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Fine-tuning of SUMOylation modulates drought tolerance of apple

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Abstract: SUMOylation is involved in various aspects of plant biology, including drought stress. However, the relationship between SUMOylation and drought stress tolerance is complex; whether SUMOylation has a crosstalk with ubiquitination in response to drought stress remains largely unclear. In this study, we found that both increased and decreased SUMOylation led to increased survival of apple (*Malus* × *domestica*) under drought stress: both transgenic MdSUMO2A overexpressing (OE) plants and *MdSUMO2* RNAi plants exhibited enhanced drought tolerance. We further confirmed that *MdDREB2A* is one of the MdSUMO2 targets. Both transgenic *MdDREB2A* OE and *MdDREB2A^{K192R}* OE plants (which lacked the key site of SUMOylation by MdSUMO2A) were more drought tolerant than wild-type plants. However, MdDREB2AK192R OE plants had a much higher survival rate than *MdDREB2A* OE plants. We further showed SUMOylated MdDREB2A was conjugated with ubiquitin by MdRNF4 under drought stress, thereby triggering its protein degradation. In addition, *MdRNF4* RNAi plants were more tolerant to drought stress. These results revealed the molecular mechanisms that underlie the relationship of SUMOylation with drought tolerance and provided evidence for the tight control of MdDREB2A accumulation under drought stress mediated by SUMOylation and ubiquitination.

1. Expression patterns and localization of SUMO2s in apple

The apple genome contains six SUMO2 genes (Fig. 1A). Due to genome duplication, each pair of genes on different chromosomes has almost identical coding sequences, and we therefore named the three pairs MdSUMO2A, MdSUMO2B, and MdSUMO2C. We found that the *MdSUMO2*s had similar expression patterns in response to drought (Fig. 1B), suggesting that they may have similar functions under drought stress. Co-localization with mCherry suggested that apple SUMO2A, SUMO2B, and SUMO2C are localized in the nucleus, plasma membrane, and cytoplasm (Fig. 1C).

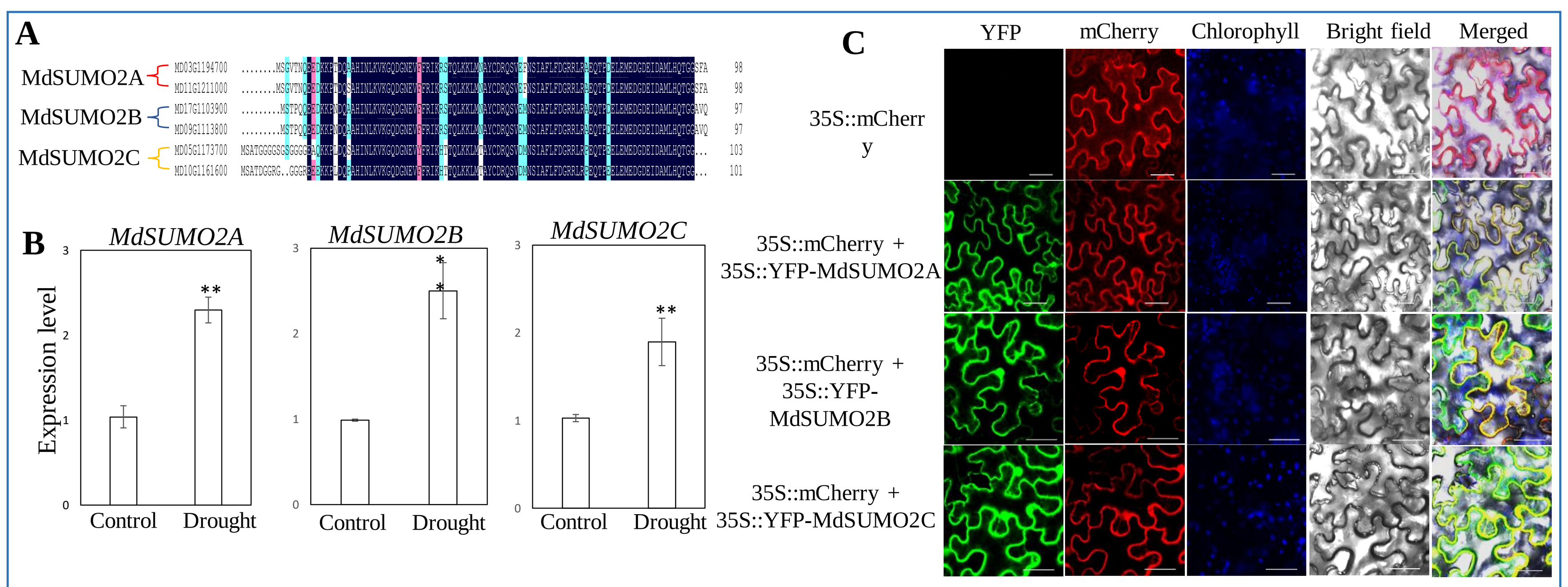

Fig. 1 Sequences, responses to drought stress, and localization of MdSUMO2s.

2. Knocking down *MdSUMO2*s or knocking in one *MdSUMO2* gene leads to drought stress tolerance

Both *MdSUMO2A* OE plants and *MdSUMO2* RNAi plants were more drought tolerant than the wild type (Fig. 2A). The *MdSUMO2A* OE plants exhibited greater root system, more vigorous growth, and higher photosynthetic capacity and hydraulic conductivity (Fig. 2B-C). The *MdSUMO2* RNAi transgenic plants had smaller but thicker leaves, much lower stomatal conductance, and higher water use efficiency (Fig. D-F).

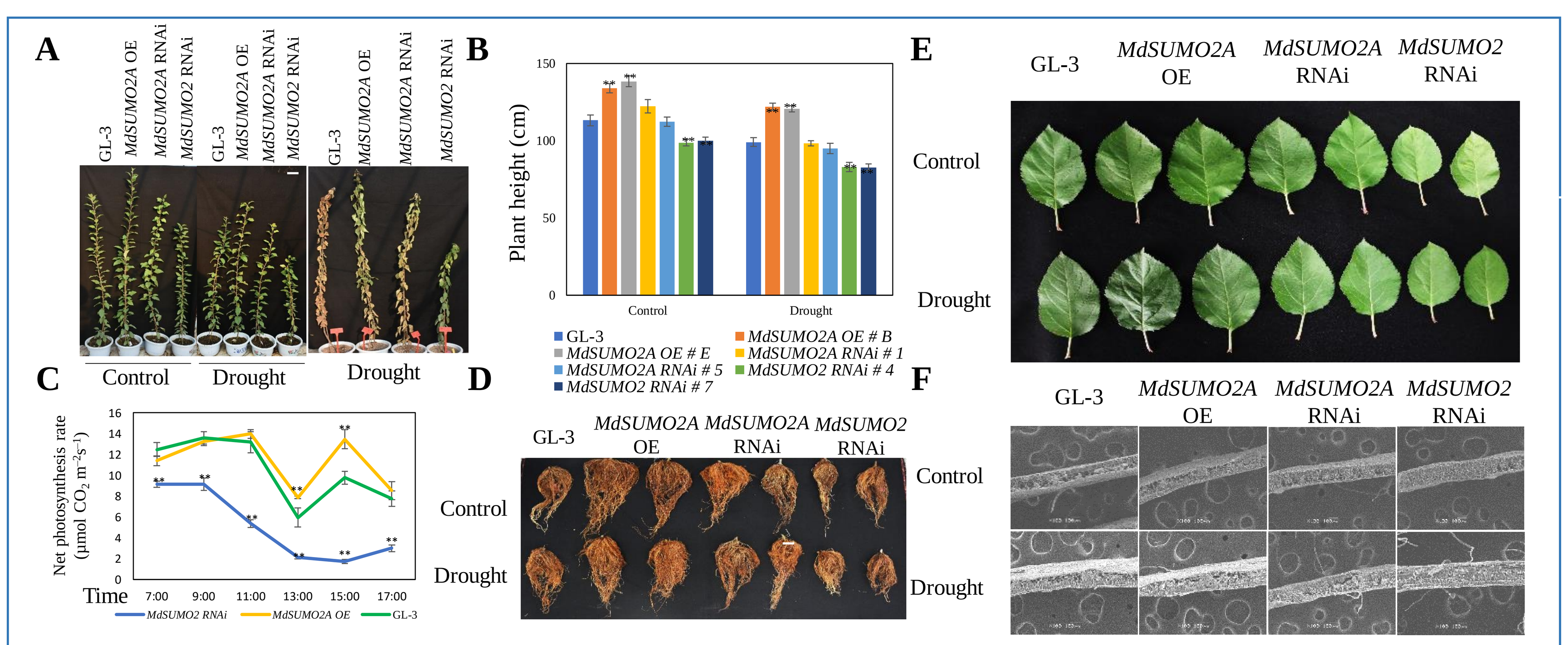

Fig. 2 Both *MdSUMO2A* OE plants and *MdSUMO2* RNAi plants were drought tolerance

3. Identification of MdSUMO2 targets reveals SUMOylation of MdDREB2A by MdSUMO2s

To obtain reliable MdSUMO2 targets, we generated transgenic apple calli carrying 6His-H89R-MdSUMO2A and performed affinity purification with three steps as described previously. To determine the actual SUMOylation sites, each candidate lysine (K) was replaced by arginine (R) singly or in combinations. SUMOylation assays using the *E. coli* system showed that K192 was the main mapped SUMOylation stie for the MdSUMO2A-mediated SUMOylation of MdDREB2A (Fig. 3).

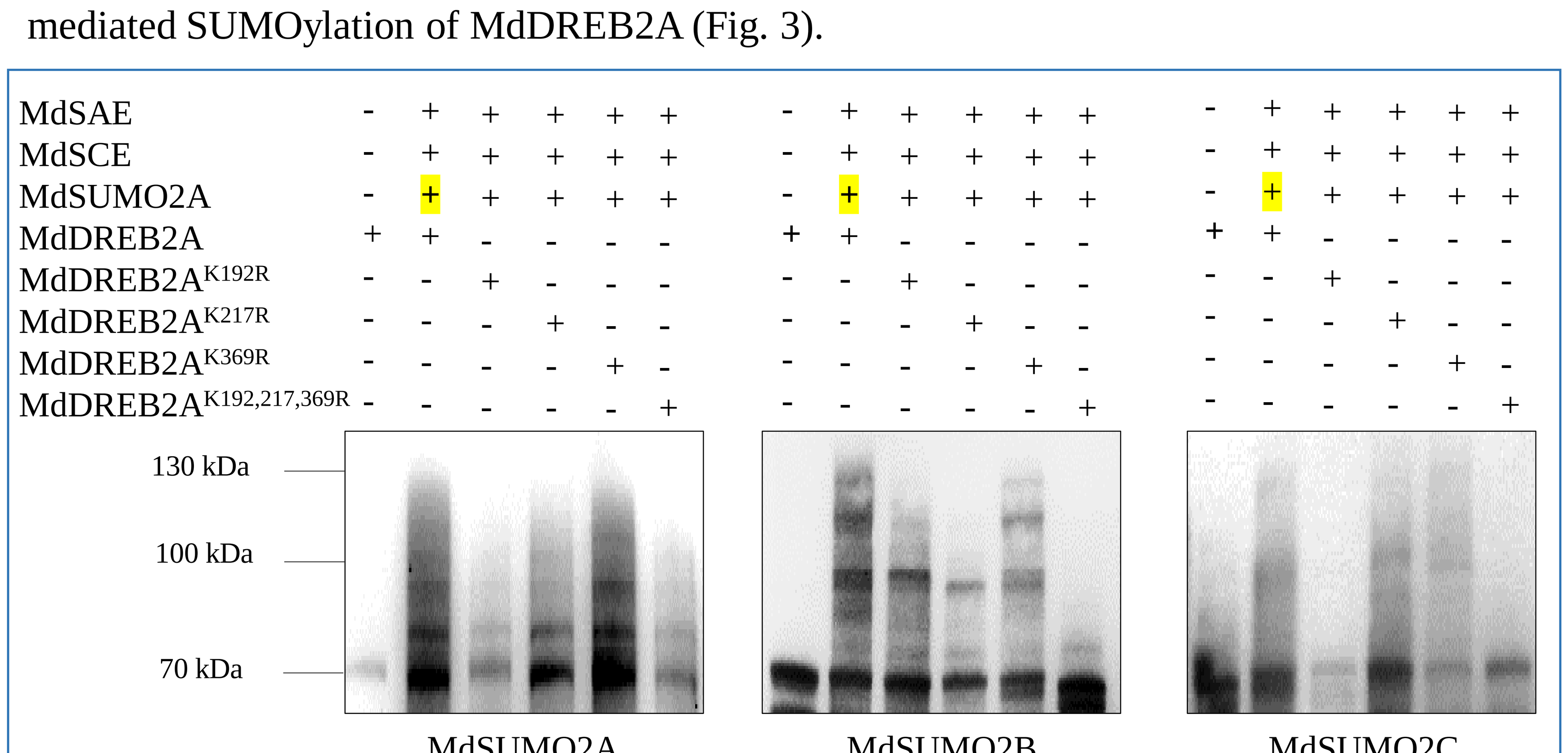

Fig. 3 MdDREB2A was SUMOylated by MdSUMO2A, MdSUMO2B, and MdSUMO2C. Putative SUMOylation sites (K) of MdDREB2A were mutated to arginine (R).

4. SUMOylation of MdDREB2A is critical for drought stress tolerance and is coupled with ubiquitination during drought

MdDREB2A^{K192R} OE plants had a higher survival rate than *MdDREB2A* OE plants (Fig. 4A-B). Under drought conditions, *MdDREB2A* OE plants accumulated more MdDREB2A protein than GL-3 plants, but less than transgenic plants carrying 35S::*MdDREB2A^{K192R}* (Fig. 4C). Compared with that of *MdDREB2A* OE plants, the SUMOylation level of MdDREB2A^{K192R} OE plants was slightly lower. However, their ubiquitination level was also lower (Fig. 8B), suggesting that SUMOylated MdDREB2A may undergo ubiquitination in response to drought in MdDREB2A OE plants (Fig. 4D).

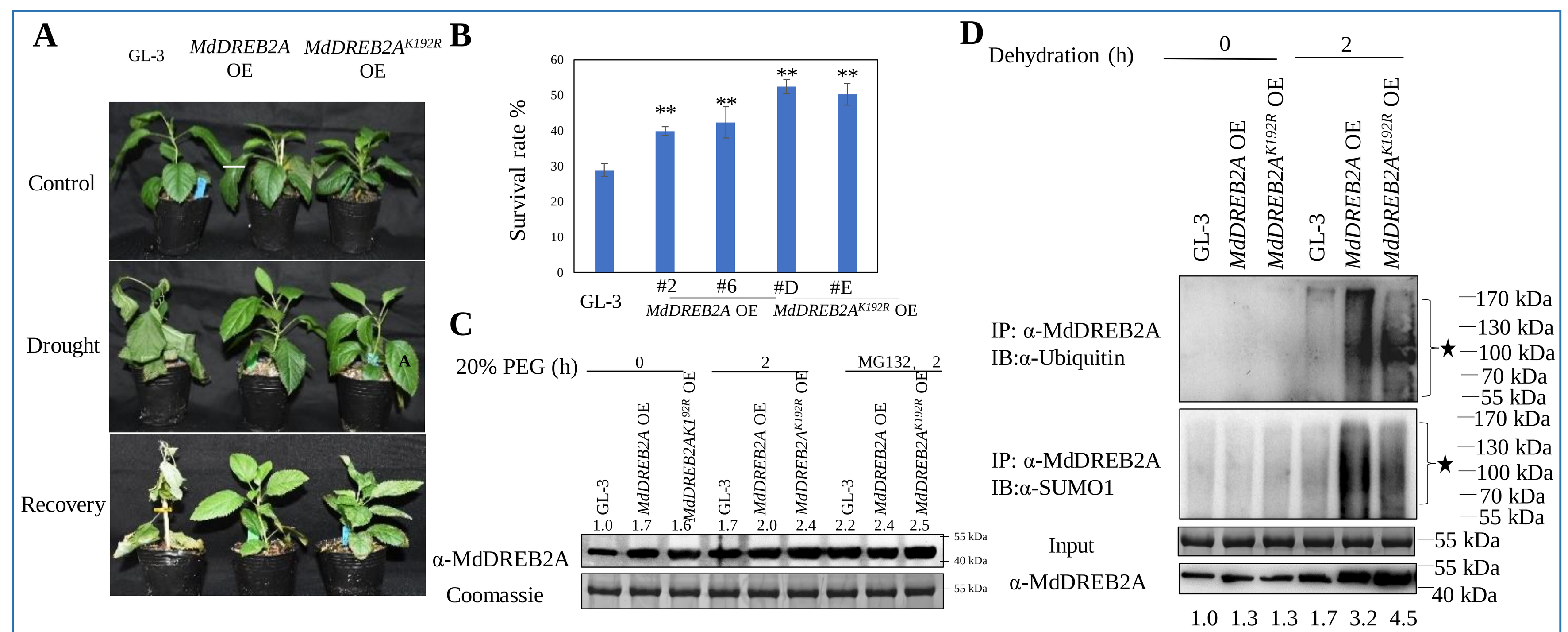

Fig. 4 SUMOylation of MdDREB2A is critical for drought stress tolerance.

5. MdRNF4 mediates ubiquitination of SUMOylated MdDREB2A

One of the potential MdDREB2A interacting proteins under drought stress was MdRNF4, which encodes an E3 ubiquitin ligase. And further Y2H, MST and Co-IP assay verified the interaction between MdSUMO2A and MdRNF4 (Fig. 5A-C). MdRNF4 mediated the ubiquitination of SUMOylated MdDREB2A (Fig. 5D). In addition, the *MdRNF4* RNAi plants were more tolerant to drought stress (Fig. 5E).

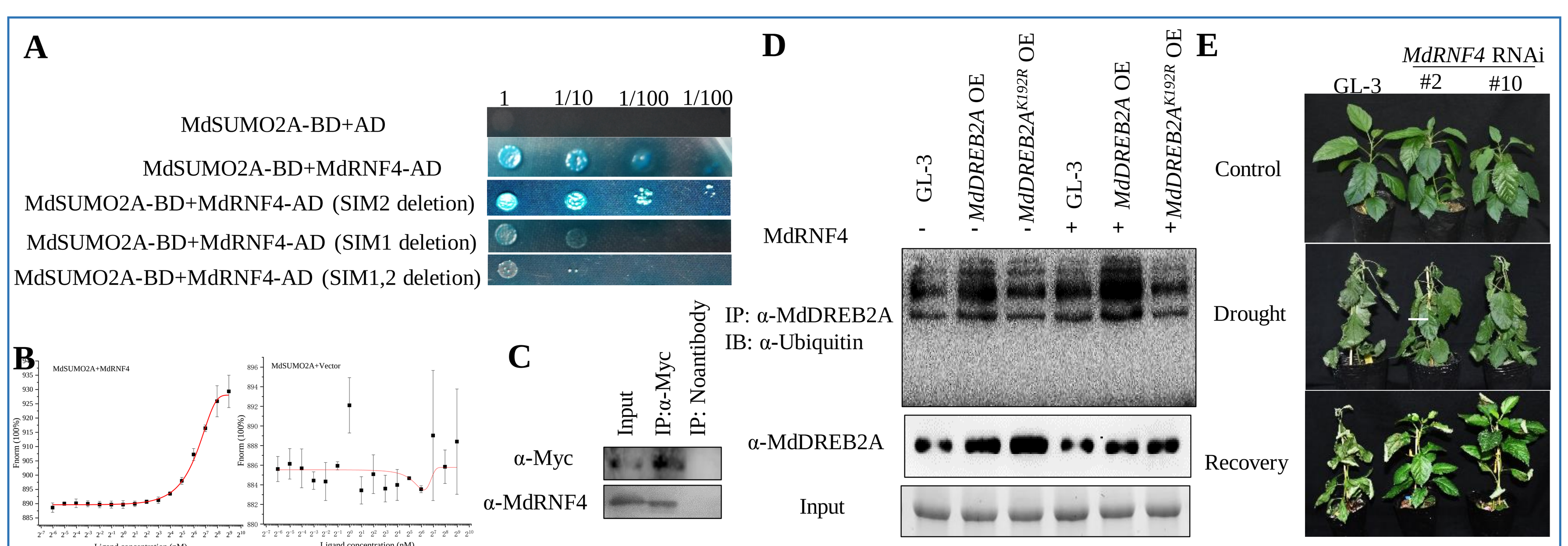

Fig. 5 MdRNF4 mediates degradation of SUMOylated MdDREB2A under dehydration conditions.

6. In conclusion

SUMOylation level modulates apple tree drought tolerance by a fine-tuning way. Both increased and decreased SUMOylation level leads to enhanced drought tolerance of apple under water deficit stress. The *MdSUMO2A* overexpression (OE) plants act as water spenders with more vigorous growth, including greater root system, higher photosynthetic capacity, and hydraulic conductivity; and the RNAi lines (RNAi) act as water savers with smaller but thicker leaves, much lower stomatal conductance, and higher water use efficiency.

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