Development and Characterization of Highly Efficient Mimics of the Rice Growth Regulator Zaxinone for Basic Research and Agricultural Applications

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The 17th International Symposium on Rice Functional Genomics
November 4 - 6, 2019 | Taipei, Taiwan

SUMMARY

Carotenoids are the precursor of several plant hormones and signaling molecules, which play important roles in literally all aspects of plants' life. Recently, we have identified zaxinone, an apocarotenoid formed by the carotenoid cleavage enzyme Zaxinone Synthase (ZAS), as a novel-signaling molecule required for rice growth and development. Investigation of a rice *zas* mutant and zaxinone treatment demonstrated that zaxinone is a negative regulator of SL biosynthesis, lowering SL content and alleviating infestation by the parasitic weed *Striga*. Now we further developed zaxinone-mimics with relatively simple structure that can be employed in fundamental research and large scale agriculture applications. Comparative studies demonstrated the two identified mimics of zaxinone (MiZaxs) appeared highly efficient that can cause a significant increase in root growth and biomass in wild type rice and restored the growth retardation and lowered SL content in the *zas* mutant. Treatment with MiZaxs significantly decreased SL release in wild-type rice and, accordingly, suppressed the infestation with the parasitic plant *Striga*.

INTRODUCTION

- Strigolactones (SLs) are carotenoid-derived plant hormones (for biosynthesis, see Fig.1, Fiorilli & Wang *et al.*, 2019) that regulate plant architecture and also act as rhizosperic signaling molecules inducing hyphal branching in symbiotic mycorrhizal fungi and triggering seed germination in root parasitic plants.
- Zaxinone, a recently discovered rice apocarotenoid growth regulator, is formed by the zaxinone synthase (ZAS) that represents a novel, sixth plant carotenoid cleavage dioxygenase (CCD) subfamily (Fig 2-3, Wang *et al.*, 2019).
- Zaxinone has a large application potential in agriculture, due to its growth promoting activity. Zaxinone is a negative regulator of SL biosynthesis and, hence, can also alleviate the infestation by the root parasitic plant *Striga*. However, chemical synthesis of zaxinone is laborious and expensive.
- We performed a structure-activity relationship (SAR) study and developed, based on the SAR results, simple zaxinone mimics that show high efficiency in different bioassays (Fig. 4-7).

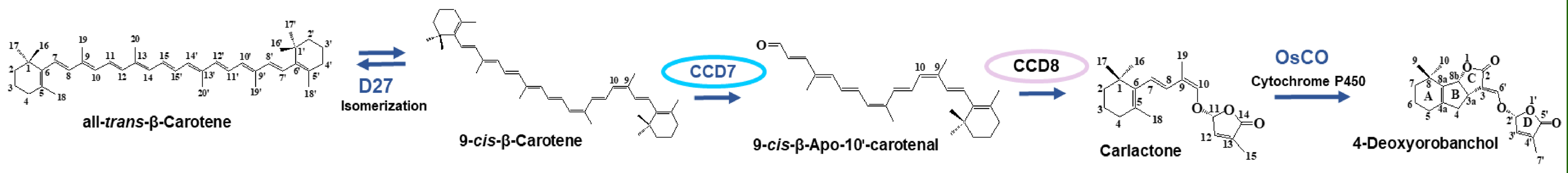

Fig. 1: SL biosynthetic pathway.

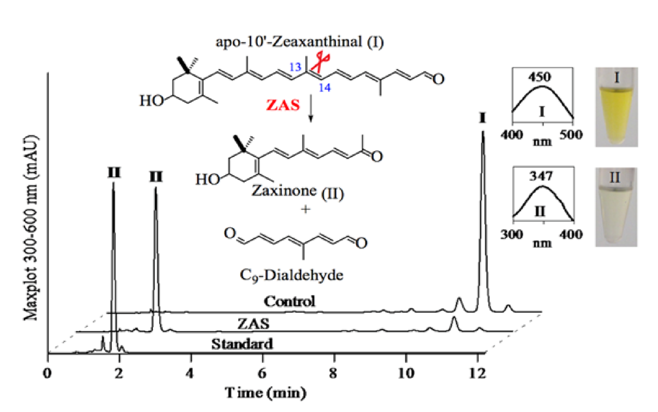

Fig. 2: HPLC analyses of *in vitro* incubation of ZAS.

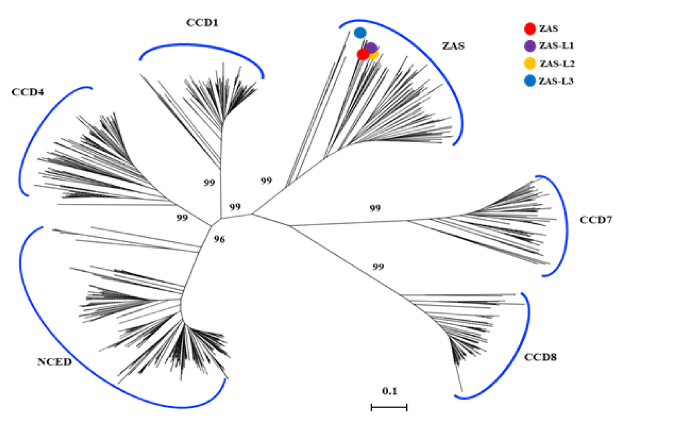

Fig. 3: Phylogenetic characterization of Plant CCDs.

METHODS

Structure activity relationship (SAR) of Zaxinone :

Three-weeks-old WT rice (Nipponbare) seedlings were treated with 5 μM zaxinone and with zaxinone-like compounds for 6 hours.

Synthesis of zaxinone-mimics (MiZaxs).

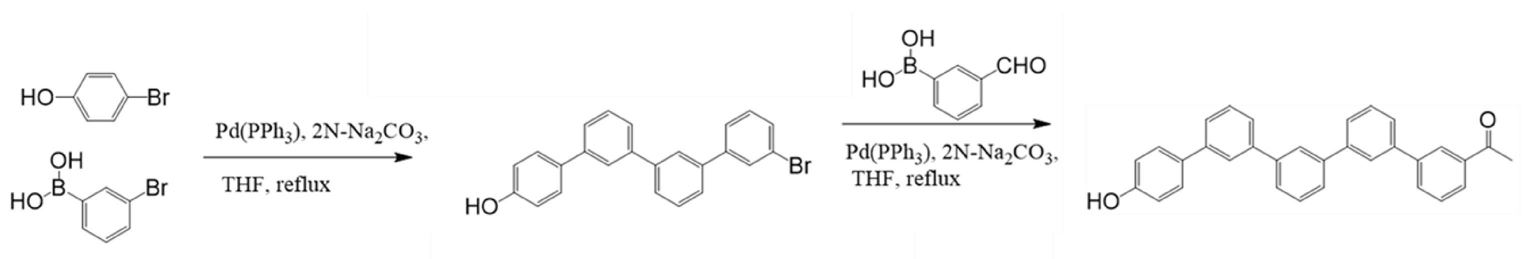

Fig. 4: Synthesis scheme of MiZax.

Phenotypic characterization of MiZaxs :

Rice WT and *zas* mutant seedlings were grown hydroponically and treated with a 2.5 μM concentration of each of the tested compounds for two weeks. Rhizotron assays were performed with a 5 μM concentration of each of the tested compounds for two weeks.

Effect of MiZaxs on rice SL biosynthesis and release :

Two-weeks-old Rice WT seedlings were exposed to 1 week phosphate starvation and treated with 5 μM of Mizaxs for 6 hours. Root tissues and exudates were used to quantify SLs by LC-MS, determine SL transcript level (Fig.7), and to perform *Striga* seed germination bioassay.

Striga plant emergence examination:

Rice cv. IAC-165 *Striga*-susceptible plants were grown in *Striga* infestated soil. Zaxinone and MiZaxs were applied to soil at a 5 μM concentration for four weeks (twice a week). The study was conducted according to Boubacar *et al.*, 2019.

RESULTS

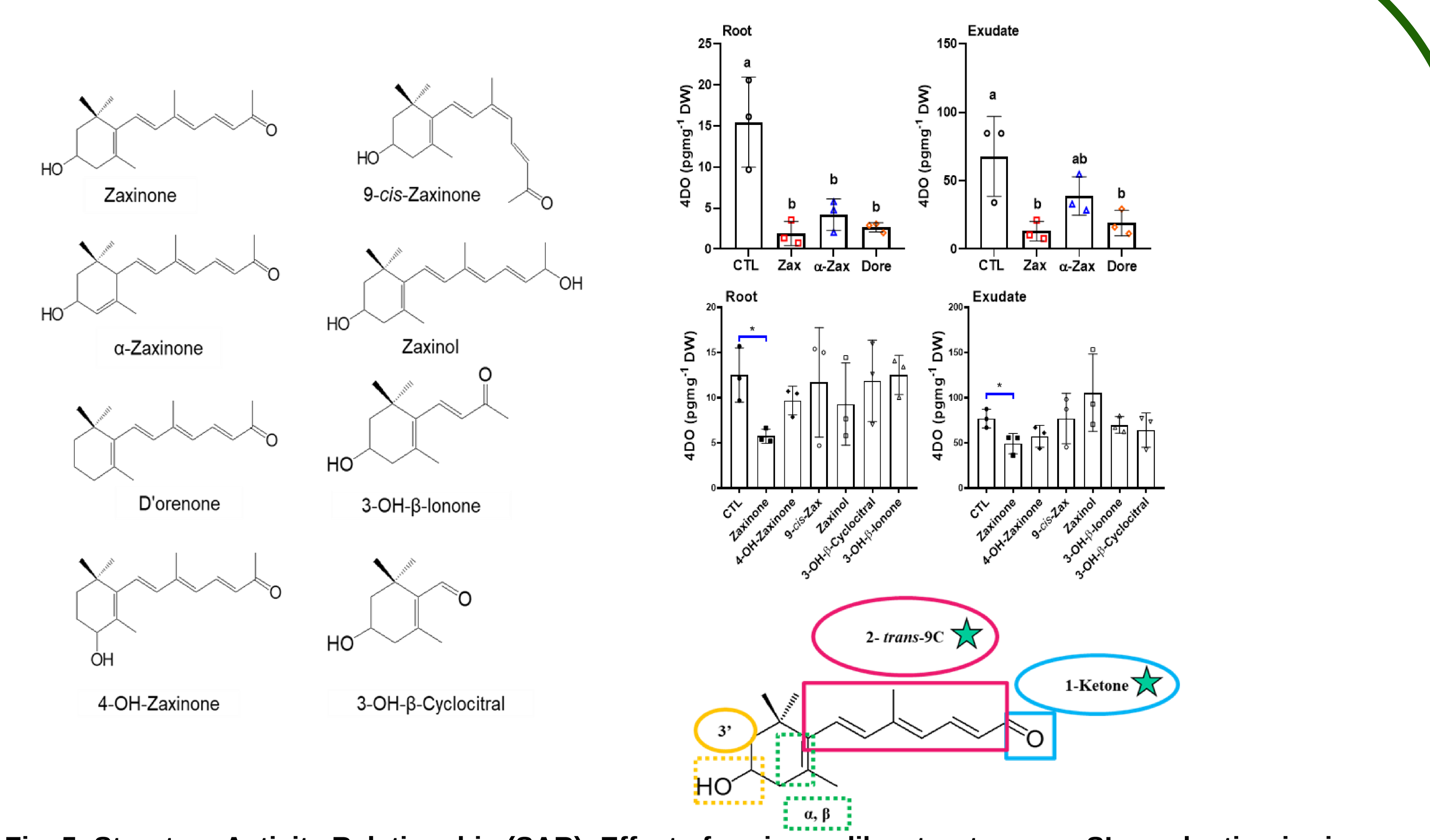

Fig. 5: Structure Activity Relationship (SAR): Effect of zaxinone-like structures on SL production in rice.

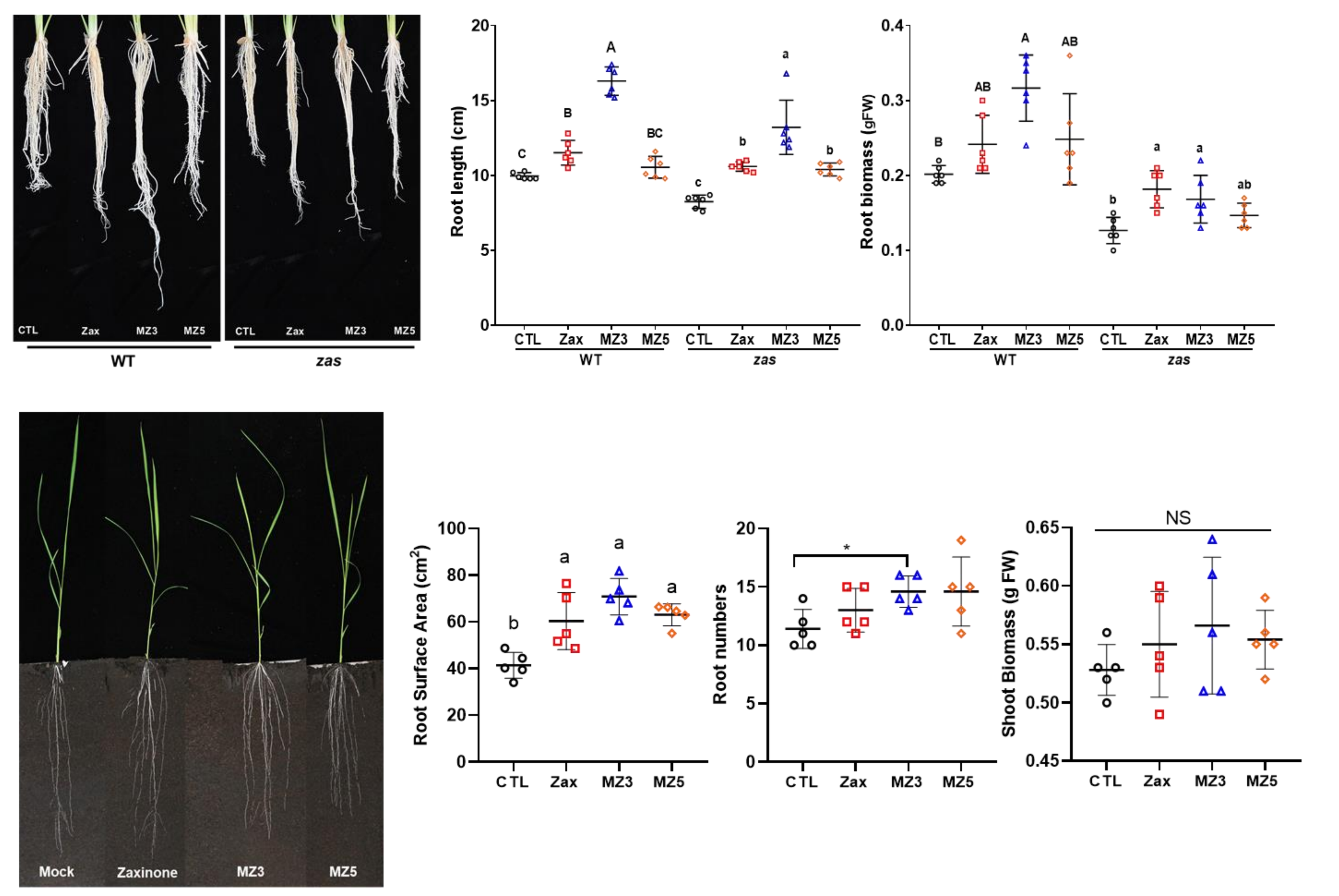

Fig. 6: Phenotypic characterization of MiZaxs (MZ) activity in hydroponic and rhizotrone systems.

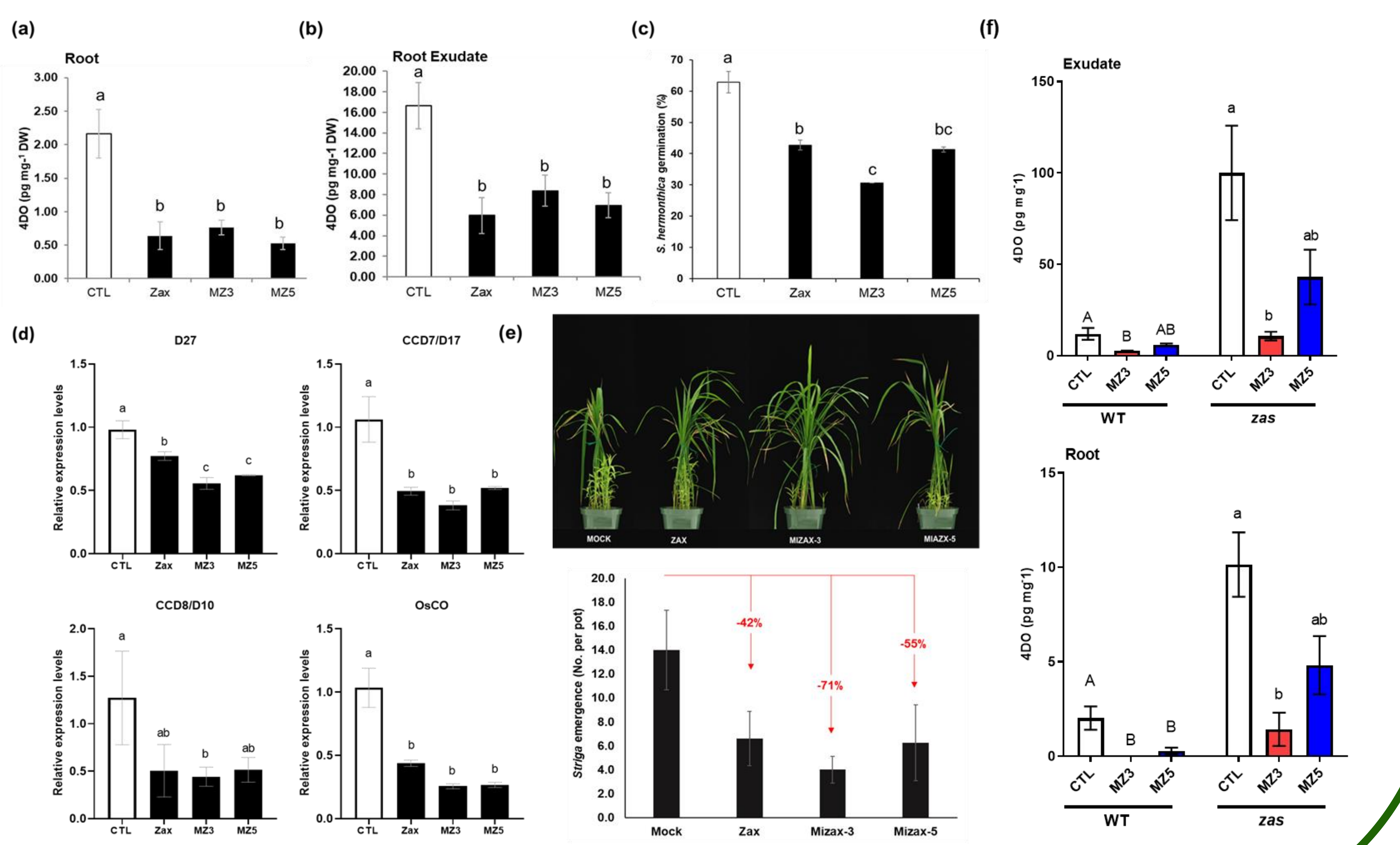

Fig. 7: Effect of MiZaxs (MZ) on rice SL biosynthesis and release, and *Striga* infestation.

CONCLUSION

Our findings demonstrate MiZaxs have a large agricultural application potential as growth stimulants and a novel tool to combat root parasitic plants, a major threat to global food security. In addition, MiZaxs are expected to be very helpful for understanding the biology of zaxinone and the regulation of SL biosynthesis.

FUNDING

This work has been financially supported by Competitive Research Grant (CRG2017) given to Prof. Salim Al-Babili from King Abdullah University of Science and Technology, and by the Bill & Melinda Gates Foundation (Grant number: OPP1194472).

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