

# The RNA-binding protein RGGA is a sugar-responsive cofactor of the 5'-3' exonuclease XRN4 that post-transcriptionally mediates plant growth

**Hongmei Liu** (✉ [hmliu@sdau.edu.cn](mailto:hmliu@sdau.edu.cn))

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

<https://orcid.org/0000-0002-1818-8334>

**Ru Yu**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Hong Zhang**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Xinlei Gao**

School of Life Sciences, University of Science and Technology of China

**Changxiang Zhu**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Changai Wu**

Shandong Agricultural University

**Liwei Geng**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Jinwen Du**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Haoran Ma**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Yunzhi Song**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

**Shumei Zhou**

State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University

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## Article

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# Abstract

Sugar-regulated gene expression is a ubiquitous mechanism for carbohydrate allocation and utilization by keeping a balance among source and sink organs in plants. Previous studies have shown that excess sugar represses the expression of the genes implicated in photosynthesis and sugar metabolism. However, the mechanism is still largely unknown. Here, we found that the mutant of *Arabidopsis* RGGA, RGG repeats RNA-binding protein A coding gene, grew faster than wild type (Col-0) in MS medium. In *rgga*, mRNA half-life of the genes related to sucrose transport and metabolism, chlorophyll synthesis, root development as well as certain transcription factors was obviously longer than those of Col-0. Further study revealed that AtRGGA could interact with 5'-3' exonuclease AtXRN4, and guide it to the target mRNAs for their degradation. When AtRGGA is absent or its interaction domain is deleted, AtXRN4 self can't recognize the target mRNAs, which leads to a dramatically increase in transcript levels of the above gene subsets, and thus promotes the growth of *Arabidopsis* with exogenous sucrose supply. And only 5-day sucrose supply could trigger the vigorous growth of *rgga*. These findings suggest that the regulation of mRNA stability mediated by RGGA plays a critical role in sugar suppression, and implicates a possibility to unlock the growth potential by modulating sugar utilization at post-transcriptional level in plants.

## Introduction

In plants, sugars function as metabolic resources and structural constituent, and also function as signals that trigger gene expression changes associated with plant growth and development ([Eveland and Jackson, 2012](#); [Ruan, 2014](#)). Indeed, we know that sugars are components of complex and interconnected signaling networks that allow plants to control cell division, growth, differentiation, and programmed cell death, including in responses to metabolic and environmental cues ([Ho et al., 2001](#); [Koch, 1996](#)). The differential availability of soluble sugars is known to induce or repress the expression of a large variety of genes. The nature of sugar-mediated changes in gene expression is unique in plants, as these autotrophic organisms because their synthesis and allocation of carbohydrates can be modulated through photosynthesis/utilization balance ([Koch, 1996](#)). That is, plants can be considered as carbon heterotrophs at some stages during their life cycle, and some of their non-green organs like roots, stems, and flowers consistently function as sinks for carbon.

The photosynthetic and sugar metabolism genes have early been demonstrated to be repressed by photosynthetic product sucrose and glucose. That is known as sugar repression or sugar catabolite repression. And most studies on the mechanisms of sugar repression in plants have emphasized regulation at the transcriptional level ([Chan et al., 1994](#); [Graham et al., 1994](#); [Sheen, 1990](#)). Yet there is increasing awareness that sugar repression involves both transcriptional and mRNA stability related regulatory mechanisms. For example, three essential motifs in the promoter of the starch metabolism  $\alpha$ -amylase gene are responsible for its strong transcription, and the 3'-untranslated region (3'-UTR) of  $\alpha$ -amylase and its subdomains function independently as sugar-dependent mRNA stability determinants ([Chan and Yu, 1998a, b](#); [Lu et al., 1998](#); [Sheu et al., 1996](#)).

Regulated stability *vs.* decay of mRNA transcript is now understood as a fundamental aspect for controlling eukaryotic gene expression. The 5'-3' and 3'-5' mRNA decay pathways were first identified in yeast and are now known to be conserved in multicellular eukaryotes (Chiba and Green, 2009; Mitchell and Tollervey, 2000). Highly unstable mRNA species in the cytoplasm are typically degraded through the 5'-3' mRNA decay pathway, which occurs in cytoplasmic foci called processing bodies (P-body) (Xu and Chua, 2011), whereas stable mRNAs are often degraded through the 3'-5' mRNA decay pathway occurs in both cytoplasmic and nuclear RNA exosomes (Puno et al., 2019; Song et al., 2013). The 5'-3' mRNA decay pathway is initiated upon the deadenylation and subsequent decapping of transcripts, which is catalyzed by the conserved eukaryotic decapping complex VARICOSE (VCS)-DCP1-DCP2; the decapped mRNAs are subsequently digested by 5' to 3' exoribonucleases (Rymarquis et al., 2011; Xu et al., 2006). *Arabidopsis* XRN4 is the functional homolog of yeast XRN1 and is known to degrade mRNAs and 3' cleavage products generated by the microRNA (miRNA) pathway (Rymarquis et al., 2011; Souret et al., 2004). Mutant analysis of *Arabidopsis* P-body components including DCP1, DCP2, VCS, and the exoribonuclease XRN4 have implicated 5'-3' mRNA decay was involved in postembryonic development, seed germination, thermal stress, as well as pathogen response (Basbouss-Serhal et al., 2017; Merret et al., 2013; Nguyen et al., 2015; Xu et al., 2006; Zhang et al., 2020).

Elevated sugar levels in plant cells have been shown to suppress the expression of a set of genes known to function in photosynthesis, reserve mobilization, and the export of sugars (Sheen, 1994; Thomas and Rodriguez, 1994; Yu, 1999). However, how this set of genes are coordinately regulated by sugar is still unclear. Here, in a screen for sucrose-responsive genes, we found that *rgga*, a mutant of *Arabidopsis* glycine-rich RNA binding protein, grew obviously faster in sucrose containing medium than wild type plants. We found that AtRGGA guides the 5'-3' exonuclease AtXRN4 to a subset of sucrose-response-related mRNAs for selective degradation. These transcripts are of genes with diverse functions including sugar carbohydrate metabolism, chlorophyll synthesis, and root development, and all detected mRNAs have common three motifs (UCUCWCUYUCDC, AGGAARRGRAA, YUUCUUCHCCU) in their 5' UTRs and start codon near regions. When *AtRGGA* is absent, these mRNAs accumulate and trigger the vigorous long-term growth phenotypes we observed. Beyond demonstrating how RNA-binding proteins can act as cofactors to direct specific degradation *via* the 5'-3' mRNA decay pathway, and given that RGGA orthologs are widely present in both eudicots and monocots, our work suggests that combining disruption of RGGA function with sugar supplementation during planting represents a promising strategy for increasing the productivity of diverse crop species.

## Results

### Exogenous sugar supplementation causes *Arabidopsis rgga* mutant plants to grow obviously faster than WT (Col-0) plants.

We conducted a screen for sucrose responsive genes using a series of *Arabidopsis* mutant lines grown in MS medium supplemented with sucrose. *rgga* (SALK\_143514), a deletion mutant of *AtRGGA* with a single T-DNA insertion located in its second exon, grew obviously faster than Col-0 in 2-3% (w/v) sucrose MS

medium (Fig. 1a). *AtRGGA* encodes a glycine-rich RNA binding protein (RBP), and its expression is induced by sucrose (Fig. 1b,c). *AtRGGA* is widely conserved in monocots and eudicots (Supplementary Fig. 1). After 2 weeks growth on MS media with 2% sucrose or then transported to vermiculite for additional 2 weeks growth, the biomass of *rgga* seedlings was significantly elevated compared to Col-0 seedlings (Fig. 1d,e).

To investigate whether the growth phenotypes result from sucrose specifically or sugar supplementation generally, *rgga* and Col-0 plants were grown in media supplemented with diverse sugars (2% w/v), including three pentoses (ribose, xylose, and arabinose), three hexoses (glucose, mannose, and fructose), two disaccharides (sucrose and maltose), and one polysaccharide (soluble starch). Besides sucrose, glucose, mannose, fructose, and maltose also caused the significant rapid growth phenotypes for *rgga* seedlings (Fig. 1f). qRT-PCR analysis showed that the expression of *AtRGGA* was induced by all of the sugars used in this study (Supplementary Fig. 2).

We also conducted experiments in which seedlings were transplanted into vermiculite and allowed to developed through their reproductive growth stage without further exogenous sucrose supply. These experiments revealed that the growth advantage we observed for 2% sucrose supplemented *rgga* seedlings actually persisted throughout the whole *Arabidopsis* life cycle (Fig. 1g). The grain yield of *rgga* plants was significantly higher than Col-0 plants (Fig. 1h-j), and *rgga* seeds were larger in size and deeper in color than Col-0 seeds (Fig. 1k). Taken together, these results support that both the vegetative growth and reproductive growth capacities of *Arabidopsis* plants lacking the glycine-rich RNA binding protein *AtRGGA* can be increased beyond the capacities of WT plants (“exuberant growth”) *via* supplementation with selected exogenous sugars during the seedling stage.

#### **Application of exogenous sucrose to *rgga* plants promotes root elongation and chlorophyll synthesis and increases the activity of sugar metabolism enzymes.**

To determine the minimum required time to induce the exuberant growth of *rgga* plants, we compared the growth of *rgga* and Col-0 plants that were initially grown in 2% sucrose MS for 2, 4, 5, or 6 d and then transferred to vermiculite and grown until they were assessed at day 28. No exuberant growth phenotypes were observed for the plants transplanted after 2 or 4 days of sucrose supplementation, but these phenotypes were evident for the plants transplanted after 5 or more days of supplementation. (Fig. 2a). We also conducted experiments further exploring the age window for *rgga* sensitivity to growth promoting effects from exogenous sucrose and detected exuberant growth phenotypes for *rgga* plants that were initially grown in sucrose free MS (for up to 8 d) before being transplanted to 2% sucrose MS for continued growth (Supplementary Fig. 3). Thus, *rgga* plants are responsive to exogenous sucrose within a relatively large time window. However, it should be noted that the earlier and the longer the duration of the sucrose supplementation, the greater the extent of the detected growth differences between *rgga* and Col-0 plants.

To better understand the effects of *AtRGGA* mutation on growth and development, the roots of Col-0 and *rgga* which grown in 2% sucrose MS were observed. The root length of *rgga* is much longer than Col-0,

with especially evident increases for the 6 d and 14 d supplementation samples. Further, we observed the presence of lateral roots in 14 d *rgga*, but not in 14 d Col-0 seedlings (Fig. 2b). Quantitative analysis showed that the 6 d and 14 d supplementation *rgga* seedlings had significant increases in both root length (Fig. 2c) and root meristem width (Supplementary Fig. 4a) compared to Col-0 seedlings. Observation of the roots of *wox5::GFP,rgga* seedlings—in which quiescent center cells are marked by GFP—showed that the number and layer number of root apical meristem cells of *wox5::GFP,rgga* seedlings were obviously increased compared to *wox5::GFP* seedlings (Supplementary Fig. 4b). These results indicate *AtRGGA* deletion also enhances the root development of *Arabidopsis* plants given an exogenous sucrose supply.

In addition to exuberant growth, the *rgga* seedlings also had obviously greener leaves compared with Col-0. Chlorophyll content analysis showed that day 28 *rgga* plants with  $\geq 5$  d sucrose supplementation had significantly higher chlorophyll levels than similarly treated Col-0 plants (Fig. 2d). Sugar metabolism is closely related to plant growth and development, and we analyzed the activities of several enzymes involved in sugar metabolism in the plants that were initially exposed to exogenous sucrose and then transplanted to vermiculite. Classic *in vitro* assays with total protein extracts from leaves showed that at day 28, the activities detected for neutral invertase (NI), acid invertase (AI), and hexokinase (HK) were all significantly higher in extracts from the  $\geq 5$  d sucrose supplemented *rgga* plants than in Col-0 or  $< 5$  d supplemented *rgga* plants (Fig. 2e-g). Note that these observations for the increased activity of sugar metabolism enzymes are consistent with the exuberant growth phenotype data for the corresponding plants (Fig. 2a). These results indicate that *AtRGGA* deletion promotes chlorophyll levels and the activity of sugar metabolism enzymes in *Arabidopsis* plants given exogenous sucrose.

#### ***rgga* plants given exogenous sucrose have strongly elevated expression of genes related to carbohydrate metabolism, chlorophyll synthesis, and root development.**

To investigate how *AtRGGA* functions in growth, we conducted transcriptome analysis of Col-0 and *rgga* seedlings that were cultivated for 6 d or 14 d in 2% sucrose MS. Notably, there were relatively more up-regulated *rgga* DEGs at the 14 d supplementation time point as opposed to that of Col-0, although there were many more down-regulated genes among 987 at 6 d and 110 at 14 d differentially expressed genes (DEGs) in *rgga* seedlings compared with Col-0 (Fig. 3a). A GO analysis comparing the DEGs for the 6 d *rgga* and Col-0 samples indicated enrichment in among the up-regulated *rgga* DEGs for annotations related to root meristem growth, transcription regulation, ubiquitin protein ligase activity regulation and carbohydrate transport (Supplementary Fig. 5). The expression levels of 19 transcript factors (TFs) were increased in *rgga*, among which 7 have been previously implicated in growth regulation including GRF1, SPL2, SPL10, AN3, SAP, PLT1, and PLT2 (Galinha et al., 2007; Kim et al., 2003; Shikata et al., 2009; Shishkova et al., 2008; Vercruyssen et al., 2014; Wang et al., 2016) (Fig. 3b).

The *rgga* root growth phenotypes focused our attention on the DEGs that have been previously reported to exhibit root-specific expression profiles. There were 25 such genes among the DEGs (Supplementary Fig. 6), including *PLT1* and *PLT2*, two master transcription factors known to promote root development

(Galinha et al., 2007). Recalling the increased chlorophyll levels in *rgga* seedlings, we also analyzed the differential expression of genes with functional annotations related to chlorophyll synthesis and photosynthesis. Few DEGs in *rgga* compared to Col-0 with such annotations including *HEMF2*, *HEMA2*, *Heme2*, and *PsbP-2* were identified.

Beyond sucrose metabolism, chlorophyll content, and root development, and recalling the exuberant growth phenotypes in the sucrose-supplemented *rgga* plants, it was highly interesting that we found that 33 DEGs with functional annotations related to plant cell wall organization were down-regulated in *rgga* (Supplementary Tab. 1). As one example, the function of *PRX71* is to strengthen cell wall and thus limit cell expansion during normal growth and in response to cell wall damage (Raggi et al., 2015). Further investigation reveals that 22/33 genes involved in cell wall organization are enriched in root, and 5/22 have proven to be involved in root elongation (*PRX44*, *PRX57*, *EXT6*, *EXT13*, *EXPA7*) (Kwon et al., 2015; Lan et al., 2013; Petricka et al., 2012). This suggest that the root growth of *rgga* plants might be affected under this condition.

To validate both the previous phenotypes and expression trends from our transcriptome analysis, we used qPCR to assess the expression levels of genes including *NI*, *AI*, *HK*, sucrose transporter genes, chlorophyll synthesis related genes, root development related genes, growth regulation TFs and plant cell wall organization genes in *rgga* and Col-0 seedlings grown on 2% sucrose or sucrose-free MS for 14 d. In the absence of sucrose supplementation, we detected no significant differences in expression levels of the detected genes between *rgga* and Col-0 seedlings. Upon exogenous sucrose supply, we found significantly elevated expression levels in sucrose-supplemented *rgga* seedlings for genes including the *NI* genes (*INVC* and *INV*), *AI* genes (*CWIN2*, *CWIN4*, and *CWIN5*), *HK* genes (*FLN2*, *HXK3*, *HKL1*), sucrose transporter genes (*SUC3*, *SUC4*, *SUC7*, *SGB1*), chlorophyll synthesis related genes (*PORB*, *PORC*, *CAO*), and growth regulation TFs (*PLT1*, *PLT2*, *GRF1*, *SPL2*, *SPL10*, *AN3*, and *SAP*) compared to similarly treated Col-0 plants among the detected genes (Fig. 3c,d and Supplementary Fig. 7). Notably, the expression of the above genes was induced by 2% sucrose both in Col-0 and *rgga* plants (Fig. 3c,d). And qPCR analysis showed that the cell wall organization genes *PRX44*, *PRX57*, *EXT6*, *EXT13*, *EXPA7*, *PRX71* were induced by sucrose in Col-0 plants and down-regulated in *rgga* plants grown on 2% sucrose MS (Supplementary Fig. 8).

### ***AtRGGA* mutation increases the mRNA stability of carbon metabolism chlorophyll synthesis, and root development related genes.**

As an RNA binding protein, AtRGGA may affect the expression of its apparent target genes by affecting the stability of their transcripts. Pursuing this speculation, we carried out mRNA stability assays for DEGs in *rgga* and Col-0. Two-week-old seedlings growing in 2% sucrose MS were treated with the transcription inhibitor Actinomycin D (Act D), and then the mRNA stability of *INVC*, *INV*, *CWIN2*, *CWIN4*, *HXK3*, *FLN2*, *SUC3*, *SGB1*, *PORB*, *CAO*, *PLT1*, and *PLT2* were examined across a time course of one hour. The remaining transcripts of these genes of Col-0 decreased faster than that of *rgga* as time went on (Fig. 4a). Additionally, the mRNAs of four TFs of *rgga* which involved in growth regulation were proved to be more

stability than that of Col-0 (Supplementary Fig. 9). These results suggest *AtRGGA* indeed reduces the mRNA stability of the detected genes.

To further explore how *AtRGGA* influences the mRNA stability of the carbon metabolism, chlorophyll synthesis, and root development related genes, RNA immunoprecipitation (RIP) combined with qPCR was conducted using GFP and AtRGGA-GFP transgenic plants grown on 2% sucrose MS for 14 d. The results showed that AtRGGA specifically bound to the 5'-UTR and the downstream regions close to the start codon of *INV*, *CWIN2*, *HXK3*, *SUC3*, *PORB*, and *PLT1* transcripts (Fig. 4b and Supplemental Fig. 10). We also searched the motifs overrepresented in the 5' terminus for 16 transcripts (AtRGGA binding regions identified by RIP for 6 transcripts and 1-800bp for 10 transcripts of which stability detected) specifically more abundant and stable in *rgga* mutant using MEME (Version 5.1.1) (Bailey and Elkan, 1994), and three common motifs (UCUCWCUYUCDC, AGGAARRGRAA, YUUCUUCHCCU) were invariably present in these transcripts (E values=7.2e-007, 1.2e-002, 4.7e-002 respectively) (Fig. 4c and Supplemental Fig. 11).

Note that we transformed a combined  $\Delta$ RGG1&2/*rgga* deletion variant of AtRGGA into the *rgga* background *Arabidopsis* and grew these plants in 2% sucrose MS for 14 days, the growth phenotypes of  $\Delta$ RGG1&2/*rgga* plants were similar to *rgga* (Fig. 5f). This is unsurprising, given the roles of the RGG1 and RGG2 domains in RNA binding (Landsberger et al., 2002). The mRNA levels of *INV*, *CWIN2*, *HXK3*, *SUC3*, *PORB*, and *PLT1* in  $\Delta$ RGG1&2/*rgga* and *rgga* plants were all significantly higher than those in Col-0 (Fig. 4d). These results indicate that the sucrose-dependent, suppressive regulation of AtRGGA on growth depends on its binding with mRNA targets.

#### **AtRGGA interacts with the 5'-3' exonuclease AtXRN4.**

RGGA binding to the 5' terminus of mRNAs raises the possibility that RGGA may regulate mRNA stability through a 5' to 3' mRNA decay pathway (Braun et al., 2016; Merret et al., 2013). We therefore used Y2H assays to explore interactions between AtRGGA and known proteins involved in P-body formation including *Arabidopsis* DCP1, DCP2, VCS and exoribonuclease XRN4. Strong activation of the reporter gene was detected in yeast cells co-expressing AtRGGA-BD and AtXRN4-AD, indicating that AtRGGA can physically interact with AtXRN4 (Fig. 5a). AtXRN4 was shown to interact with AtDCP1 and AtDCP2 as previously reported, but AtRGGA did not interact with them (Supplemental Fig. 12). Subsequent bimolecular fluorescence complementation (BiFC) assays and co-immunoprecipitation (Co-IP) further supported the interaction between AtRGGA and AtXRN4 in plant (Fig. 5b,c).

Primary structure analysis using a web-based tool SMART as well as the structure reported previously (Landsberger et al., 2002), five functional domains in AtRGGA, including Stm1, HABP4-PAI-RBP1, two RGG boxes, and two resemble bipartite nuclear localization signals (NLSSs) were predicted (Fig. 5d). Notably, although having two presumed NLSSs, AtRGGA is located perinuclear region of the cytoplasm rather than nucleus according previous study (Ambrosone et al., 2015) and our observations (Supplemental Fig. 13). And the perinuclear localization of AtRGGA can also be seen in our BiFC results (Fig. 5b). Co-IP assays with AtRGGA domains deletion variants (transiently expressed in *N. benthamiana* leaves) showed that NLS1 (EKKKALQSLTTSER KV) was essential for the AtRGGA–AtXRN4 interaction. Deletions of Stm1, the

two RGG boxes, HABP4-PAI-RBP1, and NLS2 did not influence the interaction (Fig. 5e). We stably transformed the AtRGGA NLS1 deletion variant into the *rgga* background, and cultivated these deletion variant lines in 2% sucrose MS for 14 d. The  $\Delta$ NLS1/*rgga* line grew significantly faster than Col-0, and exhibited the same growth phenotypes as the *rgga* plants (Fig. 5f). The mRNA levels of *INVC*, *CWIN2*, *HXK3*, *SUC3*, *PORB*, and *PLT1* in  $\Delta$ NLS1/*rgga* and *rgga* plants were all significantly higher than those in Col-0 (Fig. 5g). These results confirm that AtRGGA's NLS1 domain is essential for its sucrose-dependent suppression of seedling growth.

### The exoribonuclease XRN4 degrades RGGA-bound mRNAs.

Given our identification of AtRGGA target mRNAs and their common motifs located in the 5' UTRs and their adjacent downstream regions, and considering the known mRNA digestion role of XRN4 in the 5' to 3' mRNA decay pathway, it seems plausible that the AtRGGA-AtXRN4 interaction may facilitate the selective degradation of AtRGGA-targeted mRNAs. Pursuing this, we generated tagged materials and conducted RIP assays using HA and GFP antibodies; specifically, we generated AtXRN4-HA/*rgga*, AtRGGA-GFP/*xrn4*, and AtXRN4-HA lines, and also used the aforementioned AtRGGA-GFP line. Seedlings of these lines were grown in 2% sucrose MS for 14 d and assessed with RIP assays. Fragment enrichment in RIP products was detected by qPCR, with segmented primer pairs for AtRGGA target mRNAs including *INVC*, *CWIN2*, *HXK3*, *SUC3*, *PORB*, and *PLT1* (Fig. 6a and Supplemental Fig. 14).

AtXRN4 did not bind to these AtRGGA target mRNAs in the absence of RGGA. Further, AtXRN4 was not required for AtRGGA binding to its target mRNAs. Additionally, these assays showed that, for all of its target mRNAs, AtRGGA bound to a 5'UTR and its adjacent region that contained the common motifs. Viewed alongside our AtRGGA-AtXRN4 interaction data, these results support a model wherein AtRGGA binds its targets in their 5'UTRs and 5'UTR near regions, and the exoribonuclease AtXRN4 can then recognize these AtRGGA-bound mRNA substrates for degradation. Pursuing this, we tested the growth of *rgga*, *xrn4-5*, AtXRN4-HA/*rgga*, AtRGGA-GFP/*xrn4-5* and *rgga,xrn4-5* plants cultured in 2% sucrose MS for 14 d. The rosettes size of AtXRN4-HA/*rgga*, AtRGGA-GFP/*xrn4-5* and *rgga,xrn4-5* was not different to that of *rgga*, but obviously larger than that of Col-0 (Fig. 6b).

The deletion of NLS1 which destroying the interaction of AtRGGA and AtXRN4 increased the accumulation of carbon metabolism, chlorophyll synthesis, and root development mRNAs (Fig. 5e), and AtXRN4 did not bind to these mRNAs in the absence of AtRGGA (Fig. 6a). It suggested that AtRGGA is probably responsible for the specificity of the mRNA degradation conducted by AtXRN4. Given the RGGA target mRNAs we identified, and considering the known digestion role of XRN4 in the 5' to 3' mRNA decay pathway, it seems plausible that the AtRGGA-AtXRN4 interaction may facilitate the selective degradation of RGGA-targeted mRNAs.

## Discussion

As the main transport form of photosynthate, sucrose participates in the balance regulation of source and sink to meet the needs of growth and stress resistance in plants (Chan and Yu, 1998a; Ruan, 2014).

Studies have established that elevated sucrose decreases the expression of genes that function in photosynthesis, sugar catabolism, and sugar transport (Koch, 1996; Paul and Pellny, 2003; Sheen, 1994). However, the mechanism through which these diverse genes are rapidly and simultaneously repressed remains unclear. In the present study, we show that the RNA-binding protein AtRGGA functions to allow the 5'-3' exonuclease AtXRN4 to recognize a subset of sucrose transport and metabolism, chlorophyll synthesis, and root development related mRNA transcripts for degradation. When AtRGGA or AtXRN4 is absent, the accumulation of these mRNAs increases substantially, thus enabling the observed rapid growth of mutant plants given an exogenous sucrose supply (Fig. 6c).

Among the three XRN enzymes of *Arabidopsis*, AtXRN4 is the only one that functions in cytoplasmic 5'-3' mRNA degradation (Kastenmayer and Green, 2000). AtXRN4 has been shown to promote the decay of some but not all mRNAs, including transcripts that code for proteins of ethylene signaling, light signaling, seed germination, and ABA/GA metabolism, as well as heat stress and nitrogen stress responses (Basbouss-Serhal et al., 2017; Estavillo et al., 2011; Merret et al., 2013; Nagarajan et al., 2019; Nguyen et al., 2015; Olmedo et al., 2006; Rymarquis et al., 2011; Wawer et al., 2018). Our results demonstrate that AtXRN4 recognizes subsets of mRNAs related to sucrose transport and metabolism, chlorophyll synthesis, and root development, and we show that this specific recognition is mediated by the RNA-binding protein AtRGGA. This echoes previous demonstrations that AtXRN4's recognition of a subset of mRNAs under thermal stress requires help from the RNA-binding protein AtLARP1 (Merret et al., 2013). Notably, the yeast RNA-binding protein VTS1 was shown to function in glucose-induced mRNA decay (Braun et al., 2016). Viewed together, these results clearly indicate that the specificity of XRN-mediated RNA decay is mediated by particular RNA-binding cofactor proteins. Thus, the further identification and functional characterization of such cofactors will almost certainly deepen our understanding of specific mRNA degradation programs triggered by particular conditions, including environmental stresses, nutrients, and specific developmental stages.

Biochemically, we discovered that AtRGGA guides AtXRN4 to interact with target mRNAs *via* binding to their 5' UTRs and 5' UTR downstream regions. This binding pattern matches our understanding of AtXRN4 as a 5'-3' exonuclease; however, it is not yet clear how the AtXRN4 cofactor AtRGGA recognizes its own target transcripts. Previous works has demonstrated specific mRNA sequences especially some hexamer motifs from select functional categories were attractive targets for XRN4-mediated decay (Basbouss-Serhal et al., 2017; Rymarquis et al., 2011). In addition, the factors known to influence AtXRN4's binding with degradation substrate mRNAs also include the GC content of 5' UTR, the poly (A) tail at the 3' end, as well as N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) modification of transcripts (Basbouss-Serhal et al., 2017). We discovered here that all of the transcripts whose stability increase in *rgga* contain three motifs in their 5' UTRs and adjacent regions, suggesting that AtRGGA might recognize its target mRNAs through these common motifs. It should be interesting to elucidate precisely which structural and biochemical factor(s) of these common motifs mediate AtRGGA's recognition of its target mRNAs.

High yield is obviously a major target of crop breeding efforts, and we know that yield is strongly impacted by source-sink balance for sugars (Smith et al., 2018). Given the strong developmental impacts

of sugar repression in plants, simply increasing source strength does not work particularly well to obtain yield improvements (Li et al., 2018; Rodrigues et al., 2019). In the present study, both *rgga* and *xrn4-5* plants showed rapid growth phenotypes upon exogenous sucrose supply. Compared with *XRN4*, which is widely involved in global signal responses in plant cells, *RGGA* may be a more suitable candidate gene for growth improvement manipulation. For example, *Arabidopsis* mutants of *XRN4* could not survive a long period of moderately high temperature, whereas mutation of LARP1, a heat-specific cofactor of AtXRN4, did not cause a similar extent of heat-induced damage (Merret et al., 2013). RGGA specifically interacts with (and thus regulates) sugar metabolism, chlorophyll synthesis, and root development related genes, making it an excellent candidate for yield improvement. Recall our finding that sucrose supply did not trigger faster growth of *rgga* over Col-0 when the treated seedlings were younger than 5 d. We suspect this is because the heterotrophy-autotrophy transition—which enables *Arabidopsis* seedlings to gain their photosynthetic competence (Chen and Thelen, 2010)—occurs at this time. The growth effects of the *rgga* mutation seem to follow the "Matthew effect"; that is, early exogenous supplementation with sugar promotes root development and chlorophyll synthesis, thereby greatly improving nutrient absorption and photosynthetic efficiency, thus increases the overall biomass of *Arabidopsis*.

Plant molecular physiology studies have shown that both transcriptional and post-transcriptional regulation mechanisms mediate sugar repression effects. Our results suggested that mRNA stability regulation even played a more crucial role in sugar repression given the coverage of the involved genes. Beyond the role we demonstrate for AtRGGA in sugar repression, it is known that both drought and salt stress induce the accumulation of the AtRGGA protein (Ambrosone et al., 2015). Thus, it seems plausible that the specific target mRNA binding and AtXRN4 cofactor model we illustrate here for *Arabidopsis* in the present study may help explain post-transcriptional mechanisms underlying plant responses to these major forms of environmental stress. Our results suggest that *rgga* does not obviously effect drought resistance compared to Col-0 when sucrose is supplied at an early growth stage. Additionally, it bears emphasis that salt and drought stress are not major limits to plant growth in many agricultural production regions. Thus, given the ubiquitous conservation of the *RGGA* gene among eudicots and monocots, it seems that combined manipulation of the *RGGA* gene alongside adjusted cultivation measures (*i.e.*, sucrose supplementation during planting and photosynthesis improvement) could result in major gains in the productivity of many crop plants.

## Methods

**Plant materials.** All the wild-type, mutant and transgenic *Arabidopsis* lines used throughout this study are Columbia (Col) ecotype. Seeds of *rgga* (SALK\_143514), *xrn4-5* (CS68822) are purchased from *Arabidopsis* Biological Resource Center. Both *AtRGGA* and *AtXRN4* genes with corresponding tags are amplified from Col-0 cDNA and ligated into pBI121 and pBI121-GFP vectors through appropriate restriction sites. The 35S::*AtRGGA*, 35S::*AtXRN4*, 35S::*AtRGGA-GFP*, and 35S::*AtXRN4-HA* transgenic lines are obtained by floral dipping of Col-0. 35S::*AtXRN4-HA/rgga* and 35S::*AtRGGA-GFP/xrn4* lines are obtained by floral dipping of *rgga* and *xrn4* with corresponding structure-containing *Agrobacterium*, respectively. *WOX5::GFP/rgga* line was acquired by hybridizing *WOX5::GFP* and *rgga*. *WUSCHEL-*

*RELATED HOMEOBOX* gene (*WOX5*) is specially expressed in the quiescent center of *Arabidopsis* root. The transgenic lines are identified by antibiotic screening and PCR amplification using specific primers (Supplemental Table 2). All plants are grown on MS medium or vermiculite under standard long-day condition (16 h of light, 8 h of dark) at 22°C.

**Growth phenotype analysis.** Fresh weight of Col-0 and *rgga* plants which grown on 2% sucrose MS for 2 weeks or then transported to vermiculite for further 2 weeks growth is weighed. After drying in an oven at 80°C for 2 days, dry weight of the above seedlings is weighed. Three biological replicates are set up, and each replication including 50 plants. Other groups of Col-0 and *rgga* firstly grown on 2% sucrose MS for 2 weeks and then transported to vermiculite for further growth until they make seeds. And three sets of 100 seeds are weighed to obtain the data of hundred-grain weight. In addition, the number of seeds per plant of *rgga* and Col-0 is separately counted. It replicates for three times, and each replication includes 5 plants.

**Chlorophyll content assays.** 0.2 g leaves are soaked in 80% acetone solution for 48 h, and then the absorbance values at 645 nm and 663 nm of the extract are measured by using a spectrophotometer. Three biological replicates are set up. According to the absorption value of chloroplast pigment and the Lambert-Beer law, the pigment content is calculated using the following formulas.

$$C = 20.3 \cdot A_{645\text{nm}} + 8.04 \cdot A_{663\text{nm}}$$

$$\rho = C \cdot V / (W \cdot 1000)$$

Where A indicates the absorption of the extract at the corresponding wavelength,  $\rho$  is the chlorophyll content (mg·g<sup>-1</sup>); C is the corresponding chlorophyll concentration in the acetone extract (mg·L<sup>-1</sup>); V is the solution volume (mL); W is the sample fresh weight (g).

**Enzyme activity analysis.** The activities of neutral invertase (NI), acid invertase (AI), and hexokinase (HK) of Col-0 and *rgga* which grown on 2% sucrose MS for a few days and then transported to vermiculite for further growth till 28 d age were assayed. 1 g leaf is ground with 3 mL extract buffer (PH 7.5, 0.05 M phosphate buffer, 0.1 mM EDTA, 1 mM cysteine, and 10 mM NaSO<sub>3</sub>). Then the extract is centrifuged at 20,000 rpm for 15 min at 4°C. The supernatant liquid is regarded as crude enzyme. NI activity is determined as following. 0.2 mL of crude enzyme is added into 1.2 mL of 0.2 M phosphate buffer (pH=7.5) and 0.2 mL of 0.3 M sucrose. The above solution added 2 mL of 1 M NaOH immediately is taken as the control. The above solutions are incubated at 35°C for 30 min, then 2 mL of 1 M NaOH and 5 mM NDS are added. The mixtures are boiled in a water bath for 5 min and the absorbance is measured at 540 nm. The enzyme activity is calculated through a standard curve and in μmol Suc/mg Pr·h. In the case of AI activity assay, 0.2 M acetate buffer is used instead of 0.2 M phosphate buffer (pH 7.5). And HK activity is analysed with Hexokinase assay kit (Cominbio). All measurements are replicated three times.

**Transcriptome sequencing.** Transcriptome sequencing for *rgga* and Col-0 whole plants grown on 2% sucrose MS for 6 d and 14 d respectively is carried out using Illumina HiSeqX10/HiSeq4000 platform.

And three biologically replicated materials are provided.

**qRT-PCR.** Total RNA is extracted from leaves by using TRIzol Reagent (Invitrogen), treated with DNase $\ddagger$ . Complementary DNA (cDNA) is synthesized from 1  $\mu$ g of total RNA by using FastKing gDNA dispelling RT SuperMix (Tiangen). Real-time PCR is performed with the LightCycler 480 SYBR Green I Master (Roche) in a 15  $\mu$ L reaction volume. As a reference gene, *actin* is amplified. The primers are presented in Supplemental Table 2. The instrument used for detection is LightCycler 96 (Roche). Results presented are means of three biological replicates.

**RNA immunoprecipitation assays.** 3 g whole plant of *Arabidopsis* is harvested into a centrifuge tube containing 37 mL of 1.0% formaldehyde and 2.5 mL of 2 M glycine, and then infiltrated for 15 min in a desiccator attached to a vacuum pump. Then RNA samples are extracted and purified. qPCR is used to detect the expression level of mRNAs ([Zhang et al., 2016](#)).

**mRNA stability analysis.** The *rgga* and Col-0 seedlings grown in MS medium (2% sucrose) for 14 d are treated with actinomycin D (Act-D) and collected every 15 min across one hour. mRNAs of the treated plants are extracted and reverse-transcribed to cDNAs for qPCR assay. This experiment is replicated for three times. Primers are mentioned in Supplemental Table 2.

**Apical structure observation.** *WOX5::GFP/rgga* lines are obtained by hybridizing *WOX5::GFP* and *rgga* lines. The *WOX5::GFP* and *WOX5::GFP/rgga* seedlings grown in 2% sucrose MS medium for 4 d, 6 d and 14 d, and the apical structures were observed using two-photon laser confocal microscope (TPLSCM).

**Protein interaction analysis.** Y2H assays are carried out using the GAL4-based two-hybrid system as described in the Yeast maker Yeast Transformation System 2 User Manual (Clontech). The cDNAs of *AtRGGA*, *AtRGGA* deletion mutants and *AtXRN4* are cloned into pGBK7 and pGADT7 respectively. And the recombination plasmids are co-transformed into AH109, followed by incubation on the QDO/X-a-gal/AbA medium.

*AtRGGA* and *AtXRN4* are cloned by Gateway technology to obtain BiFC test vectors AtRGGA-YFPC, AtRGGA-YFPN, AtXRN4-YFPC, AtXRN4-YFPN. And these recombination vectors are transformed to *Agrobacterium* GV3101 and used to carry out transient infiltration. About 1 cm<sup>2</sup> infiltrated tobacco leaf at a distance of 2-3 mm from the pinhole is cut, placed flat on a glass slide, and then observed with TPLSCM for YFP fluorescent signals ([Tang et al., 2020](#)).

Co-IP assay is performed as described by Tang *et al.* ([Tang et al., 2020](#)) and the films are washed by TTBS (Tris Buffered Saline, with Tween-20, pH 8.0) and then photographed using Tanon-5500 (Tanon). Bound proteins are detected by immunoblotting using anti-His, anti-HA, anti-GFP (Beyotime) antibodies respectively.

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## Declarations

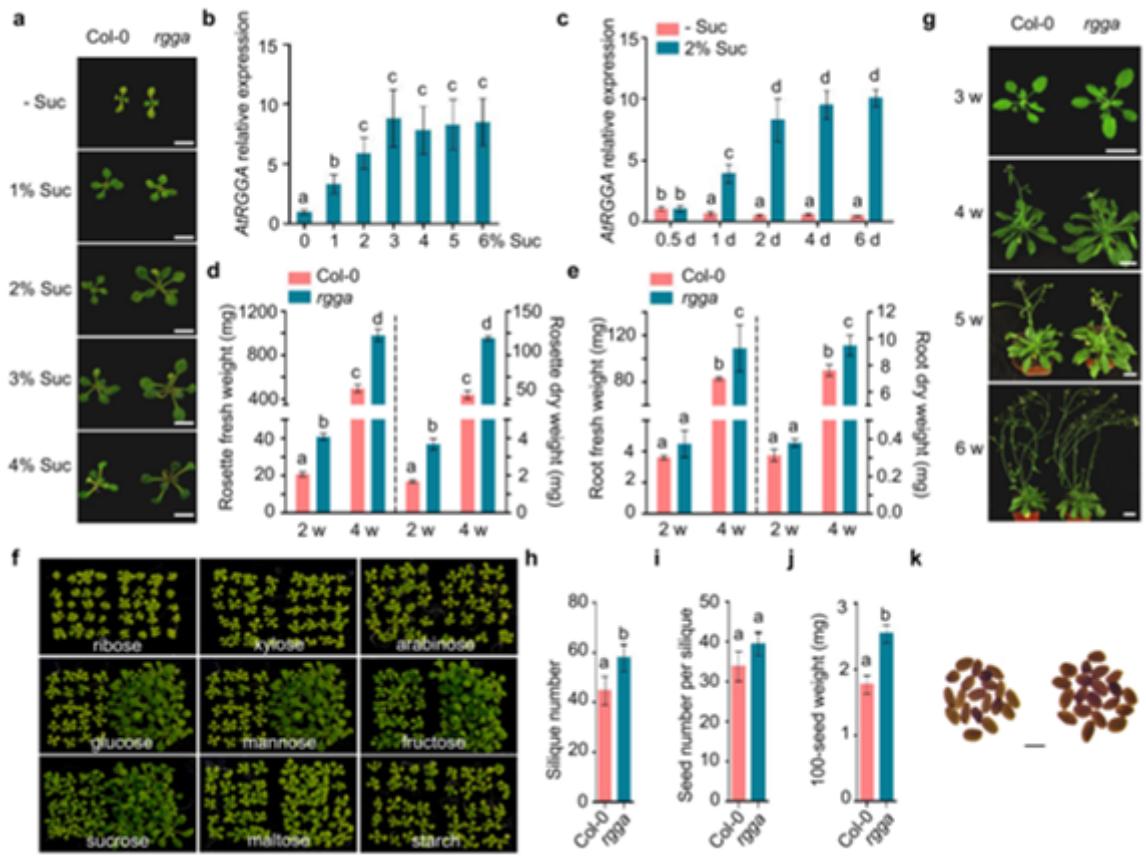
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**Author Contributions** H.L. and R.Y. conceived the project. R.Y. performed most of the experiments. H.Z. carried out phenotypic analysis experiments. X.G. and H.L. conducted bioinformatics analysis. R.Y., L.G., C.Z., C.W., L.G., J.D., H.M., Y.S., S.Z. analysed data. R.Y. and H.L. wrote the manuscript.

**Competing Interests Statement** The authors declare that they have no competing interests.

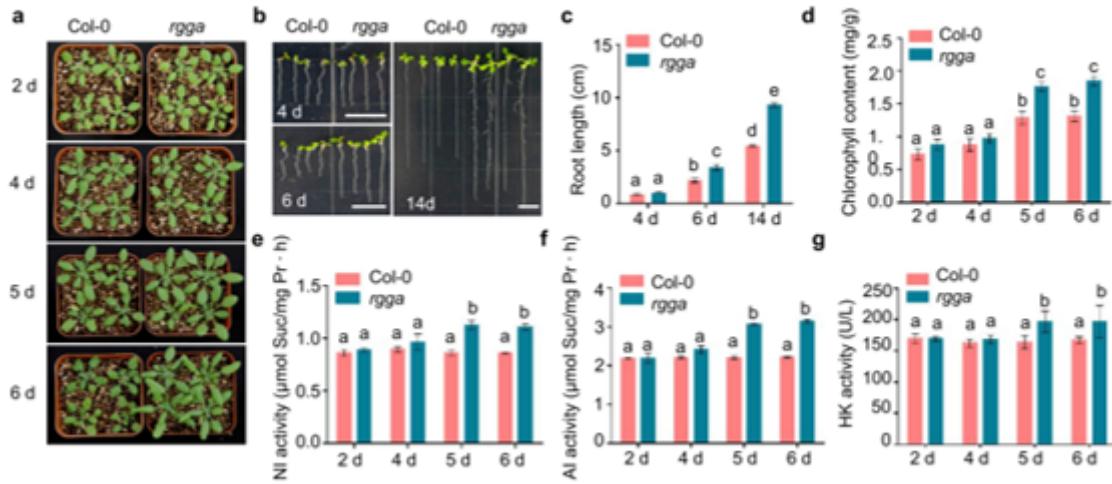
**Correspondence and requests for materials** should be addressed to H.L.

## Figures



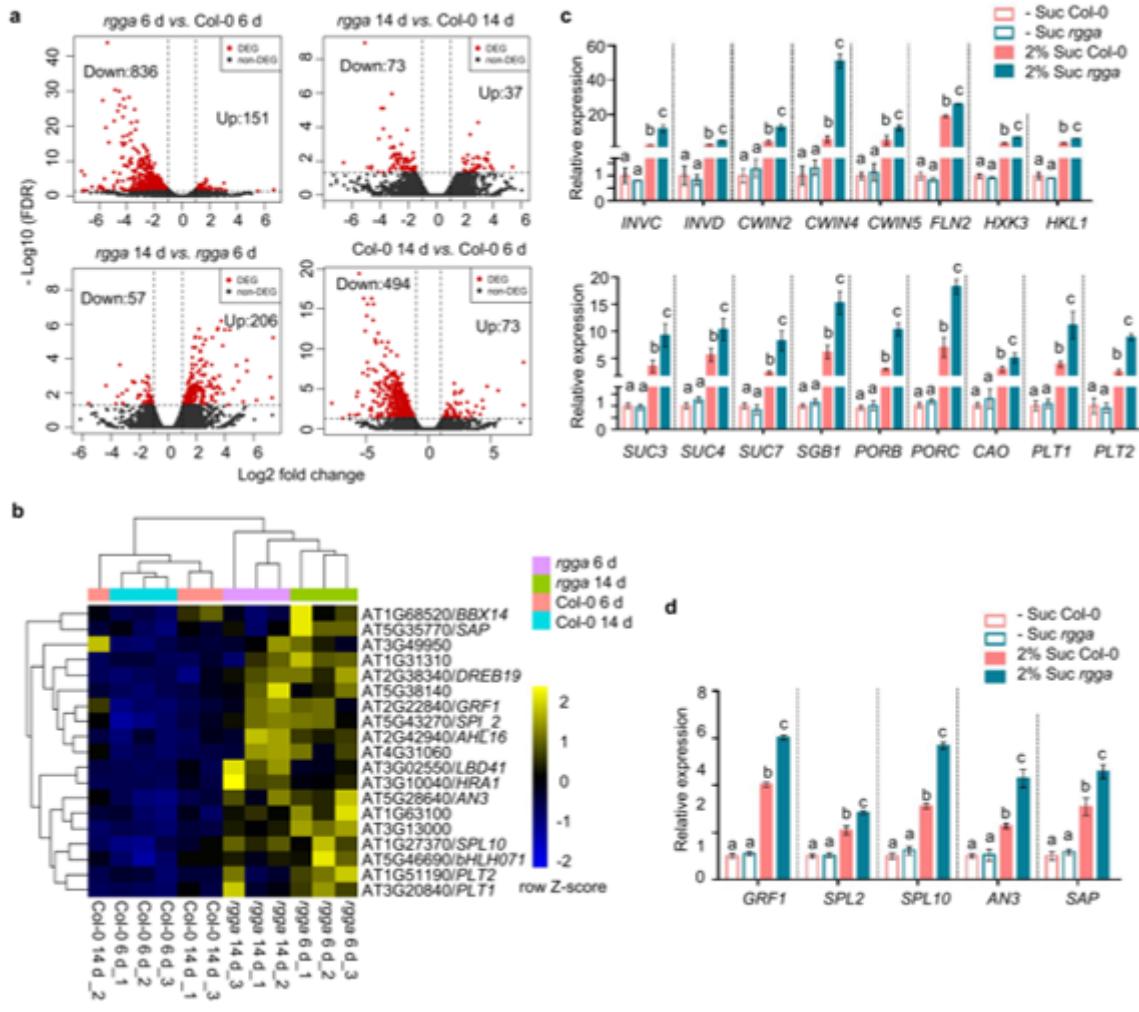
**Figure 1**

rgga seedlings grow faster than Col-0 when supplied with exogenous sucrose. a, Phenotypes of Col-0 and rgga seedlings grown in MS media with sucrose (1%, 2%, 3%, or 4%) for 2 weeks. Scale bar, 1 cm. Suc, sucrose; - Suc, no sucrose. b, qPCR-based analysis of AtRGGA expression in Col-0 seedlings cultured in MS with different sucrose concentrations for 2 weeks. The AtRGGA expression level for the - Suc treatment was set to a value of 1.0. c, AtRGGA expression analysis in Col-0 seedlings cultured on 2% sucrose MS media for the indicated times; the level detected at 0.5 days was set as 1.0. One-Way Anova, P<0.05, n=3 biological replicates. d-e, Biomass analysis of Col-0 and rgga seedlings grown on 2% sucrose MS for 2 w and the seedlings grown on 2% sucrose MS for 2 w and then transferred to vermiculite for another 2 w (n=50). d, rosette fresh weight and dry weight values; e, root fresh weight and dry weight values. f, Growth comparison for Col-0 and rgga seedlings grown on MS medium containing 2% ribose, xylose, arabinose, glucose, mannose, fructose, sucrose, maltose, or starch for 14 days. g-k, Rapid growth phenotypes were evident throughout the life cycle of rgga plants. Col-0 and rgga plants were cultured on 2% sucrose MS media for 2 w, and then transferred to vermiculite and grown until they produced seeds. Images of Col-0 and rgga plants at their indicated seedling ages (scale bar, 1 cm) (g). Siliques number per plant (h), seed number per siliques (i), 100-seed weight (j), seed size (scale bar, 500 µm) (k) of Col-0 and rgga.



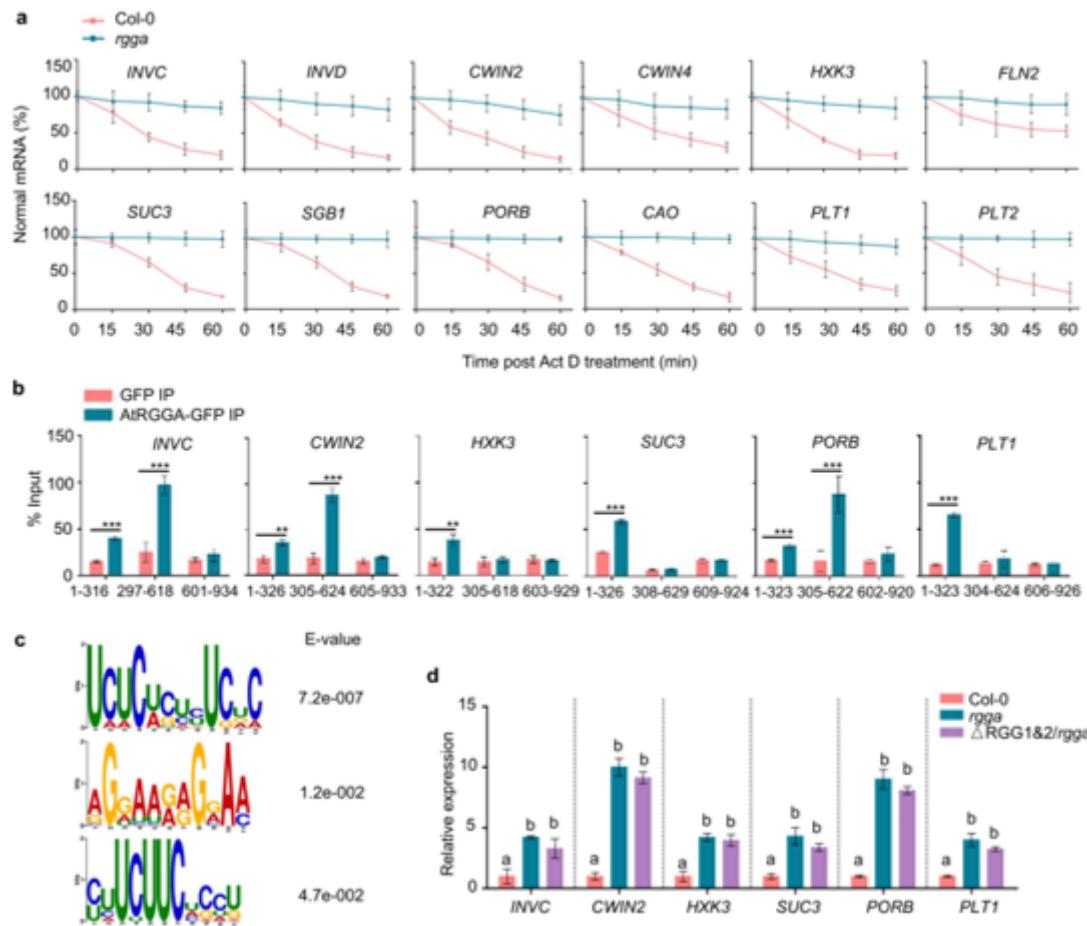
**Figure 2**

Exogenous sucrose supply could promote sugar metabolism, chlorophyll synthesis, and root development of rgga. Growth phenotypes (a), root images (b), primary root length determination (c), chlorophyll content (d), activity of NI (neutral invertase) (e), AI (acid invertase) (f), and HK (hexokinase) (g) of Col-0 and rgga seedlings which were cultured on 2% sucrose MS media for 2, 4, 5, or 6 d, and then transferred to vermiculite until phenotypic assessment at the 4 w. One-Way Anova, P<0.05, n=3 biological replicates. Scale bar, 1 cm. Each replicate including 50 plants.



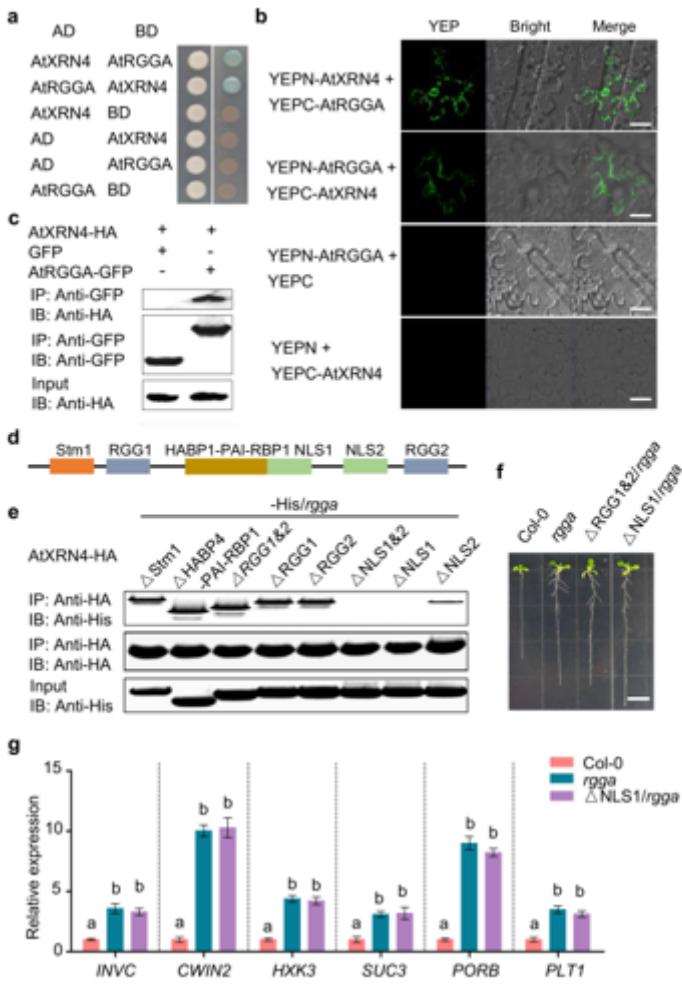
**Figure 3**

Transcriptome analysis of the differential expression of growth-related genes. a, Volcano plot of differential gene expression in Col-0 and rgga seedlings cultured in 2% sucrose MS for 6 d or 14 d. FDR, false discovery rate; DEG, differentially expressed genes. b, 19 transcription factor genes, including PLT1 and PLT2, were up-regulated in rgga compared with Col-0. Col-0 and rgga seedlings cultured on 2% sucrose MS for 6 d or 14 d. c, Gene expression analysis by qPCR for genes encoding neutral invertase, acid invertase, hexokinase, and sucrose transporters, as well as chlorophyll synthesis and root development related genes in Col-0 and rgga seedlings cultured in sucrose free or 2% sucrose MS for 14 days. One-Way Anova, P<0.05, n=3 biological replicates. Suc, sucrose; - Suc, no sucrose. d, Expression verification of up-regulated transcription factor genes which were related to growth. rgga and Col-0 cultured in sucrose free and 2% sucrose MS for 14 days respectively. One-Way Anova, P<0.05, n=3 biological replicates. Suc, sucrose; - Suc, no sucrose.



**Figure 4**

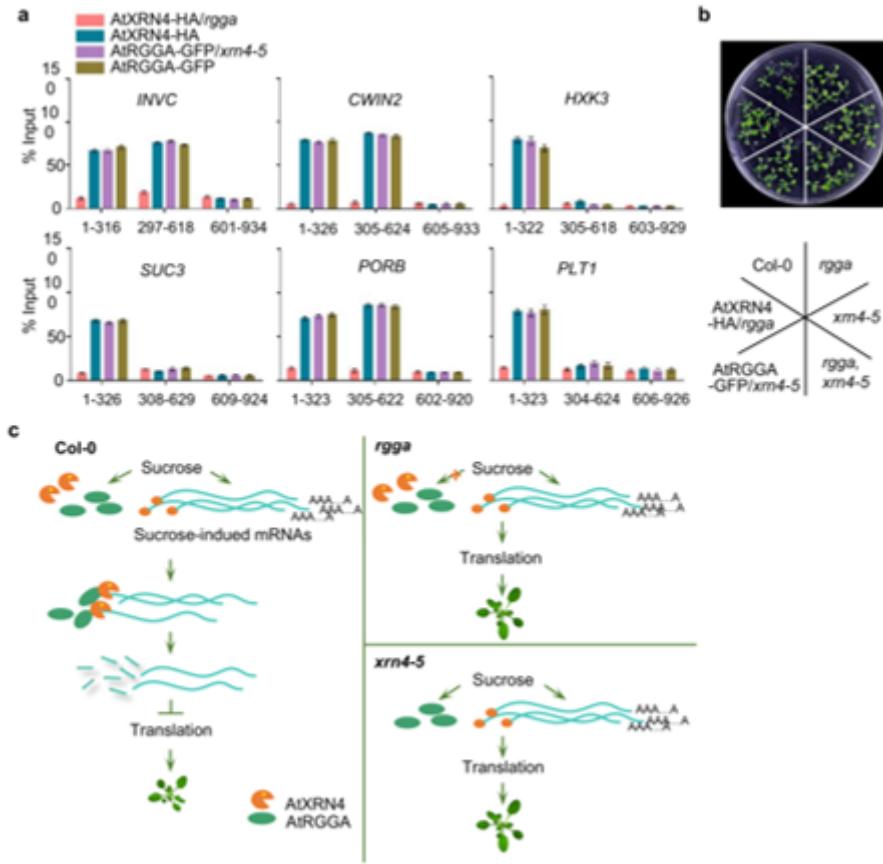
AtRGGA binds to 5' end of target mRNAs and thus affects their stability. a, mRNA stability analysis of certain genes related to sucrose metabolism and transport, chlorophyll synthesis and root development in Col-0 and rgga. Seedlings grown in 2% sucrose MS for 14 days were treated with Act D and then sampled for qPCR analysis at 15, 30, 45, and 60 min post ActD treatment. min, minutes. b, Binding region analysis of AtRGGA target mRNAs by using RIP combined with qPCR. GFP transgenic and AtRGGA-GFP transgenic Arabidopsis seedlings cultured in 2% sucrose MS for 14 d were sampled. Each detected mRNA was divided to several segments each about 300bp to detected by qPCR. Only the results of 5' terminus of each target mRNA were showed here, and other results were showed in Fig. S10. One-Way Anova, P<0.05, n=3 biological replicates. RIP, RNA immunoprecipitation. c, Common motifs identified in the 5' terminus of the transcripts using MEME (Version 5.1.1). Analysis was performed on subsets of 6 transcripts (INVc 1-618, CWIN2 1-624, HXK3 1-322, SUC3 1-326, PORB 1-622, PLT1 1-323) of which AtRGGA binding regions were verified by RIP-qPCR and 10 transcripts (1-800) of which stability increased in rgga. d, Expression levels of INVc, CWIN2, HXK3, SUC3, PORB and PLT1 in Col-0, rgga and  $\Delta$ RGG1&2/rgga plants. One-Way Anova, P<0.05, n=3 biological replicates.



**Figure 5**

AtRGGA interacts with AtXRN4. a, Yeast two-hybrid (Y2H) assay of AtXRN4 and AtRGGA. Transformed yeasts were spotted on control medium (DDO) or selective medium (QDO). Empty vector transformed yeasts were used as controls. BD, DNA binding domain vector; AD, activation domain vector. b, Bimolecular fluorescence complementation (BiFC) assays of AtXRN4 and AtRGGA. Co-infiltrated leaves of *N. benthamiana* with YEPN-AtXRN4/YEPC-AtRGGA and YEPN-AtRGGA/YEPC-AtXRN4 at 48 hours post infiltration (hpi) were sampled. Empty vectors were used as negative controls. Scale bar, 20 μm. c, Co-immunoprecipitation (Co-IP) assays of AtXRN4 and AtRGGA. AtXRN4-HA was co-infiltrated with AtRGGA-GFP or GFP in *N. benthamiana* leaves. Then the infiltrated leaves were sampled at 48 hpi for Co-IP assay. IP, immunoprecipitation; IB, immunoblotting. d, The linear diagram represents the domain structure of AtRGGA protein. Stm1, Stm1 N-terminal domain; HABP4-PAI-RBP1, hyaluronan/mRNA-binding domain; RGG, arginine glycine glycine box; NLS, supposed nuclear localization signal. e, Key domain analysis of AtRGGA interacted with AtXRN4 by using Co-IP. The *N. benthamiana* leaves for Co-IP assay were co-infiltrated AtXRN4-HA with AtRGGA, △Stm1-His, △RBP-His, △RGG1-His, △RGG2-His, △RGG1&2-His, △NLS1-His, △NLS2-His and △NLS1&2-His, respectively. f, Comparison of △RGG1&2, △NLS1 lines with Col-0 and rgga. These *Arabidopsis* lines were vertically cultured in 2% sucrose MS for 14 d. Scale bar, 1

cm. g, Expression levels of INVC, CWIN2, HKX3, SUC3, PORB and PLT1 in Col-0, rgga and  $\Delta$ NLS1/rgga lines. One-Way Anova, P<0.05, n=3 biological replicates.



**Figure 6**

Sucrose promotes the growth of xrn4-5. a, AtRGGA addresses AtXRN4 to recognize the target mRNAs. The seedlings of AtXRN4-HA/rgga, AtRGGA-GFP/xrn4-5, AtXRN4-HA and AtRGGA-GFP grown in 2% sucrose MS for 14 d were sampled for binding region analysis for AtRGGA targets including INVC, CWIN2, HKX3, SUC3, PORB and PLT1 mRNAs using RIP combined with qPCR. The enrichment of mRNA fragments was detected by the primer pairs each covering about 300bp. Only the results of 5' end of each target mRNA were showed here, and other results were showed in Fig. S13. One-Way Anova, P<0.05, n=3 biological replicates. RIP, RNA immunoprecipitation assay. b, Comparison of Col-0, rgga, xrn4-5, XRN4-HA/rgga, RGGA-GFP/xrn4-5 and rgga,xrn4-5 lines which were grown in 2% sucrose MS for 14 days. c, Proposed model for the regulation mechanisms of AtRGGA on Arabidopsis growth. AtRGGA interacts with AtXRN4, and guides the degradation of mRNA subsets which related to sucrose transport and metabolism, chlorophyll synthesis, and root development by recognizing the common motif(s) located in the 5'UTRs and 5'UTR adjacent regions of mRNAs. In the absence of AtRGGA or the interaction domain, AtXRN4 itself no longer recognizes the target mRNAs, leading to a significant increase in mRNAs accumulation, thus promoting Arabidopsis growth with exogenous sucrose supplement. AtXRN4 mutant line xrn4-5 shows similar phenotypes with rgga line. The Arabidopsis images represent the relative rosette size of the seedlings grown in 2% sucrose MS for 14 days.

# Supplementary Files

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- [SupplementaryInformation.pdf](#)