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Research Article

Interfacial mechanisms involved in the interaction between *Fusarium oxysporum* f. sp. *albedinis* and date palm root

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Abstract: Our study aims to evaluate the physicochemical mechanism involved in the adhesion of *Fusarium oxysporum* f. sp. *albedinis* (Foa) on date palm root of resistant (Aziza M) and susceptible (Boufegouss) varieties by determining their surface properties. Hydrophobicity of Date palm root and Foa was evaluated by contact angle measurement (θ_w) and free energy of interaction determination (ΔGiwi). Our results showed that Foa surface is hydrophilic (θ_w = 30.57° and ΔG_{iwi} = 15.51 mj/m^2) and has an important electron donor character (γ^- = 53,99mj/m^2), whereas its electron acceptor property is low (γ^+ = 8.95 mj/m^2). Regarding date palm, the surface of sensitive variety's root is hydrophilic (θ_w = 62.97°), while that of resistant variety is hydrophobic (θ_w = 69.50°). This character was confirmed by quantitative approach (ΔG_{iwi} = 6.84 mj/m^2 for sensitive variety and ΔG_{iwi} = -20.61 mj/m^2 for resistant variety). Also, it was noted that both resistant and sensitive varieties are weak electron acceptors (γ^+ = 0.15 mj/m^2 and γ^+ = 0.08 mj/m^2 for resistant and sensitive varieties respectively). The two varieties are relatively important electron donors, but the sensitive variety is more donor (γ^- = 30.5 mj/m^2) than the resistant one (γ^- = 16.57 mj/m^2). These results suggest that hydrophilic character and electron donor/acceptor character may be responsible for the adhesion of Foa on sensitive date palm root and therefore causes its susceptibility to bayoud disease. In contrast, the hydrophobic character of the resistant variety could explain its resistance.

Keywords: Fusarium, Date Palm, Physicochemical, Adhesion, Resistance, Sensitivity

Introduction

Fusarium oxysporum f. sp. *albedinis* (Foa) is a fungus responsible for the vascular wilt of date palm *Phoenix dactylifera*, known under the name of Bayoud disease. The Bayoud is the most important disease of date palm in Morocco and around the world. Foa has destroyed about 60% of Moroccan palm plantations causing significant economic, ecological, and social damages (Sedra, 2005; Fernandez, 1995; El Modafar, 2010). In fact, over the last decades, this disease has caused a reduction in date production, the principal food of humans and animals in the desert, desertification, the disappearance of subjacent cultures like cereals, fodder, vegetables, and fruit tree production.

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Foa attacks the plant through roots and spreads in all the other parts of the tree through the vascular system producing foliar withering and leading to the death of the date palm tree (Belarbi-Halli and Mangenot, 1985; El Modafar, 2010). Foa produces typical micro- and macroconidia and chlamydospores, giving pathogen fungus the capacity to transmit and survive under adverse environmental conditions (Saleh *et al*., 2017). The releases of propagules ensure dissemination of the fungus through infested soil, irrigation water, and infected date palm tissues. No invasion of flower heads or fruits has been demonstrated, and there is no transmission by seeds. Chemical, prophylactics, genetic, and biological controls have been used to reduce the dissemination of this disease but without satisfactory results (Essarioui and Sedra, 2017).

The initial interaction between microorganisms and substrata is mediated by physicochemical force, which ensues from the physicochemical surface properties of both interacting phases. The adherence of microorganisms to different surfaces, including their hosts, is a complicated process impacted by various physicochemical properties of both substrata and microbial surfaces. These interactions can be classified into three classes: Lifshitz van der Waals interactions, electrostatic interactions, and polar or Lewis acid-base interactions (i.e., electron donor/electron acceptor) (Gannon *et al.*, 1991; Vernhet and Bellon-Fontaine, 1995; Tuson and Weibel, 2013). Reports in the literature have shown that parameters such as hydrophobicity, surface charge, and electron donor/electron acceptor (acid-base) properties may have a significant effect on microbial adhesion (Oliveira *et al*., 2001; Zhao *et al.*, 2007; Asaaidi *et al.*, 2018; El Fazazi *et al*., 2018;).

All surfaces are potential sites for biofilm formation after the initial attachment of microorganisms. Once established, the biofilm can be responsible for the spoilage of engineering materials and can lead to product contamination and surface destruction (Zottola, 1994, Wirtanen *et al*., 1996). The capacity of microorganisms to adhere rapidly to surfaces such as plastics, polypropylenes, rubbers, stainless steel, glass, and wood is now well established (El abed *et al.*, 2010).

To our knowledge, no works have been published on the physicochemical studies of "BAYOUD" to understand fungus-host interaction, and therefore, to explain the resistance or sensitivity of date palm varieties to bayoud. Understanding the interaction between microbial biofilms and date palm root surface may play a significant role in developing strategies to reduce the adherence of the fungus on date palm roots. The purpose of the present work is to study, using the thermodynamic approach, the surface's physicochemical properties of both Foa and date palm root to explain, first, the adhesion of Foa on date palm root, and, second, the involvement of these surface characteristics in resistance and sensitivity phenomenon of Date Palm to bayoud disease. We are convinced that elucidating this interaction mechanism may give us information that could help develop control measures to fight this disease.

Materials and Methods

isolation, purification, and identification of microorganisms

Isolation: *Fusarium oxysporum* f. sp. *albedinis* strain was isolated from infected fragments of the date palm spine. The purification was realized using culture on PDA solid medium. Small fragments of date palm spine presenting bayoud symptoms were disinfected by alcohol for one minute and dried by flame. Then, they were deposited in PDA medium and incubated for 48h at 25 °C. A delicate and pink mycelia carpet was obtained.

Purification: After 5 to 7 days of incubation, mycelia hyphae appear around each spine fragment. Explants of the uncontaminated peripheral zone of mycelia were drawn and transferred on a new Petri dish with PDA medium. These mycelia were subcultured for about three weeks until obtaining a pure culture.

Identification: First, macroscopic identification was realized by observation of colonies and their pigmentation. Then, microscopic identification was performed by observation in optic microscopic at x400.

Sampling and cleaning of date palm root

Date palm root samples were obtained from the Feguig palm plantation in the East of Morocco. Our study was performed on Aziza Manzo (Resistant variety) and Boufeggouss (Sensitive variety). The root pieces were cleaned by soaking them for 15 min in ethanol and rinsing them six times with distilled water.

Contact angle measurements (CAM)

Usually, the method described by Busscher *et al.* (1984) is used for measuring contact angles on bacterial layers. Since our fungus causes clogging at the filtration stage, this method is not suitable for our study. Thus, we have implemented a new method of performing contact angle measurements on1 cm^2 pieces cut on agar containing mycelia. This technique is realized for the first time in our laboratory. Regarding date palm, 1 cm^2 piece were cut out of the outer part of the date palm root used for contact angle measurements.

The contact angle (θ) was then measured. Distilled water, formamide (99%), and diiodomethane (99%) were used as reference solvents. A drop was formed at the end of a syringe to be deposited on the sample surface. A sequence of digital images was immediately acquired (Windrop) using a CCD camera placed on a goniometer (GBX Instruments, France). Three measurements were made for each sample and each solvent. The experiment was repeated three times. The free surface energies were determined: the Lifshitz-Van der Waals γ^{LW}, electron acceptor γ^{+}, and electron donor γ^{-} using Van Oss *et al.* (Van Oss *et al.*, 1988). In this approach, the contact angles (θ) can be expressed as

$$\gamma_L(1+\cos\theta) = 2.\left(\sqrt{\gamma_S^{LW}.\gamma_L^{LW}} + \sqrt{\gamma_S^{+}.\gamma_L^{-}} + \sqrt{\gamma_S^{-}.\gamma_L^{+}}\right)$$

and Quantitative hydrophobicity can be estimated by the following equation:

$$\Delta G_{iwi} = -2\left[\left((\gamma_i^{LW})^{\frac{1}{2}} - (\gamma_w^{LW})^{\frac{1}{2}}\right)^2 + 2((\gamma_i^{+}\gamma_i^{-})^{\frac{1}{2}} + (\gamma_w^{+}\gamma_w^{-})^{\frac{1}{2}} - (\gamma_i^{+}\gamma_w^{-})^{\frac{1}{2}} - (\gamma_w^{+}\gamma_i^{-})^{\frac{1}{2}})\right]$$

The surface free energy characteristics were measured using the sessile drop technique (Blanco *et al.*, 1997). Three to six contact angle measurements were made on each substratum surface for all probes using three pure liquids with known energy characteristics (Table 1).

Table 1 Surface tension properties [Lifshitz-van der Waals (γLW), electron donor (γ−) and electron acceptor (γ+)] of pure liquid used for contact angles measurements (Van Oss, 1998).

Liquids	γ^{Total}(mJ/m^2)	γ^{LW}(mJ/m^2)	γ^{+}(mJ/m^2)	γ^{-}(mJ/m^2)
Water (H_2O)	72.8	21.8	25.5	25.5
Formamide (CH_3NO)	58.0	39.0	2.3	39.6
Diiodomethane (CH_2I_2)	50.8	50.8	0	0

Statistical analysis

The individual data values are presented as the arithmetic mean ± SD (standard deviation). The statistical significance of the results obtained from *in vitro* studies was evaluated by the Student's t-test or by ANOVA at $P < 0.05$, using STATISTICA software.

Results

1. Surfaces characterization by contact angle measurement

Contact angle measurement was used to determine the surface characteristics of both Date palm root (resistant and sensitive varieties) and *Fusarium oxysporum* f. sp. *albedinis* (Foa) to explore their physicochemical interaction and the possible involvement of their surface properties in the resistance and the sensitivity of date palm to bayoud disease. Results of qualitative (θw), quantitative (ΔGiwi), and electron donor/ electron acceptor character tests are summarized in table 2 and Figs. 1, 2, and 3.

Table 2 Contact angle measurements of date palm and Foa and Lifshitz-van der Waals (γLW), electron acceptor (γ−), electron–donor (γ+) parameters and free energy of interaction (ΔGiwi).

Samples	Contact angles θ (°)			Surface tension: components and parameters (mJ.m^{-2})				
	θw	Θf	θd	γLW	γ+	γ−	γab	ΔGiwi
F. o. a	30.57 ± (2.35)	41.27 ± (1.15)	93.13 ± (2.85)	11.33 ± (0.43)	8.95 ± (0.92)	53.99 ± (4.01)	43.98 ± (0.27)	15.51
Sensitive variety (Bouf)	62.97 ± (1.09)	63.17 ± (2.40)	52.77 ± (2.21)	32.65 ± (0.10)	0.08 ± (0.11)	30.50 ± (3.20)	3.07 ± (0.29)	6.84
Resistantvariety Az. M.	69.50 ± (1.9)	58.10 ± (0.91)	52.00 ± (3.63)	33.08 ± (0.97)	0.15 ± (0.09)	16.57 ± (2.36)	3.15 ± (0.28)	-20.61

Foa: *Fusarium oxysporum* f. sp *albedinis.*

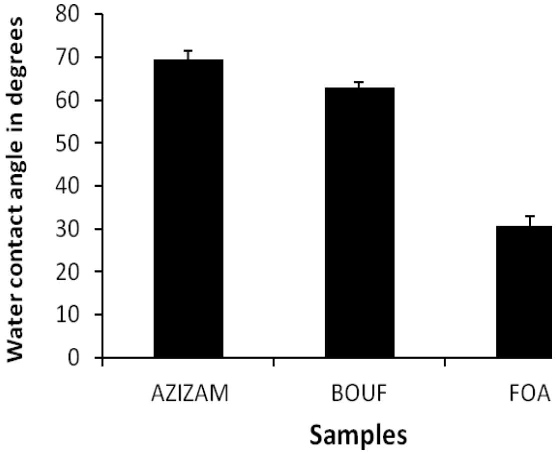

Figure 1 Water contact angle (θw°**)** of Foa and date palm root of sensitive (Bouf) and resistant varieties (Aziza M). FOA: *Fusarium oxysporum* f. sp *albedinis.*

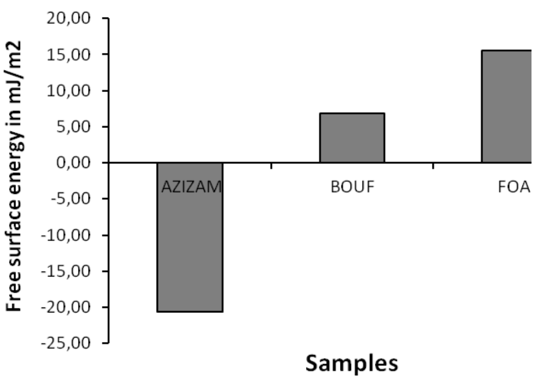

Figure 2 Free surface energy (ΔGiwi) of Foa and date palm root of sensitive (Bouf) and resistant varieties (Aziza M). FOA: *Fusarium oxysporum* f. sp *albedinis.*

Analysis of hydrophobicity shows that the water contact angle of Foa surface is θ_w = 30.57°, which means that the fungus strain tested has a hydrophilic character. The quantitative approach asserts this suggestion since it is found that the tested strain has positive free surface energy (ΔG_{iwi} = 15.51 mj/m^2). Moreover, it is noted that this strain has a high electron donor character (γ^- = 53, 99 mj/m^2), whereas its electron acceptor property is low (γ^+ = 8.95 mj/m^2).

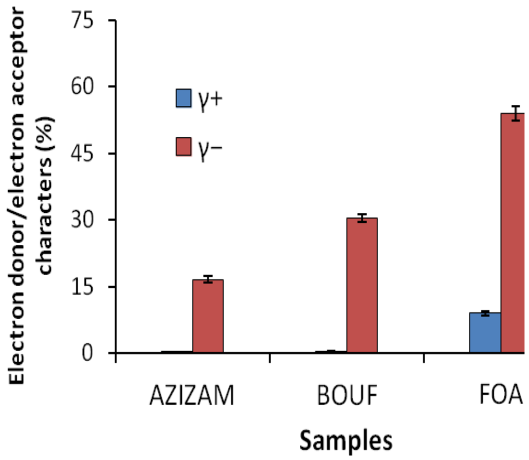

Figure 3 Donor/acceptor electron character of date palm root (Aziza and Boufegouss varieties) and Foa. FOA: *Fusarium oxysporum* f. sp *albedinis.*

Regarding date palm root, two varieties, sensitive variety (Boufegouss) and resistant variety (Aziza M) were studied. Results of contact angle measurements show that the surface of sensitive variety's root has a hydrophilic character (θ_w = 62.97°), while that of resistant variety is hydrophobic (θ_w = 69.50°). The quantitative approach confirms this character since date palm root of sensitive variety has positive free surface energy (ΔG_{iwi} = 6.84 mj/m^2), whereas the resistant variety has a negative free surface energy (ΔG_{iwi} = -20.61 mj/m^2). These values confirm the hydrophobic character of the resistant variety and the hydrophilic character of the sensitive one. Regarding electron donor character, the two varieties have relatively moderate electron donor character. Moreover, the sensitive variety (Boufegouss) shows an electron donor character two times greater (γ^- = 30.5 mj/m^2) than the resistant one (Aziza M) (γ^- = 16.57 mj/m^2). Also, it is noted that both resistant and sensitive varieties have a weak electron acceptor character. In fact their values are, γ^+ = 0.15 mj/m^2 and γ^+ = 0.08 mj/m^2 for resistant and sensitive varieties respectively.

2. Theoretical prediction of Foa adhesion on date palm root

One of the objectives proposed in this study was to predict the ability of Foa to adhere to the date palm root surface. In order to do this, total interactions free energy of adhesion process has been calculated (Table 3). It can be seen here that, from a thermodynamical point of view, the adhesion of Foa to date palm root is unfavorable, for both sensitive and resistance varieties, since the values of the ΔG_{Total} are positive, which means that other factors, like electrostatics strengths, may explain the adhesion of Foa on date palm root.

Table 3 Lifshitz_van der Waals ΔGLW (mJ. m^{-2}), acid-base ΔGAB (mJ. m^{-2}) and total ΔG^{Tot} (mJ. m^{-2}) interaction free energy for the adhesion between Foa and date palm species.

Date palm root	*Fusarium oxysporum* f. sp. *albedinis*		
	ΔG^{LW} (mj/m^2)	ΔG^{AB} (mj/m^2)	ΔG^{Total}(mj/m^2)
Boufegouss	2.82	17.41	20.23
Aziza M	2.72	23.88	26.60

Foa: *Fusarium oxysporum* f. sp *albedinis*.

Discussion

Understanding the physicochemical interaction between microorganisms and their host goes through knowing their respective surface characteristics. Several works have evaluated the potentiality of adhesion in various surfaces using a thermodynamic approach (Sharma and Hanumantha, 2003; Hamadi and Latrache, 2008).

Several techniques are usually employed to assess cell surface properties. Cell surface hydrophobicity was evaluated by hydrophobic interaction chromatography, bacterial adhesion to hydrocarbon, salting out, and water contact angle (Stenstrom, 1989; Lindhal *et al.*, 1981; Absolom *et al.,* 1983). Hydrophobicity has always been considered the primary factor in microorganisms’ adhesion to their host surfaces and their environment. According to Vogler (1998), hydrophobic surfaces exhibit water contact angle values higher than 65°, whereas hydrophilic ones exhibit water contact angle values lower than 65°. However, it is possible to assess hydrophobicity qualitatively (Oliveira *et al.,* 2001). Using the approach of Van Oss and coworkers (Van Oss, 2007), it is possible to determine the absolute degree of hydrophobicity of any substance (i) vis-a-vis water (w), which can be precisely expressed in an applicable International System.

We reported in our study that Foa and the root of date palm sensitive variety both have hydrophilic character, while the root of resistant variety has a hydrophobic character. The hydrophilic character of other fungi has been reported in the literature. It has been shown that *Penicillium commune*, *Penicillium crustosum,* and *Penicillium chrysogenum* spores have high hydrophilicity (El Abed *et al.,* 2010, Barkai *et al*., 2015; Jeffs *et al.,* 1999). Also, it has been reported that other microorganisms like bacteria (Hamadi *et al*., 2008) and actinomycetes have a hydrophilic character (Zahir *et al.,* 2015). Regarding date palm root, we reported that the surface of the sensitive variety’s root has a hydrophilic character, while that of resistant variety is hydrophobic. The two varieties have relatively moderate electron donor character. These results are in agreement with those of other studies. This weak electron-accepting donating character of resistant date palm root has been reported in other trees like cedar, oak, and beech. Some other trees have a relatively important electron donor character like teak and pine (De Meijer *et al.*, 2000; El Abed *et al.*, 2011; Gerardin *et al.*, 2007).

Our results suggest that hydrophilic character may be responsible for the adhesion of Foa on sensitive date palm root. Therefore, the sensitivity of this variety to bayoud disease, while the hydrophobic character of the resistant variety, could explain its resistance to this disease. The microbial surface properties depend essentially on the chemical compositions of the cell surface. The residues and the structures on the cell surface largely influence the character of a bacterial cell, which can be hydrophilic or hydrophobic (Aguedo *et al.*, 2005; Hamadi *et al.*, 2008, 2012; Busscher and Van der Mei, 2012). In fact, according to the physicochemical approach, the adhesion process results from intermolecular interactions between microorganisms and host surfaces: electrostatic, Van der Waals, and polar interactions (hydrophilic/hydrophobic) (Van Oss, 2007). It has been shown that the hydrophobicity measured by the contact angle is directly correlated with the high ratio of N/C and inversely correlated with that of O/C ratio (Latrache *et al.*, 2002).

Moreover, since palm root of sensitive variety is a vital electron donor comparing to that of resistant variety, and Foa surface has a relatively important acceptor character, it could be said that the electron donor/electron acceptor character may also play an essential role in the adhesion of Foa on date palm root of sensitive variety. The importance of the electron donor character has been attributed to the presence of primary groups exposed at the cell surface, such as carboxyl groups (COO^-), phosphate (PO_4) phospholipids, lipoproteins and lipopolysaccharides, amines (NH_2) (Briandet *et al.*, 1999) or sulfate groups (SO_3) (Skinner *et al.*, 1997). The importance of the electron acceptor character has been attributed to the presence of groups, such as R-NH or R-OH, exposed on the cell surface. The work of Hamadi *et al.* has correlated the electron-donating character of the surface of E. coli to a combination of the carboxyl group and the amino group and a combination of proteins and polysaccharides (Hamadi *et al.*, 2012).

Referring to these results, we could say that adhesion of Foa to a sensitive variety of date palm is possible since they have both a hydrophilic character, while this adhesion could not be possible to the resistant variety because it has a hydrophobic character.

Although Bayoud is a severe threat to date production in Morocco and other countries, and knowing that the adhesion phenomenon is strongly involved in the infection process, it appears that no studies have investigated the potentiality of Foa to adhere to date palm root. Thus, we tried in this study to predict the ability of microorganisms to adhere to Aziza (resistant variety) and Boufegouss (sensitive variety) root surfaces and to have indications on the involvement of these surface characters in the infection process and resistance and susceptibility phenomenon of the date palm to bayoud. Several works have evaluated the potentiality of microorganisms' adhesion on various surfaces using a thermodynamic approach (Sharma and Hanumantha, 2003; Li and Amirfazi, 2005; Hailiang *et al.*, 2002). From a thermodynamical point of view, we reported that the adhesion of Foa to date palm root is unfavorable. This phenomenon of unfavorable adhesion of fungi on wood has been reported in the literature for other fungi like *Penicillium commune* (SS10) and *Penicillium chrysogenum* (SS11) (El abed *et al.*, 2011).

Conclusion

Our work shows that the Foa surface has a hydrophilic character and a high electron donor character. The root surface of the susceptible variety of date palm has a hydrophilic character, while that of resistant variety is hydrophobic. Also, it was noted that both resistant and susceptible varieties have a weak electron acceptor character. Regarding electron donor character, the two varieties are relatively significant donors, but the sensitive variety

(Boufegouss) shows a strong electron donor character compared to the resistant one (Aziza M). These results suggest that the adhesion of Foa on sensitive date palm root may be caused by the hydrophilic character and electron donor/acceptor character of fungus and host surfaces, which may be responsible for its susceptibility to Bayoud disease. The hydrophobic character of the resistant variety, on the other hand, could explain its resistance to this disease.

Statement of conflicting interests

The authors state that there is no conflict of interest.

Authors' contributions

Souad Lekchiri (Methodologist, Writer, Data Analyser), Taoufik hakim (Subsidiary researcher), Hajida Zahir (Data analyser), Redouane Benabbes (Subsidiary researcher), Kaoutar El Fazazi (Subsidiary researcher), Chourouk Zanane (Data analyser), Abdeslam Jaafari (writer of introduction), Mostafa Elouali (Methodologist), Hassan Latrache (Methodologist).

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مکانیسم‌های کشش سطحی در تعامل بین قارچ ***Fusarium oxysporum* f. sp. *albedinis*** و ریشه نخل خرما

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چکیده: این مطالعه با هدف ارزیابی مکانیسم فیزیکوشیمیایی دخیل در چسبندگی *Fusarium oxysporum* f. sp *albedinis* (Foa) با تعیین خصوصیات سطحی آنها در ریشه نخل خرما در رقم مقاوم (Aziza M) و رقم حساس (Boufegouss) انجام شد. آب‌گریزی ریشه نخل خرما و Foa با اندازه‌گیری زاویه تماس (θw) و انرژی آزاد تعیین تعامل (ΔGiwi) ارزیابی شد. نتایج نشان داد که سطح Foa آب دوست است ($\theta_w = 30.57$ and $\Delta G_{iwi} = 15.51$ mj/m^2) و دارای یک ویژگی مهم‌دهنده الکترون است ($\gamma^- = 53$، 99mj/m^2)، درحالی‌که خاصیت گیرندگی الکترون آن کم است ($\gamma^+ = 8.95$ mj/m^2). در مورد نخل خرما، سطح ریشه رقم حساس آب دوست است ($\theta_w = 62.97°$)، درحالی‌که رقم مقاوم آب‌گریز است ($\theta_w = 69.50°$). این ویژگی با اندازه‌گیری $\Delta Giwi = 6.84$ mj / m^2 برای رقم حساس و $\Delta Giwi = -20.61$ mj / m^2 برای رقم مقاوم تأیید شد. هم‌چنین، هر دو رقم مقاوم و حساس، گیرنده الکترون ضعیفی دارند ($\gamma^+ = 0.15$ mj/m^2 و $\gamma^+ = 0.08$ mj/m^2 به‌ترتیب برای ارقام مقاوم و حساس). این دو رقم دهنده الکترون نسبتاً مهمی هستند، اما رقم حساس ($\gamma^- = 30.5$ mj/m^2) بیش‌تر از رقم مقاوم ($\gamma^- = 16.57$ mj/m^2) اهداکننده است. این نتایج نشان می‌دهد که شخصیت آب‌دوست و شخصیت دهنده/گیرنده الکترون ممکن است مسئول چسبندگی Foa روی ریشه خرما در رقم حساس باشد و بنابراین باعث حساسیت آن به بیماری فوق می‌شود. در مقابل، ویژگی آب‌گریز رقم مقاوم می‌تواند مقاومت آن را توجیه نماید.

واژگان کلیدی: فوزاریوم، خرما، فیزیکوشیمیایی، چسبندگی، مقاومت، حساسیت