron microscopy (SEM) operating at an acceleration voltage of 5 kV (HITACHI S-3500). To avoid charging effects, samples were coated with a layer of gold (thickness ~ 20 nm) making use of a Quorum Q150RS gold sputter coater. In order to have some chemical information of the visualized images, SEM was coupled with energy dispersive X-ray (SEM/EDX) spectroscopy and elemental mapping of the wheat seed interior was performed before and after plasma treatment on cross-sectioned seeds. No metal coating was applied during the sample preparation for these SEM/EDX analyses.

X-ray photoelectron spectroscopy (XPS) measurements of untreated and plasma-treated seeds were acquired using the PHI 5000 Versaprobe II spectrometer equipped with a monochromatic Al K_α X-ray source (hυ = 1486.6 eV) and operated at 25 W. All measurements were conducted at a pressure of at least 10^{-6} Pa and photoelectrons were detected by a hemispherical analyzer positioned at an angle of 45° with respect to the normal of the sample surface. A survey scan and individual high resolution C_{1s}, O_{1s} and N_{1s} spectra were recorded with a pass energy of 187.85 eV (step size = 0.8 eV) and 23.5 eV (step size = 0.1 eV) respectively. Binding energies were calibrated with respect to the C−C/C−H peak of the C1s spectrum at 285.0 eV. Surface composition was quantified via the analysis of the survey scans by means of the Multipak software (V 9.6) while using a Shirley background and complying with the relative sensitivity parameters allocated by the manufacturer.[30] To do so, Gaussian-Lorentzian peak shapes (80–100% Gaussian) were employed and a full width at half maximum (FWHM) set between 1.3 eV and 1.4 eV was adopted for each line shape. In this work, all reported XPS values are equal to the average of 3 values derived from the measurement of 3 random surface spots per condition.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis of seeds was carried out using a Nicolet AVATAR 360 spectrometer in the range of 400 - 4000 cm^{-1}. Measurements were performed using the Smart iTR sampling Accessory (Thermo Scientific Inc., USA). Spectra were obtained from an average of 32 scans using a resolution of 4 cm^{-1}. An advanced ATR correction algorithm (OMNIC 7.3 from Thermo Electron Corporation) was used to correct the band intensity distortion, peak shifts and polarization effects. Corrected ATR spectra were found to properly reproduce band position and shape of their transmission equivalents.[31] The background signal was extracted by means of linear backgrounds applied to the ranges 4000 - 3665 cm^{-1}, 3665 - 3000 cm^{-1}, 3000 - 2650 cm^{-1}, 2650 - 2412 cm^{-1}, 2412 - 2271 cm^{-1}, 2271 -

1800 cm^{-1}, 1800 - 1486 cm^{-1}, 1486 - 1186 cm^{-1} and 1186 - 830 cm^{-1}. Spectra were normalized based on the C-O-C stretching vibration peak located at 1031 cm^{-1}. An averaged ATR-FTIR spectrum was obtained from 5 replicas per one experimental point.

The wetting behavior of wheat seeds was characterized before and after plasma treatment by measuring with a goniometer the static contact angle of a small droplet carefully deposited onto seed surface (~2 μl of 0.5μM water diluted methylene blue solution (Certified by Biological Stain Commission, Sigma-Aldrich)). Methylene blue solution was used in order to better visualize the water drop for a more accurate angle measurement. The surface tension of methylene blue solutions at the micromolar level (71.0 mN/m) is practically identical to that of deionized water (72.0 mN/m).[32] Results are presented as the average of 5 different measurements for a given seed treatment.

2.4. Water uptake measurements and germination

The percentages of water absorption (imbibition) and germination of untreated and plasma-treated seeds were assessed under different irrigation conditions based on a previously reported multi-step methodology.[3,7] In short, for each condition, 55 seeds were placed in plastic Petri dishes containing 4 layers of filter paper (~ 1.25 gr) at 21 ºC. Ionized water was then added to each Petri dish simulating dry (3 ml), medium (6 ml) and wet (12 ml) irrigation conditions. Water absorption was monitored at different time intervals up to 72 h of water exposure and was calculated from 3 replicas of 55 seeds per experimental point using the following equation: where *Wf* and *Wo* correspond to the seed weight after and before water immersion respectively:

$$Water\ absorption(\%) = 100 \times \frac{Wf - Wo}{Wo}$$

It must be pointed out that water uptake data disregarded the cases in which a radicle was already observed since under these conditions the increase in weight corresponds partially to the growth of the plant.

Germination tests were performed under the same experimental conditions used for the water uptake tests and were repeated 6 times for each experimental condition. The dishes were kept in the dark to avoid any influence of light on germination and were closed with Petri covers to avoid any water evaporation. The number of germinated seeds was counted every 20 h, 24 h, 48 h and 72 h. A seed is considered as germinated when a white radicle larger than 1 mm has developed. The germination percentage was calculated using the following equation:

$$Germination\ (\%) = 100 \times \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}$$

3 RESULTS AND DISCUSSION

The first part of this study was devoted to characterize in-situ the active species present in the He plasma with and without wheat seeds inside the DBD reactor. The second part of this work was dedicated to study the physicochemical changes induced at the surface of wheat seeds when exposed to atmospheric pressure DBD plasmas for increasing periods of time. Lastly, the third part was devoted to examine the possible effects of the observed plasma-induced chemical and morphological surface changes on the water uptake and germination capacity of the seeds at different irrigation conditions.

3.1 OES results

The optical characterization of the He discharge is a powerful technique as it unravels insights into the occurring plasma/gas interactions. In fact, OES is able to identify the active species present in the discharge thus shortlisting the potential candidates that could be implicated in the observed surface modifications. The determination of such species is therefore of great relevance in this study. In order to examine whether the presence of wheat seeds in the reactor would cause any variations in the resultant plasma active species, OES spectra were recorded in a seedless and a seed-containing plasma-on reactor (Figure 2). Several emission lines attributed to atomic helium (He I transition $3p^1\ P^0 \rightarrow 2s^1S$ at 501.5 nm, He I transition $3d^3D \rightarrow 2p^3P^0$ at 587.5 nm, He I transition $3d^1D \rightarrow 2p^1P^0$ at 667.8 nm, He I transition $3s^3S^1 \rightarrow 2p^3P^0$ at 706.5 nm and He I transition $3\ s^3S^0 \rightarrow 2p^1P^0$ at 728.1 nm) were detected in the emission spectrum recorded in the seedless reactor. Moreover, emission bands corresponding to molecular N_2 (C-B) second positive system (315.9 nm, 337.1 nm and in the range of 357 nm-410.0 nm), N_2+ (B-X) first negative system (391.4 nm, 427.8 nm and 470.0 nm), OH (A-X) radicals (308.9 nm) and NOx species (NO-γ system in the range of 280.0 nm-300.0 nm) and emission lines attributed to atomic oxygen (O I transition $3p^5P \rightarrow 3s^5S^0$ at 777.4 nm) and atomic hydrogen (Hβ line at 486.1 nm, Hγ at 434.0 and Hα line at 656.3 nm) could be also visualized. All detected species are in perfect conformity with previously recorded OES spectra of He plasmas generated at atmospheric pressure.[33–37] The presence of these non-helium species in a He plasma can be mainly accredited to the dissociation of humid air contaminations lingering in the reactor.[34,36] The characteristic presence of N_2^+ (B-X) in helium discharges stemmed from reactions between highly energetic He metastables and ground state nitrogen in a process known as the penning effect. The second positive system of N_2 (C-B) was mainly engendered by direct energetic electron excitations.[38,39] In fact, the presence of an intense He emission line at 706.5 nm can corroborate the generation of N_2 (C-B) since this emission line is particularly indicative of energetic electrons.[39,40] These results revealed that air impurities could not be completely purged from the plasma reactor at the maximal He flow rate of 5 ln/min.

In the presence of wheat seeds in the reactor, the same emission bands/lines corresponding to He, H and OH were mainly detected in the OES spectrum with the complete absence of the line at 777.4 nm corresponding to atomic oxygen. Moreover, in contrast to the OES spectrum recorded in the seedless rector, low intensities of the bands attributed to N_2 were visualized and extremely small bands accredited to N_2^+ were barely seen. Similar trends in the OES spectra were previously observed in He discharges in the presence of water or water-containing samples.[29,36] Therefore, one can conclude that plasma was desorbing water from the wheat seeds present inside the reactor. In actual fact, the OES spectrum obtained in presence of seeds came to corroborate this hypothesis since significantly more intense OH signal was detected compared to the OES spectrum recorded in the seedless reactor. This is due to a dissociative excitation of the desorbed water. The intensity of the emission line at 706.5 nm corresponding to highly energetic He was lower in the seed-containing reactor. This can be also attributed to the fact that when seeds are present, plasma has a tendency to shift from a diffuse to a filamentary regime. This change in the He discharge mode was also previously perceived when water-containing samples such as H_2O impregnated cotton were put inside the reactor.[29,36]

Overall, when comparing between the OES spectra recorded with and without seeds in the reactor, one can conclude that the dissociation of water molecules desorbed from the wheat seeds predominated over other reactions involving air impurities given the higher intensity of OH band, lower intensities of N_2 and N_2^+ bands and absence of O emission line. OES spectra recorded at different helium flow rates with and without seeds in the reactor can be found in the supporting information (Figures S1 and S2).

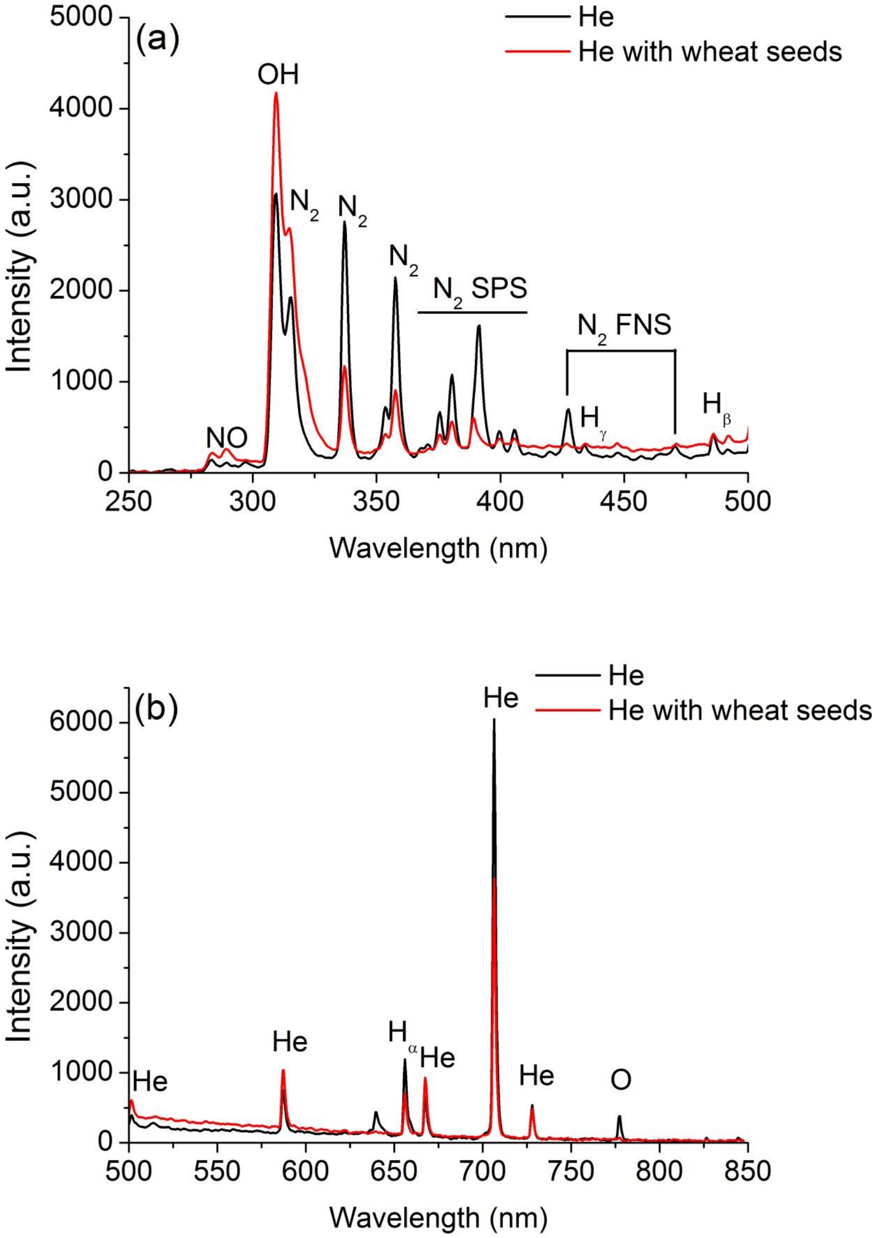

FIGURE 2. *UV-VIS emission spectra obtained during a He plasma treatment with and without wheat seeds inside the plasma reactor. (a) Wavelength range: 250-500 nm; (b) Wavelength range: 500-850 nm.*

3.2 Weight loss and surface modifications promoted by DBD plasma treatment

Biopolymeric materials exposed to air plasmas usually exhibit a measurable weight loss due to different physical or chemical processes such as water desorption and/or hydrocarbon oxidative etching.[41,42] Weight loss of wheat seeds increased almost linearly with plasma treatment time (Figure 3) reaching a noticeable 2.5 % weight loss after 900 s of plasma exposure.

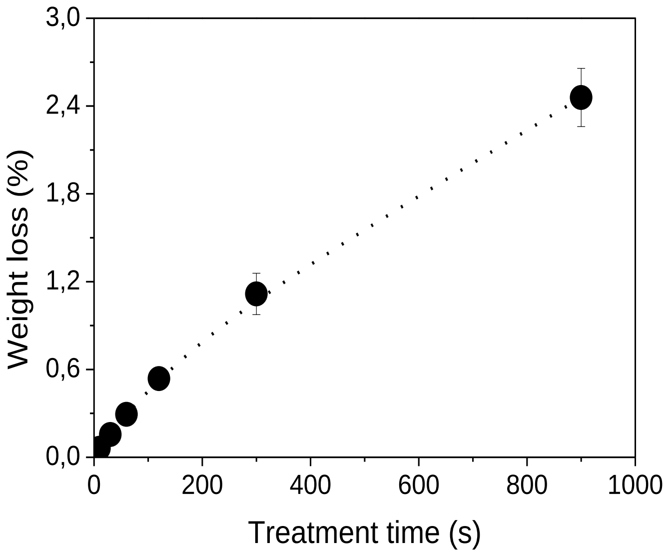

FIGURE 3. *Weight loss of wheat seeds as a function of DBD plasma treatment time.*

This quasi-linear behavior differs from a certain tendency to an asymptotic behavior previously observed for other seeds such as, for example, nasturtium seeds plasma-treated using the same reactor and under the same experimental conditions.[7] In order to have clear view whether this linear weight loss is due to water desorption and/or changes in surface roughness/morphology resulting from plasma-induced etching processes, surface and cross sections of wheat seeds were studied by means of SEM and SEM/EDX.

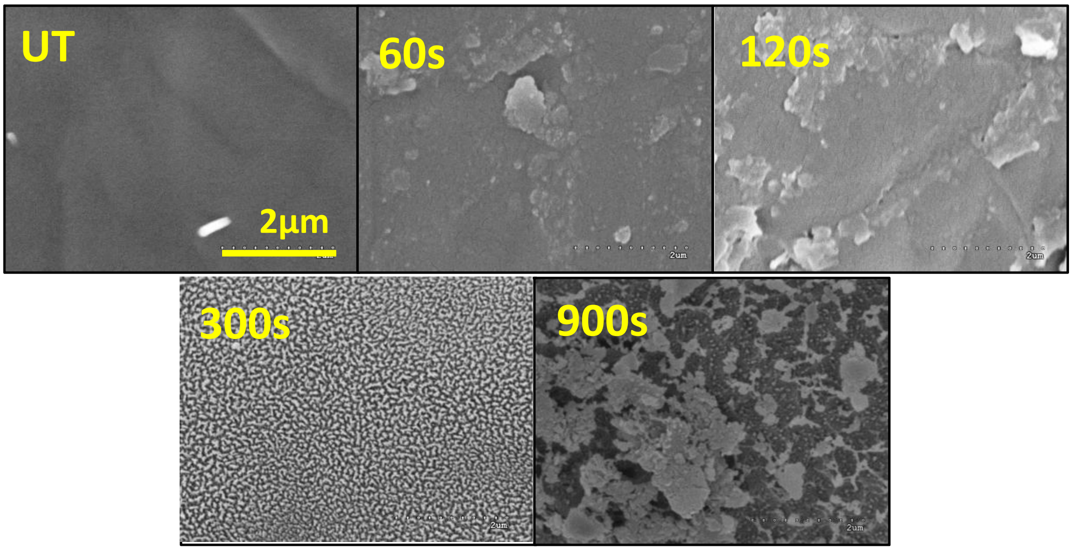

FIGURE 4. *SEM images of the surface of untreated and plasma-treated wheat seeds at different plasma treatment times.*

Figure 4 evidences that the seed pericarp was progressively etched and damaged by increasing the plasma exposure. The very smooth surface of an untreated seed started to be eroded until a randomly nano-grooved outer layer was observed at a treatment time of 300 s. When further extending the treatment time to 900 s, the outer layer was highly grinded down with some parts delaminating and another layer located beneath the first one appearing. This surface erosion is engendered by the increasing amount of chain scissions at the wheat seed surface induced by the bombardment of the highly energetic plasma reactive species of which He species. In fact, the numerous chain scissions occurring at extended treatment times are actually known to cause the generation of oligomers and the desorption of volatile compounds from a biopolymer or a synthetic polymer surface, thus etching it.[43,44] Therefore, the weight losses seen in Figure 3 can be associated with the resulting effects of both water desorption and etching processes delaminating parts of the outer seed layer. The linear aspect of the weight loss curve is thus due to the gradually more pronounced etching effect occurring when increasing the treatment time and most likely to the softer wheat seed pericarp compared to other seeds such as the nasturtium seeds. In fact, since the pericarp of wheat seeds is not chemically uniform and has different compositions in its internal and external parts, the modification and/or the elimination of the outer carbon reach layer can also alter the diffusion of water molecules from the inner parts of the seeds to the ambient atmosphere. In contrast, the pericarp of nasturtium seeds is less prone to etching which results in a considerably less pronounced elimination of the outer surface layer responsible of the water diffusion from the inner parts of the seed to the ambient atmosphere. Therefore, it is suggested that the previously observed more asymptomatic weight loss could be related to an incomplete removal of the pericarp outer layer and to the cessation of water release after a certain plasma treatment time.[7] When taking a look at the SEM images of the cross-sectioned seeds, one can clearly discern the noteworthy surface character of the etching processes as evidenced by the similar microstructure of the inner part of seeds before and after plasma treatment (Figure 5).

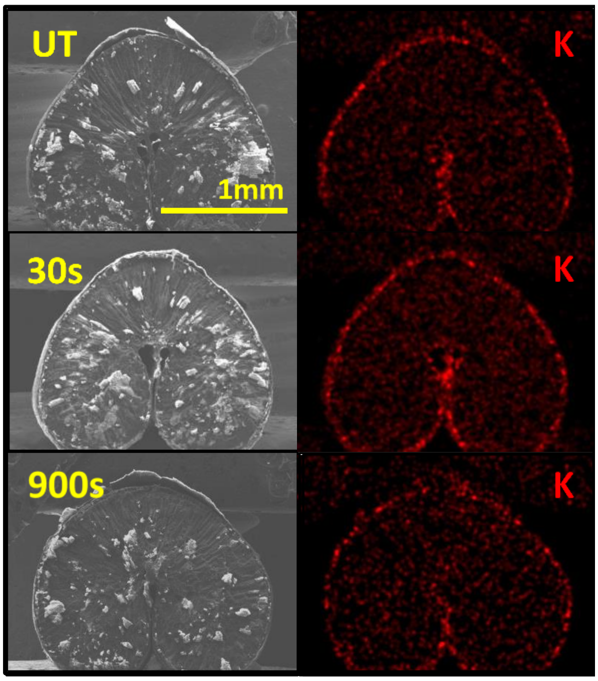

FIGURE 5. *SEM images and potassium EDX mapping of the interior of cross-sectioned wheat seeds before and after plasma treatments of 30 s and 900 s.*

Moreover, EDX micro-analysis of the distribution of some elements (potassium, magnesium, phosphorus and nitrogen) on cross-sectioned seeds confirmed that no observable chemical changes are induced in the interior of the seeds after plasma treatment (see the EDX maps of potassium in Figure 5 and the EDX maps of the other elements in Figure S3 of the supporting information). Therefore, the DBD plasma treatments only affected, as expected, the top layers of the pericarp and induced, in contrast to the seed bulk, only significant chemical modifications at the seed surface as can be concluded from the XPS analysis. Table 1 shows the surface atomic chemical composition of wheat seeds as a function of plasma treatment time. Results revealed that untreated wheat seeds have a carbon-rich surface (≈ 91 %) with a relatively small oxygen content (≈ 7 %) and a small nitrogen content (≈ 1 %). It should be noted that in the first two nanometers of wheat seed surface (penetration depth of XPS technique) few amount of oxygen atoms corresponding to hemicellulose, cellulose or lignin can be detected.

TABLE 1. *Surface atomic chemical composition (%) of untreated and plasma-treated wheat seeds determined by XPS.*

Plasma treatment time (s)	**Atomic concentration (%)**			
	C_{1s}	O_{1s}	N_{1s}	K_{2p}
0	91.3 ± 0.8	7.3 ± 0.5	1.4 ± 0.4	_
10	83.7 ± 0.5	14.8 ± 0.7	1.1 ± 0.5	0.4 ± 0.2
30	80.4 ± 0.6	17.7 ± 0.2	1.4 ± 0.2	0.5 ± 0.2
60	79.0 ± 0.8	19.1 ± 0.9	1.1 ± 0.1	0.7 ± 0.3
120	79.5 ± 1.4	16.8 ± 1.5	1.5 ± 0.3	2.2 ± 0.2
300	73.6 ± 6.9	23.4 ± 6.5	1.9 ± 0.3	1.1 ± 0.2
900	60.3 ± 1.0	34.6 ± 1.6	1.3 ± 0.7	3.8 ± 0.4

According to table I, the high carbon content decreased progressively while, in return, the oxygen content increased as a function of plasma treatment time. In fact, helium plasma contains several non-reactive species and ions such as excited atoms, electrons, photons and molecules that are able to break down C-H and C-C chemical bonds thus forming free radicals on the wheat seed surface. Chemically reactive oxygen and nitrogen species originating from air impurities lingering inside the reactor and from water molecules that plasma desorbs from the seeds are also generated. Such species can then interact with the plasma-induced free radicals to oxidize the pericarp top layers through the insertion of some oxygen-containing functional groups.[43] When increasing the plasma treatment time to 120 s, the oxygen content slightly decreased to approximately 17 % suggesting a competitive aspect between the incorporation of functional groups and chain scissions these latter due to other energetic species (Helium, electrons, etc.). Then, for 300 s or longer treatment times oxygen content significantly increased, precisely when SEM micrographs in Figure 4 reveal a substantial erosion of the seed surface by the plasma etching processes. This tendency must be associated with the progression of the oxidative etching process that would become more prominent and lead to numerous random chain scissions. When further treating the seeds to reach an exposure time of 900 s, the carbon content decreased considerably to reach 60 % suggesting the occurrence of even more chain scissions leading to a degradation of the carbon-rich backbone of the wheat seed that got in return exceedingly oxidized. It is worth mentioning that no nitrogen-containing functionalities were incorporated onto the wheat seed surface since no significant changes in the nitrogen content was perceived. This can be correlated with the relatively low intensity bands attributed to active nitrogen species in the OES spectrum recorded when seeds were present in the reactor (Figure 2). A small concentration of potassium atoms also appeared at the surface of the plasma-treated wheat seed and increased with the plasma treatment time to reach approximately 4 % after 900 s. Surface diffusion of potassium or calcium has been reported for quinoa and Arabidopsis seeds exposed to plasma in studies that illustrated how the surface state of plasma-treated seeds becomes activated by the accumulation of ions and other charged groups.[6,45] High resolution K2p spectra (Figure S4 in supporting information), clearly revealed the increase in the amount of segregated potassium as a function of the treatment time. The diffusion of potassium from the interior to the outer layers of the wheat seed is presumably propelled by the polar character of the plasma-generated oxygen-containing functionalities groups on the seed surface and/or by variations in the surface potential during plasma treatment.[6]

For a more detailed analysis of the particular types and relative concentrations of the surface chemical groups generated by the different plasma treatments, high resolution C1s spectra were deconvoluted (non deconvoluted high resolution spectra corresponding to O1s and N1s can be shown in Figure S4 in supporting information). The C1s envelope of the wheat seed surface was decomposed into 4 different components as shown in Figure 6: a peak at 285.0 eV ascribed to **C**-C/**C**-H bonds, a peak at 286.4 eV corresponding to **C-**O bonds, a peak at 287.7 eV assigned to **C=**O bonds and a peak at 289.1 eV accredited to O-**C=**O bonds. Based on these C1s curve fittings, the relative amount of every carbon-containing surface functionality was acquired and the averaged data are summarized in Table 2. Results revealed that all plasma treatments concurrently incorporated C-O, C=O and O-C=O bonds at the expenses of C-C and C-H groups. In fact, the high relative concentration of C-C/C-H bonds on the surface of the untreated seeds (≈ 85 %) progressively decreased with increasing the treatment time to reach approximately 70 % after 60 s of plasma exposure. This confirms again our previous hypothesis stating that plasma attacks C-C and C-H bonds to form free radicals able to react with oxygen. The relative amounts of C-O, C=O and O-C=O bonds reaching values of 16 %, 7 % and 6 % respectively after 60 s of plasma exposure, slightly decreased when increasing the treatment time to 120 s. This small decline in the plasma-induced functional groups corroborates the survey scan results that suggested the occurrence of some chain scissions. A significant increase in the relative amount of C-O and C=O bonds to 22 % and 12 % respectively is then observed when increasing the treatment time to 120 s. However, the associated high standard deviations validate again the random competitive bond scissions and insertions caused by the highly energetic plasma species, particularly for a He rich plasma as the one utilized in this work. At 900 s of plasma treatment, the relative concentration of C-O functionalities (≈ 38 %) reached almost that of C-C functionalities (≈ 40 %) with a further increase in C=O groups (≈ 18 %) suggesting that the original carbon-carbon backbone is being markedly degraded by a pronounced oxidative etching effect and consequently chemical compounds located below the original carbon rich layer, such as cellulose and hemicellulose compounds rich in C-O functionalities, are now appearing in the outermost part of the plasma treated wheat surface.

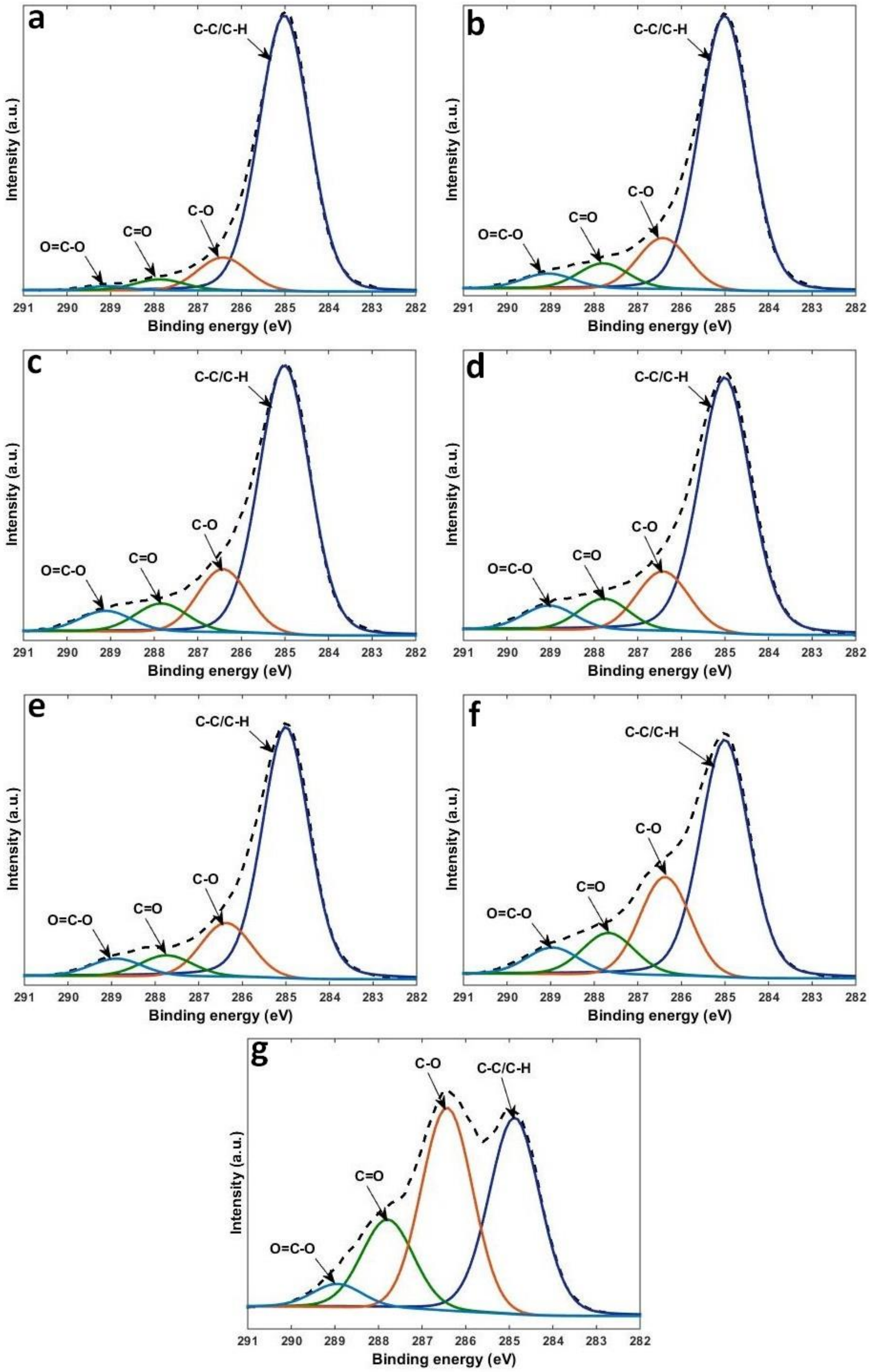

FIGURE 6. *C1s curve deconvolution of untreated and plasma-treated wheat seeds for different plasma treatment time (a: untreated, b: 10 s, c: 30 s, d: 60 s, e: 120 s, f: 300 s and g: 900 s).*

TABLE 2. *C1s curve fitting results of untreated and plasma-treated wheat seeds for different plasma treatment times.*

Plasma treatment time (s)	C1s curve fit (%)			
	C-C/C-H 285.0 eV	C-O 286.4 eV	C=O/O-C-O 287.7 eV	O-C=O 289.1 eV
0	85.6 ± 1.4	9.8 ± 0.2	3.6 ± 0.1	0.9 ± 0.1
10	75.9 ± 1.4	13.7 ± 0.7	6.8 ± 0.2	4.1 ± 0.5
30	71.4 ± 1.1	15.6 ± 0.6	7.5 ± 0.4	5.4 ± 0.1
60	70.7 ± 0.9	16.1 ± 0.9	7.4 ± 0.5	5.8 ± 0.4
120	75.0 ± 2.3	14.9 ± 0.8	4.5 ± 1.5	5.6 ± 0.4
300	60.5 ± 13.0	22.1 ± 6.5	12.1 ± 6.9	5.3 ± 0.5
900	40.1 ± 4.4	37.7 ± 3.6	17.6 ± 1.4	4.6 ± 1.0

The surface chemical modification of plasma-treated wheat seeds has been also studied by FTIR-ATR, which has a different penetration depth of analysis than XPS that is only affected by the outmost layers of the pericarp (~1.5 µm for FTIR-ATR versus ~2 nm for XPS).[46] Knowing that the wheat seed pericarp thickness is around 40 µm, the information retrieved by FTIR-ATR would still be restricted to the external layers of the pericarp, with no contribution of the inner parts such as the testa, the aleurone layer, the endosperm or the embryo. The relatively short etching time of the experiments does not alter this basic principle as proved by the SEM observation of cross-sectioned seeds in Figure 5. Nevertheless, this analysis can indicate whether the plasma-induced etching effect is harsh enough to affect deeper layers than those affected by plasma-functionalization (a few nanometers).

Figure 7 shows the normalized FTIR-ATR spectra of untreated and plasma-treated wheat seeds at different exposure times. The spectrum of the untreated seed is characterized by different peaks that can be attributed to OH stretching bands, C-H symmetrical and asymmetrical stretching bands of cellulose and lignin, C=O and C=C stretching bands of lignin, aromatic skeletal vibrational bands of lignin, C=C aromatic ring of lignin, OH bending bands of cellulose, hemicellulose and lignin, C-H aromatic skeletal bands of lignin, C-H bending bands of lignin, cellulose and hemicellulose and C-O and C-O-C stretching bands of lignin, cellulose and hemicellulose. These contributions due to hemicellulose (H), cellulose (C) and lignin (L) compounds that are present in the pericarp are identified and assigned to their characteristic bands in the plots of Figure 7. [47–50] After plasma treatment, wheat seed spectra exhibited a decrease in the intensity of the bands attributed to -OH and -CH groups (Figure 7a) whereas no significant changes were observed after short plasma treatment times (≤ 120 s) in the spectral region of 1800-650 cm^{-1} (Figure 7b). For longer treatment times (300 s and 900 s), a net decrease in C=O (1730 cm^{-1}) and C=C (1635 cm^{-1}) chemical groups, which are associated with lignin, can be detected in the spectra showing a shape that resembles that of the inner part of the pericarp.[49] This evolution in the FTIR spectral shape agrees with the progressive etching of the wheat pericarp observed by SEM analysis (Figure 4) and confirms that the outermost carbon-rich layer is being markedly etched away by the plasma active species, particularly for long plasma exposure times.

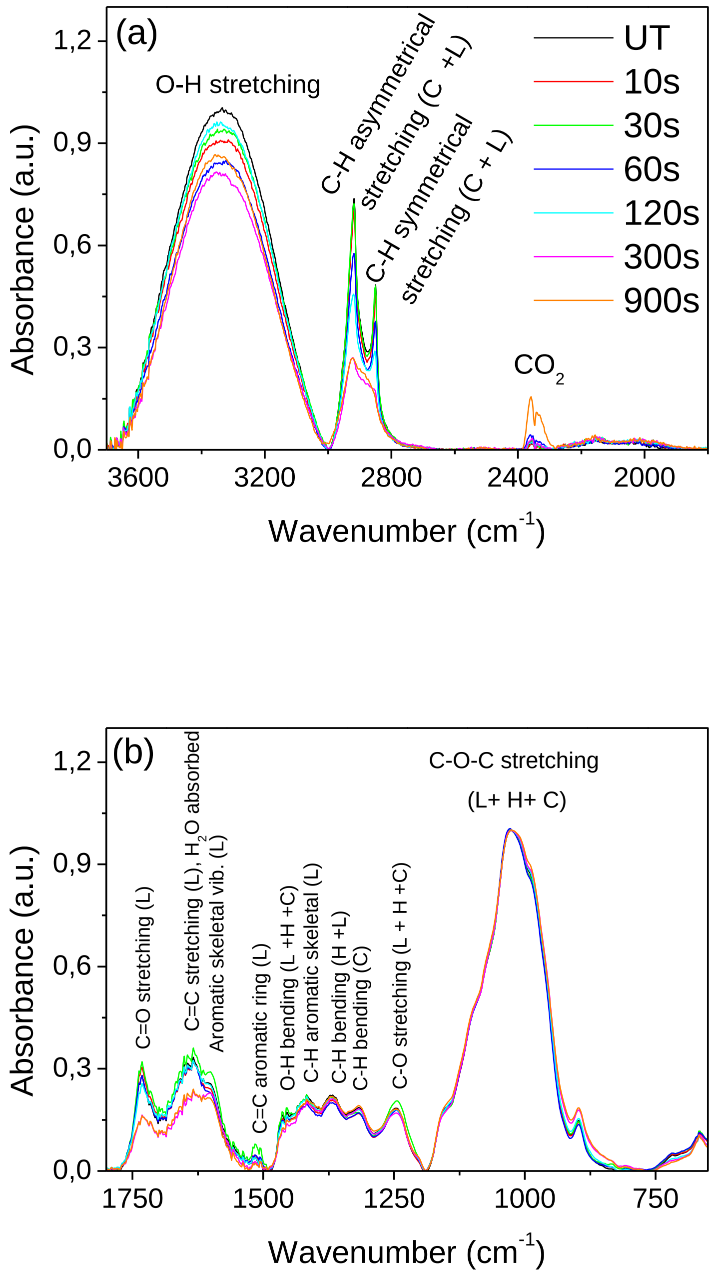

FIGURE 7. *Normalized FTIR-ATR spectra (average of 5 replicas) of untreated and plasma-treated wheat seeds for increasing periods of time. The assignation of specific bands due to H, C and L compounds is included in the graphs ((a) Range: 3700-1800 cm^{-1}; (b) Range: 1800-650 cm^{-1}).*

3.3 Water uptake under different irrigation conditions

A common result of plasma treatment is an increase in the seed hydrophilicity; an effect that is usually accompanied with a net increase in water uptake capacity. Examples of this behavior have been reported for quinoa, nasturtium, mulungu and other seeds.[1,6,7,10] In the present work, the water contact angles of wheat seeds subjected to plasma treatments for increasing periods of time were first determined.

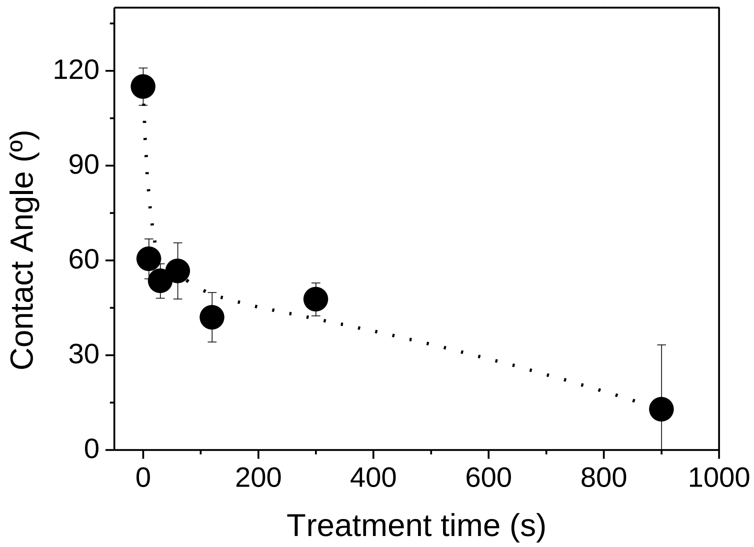

FIGURE 8. *Apparent water contact angle (average of 5 replicas) of wheat seeds as a function of DBD plasma treatment time.*

According to Figure 8, untreated wheat seeds have a hydrophobic surface since they presented an apparent water contact angle close to 120 º; a value similar to that found by other authors.[18,23] Just after 10 s of plasma treatment, the apparent contact angle decreased noticeably to reach a value of 55 º. A progressive further decrease in the apparent contact angle continued to be observed when increasing the treatment time to reach a very hydrophilic surface (i.e., apparent contact angle of 10 º or less) after 900 s of plasma exposure. This gradual increase in the wettability is due to the incorporation of increasing amounts of polar oxygen-containing functionalities as a function of the plasma treatment time. Moreover, the pronounced etching effect eroding the pericarp at extended treatment times might have an additional role in the decrease of the water contact angle. In fact, the creation of surface grooves and the delamination of some parts of the pericarp increase the roughness of the wheat seed surface, which in turn increases the surface wettability because of the water penetration in the roughness furrows. This behavior, so called homogeneous wetting, can be described by the Wenzel equation.[51]

In order to study the interplay between the surrounding water availability and the plasma treatment effects, a quantitative analysis of the seed water uptake capacity was carried out for 3 water dosages aimed at reproducing dry (3 ml), medium (6 ml) and wet (12 ml) irrigation conditions. The results of these experiments are reported in Figure 9. Unlike the significant variation in surface wettability (c.f. Figure 8) and the large changes in the pericarp surface composition induced by plasma treatment (c.f., Figures 6 and 7), only slight or negligible differences were observed in the final water uptake of untreated and plasma-treated seeds (imbibition of 72 h), independently of the treatment time. Nonetheless, the irrigation conditions had a significant effect on the water adsorption after 72 h of imbibition with large differences in the water uptake capacities of untreated seeds reaching approximately 34 %, 48% and 84% at dry (3 ml), medium (6 ml) and wet (12 ml) water dosages respectively. When taking a look at shorter imbibition times (< 4 hours), one can however notice that plasma treatments could ameliorate the water adsorption at dry (3 ml) and medium (6 ml) irrigation conditions. The water uptake capacities increased with increasing the plasma treatment time as a result of the more hydrophilic surface permitting more water diffusion.

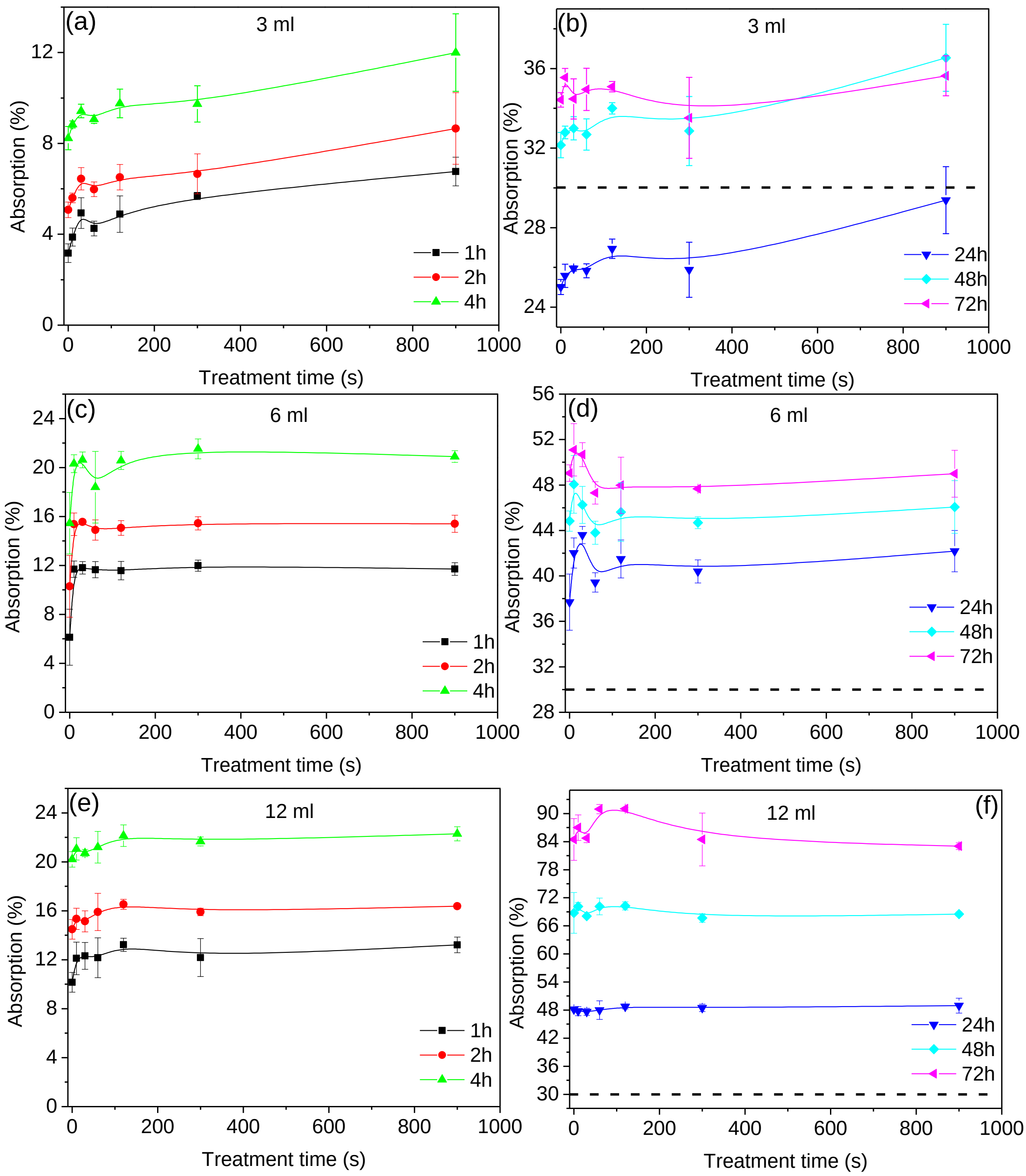

FIGURE 9. *Water uptake of wheat seeds in dry, medium and wet irrigation conditions as a function of plasma treatment time for different imbibition periods (a, b: dry condition (3 ml); c, d: medium condition (6 ml); e, f: wet condition (12 ml); a, c, e: short imbibition time (1-4h); b, d, f: long imbibition times (24-72 h)). The dotted line represents the threshold of water imbibition required to induce germination, according to Evans et al.* [28]

The abundance of water in the wet condition (12 ml) seems to neutralize any possible difference that could have been distinguished between untreated and plasma-treated samples even at short imbibition times. Taken into account the initial moisture content of wheat seeds (~ 12 %) and the requirement of an average value of 40 % of water content to initiate germination, a seed water uptake close to 30 % can be estimated as a threshold triggering germination.[28] This limit has been highlighted as a dashed line in the plots of Figure 9. In principle, the availability of water seems to be the key factor determining the capacity of seeds to accumulate this 30 % of water independently on the plasma treatment. This threshold was attained at 48 h of imbibition in case of the dry state (3 ml) and was already exceedingly surpassed at 24 h of imbibition in case of the medium (6 ml) and wet (12 ml) conditions.

3.4 Germination of untreated and plasma-treated wheat seeds

In the final part of this study, the germination of wheat seeds as a function of plasma treatment time and for increasing periods of water imbibition (20 h – 72 h) was assessed in case of the 3 irrigation conditions (dry, medium and wet) previously utilized in the water uptake experiments (Figure 10). Results firstly evidenced that the germination primarily depends on the irrigation conditions and the associated times needed to incorporate the minimum water content threshold required for germination.[28] In dry conditions (3 ml), the germination required periods longer than 24 h while in medium and wet conditions the germination started already at 20 h after seeding or earlier. One can therefore conclude that in the abundance of water, 24 h is more than sufficient to induce a significant germination of untreated seeds, but with a much higher percentage in case of the wet environment (≈ 90 %) than in case of the medium conditions (22%).

However, regardless of the irrigation condition, results revealed that after 72 h of water imbibition, the overall germination percentage reached over 95 % for untreated and plasma-treated seeds. This observation also indicated that the maximal germination capacity could be attained with or without the influence of plasma treatment. Nonetheless, plasma was clearly shown to have a net effect in accelerating the germination during the first 48 h after seeding, particularly for short treatment times (less than 120 s) and in case of dry and medium irrigation conditions. For instance, in the dry condition and after an imbibition of 48 h, the germination percentage gradually increased with increasing the plasma exposure from approximately 20 % for the untreated seeds to 40 % for the seeds plasma-treated for 120 s, after which this percentage slightly decreased (Figure 10.a). When comparing the germination percentage after 24 h of imbibition of the seeds in 6 ml of water, one can also notice an increase from 22% to 65% and then a decrease to 30 % for untreated seeds and plasma-treated seeds subjected to 60 s and 900 s of plasma exposure respectively (Figure 10.b). This positive effect seems to coincide with the increase in the water uptake capacity observed at short imbibition times in seeds plasma-treated for short times (Figure 9). The decelerated germination that was again noted at extended treatment times is also probably caused by the extensively damaged pericarp resulting from the plasma-induced etching effects. Several studies have indeed shown that short plasma treatment times are associated with better germination results than extended plasma exposures.[52–54] In particular, Filatova et al. have particularly detected an increased germination of wheat seeds subjected to a plasma treatment for short times up to 7 min compared to longer times that drastically impaired the germination because of the induced seed damage. It is worth mentioning as well that an eroded seed surface was previously demonstrated to be more prone to infections.[54]

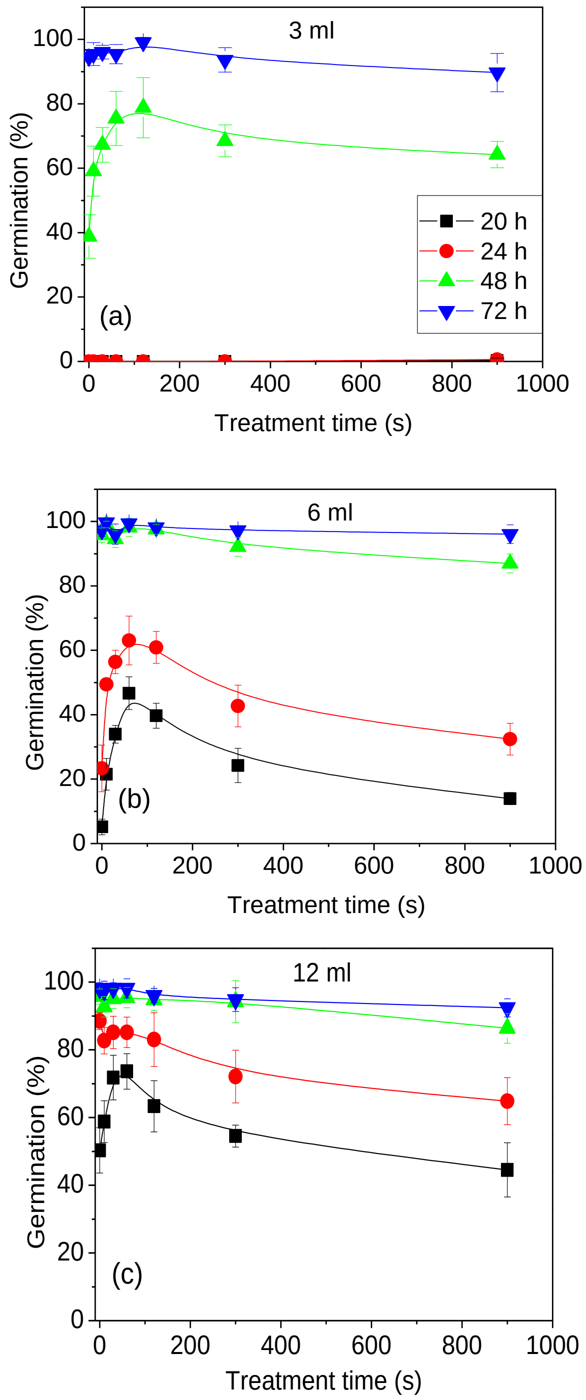

FIGURE 10. *Germination ratios of wheat seeds in dry, medium and wet irrigation conditions as a function of plasma treatment time for different imbibition periods (a: dry condition (3 ml); b: medium condition (6 ml); c: wet condition (12 ml)).*

In line with the ample evidence in literature about the positive effect of plasma treatments in improving seed germination, a similar effect can be noticed in this study for wheat seeds with extra findings showing that: 1) water scarcity (dry and medium environments) that drastically conditions the germination can be compensated by short plasma treatments while 2) water abundance significantly accelerates the germination that becomes less affected by plasma treatment.[7]

4 CONCLUSION

The ultimate goal of this present paper was to examine the synergetic effect of plasma treatment and irrigation condition on the water uptake and germination capacity of wheat seeds. In addition to the traditional methodologies commonly used in the literature to study the effects of plasma treatments on seeds such as water contact angle goniometry and SEM, this paper used XPS to more extensively investigate the chemical variations taking place at the wheat seed surface. To do so, wheat seeds were firstly subjected to an atmospheric DBD plasma treatment for different exposure times ranging from 10 s to 900 s. Results revealed that plasma treatment caused a gradual decrease in the wheat seed weight as a function of treatment time because of the oxidative plasma etching effect eroding and damaging the seed pericarp. In fact, XPS results showed that plasma treatment significantly increased the surface oxygen content by triggering the incorporation of oxygen-containing functionalities in the form of C-O, C=O and O=C-O bonds. At extended treatment time, the relative concentration of C-O groups reached almost that of C-C groups suggesting that the initial carbon-carbon backbone of the pericarp is being degraded by a pronounced etching effect. This finding was further confirmed by FTIR measurements showing that the lignin of the outer pericarp layers became partially depleted after long treatments. Plasma-induced polar groups greatly enhanced the surface wettability of wheat seed that was reflected by an increase in the water uptake of seeds treated for short times. The positive plasma effect on water uptake was however less perceived when water was abundant. Germination results showed that, regardless of the irrigation conditions, the maximal seed germination capacity was reached after long imbibition times for untreated and plasma-treated seeds. Therefore, surface modifications affecting the pericarp at long plasma treatment times did not seem to affect the embryo germination capacity. Nonetheless, short plasma treatments considerably accelerated the seed germination especially in dry and medium water content conditions as a result of the pericarp oxidation. A question about whether this oxidation affects the diffusion rate of germination factors such as oxygen or other components is still open and should be further analyzed in the future.

ACKNOWLEDGEMENTS

We thank EU-FEDER funds and the MINECO (projects MAT2016-79866-R, MINECO-CSIC 201860E050) and Junta de Andalucía (project P12-2265 MO). Rouba Ghobeira would also like to thank the Research Foundation Flanders (FWO-grant number 12ZC720N) for financing her post-doctoral position. The authors would also like to thank the contribution of the scanning electron microscopy service of the Institute of Marine Sciences (ICM-CSIC).

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests

Received: ((will be filled in by the editorial staff)); Revised: ((will be filled in by the editorial staff)); Published online: ((please add journal code and manuscript number, e.g., DOI: 10.1002/ppap.201100001))

HIGH RESOLUTION FIGURE FILES

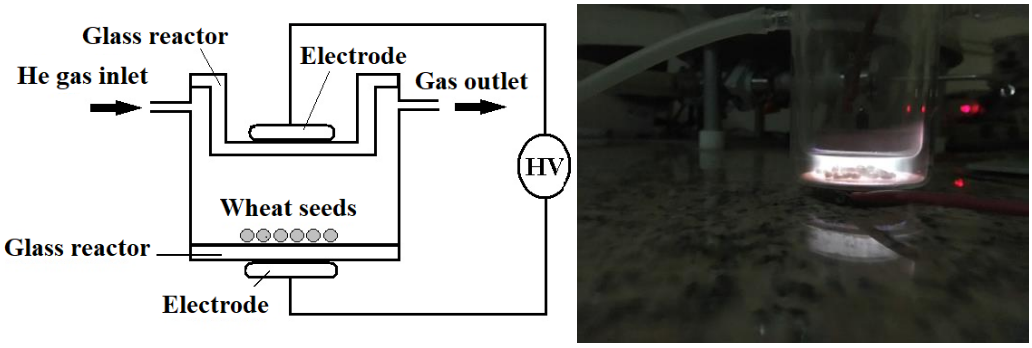

FIGURE 1. *Scheme of the experimental set-up used for the atmospheric plasma treatment of wheat seeds (Left) and photograph of the reactor under operation conditions (Right).*

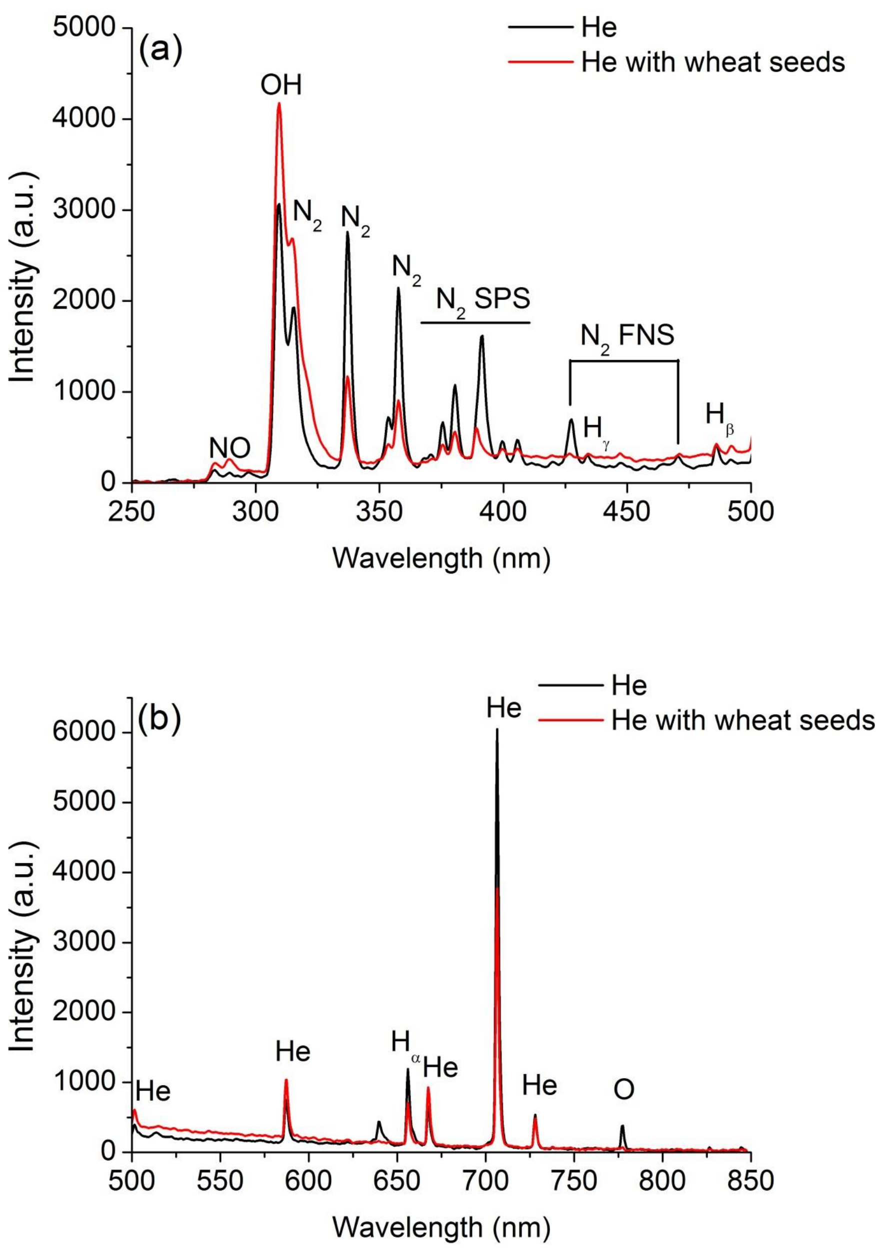

FIGURE 2. *UV-VIS emission spectra obtained during a He plasma treatment with and without wheat seeds inside the plasma reactor. (a) Wavelength range: 250-500 nm; (b) Wavelength range: 500-850 nm.*

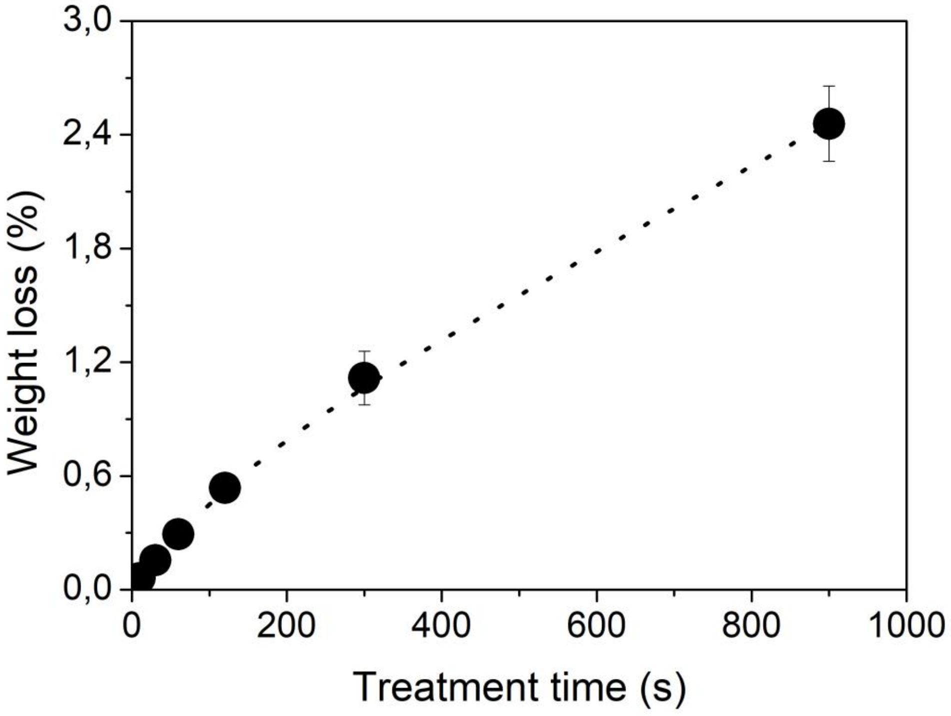

FIGURE 3. *Weight loss of wheat seeds as a function of DBD plasma treatment time.*

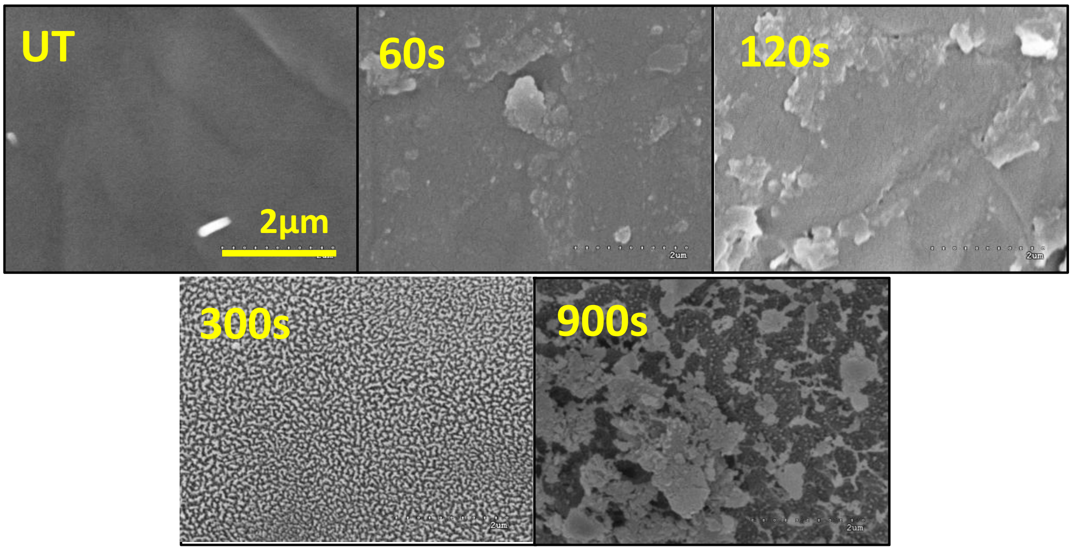

FIGURE 4. *SEM images of the surface of untreated and plasma-treated wheat seeds at different plasma treatment times.*

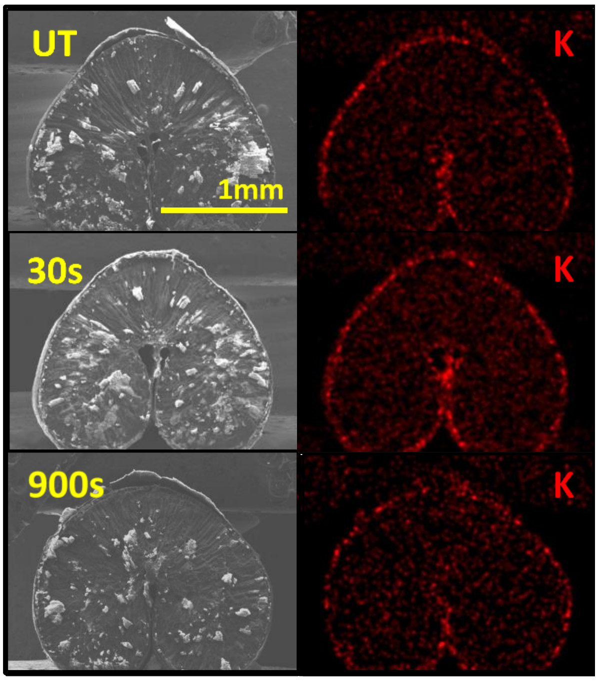

FIGURE 5. *SEM images and potassium EDX mapping of the interior of cross-sectioned wheat seeds before and after plasma treatments of 30 s and 900 s.*

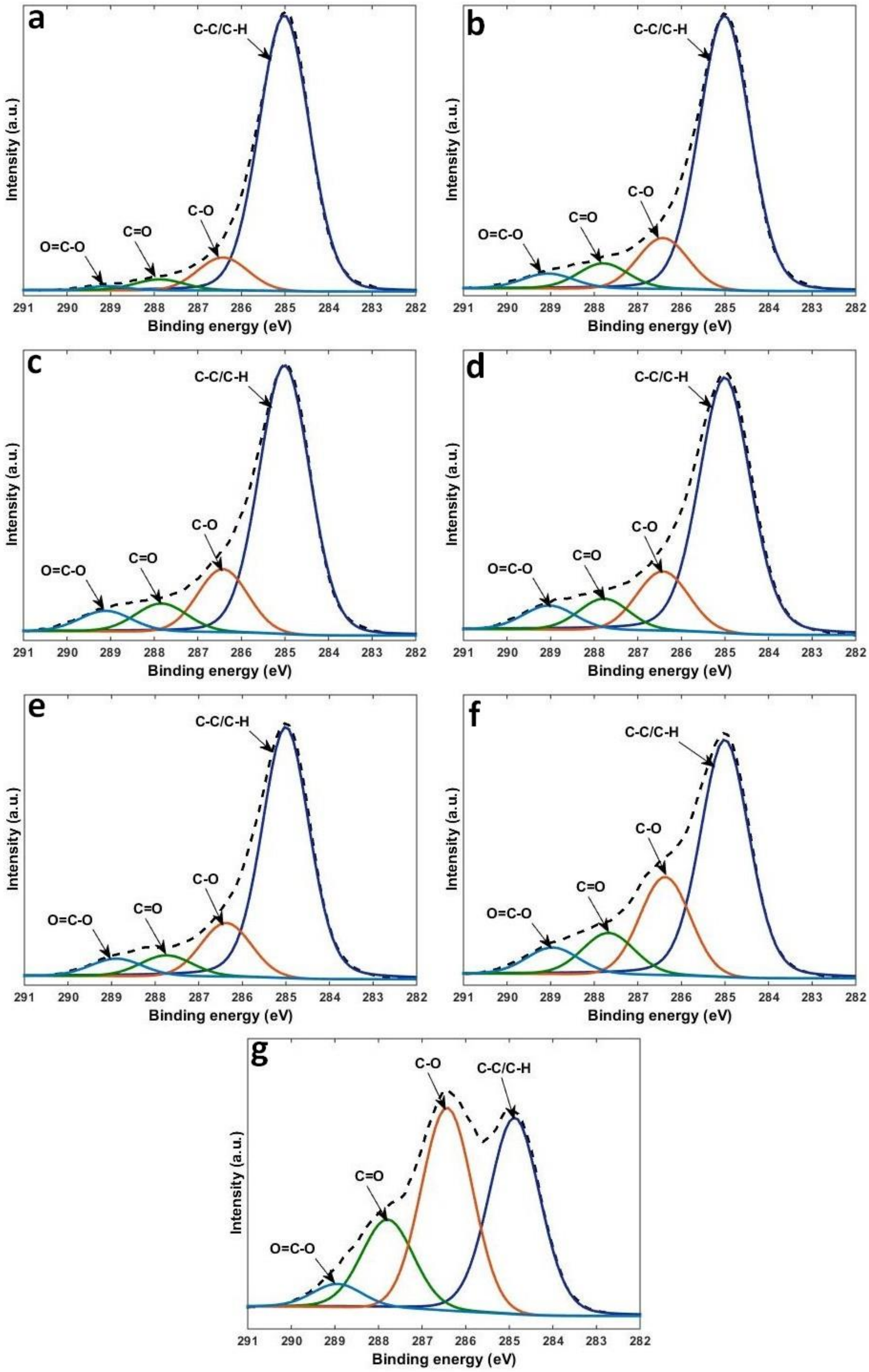

FIGURE 6. *C1s curve deconvolution of untreated and plasma-treated wheat seeds for different plasma treatment time (a: untreated, b: 10 s, c: 30 s, d: 60 s, e: 120 s, f: 300 s and g: 900 s).*

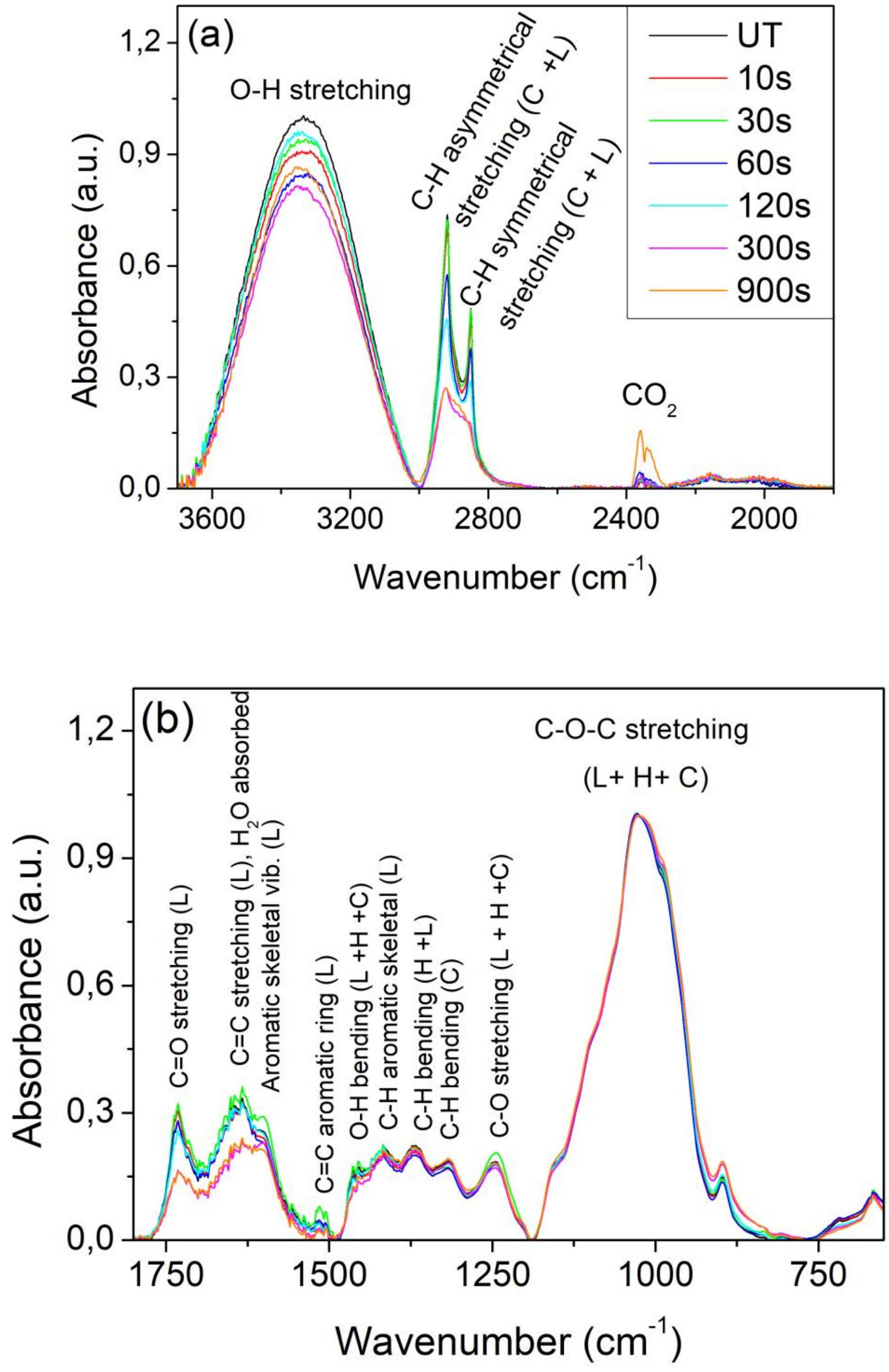

FIGURE 7. *Normalized FTIR-ATR spectra (average of 5 replicas) of untreated and plasma-treated wheat seeds for increasing periods of time. The assignation of specific bands due to H, C and L compounds is included in the graphs ((a) Range: 3700-1800 cm^{-1}; (b) Range: 1800-650 cm^{-1}).*

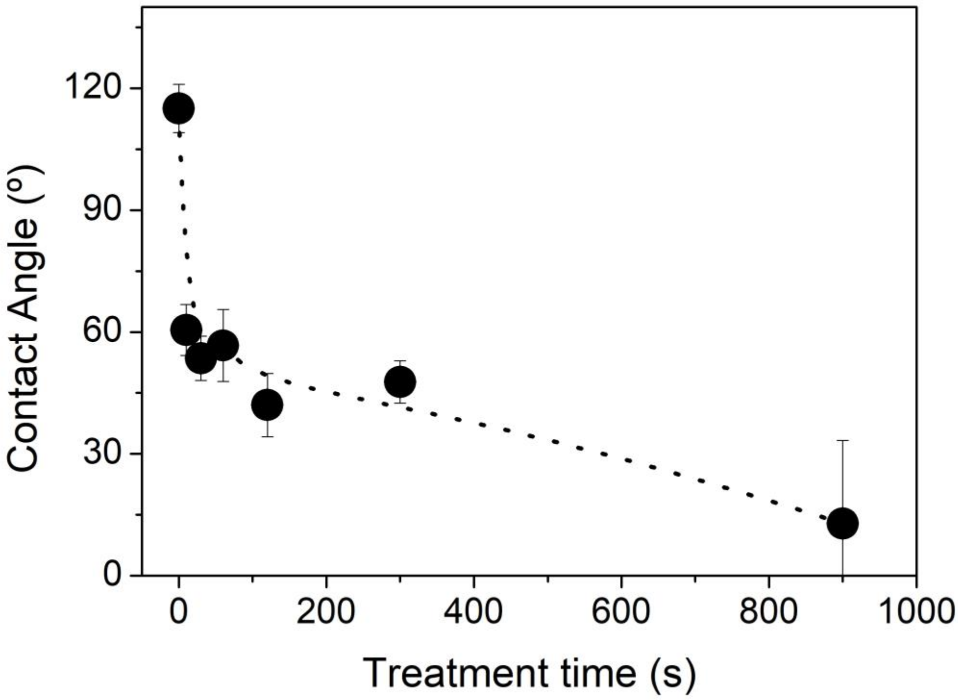

FIGURE 8. *Apparent water contact angle (average of 5 replicas) of wheat seeds as a function of DBD plasma treatment time.*

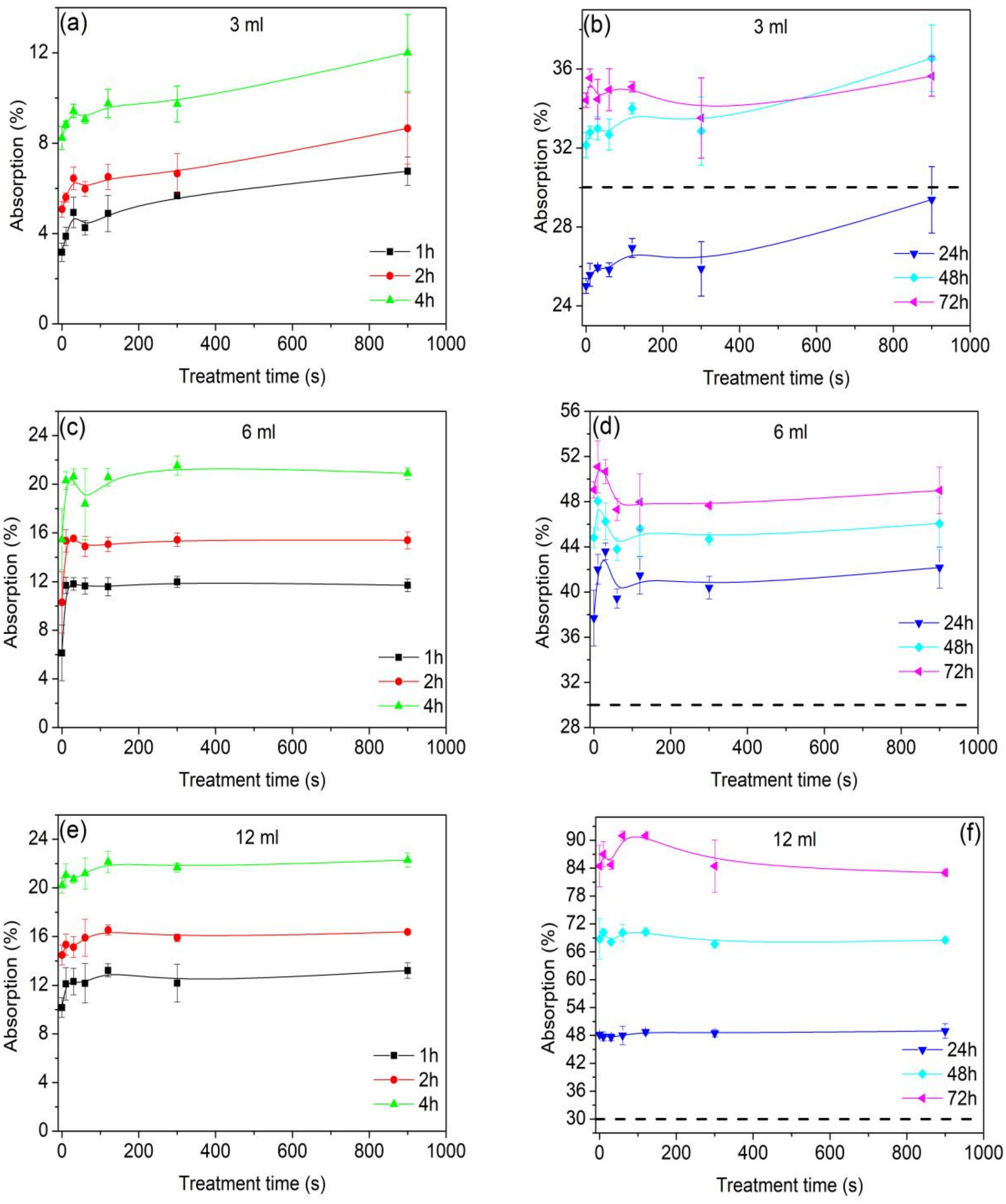

FIGURE 9. *Water uptake of wheat seeds in dry, medium and wet irrigation conditions as a function of plasma treatment time for different imbibition periods (a, b: dry condition (3 ml); c, d: medium condition (6 ml); e, f: wet condition (12 ml); a, c, e: short imbibition time (1-4h); b, d, f: long imbibition times (24-72 h)). The dotted line represents the threshold of water imbibition required to induce germination, according to Evans et al..* [28]

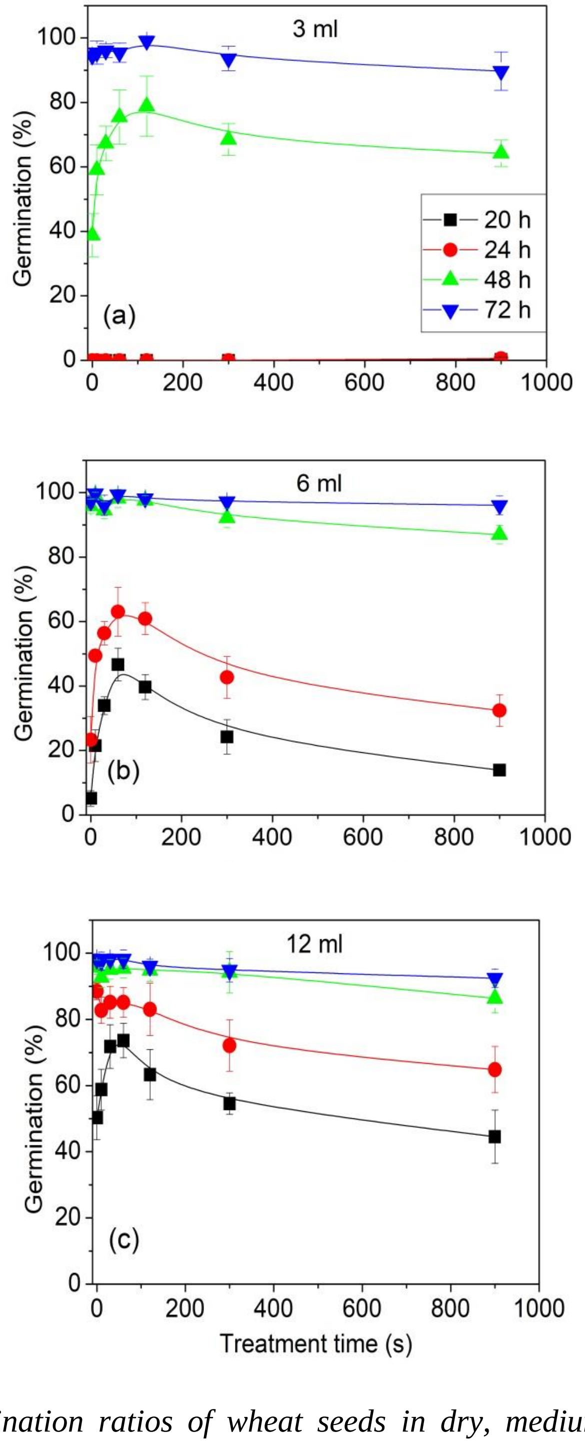

FIGURE 10. *Germination ratios of wheat seeds in dry, medium and wet irrigation conditions as a function of plasma treatment time for different imbibition periods (a: dry condition (3 ml); b: medium condition (6 ml); c: wet condition (12 ml)).*

GRAPHICAL ABSTRACT

DBD plasma treatments increase water uptake velocity and independent of the irrigation conditions plasma treated wheat seeds reach the initial water content required to initiate germination faster without affecting the embryo germination capacity. Surface analysis reveal that modifications in the composition and morphology of the outermost seed surface layers contribute to accelerate the germination processes.