Gibberellin (GA) regulates plant development primarily by triggering the degradation/deactivation of the DELLA proteins. However, it remains unclear whether all GA responses are regulated by DELLAs. Tomato (Solanum lycopersicum) has a single DELLA gene named PROCERA (PRO), and its recessive pro allele exhibits constitutive GA activity but retains responsiveness to external GA. In the loss-of-function mutant pro^GRAS, all examined GA developmental responses were considerably enhanced relative to pro and a defect in seed desiccation tolerance was uncovered. As pro, but not pro^GRAS, elongation was promoted by GA treatment, pro may retain residual DELLA activity. In agreement with homeostatic feedback regulation of the GA biosynthetic pathway, we found that GA20oxidase expression was suppressed in pro^GRAS and was not affected by exogenous GA. In contrast, expression of GA20oxidase was not affected by the elevated GA signaling in pro^GRAS but was strongly induced by exogenous GA. Since a similar response was found in Arabidopsis thaliana plants with impaired activity of all five DELLA genes, we suggest that homeostatic GA responses are regulated by both DELLA-dependent and -independent pathways. Transcriptome analysis of GA-treated pro^GRAS leaves suggests that 5% of all GA-regulated genes in tomato are DELLA independent.

INTRODUCTION

The phytohormone gibberellin (GA) regulates numerous developmental processes throughout the plant life cycle, including seed germination, stem elongation, flowering, and fruit set (Yamaguchi, 2008). The signaling pathway from GA perception to transcriptional activation has been intensively studied over the past two decades and its major components have been identified. The nuclear DELLA proteins, a subgroup of the GRAS transcription factors family, suppress GA signaling (Locascio et al., 2013). GA binding to the soluble GIBBERELLIN INSENSITIVE DWARF1 (GID1) receptor triggers GID1 interaction with the DELLA proteins (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006; Griffiths et al., 2006), which then stimulates assembly of the DELLA proteins into an SCF E3 ubiquitin ligase complex via the GID2/SLEEPY1 F-box proteins. The SCF complex polyubiquitinates the DELLA proteins, targeting them for destruction by the 26S proteasome (Sasaki et al., 2003; Dill et al., 2004; Griffiths et al., 2006; Harberd et al., 2009; Hauvermale et al., 2012). GA, via GID1, can also reduce DELLA activity through a degradation-independent mechanism (Ariizumi et al., 2008, 2013; Ueguchi-Tanaka et al., 2008).

Despite the central role of DELLAs in GA signaling, the mechanism underlying this regulation is not fully understood. Several studies have shown that protein-protein interactions play a major role in DELLA function. DELLAs bind to various transcription factors and proteins affecting transcription, including PHYTOCHROME-INTERACTING FACTORS (PIFs), ALCATRAZ, MYC2, JASMONATE-ZIM-DOMAIN PROTEIN9, SCARECROW LIKE3 (SCL3), and TCP transcription factors (de Lucas et al., 2008; Feng et al., 2008; Arnaud et al., 2010; Gallego-Bartolomé et al., 2010; Hong et al., 2012; Yang et al., 2012; Hou et al., 2010; Zhang et al., 2011; DAVIÈRE et al., 2014). The interaction between DELLA and PIFs, for example, suppresses the binding of the latter to target promoters and thus inhibits their activity. Although DELLAs lack a DNA binding domain, they possess transactivation properties (Hirano et al., 2012), and several studies have shown that DELLAs can act as coregulators when interacting with transcription factors and directly regulate gene expression (Zentella et al., 2007; Hirano et al., 2012; Yoshida et al., 2014).

The DELLA N-terminal region consists of the conserved DELLA and VHYNP motifs (Locascio et al., 2013). These motifs interact with the GID1 N-terminal arm to form the GID1-GA-DELLA complex (Murase et al., 2008). The C-terminal region of DELLAs consists of several distinct motifs comprising the GRAS domain. These motifs include two leucine heptad repeats (LHRI and LHRII) with putative nuclear localization signals, flanking a VHIIID motif, forming the LHRI-VHIIID-LHRII domain said to be involved in protein-protein interactions (Sun et al., 2012). Hirano et al. (2010) have shown that the SLENDER RICE1 (SLR1; the rice [Oryza sativa] DELLA protein) GRAS domain is also required for a stable interaction between DELLA and GID1. Recently, Sato et al. (2014) confirmed this observation and demonstrated an interaction between the purified SLR1 GRAS domain and GID1.

Arabidopsis thaliana has five DELLA proteins (Repressor of ga1-3 [RGA], GA-INSENSITIVE [GAI], RGA-LIKE1 [RGL1], RGL2,
and RGL3), whereas rice, barley (Hordeum vulgare), and tomato (Solanum lycopersicum) each have only one, called SLR1, SLLEN1, and PROCERA (PRO), respectively (Ikedaa et al., 2001; Chandler et al., 2002; Jasinski et al., 2008; Harberd et al., 2009). The one well-studied recessive tomato pro allele contains a point mutation within the VHIID domain (Val [V] to Glu [E] at position 273; Basell et al., 2008). Creating a similar mutation in gai, an Arabidopsis gain-of-function DELLA allele, completely abolished its growth-suppressing activity (Jasinski et al., 2008), suggesting a loss-of-function allele. The pro phenotype resembles wild-type plants treated with GA and includes elongated internodes, thinner leaves, and reduced lobing of the main leaflets (George Jones, 1987; Jupe et al., 1988; Van Tuinen et al., 1999; Basell et al., 2008; Jasinski et al., 2008). Antisense suppression of PRO also promoted GA responses, including pollination-independent ovary growth, resulting in parthenocarpic fruit formation (Marti et al., 2007).

In striking contrast with other plants with a single DELLA, such as barley and rice, pro plants respond to GA treatment and the pro mutation does not completely suppress chemicals or mutations that inhibit GA biosynthesis (George Jones, 1987; Jupe et al., 1988; Van Tuinen et al., 1999; Basell et al., 2008; Jasinski et al., 2008; Fleishon et al., 2011). The responsiveness of pro to GA might be due to an incomplete loss of DELLA function (Van Tuinen et al., 1999) or due to the activity of a DELLA-independent response pathway (Fleishon et al., 2011).

While the central role of DELLA in the regulation of GA responses is indisputable, it is not yet clear if DELLA mediates all GA responses. Recently, Yano et al. (2015) have shown that GID1-DELLA is the sole mechanism for GA regulation of gene expression in rice aleurone cells. On the other hand, results from a number of studies support the existence of a DELLA-independent GA signaling pathway. Our earlier work in Arabidopsis has suggested the existence of a cytosolic, SPINDLY-dependent, DELLA-independent GA response pathway (Maymon et al., 2009). These findings stood in line with those reported by Cao et al. (2006), who demonstrated that some GA-regulated genes are not regulated by DELLA. Moreover, GA-induced increases in cytosolic calcium concentrations, detectable within ~2 min of exposure to GA (Bush, 1998), have been suggested to occur too rapidly to be regulated by DELLA proteins, whose levels are only significantly reduced 5 to 10 min after GA treatment (Gubler et al., 2002). Furthermore, cytosolic activity of DELLA has never been detected, thereby challenging attempts to ascribe it a regulatory role in cytosol-emanating responses. Finally, application of GA to emasculated pistils of global (an Arabidopsis mutant that lacks the activities of all five DELLA proteins) resulted in significant promotion of their growth (Fuentes et al., 2012). This DELLA-independent response is mediated by the basic helix-loop-helix transcription factor SPATULA, which suppresses fruit growth. Despite these findings and other evidence of the existence of a DELLA-independent, GA response pathway, its significance remains unclear.

Here, we present a pro loss-of-function mutant tomato line named proGRAS. All examined GA-dependent developmental responses were much stronger in proGRAS than in pro. In addition, roles of PRO in seed desiccation tolerance and pollen tube elongation were uncovered. The presented results suggest that while GA regulation of tomato plant development is primarily DELLA dependent, ~5% of all identified GA-regulated genes are DELLA independent. Our results indicate that feedback regulation of GA catabolism is at least partially DELLA independent.

RESULTS

Identification and Characterization of a pro Mutant

In a visual screen of a tomato activation tagging population, a slender elongated mutant was identified. This mutant population was produced in the dwarf Micro-Tom tomato background by a maize Ds transposon element containing an enhancer sequence (see Methods). Backcross analysis of the newly identified mutant showed a recessive mode of inheritance, suggesting a loss-of-function mutation. After introgressing the mutant into the M82 (SP+) background by four successive backcrosses, the homozygous progeny exhibited similar slender-elongated growth. Since the mutant phenotype resembled that of pro, we sequenced the PRO gene and found a mutation likely to be caused by excision of a transposon used for activation tagging. The mutation created a stop codon downstream to the VHIID domain (position 339, Glu to stop); thus, the allele was predicted to encode a truncated protein lacking most of the GRAS domain (Figure 1A; Supplemental Figure 1). These proGRAS plants were extremely slender and tall compared with M82 and the pro mutant. Four-week-old proGRAS plants were ~3 times taller than M82 plants and twice as tall as M82 with pro introgressed into it (Figures 1B and 1C). The leaf phenotype of proGRAS was also stronger than that of pro, with larger, smoother, and curlier leaves (Supplemental Figures 2A and 2B). This includes longer stem, simpler leaves with smoother leaflets lacking intercalary leaflets (Figure 2A). In addition, flowering time was delayed and first inflorescence emerged after the production of 8 to 10 leaves rather than 5 to 7 and 7 to 8 leaves in M82 and pro, respectively (Figure 2B). The stigmas of the proGRAS pistils protruded above the staminal cone due to the long style (Figure 2C), and when fruits were made, they were all seedless, small, and oval (Figure 2D). Notably, the development of parthenocarpic fruits in tomato can be triggered by constitutive GA signaling (Carrera et al., 2012).

Recently, new pro alleles were produced using a transcription activator-like effector nuclease (TALEN; Lor et al., 2014), proΔGRAS plants were similar to proGRAS and had stronger defects than pro (Supplemental Figure 2A and 2B). This includes longer stem, simpler leaves with smoother leaflets, long styles, and production of small parthenocarpic fruits. When pro, proΔGRAS, and proΔGRAS plants (Supplemental Figure 3) were grown side by side for 4 weeks and their phenotypes were compared, proΔGRAS plants were indistinguishable from homozygous proΔGRAS plants (Supplemental Figure 2B), indicating that both are strong alleles that are likely null.

Loss of PRO Activity Affects Fertilization and Seed Set

As lack of fertilization in proGRAS and proΔGRAS flowers could stem from the long styles that prevent self-pollination (Figure 2C), proGRAS flowers were hand-pollinated with proGRAS pollen. Fertilization was rarely observed, suggesting a physiological barrier that prevents the fertilization process. This differs from the pro mutant that exhibits facultative parthenocarpy (Carrera et al., 2012). Pollination of proGRAS flowers with M82 pollen grains
experiment, proGRAS pollen tubes stopped elongating shortly after germination (Figure 3B). This growth suppression of proGRAS pollen tubes may explain the obligatory parthenocarpy observed in this mutant.

**Seed Viability and Segregation Distortion in Progenies of proGRAS/+ Plants**

Since homozygous proGRAS did not produce seeds, we had to use progenies of heterozygous plants to obtain homozygous plants. When sowing these seeds after a short period of storage (2 to 5 weeks of dry storage), ~2 to 8% of the seedlings were homozygous, and not the 25% expected by the Mendelian segregation ratio (Supplemental Figure 5A). To test if PRO activity is required for embryo vitality or for embryo survival under dry storage conditions, we extracted seeds from red fruits and sowed them either immediately or after longer periods of dry storage. The expected ratio of 25% seedlings with a proGRAS phenotype was obtained for fresh seeds. In contrast, only 8% of the seeds from seeds that were stored for 10 d exhibited the proGRAS phenotype, while ~18% of the seeds did not germinate (Figure 4A). After 2 months of dry storage, only 5% of the seedlings exhibited the proGRAS phenotype. These results led us to speculate that proGRAS seeds are intolerant to desiccation. However, it should be mentioned that when seeds were sown, proGRAS seedlings were the first to germinate, pointing at a promoting effect of the constitutive GA signaling on germination (Supplemental Figure 5B). To further examine this phenomenon, we conducted the same experiment with pro and proTALEN,2 seeds. Dry storage of pro seeds (5 months) did not affect their germination (Supplemental Figure 6A), while proTALEN,2 seeds, similar to proGRAS, exhibited reduced germination after short periods of dry storage (Supplemental Figure 6B).

**Abscisic Acid Responses in proGRAS Seeds**

To understand how PRO promotes desiccation tolerance, we followed the expression of desiccation-related genes by quantitative RT-PCR (qRT-PCR) analysis of RNA extracted from both M82 and the scarce fresh homozygous proGRAS seeds. To this end, we collected pollen from a large number of proGRAS anthers and pollinated many proGRAS flowers that eventually produced a few homozygous seeds. We analyzed the expression of the tomato ABA INSENSITIVE3 (ABI3), LATE EMBRYOGENESIS25 (LE25), and GALACTINOL SYNTHASE1 (GOLS1) genes, all of which are known to be regulated by abscisic acid (ABA) and to be involved in the acquisition of seed desiccation tolerance (Cohen and Bray, 1992; Downie et al., 2003; Bassel et al., 2006; To et al., 2006). In addition, we analyzed the expression of the tomato FUSCA3-like (FUS3-like) homolog, a major player in the acquisition of desiccation tolerance (To et al., 2006). All four genes exhibited significantly lower levels of expression in proGRAS compared with M82 seeds (Figure 4B), suggesting that the machinery to induce desiccation tolerance is suppressed in proGRAS seeds. Since ABA has a major role in the acquisition of desiccation tolerance during seed maturation (Ooms et al., 1993; Koornneef et al., 2002; Finkelstein et al., 2008), and DELLA positively regulates ABA accumulation via the transcriptional activation of XERICO, a RING-E3 ligase (Zentella et al., 2007; Ariizumi et al., 2013), we analyzed the
expression of the tomato XERICO homolog in fresh proGRAS seeds. XERICO-like expression was lower in proGRAS compared with M82 seeds (Supplemental Figure 7), implying that the lack of desiccation tolerance in proGRAS seeds may result from reduced ABA levels.

DELLA-Independent GA Responses

Our data suggest that the proGRAS allele is much stronger than pro and may represent a null allele. Thus, we next tested whether the well-documented responsiveness of pro to GA (Van Tuinen et al., 1999) is due to a partial loss of DELLA function or due to the activity of a DELLA-independent GA signaling pathway (Fleishon et al., 2011). To this end, we first treated M82, pro, and proGRAS seedlings with the GA biosynthesis inhibitor paclobutrazol (PAC), followed by application of GA3. PAC treatment of M82 and of the pro mutant suppressed stem elongation (Figures 5A and 5B), an effect that was reversed by application of GA3. However, PAC, GA3, or their sequential application did not alter elongation of proGRAS or proTALEN,2 stems (Supplemental Figure 8). Likewise, chlorophyll content was elevated by PAC and reduced by GA3 in M82 and pro but not in proGRAS leaves (Figure 5C). These results suggest that proGRAS and proTALEN,2 plants are largely insensitive to GA, while pro plants retain some DELLA activity.

To examine the molecular responses of proGRAS to GA, we compared the regulation of GA metabolism and catabolism genes by GA. GA homeostasis is regulated by a negative feedback loop, where high GA levels/signals suppress GA production via the inhibition of the GA biosynthetic gene GA20oxidase (GA20ox) and promote GA deactivation by the induction of the GA deactivation gene, GA2oxidase (GA2ox; Yamaguchi, 2008). M82 and proGRAS seedlings were treated with PAC for 3 d and then treated with 0, 1, or 100 μM GA3. Three hours after the GA treatment, RNA was extracted from young leaves and the expression levels of GA20ox1 and GA2ox4 were analyzed by qRT-PCR. We would like to emphasize that the names of these and other tomato GA metabolism and catabolism genes do not necessarily reflect their relatedness to the Arabidopsis genes. The accession numbers of all the tomato genes used in this study can be found in Methods. As expected, GA20ox1 expression was promoted by PAC and suppressed by GA3 in M82 leaves. In agreement with the constitutive GA signaling and insensitivity to GA, GA20ox1 expression was extremely low in proGRAS and neither affected by PAC nor by GA3 treatment (Figure 6A). GA2ox4 expression was low in mock-treated M82 and induced by GA3 treatment. However, the GA2ox4 expression level in proGRAS remained low, similar to the level found in M82 leaves, indicating that it was not affected by the endogenous constitutive GA signal. Moreover, expression of this gene in proGRAS was strongly induced by exogenous GA3 (Figure 6B). As these results were unexpected, the experiment was repeated six times and similar results were obtained (Supplemental Figure 9). However, it should be noted that in some experiments, the GA induction of GA2ox4 was stronger in proGRAS than in M82, but not in others (Figure 6B versus Supplemental Figure 9). We next examined the impact of GA3 treatment of pro leaves on the expression of these two genes. GA20ox1 expression was low in
mock-treated pro (due to the constitutive GA responses) but was further inhibited by treatment with 10 \text{ \( \mu \text{M} \) GA3} (Supplemental Figure 10), indicating partial PRO activity. GA2ox4 expression, on the other hand, was not affected by the constitutive GA signaling in pro but was induced by exogenous GA3 treatment. We next analyzed the expression levels of other GA2ox genes, GA2ox2 and GA2ox5. GA2ox2 expression was not altered by GA application to the wild-type M82; therefore, its expression was not examined in pro\text{\( \Delta \)GRAS} seedlings (Supplemental Figure 11A). The expression profile of GA2ox5, on the other hand, in response to GA was similar to that of GA2ox4, i.e., induced by exogenous GA3 in pro\text{\( \Delta \)GRAS} (Supplemental Figure 11B).

The strong induction of GA2ox4 by exogenous GA3 in pro\text{\( \Delta \)GRAS} combined with the lack of effect of the constitutive endogenous GA signaling in this mutant suggest a GA response that is DELLA independent. However, it should be noted that GA2ox4 did not respond to application of GA3 in M82 and pro\text{\( \Delta \)GRAS}, without prior exposure to PAC, and the PAC treatment itself, typically weakly promoted expression. Similar results were found previously in rice (Huang et al., 2010).

To further investigate this possible DELLA-independent GA response, we generated transgenic rga\text{\( \Delta \)17} tomato plants (M82 background) overexpressing the Arabidopsis DELLA RGA lacking the DELLA domain (Dill et al., 2001). The 17-amino acid deletion in RGA inhibits the degradation of the protein in response to GA and, therefore, when overexpressed, constitutively suppresses GA responses (Dill et al., 2001). We used the Arabidopsis gene to bypass possible cosuppression. 35S:rga\text{\( \Delta \)17} tomato lines with high rga\text{\( \Delta \)17} expression levels (Figure 7A) and a severe dwarfism were self-pollinated and homozygous lines were generated. These lines also had small dark-green leaves, typical of tomato plants with reduced GA activity (Nir et al., 2014). Application of exogenous

Figure 3. The Effect of pro\text{\( \Delta \)GRAS} and pro on Anther Development, Pollen Production, and Pollen Tube Elongation.

(A) Scanning electron microscopy images of M82, pro, and pro\text{\( \Delta \)GRAS} anther cones and single anthers. Flowers were detached prior to anthesis and cut widthwise. Bars in the upper panels = 500 \text{ \( \mu \text{m} \)}; bars in the lower panels = 250 \text{ \( \mu \text{m} \)}.
(B) Real-time observation of in vitro germination of M82, pro, and pro\text{\( \Delta \)GRAS} pollen. Flowers were detached at anthesis and pollen was incubated in germination solution. Germination and tube elongation were monitored for 6 h using a light microscope. Bar = 50 \text{ \( \mu \text{m} \)}.
The expression of this gene was suppressed by GA3 treatment in tomato plants, following PAC and GA treatments, as described above. As expected in cases of feedback regulation, At-GA20ox2 exhibited normal feedback regulation in the wild type but expression was unaffected by PAC or GA in dellaP (Figure 8A). At-GA20ox4 was induced by GA3 in both the wild type and dellaP (Figure 8B). We also tested the response of these two genes to GA3 in flowers. To this end, seedlings were treated with PAC (5 mg/L) twice a week until flowering and then treated once with 10 µM GA3. Three hours after the GA treatment, RNA was extracted from seedling shoots and analyzed for At-GA20ox2 and At-GA20ox4 expression. While At-GA20ox2 exhibited normal feedback regulation in the wild type, in dellaP its basal expression was low and was unaffected by either PAC or GA (Figure 8C). At-GA20ox4 was not affected by the endogenous constitutive GA signaling in dellaP but was induced by GA3 in both the wild type and dellaP (Figure 8D). These results suggest that in Arabidopsis, the regulation of At-GA20ox4 by GA is also DELLA independent.

To further explore DELLA-independent GA responses in Arabidopsis, we examined the expression of At-GA2ox1 in the flowers. At-GA2ox1 behaved as expected of a DELLA-regulated gene, i.e., high expression in dellaP and lack of response to GA3 (Figure 8E). To examine whether the activation of At-GA20ox4 by GA is initiated by the GA receptor GID1, we treated wild-type and gid1ac (loss of two of the three GID1 receptor genes; Griffiths et al., 2006) seedlings with PAC (5 mg/L) once a day for 3 d followed by a single GA3 application (10 µM). Three hours after the GA treatment, RNA was extracted and analyzed for At-GA20ox4 expression. The lack of GID1a and GID1c activity significantly reduced the response of At-GA20ox4 to GA3 (Figure 8F), suggesting that this DELLA-independent GA response is initiated by GA binding to the GID1 receptors. The observed weak response of At-GA20ox4 to GA3 in gid1ac was probably mediated by GID1b.

**Global Analysis of DELLA-Independent GA Responses**

To understand the scope of DELLA-independent GA-regulated genes, deep sequencing (RNA-seq) was performed to RNA samples extracted from GA-treated M82 and proGRAS plants. M82 and proGRAS seedlings were treated with PAC (10 mg/L) once a day for 3 d followed by a single GA3 application (100 µM). Three hours after the GA treatment, young leaves were collected, RNA was extracted, and cDNA libraries were sequenced by Illumina HiSeq2500. A total of eight samples were analyzed, and each treatment had two biological replicates. TopHat was used to align the reads to the tomato genome SL2.50 (Trapnell et al., 2009). Counts of aligned reads per gene were obtained using HTSeq-count (Anders et al., 2015), and the DESeq2 package was used to identify genes that were differentially expressed between PAC and PAC + GA3 treated leaves. Using a 2-fold increase or decrease cutoff (adjusted P value for multiple comparisons ≤0.05), we identified 81 GA-upregulated and 15 GA-downregulated genes (Tables 1 and 2; Supplemental Table 2). The majority of these genes were DELLA dependent, i.e., their expression was unaffected by GA3 in proGRAS. These include some well-characterized GA-regulated genes, such as GA20ox.
GID1, SCL, GAST1, and EXPANSINS (Shi et al., 1992; Chen et al., 2001; Zentella et al., 2007). Five of the GA-regulated genes (four upregulated and one downregulated) were DELLA-independent, i.e., they were similarly induced or suppressed by GA3 in M82 and proΔGRAS (Table 3). It should be noted that in this experiment, all GA2ox genes were expressed at low levels and none of them was affected significantly by GA3 in M82 or proΔGRAS. To confirm the results, we analyzed the expression of the identified GA-regulated DELLA-independent genes, Solyc07g064600.2 (encoding Endoribonuclease) and Solyc09g008670.2 (encoding Thr ammonia lyase) by qRT-PCR. The results confirm those of the RNA-seq and show that GA induces both in a DELLA-independent manner (Supplemental Figure 13).

DISCUSSION

The tomato genome contains a single DELLA gene, named PRO, and a pro mutant has been extensively characterized (George Jones, 1987; Jupe et al., 1988; Van Tuinen et al., 1999; Jasinski et al., 2008; Bassel et al., 2008; Carrera et al., 2012). pro exhibits constitutive GA activity but retains some responsiveness to the hormone, either due to incomplete loss of DELLA activity (Van Tuinen et al., 1999) or due to activity of a DELLA-independent GA response pathway (Fleishon et al., 2011). Here, we describe pro mutants, proΔGRAS and proTALEN_2 (Lor et al., 2014) that are likely null or close to null alleles. Our study suggests that the responsiveness of the “classic” pro mutant to GA is due to residual DELLA activity but also uncovers DELLA-independent GA responses.

The phenotype of proΔGRAS and proTALEN_2 plants resembles that of tomato plants treated with high doses of GA. In tomato, exogenous GA application has a dramatic effect on stem elongation. In Arabidopsis, on the other hand, application of GA or lack of DELLA activity has only a mild effect on final stem length (King et al., 2001). A strong effect is found only when the hormone is applied to GA-deficient mutants. This difference between Arabidopsis and tomato...
may be due to differences in basal levels of endogenous active GAs. A rapid stem elongation (bolting) in Arabidopsis occurs after the floral transition and is associated with a dramatic increase in GA level ( Eriksson et al., 2006 ). Thus, GA activity may be saturated and the loss of DELLA or addition of exogenous GA has only a mild effect. On the other hand, the tomato stem elongates slowly but continuously throughout the life of the plant. It is possible that this slow elongation requires intermediate GA levels, below saturation; therefore, loss of PRO activity or application of high GA doses has a dramatic effect on stem elongation.

All analyzed GA-related phenotypes were more severe in pro GRAS and pro TALEN,2 plants than in pro, suggesting that pro is a “leaky” mutant, as previously proposed (Van Tuinen et al., 1999). While pro exhibits facultative partenocarpy ( Carrera et al., 2012 ), pro GRAS and pro TALEN,2 did not produce seeds even after hand-pollination, suggesting obligatory partenocarpy. Previous studies suggested that the facultative partenocarpy of pro is due to the longer style, which prevents self-pollination ( Bassel et al., 2008; Carrera et al., 2012 ). While the pro GRAS and pro TALEN,2 styles are longer than that of pro, it cannot explain the obligatory partenocarpy. Since pollination of pro GRAS and pro TALEN,2 flowers with M82 pollen resulted in an almost normal seed set, the lack of fertilization in homozygous pro GRAS or pro TALEN,2 flowers is probably due to male sterility. An in vitro pollen germination assay showed that the elongation of pro GRAS pollen tube, but not that of pro, is arrested shortly after germination. Previous studies in Arabidopsis and rice suggested that while GA is required for pollen tube elongation, GA concentrations higher than optimal inhibit this process ( Singh et al., 2002; Chhun et al., 2007 ). This can explain why pro GRAS but not pro, inhibited pollen tube elongation. The suppression of pollen tube elongation in pro GRAS is probably not a cell-autonomous effect. If it was, homozygous seeds would not be obtained by self-pollination of heterozygous plants, since haploid pro GRAS pollen would not elongate to fertilize the pro GRAS egg cells. Thus, it is possible that the effect of pro GRAS on the ability of the pollen cells to elongate is via the supporting tissues, the connective and tapetum cell layers. Indeed, scanning electron microscopy analysis showed malformation of these tissues in pro GRAS .

Tomato seeds can be considered “orthodox” seeds ( Angelovic et al., 2010 ), since they can tolerate desiccation and can be stored in a dry state for years ( Priestley et al., 1985 ). Our results show that homozygous pro GRAS and pro TALEN,2 seeds lose their ability to germinate shortly after harvest and cannot survive even short periods (days) of dry storage. Analysis of desiccation tolerance-related genes ( ABI3, FUS3, LE25, and GOLS ) in pro GRAS seeds revealed reduced expression levels, suggesting that PRO is required for activation of the machinery that acquire tolerance. The germination of pro seeds, on the other hand, was not affected by long dry storage, suggesting that residual DELLA activity is sufficient to acquire desiccation tolerance.

ABA plays a major role in the acquisition of desiccation tolerance as well in the induction of dormancy during the late stages of seed maturation ( Oorns et al., 1993; Koornneef et al., 2002; Finkelstein et al., 2008 ). Previous studies have shown that DELLA regulates ABA synthesis in seeds via the transcriptional activation of the RING ubiquitin E3 ligase XERICO, an inducer of ABA synthesis ( Zentella et al., 2007; Piskurewicz et al., 2008; Arizumi et al., 2013 ). We found reduced expression of the putative tomato homolog of XERICO in pro GRAS seeds, suggesting that PRO increases desiccation tolerance by promoting ABA synthesis. Although desiccation tolerance is tightly associated with dormancy and both are regulated by ABA, previous studies linked DELLA activity in seeds with dormancy only ( Lee et al., 2010; Arizumi et al., 2013 ). Our results suggest that the loss of PRO activity suppresses both processes; while homozygous pro GRAS seeds had reduced desiccation tolerance, they germinated much faster than M82 seeds, suggesting weaker dormancy.

It is possible that the loss of seed viability during dry storage prevented the identification of strong pro alleles in all previous tomato mutant screenings. It is also possible that the Micro-Tom background, which has a mutation in the DWARF ( D ) gene, allowed the identification of this allele in our screening. D encodes a P450 protein involved in brassinosteroid biosynthesis ( Bishop et al.,

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6.png}
\caption{Regulation of GA20ox and GA2ox Expression by GA in pro GRAS .}
\end{figure}
Since GA and brassinosteroids act synergistically (Bai et al., 2012), and the response to GA in Micro-Tom partially depends on brassinosteroids (Martí et al., 2006), it is possible the GA responses are partially suppressed in Micro-Tom, improving seed tolerance to desiccation.

Our results suggest that the reported, relatively strong response of *pro* to GA (Van Tuinen et al., 1999) is due to the “leaky” nature of the *pro* allele and not due to the activity of an alternative GA signaling pathway. In parallel, while the null mutants *pro^GRAS* and *pro^TALEN_2* exhibited insensitivity of growth to GA and PAC, a DELLA-independent GA response in *pro^GRAS* plants, namely, the feedback regulation of GA catabolism, was discovered. As expected, the expression level of GA20ox1 was lower in *pro^GRAS* than in M82 and was not affected by GA or PAC treatments. On the other hand, the expression of GA2ox4 and GA2ox5 was unexpectedly low in *pro^GRAS* and was strongly induced by GA. These findings suggest that GA2ox4 and GA2ox5 do not respond to the endogenous constitutive GA signaling produced by the loss of *PRO*, but rather, are induced by exogenous GA treatment. In addition, although transgenic tomato plants overexpressing the
Figure 8. Regulation of Arabidopsis GA20ox2, GA2ox4, and GA2ox1 Expression by GA in Arabidopsis.
Arabidopsis gain-of-function DELLA protein RGA\(\Delta 17\) were insensitive to GA in terms of growth, GA2ox4 was strongly induced by GA3 treatment in these plants. In summary, these findings suggest that expression of tomato GA2ox4 is activated by GA via a DELLA-independent pathway. Similar results were found in the Arabidopsis delfap mutant. While At-GA2ox1 behaved as expected, i.e., exhibited high levels of expression in delfap and insensitivity to GA treatment, the expression of At-GA2ox4 was strongly induced by exogenous GA3 in this mutant. While numerous studies have shown that the expression of GA2ox3 is suppressed, and that of GA2ox3 is promoted by GA (Yamaguchi, 2008), Zentella et al. (2007) suggested that At-GA2ox3 genes, but not At-GA2ox2, are regulated directly by DELLA. The mechanism by which GA promotes GA2ox expression in a DELLA-independent manner is yet unknown, but our results imply that GA binding to the GID1 receptor is required. High GA activity increases plant susceptibility to various biotic and abiotic stresses (Achard et al., 2006; Nir et al., 2014) and therefore can be destructive to plants. Thus, it is possible that both DELLA-dependent and -independent induction of GA catabolism by increased GA signal evolved to ensure efficient regulation of GA homeostasis.

Our results suggest that ~5% of all tomato GA-regulated genes are DELLA-independent (Tables 1 to 3). Similarly, Cao et al. (2006) suggested that only a portion of the GA-regulated genes in Arabidopsis are DELLA dependent. In tomato, the strongest DELLA-independent induction by GA was on a ribonuclease (RNase) gene (SolyC05g007950.2, 15-fold change). A previous study in barley aleurone identified RNase as a GA-induced gene (Rogers and Rogers, 1999). Tomato GA-regulated genes include homologs of well-characterized Arabidopsis genes: GA downregulated, such as GA2ox3, GID1, and SCL, and GA upregulated genes, such as GASA-like (GAST1) and EXPANSIN (Shi et al., 1992; Chen et al., 2001; Zentella et al., 2007). Thus, while many “classic” GA-associated genes are common to distantly related plants, others, e.g., bZIP transcription factor (Solyc12g010800.1, 10-fold induction), may

| Table 1. GA Upregulated, DELLA-Dependent Genes (Fold Change > 4) |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| SolyC Locus       | Description     | Mean Pac         | Mean Pac+GA     | Fold Change     | Adj. P Value    |
| SolyC05g007950.2  | Ribonuclease T2 | 68              | 1068            | 15.78           | 5.62E-08        |
| SolyC12g010800.1  | BZIP transcription factor | 10          | 106             | 10.29           | 7.15E-06        |
| SolyC03g025380.2  | Peroxidase      | 24              | 243             | 9.98            | 0.000267        |
| SolyC03g005320.2  | 3-Ketoacyl-CoA synthase | 60          | 379             | 6.36            | 1.64E-06        |
| SolyC01g110630.2  | Auxin-induced SAUR-like | 18          | 103             | 5.95            | 0.000945        |
| SolyC04g017720.2  | GAST1           | 55              | 312             | 5.71            | 1.22E-07        |
| SolyC12g056250.1  | Glutathione S-transferase | 341         | 1917            | 5.62            | 2.76E-06        |
| SolyC07g062710.1  | BZIP transcription factor | 64          | 342             | 5.38            | 6.02E-08        |
| SolyC04g081790.2  | GDSL esterase/lipase | 54           | 290             | 5.38            | 6.02E-08        |
| SolyC04g161901.2  | Glucosyltransferase | 87            | 465             | 5.33            | 0.001001        |
| SolyC03g097170.2  | Cinnamoyl-CoA reductase | 140         | 739             | 5.29            | 8.31E-07        |
| SolyC03g078090.2  | Pectinesterase   | 19              | 89              | 4.80            | 0.033812        |
| SolyC10g005210.2  | Methyladenine glycosylase | 42         | 195             | 4.60            | 1.64E-06        |
| SolyC10g011730.2  | Arabinogalactan peptide | 49          | 219             | 4.49            | 3.75E-05        |
| SolyC03g006100.2  | Receptor-like kinase, RLK | 144         | 633             | 4.41            | 0.000322        |
| SolyC08g075210.1  | Acp4transferase-like protein | 60          | 259             | 4.31            | 0.011786        |
| SolyC03g114710.2  | Glucosyltransferase | 33            | 141             | 4.30            | 0.00217         |
| SolyC10g025301.2  | Auxin-responsive protein | 544         | 2301            | 4.28            | 0.00546         |
| SolyC11g069601.2  | Receptor-like kinase, RLK | 32           | 137             | 4.22            | 0.000267        |
| SolyC04g081870.2  | Expansin         | 467             | 1964            | 4.20            | 1.98E-07        |
| SolyC02g088100.2  | Expansin         | 297             | 1232            | 4.15            | 0.000293        |
| SolyC07g008560.2  | Purple acid phosphatase | 25           | 103             | 4.04            | 0.010653        |

aMean value of two biological replicates.
bFold change is the ratio mean Pac + GA/mean Pac.
cCorrected P values were calculated using the Benjamini and Hochberg (1995) false discovery rate approach.

Figure 8. (continued).

(A) and (B) Seedlings of wild-type Col-0 and delfap mutant Arabidopsis plants were treated with PAC (5 mg/L) once a day for 3 d followed by a single GA3 application (10 \(\mu\)M). Three hours after the GA treatment, RNA was extracted from the seedlings and analyzed by qRT-PCR for At-GA2ox2 (A) and At-GA2ox4 (B) expression.

(C) to (E) Plants (wild-type Col-0 and delfap) were treated with PAC (5 mg/L) twice a week until flowering and then treated once with 10 \(\mu\)M GA3. Three hours after the GA treatment, RNA was extracted from the flowers and analyzed by qRT-PCR for At-GA2ox2 (C), At-GA2ox4 (D), and At-GA2ox4 (E).

(F) Wild type (Col-0) and gid1ac seedlings were treated with PAC (5 mg/L) once a day for 3 d followed by a single GA3 application (10 \(\mu\)M). Three hours after the GA treatment, RNA was extracted and analyzed qRT-PCR for At-GA2ox4 expression.

Values (gene-to-TUBULIN ratios) in (A) to (F) are means of three biological replicates ± se.
represent tomato-specific GA responses. For the five DELLA-independent genes, we were not able to find a common theme that characterizes their specific regulation.

In summary, this work presents new tomato DELLA loss-of-function mutants. Phenotypic, physiological, and molecular analyses of these pro mutants uncovered DELLA-regulated processes and identified GA-regulated, DELLA-independent responses, providing a powerful tool to study GA physiology and the role of DELLA in plant biology.

METHODS

Plant Materials and Growth Conditions

*Tomato (Solanum lycopersicum)* plants were in the M82 background (SP*). The recessive pro GRAS allele was isolated from an activation-tagging population of Micro-Tom, mutagenized with an Ac/Ds system carrying a 4 × 3SS enhancer element in the Ds transposon (MacAlister et al., 2012). The pro GRAS line used in this study was backcrossed with M82 (SP* ) plants four times. pro (Bassel et al., 2008) and pro TALEN (Lor et al., 2014) were in the M82 (SP*) background. Plants were grown in a greenhouse under 24/20°C (day/night) at natural daylength conditions. *Arabidopsis thaliana* plants were grown in a growth room under controlled temperature (22°C) and long-day (16 h light/8 h dark) conditions. The Arabidopsis DELLA pentuple mutant (deltaP; Park et al., 2013) and gid1ac double mutant (Griffiths et al., 2006) were in the Col-0 background. Tomato seeds were harvested from ripe fruits, incubated with 10% sucrose overnight at 37°C, and then treated with 1% sodium hypochlorite followed by 1% Na3PO4. Seeds were stored dry at room temperature.

**Molecular Cloning/Constructs and Plant Transformation**

The RGA Δ17 coding sequence (Zentella et al., 2007) was fused to the 5′ of the enhanced GFP coding sequence, in a KpnI site. The GFP-RGA Δ17 fusion was inserted to a partT7 plasmid downstream of the 3SS promoter, into XhoI and BamHI sites, to create 3SS:GFP-RGA Δ17. The construct was subcloned into the partT27 binary vector and was introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. The construct was transferred to *S. lycopersicum* variety M82 cotyledons, using the transformation and regeneration methods described by McCormick (1991). Kanamycin-resistant T0 plants were grown in the greenhouse, and three independent transgenic lines were selected and self-pollinated to generate homozygous transgenic lines. All primer sequences are presented in Supplemental Table 3.

**Hormone Treatments**

Tomato seedlings with two true leaves were sprayed with PAC (10 mg/L) three times a week for 2 weeks, followed by GA3 (Sigma-Aldrich) application (100 μM), throughout the experiment. For the analysis of GA biosynthesis gene expression, young tomato seedlings were sprayed for 3 d with PAC (10 mg/L) and on the fourth day, immersed in 10 or 100 μM GA3 for 30 min.

### Table 2. GA-Downregulated, DELLA-Dependent Genes

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<th>Description</th>
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<th>Mean Pac+GAb</th>
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<th>Adj. P Valuec</th>
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<td>Solyc01g008910.2</td>
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a Mean value of two biological replicates.

b Fold change is the ratio mean Pac + GA/mean Pac [value is presented as: −1(Pac + GA/Pac)].

c Corrected P values were calculated using the Benjamini and Hochberg (1995) false discovery rate approach.

### Table 3. GA-Regulated, DELLA-Independent Genes

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<tr>
<th>SolyC Locus</th>
<th>Description</th>
<th>Fold Changea</th>
<th>Adj. P Valueb</th>
<th>Fold Changea</th>
<th>Adj. P Valueb</th>
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<td>Threonine ammonia-lyase</td>
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<td>Solyc03g121270.2</td>
<td>IAA-amino acid hydrolase</td>
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a Fold change is the ratio mean Pac + GA/mean Pac [for fold change <1, the value is presented as: −1(Pac + GA/Pac)].

b Corrected P values were calculated using the Benjamini and Hochberg (1995) false discovery rate approach.
Leaves were collected after 3 h and RNA was extracted. Arabidopsis seedlings were treated with PAC (5 mg/L) once a day for 3 d followed by a single GA\(_2\) application (10 \(\mu\)M) or twice a week with PAC (5 mg/L) until flowering and then immersed in 10 \(\mu\)M GA\(_2\) for 30 min. Seedlings or flowers were collected 3 h after the GA treatments and RNA was extracted.

**Chlorophyll Measurements**

Chlorophyll was extracted from fresh leaves in acetone (100%) and spectrophotometrically measured at 645 and 663 nm (Arnon, 1949). Chlorophyll concentrations were calculated using the formula: (\(20.2 \times A_{663} + 8.02 \times A_{645}\))/cm².

**RNA Extraction and cDNA Synthesis**

Total RNA was isolated using the GBC-phenolchloroform method: Frozen tissues were ground and resuspended in guanidine HCl and then phenol/chloroform was added. Samples were mixed by vortexing for 30 s and after 30 min were centrifuged at 4°C for 45 min. Ethanol (100%) and 1 M acetic acid were added, and the samples were mixed and stored overnight at −80°C. NaCl (3 M) was added and samples were washed with cold 70% ethanol. For the synthesis of cDNA, we used SuperScript II reverse transcriptase (Invitrogen) and 3 \(\mu\)g of total RNA, according to the manufacturer's instructions.

**qRT-PCR Analyses**

qRT-PCR analysis was performed using the Absolute Blue qPCR SYBR Green ROX Mix (AB-41628/2) kit (Thermo Fisher Scientific). Reactions were performed using a Rotor-Gene 6000 cycler (Corbett Research). A standard curve was obtained for each gene using dilutions of a cDNA sample. Each gene was quantified using Corbett Research Rotor-Gene Gene-Software. At least three independent technical repeats were performed for each cDNA sample. Relative expression of each sample was calculated by dividing the expression level of the analyzed gene by that of TUBULIN. Gene-to-TUBULIN ratios were then averaged. All primer sequences are presented in Supplemental Table 3.

**Library Construction and Sequencing**

Total RNA (0.5 \(\mu\)g) was processed using the TruSeq RNA Sample Preparation Kit v2 protocol (Illumina). Libraries were evaluated by Qubit and TapeStation. Sequencing libraries were constructed with barcodes to prepare multiplexing of eight samples on one lane. Twenty to twenty-and-one million single-end 60-bp reads were sequenced per sample on an Illumina HiSeq2500 V4 instrument.

**Sequence Data Analysis**

TopHat (v2.0.10) was used to align the reads to the tomato genome sequence SL2.50 (downloaded from the Sol genomics network http://solgenomics.net/organism/Solanum_lycopersicum/genome) (Trapnell et al., 2009). The percentage of the reads that were aligned uniquely to the genome was between 85 and 91%. Counting reads on ITAG2.4 genes (downloaded from Sol genomics network) was done with HTSeq-count (version 0.6.1p1) (Anders et al., 2015). Differential analysis was performed using DESeq2 (1.6.3) (Anders and Huber, 2010). Raw P values were adjusted for multiple testing using the procedure of Benjamini and Hochberg (1995). Genes with a false discovery rate of <0.05 and fold changes >2 were regarded as differentially expressed genes.

Expression data were submitted to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/; accession number GSE68018).

**Genotyping**

DNA was extracted from cotyledons of progenies of the proTALEN-2/ proGRAS crosses that exhibited elongated hypocotyls using the DNeasy Plant Mini Kit (Qiagen). To identify the proTALEN-2 allele, the forward primer proTALEN_dF1 and reverse primer proTALEN_dR1 (Supplemental Table 3) were used to amplify the region encompassing the proTALEN-2 deletion site (Lor et al., 2014). Each PCR reaction used 50 ng of genomic DNA template in a 50-\(\mu\)L volume using ExTaq polymerase (Clontech). Thermocycler conditions were set according to the manufacturer's recommendations with the annealing temperature set for 55°C and elongation time set for 1 min. proTALEN-2 PCR amplicons were digested with SmFI, which cuts the wild-type sequence but not the proTALEN-2 mutant sequence, and 10 \(\mu\)L of the digestion was run on a 0.8% agarose gel. To identify the proGRAS allele, we designed derived cleaved amplified polymorphic sequence (Neff et al., 1998) primers proGRAS_dF1 and proGRAS_dR1 using diCAPS Finder 2.0 (http://helix.wustl.edu/dicapsc). The resulting primers produce a wild-type PRO amplicon that is digested with PvuII to produce 302- and 27-bp products, while the proGRAS amplicon is resistant to digestion. PCR reaction mixtures and conditions are similar to the proTALEN-2 PCR conditions except for the annealing temperature that was set at 65°C. proGRAS PCR amplicons were digested with PvuII and separated on 1.5% agarose gel.

**Microscopy**

Samples for scanning electron microscopy were immersed in increasing concentrations of ethanol (25% up to 100%) and critical-point dried with liquid carbon dioxide in a CPD 750 (Bio-Rad), sputter-coated with gold, and photographed with a Jeol scanning electron microscope (JSM-5410 LV).

**In Vitro Pollen Germination Assay**

Flowers were detached at anthesis and shaken with a pollen buzzer into a microfuge tube containing germination solution (100 g L⁻¹ sucrose, 40% polyethylene glycol 4000, 0.01 M HEPES, pH 6, 2 mM boric acid, 2 mM calcium nitrate, 2 mM magnesium sulfate, and 1 mM potassium nitrate); tubes were shaken well to release the pollen grains. The final solution with the pollen grains was transferred to a slide covered with glass slip and sealed with grease. Germination and tube elongation were monitored for 6 h under a light microscope.

**Accession Numbers**

Sequence data from this article can be found in the GenBank/EMBL libraries under the following accession numbers: PROCERA, Solyc11g011260; AB32, Solyc06g038590; GOL1, Solyc01g008301; L235, Solyc09g077701; Fus3-1, Solyc02g044601; XERICO-1, Solyc07g045190; GA2ox2, Solyc07g056670; GA2OX4, Solyc07g061720; GA2OX5, Solyc07g061730; GA2OX1, Solyc03g006880; At-GA20X2, AT5G51810; At-GA2OX1, At1G78440; At-GA2OX4, At1G79901; At-RGA, At2G01570; ENDRIBONUCLEASE, Solyc07g064600; THREONINE AMMONIA LYASE, Solyc09g008670. In addition, sequence data and their sources are provided in Tables 1 to 3 and Supplemental Table 2.
Supplemental Figure 6. proTALEN, proGRAS, but not proTALens are sensitive to desiccation.

Supplemental Figure 7. qRT-PCR expression analysis of the putative XERICO gene in tomato.

Supplemental Figure 8. proGRAS and proTALEN, proGRAS, and proTALEN, proGRAS, are insensitive to PAC and GA₃.

Supplemental Figure 9. Regulation of GA2ox4 expression by GA in proGRAS.

Supplemental Figure 10. Expression analyses of GA2ox1,3, and GA2ox4,5 in M82 and pro.

Supplemental Figure 11. Expression analyses of GA2ox1 and GA2ox4 in M82 and pro.

Supplemental Figure 12. Expression analyses of GA2ox2 and GA2ox5 expression.

Supplemental Figure 13. Expression analyses (qRT-PCR) of Solyc07g064600.2 (Endoribonuclease) and Solyc09g008670.2 (Thrp ammonia lyase) in M82 and proGRAS leaves.

Supplemental Table 1. proTALEN, proGRAS plants are male, but not female, sterile.

Supplemental Table 2. Complete list of GA upregulated genes

Supplemental Table 3. Primers used in this study.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS


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REFERENCES


Arnon, D.I. (1949). Cooper enzymes in isolated chloroplasts poly- 


Cohen, A., and Bray, E.A. (1992). Nucleotide sequence of an ABA-
duced tomato gene that is expressed in wilted vegetative organs and developing seeds. Plant Mol. Biol. 18: 411–413.


Uncovering DELLA-Independent Gibberellin Responses by Characterizing New Tomato *procera* Mutants
Sivan Livne, Vai S. Lor, Ido Nir, Natanella Eliaz, Asaph Aharoni, Neil E. Olszewski, Yuval Eshed and David Weiss

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DOI 10.1105/tpc.114.132795

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