ABSCISIC ACID-INSENSITIVE 5-KIP-RELATED PROTEIN 1-SHOOT MERISTEMLESS modulates reproductive development of Arabidopsis

Abscisic Acid Signaling Determines Reproductive Development under Water-deficiency

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Abstract

Soil (or plant) water deficit accelerates plant reproduction. However, the underpinning molecular mechanisms remain largely unknown. By modulating cell division/number, ABSCISIC ACID-INSENSITIVE 5 (ABI5), a key bZIP transcription factor, regulates both seed development and abiotic stress responses. The KRPs/ICKs cyclin-dependent kinases (CDKs) play an essential role in controlling cell division and SHOOT MERISTEMLESS (STM) plays a key role in the specification of flower meristem identity.

Here, our findings show that abscisic acid (ABA) signaling in *Arabidopsis* plants adjusts reproductive outputs (rosette leaf number and open flower number) under water-deficient conditions. Reproductive outputs increased under water-sufficient conditions but decreased under water-deficient conditions in the ABA signaling/metabolism mutants *aba2-1*, *aba2-11*, *abi3-1*, *abi4-1*, *abi5-7*, and *abi5-8*. Further, under water-deficient conditions, ABA induced-ABI5 directly bound to the promoter of *KRP1*, which encodes a cyclin-dependent kinase (CDK) that plays an essential role in controlling cell division, this binding subsequently activatived *KRP1* expression. In turn, KRP1 physically interacted with SHOOT MERISTEMLESS (STM), which functions in the specification of flower meristem identity, promoting STM degradation. We thereby demonstrate that reproductive outputs are adjusted by the ABI5–KRP1–STM molecular module under water-deficient conditions. Together, our findings reveal the molecular mechanism by which ABA signaling controls reproductive development under water-deficient conditions. These findings provide insights that may help guide crop yield improvement under water deficiency.

Introduction

Drought stress has a major impact on crop production outweighing crop yield losses from all other natural hazards (Lv et al., 2019). Under drought stress, a delay of reproductive timing can sometimes be observed (Zhang et al., 2016). However, the most prevalent response to terminal drought stress is to accelerate reproductive outputs (Su et al., 2013; Brodribb et al., 2016; Meng, 2018; Du et al., 2018). *Arabidopsis* reproductive outputs are determined by multiple factors including rosette leaf number, the number of flowers produced, flower and ovary development, open flower number, fruit maturation, ovary abortion, seed size reduction (seed filling rate), and

fruit drop] (Su et al., 2013). All these factors determine a final total seed weight after completion of the *Arabidopsis* life cycle. In this study, for simplification, we only focused on the two most relevant factors, rosette leaf number and open flower number, to determine a final total seed weight (plant reproductive outputs).

The molecular mechanisms triggering the modulation of reproductive outputs are complex. Firstly, the term, drought stress, is loosely used and may denote many different types of stresses including those resulting from shadow, light intensity, temperature and wind. In this context, abundant literature shows that both soil water deficit and plant water status are typically measured to score for "water deficiency"(Su et al., 2013). Understanding these physiological responses may help identify approaches that may contribute to improving crop yield under specific drought regimes (Su et al., 2013; Meng, 2018; Du et al., 2018; Yu et al., 2020a).

ABA is an important plant hormone, involved in a wide variety of regulatory processes, including growth, development and responses to stress. Changes in resource and energy allocation toward processes that increase survival are integrated into these stress responses (Robinson and Hill, 1999; Yamaguchi-Shinozaki and Shinozaki, 2005; Su et al., 2013; Du et al., 2018). The evolutionary role of ABA has evolved to help improve plant fitness under stress (including water deficiency), where it integrates many different processes. Adjustments to plant reproductive outputs are complex under unpredictable environmental changes, including water deficiency. Therefore, the functions of ABA signaling in the context of water deficiency and associated responses are intricate and multifarious.

Arabidopsis lines defective in the function of the basic leucine zipper transcription factor ABSCISIC ACID-INSENSITIVE 5 (ABI5), involved in ABA signal transduction, presented large seed size (Wang et al., 2013; Cheng et al., 2014) and is involved in seed development and the regulation of maturity and subsequent plant cell death (Sakuraba et al., 2014; Su et al., 2016; Zinsmeister et al., 2016).

The KRPs/ICKs cyclin-dependent kinases (CDKs), cyclin-dependent kinase inhibitor proteins, also play a key regulatory role in cell size. These proteins have essential functions in the regulation of cell division and cell cycle progression (Wang et al., 1998, 2000). Therefore, KRPs/ICKs function as

a negative regulators of cell proliferation, for example, regulation of seed size (Cheng et al., 2013) and mitotic cell cycle progression during *Arabidopsis* gametogenesis (Liu et al., 2008) and both ABI5 and KRPs/ICKs are closely connected to adjustments of reproductive outputs. Also, the functions of both ABI5 and KRPs/ICKs are associated with ABA signaling (Wang et al., 1998; Cheng et al., 2014; Su et al., 2016). However, whether either ABI5 or KRP1 are involved in ABA signaling mediated-adjustment of reproductive outputs under water-deficient conditions remains to be determined.

In this study, we adopted a method for adjusting plant survival and plant reproductive outputs under constant water-deficient conditions (25%–35% soil water content, which was maintained by using frequent irrigation) to explore the molecular mechanism of associated reproductive outputs (Su et al., 2013). Genotypic variation in “drought stress” requires carefully interpretation because frequent irrigation to "constant" low soil water content attenuates ABA accumulation (Pu értolas et al., 2017). Our results indicate that under constant water-deficient conditions, leaf relative water content (RWC) was closely related to relative soil water content (SWC), leaf ABA status, transpiration rate, daily water loss and stomatal conductance. This suggests that plant water relations are coordinated with leaf ABA status. Therefore, constant water-deficient conditions may be suitable for studying adjustment of reproductive outputs in plants. We therefore employed this approach to test the hypothesis that ABA biosynthesis and associated signaling can adjust *Arabidopsis* reproductive outputs under different water stress environments (soil water deficit and plant water status). Our results indicated that under constant water-deficient conditions, ABA signals to decrease plant reproductive outputs, namely, rosette leaf number, open flower number and total seed weight. Further analysis revealed that under constant water-deficient conditions, ABI5 activated *KRP1* transcription by direct binding to the *KRP1* promoter. In turn, KRP1 interacted with STM to promote STM degradation and thereby inhibit inflorescence meristem activities. Our findings therefore reveal a molecular mechanism by which plant reproductive outputs are adjusted under constant water-deficient conditions. Moreover, this study identified several cognate components of this regulatory machinery that may have utility to help guide the breeding drought-tolerant crops.

RESULTS

ABA biosynthesis and associated signaling mediates plant reproductive outputs under water-deficient conditions

Plant reproductive outputs are determined by multiple factors, which determine a final total seed weight after completion of the *Arabidopsis* life cycle. In this study, for simplification, we only focused on two key factors, rosette leaf number and open flower number, to determine a final total seed weight.

We employed the loss-of-function mutants *abi3-1*, *abi4-1*, *abi5-7*, *abi5-8*, *aba2-1*, *aba2-11*, and an *ABI5*-overexpression (*ABI5ox*) lines grown under long-day conditions (16-h light/8-h dark). Wild-type, *abi5-7*, *abi5-8*, *ABI5ox*, *abi4-1*, *aba2-1* and *aba2-11* plants all survived at high (~91%—~78% considered as water-sufficient conditions) or low (34%—~29% considered as water-deficient conditions) relative SWC?????? (Supplemental Figures 1A and 1B). Under water-sufficient conditions, leaf RWC?????? (~83%—~74%) was not significantly different within all genotypes (Supplemental Figure 1C), whereas under water-deficient conditions, this parameter was increased in the *ABI5ox* plants (~65%) and decreased in *abi5-7*, *abi5-8*, *abi4-1*, *aba2-1* and *aba2-11* (~29%—~24%) mutants relative to wild-type (~45%) (Supplemental Figure 1C). This might be caused by different transpiration rates. Indeed, the transpiration rates were consistently decreased in the *ABI5ox* plants and increased in the *abi5-7*, *abi5-8*, *abi4-1*, *aba2-1*, and *aba2-11* lines relative to wild-type, at the three time points assayed during the day (Supplemental Figure 1D). This resulted in decreased daily water loss from *ABI5ox* plants and in contrast, increased daily water loss from *abi5-7*, *abi5-8*, *abi4-1*, *aba2-1*, and *aba2-11* mutants relative to wild-type (Supplemental Figure 1E). These findings may result from changes in stomatal conductance (Supplemental Figure 1F) but not leaf area (Supplemental Figure 1G).

Rosette leaf number is slightly reduced with increasing age (Robinson and Hill., 1999). Under water-sufficient conditions, rosette leaf number was decreased in *abi5-7*, *abi5-8*, and *abi4-1* plants and increased in *ABI5ox*, *aba2-1*, and *aba2-11* lines relative to wild-type (Figure 1A; Supplemental Figure 1A). However, under water-deficient conditions, rosette leaf number was decreased in *abi5-7*, *abi5-8*, *aba2-1*, *aba2-11* and *abi4-1* plants and increased in the *ABI5ox* line relative to wild-type

(Figure 1B; Supplemental Figure 1B). Further, the expression of the flowering repressor *FLOWERING LOCUS C* (*FLC*) (Xiong et al., 2019) was consistent with rosette leaf number (Figures 1A—D). Moreover, under water-sufficient conditions, *aba2-1*, *aba2-11*, *abi4-1*, *abi5-7* and *abi5-8* plants presented an increased open flower number, whereas *ABI5ox* plants exhibited a decreased open flower number (Figure 1E; Supplemental Figure 1C). By contrast, under water-deficient conditions, our findings were the complete opposite (Figure 1F; Supplemental Figure 1D). As a result, under water-sufficient conditions, a final total seed weight after completion of the *Arabidopsis* life cycle was increased in *abi5-7*, *abi5-8*, *aba2-1*, *aba2-11* and *abi4-1* plants but decreased in the *ABI5ox* line relative to wild-type (Figure 1G; Supplemental Figure 1E). By contrast, under water-deficient conditions, total seed weight was decreased in *abi5-7*, *abi5-8*, *aba2-1*, *aba2-11*, and *abi4-1* plants and increased in the *ABI5ox* lines relative to wild-type (Figure 1H; Supplemental Figure 1F).

abi3-1 plants partitioned more resources for seed development than wild-type (L*er*) plants (Robinson and Hill, 1999). Time–course analysis revealed that the dry weight and number of fruits per plant, total leaf area per plant and the number of rosette and caulinar leaves per plant in *abi3-1* mutants were all higher than those in the wild-type (L*er*) plants under water-sufficient conditions (Robinson and Hill, 1999), indicating that *abi3-1* plants may have more reproductive output than wild-type. Indeed, the *abi3-1* plants had more reproductive outputs under water-sufficient conditions but less reproductive outputs under water-deficient conditions relative to wild-type (Supplemental Figure 3). Therefore, ABI3 adjusts *Arabidopsis* reproductive outputs under water-deficient conditions.

We speculate that *abi* mutations in turn may elevate ABA levels under water-sufficient conditions. In this context, ABA could influence some developmental pathways independently of *ABI4* or *ABI5*, thus explaining why we observed a phenotypic response mimicking that established by high ABA levels in these mutants.

In sum, our findings indicate that ABA biosynthesis and associated signaling mediates plant reproductive outputs under water-deficient conditions.

ABI5 adjusts reproductive outputs by controlling inflorescence meristem activity under water-deficient conditions

In this study, for simplification, we only focused on ABI5, not ABI3 and ABI4. This is because ABI5 is involved in the regulation of seed development and maturity and subsequent plant death by modulating cell division/number and thus is a key regulator of reproductive output (Cheng et al., 2014; Zinsmeister et al., 2016). Further, flower/fruit number is determined by shoot apical meristem (SAM) activities (Balanz et al., 2018), suggesting that reproductive outputs might be affected by inflorescence meristem activities. Therefore, we determined whether the ABI5 genotype adjusts reproductive outputs by regulating inflorescence meristem activities under water-deficient conditions.

WUSCHEL (*WUS*), a homeodomain transcription factor, can be clearly detected in the SAM, where this factor is essential in maintaining the stem cell pool (Laux et al., 1996; Mayer et al., 1998). The *SHOOT MERISTEMLESS* (*STM*) gene encodes a class I knotted-like homeodomain protein required for SAM formation during embryogenesis and has a key function in the specification of flower meristem identity (Kirch et al., 2003). Therefore, *WUS* and *STM* can be considered as marker genes to detect inflorescence meristem activities under water-deficient conditions.

We thus determined whether ABI5 adjusts reproductive outputs by regulating inflorescence meristem activities under a given water status. Indeed, *WUS* expression in inflorescence meristems in the *abi5-7* plants at 10-, 15-, 20- days after germination (DAG) was elevated under water-sufficient conditions and decreased under water-deficient conditions relative to wild-type (Figures 2A and B). Consistent with these data, quantitative PCR (qPCR) analysis of wild-type and *abi5-7* inflorescence meristems at 30-, 40-, 50-DAG (water-sufficient conditions) or at 25-, 30-, 35-DAG (water-deficient conditions) produced similar results (Figures 2C—F). Therefore, under water-sufficient conditions, the enhancement of *abi5-7* inflorescence meristem activities may elevate open flower number, and thus raise a final total seed weight relative to wild-type, whereas under water-deficient conditions, the opposite is the case (Figure 1).

Overall, our findings suggest that ABI5 adjusts reproductive outputs by regulating inflorescence meristem activities under water-deficient conditions.

Transcription factor ABI5 activates *KRP1* expression

We firstly analyzed the expression levels of key genes, *KRP1*, *KRP2* and *KRP3*, involved in regulating cell proliferation (Wang et al., 1998) in the *abi5-7* and *35S*: *ABI5* lines to identify the downstream target genes of ABI5. qPCR analysis indicated that the *KRP1/2/3* transcripts were less abundant in *abi5-7* plants but more abundant in the *35S*:*ABI5* line relative to wild-type (Figure 3A). Time-course qPCR analysis of the wild-type plants showed that *ABI5* transcripts increased with age under water-sufficient or -deficient conditions (Figure 3B). A similar analysis of wild-type and *abi5-7* plants indicated that *KRP1* transcript abundance over time was considerably reduced in both *abi5-7* whole plants (Figure 3C) and *abi5-7* inflorescence meristems (Figure 3D) relative to that in wild-type, implying that ABI5 may promote *KRP1* expression with increasing age. Also, both *KRP1* and *ABI5* were expressed in SAMs (Ren et al., 2008; Yuan et al., 2014), suggesting that KRP1 and ABI5 functions overlap. Further, the transient expression data in *Nicotiana benthamiana* showed that *ABI5* co-expression increased the expression of a *KRP1pro-LUC* reporter gene (Figure 3E), indicating that ABI5 activates *KRP1* expression. Overall, our findings suggest that ABI5 activates *KRP1* expression.

ABI5 directly binds to the promoter of *KRP1*

Our findings prompted us to examine whether ABI5 directly regulates *KRP1* transcription. Examination of the *KRP1* promoter revealed the presence of putative ABI5 binding sites (ABREs) (ACGT) (Cheng et al., 2014) at three or four possible locations in the *KRP1* or *KRP3* promoters that we labeled as sequences K1–K4 and K1–K3, respectively (Figure 4A; Supplemental Figure 4A). These DNA sequences within the *KRP1* or *KRP3* promoters were utilized for chromatin immunoprecipitation (ChIP) analyses. We employed transgenic plants expressing a MYC-tagged ABI5 for ChIP-qPCR assays. The DNA primers located at regions K3 and K4 of the *KRP1* promoter (Figure 4A) produced transcripts of greatest abundance (Figure 4B). However, a *KRP1* construct, *35S*:*ABI5–GFP/KRP1*(*mABREs*)*pro*:*KRP1* (which was transformed into the *krp1/3* mutant with a

mutant binding site for ABI5 [mABREs]) decreased ABI5 binding at the K3 and K4 regions of the *KRP1* promoter containing mABREs (Figure 4C).

Further, an electrophoretic mobility shift assay (EMSA) was performed to determine if ABI5 can directly bind to the K3 and K4 sequences of *KRP1 in vitro*. The recombinant ABI5 protein bound to the labeled K3 and K4 elements *in vitro* (Figure 4D). An excessive amount of unlabeled competitor K3 and K4 DNA effectively abolished this binding in a dose-dependent manner (Figure 4D). The GST–ABI5 protein was unable to bind to mutated DNA probes of *KRP1* (Figure 4E). These data indicated that ABI5 directly bound the *KRP1* promoter at ACGT motifs between the K3 and K4 sequences *in vitro*. Whereas K1–K3 sequences in the *KRP3* promoter contained at least one ABRE (Supplemental Figure 4A), our ChIP-qPCR assays could not detect ABI5 association with this sequence of the *KRP3* promoter (Supplemental Figure 4B).

We further utilised a transgenic line containing the *GUS* reporter gene driven by tandem repeats of the ABRE sequence (ABI5 binding site), followed by a minimal *35S* promoter (*5 × ABREs:GUS*/wild-type), which was utilized to check ABI5 transcriptional activities. GUS staining intensity in the flowers of the same stage of the plants at 8, 13, 18 and 23 day after bolting (DAB) gradually increased over time under constant water-sufficient conditions (Figures 4F and G). Together, these data suggest that ABI5 directly binds to the promoter of *KRP1* gene.

KRP1 is epistatic to *ABI5* in the regulation of reproductive outputs

Since ABI5 directly binds to the *KRP1* promoter to transcriptionally activate *KRP1* expression (Figures 3 and 4) and ABI5 function was associated with reproductive outputs of *Arabidopsis* plants (Figure 1), we determined if KRP1 activity might regulate reproductive outputs.

The *krp1-KO* and *krp3-KO* single mutants were crossed to generate the *krp1/krp3* double mutant line. Under water-sufficient conditions, a final total seed weight after completion of the plant life cycle was increased in *krp1/krp3* plants but not in *krp1-KO* and *krp3-KO* plants. Further, total seed weight was decreased in the *KRP1ox* line relative to wild-type (Supplemental Figure 5A). By contrast, under water-deficient conditions, total seed weight was decreased in *krp1/krp3* plants

and increased in the *KRP1ox* line relative to wild-type (Supplemental Figure 5B). Together, our results indicate that KRP1/3 mediates the adjustment of plant reproductive outputs under water-deficient conditions.

We next examined the genetic interaction between *ABI5* and *KRP1*. Under water-sufficient conditions, *abi5-7* plants increased total seed weight relative to wild-type, whereas *35S:KRP1* reduced total seed weight (Supplemental Figure 6A). Further, *abi5/35S:KRP1* plants decreased total seed weight relative to wild-type, in a similar fashion to the *35S:KRP1* line (Supplemental Figure 6A). These results indicate that over-expression of *KRP1* inhibits the increase in total seed weight observed in the *abi5-7* mutant. However, under water-deficient conditions, this phenomenon was reversed (Supplemental Figure 6B).

Together, our findings indicate that *KRP1* is epistatic to *ABI5* in the adjustment of reproductive outputs under water deficient conditions.

KRP1/3 adjusts plant reproductive outputs by controlling inflorescence meristem activities under water-deficiency

To determine whether KRP1/3 adjusts plant reproductive outputs by controlling inflorescence meristem activities under water-deficient conditions, we scored *WUSpro–GUS* expression in reproductive tissues of wild-type and *krp1/3* plants under water-sufficient or -deficient conditions. *WUS* expression was either increased or decreased in *krp1/3* inflorescence meristems of *Arabidopsis* plants at 10-, 15-, 20- DAB under water-sufficient or -deficient conditions, respectively (Figures 5A and B). Consistent with these data, qPCR analysis of wild-type and *krp1/3* inflorescence meristems at 30-, 40-, 50-DAG (water-sufficient conditions) or at 25-, 30-, 35-DAG (water-deficient conditions) produced similar results (Figures 5C—F), which were similar with *WUS* and *STM* expression patterns in *abi5-7* plants (Figure 2).

Together, our findings suggest that KRP1/3 adjusts plant reproductive outputs by controlling inflorescence meristem activities under water-deficient conditions.

KRP1 physically interacts with STM to regulate reproductive outputs under water-deficient conditions

We next determined if the cyclin-dependent kinase KRP1 directly interacts with WUS and STM, or whether the transcription factor ABI5 directly binds to *WUS* and *STM* promoters, to regulate plant reproductive outputs under water-deficient conditions. Our ChIP-qPCR assays indicated that ABI5 did not directly bind to either of the *WUS* and *STM* promoters (Supplemental Figure 7).

Full-length KRP1 was expressed as a HIS fusion protein and full-length WUS and STM were expressed as glutathione S-transferase (GST) fusion proteins. Following mixing of the fusion proteins, sefinose resin was used to bind selectively to the HIS–KRP1 fusion protein. The potential presence of the co-precipitated GST fusion protein was examined using a GST antibody. Our findings indicate that KRP1 may interact specifically to STM, but not to WUS, *in vitro* (Figures 6A and B).

Further, we performed *in vivo* co-immunoprecipitation experiments. Following transformation and associated genetic crosses, transgenic plants expressing both HA–KRP1 and GFP–STM were generated. Subsequently, total soluble protein in wild-type and GFP–STM transgenic seedlings was isolated and an anti-GFP antibody was utilized to potentially immunoprecipitate GFP–STM. Our findings indicated that the STM–green fluorescent protein (GFP) fusion interacted with KRP1 in these transgenic *Arabidopsis* plants *in vivo*, but not in wild-type (Figure 6C). KRP1 has been reported to locate to the nucleus (Bird et al., 2007) and STM is a homeodomain transcription factor (Kirch et al., 2003). To confirm that KRP1 interacts specifically to STM, we performed bimolecular fluorescence complementation (BiFC). Our results indicated that KRP1 specifically interacted with STM in BiFC experiments (Figure 6H). Therefore, KRP1 may physically interact with STM *in vivo*.

To investigate if KRP1 impacts on the stability of STM, the recombinant expressed and purified GST-STM fusion proteins were incubated with plant total proteins extracted from *KRP1ox*, *krp1-KO* and wild-type plants. Subsequently, protein degradation assays were performed. Our findings indicated that GST-STM proteins were degraded at a more rapid rate in the protein extracts from *KRP1ox*

transgenic plants relative to the wild-type control (Figures 6D, E and G). By contrast, GST-STM was more stable in the protein extracts derived from *krp1-KO* plants relative to wild-type (Figures 6F and G). Together, these findings indicate that KRP1 may decrease STM stability following their interaction.

To demonstrate *STM* is epistatic to *KRP1* in the regulation of reproductive outputs, we performed further experiments. Under water-sufficient conditions, whereas *krp1/3* double mutant plants increased total seed weight, the loss-of-function mutant of *STM*, *bum1-3*, decreased total seed weight (Figure 6I). Further, the *krp1/krp3/bum1* triple mutant plants also reduced total seed weight under water-sufficient conditions (Figure 6I). However, our findings were the opposite under water-deficient conditions (Figure 6J). These findings indicate that *STM* acts downstream of *KRP1* in the regulation of reproductive outputs under water-deficient conditions.

Together, our findings suggest that KRP1 physically interacts with STM to regulate reproductive outputs under water-deficient conditions.

DISCUSSION

In this study, we focused only on rosette leaf number and open flower number to determine a final total seed weight after completion of the plant life cycle. Further, we applied an approach (Su et al., 2013) that increased plant survival and elevated plant reproductive outputs under constant water-deficient conditions (~25%–35% soil water content). Under these parameters, we identified several key molecular components, ABI3, ABI4, ABI5 and KRP1, and an associated regulatory pathway, the ABI5—KRP1—STM module, involved in the control of plant reproductive outputs under water-deficient conditions. The cognate components and associated regulatory pathway can be considered as potential targets within the evolutionary strategies for adaptation to water-deficient conditions or specific drought regimes. Therefore, the molecular pathway identified ABA - KRP1 – STM may provide opportunities to increase crop yields under water-deficient conditions or specific drought regimes.

Endogenous ABA is rapidly accumulated under water-deficient conditions (Supplemental Figure 8D;

Knight and Knight, 2001), which in turn promotes the expression of the gene encoding the bZIP transcription factor, ABI5 (Wang et al., 2013). Subsequently, ABI5 directly binds to the *KRP1* promoter, thereby activating its expression (Figures 3 and 4) and in turn KRP1 interacts with STM (Figure 6), to inhibit activities of inflorescence meristems (Figures 2 and 5). These components thus form an ABI5–KRP1–STM module. Therefore, under water-deficient conditions, the ABI5–KRP1–STM module decreases reproductive outputs through inhibiting inflorescence meristem activities.

Under constant water-deficient conditions, stomatal aperture, stomatal conductance and transpiration were elevated in *abi5-7* and *krp1/3* mutant plants relative to wild-type (Supplemental Figures 8A-C), which leads to more water loss in *abi5-7* and *krp1/3* mutant plants. The resulting water-deficiency subsequently generated slightly more ABA in these mutant plants (Supplemental Figure 8D; Léon-Kloosterziel et al., 1996) and in turn decreased inflorescence meristem activities through the ABI5-KRP1-STM module. The resulting decreased reproductive outputs in these mutants are produced under water-deficient conditions (Figure 1J; Supplemental Figure 5B). However, we have demonstrated that ABA biosynthesis and associated signaling mediates these reproductive outputs under water-deficient conditions (Figure 1). Therefore, the ABI5-KRP1-STM module regulates inflorescence meristem activities predominantly through ABA biosynthesis and associated signaling.

Previous observations lend some support to our model. ABA is a general negative regulator of *Arabidopsis* axillary bud growth and the *aba2-1* mutant has more branching and bud outgrowth under water-sufficient conditions (Yao and Finlayson., 2015), suggesting the meristematic activity of the shoot apex is increased in *aba2-1* mutant plants under water-sufficient conditions. ABA may inhibit plant cell division (Wang et al., 1998; Mcadam et al., 2016), implying cell division is promoted in organs such as the SAM in either *aba3* or *abi5* mutant plants under water-sufficient conditions. Further, time–course analysis (Robinson and Hill., 1999) revealed that the dry weight and number of fruits per plant, total leaf area per plant and the number of rosette and cauline leaves per plant in *abi3-1* mutants all were higher than those in the wild-type plants under water-sufficient conditions. Thus, suggesting that *abi3-1* mutants have increased reproductive outputs under water-sufficient conditions, similar to that of the *abi5-7* line (Figure 1G). In addition, the absence of ABI5 function delayed senescence (Sakuraba et al., 2014) and increased seed size

(Cheng et al., 2014) and the overexpression of *KRP1* resulted in fewer flowers and fruits relative to wild-type (Wang et al., 2000). Also, KRP1 is known to negatively regulate seed size (Cheng et al., 2013). Therefore, based on our data, we concluded that reproductive outputs are adjusted by the ABI5 – KRP1 – STM molecular module under water-deficient conditions.

In this study, we observed that the differences in soil or plant water status does not result in genotypic differences in reproductive outputs under water-sufficient conditions (Figure 1; Supplemental Figures 1 and 2), suggesting a direct impact of ABA signaling. By contrast, under water deficit conditions, genotypic differences in reproductive outputs had a direct correlation with variation in plant water status (Figure 1; Supplemental Figures 1 and 2). Thus, an alternative explanation is that differences in leaf water status may modulate reproductive outputs, rather than ABA signaling per se (Sharp et al., 2000).

Experimental procedures

Plant Materials and Growth Conditions

The *aba2-1* (Cheng et al., 2014), *aba2-11* (Gonzalez-Guzman et al., 2002), *abi5-7* (Wang et al., 2013; Yu et al., 2020b), *abi3-1* (CS24) (Robinson and Hill., 1999), and *abi4-1* (Shkolnik-Inbar et al., 2013) mutations were described previously.

The *krp1/krp3* [*krp1-KO1* (SALK_026391C) *krp3-KO1* (KRP3B_144H6)] double mutant was obtained from adult-fertile F2 plants, which were *krp1-KO1* (SALK_026391C) crossed with *krp3-KO1* (KRP3B_144H6). The genotype was confirmed by PCR. The *abi5-7/35S:KRP1* line was obtained from F2 plants, which were *abi5-7* crossed with *35S:KRP1*. The *abi5-7/WUSpro-GUS*, *krp1/krp3/WUSpro-GUS*, *abi5-7/STMpro-GUS*, and *krp1/krp3/STMpro-GUS* lines were obtained from F2 plants, which were *WUSpro-GUS/*wild-type (+) was crossed with *abi5-7* and *krp1/krp3* (-). These crossed lines were identified by GUS staining and the genotype confirmed by qPCR. *pCB308R-ABE-GUS* and *pMD111-KRP1pro-GFP* were introduced into the wild-type background by *Agrobacterium* mediated-transformation and were identified and characterized. *35SABI5-GFP/KRP1(mABREs)pro::KRP1* were introduced into the *krp1/krp3* background by *Agrobacterium*

mediated-transformation and then were identified and characterized, and used as material for ChIP-qPCR. *35S:ABI5-MYC* were introduced into the *abi5-7* background by *Agrobacterium* mediated-transformation, and then were identified and characterized, and used as material for ChIP-qPCR. The *bum1-3* (CS3781) lines were identified by qPCR. All transgenic plants were generated using the *Agrobacterium tumefaciens*-mediated floral dip method (Meng and Yao., 2015).

Plants grown in soil were maintained in a growth room under 16/8 h of light/dark cycle with cool white fluorescent light (140 μmol quanta PAR m^{-2} s^{-1}) at 21 ± 2°C. Seedlings grown on MS medium were maintained in a controlled environment growth chamber under 16/8 h light/dark cycle with cool white fluorescent light at 21 ± 2°C.

GUS Assay and Analysis of GUS Activity

The GUS assays were described previously (Meng et al 2018c; Mu et al., 2022, 2023; Bao et al., 2023).

Water-Deficient Experiments

Experiments were undertaken as has been described as Su et al (2013) with minor revisions. For plants, wild-type and relative mutant/transgenic seeds were separately germinated at normal density in soil in 10cm×10cm pots. All mutant/transgenic plants were well watered before bolting (the main stem was ~1 cm high). To assay drought stress responses, two treatments (WS-water sufficiency, ~80~90% soil moisture or field capacity; WD-water deficiency, ~25~35% soil moisture or field capacity) were performed. Pots were randomly arranged. To WS plants (control), ~80~90% of the soil moisture was always kept until seed maturation or fruit dry. To WD plants, the content of the relative soil moisture rapidly declined; drought treatment was initiated by withholding water at 15 days after germination, the content of the relative soil moisture arrived at ~25~35% of the soil water-holding capacity. A controlled environment growth chamber (temperature: 23 ℃ [light]/19℃ [dark]; light intensity: ~130 μmol quanta m^{-2} s^{-1} provided via fluorescent bulbs; relative humidity: ~65%) was altered back to ~65%. The soil water status was maintained via daily watering. In this context, pots were weighed and watered once per day) until almost all fruits

became dry and mature to harvest. During this process, the rosette leaf number and open flower number of wild-type and mutant/transgenic plants at different rosette leaf number or sowing times was counted and analyzed. Mature seeds were weighted to gain a final total seed weight.

Assays of Stomatal Aperture, Stomatal Conductance, and Transpiration

Stomatal aperture, soil RWC, leaf RWC, stomatal conductance, water loss, leaf area, and transpiration were tested, as was previously described (Meng and Yao., 2015; Meng et al., 2015).

Transient Assays

To produce *KRP1-LUC*, its promoter was amplified by PCR. used primers were seen in Supplemental Table 1. To examine ABI5 activates *KRP1* expression by LUC activity assay, we performed the below experiments. To generate *proKRP1-LUC*, the promoter was PCR amplified with primers *proKRP1-F* and *proKRP1-R* (*ProKRP1*-F-ttcctgcagcccgggggatccatgtattgatgcatgaaacctct; *ProKRP1*-R-tgtttttggcgtcttccatggcttcgatttaggttacgtgtgcgtg) for the gnomic DNA of *Arabidopsis* and inserted into the cloning site of the *pGreen0800-LUC* vector.

The *MproKRP1-LUC* construct containing mutations in the S2 sequence of the *KRP1* promoter was generated using overlap extension PCR with primers (*proKRP1-F*: ttcctgcagcccgggggatccatgtattgatgcatgaaacctct, KRP1-mR1: gatgtcaagttgtcaacagcaGCtgtcgatcgtgatatattgggc, *KRP1-mF2*: gcccaatatatcacgatcgacaGCtgctgttgacaacttgacatc, KRP1-mR2:gttacggatagcctaaaaactaGCtacctgtgagtaaaataagg, KRP1-mF3: ccttattttactcacaggtaGCtagtttttaggctatccgtaac *proKRP1-R*: tgtttttggcgtcttccatggcttcgatttaggttacgtgtgcgtg,) and inserted into *pGreen0800-LUC* vector.

Transient assays were performed as has been previously described (Meng et al., 2020).

Plasmid Constructs

For obtaining 5×ABREs binding site +minimal CaMV 35S promoter: this fragment (GACGCTCGTCATGCGGTACACGTGGCAATCTTGACGCTCGTCATGCGGTACACGTGGCAATCTTGACGCTC GTCATGCGGTACACGTGGCAATCTTGACGCTCGTCATGCGGTACACGTGGCAATCTTGACGCTCGTCATGCG

GTACACGTGGCAATCTTGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACGGGGG ACTCTTGA) was inserted into EcoRI+NcoI site of Pcambial301.

For obtaining plasmid of *pMD111-KRP1pro-GFP*, *PHB-35S:KRP1-HA*, *pCB308R-KRP1-GUS*, *pCB308R-STM-GUS* and *PHB-35S:STM-GFP*, used primers were seen in Supplemental Table 1.

Endogenous ABA assay

To assay endogenous ABA contents under constant water-deficient conditions (~25% – 35% soil water content), a radioimmunoassay method was performed as previously described via Wang et al. (2011).

ChIP-qPCR

The *35S:ABI5-MYC* line was used in this experiment. ChIP was performed using 6-week-old seedlings as materials, as has been described by Meng and Yao (2015). MYC tag-specific monoclonal antibody and Anti-GFP antibody were used for ChIP analysis. The ChIP DNA products were analyzed through qPCR using a few pairs of primers that were synthesized to amplify ~100-300-bp DNA fragments in the promoter of *KRP1*, *KRP3*, *STM*, and *WUS* in ChIP analysis. Used primers are listed in Supplemental Table 1.

Quantitative PCR

Total RNA was extracted from tissues indicated in the figures by the TRIZOL reagent (Invitrogen), as has been described in Meng et al (2018b). SYBR green was used to monitor the kinetics of PCR product in real-time RT-PCR, as has been described in Meng et al (2018b).

For analyzing *KRP1*, *KRP2* and *KRP3* expression in wild-type and mutant seedlings, used primers are listed in Supplemental Table 1.

Protein Expression and Purification

The plasmid *pGEX-5X-1* was used in this experiment. The coding sequence of *ABI5* was amplified by the primer pair. Used primers are shown in Supplemental Table 1. Protein expression and purification was described (Meng et al., 2020).

Electrophoretic Mobility Shift Assay (EMSA)

The electrophoresis mobility shift assay (EMSA) was performed by using the LightShift Chemiluminescent EMSA Kit (Pierce, 20148) according to the manufacturer's instructions. The biotin-labeled *KRP1-K3-K4* DNA fragments (5'-ac**ACGT**gctgttgacaacttgacatcaggctaagcaaatacgcagggataacaaaaatataatttctagtgtcagttttacttttaccttatttactcacaggt**ACGT**a-3',5'-t**ACGT**acctgtgagagtaaaataaggtaaaagtaaaactgacactagaaattatatttttttgttatccctgcgtatttgcttagcctgatgtcaagttgtcaacagc**ACGT**gt-3') and mutated *KRP1-K3-K4* DNA fragments(5'-ac**ACCT**gctgttgacaacttgacatcaggctaagcaaatacgcagggataacaaaaatataatttctagtgtcagttttacttttaccttatttactcacaggt**ACCT**a-3',5'-t**AGGT**acctgtgagagtaaaataaggtaaaagtaaaactgacactagaaattatatttttttgttatccctgcgtatttgcttagcctgatgtcaagttgtcaacagc**AGGT**gt-3') were synthesized, annealed and used as probes, and the biotinunlabeled same DNA fragments as competitors in this assay. EMSA was performed, as was previously described (Meng et al., 2020).

GFP or YFP Imaging

Confocal laser scanning microscope for GFP or YFP imaging was performed, as was previously described (Meng et al., 2018c).

BiFC assays

To perform BiFC assays, pBI121-YFPC was generated through using primers; and pBI121-YFPN was produced by using; and pBI121-YFPN-ABI5 was generated through using primers; and pBI121-YFPC-STM was generated through using primers. Used primers were seen in Supplemental Table 1. BiFC assays were performed, as has been described (Meng et al., 2018c).

In vitro pull-down assay

HIS-KRP1 and GST-WUS or GST-STM expression constructs were prepared as described (Meng et al., 2018c). The potential for in vitro interaction between KRP1 and these GST fusion proteins was tested. *In vitro* pull-down assays were performed, as has been described (Meng et al., 2018). Used

primers are listed n in Supplemental Table 1.

In vivo pull-down assay (Co-immunoprecipitation)

In vivo pull-down assays using wild-type and transgenic plant extracts were performed according to (Meng et al., 2018). Transgenic plants containing both HA-KRP1 and GFP-STM expression constructs were harvested. *In vivo* pull-down assay was performed, as has been described (Meng et al., 2018c).

Statistical Analysis

Three independent experiments were performed with similar results and error bars represent SD. Student's *t* test was used to analyze the significance between two indicated samples at a significance level of 0.05 (***$p < 0.001$; **$0.001<p< 0.01$; *$0.01<p<0.05$).

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Supporting Information

Supplemental Figure 1. Relative SWC, leaf RWC, transpiration rates, water loss, and stomatal conductance were assayed.

Supplemental Figure 2. Reproductive outputs of ABA signaling mutant plants were adjusted under water-deficient conditions.

Supplemental Figure 3. Reproductive outputs of *abi3-1* plants were adjusted under water-deficient conditions.

Supplemental Figure 4. ABI5 is not able to directly bind to promoters of *KRP3* and *CYCD3:1*.

Supplemental Figure 5. Reproductive outputs of *krp1/krp3* plants were adjusted under water-deficient conditions.

Supplemental Figure 6. *ABI5* acts upstream of *KRP1* in the regulation of reproductive outputs under water-deficient conditions.

Supplemental Figure 7. ABI5 is not able to directly bind to promoters of *WUS* and *STM*.

Supplemental Figure 8. Stomatal aperture, stomatal conductance, and transpiration in *abi5-7* and *krp1/3* mutant plants were enhanced under constant water-deficient conditions.

Supplemental Table 1. All used primers in this work.

Author Contributions

L.S. Meng designed experiments. L.S. Meng, S.T Zhao, X.R. Mu, Y.T Wei, Q.X. Bao, W.N Wang, J.W. Wang, C.Y. Gu, X.R. Liu, Q.M. Wang, Y.X. Liu, J.L Zhu, N Sai, F.H and Yu performed the experiments. L.S.Meng and W.N Wang completed statistical analysis of data. L.S. Meng, J.H Jiang, and G.J. Loake wrote, edited and revised this manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

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Figure legends

Figure 1. Reproductive outputs of ABA signaling mutant plants were adjusted under water-deficient conditions.

A and **B,** Bar graph illustrating rosette leaf number of wild-type (Col-0), *aba2-1*, *abi5-7*, and *35S:ABI5-MYC* (*ABI5ox*) plants by using a time course analysis. Seedlings were grown in soil under water-sufficient (A) or -deficient (B) conditions.

C and **D,** Bar graph illustrating *FLC* expression in all genotypes (wild-type (Col-0), *aba2-1, aba2-11, abi4-1, abi5-7, abi5-8,* and *35S:ABI5-MYC (ABI5ox)*) for 40 days after

germination were grown in soil under water-sufficient (C) or -deficient (D) conditions. The expressions of *FLC* gene in wild-type plants were set as 1.0. Quantification was normalized to the expression of *UBQ5.*

E and **F,** Bar graph illustrating open flower number of all genotypes by using a time course analysis. Seedlings were grown in soil under water-sufficient (E) or -deficient (F) conditions.

G and **H,** Bar graph illustrating total seed weight of all genotypes by using a time course analysis.

Seedlings were grown in soil under water-sufficient (G) or -deficient (H) conditions. Seeds were harvested from wild-type and different genotypes determined.

Please note: total seed weight indicates a final total seed weight after completion of plant life cycle.

The ANOVA of two-way was utilized to calculate significant differences (n=3). Tukey's post-test was used, and different lowercase letters above the bars indicate significant differences at $*p < 0.05$.Three replicate samples of 18 seedlings were harvested for analysis.

Figure 2. The activities of *abi5-7* inflorescence meristems were raised or reduced under water-sufficient or -deficient conditions, respectively.

A and **B,** Images illustrating expressions of *WUSpro-GUS* in SAMs of inflorescences from wild-type and *abi5-7* plants at 10, 15, 20 DAB grown in soil under water-sufficient (WS) (A) or -deficient (WD) (B) conditions. Arrows indicate SAM. Bar=200 μm.

C—F, Bar graph illustrating time course qPCR for the differential expression of *WUS* and *STM* genes between wild-type and *abi5-7* plants at 30-, 40- and 50-DAG (Day after germination) grown in soil under water-sufficient (WS) (C and D) or 25-, 30- and 35-DAG grown in soil under water-deficient (WD) (E and F) conditions. Inflorescence meristems were collected as materials for time course qPCR. The expressions of the

WUS and *STM* genes in wild-type plants were set as 1.0. Quantification was normalized to the expression of *UBQ5.* Error bars represent SD (n=3). Student's *t* test (**p*< 0.05; ***p*< 0.01).

Figure 3. ABI5 activates *KRP1* expression.

A, Bar graph illustrating the differences in the expression levels of *KRP1*, *KRP2*, and *KRP3* among wild-type, *35S:ABI5* and *abi5-7* plants for 45 DAG. Quantifications of the expression levels of these genes in wild-type plants were set as 1.0.

B, Bar graph illustrating differential expressions of *ABI5* gene in wild-type plants at 25-, 30- and 35-DAG grown in soil under water-sufficient or deficient conditions. Quantifications of wild-type plants at 25-DAG were set as 1.0 in qPCR analysis. Relative soil water content was provided in Supplemental Figure 1. Inflorescence meristems were collected as materials for time course qPCR.

C and **D,** Bar graph illustrating differential expression of *KRP1* in wild-type and *abi5-7* whole plants (C) and inflorescence meristems (D) at 25-, 30- and 35-DAG grown in soil under water-sufficient conditions. Quantifications of wild-type plants at 25-DAG were set as 1.0 in qPCR analysis. Inflorescence meristems were collected as materials for time course qPCR.

E, Bar graph illustrating transient expression of the *35S:ABI5* effector construct with the *KRP1pro-LUC* reporter construct in *N. benthamiana* leaves. Note *PGreen-mKRP1* indicates the conserved sites (ACGT) of the K3-K4 region in *KRP1* promoter were mutated. The activity of relative LUC represents arbitrary luminescence units, that is, expressing *KRP1-LUC* is ~5, other expressions was quantified by using Adobe Photoshop CS (AdobeSystems) software, as described previously by Meng et al (2015).

Quantifications were normalized to *UBQ5* expression. Error bars represent SD (n=3). Student's *t* test (****p*< 0.001; ***p*< 0.01; **p*< 0.05).

Figure 4. ABI5 directly binds to *KRP1* promoter.

A, Schematic of the *KRP1* loci and a few amplicons initiating from ATG of *KRP1.* K1: -1 — -150 bp; K2: -120 — -280 bp; K3-K4: -540 — -690 bp. Red bars indicate that potential ABI5-binding sites.

B, Bar graph illustrating ChIP-qPCR. ChIP-qPCR was performed to analyze the *in vivo* interaction between ABI5 and the *KRP1* promoter. Anti-MYC antibody was used to facilitate precipitation of chromatin associated with *35S:ABI5-MYC/abi5-7*. A two-way ANOVA was utilized to calculate significant differences (n=3). Tukey's post-test was used, and different lowercase letters above the bars indicate significant differences at $^{**}p < 0.01$.

C, Bar graph illustrating ChIP-qPCR. ChIP-qPCR was performed to analyze the *in vivo* interaction between ABI5 and K3-K4 of *KRP1* promoter. Enrichment of particular chromatin regions of K3-K4 in *KRP1* promoter with anti-GFP antibody or anti-HA antibody (control) by using *35S:ABI5-GFP/KRP1(mABREs)pro::KRP1/krp1/krp3* or *35S:ABI5-GFP/abi5-7* seedlings at 8 DAB as materials, as detected by qPCR analysis.

D and **E,** Images illustrating unlabeled *KRP1* promoter and unlabeled probes were used as competitors to determine the specificity of DNA-binding activity for ABI5. Free probe and ABI5 probe complexes are indicated by an asterisk and arrows, respectively (D). A mutant version of the *KRP1* promoter (TGCA) was labeled with biotin and used for EMSA with ABI5 polypeptides (E).

F, Images illustrating *5×ABREs* (*5×ABI5 binding site*)*:GUS* flowers of seedlings at 8, 13, 18, 23 DAB grown on soil under constant water-sufficient conditions. GUS staining was performed to mark associated gene expression. 20 flowers were hand pollinated at identical positions on secondary inflorescences of *5×ABREs:GUS* plants to generate even-aged fruits. Bar= 1.0 cm.

G, Bar graph illustrating ABI5 activities (indicated through 5×ABS-GUS activity) of the indicated seedlings in F were assayed quantitatively at the indicated time point. GUS activity was measured by picomoles of 4-methyl umbelliferone (4-MU) per mg protein

per min. GUS activity of 8 DAB was used as control.

Quantifications were normalized to the expression of *UBQ5*. Error bars represent SD (n=3). Student's *t* test (**p< 0.01; *p< 0.05).

Figure 5. The activities in *krp1/3* inflorescence meristems were enhanced or reduced under water-sufficient or -deficient conditions, respectively.

A and **B,** Images illustrating the expression of *WUSpro-GUS* in SAMs of inflorescences from wild-type and *krp1/3* plants at 10, 15, 20 DAB grown in soil under water-sufficient (WS) (A) or -deficient (WD) (B) conditions. Arrows indicate SAM. Bar=200 μm.

C—F, Bar graph illustrating time course qPCR for the differential expression of *WUS* and *STM* genes between wild-type and *krp1/3* plants at 30-, 40- and 50-DAG grown in soil under water-sufficient (WS) (C and D) or 25-, 30- and 35-DAG grown in soil under water-deficient (WD) (E and F) conditions. Inflorescence meristems were collected as materials for course time qPCR.

The expression of the *WUS* and *STM* genes in wild-type plants were set as 1.0. Quantification was normalized to the expression of *UBQ5.* Error bars represent SD (n=3). Student's *t* test (***p< 0.001; **p< 0.01; *p< 0.05).

Figure 6. KRP1 interacts physically with STM, to inhibit STM stability.

A, Images illustrating *in vitro* GST pull-down assays of the interaction between KRP1 and WUS. Anti-His immunoblot (IB) showed KRP1-His cannot be bound by the indicated WUS-GST protein. HIS or GST fusion proteins were mixed at 4 ℃ for 3 h (to guarantee full interaction between HIS and GST fusion proteins). Then, sefinose resin was added to obtain the interacting HIS fusion proteins. Western blot analysis was performed by using these purified materials. Anti-GST antibody (Abcam, China) was used for protein detection. The input (crude protein containing the GST–WUS protein) was used as the positive control. Non-specific bands were used a control.

B, Images illustrating *In vitro* GST pull-down assays of the interaction between KRP1

and STM. Anti-His immunoblot (IB) shows the amount of KRP-His bound by the indicated STM-GST protein. The input (crude protein containing the GST–STM protein) was used as the positive control. Non-specific bands were used a control.

C, Images illustrating *In vivo* put-down assays of the interaction between KRP1 and STM in transgenic *Arabidopsis* plants. Transgenic *Arabidopsis* plants containing *KRP1ox–GFP* (positive control) or *KRP1ox–GFP/STMox-HA* were extracted by using a protein extraction kit. The obtained crude protein was enriched through the addition of beads (Anti-HA Magnetic Beads, ChromoTeK, Germany) at 4 ℃ for 2 h. Western blot analysis was performed by using these materials. GFP-KRP1 was associated with membranes and can be detected with an anti-GFP antibody (Abcam, China). Tubulin was used a control.

KRP1ox-GFP as materials for Western blot analysis was used as the positive control. Wild type was considered as the negative control in Western blot analysis.

D—F, Images illustrating cell-free degradation assays proved that KRP1 destabilizes the STM protein. The recombined STM-GST protein was co-incubated with the isolated total proteins extracted from the the *KRP1ox* (D), wild-type (WT) control (E), and *krp1-KO1* (F) *Arabidopsis* plants. Moreover, the mixed proteins were treated with 25 μg /mL cycloheximide for 0, 1 or 3 hours. The degradation of STM protein was assayed by using western blotting with an anti-GST antibody. MG132 was used as a positive control for stabilizing the STM protein.

G, Bar graph illustrating the quantitation of the western blot data in D—F.

H, Images illustrating BiFC assay. Pairs of proteins of KRP1 and STM fused with halves of the YFP molecule were transiently expressed in wild-type tobacco leaves and reconstituted YFP fluorescence was imaged in epidermis on the abaxial leaf blades. Plasmid containing YFP was used as a positive control. Only KRP1 fused with halves of the YFP molecule was used as a negative control. Bars = 40μm.

I and **J,** Bar graph illustrating total seed weight in in wild-type, *krp1/3*, *bum1-3*,

krp1/krp3/bum1 plants were grown in soil under water-sufficient (I) or -deficient (J) conditions. Please note: total seed weight indicates a final total seed weight after completion of plant life cycle. Wild-type was used as a control in I and J. Error bars represent SD (n=14 in I and J; n=3 in G). Student's *t* test (**$p < 0.01$; *$p < 0.05$).

Figure 7. Proposed model is shown to illustrate how reproductive outputs in *Arabidopsis* plants are adjusted under constant water deficient conditions.

Under constant water-deficient conditions, ABA levels are enhanced and ABA signaling is transduced by the *ABA2-ABA-ABI5-KRP1-STM/WUS* pathway, leading to *KRP1* upregulation. *KRP1* upregulation with high ABA level inhibits cell proliferation, which in turn inhibits inflorescence meristem activity by directly suppressing the expression *STM* and *WUS* genes and thereby lowering flower number and a final total seed weight. As a result, reproductive outputs in plants are decreased under constant water-deficient conditions. Further, flowering time is inhibited by the ABA-U2AF65b-ABI5-FLC pathway (Xiong et al., 2019).

Arrows and bars represent positive and negative regulation, respectively. Solid lines indicate direct regulation, whereas dotted lines indicate either indirect regulation or regulation in an unknown manner.