American Journal of Plant Physiology

ISSN 1557-4539

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS American Journal of Plant Physiology

ISSN 1557-4539
DOI: 10.3923/ajpp.2017.10.19

Research Article
Phenotypic Characterization of the *Arabidopsis ufm1* (Ubiquitin Fold Modifier) Gene Involved in Seed Development

Cornejo Paula and Masuelli Ricardo Williams

Laboratorio de Biología Molecular, Instituto de Biología Agrícola de Mendoza (IBAM), Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, A. Brown 500 (M5528AHB) Chacras de Coria, Mendoza, Argentina

Abstract

Background and Objective: With the completion of the *Arabidopsis* genome sequencing, the next challenge is the determination of gene function. Post-translational modifications of proteins by small polypeptide are implicated in plant growth and development. Ubiquitin fold modifier 1 is a member of the ubiquitin like protein family. While, the enzymatic conjugation cascade of ubiquitin fold modifier 1 has been elucidated in recent years, its biological role is still unknown. The present study focuses in elucidate the role of the *ufm1* in plants development. **Materials and Methods:** The researchers performed analyses of the development of wild-type Columbia plants and mutants of the ubiquitin fold modifier 1 gene to identify and interpret phenotypic changes in plants and seeds. Data were statistically analyzed with the Info Stat software. **Results:** In this study, evidence suggesting that ubiquitin fold modifier 1 is involved in the normal development of the seeds. **Conclusion:** The *ufm1* gene would affect the normal development of the seed, particularly of the embryo, causing high percentage of seed abortion.

Key words: UBLs, embryo, endosperm, seed abortion, development, cell cycle, post translational modifications, embryogenesis

Received: May 03, 2016 Accepted: September 18, 2016 Published: December 15, 2016

Citation: Cornejo Paula and Masuelli Ricardo Williams, 2017. Phenotypic characterization of the *Arabidopsis ufm1* (Ubiquitin fold modifier) gene involved in seed development. Am. J. Plant Physiol., 12: 10-19.

Corresponding Author: P. Cornejo, Laboratorio de Biología Molecular, Instituto de Biología Agrícola de Mendoza (IBAM), Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, A. Brown 500 (M5528AHB) Chacras de Coria, Mendoza, Argentina Tel: 0549-0261-4135000-1232

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Post-translational modifications to proteins are one of the principal components of the organization and regulation of function and cell structure as well as the development of organism, refer to covalent and generally enzymatic modification of proteins during or after its biosynthesis. The protein modification systems produce a functional change in target proteins. The 1st protein modifier system discovered was ubiquitin (Ub). Ubiquitin is a highly conserved 76 amino acid polypeptide ubiquitously present in eukaryotes, from yeast to human[1-3]. It is covalently ligated to a wide variety of target proteins through the action of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein isopeptide ligase (E3)[3-5]. This addition can be reversed by the action of deubiquitylating enzymes (DUBs)[6]. Ubiquitin can participate in a wide variety of cellular processes like protein degradation pathways, histone regulation, transcriptional regulation, RNA metabolism, chromatin remodeling and cell cycle control. Besides ubiquitin, a number of other ubiquitin like proteins (UBLs) have been identified[7-12]. These proteins do not necessarily share a high similarity to ubiquitin, but are structurally related and can be conjugated to target proteins in a similar manner than ubiquitin[2,13-16].

The most recently identified ubiquitin like protein, *ufm1* (ubiquitin fold modifier 1), shares only 14% sequence identity with the ubiquitin proteins family but adopts the typical β-grasp fold[17]. The *ufm1* is synthesized as a precursor protein and is activated by an E1-like protein, *Uba5*, while conjugation is conducted by an E2-Like protein, *ufc1*[18]. Recently, an E3-Like ligase protein termed *ufl1* has been identified[19]. Finally specific *ufsps* can also cleave *ufm1* from its target proteins[20,21]. Cascade of *ufm1* is conserved among nearly all the eukaryotic organisms except yeast[18]. Studies on mammalian cells showed that the only target of *ufm1* identified, until now is the *ufbp1* protein and suggested a limited number of substrates[21,22]. Mutant mice of *Uba5* -/- are embryonic lethal[23] and cultured human cells silenced by *ufm1* and *ufl1* showed accelerated apoptosis[22]. Until now, there are no studies on the role of *ufm1* in plant development. The aim of this study is conduct a functional analysis to understanding the role of *ufm1* gene in plants. The phenotypic analysis described here, in conjunction with gene expression profiling, suggest that *ufm1* protein has a fundamental role in development of plant primordial in seed, particularly embryo development.

MATERIALS AND METHODS

Plant material and growth conditions: Plants of *Arabidopsis thaliana* were derived from the Columbia-0 (Col-0) mutant lines *ufm1* (SALK-040955C), locus AT1G77710, obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH), from de SALK T-DNA insertion collection[24]. Surface-sterilized seeds were placed in petri dishes containing 15 mL of agar 0.8% w/v. Petri dishes were placed in a cold room for 3 days at 4°C to synchronize germination. Plates with cold stratified seeds were transferred into a room with day and night temperatures of 22 and 20°C, respectively. The average light intensity at the level of the rosette was ~65 μmol m^{-2} sec^{-1} supplied by fluorescent tubes. Seedlings were transferred to soil when 2 rosette leaves were greater than 1 mm in length. Plants were watered by sub irrigation every 2-3 days, depending on the growth stage. The plants, wild type and mutant were sown in 2 subgroups of 12 individuals each.

Phenotypic characterization: All plants were analyzed for the following phenotypic characteristics: Leaf morphology, rosette radius, flower morphology, length of major axis of silique and size of embryos. The phenotype of the mutant plants were compared to that of control plants during the different stages of growth.

All measurements were performed by analysis of digital images using AxioVision Rel. 4.8 software (Carl Zeiss).

Histology: For whole-mount preparations of embryos, silique were dissected with hypodermic needles. Dissected siliques were fixed on ice with FAA (10:7:2:1 ethanol:distilled water:acetic acid:formaldehyde 37%) for 30 min, hydrated in a graded ethanol series to 50 mM $NaPOH_4$ buffer, pH 7.2. Embryos were excised using fine forceps and surgical blades under a magnifying scope and mounted on a microscope slide in a clearing solution 8:2:1 chloral hydrate:distilled water:glycerol. Samples were cleared for 1 h at 4°C before inspection. Light microscope preparations were examined using a Zeiss Axioplan microscope (Jena, Germany). Pictures were taken with a Cannon PowerShot G11 camera and edited with Adobe Photoshop.

Tetrazolium staining: Seeds were imbibed for 2 h and embryos were excised using fine forceps and surgical blades

under a magnifying scope. Embryos were subjected to staining in 0.5% w/v 2, 3, 5-triphenyl tetrazolium chloride solution.

Germination assay: Seed lots were harvested from individual plants grown under identical conditions. Each plant population was sown in 5 petri dishes (20 seeds from each individual plant per petri dish) on agar and incubated in a climate-controlled room. Germination was measured 5 days after imbibition and was expressed as the percentage of plants with emerged roots in each plant population (germination percentage).

Quantification of DNA: Excised embryos were stained in the dark with DAPI dihydrochloride (Invitrogen, San Diego, Calif) 300 nM. Images were taken in a fluorescence Zeiss Axioplan microscope (Jena, Germany) with 358 nm excitation and 461 nm emission wavelengths, recorded with Cannon Power Shot G11 camera and processed using Image J.

RNA extraction: Siliques of mutant *ufm1* and wild type plants were dissected to isolate the seeds. Total RNA was extracted from a pool of seeds per plant using the TRIZOL reagent (Invitrogen GIBCO/Life Technologies, Grand Island, NY) according to the protocol by Meng and Feldman[25].

Semiquantitative reverse transcription (RT)-PCR analysis: Equal amounts of RNA from seeds were treated with DNase and reverse transcribed using reverse transcriptase M-MLV (Invitrogen, San Diego, Calif) as indicated by the manufacturer. The cDNA was amplified with specific primers designed based on the sequence of *ufm1* transcript of *Arabidopsis thaliana* (forward primer, CGTTGGTATTATCTCTGGTTTCAC, reverse primer, CAACTCACTTCAATTGTGACGA) and specific primers of the constitutive control gene action (forward primer, CATCAGGAAGGACTTGTACGG, reverse primer, GATGGACCTGACTCGTCATAC). As template, 40 ng of cDNA were used in a 15 μL reaction volume that contained 20 mmol L^{-1} tris-HCl, pH 8.4, 50 mmol L^{-1} KCl, 1.5 mmol L^{-1} $MgCl_2$, 300 μmol L^{-1} of each dNTP, 0.4 μmol L^{-1} of each primer and 0.4 U of Taq polymerase (Invitrogen, San Diego, Calif.). The thermocycler (PTC-100 MJ Research, Watertown, Mass.) was programmed for an initial denaturalization step of 94°C for 1 min and then 35 cycles of 94°C for 30 sec, 51°C for 1 min and 72°C for 2 min. The PCR products were separated in a 1.5% agarose gel and stained with ethidium bromide. The stained gel was photographed with the Molecular Analyst Gel Photo documentation System (Bio-Rad, Calif.) and analyzed with the Image J software. The quantity of each PCR product was expressed as the ratio of the sample densitometry measurement to the corresponding internal standard (action). To evaluate the changes in relative mRNA levels, densitometry values for the target genes were normalized against those of the housekeeping action mRNA. Data were statistically analyzed with the Info Stat software (Córdoba, Argentina).

RESULTS

Phenotypic characterization of a *ufm1* mutant: To analyze *ufm1* function, a T-DNA insertion homozygous mutant of *Arabidopsis thaliana* (SALK 040955C) and wild-type plants were used. Analyzed growth stages included the development of the plant from seed through the completion of flowering and seed maturation. Figure 1 and 2 shows the vegetative growth of mutant and wild-type plants was not statistically different. Flower morphology of the mutant was normal. However, silique of the homozygous mutant plants contained approximately 75% of aborted seeds (Fig. 3).

Based on these findings, embryo development at different stages was investigated. In the mutants, the aborted seeds had embryos arrested at globular or torpedo stages. The rest of the mutant seeds presented embryos with delayed development respect to the wild type. At 6 Days After Pollination (DAP), mutant embryos were at globular, heart or torpedo stage, while wild type embryos were at torpedo stage. Between 8-12 DAP, the growth of the mutant embryos was retarded respect to the wild type. Mutant embryos at 8 DAP were arrested at heart or torpedo stage while the wild type was at cotyledon stage. At 12 DAP viable mutant seeds presented embryos at cotyledonal stage with several abnormalities, like enlarged cells and nuclei, disorganized cells in the tissue and flatten and smaller cotyledons. The mature embryos were significantly smaller than the wild type (Fig. 4a, b).

Germination: Tetrazolium staining showed that the mutant embryos had reduced germination potential, showing a reduce coloration and only morphologically mature embryos exhibited full staining by tetrazolium in wild type plants. Consistent with the viability of the embryos

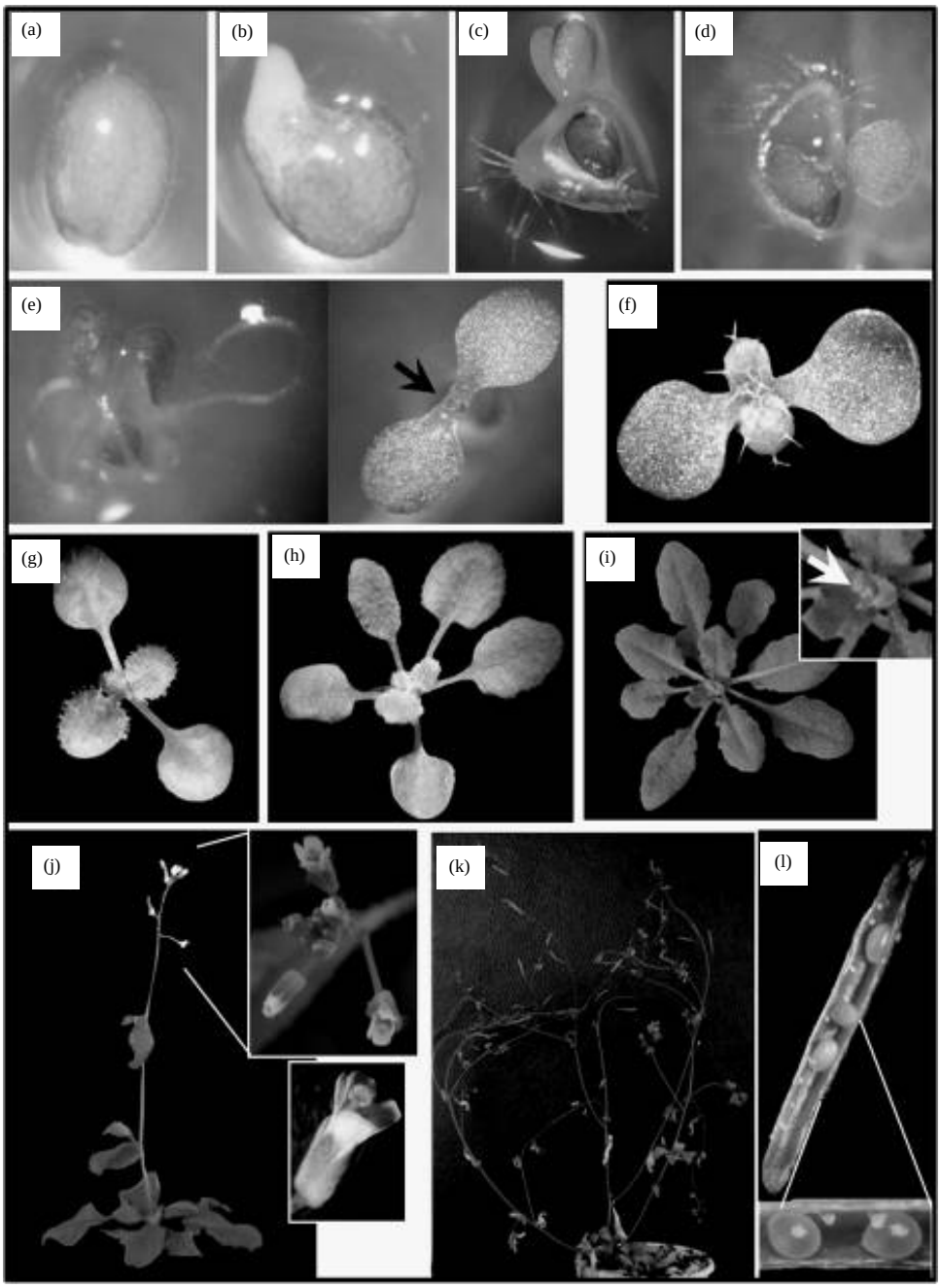

Fig. 1(a-l): Representative mutant growth stages, (a) Imbibition, (b) Radicle emergence, (c) Hypocotyl and cotyledons emerged from seed coat, (d, e) Fully opened cotyledons, (f) Two rosette leaves of 1 mm in length, (g) Four rosette leaves of 1 mm in length, (h) Ten rosette leaves of 1 mm in length, (i) First visible flower buds (indicated by arrow), (j) First open flowers, (k) Flowering complete and (l) Mature silique

using the tetrazolium staining method the seed germination percentage was significantly lower in the mutant compared to the wild type (Fig. 5).

Embryo DNA content: The DNA content in nuclei of wild type and mutant embryos was estimated by considering intensity of the DAPI staining. The signal was 1.7 times stronger in the

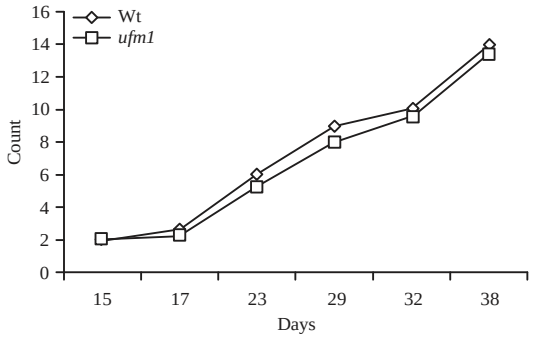

Fig. 2: Representative pdata of vegetative parts of wild-type and mutant plants, maximum rosette radius, length of the longest leaf over time and number of rosette leaves >1 mm in length over time. Data are given as average±SE for 24 individual plants. Days including a 3 day stratification at 4°C to synchronize seed germination. No significant differences were observed ($p<0.01$) between wild type and mutant plants

mutant embryos (45929 U per nucleus±1312,8, n = 60) compared with wild type embryos (25883 U per nucleus±1468,7, n = 60) (Fig. 6a, b).

Gene expression analysis: To verify the expression patterns, specific primers were designed to amplify the gene *ufm1*. The RT-PCR expression analysis was carried out on mature seeds of *ufm1* mutants and wild type plants. Figure 7 shows the results indicate that *ufm1* was expressed in seed of the wild type plants, but its expression was null in seeds of the mutant plants, which were characterized by the embryo retarded growth and seed abortion.

DISCUSSION

Post-translational modification by ubiquitin-like proteins (UBLs) plays a critical role in controlling stress responses, signaling, plant growth, cell cycle, transcription,

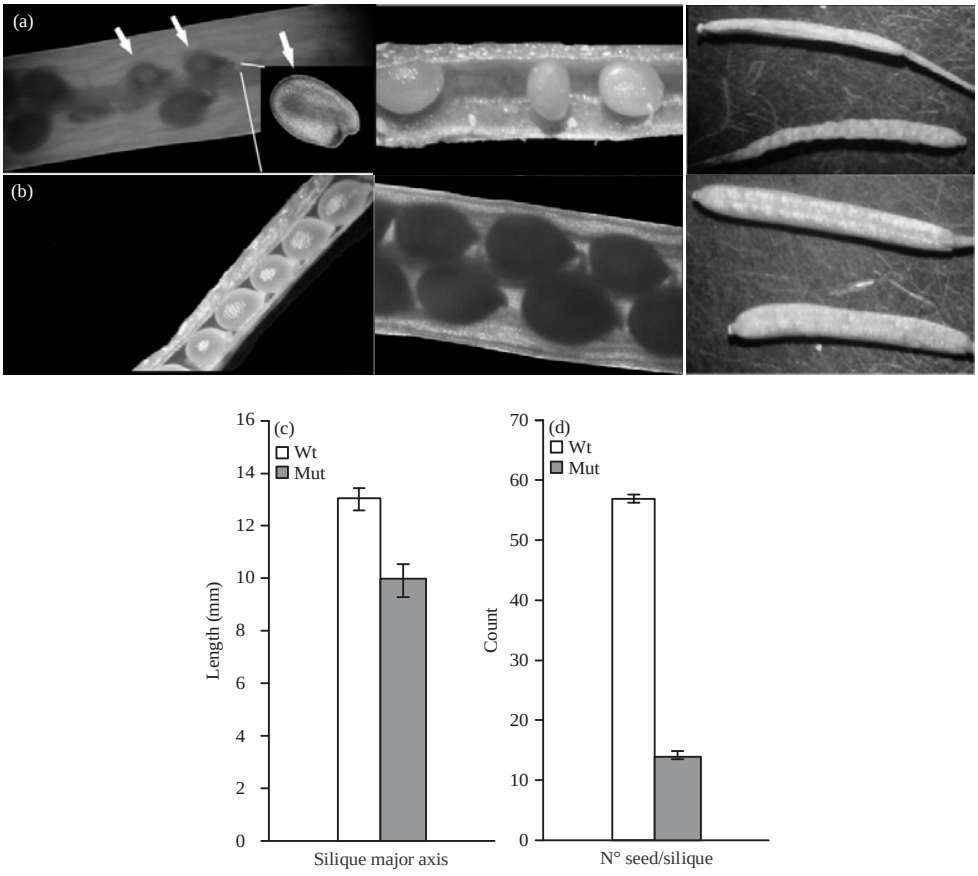

Fig. 3(a-d): Comparative analysis of siliques, (a) Siliques of homozygous *ufm1* mutant plants with early aborted seeds (arrows), (b) Siliques of wild type plants, (c) Comparison of major axis of siliques between wild type and mutant plants. Data were average from three siliques per plant and (d) Comparison of seeds per silique between wild type and mutant. The number of seeds was observed in mature siliques. Data were average from three siliques per plant. Significant differences were observed ($p<0.01$). Error bars indicate ±SE

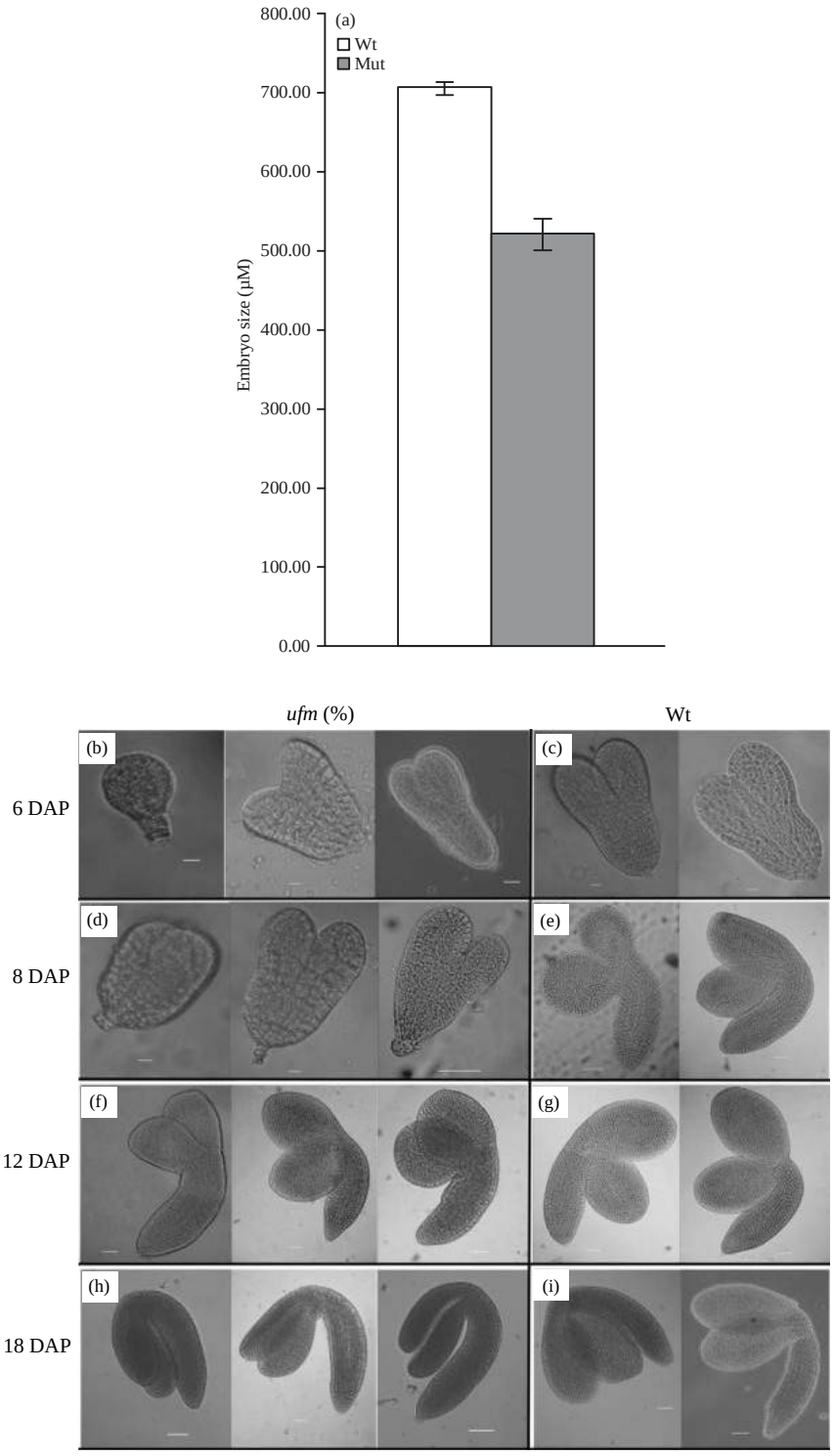

Fig. 4(a-i): Comparative analysis of embryos, (a) Comparative wild type and mutant embryo size. Significant differences were observed ($p<0.01$). Error bars indicate ±SE, (b-i) Image of cleared embryos of wild type and mutant seeds, (b) Embryos of the mutant to 6 DAP arrested at globular, heart and torpedo stage, (c) Wild type embryos to 6 DAP are uniformly developed at torpedo stage, (d) Mutant embryos to 8 DAP, (e) Wild type embryos to 8 DAP, (f) Mutant embryos to 12 DAP, (g) Wild type embryos to 12 DAP are mature, (h) Mutant mature embryos to 18 DAP present abnormalities and (i) Wild type embryos to 18 DAP are normal and mature

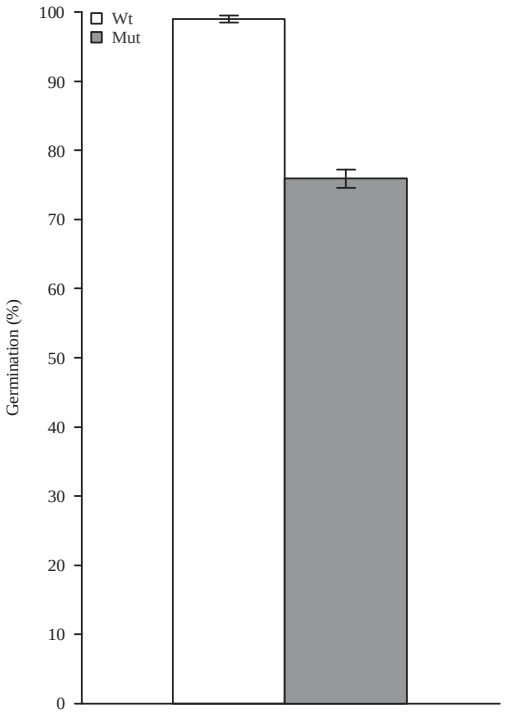

Fig. 5: Germination assay of mutant and wild type seeds. Germination was scored 5 days after imbibition. Significant differences were observed ($p<0.01$). Error bars indicate ±SE

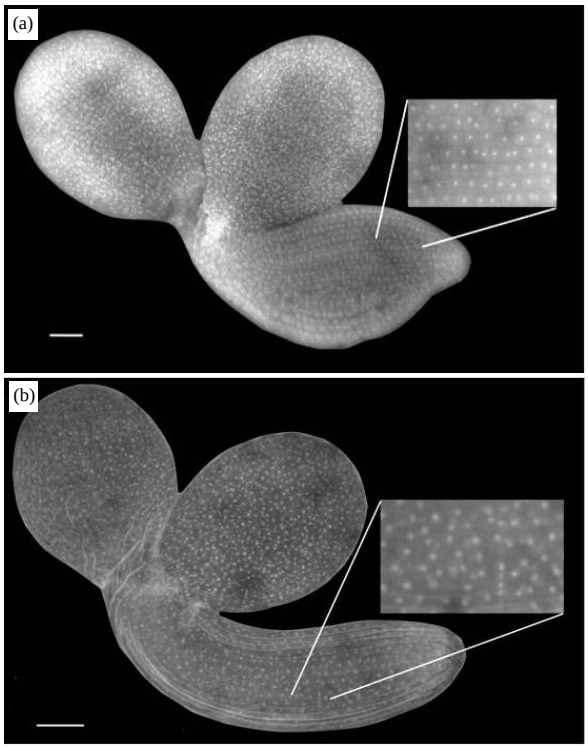

Fig. 6(a-b): Analysis of nuclear DNA content in wild type and mutant embryos. The DAPI stained nuclei in embryos were recorded by fluorescence microscopy and their nuclear DNA, defined as total pixel intensities of DAPI signals per individual nucleus, was quantified using Image J (a) Wild type embryo and (b) Mutant embryo *ufm1*. Bar = 50 μm. The horizontal lengths of the rectangle zoom section correspond to 50 μm

abnormalities and show reduced viability. A possible embryogenesis, circadian rhythms and other pathways[3,14,26-29]. Recently, the ubiquitin fold modifier 1 (*ufm1*) pathway described in mammalian cells, involves E1 activating (*Uba5*), E2 conjugating (*ufc1*) and E3 ligating (*ufl1*) enzymes, which will conjugate *ufm1* to targets both *in vitro* and *in vivo*[18,19]. The abnormal *ufm1* cascade is associated with many human diseases including cancer, ischemic heart diseases, diabetes, atherosclerosis, hip dysplasia and schizophrenia[30]. Studies on mutant mice of the enzyme *uba5* resulted lethal for the embryos. It was demonstrated that *Uba5* was indispensable for erythroid differentiation[23]. Also, cultured human cells silenced for *ufm1* and *ufl1* showed accelerated apoptosis[22].

Several genes have an essential role in plants life cycle, the absence of a functional gene product becomes critical or lethal. The distinction between embryo and gametophyte mutants is subjective. Some mutants with defects in embryos may also have defects in the gametophyte development[31]. Plant embryogenesis involves a strict synchronization of cell division, elongation and differentiation[32].

An important objective in *Arabidopsis* research is to identify minimal number of genes necessary to have a viable plant under normal growth conditions[33]. To understand the molecular bases of the embryonic development in plants, in the present study, an evidence was provided on the *ufm1* gene function and seed phenotypes. The RT-PCR analysis showed that *ufm1* is expressed in the seeds of the wild type plants and the expression is suppressed in the mutants. The use of null mutants revealed that *ufm1* is involved in the normal development of the *Arabidopsis* embryo.

To understand the role of *ufm1* in plants, reverse genetics to search for changes in phenotype of plants at different growth stages, morphology and DNA content in the nucleus of embryos in wild type (ecotype Col-0) and homozygous mutant (SALK 040955C) lines was used. Interestingly, phenotypic analysis reveal no significant differences in the vegetative parts but significant differences were observed in the number of viable seeds and size of the embryos, the mutant embryos are smaller and the number of seeds per

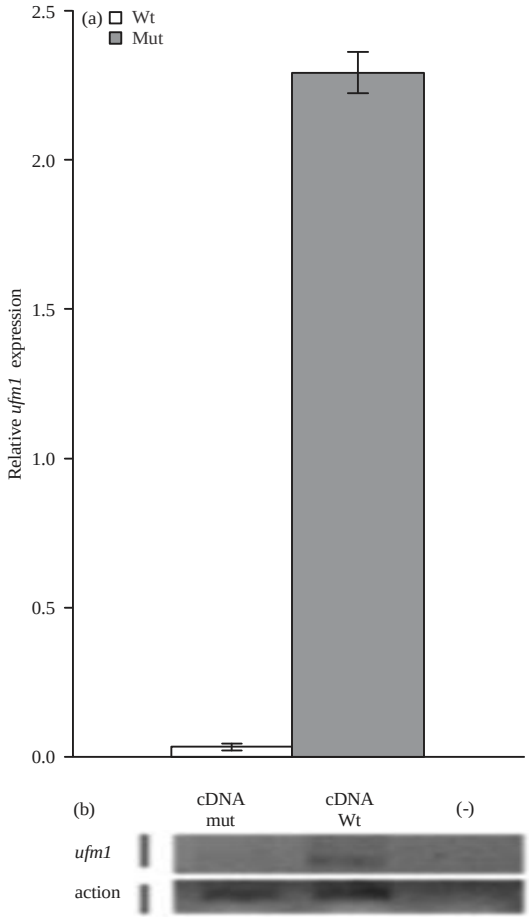

Fig. 7(a-b): RT-PCR analysis of mutant and wild type seeds of *Arabidopsis thaliana*, (a) Relative expression level of *ufm1* gene and (b) The wild type seeds amplified the expected product of 506 bp. The actin gene was used as a positive control

silique is lower than the wild type. Also was observed that mutant embryos have delayed development, present abnormalities and show reduced viability. A possible explanation for how some embryo defective mutant survive and develop normally, to complete their life cycle, involve the presence of genes with redundant function.

The results showed an increment in size and DNA content of embryo nuclei in *ufm1* mutants, denoting an alteration in the cell cycle and cellularization possibly by alteration of the ubiquitin pathway. Similar results, although more severe, were obtained in TTN6 seed mutants in *Arabidopsis*[33,34] and in seeds of incompatible crosses in potato[35-38]. This observation establishes an essential role for *ufm1* in plant development, demonstrating that *ufm1* is primordial for plant embryo genesis as observed in mammals[23,39,40]. Further studies of *ufm1* interactions and pathway should clarify the molecular basis for the phenotypes resulting in mutant seeds.

CONCLUSION

It is observed that mutant embryos, which do not express *ufm1* have delayed development, present abnormalities and show reduced viability, relative to control. This is the 1st report to establish the role of *ufm1* in plant embryogenesis. These results are important because they contribute to the understanding of the mechanisms involved in seed development.

SIGNIFICANCE STATEMENT

Little is known about the role of the *ufm1* gene in plants. This study sets the role played by the *ufm1* gene in plant development, remains essential in embryogenesis of plants as well as in mammalian.

ACKNOWLEDGMENTS

This study was funded by Agencia Nacional de Promoción Científica y Tecnológica, Argentina (ANPCyT) [BID 1201-OC-AR PICT-2007-01294]. We are greatful with Carina González member of the laboratory of deceased H. Boccalandro for helpful device and for plant material.

REFERENCES

1. Hershko, A. and A. Ciechanover, 1998. The ubiquitin system. Annu. Rev. Biochem., 67: 425-479.
2. Smalle, J. and R.D. Vierstra, 2004. The ubiquitin 26S proteasome proteolytic pathway. Annu. Rev. Plant Biol., 55: 555-590.
3. Vierstra, R.D., 2009. The ubiquitin-26S proteasome system at the nexus of plant biology. Nat. Rev. Mol. Cell. Biol., 10: 385-397.
4. Vierstra, R.D., 2012. The expanding universe of ubiquitin and ubiquitin-like modifiers. Plant Physiol., 160: 2-14.
5. Herhaus L. and I. Dikic, 2015. Expanding the ubiquitin code through post translational modification. EMBO Rep., 16: 1071-1083.
6. Sahtoe, D.D. and T.K. Sixma, 2015. Layers of DUB regulation. Trends Biochem. Sci., 40: 456-467.
7. Burroughs, A.M., L.M. Iyer and L. Aravind, 2012. The natural history of ubiquitin and ubiquitin-related domains. Front. Biosci., 17: 1433-1460.

8. Callis, J., T. Carpenter, C.W. Sun and R.D. Vierstra, 1995. Structure and evolution of genes encoding polyubiquitin and ubiquitin-like proteins in Arabidopsis thaliana ecotype Columbia. Genetics, 139: 921-939.
9. Downes, B.P., S.A. Saracco, S.S. Lee, D.N. Crowell and R.D. Vierstra, 2006. MUBs, a family of ubiquitin-fold proteins that are plasma membrane-anchored by prenylation. J. Biol. Chem., 281: 27145-27157.
10. Iwai, K. and F. Tokunaga, 2009. Linear polyubiquitination: A new regulator of NF κB activation. EMBO Rep., 10: 706-713.
11. Matunis, M.J., E. Coutava and G. Blobel, 1996. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J. Cell Biol., 135: 1457-1470.
12. Ohsumi, Y., 2001. Molecular dissection of autophagy: Two ubiquitin-like systems. Nat. Rev. Mol. Cell Biol., 2: 211-216.
13. Downes, B. and R.D. Vierstra, 2005. Post-translational regulation in plants employing a diverse set of polypeptide tags. Biochem. Soc. Trans., 33: 393-399.
14. Hochstrasser, M., 2009. Origin and function of ubiquitin-like proteins. Nature, 458: 422-429.
15. Kerscher, O., R. Felberbaum and M. Hochstrasser, 2006. Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu. Rev. Cell. Dev. Biol., 22: 159-180.
16. Shimizu, Y., Y. Okuda-Shimizu and L.M. Hendershot, 2010. Ubiquitylation of an ERAD substrate occurs on multiple types of amino acids. Mol. Cell, 40: 917-926.
17. Sasakawa, H., E. Sakata, Y. Yamaguchi, M. Komatsu and K. Tatsumi *et al*., 2006. Solution structure and dynamics of Ufm1, a ubiquitin-fold modifier 1. Biochem. Biophys. Res. Commun., 343: 21-26.
18. Komatsu, M., T. Chiba, K. Tatsumi, S.I. Iemura and I. Tanida *et al*., 2004. A novel protein conjugating system for Ufm1, a ubiquitin fold modifier. EMBO J., 23: 1977-1986.
19. Tatsumi, K., Y.S. Sou, N. Tada, E. Nakamura and S.I. Iemura *et al*., 2010. A novel type of E3 ligase for the Ufm1 conjugation system. J. Biol. Chem., 285: 5417-5427.
20. Daniel, J. and E. Liebau, 2014. The ufm1 cascade. Cells, 3: 627-638.
21. Kang, S.H., G.R. Kim, M. Seong, S.H. Baek and J.H. Seol *et al*., 2007. Two novel ubiquitin-fold modifier 1 (Ufm1)-specific proteases, UfSP1 and UfSP2. J. Biol. Chem., 282: 5256-5262.
22. Lemaire, K., R.F. Moura, M. Granvik, M. Igoillo-Esteve and H.E. Hohmeier *et al*., 2011. Ubiquitin fold modifier 1 (UFM1) and its target UFBP1 protect pancreatic beta cells from ER stress-induced apoptosis. PLoS ONE, Vol. 6. 10.1371/journal.pone.0018517.
23. Tatsumi, K., H. Yamamoto-Mukai, R. Shimizu, S. Waguri and Y.S. Sou *et al*., 2011. The Ufm1-activating enzyme Uba5 is indispensable for erythroid differentiation in mice. Nat. Commun., Vol. 2. 10.1038/ncomms1182
24. Alonso, J.M., A.N. Stepanova, T.J. Leisse, C.J. Kim and H. Chen *et al*., 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science, 301: 653-657.
25. Meng, L. and L. Feldman, 2010. A rapid TRIzol-based two-step method for DNA-free RNA extraction from *Arabidopsis* siliques and dry seeds. Biotechnol. J., 5: 183-186.
26. Li, F. and R.D. Vierstra, 2012. Autophagy: A multifaceted intracellular system for bulk and selective recycling. Trends Plant Sci., 17: 526-537.
27. Miura, K., J.B. Jin and P.M. Hasegawa, 2007. Sumoylation, a post-translational regulatory process in plants. Curr. Opin. Plant Biol., 10: 495-502.
28. Okumoto, K., S. Misono, N. Miyata, Y. Matsumoto, S. Mukai and Y. Fujiki, 2011. Cysteine ubiquitination of PTS1 receptor Pex5p regulates Pex5p recycling. Traffic, 12: 1067-1083.
29. Tompa, P., N.E. Davey, T.J. Gibson and M.M. Babu, 2014. A million peptide motifs for the molecular biologist. Mol. Cell, 55: 161-169.
30. Yoo, H.M., S.H. Kang, J.Y. Kim, J.E. Lee and M.W. Seong *et al*., 2014. Modification of ASC1 by UFM1 is crucial for ERα transactivation and breast cancer development. Mol. Cell, 56: 261-274.
31. Muralla, R., J. Lloyd and D. Meinke, 2011. Molecular foundations of reproductive lethality in *Arabidopsis thaliana*. PLoS One, Vol. 6. 10.1371/journal.pone.0028398.
32. Meinke, D.W., 1994. Seed Development in *Arabidopsis Thaliana*. In: *Arabidopsis*, Meyerowitz, E.M. and C.R. Somerville (Eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., pp: 253-295.
33. Tzafrir, I., R. Pena-Muralla, A. Dickerman, M. Berg and R. Rogers *et al*., 2004. Identification of genes required for embryo development in *Arabidopsis*. Plant Physiol., 135: 1206-1220.
34. Tzafrir, I., J.A. McElver, C.M. Liu, L.J. Yang and J.Q. Wu *et al*., 2002. Diversity of TITAN functions in *Arabidopsis* seed development. Plant Physiol., 128: 38-51.
35. Cornejo, P., E.L. Camadro and R.W. Masuelli, 2006. The importance of *Arabidopsis* seed mutants in the elucidation of the molecular basis of Endosperm Balance Number in tuber-bearing *Solanum* species. Biocell, 30: 413-421.
36. Cornejo, P., E.L. Camadro and R.W. Masuelli, 2012. Molecular bases of the postzygotic barriers in interspecific crosses between the wild potato species *Solanum acaule* and *Solanum commersonii*. Genome, 55: 605-614.
37. Masuelli, R.W. and E.L. Camadro, 1997. Crossability relationships among wild potato species with different ploidies and Endosperm Balance Numbers (EBN). Euphytica, 94: 227-235.

38. Masuelli, R.W., 2001. Mitotic-cycle time and the development of embryo and endosperm in compatible and incompatible crosses in tuber-bearing *Solanum* species. Genome, 44: 426-431.
39. Zhang, M., X. Zhu, Y. Zhang, Y. Cai and J. Chen *et al*., 2015. RCAD/Ufl1, a Ufm1 E3 ligase, is essential for hematopoietic stem cell function and murine hematopoiesis. Cell Death Differ., 22: 1922-1934.
40. Cai, Y., W. Pi, S. Sivaprakasam, X. Zhu and M. Zhang *et al*., 2015. UFBP1, a key component of the Ufm1 conjugation system, is essential for ufmylation-mediated regulation of erythroid development. PLoS Genet. 10.1371/journal.pgen.1005643.