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REVIEW

Pathogenesis-Related Proteins Dynamics during *Cochliobolus sativus* - Barley Interaction — an Updated Review

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Barley is one of the most important cereal crops grown worldwide. Spot blotch caused by the hemibiotrophic fungus *Cochliobolus sativus*, is a destructive disease of barley leading to significant yield losses globally. Barley plants have evolved complex and orchestrated defense mechanisms to protect themselves towards this disease. Therefore, understanding the molecular basis of barley - *C. sativus* interaction is crucial to efficiently breed for durable and long lasting resistance. A number of pathogenesis-related proteins (PRs) genes have been identified and studied. Overexpression of *PR* genes (chitinase, glucanase and thaumatin) individually or in combination has significantly uplifted the level of defense response in barley plants against *C. sativus* pathogen. However, the detailed comprehension of signaling pathways that regulates the expression of these PRs is critical for improving crop plants to the pathogen challenge, which is the future theme of plant stress biology. Here, we summarize the advances in studies on interactions between barley and the *C. sativus* pathogen through these *PR* genes, by reviewing a comprehensive body of research on their interaction and the advances recently made.

Key words: Barley, Cochliobolus sativus, defense signaling, PR proteins

Barley (*Hordeum vulgare* L.), a member of the grass family Poaceae, is one of the most important crops from an economic point of view, as it is widely used for breweries, animal feeds and human food, and successfully grown under a wide range of environments. According to USDA report, the global barley production in 2021/2022 amounted to about 145.10 million metric tons (USDA 2022). Nevertheless, cereals losses due to fungal diseases continue to pose a huge threat to agricultural food and impact economic decisions as well as practical developments. Using plant genotypes having genetic resistance is considered as an efficient and environmentally suitable approach to alleviate losses caused by the fungal pathogens (Jeger *et al.,* 2021). However, current technologies for molecular genetics of barley resistance towards diseases are based on the over-expression of defense signalling genes in plant (Boccardo *et al.,* 2019; Ali *et al.,* 2022).

Cochliobolus sativus (Ito & Kurib.) Drechsl. ex Dast. [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], the cause of spot blotch (SB), is an economically important disease of wheat and barley worldwide (Prasad *et al.,* 2013; Rehman *et al.,* 2020; Al-Sadi 2021). The disease is prevalent in North America, Latin America, North Africa, Asia and the Middle East, it causes up to 16%–43% yield losses in warmer areas of the world (Clark 1979; Devi *et al.,* 2018; Kumar *et al.,* 2020). The Syrian SB isolates have shown a diversity of virulence (Arabi and Jawhar 2004; Jawhar *et al.,* 2017a; Chen *et al.,* 2022). Therefore, understanding barley-*C. sativus* interaction at the genetic level is vital for identifying and deploying SB resistance.

Plants are constantly exposed to pathogens and have developed complicated mechanisms to recognize infection and trigger orchestrated defense responses including the generation of reactive oxygen species, the biosynthesis of phytoalexins, cell wall cross-linking, induction of defense enzymes, and the accumulation of pathogenesis-related proteins (PRs) (Juškyt *et al.,* 2022). PRs are a structurally diverse group of _proteins induced by plants as a defense response against the attacking fungal pathogens (Antoniw *et al.,* 1980; Alkan *et al.,* 2022). They are known to accumulate in plants post infection by various pathogens as well as upon exposed to certain abiotic stress conditions (van Loon *et al.,* 2006; Boccardo *et al.,* 2019; Ali *et al.,* 2018).

Since the detection of PRs in 1970, 17 PR families have been identified based on amino acid sequences and their biological activities (Kaur *et al.,* 2017; Jo *et al.,* 2020; Anisimova *et al.,* 2021). The majority of these families were identified in tobacco but some others were found in plant species, including monocotyledons such as barley and wheat. The exact functions of different PRs are not completely understood, but they are mainly expressed in plants as glucanases, chitinases and thaumatin-like proteins (GonzÁlez-Teuber *et al.,* 2010; Farrakh *et al.,* 2018; Sharma *et al.,* 2022). Upon pathogen challenge many PRs are located in plant cell gaps and vacuoles which are correspond to their isoelectric points and the contact to stress. Their small size 10–40 kDa leads to the accumulation in intracellular and intercellular spaces (Jo *et al.,* 2020).

This review provides an overview of our current knowledge on the barley-*C. sativus* interaction, mainly regarding pathogenesis-related proteins alterations. Understanding the molecular basis of this interaction would greatly facilitate the development of new control strategies and the identification of *C. sativus* and barley factors required for disease progression.

Infection biology

C. sativus survives as conidia on plant wastes in the field as well as on seeds or in the soil as mycelium in infected plant tissues. The infection starts within 4 hr with conidia germinating on the plant leaf, forming an appressorium after 8 hr, and penetrating the cuticle by the hyphae during 12 hr. The fungus spreads into the intercellular space of the mesophyll and its hyphae produce latter conidiophores, which emerge via the stomata carrying new conidia. The new conidial generation is created within 48h that makes the disease highly epidemic with many infection cycles during one growing season (Gupta *et al.,* 2018; Al-Sadi *et al.,* 2021).

C. sativus is well known as a hemibiotroph fungus since it has both biotrophic and necrotrophic phases (Kumar *et al.,* 2002; Shao *et al.,* 2021). It starts as a

biotroph and then switched to necrotrophic lifestyle. The biotrophic phase is mostly confined to a single epidermal cell attacked by the infection hypha, whereas the necrotrophic phase begins after infection of the mesophyll tissue followed by host cell death as a consequence of toxin secretion (Apoga *et al.,* 2002; Acharya *et al.,* 2011). Helminthosporol is the major non-specific toxin produced by *C. sativus* that affects the permeability of the leaf cell plasma membrane so that the pathogen can nourish of the electrolytes leaked and colonize the host plant tissue (Wisniewska *et al.,* 1998; Apoga *et al.,* 2002). Hence, barley plant has to deploy different defense mechanisms against pathogens with contrasting lifestyles. For this, in depth knowledge on the barley-*C. sativus* interaction at the genetic level is crucial.

Cell Death during *C. sativus* Infection

It is documented that the earliest defense responses are opening ion channels through the leaf plasma membranes, induction of active oxygen species such as O_2^- and H_2O_2, and phosphorylation and dephosphorylation of certain proteins (Doke *et al.,* 1996). These primary reactions are necessary for beginning of the signaling network that further activate the defense responses (Hammond-Kosack and Jones, 1996). Initial H_2O_2 accumulation in epidermal cell walls under host control may restrict fungal access, whereas high H_2O_2 accumulation in mesophyll tissue during the second stage results in a massive tissue collapse and fungal colonization. Kumar *et al.* (2002) and Al-Daoude *et al.* (2013; 2018) reported that H_2O_2 may play a dual role in the barley–*C. sativus* interaction. They reported that 24 and 48 hours post inoculation, H_2O_2 accumulation was detected in epidermal and mesophyll cells which were completely stained with 3,3-diaminobenzadine (DAB), indicating a hypersensitive reaction (HR) (Fig.1).

Defense responses in prepenetrated epidermal cells included cell wall apposition (CWA) formation, HR, or both, and were found to be associated with failures in fungal penetration. After fungus penetration, defense consisted of HR in epidermal cells may stop successful infection, linking invasion and collapse of mesophyll (Hückelhoven, 2007; Rodríguez-Decuadro *et al.,* 2014). On the other hand, comprehensive sequencing of expressed sequence tags (ESTs) was used to supply a global picture of barley genes differentially expressed during HR of a barley resistant genotype to *C. sativus* (AL-Daoude *et al.,* 2009; Jawhar *et al.,* 2017b). These methodologies have allowed the classification of genes assumed involved in barley resistance to *C. sativus*, including signaling, transcription factors, defense, hypersensitive response, phytohormones pathways, oxidative burst and secondary metabolites biosynthesis (Fig. 2).

Barley Signaling Network

Barley responses to *C. sativus* have been reported to be highly dependent on the balanced interplay between critical phytohormones which may act differently in several pathosystems (Al-Daoude *et al.,* 2022; Yousaf *et al.,* 2022). Some PRs are considered as the signature genes of salicylic acid (SA) and jasmonic acid (JA) pathways in model and many crop plants, in which the biotrophic pathogen activates the SA pathway, whereas the necrotrophic pathogen stimulates the JA pathway (Ali *et al.,* 2017) (Fig 3).

Recently, it has been reported that SA signaling could have an important role in defense mechanisms against *C. sativus* disease in contrasting with JA signaling, however, both hormones were induced in response to the same isolate of *C. sativus* in different barley cultivars (Popova *et al.,* 2017; Al-Daoude *et al.,* 2022). In addition, in *C. sativus*-infected wheat leaves, the SA-regulated genes *TaPAL*, *TaPR1*, and *TaPR2* genes were expressed (Zhang, 2021). However, cross talk and synergistic effects between the defense pathways mediated by SA and JA during different pathogenic infections have been proposed (Schenk *et al.,* 2000; Han and Kahmann 2019). Their pathways are linked with enhanced transcription of PRs that are particularly stimulated both around infection sites and systemically (van Loon *et al.,* 2006; Ali *et al.,* 2018). Several researches indicated that expressing SA and JA signature genes or *PR* genes in plants lead to increase the resistance to different pathogens (Pieterse *et al.,* 2012; Ali *et al.,* 2017; Anisimova *et al.,* 2021; Castro-Camba *et al.,* 2022).

PRs expression in resistant and susceptible barley genotypes

PRs are relevant elements of the defense response machinery, many with antimicrobial functions, such as, *PR2* (β -1, 3-glucanase), *PR3* (chitinases) and *PR5* (thaumatin-like) (Manandhar *et al*., 1999; Farrakh *et al.,* 2018; Sharma *et al.,* 2022). During barley-*C. sativus* interaction increased expression of *PR1, PR2, PR3* and *PR5* genes was recorded along with increasing SA and JA levels (Al-Daoude *et al.,* 2019) (Fig 4 and 5).

The *PR1* family contains the first discovered *PRs*, but, no biochemical role is known for any of the PR-1 proteins (van Loon *et al.,* 2006; Mitsuhara *et al.,* 2008; Fang *et al.,* 2019). The *PR2* proteins have functions in catalyze hydrolytic cleavage of β -1,3-D-glucosidic linkages in β -1,3-glucans, and it is supposed to operate primarily on glucans of the fungal pathogen cell wall to release oligosaccharides (Mauch and Staehelin 1989; Boccardo *et al.,* 2019, Anisimova *et al.,* 2021). The plant may then recognize these fragments as elicitors that use to activate further defense responses. Both *PR-2* and *PR-3* are likely to play double functions in plant defense both directly by hydrolyzing structural components from the cell walls of the pathogen and indirectly by releasing elicitors that might intensify the plant defense response (Stintzi *et al.,* 1993; Rebaque *et al.,* 2021). The *PR3* proteins hydrolyze β -1, 4-linkages between N-acetylglucosamines of chitin, releasing oligosaccharides from the pathogen cell walls. It has been proposed that the thinning of the fungal cell walls by *PR2* exposes the chitin located in the internal parts of the wall, making it available to chitinases to hydrolyze the fungal cell wall and release elicitors (Kombrink and Somssich 1997; Boccardo *et al.,* 2019).

Our works showed differential *PR1, PR2, PR3* and *PR5* expressions in barley leaves during the early stage of *C. sativus* infection, before any visible symptoms are apparent in the tissues (Arabi *et al.,* 2015; Al-Daoude *et al.,* 2017). However, necrosis appeared after approximately 36 hours of inoculation. The northern blot analysis revealed a biphasic accumulation of *PR1, PR3* and *PR5* mRNA in leaves before these visible symptoms (Santén 2007). On the other hand, Alkan et *al.* (2022) reported that PR3, PR5 and PR10 were strongly increased in the wheat cultivars resistance pathways after *C. sativus* infection. Moreover, Al-Daoude *et al.* (2019) demonstrated that transcripts of the *PR1, PR2, PR3* and *PR5* genes were accumulated earlier in resistant barley leaf tissues upon challenge with either the biotrophic or the necroptrophic pathogen *C. sativus*. Importantly, some *PR* genes were related with a multi-gene resistance which dispels the current belief that similar mechanisms are activated in response to pathogens *with different* lifestyle infection (Fig 5).

Figure 1. Localization of H_2O_2 (a) and SB symptoms (b) in tissues of susceptible barley cv. WI 2291 48 hours post inoculation with *C. sativus.*

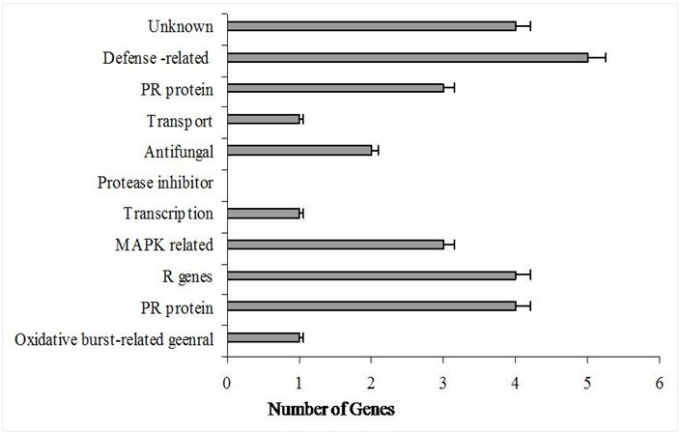

Figure 2. Transcripts expression grouped by functions in resistant barley cultivar infected with *C. sativus* (Jawhar *et al.*, 2017).

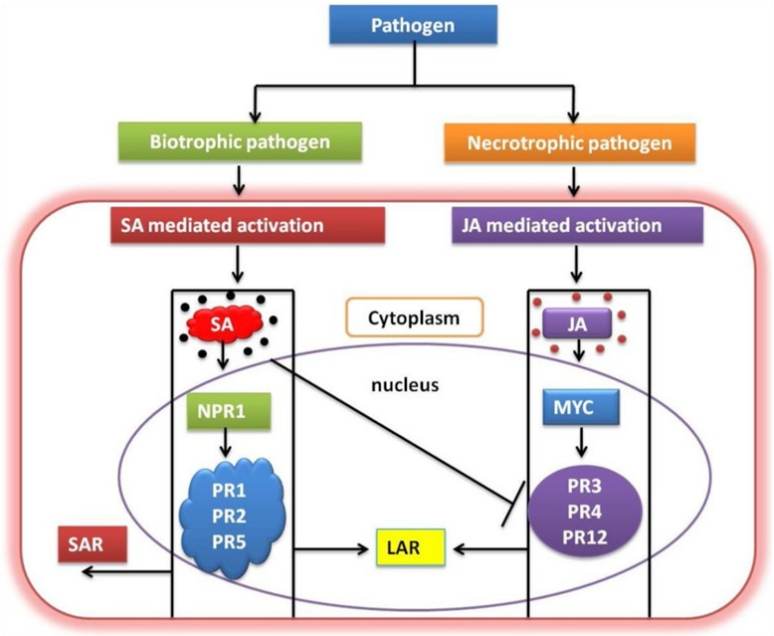

Figure. 3. An overview of activation of signaling cascades in plants after biotrophic and necrtrophic pathogenic infection (Ali *et al.,* 2017).

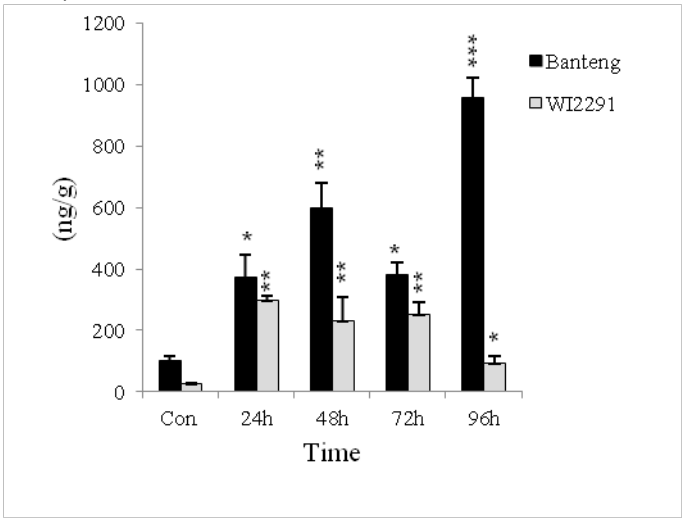

Figure 4. Quantification of total salicylic acid in barley leaves of resistant 'Banteng' and susceptible 'WI2291' cultivars 6 days post inoculation with *C. sativus*. Error bars are representative of the standard error (Mean ± SD, *n* = 3). Significance at **P* < 0.05; ***P* <0.01 and ****P* < 0.001 within each genotype during different periods comparing with the control

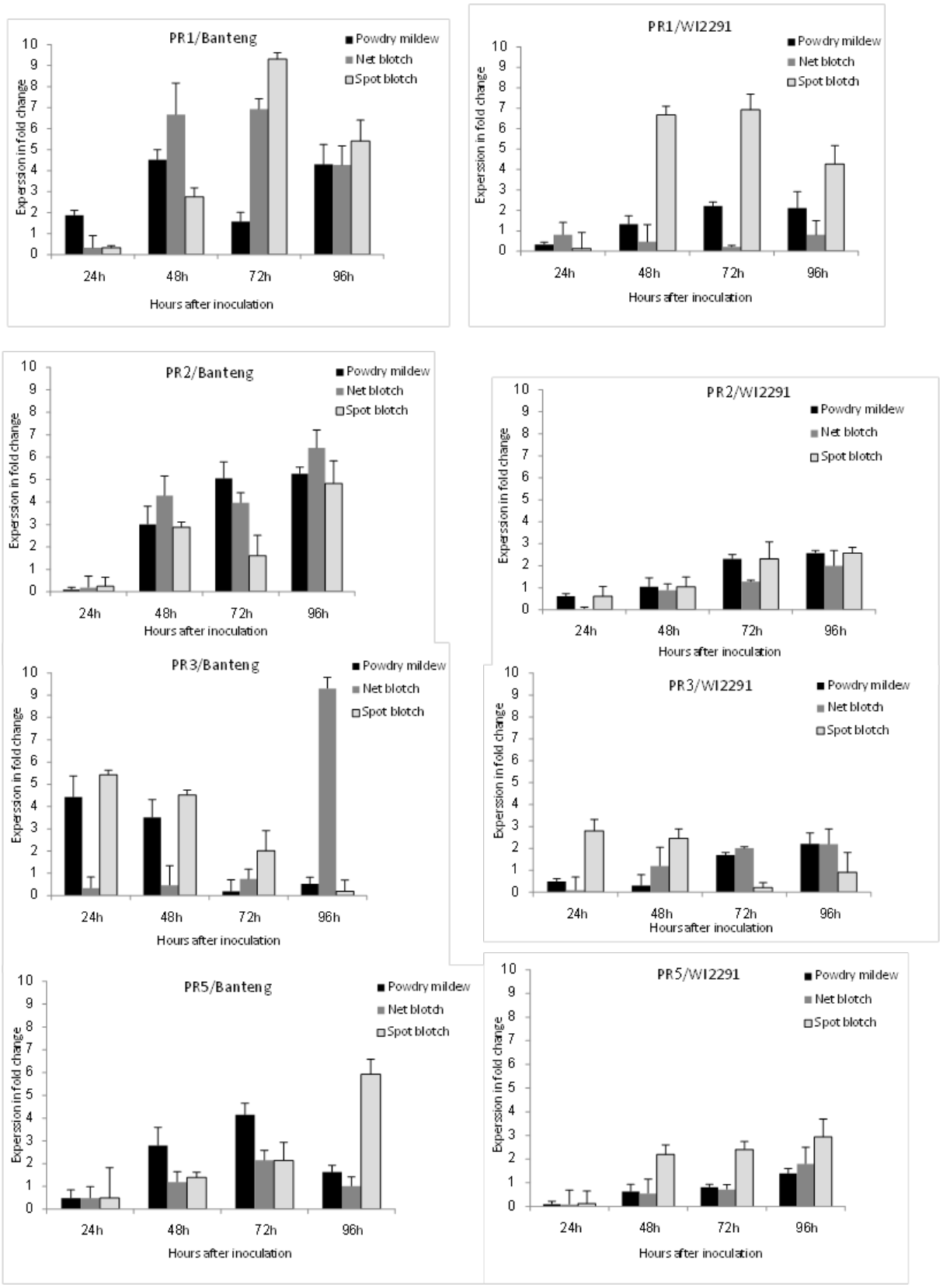

Figure 5. Relative expression profiles of marker genes in the resistant genotype Banteng and in the susceptible genotype WI2291during the time course following *infections with three diseases (Powdery mildew, net blotch and spot blotch).* Error bars are representative of the standard error (Mean ± SD, *n* = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0). (Al-Daoude *et al.,* 2019).

CONCLUSION

This review summarizes the progress made to date in the research of barley-*C. sativus* interactions through *PR* alterations. Given the current availability of genomic and EST data, *PR* genes potentially involved in these interactions have been identified, and it is expected to provide the chance to raise their numbers in a near future, permitting subsequent works at the functional genomics level. However, although a better understanding of the barley resistance mechanisms has been achieved; hard work still lies ahead for the research society working on this resistance, regarding a deep lack of information on resistance genes at different levels from identification, function and regulation.

It would be attractive to study in more details about where and when different *PRs* are transcribed to better understand how barley plants utilize and coordinate their defense mechanisms towards *C. sativus*. Also, it would be valuable to know if the pure barley PRs have antifungal activity against *C. sativus*, separately or in various combinations and also in more detailed studies to see how this pathogen is affected. Since *C. sativus* secretes the toxin prehelminthosporol it would be of interest to see where it is recovered in the tissue, and if it affects the barley response in terms of localization and accumulation of different *PRs*.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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