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Mechanisms of temperature-regulated growth and thermotolerance in crop species

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Abstract

Temperature is a major environmental factor affecting the development and productivity of crop species. The ability to cope with periods of high temperatures, also known as thermotolerance, is becoming an increasingly indispensable trait for the future of agriculture owing to the current trajectory of average global temperatures. From temperature sensing to downstream transcriptional changes, here, we review recent findings involving the thermal regulation of plant growth and the effects of heat on hormonal pathways, reactive oxygen species, and epigenetic regulation. We also highlight recent approaches and strategies that could be integrated to confront the challenges in sustaining crop productivity in future decades.

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Thermotolerance, Crops, ROS, Hormones.

Introduction

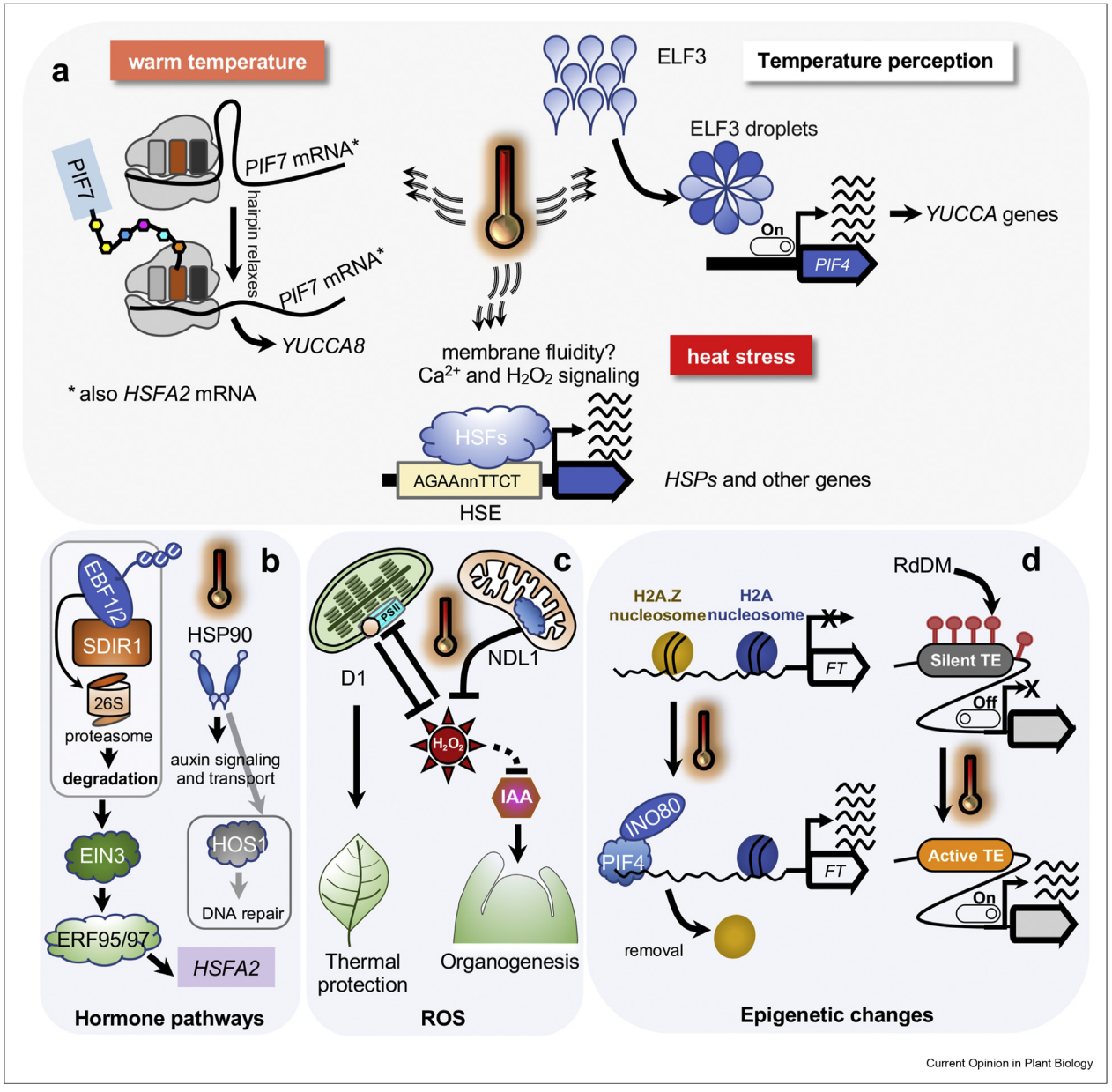
Earth is on track to reach an average increase of as much as 5.5 C in global surface temperatures by the end of the century, depending on actions taken to mitigate CO2 and other harmful emissions by human activities [1]. This will be accompanied by more frequent extreme weather events such as floods and droughts that will negatively impact crop productivity in many areas. As temperature rises, the geographical distribution of plant species is likely to shift, affecting growth in relation to day/night temperatures, light intensity, and biotic stresses, among other factors. The impact of a single degree-Celsius increase in temperature is estimated to reduce maize, rice, wheat, and soybean yields between 3.1% and 7.4% [2], and the severity of temperature-induced stresses is exacerbated by the fact that environmental stresses are often combined (e.g. heat and drought) [3]. For example, during the severe 2012 heatwave in the United States corn belt, combined with limited precipitation, maize, and soybean, yield decreased ~22% and 17%, respectively, versus trend yields [4]. Similarly, severe losses in wheat and barley yields were recorded in 2018 in Northern and Eastern Europe owing to extreme temperature and reduced precipitations [5].

Different crop species and genotypes vary in their optimal growth temperatures and heat tolerance at various stages throughout their lifecycles. However, because of natural fluctuations in daily and seasonal temperatures, crops have robust mechanisms to adjust their growth in response to temperature changes. Accelerated shoot and root growth as well as the transition to flowering are well-known responses of plants to warm ambient temperatures, collectively referred to as thermomorphogenesis. However, on exposure to heat shock, plants typically experience stress, which impairs respiration, photosynthesis, water and nutrient uptake, immunity, membrane function, protein function, and hormone and antioxidant metabolism. One of the major manifestations of heat stress on plant development is sterility, in particular male sterility [6], which causes widespread yield losses. In crops such as maize, rice, and wheat, these effects impact growth, reproductive potential, and grain filling after fertilization. Because these species together represent the largest contributors to human calorie intake, it is essential to better understand how temperature affects their growth and devise multiple integrated strategies to develop thermotolerant varieties.

Plant thermotolerance is divided into basal and acquired. Whereas basal thermotolerance refers to the ability to survive high temperatures, acquired thermotolerance refers to the ability to cope with lethal high temperatures after an acclimatization period. At the molecular level, some of the well-known responses to heat stress include the induction of heat shock transcription factors (HSFs), whose targets include heat

shock proteins (HSPs) and reactive oxygen species (ROS)-scavenging enzymes [7,8]. HSPs generally function as chaperons for denatured proteins and are important factors for thermotolerance. For example, in rice, acquired thermotolerance depends on OsHSP101 and OsHSA32 (HEAT STRESS-ASSOCIATED 32-KD PROTEIN) [9], and tomato HSP40 protects the synthesis of melatonin, a molecule involved in regulation of abiotic tolerance under heat stress [10]. At high temperatures, plants also accumulate protective proteins that target misfolded proteins in the endoplasmic reticulum, a process known as the unfolded protein response (UPR). In a recent report in maize, the UPR and heath shock response were connected by the

Figure 1



Temperature perception and influence on plant development. (**a**) Recent insights into temperature perception include mRNA conformational changes (*PIF7* [19]) and phase changes in predicted prion domain-containing proteins (ELF3 and others; [18]), both occurring at warmer temperature. (**b**) Temperature influences several hormonal pathways, including auxin signaling and transport via HSP90 [28,29], as well as ethylene signaling pathways [37,38]. HSP90 is also involved in thermostabilizing HOS1 that induces thermotolerance by activating DNA repair mechanisms [30]. (**c**) ROS production increases during heat stress, affecting core developmental pathways. Expressing the D1 subunit of photosystem II (PSII) from the nuclear genome enhances thermotolerance in different species [51]. The maize mitochondria-localized FtsH protein NDL1 protects plants from temperature stress [49]. (**d**) Temperature also influences histone H2A.Z removal [24,53] and transposable element activation via the RdDM pathway [55,61,62]. Depicted examples are discussed in detail in the main text. ELF, EARLY FLOWERING 3; ROS, reactive oxygen species; RdDM, RNA-directed DNA methylation.

activity of the transcription factor (TF) bZIP60. *bZIP60* is activated by heat-induced splicing during the UPR [11] and promotes the expression of a key *HSF* in maize heat stress response [12]. Similarly, in rice, OsbZIP74 and the membrane-localized NAC TF OsNTL3 are part of a UPR-related regulatory circuit that promotes heat tolerance [13].

Recent insights into temperature perception

Temperature sensing depends on a variety of cellular mechanisms, including the activity of phytochromes at warm temperatures, the induction of HSPs, and physical changes in lipid membranes occurring during heat stress [14]. Phytochromes are a major type of plant photoreceptors that respond to different light inputs and coordinate growth. Recently, it was reported in Arabidopsis that phytochrome B (phyB) signaling is also responsive to heat and that perturbation of phyB signaling is exacerbated by warm temperature during early night [15,16]. Additional mechanisms for temperature sensing recently uncovered in Arabidopsis include EARLY FLOWERING3 (ELF3) and the bHLH TF PHYTOCHROME INTERACTING FACTOR 7 (PIF7) (Figure 1a).

ELF3, a key component of temperature sensing that functions as a repressor and negative regulator of thermomorphogenesis and whose binding to target genes decreases as temperature rises [17], is a prion-like thermosensor that changes phases as per temperature, a process dependent on the presence and length of a predicted prion domain (PrD). However, plant species whose optimal growth is in temperate and warmer climates such as tomato and Brachypodium, either completely lack or contain a shortened PrD region, do not show phase changes and consequently lack ELF3 thermal responsiveness [18]. On the other hand, the translation of PIF7 is enhanced by conformational changes in its RNA secondary structures in response to higher temperatures. This thermosensing mechanism is also shared by the heat shock transcriptional regulator HSFA2 [19]. In both instances, it remains to be determined whether similar thermosensory mechanisms are found in crop species growing in warmer climates (e.g. presence of other PrD domain-containing proteins) and whether they also play a role in response to heat stress, as suggested by conformational changes of *HSFA2* mRNA.

Once temperatures rise above physiologically tolerated levels, most plants begin to experience the detrimental effects of heat stress. This triggers a set of signaling pathways that are largely distinct from those of thermomorphogenesis [20,21]. Upstream perception mechanisms triggering this response are not entirely clear but appear to involve calcium and hydrogen peroxide (H_2O_2) signaling [14]. Heat stress also leads to an increase in membrane fluidity and instability, and therefore these changes may be a signal themselves [16]. Regardless of the upstream signal transduction, downstream transcriptional responses are activated within minutes of heat stress and result in rapid transcriptional changes that include downregulation of growth and metabolic genes, and induction of HSPs, cochaperones and certain HSFs that bind to a highly conserved *cis*-regulatory heat shock element (HSE) containing the DNA sequence AGAAnnTTCT or similar motifs [21]. Genome-wide empirical identification of HSF binding sites in crop species could be highly beneficial because engineering of HSEs and upregulation of certain HSFs may sensitize plants to increases in heat, thereby triggering 'priming' to protect plants from subsequent stresses [22].

Temperature influence on hormonal pathways

Plants rely on phytohormones to regulate every aspect of development and to respond to constant environmental challenges. Facing daily temperature changes and fluctuations, plants actively engage hormones to regulate thermomorphogenesis (e.g. auxin, brassinosteroids, and ethylene) as well as to impart thermotolerance (e.g. ethylene and abscisic acid). Exogenous application of phytohormones can potentially mitigate heat-induced damage and improve heat tolerance to improve agricultural crop productivity and yield [23].

One of the most well-known effects of temperature on plant development is the acceleration of the floral transition in warmer temperatures. In Arabidopsis, this is owing to PIF4-mediated activation of the florigen FLOWERING LOCUS T (FT) [24]. PIF4, a bHLH TF, is also involved in promoting auxin-dependent growth in warm conditions by activating *YUCCA* flavin monooxygenase auxin biosynthetic genes and early auxin-induced *SAUR* genes [25,26]. To maintain robustness of floral formation across varied temperatures, Arabidopsis uses the meristem maintenance regulators CLAVATA2 and CORYNE, a leucine-rich repeat (LRR) receptor-like protein and a membrane-localized pseudokinase, respectively. CLAVATA2/CORYNE function in flower primordia formation at ambient and cooler temperatures; at high temperature, however, this pathway is bypassed by the activation of auxin biosynthesis via PIF4, which in turn depends on the removal of ELF3, as previously discussed [27] [17].

Another well-established connection between auxin and temperature-regulated growth concerns Arabidopsis HSP90, which promotes the stability of the auxin coreceptor TIR1 thermomorphogenesis [28] and influences the localization of the polar auxin transporter PIN1 [29] (Figure 1b). Intriguingly, HSP90 is also involved in the thermal stabilization of HOS1 (HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE

GENE1), a protein with E3 ubiquitin ligase activity with important functions in regulating temperature response. When stabilized, HOS1 increases thermotolerance by activating DNA repair components [30] and is known to negatively regulate PIF4 and repress the thermomorphogenesis response [31]. Root architecture is also known to change in response to temperature, and auxin contributes to the induction of root growth in warmer temperature [32]. A recent study showed that increased temperature decreases the abundance of the Arabidopsis intracellular auxin carrier PILS6, a negative regulator of organ growth that limits auxin availability in the nucleus, therefore increasing auxin signaling for root formation [33]. However, prolonged exposure to higher temperature (29 °C) promotes primary root growth by downregulating brassinosteroid (BR) signaling, independently of auxin [34]. The TF BRASSINAZOLE RESISTANT1 (BZR1) is a positive regulator of BR signaling, and loss of function of BZR1 in tomato reduces expression of the NADPH oxidase-encoding *RBOH1* (*RESPIRATORY BURST OXIDASE HOMOLOG1*) gene, involved in the regulation of apoplastic ROS production, and weakens heat tolerance; overexpression of BZR1, on the other hand, improves thermotolerance by enhancing H_2O_2 levels [35]. Recently, BR signaling was also shown to regulate thermomorphogenesis in conjunction with the membrane-bound MAP4K4 kinase TOT3, which regulates thermal response in wheat and Arabidopsis [36].

EIN3 (ETHYLENE-INSENSITIVE 3) is a key transcriptional regulator of the gaseous hormone ethylene signaling pathway. EIN3 protein levels are tightly controlled by EBF1 and EBF2, part of the 26S proteasome-mediated degradation pathway, which in turn are regulated by SDIR1, a RING finger E3 ligase, which targets them for ubiquitination and proteasome-dependent degradation in a temperature-dependent fashion. Consequently, higher temperatures promote EIN3 activity [37]. Among the targets of EIN3-positive regulations are the *ETHYLENE RESPONSE FACTORs ERF95* and *ERF97*. ERF95 and ERF97 are involved in basal thermotolerance of Arabidopsis and regulate heat-responsive genes including *HSFA2*, which as mentioned previously may function as a thermosensor [19] (Figure 1b). It remains to be determined how the activity of both factors is regulated by temperature, although heat stress strengthened dimerization of ERF95 and ERF97 [38].

Abscisic acid (ABA) is a stress hormone that enables plants to enhance thermal acclimation under various stresses, in particular, via its well-known role in stomata regulation in connection with ROS [39]. Under heat stress, ABA concentrations increase owing to enhanced biosynthesis, reduced degradation, or release from conjugated forms [39]. ABA is an inducer of the transcription factors ERF74 and ERF75, which on heat stress are released from the plasma membrane and translocate in the nucleus to directly regulate *RBOHD* and induce thermotolerance [40]. ABA also induces the expression of certain *HSF* and *HSP* genes, contributing further to a complex network of thermotolerance pathways [41,42].

Reactive oxygen species and temperature

ROS are toxic by-products of aerobic metabolism but also serve as important signaling molecules that increase under stress conditions and trigger acclimation responses [43,44]. Modulation of ROS levels via ROS-scavenging enzymes in rice was shown to lead to stress tolerance by affecting H_2O_2 accumulation and consequently regulating stomata aperture [45,46].

Recently, ROS have emerged as key regulators of stem cell proliferation and differentiation in meristems. In meristems, the superoxide anion (O_2^-) is enriched in the central zone of shoot apical meristems to maintain stem cell fate, and H_2O_2 accumulates primarily in the peripheral zone for stem cell differentiation [47,48]. In the tomato shoot apical meristem, the distribution of H_2O_2 resembles the expression pattern of the *TERMINATING FLOWER* (*TMF*) gene, which encodes a TF that promotes flowering independently of the florigen pathway. The cysteine residues on the TMF protein are oxidized by H_2O_2 to form disulfide bonds that drive the formation of the phase-separated TMF transcriptional condensate, which reinforces the repression of an important floral identity gene, directly linking ROS to the regulation of floral transition [48]. It remains to be determined whether rapid fluctuations in ROS levels caused by changing temperatures affect TMF function and meristem activity.

In a recent study, the maize temperature-sensitive mutant *needle1* (*ndl1*) was reported to hyper-accumulate ROS owing to respiratory defects in mitochondria [49]. NDL1 is an FtsH mitochondria-localized ATP-dependent metalloprotease that participates in the quality control of oxidative phosphorylation complexes. *ndl1* mutants showed defective meristem development and markedly high levels of H_2O_2 in inflorescence meristems, owing to increased alternative respiration [50]. Elevated H_2O_2 levels correlated with decreased endogenous auxin concentrations, leading to defective initiation of lateral primordia and meristems in inflorescences. Interestingly, *ndl1* mutants carry transcriptional signatures of enhanced stress response when grown in warm temperature, revealing many genes that may work under heat stress during maize inflorescence development, including several *RBOHD* genes, antioxidant enzymes, and transcriptional regulators [49] (Figure 1c).

ROS are also produced in chloroplasts under heat stress and mainly prevent the translation of the D1 subunit by the chloroplast *psbA* gene. D1 functions to repair and sustain photosystem II activity against thermal damage (Figure 1c). Supplementing the chloroplast pool with D1 of nuclear origin using the *HSFA2* promoter was shown to enhance thermotolerance in Arabidopsis, tobacco, and rice. Noticeably, the increased rate of photosynthetic carbon fixation in rice transgenic plants translated into enhanced biomass at harvest and increased grain yield in field-grown plants [51]. These results show how the use of a clever biotechnological strategy can bypass the negative effect of excessive ROS production in chloroplasts under heat stress.

Temperature-induced epigenetic changes

Epigenetic regulation plays an indispensable role in plant development and in response to environmental challenges. The plant epigenome appears to be highly dynamic, and a variety of biotic and abiotic stimuli can rapidly reshape genome-wide epigenetic modifications [52]. In Arabidopsis, the removal of the histone variant H2A.Z in warm temperatures, which allows PIF4 binding and the transcriptional activation of *FT* as part of the thermomorphogenesis response [24], is driven by the association of the chromatin-remodeling complex INO80 with PIF4 itself [53] (Figure 1d). Although originally described as a thermosensor, this and other evidence instead suggest that H2A.Z lacks the property of a true thermosensor [16].

RNA-directed DNA methylation (RdDM) is a unique pathway in plants involving noncoding RNA that directs DNA methylation to specific DNA sequences and establishes H3K9me2 histone methylation [54]. Plants use the RdDM pathway to cope with heat stress, and mutations in components of the RdDM pathway are hypersensitive to heat exposure [55]. The Arabidopsis *FLOWERING WAGENINGEN* (*FWA*) gene is silenced in wild-type plants owing to RdDM-mediated DNA methylation of a transposable element in its promoter region; and the removal of DNA methylation reactivates its expression, leading to a late-flowering phenotype [56,57]. Intriguingly, the activation of the *FWA* gene is also observed in wild-type plants under heat stress [58]. Normally, transposable elements are partially suppressed by the RdDM pathway in plants [59], but their expression can be upregulated under abiotic stress conditions, including heat stress [60,61] (Figure 1d), suggesting that the heat-driven activation of the *FWA* gene could be owing to epigenetic changes. A recent maize study also showed that the RdDM pathway can buffer the effects of heat stress on transposable elements [62]. Another study in maize inbred lines exposed to heat and cold stresses found that only certain transposable elements show expression changes in response to heat stress, and this response depended on methylation status as well as genomic variation [61], highlighting the complexity of the response to stresses in species with genome-wide proliferation of transposable elements.

Heat shocks are also known to impact chromatin accessibility, and genes with heat-activated DNase I hypersensitive sites include those encoding HSPs and TFs involved in heat stress response. Analysis of enriched elements in their promoters revealed HSEs, highlighting the importance of HSFs in driving the heat shock response [21].

Conclusions and perspectives: improving thermotolerance in crop species

As agriculture faces the pressing challenges forced by climate change, different approaches should be simultaneously pursued to develop thermotolerant crop varieties. These should include biotechnological strategies targeted at increasing thermotolerance, the genetic and genomic selection of tolerant varieties, as well as the widespread adoption of resilient species. From perception to downstream temperature response regulation, our increasing mechanistic knowledge provides opportunities to develop heat-tolerant crop varieties. Although transcriptomic studies have been extensively used to characterize the response to abiotic stresses, a deeper mechanistic understanding of temperature regulation and stress response specifically in crop species grown under natural environmental conditions [63] provides the potential to devise *ad hoc* biotechnological strategies. For example, in potatoes, the FT homolog SP6A was shown to be under control of a small RNA upregulated by heat which inhibited tuberization. The expression of a target mimicry construct to sequester the endogenous small RNA promoted tuberization even in the presence of continuous heat [64]. In rice, mutations in the cytosolic tRNA 2-thiolation protein SLENDER GUY1 involved in the post-transcriptional modification of a tRNA showed enhanced sensitivity to high temperature in both vegetative and reproductive development, whereas overexpression and natural variation existing in both promoter and coding sequences of *SLENDER GUY1* in *indica* varieties promoted thermotolerance [65]. This study adds to the growing evidence for the role of RNA modifications in promoting thermotolerance [66,67]. Additional examples of the importance of exploiting natural variation in developing high-temperature tolerant varieties as well as the adoption of heat-tolerant crops include Arabidopsis accessions with distinct *ELF3* variants [68], the African rice proteasome α2 subunit involved in the degradation of ubiquitinated proteins [69], the alternative splicing of the tomato *HSFA2* pre-mRNA [70], and the recent characterization of a wild species of coffee tolerant to high temperature, even though the basis for such tolerance is presently unknown [71].

As shown for developmental regulators in tomatoes and maize [72,73], generating new *cis*-regulatory variants presents another attractive avenue to improve crop resilience. Recently, a machine learning approach was applied to identify *cis*-regulatory elements associated with heat-responsive gene expression and predict expression changes in different maize inbreds [74]. Such loci could ultimately be targeted for specific modifications using base editing to improve thermotolerance in various germplasms. The expanding genomic and pangenomic resources, single-cell genomics, and machine learning approaches coupled with precision editing in crop species are likely to aid the breeding of stress-tolerant varieties to confront the daunting challenges of the incoming decades.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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