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The importance of *Arabidopsis* seed mutants in the elucidation of the molecular basis of Endosperm Balance Number in tuber-bearing *Solanum* species

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ABSTRACT: The Endosperm Balance Number (EBN) is an important concept for potato breeding and has evolutionary importance in tuber-bearing *Solanum* species. The EBN is part of the post-zygotic hybridization barriers in the group and represents a reproductive isolating mechanism. Few genes have been proposed to be involved in its genetic control; until now, however, neither specific genes nor its molecular basis have been well established. Histological observations of embryo and endosperm development in inter-EBN crosses in tuber-bearing *Solanum* revealed phenotypes similar to those recently described in *Arabidopsis* seed mutants. The common feature between them is that the endosperm nuclei become greatly enlarged and that embryos are arrested at the globular stage. The proteins encoded by the *Arabidopsis TITAN* genes are related to chromosome dynamics and cell division. Based on the sequence of *titan* mutants, genes in potato species related to cell cycle and microtubule assembly were isolated. In this article a perspective model is proposed to explore the utility of *Arabidopsis* mutants associated with cell cycle control as a tool to elucidate the molecular basis of EBN in potato. Further research focused on the expression pattern of these genes in intra- and inter-EBN crosses in potato species will be performed.

Introduction

The endosperm is a very singular tissue of the seed that supports the development of the embryo and is viewed as a fundamental component in the evolutionary success of the Angiosperms. It results from the fusion of a haploid sperm nucleus and the two haploid nuclei of the central cell in the embryo sac. Failure in the development of the endosperm results in embryo abortion. In potato and other related tuber-bearing *Solanum* species, hybridization barriers at the endosperm level act as an effective force in speciation. As Birchler (1993) has pointed out, mutations in genes that cause endosperm failure will result in reproductive isolating mechanisms.

Several hypotheses have been put forward to explain abnormal seed development in interploid and interspecific crosses in Angiosperms. These hypotheses go from the proportion of genomes in (a) the three tissues that compose the seed: maternal, embryo and endosperm (Müntzing, 1933), (b) endosperm and embryo (Watkins, 1932) and (c) endosperm, of maternal and paternal origin (Nishiyama and Inomata, 1966), to the proportion of qualitative factors in the endosperm con-

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tributed by the male and the female parents (Nishiyama and Yabuno, 1978; Johnston *et al*., 1980). The overgrowth of the maternal tissue or "somatoplastic sterlity" (Brink and Cooper, 1947) and the quantitative balance between nucleus and cytoplasm (von Wangenheim, 1957) have also been considered. Most authors agree that the 2:1 ratio of maternal to paternal genomes in the endosperm is crucial for normal development of this tissue; the endosperm collapses, therefore, when this ratio is disturbed (Katsiotis *et al*., 1995). This concept was clarified by Lin (1984) in diploid maize, by generating endosperms with different ploidy levels (2x to 8x) using an indeterminate gametophyte (ig) mutant -that displays various incomplete penetrance irregularities in seed formation- as the female parent. Invariably, endosperm developed normally when the maternal:paternal genome ratio was 2:1.

All crossing results observed in the tuber-bearing *Solanum* species were not supported by the preceding hypothesis. Thus, the Endosperm Balance Number (EBN) hypothesis was proposed by Johnston *et al*. (1980). According to this hypothesis, normal seed will develop when the maternal to paternal dosage of hypothetical genetic factors in the endosperm reaches a 2:1 ratio. Each species is assigned an EBN or "effective" ploidy on the basis of its crossing behavior in pollen-pistil compatible crosses with a species taken as a standard. Although most diploid species have been assigned 2EBN and most tetraploid species have been assigned 4EBN, some diploid species (i.e. *S. commersonii*) behave as having 1EBN and some tetraploid species (i.e. *S. acaule*) as having 2EBN, whereas hexaploids (i.e. *S. oplocense*) behave as having 4 EBN. Crosses are compatible at the endosperm level between species with the same EBN, but not between species with different EBN. More recently, a good correlation was found between the EBN and the postulated evolution of the species in the group (Hawkes and Jackson, 1992).

Several efforts were made to try to understand the genetic bases of EBN in potatoes. Ehlenfeldt and Hanneman (1988) proposed a genetic model with three unlinked genes that operate in a threshold-like system. Two independent loci with two alleles in homozygosity were proposed by Camadro and Masuelli (1995) to explain the behavior of *Solanum acaule* in intra- and inter-EBN crosses. Neither a single chromosome nor a gene involved in the determination of EBN were found using trisomic and induced mutation analyses (Johnston and Hanneman, 1996). In maize, more than two genes have been involved in the EBN genetic control and the cause of endosperm failure was proposed to be a consequence of a gene regulatory system (Birchler, 1993). Due to methodological problems, the molecular bases underlying the EBN hypothesis have not been characterized.

The aim of this article is to discuss the last findings obtained recently using *Arabidopsis* seed development mutants and to propose a perspective model to be used in the elucidation of the molecular basis of EBN in potatoes. Preliminary results are also presented.

Materials and Methods

Seeds of *Solanum commersonii* (cmm, 2n=2x=24), *S. acaule* (acl, 2n=4x=48) and *S. gourlayi* (grl; 2n=2x=24) were provided by the Potato Germplasm Bank of the Agriculture Experimental Station (E.E.A.) Balcarce, National Institute for Agriculture Technology (INTA), Argentina.

Seeds were sown in Petri dishes and seedlings were transplanted into pots in a screenhouse. Controlled crosses were performed one or two days before anthesis, on a buds previously emasculated. Pollinated pistils were fixed in FAA (8:1:1 v/v ethanol:glacial acetic acid: 40% formaldehyde) for 24 hr, at intervals of 3, 5, 7 and 9 days after pollinization. Fixed ovaries were dehydrated through a tertiary butyl alcohol series, embebded in paraffin, cut into 10 μm section with microtome and stained following a safranin-fast green series (Sass, 1958).

Gene amplification and sequencing

DNA was extracted from leaves according to Dellaporta *et al*. (1983). After spectrophotometric measurement of DNA concentration (GeneQuant RNA/ DNA Calculator, Pharmacia Biotech), DNA was diluted in 1x TE buffer to 100 ng μl^{-1} for use in degenerate PCR. The upstream primer 5′-TGGGACTGCTGA CTATTATTAAAAAGATNAARMARA-3′ and the downstream primer 5′-AGTCAGAGCTCCTGGAA TATCTTGYTTRTTNGC-3′ were used to amplify the *Arabidopsis* mutant *ttn5* orthologous gene in cmm and acl. Between 10 and 20 ng of DNA was used as a template in a 20 μl reaction volume that contained 10mM Tris-HCl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Tween-20, 0.01% Triton X-100, 0.2 μM of each primer, 100 μM of each dNTP and 1 unit of Taq DNA Polymerase (Promega, Madison, Wis.). Amplifications were performed in a PTC-100 MJ Research (Watertown, Mass.) thermocycler, programmed for a first denatur-

ation step at 94ºC for 3 min followed by 30 cycles at 94ºC for 3 min, 48ºC for 1 min, and 72ºC for 2 min. After completion of the 30 cycles, the reactions were kept at 72ºC for 5 min and then held at 4ºC until the tubes were removed. PCR products were separated on a 1.2% agarose gel and stained with ethidium bromide. Species-specific PCR fragments were eluted from the gel and were re-amplified with the appropriate primers and finally cloned into pGEM-T Easy vector (Promega, Madison, WS) and sequence. Further analysis of DNA similarity was performed using the BLAST program searching the TIGR Potato (*Solanum tuberosum*) database.

Accession numbers

The accession numbers for the sequences described in this article are DQ975206 (*S. acaule* genomic DNA) and DQ975207 (*S. commersonii* genomic DNA).

Results and Discussion

Intraspecific, interspecific and interploidy crosses in Solanum and the EBN hypothesis

The EBN is a unifying concept for predicting endosperm function in intraspecific and interspecific crosses between and within ploidy levels (Ortiz and Ehlenfeldt, 1992). Crosses between species with the same EBN (intra-EBN crosses) and those with different EBN (inter-EBN crosses) are, respectively, compatible and incompatible at the endosperm level notwithstanding the functioning of gametes with unexpected chromosome numbers (i.e. 2n gametes). The genetic control of the EBN should be similar in interploidy intraspecific and interploidy interspecific crosses. The difference between both types of crosses is that in interploid intraspecific crosses, i.e. *S. gourlayi* (2x, 2EBN) x *S. gourlayi* (4x, 4EBN), only a dosage effect is operating. However, in interploidy interspecific crosses, i.e. *S. acaule* (4x, 2EBN) x *S. commersonii* (2x, 1EBN), two types of effects are operating, dosage and hybridization effects. In some interspecific crosses in *Solanum,* a gradation of crossabilities was observed among species and genotypes within species. Thus, the EBN appeared to be insufficient to explain the crossability relationship between species and it was suggested that it was part of a more complex system of interspecific barriers (Masuelli and Camadro, 1997).

The EBN hypothesis is stressed to explain two different phenomenons: dosage effect and, in interspecific crosses, post-zygotic incompatibility barriers, rendering the genetic and molecular analyses very difficult. A strategy is clearly needed to differentiate these two phenomena. However, imbalance between maternal and paternal genomes in *Solanum* hybrids can sometimes be corrected by a change in the numerical ploidy of one of the parents (Johnston *et al*., 1980). These results suggest that if several genes are involved in the EBN they might be acting in the same "developmental pathway". This is the only way to explain the restoration of the balance by a change in ploidy.

According to Dilkes and Comai (2004), similarities between interploidy crosses and interspecific hybridizations will be better explained by differential expression of dosage-sensitive genes. They propose a model in which differentially contributed dosage-sensitive gene products interact to produce a viable endosperm. Moreover, Josefsson *et al*. (2006) recently found that in incompatible *A. thaliana* x *A. arenosa* crosses three Polycomb-regulated genes (*PHERES1*, *MEIDOS* and *MEDEA*) were induced from the paternal genome and the rate of hybrid seed lethality was sensitive to parental genome dosage.

Interploidy crosses and parent-of-origin effects

The abnormal development of the seed in interploidy crosses has been investigated in *Arabidopsis* using diploids and their autopolyploids. In crosses 2x x 4x, 2x x 6x and their reciprocals, a dramatic effect on seed growth was observed when the paternal genome double the dose of the maternal genome (Scott *et al*., 1998). The endosperm showed accelerated mitotic times and delayed cellularisation. On the other hand, seeds with maternal excess were smaller and showed reduced endosperm mitosis. A possibly explanation for these results is that the dosage effect observed in interploidy crosses is produced by expressed imprinted loci affecting endosperm development. To test this hypothesis Adams *et al*. (2000) crossed hypomethylated and wild type diploid plants. The phenotypes observed were similar to the crosses between plants with normal methylation but different ploidies. These results might be interpreted as follow, hypomethylation of one parental genome deregulates genes that are usually active in the other parent, emulating the effect of extra doses produced by interploidy crosses.

Evidence of parent-of-origin-effect in *Solanum* were obtained by Ehlenfeldt and Hanneman (1988) crossing a diploid F1 hybrid between *S. commersonii* (2x, 1EBN) and *S. chacoense* (2x, 2EBN). Crosses with

maternal excess, between *S. chacoense* (2 EBN) x F1 (1.5 EBN) and F1 (1.5 EBN) x *S. commersonii* (1 EBN) produced average to small sized seeds. Crosses in which the male parent had higher EBN value, *S. commersonii* (1 EBN) x F1 (1.5 EBN) and F1 (1.5 EBN) x *S. chacoense* (2 EBN) produced average to large sized

TABLE 1.

Embryos diameter of intra and inter-EBN crosses in *Solanum*.

Cross	Endosperm EBN ratio	D.A.P.[a]	Diameter of the embryo at globular stage (μm)			No. of embryo sac
			Average	SD[c]	Range	
Intra-EBN						
cmm x cmm[b]	2:1	15	109.5	20.4	80-132.5	5
acl x acl	2:1	9	67.5	18.3	37.5-85	9
acl x grl	2:1	11	70	6.32	60-75	6
Inter-EBN						
acl x cmm	2:1/2	11	34.7	5.4	27.5-45	9
cmm x grl	1:1	11	56.8	9.1	50-72.5	4

a. D.A.P. Days After Pollination.
b. cmm: *S. commersonii* (2x, 1EBN); acl: *S. acaule* (4x, 2EBN); grl: *S. gourlayi* (2x, 2EBN).
c. SD: Standard deviation

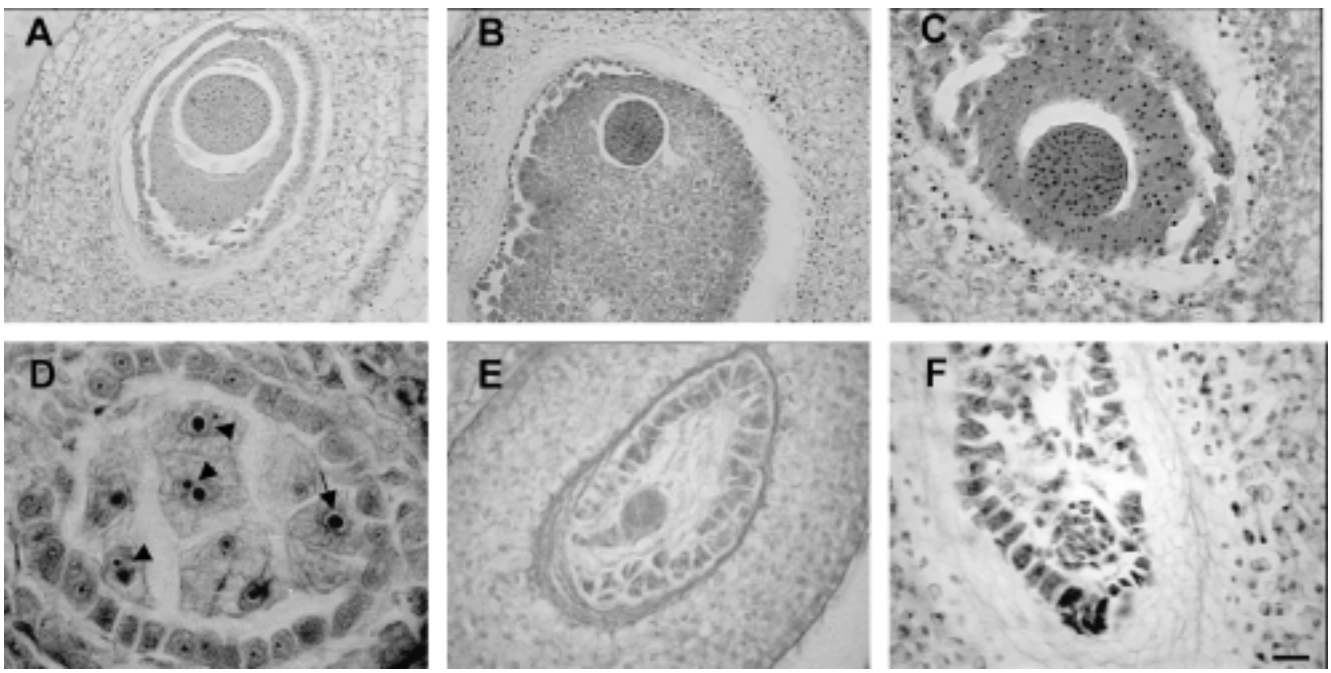

FIGURE 1. Embryo and endosperm phenotypes in intra- and inter-EBN crosses in potato. (A-C) Intra-EBN crosses. (A) *S. commersonii* (2x, 1EBN) crosses at 15 days after pollination (DAP) ; (B) *S. aca*ule (4x, 2EBN) crosses at 9 DAP; (C) *S. acaule* (4x, 2EBN) x *S. gourlayi* (2x, 2EBN) at 11 DAP. (D-F) Inter-EBN crosses.(D) *S. acaule* (4x, 2EBN) x *S. commersonii* (2x, 1 EBN) at 9 DAP, giant nuclei (arrow) and nuclei of different sizes (arrowheads) ; (E) *S. acaule* (4x, 2EBN) x *S. commersonii* (2x, 1EBN) at 11 DAP ; (F) *S. commersonii* (2x, 1 EBN) x *S. gourlayi* (2x, 2EBN) at 11 DAP. In E and F see the disintegration of embryo and endosperm tissue. Bar = 50 μm.

seeds. Haig and Westoby (1989) interpreted these results as a conflict of interest for allocation resources between maternally and paternally derived alleles. The authors propose the parental-conflict theory, in which the species evolve by generating a balance between the activity of the male genome in acquiring resources for its offspring and the activity of the female genome in inhibiting resource acquisition.

Arabidopsis seed development mutants and EBN phenotypes

Several genes involved in embryo and endosperm development and that also exhibit gametophytic maternal effects have been described in *Arabidopsis*. Mutations in two loci, Fertilization Independent Endosperm (*FIE*) and Fertilization Independent Seed (*FIS*), activate the onset of central cell proliferation and endosperm development prior to fertilization (Luo *et al*., 1999; Ohad *et al*., 1999). Recently, Nowack *et al*. (2006) showed that pollen mutation in the A-type cyclin-dependent kinases (CDK) called CDC2A in *Arabidopsis thaliana* produced a single sperm cell that fertilized exclusively the egg cell whereas the unfertilized endosperm developed autonomously. The authors concluded that the fertilized egg cell stimulated in some way the initiation of cell proliferation in the central cell. Mutants in other loci, such as Medea (*MEA*), resulted in embryo lethality with mutant embryos showing excess cell proliferation and containing extra cell layers. At the time the wild-type embryos reach the late heart stage, *mea* embryos are still at the globular stage and endosperm development is indistinguishable from that of the wild type at earlier stages (Grossniklaus *et al*., 1998). Similarly to the *mea* phenotype, in inter-EBN crosses in *Solanum* we observed that embryos developed until the globular stage but their diameters were always smaller than in intra-EBN crosses (Table 1 and Fig. 1). Differences between both types of crosses are at the level of integrity of embryo and endosperm tissues. The globular embryos and the endosperms of incompatible (inter-EBN) crosses showed disintegration and enucleated cells (Figs. 1 E and F). Therefore, as discussed for *Arabidopsis* by Scott and Vinkenoog (1998), it is likely that orthologous *MEA* genes in *Solanum* might be acting at the endosperm level with direct effects on embryogenesis.

The *titan* mutants described in *Arabidopsis* cause dramatic enlargement of endosperm nuclei (Liu and Meinke, 1998). The seed phenotype of each mutant depends on the locus involved: giant endosperm nuclei, and enlarged nuclei and embryo cells in *ttn1*, early embryo abortion in *ttn2*, enlarged endosperm nuclei and well developed embryos in *ttn3*. At the moment, a total of 17 *titan* mutants in nine different genes (*TTN1* to *TTN9*) have been described, encoding proteins related to: chromosome scaffold (*ttn3*), GTP-binding protein (*ttn5*), tubuling-folding cofactor D (*ttn1*) and deubiquitinating enzyme (*ttn6*). These proteins are related to the regulation of endosperm

TABLE 2.

Comparison of seed phenotypes of *titan* mutants in *Arabidopsis* and inter-EBN crosses in *Solanum*.

Seed phenotype	Arabidopsis mutants					Solanum Inter-EBN crosses[a]	
	mea-1	*titan 1,5*	*titan 2,7,8,9*	*titan 6*	*titan 3*	acl x cmm	cmm x grl
Endosperm							
Nuclear size	Normal	Giant	Giant	Giant	Giant	Giant	Normal
Number of nuclei	Reduce	Reduce	Reduce	Reduce	Reduce	Reduce	Reduce
Embryo							
Nuclear size	Normal	Giant	Normal	Giant	Normal	Normal	Normal
Morphology	Giant	Giant cells	Small cells	Small cells	Normal	Irregular	Irregular
Arrest Stage	Late heart	1 to 4 cells	1 to 2 cells	Globular	Normal	Globular	Globular
Viability	Inviable	Inviable	Inviable	Inviable	Viable	Inviable	Inviable

[a] cmm, *S. commersonii* (2x, 1EBN); acl, *S. acaule* (4x, 2EBN); grl, *S. gourlayi* (2x, 2EBN).

nuclear division and cellularization (Tzafrir *et al*., 2002). In inter-EBN crosses in *Solanum* we observed phenotypes similar to *titan* mutants. Crosses between *S. gourlayi* (2x, 2EBN) x *S. gourlayi* (4x, 4EBN) and *S. acaule* (4x, 2EBN) x *S. commersonii* (2x, 1EBN) showed giant endosperm nuclei that could reach an area of 41.5 μm^2, whereas the average area of the endosperm nuclei in intra-EBN crosses ranged from 3.1 to 23.1 μm^2 (Masuelli, 2001) (see Fig. 1D).

A comparison of the phenotypes of the *Arabidopsis* mutants and the *Solanum* inter-EBN crosses are summarized in Table 2. Among the mutants described, the phenotypes of *ttn5* and *ttn6* are similar to the inter-EBN phenotypes (giant endosperm nuclei, embryo arrest at the globular or four cell stage and inviable seeds). Therefore, it is likely that the expression of genes orthologous to these in Arabidopsis could be altered in inter-EBN potato crosses.

Control of cell cycle and giant endosperm nuclei

Endosperm development in tuber-bearing *Solanum* species corresponds to the cellular type. Mitosis and cytokinesis occur after the first division of the primary

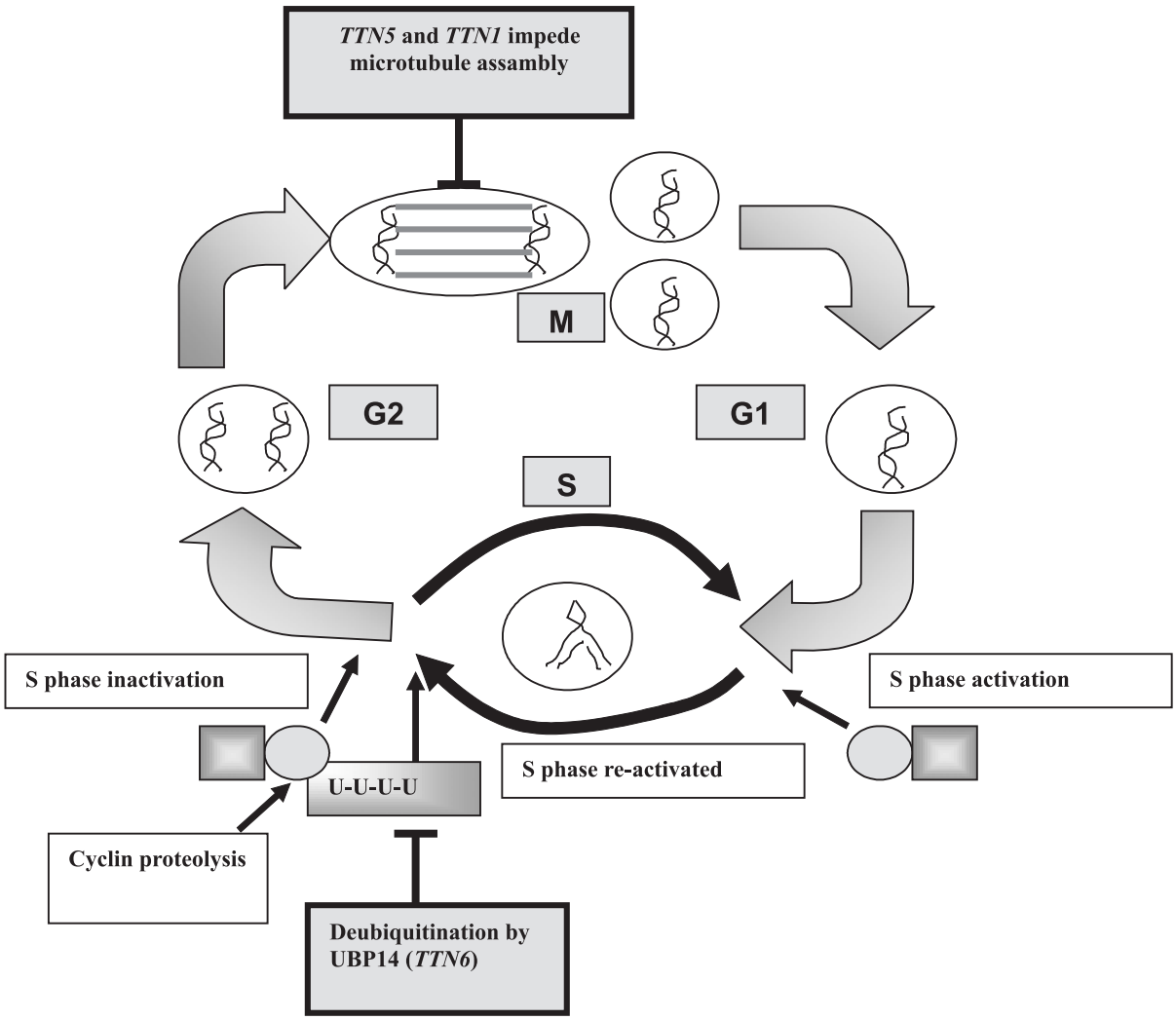

FIGURE 2. Proposed role of potato orthologous to *titan* mutants in the enlargement of the endosperm nuclei in inter-EBN crosses in *Solanum*. Giant nuclei could be produced by several cycles of endoreduplication without cytokinesis that involve TITAN proteins. We isolated potato sequences homologues to *TTN5* related to chromosome assembly and dynamics. The expression of these genes could be altered in inter-EBN crosses interfering the formation and dynamics of mitotic microtubules. According with Tzafrir *et al*. (2002) the *TTN6* gene could alter the ubiquitin-dependent proteolysis of the S-phase cyclins maintaining the S phase active. Square, cyclin-dependent kinase. Circle, cyclin.

endosperm nucleus to form a multicellular endosperm (Lopes and Larkins, 1993).

In plants, cell cycle is tightly regulated and many of the genes involved in its control were recently discovered (Mironov *et al*., 1999). Progression through the different stages (G1, S, G2 and M) of the cell cycle division depends on the interplay of CDKs and ubiquitin-dependent proteases. In plants, certain CDKs interact with specific cyclins determining the activity and specificity of the enzyme complexes at particular points of the cell cycle. The progression to the next cell division-cycle stage is mediated by the ubiquitination and destruction of the cyclins needed in the previous one. De-ubiquitinating enzymes can reverse the effects caused by ubiquitin modification (Doelling *et al*., 2001). In *Arabidopsis*, *TTN6* gene encodes a large protein (AtUBP14) with ubiquitin-specific protease (UBP) activity. AtUBP14 is disrupted in *ttn6* mutant resulting in the accumulation of multi-ubiquitin chains (Tzafrir *et al*., 2002).

The enlarged endosperm nuclei observed in *Arabidopsis* mutants and in *Solanum* inter-EBN crosses must be caused by multiple cycles of DNA replication without cytokinesis and the ubiquitin pathway might affected in these phenotypes. In inter-EBN crosses, accumulation of free multi-ubiquitin chains due to dysfunction of orthologous genes to *TTN6*, reduces the free ubiquitin monomers for re-use to target S-phase cyclins. Therefore, the proteins are not degraded by the 26S proteosome inducing several cycles of DNA replication (Fig. 2). Recently, it was shown that the inactivation of an ubiquitin ligase protein (CUL-4) in *Caenorhabditis elegans* causes massive DNA re-replication (Zhong *et al*., 2003). Other possible explanation for the phenotypes observed might be related to alterations in microtubule dynamics during mitosis by action of the *TTN5* and *TTN1* genes (Tzafrir *et al*., 2002) (Fig. 2).

Characterization of orthologous sequences to Arabidopsis TTN5 in potato. Preliminary results

Genome databases from model organisms like *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Sacharomyces cerevisiae* among others, have revealed that many genes and proteins are conserved in most living cells. The biological function of potato proteins might be inferred, therefore, from their role in a model organism. To study the role of *TITAN* genes in potato, we aligned the sequences of *TTN5* with ADP-ribosylation factor (ARF) and ADP-ribosylation factor-like (ARL) related proteins, and searched for conserved domains using BLOCKS (Henikoff *et al*., 1995). Five conserved blocks were generated and primers were designed with the CODEHOP program. DNA from *S. acaule* and *S. commersonii* were amplified by using degenerated primers. BLASTN search against The Institute for Genomic Research (TIGR) potato sequences of *S. commersonii* fragment reveled a high level of sequence identity to the potato expressed sequence tag (EST) TC73296 (96% identity, e = -101). This sequence has an open reading frame of 513 base pairs, whereas the predicted protein product contains 169 amino acids. The EST found in the potato database confirmed the expression of this gene in potato stolons. The analysis of the predicted protein against the InterPro database aligned to the NAD-dependent DNA ligase family (Mulder *et al*., 2003). This domain is related to the replication factor C conserved domain and kinesin

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cmm   96 PSVLDPHLRQFHLVMKKGLIPSVKVSIKHSSKRNVMIQSGLVIRGN
At    51 PEGTPDCLAGLTFVISGTLDSLEREEAEDLIKRHGGRITGSVSKKT
Hs    40 PKGAENCLEGLIFVITGVLESIERDEAKSLIERYGGKVTGNVSKKT
Mm    38 PKGAENCLEGLTFVITGVLESIERDEAKSLIERYGGKVTGNVSKKT
Nc    56 PEGEIDCLAGKTFVFTGLLKTIAREEAQALVKRYGGKVTGAPSSKT
Ssp  100 KLVQLNSLVTRNQQLSDQLSYVEQNQAKAIEQRLASQEKLWQQRHE

cmm  100 AREHAIHESLTYREV
At    96 AKLVSQMLGFQAVEV
Hs    54 ASSVSTKHALIMDEV
Mm    60 APSVSARHALIMDEV
Nc    76 ADARKKKIVLVMDEV
Ssp   83 ASPAKKSTAKAVDEV
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FIGURE 3. Sequence alignment and conserve domains, identified by BLOCKS (Henikoff 1995), of *S. commersonii* (cmm) sequence with related replication factor C proteins. Bold letters represent identical amino acids found in at least five sequences. Species and Swiss-Prot/TrEMBL accession numbers: Arabidopsis (Q9C587), human (P35251), mouse (P35601), Neurospora crassa (Q8X080), Synechocystis sp. (P73196).

motor domain, proteins associated with cell division and the formation and dynamics of the microtubules, respectively. Two conserved domains were found when the *S. commersonii* sequence was compared against replication factor C proteins, showing a high degree of sequence conservation (Fig. 3). Similarly, the TBLASTX search for the *S. acaule* PCR fragment resulted in a similarity to an *Arabidopsis thaliana* kinesin-related protein (At3g45850) (e = -31). The analysis using InterPro database showed that the *S. acaule* sequence has a domain (PF00225) related to a microtubule-associated force-producing protein that may play a role in organelle transport. Similar domains are present in several species including *D. melanogaster*, *S. cerevisie* and *Arabidopsis*, and are required for the assembly of the mitotic spindle and normal chromosomal segregation during meiosis. In humans this domain is related to CENP-E, a protein that associates with kinetochores during chromosome congression. CENP-E is probably an important motor molecule in chromosome movement and/or spindle elongation.

Our results in potato are in accordance with the model of the *TITAN* gene functions proposed by Tzafrir *et al*. (2002) in which ARL2 proteins, encoded by *TTN5* gene, regulate microtubule assembly in *Arabidopsis*. Therefore, it is likely that the *S. commersonii* and *S. acaule* sequences homologue to TTN5 may be involved in microtubule assembly in potato. The analysis of the expression profiles of the genes described above in the endosperm tissue of intra and inter-EBN potato crosses might help to elucidate what genes are related to the control of the EBN.

Conclusions

The EBN is an important tool for introgression of germplasm from wild tuber-bearing *Solanum* species to cultivated potato. Moreover, as a part of the post-zygotic barriers to hybridization, it is an important reproductive isolating mechanism in nature. However, little is known about the molecular and genetic basis of the EBN and other post-zygotic barriers in *Solanum*. The post-zygotic barriers are acting mainly at the endosperm level, producing the collapse of the endosperm and, consequently, of the embryo. Cytological abnormalities have been observed in the hybrid endosperm of inter-EBN crosses, including giant nuclei and nuclei of irregular sizes. Apparently, mitosis is altered in the endosperm of the hybrid seeds and enlarged nuclei are generated by cycles of endoreduplication without cytokinesis. Therefore, it is likely that genes controlling the cell cycle might be responsible for the collapse of the hybrid endosperm. The generation and molecular characterization of *Arabidopsis* seed mutants with phenotypes similar to those observed in inter-EBN crosses open a door for further molecular characterization of post-zygotic barriers in potato. In fact, we were able to identify potato homologues to *Arabidopsis* genes involved in the cell cycle and chromosome function. In addition, progression of the cell cycle in the endosperm might be studied using molecular markers to monitor the expression patterns of cyclin-dependent kinases and cyclins.

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