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REVIEW

The tomato seed as a model system to study seed development and germination.

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INTRODUCTION

Over the past decades a number of plant species have developed into models to study different aspects of seed biology. Cereal grains have long been used to unravel the pathways and regulation of endosperm mobilization by the aleurone layer (Fincher 1989; Jones & Jacobsen 1991). Seeds of pea (*Pisum sativum*) have been used extensively for the study of seed development and assimilate partitioning (Wang & Hedley 1991). *Arabidopsis thaliana* seeds are in use for molecular and genetic studies, employing large collections of mutants (Feldmann *et al.* 1994). In several cereals, such as maize (*Zea mays*) and wheat (*Triticum aestivum*), extensive studies have been undertaken to improve seed quality, both for stand improvement and nutritional value. However, the tomato seed has been used most extensively to study the physiology and biochemistry of seed development, germination and dormancy.

The tomato is a member of the nightshade family (Solanaceae) and belongs to the genus *Lycopersicon*. The fruits of the tomato plant have been used for human consumption since the sixteenth century. Today it is one of the major horticultural cash crops in the world and many new varieties are created each year by intensive breeding efforts to meet the different requirements all over the world. Because of its high economic value, many studies have been devoted to the improvement of its seed quality.

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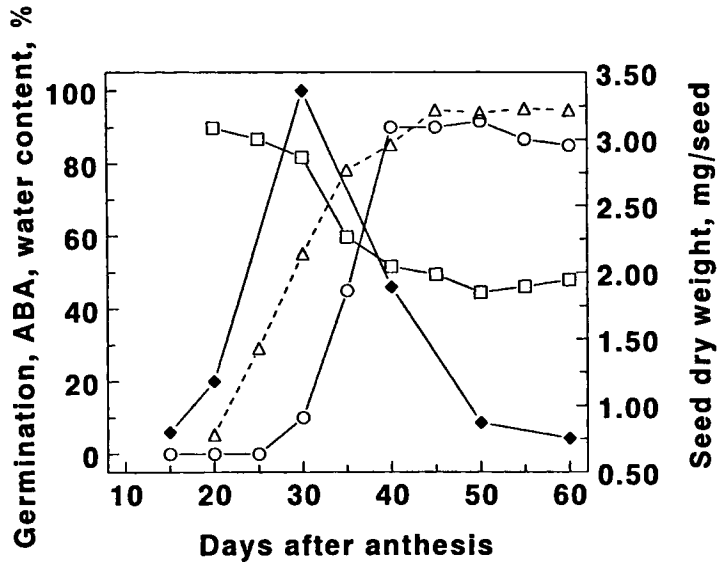

Fig. 1. Germinability (○), relative ABA content (◆), water content on fresh weight basis (□) and dry weight (△) of developing tomato seeds. Data adapted from Groot & Karssen (1987) and Liu *et al.* (1996).

The tomato seed is a convenient research object because of its relatively simple structure. The mature seed consists of a full-grown embryo embedded in a thick-walled endosperm and is surrounded by a thin testa. Its size, in the order of 2–5 mm, allows for easy manipulation and dissection. In the present review we will focus on the recent developments in our understanding of the processes regulating maturation and germination of the tomato seed.

SEED DEVELOPMENT

General characteristics

Tomato seeds develop and mature in the moist environment of a developing fruit. Fruit growth is governed by the developing seeds. The final weight and size of the fruit is largely determined by the number of seeds per fruit (Varga & Bruinsma 1986). During development seeds are embedded in the locular tissue of the fleshy fruit. When the seeds attain maturity the senescing locular tissue consists of lysed cells, but the seeds remain attached to a sheath of locular tissue which consists of intact cells (Berry & Bewley 1993). It has been suggested that the sheath and locular tissues play a role in seed development and prevent precocious germination (Berry & Bewley 1992).

Fruits are red and ripe after approximately 60 days after pollination (DAP). A number of significant events occur during the period 35–45 DAP: seeds reach maximum dry weight, seed water content drops to 40–50 % (fresh weight basis) and germinability of the seeds, when taken from the fruit, reaches a maximum (Fig. 1). Simultaneously, ABA contents show a transient rise, the endosperm solidifies, the funiculus is abscised and the testa turns brown (Smith 1935; Berry & Bewley 1993; Liu et al. 1996). Many

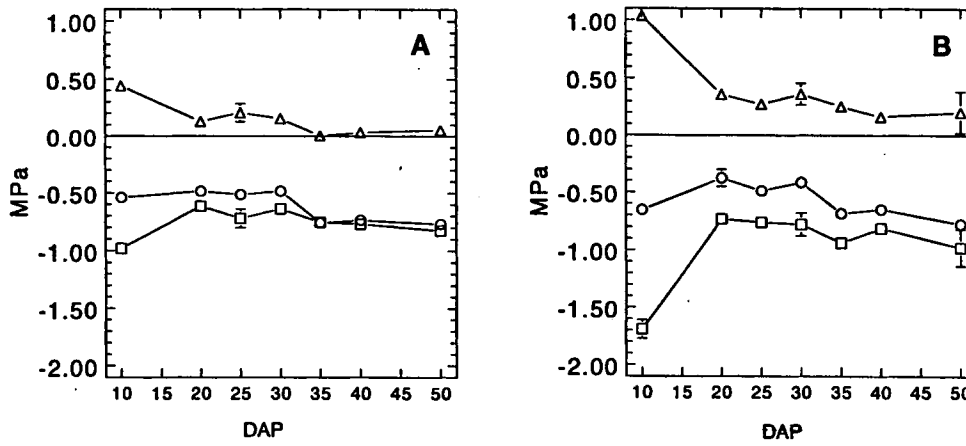

Fig. 2. Osmotic potential (□), water potential (○) and calculated pressure potential (△) of developing tomato fruits (A) and seeds (B). Redrawn from Liu *et al.* (1996).

studies of tomato seed development have been undertaken to explain how precocious germination is prevented in a wet environment. The following paragraph centres around this theme and explores the possible contributions of these simultaneously occurring events.

The prevention of precocious germination

The germination inhibiting capacity of tomato fruit tissues was recognized as early as the beginning of this century (reviewed by Dörffling 1970). Köckemann (1934, 1936) isolated an ether soluble substance from fleshy fruits that inhibited germination. This substance was called 'Blastokoline' and was later identified as the plant hormone abscisic acid (ABA) (Dörffling 1970). The latter author indicated that the concentration of ABA in the fruit was not sufficiently high to account for the absence of precocious germination. This was confirmed by Berry & Bewley (1992), who demonstrated that the germination of developing seeds, when dissected from the fruit tissues, could be inhibited by placing them on an osmoticum such as polyethylene glycol, whereas ABA was less effective. However, viviparous germination frequently occurs in overripe fruits of the ABA-deficient *sit*^{w} mutant (Groot & Karssen 1992). Thus, ABA appeared to play a role in the prevention of precocious germination, other than direct inhibition. Several studies have focused on the water relations of both fruit and seed tissues during development to account for this discrepancy (Berry & Bewley 1992; Liu et al. 1996). During growth of wild-type fruits and subsequent ripening, the water potential of the locular tissues decreases from approximately −0·50 MPa to −0·75 MPa (Fig. 2). Concomitantly, the water potential of the seeds also decreases to approximately −0·75 MPa at 50 DAP (Fig. 2). As the differences in water potential between seed and fruit are only marginal, these results would predict a frequent occurrence of precocious germination in wild type seeds. The water potential of fruit tissues and seeds from the *sit*^{w} mutant also do not differ significantly at 50 DAP. However, the mutant genotype is viviparous. It has been shown that mature seeds of the *sit*^{w} mutant have the capacity to germinate in osmoticum at considerably lower osmotic potential than wild-type seeds

(Groot & Karssen 1992; Hilhorst & Downie 1995). Apparently, the absence of ABA during seed development has led to a persistent property of the mutant seeds, expressed as a higher resistance to osmotic stress. Groot & Karssen (1992) hypothesized that the high levels of ABA that occur during seed development induced an inhibition of embryo cell elongation that was persistent, even after years of dry storage. However, a re-examination of the phenomenon revealed that the difference in the response to osmotic stress was due solely to differences in testa thickness (Hilhorst & Downie 1995). Wild-type seeds possessed a testa with a thickness of 4–5 cell layers, whereas the *sit*^{w} testa consists of only one cell layer. Germination experiments with both genotypes showed that the differences in resistance to osmotic stress diminished when the testa is removed from the micropylar side of the endosperm. Expansion in osmoticum of dissected embryos from both genotypes was similar (P. E. Toorop, personal communication), supporting the notion that the differential reponse to osmotic stress was located in the endosperm and/or testa. For radicle protrusion to occur, enzymatic degradation of the micropylar endosperm is a prerequisite (Groot *et al.* 1988, and see below). Endo-β-mannanase activity in the micropylar tomato endosperm is not affected by ABA (Toorop *et al.* 1996) and is partly inhibited by osmotic stress, but equally in both genotypes (Hilhorst & Downie 1995). However, ABA is able to inhibit a second step in endosperm weakening, which is independent of mannanase activity (P. E. Toorop, personal communication). These results suggest an important role for testa strength to resist expansion of the embryo during development. Also in mature seeds the resistance of the testa plays a small but significant role in germination. The lag time to germination at 12°C of a cold-resistant tomato line was decreased from 9 to 6 days after removal of the testa (Leviatov *et al.* 1994). Thus, after attaining full germinability in the fruit, the expansion force of the embryo is in delicate balance with the resistance of endosperm and testa in addition to the osmotic environment and ABA in seed and fruit tissues. In this respect we may speculate on the timing of solidification of the endosperm, which occurs when germinability increases between 30 and 40 DAP. Solidification of the endosperm evidently increases its mechanical restraint. Also a slight inhibitory effect of ABA in the seed and fruit on embryo expansion cannot be precluded. Welbaum *et al.* (1990) reached a similar conclusion for the prevention of precocious germination of developing muskmelon (*Cucumis melo*) seeds. Developing alfalfa (*Medicago sativa*) seeds in pods on plants treated with fluridone, an inhibitor of ABA-synthesis, did not germinate viviparously, which is an indication that also in alfalfa ABA alone is not responsible for the suppression of precocious germination (Xu *et al.* 1990). In this species only osmoticum was able to maintain the synthesis of developmental proteins.

Another possible role for ABA during seed development is to induce desiccation tolerance. This was convincingly shown in *Arabidopsis thaliana* (Ooms *et al.* 1994). In this study the ABA-insensitive *abi-3* and *abi-5* mutants, as well as the *aba-1,abi-3* double mutant, which is both deficient in and insensitive to ABA, failed to acquire desiccation tolerance during the second half of seed development. As these were severe mutants, the results indicated that the induction of desiccation tolerance is highly sensitive to ABA. The less severe mutants acquired at least moderate levels of desiccation tolerance. Thus, in general, a probable role of ABA during seed development is to maintain the seed in a developmental mode, i.e. to sustain accumulation of storage proteins and not primarily to suppress precocious germination. As an alternative, ABA may induce desiccation tolerance.

Primary dormancy

Depending on growth conditions, developing tomato seeds may enter a state of dormancy, called primary dormancy, resulting in a decrease in germinability around mid-development (30–40 DAP) which may or may not be sustained after maturity. Which environmental conditions are responsible for the induction of developmental dormancy in tomato or other species remains largely unknown. ABA-deficient mutants of tomato and *Arabidopsis thaliana* essentially lack primary dormancy (Karssen *et al.* 1983; Koornneef *et al.* 1985). This has led to the conclusion that the transient rise in ABA content during mid-development is responsible for the induction of primary dormancy. However, as pointed out by Hilhorst (1995), evidence for this is at best circumstantial. First, there is often a lack of correlation between ABA content and depth of dormancy. For instance, the tomato cultivar Caruso has more than 10 times higher levels of ABA than the cultivar Moneymaker, yet the latter shows a much deeper dormancy (Berry & Bewley 1992; Hilhorst, 1995). Secondly, it has been shown that complementation of the ABA mutation in *Arabidopsis thaliana* by the admission of ABA reversed all mutant traits to the wild-type phenotype, except for the dormancy (Koornneef *et al.* 1989). Thirdly, in *Arabidosis thaliana,* mutants have been isolated that lack dormancy but possess wild-type ABA levels (Léon-Kloosterziel *et al.* 1996). Apparently, the sensitivity to the hormone plays an important role. ABA-insensitive mutants of *Arabidopsis thaliana*, containing wild-type ABA contents, also lack primary dormancy (Koornneef *et al.* 1984). Vivipary or pre-harvest sprouting in wheat has been demonstrated to be paralleled by a reduced sensitivity to ABA (Walker-Simmons 1987). During the second half of seed development sensitivity to ABA decreased in seeds of muskmelon (Welbaum *et al.* 1990) and alfalfa (Xu & Bewley, 1991, Xu *et al.* 1990). It is likely that this is also the case in tomato, but data are not available. Only when the sensitivity to ABA among seed batches is similar, can ABA content be correlated with germination, as was shown for tomato and *Arabidopsis thaliana* seeds (Hilhorst 1995). Apparently, the remaining amounts of ABA in the mature seeds inhibited or delayed germination. This would also explain why either dry storage or cold imbibition can break dormancy. It has been shown in tomato (Groot & Karssen 1992) and lettuce (Dulson *et al.* 1988) that both treatments may lead to a decrease in endogenous ABA and an increase in germination.

DNA replication during seed maturation

At around 30 DAP the tomato embryo is fully developed and this marks the end of the histo-differentiation phase of embryo development. At that stage, the fruit is still green and the endosperm is liquid. The histo-differentiation phase is characterized by high cell division activity. Between 30 and 45 DAP embryo development is arrested (see above). The transition from the developmental to the maturation phase is also characterized by an arrest of embryonic cell cycle activities. By employing flow cytometry it was demonstrated that at 30 DAP embryo radicle cells contained 2C DNA from diploid nuclei at the pre-replication stage of nuclear division (G_1 phase), 4C DNA of diploid nuclei at the G_2 phase and the 8C DNA amount of polyploid nuclei (Liu *et al.* 1997). Between 30 and 45 DAP, the 4C DNA signal is reduced, and radicle cells predominantly show 2C DNA signals, indicating that most cells arrest in G_1 phase. At the same time, seeds start to gain desiccation-tolerance and germinability. At 60 and 75 DAP, most embryo radicle

tip cells of wild-type, *gib-1* (gibberellin-deficient), and *sit*^{w} seeds arrest at G_1 phase. However, in ripe fruits, *sit*^{w} seeds contain a significantly higher proportion of G_2 cells compared to the other two genotypes. ABA has been shown to be an inhibitor of cell division (Evans 1984) and is claimed to play an important role in the arrest of cell cycle activities during seed maturation (Bouvier-Durand *et al.* 1989). As mentioned above, during late stages of fruit maturation *sit*^{w} seeds may shift into the germination mode inside the fruit, eventually leading to precocious germination. Concomitantly, nuclear replication activities start, leading to the augmentation of 4C DNA signals inside the seed.

SEED GERMINATION

For radicle protrusion to occur the expansion force of the embryonic radicle must exceed the mechanical restraint of the surrounding endosperm and testa. This can be accomplished when the water potential of the embryo becomes more negative, when the resistance of endosperm and testa decreases, or when both events occur simultaneously. Thus the simplest mechanistic model of (tomato) seed germination is based on a balance of opposing forces (Pavlista & Haber 1970). During the last decade this model has been considerably refined, based on a better understanding of the processes regulating the switch between quiescence and germination. Here, quiescence is defined as developmental arrest caused by dehydration. Of all germination models described for seeds from various species, the current model for tomato is considered the most sophisticated. Below we will discuss the evidence that has contributed to the model.

Cell cycle-related events during seed germination

An induction of G_2 phase in radicle cells of wild-type and *gib-1* seeds is only found after imbibition and breakage of dormancy. In wild type seeds 4C DNA signals increase 16–24 h after the imbibition of water. This increase in 4C signal precedes visible germination, which starts 48 h after imbibition. Seeds of *gib-1* show no induction of nuclear replication activity upon imbibition in water. Only when GAs are supplemented to the germination medium, are 4C DNA signals found and germination starts. Apparently, GAs are necessary to initiate DNA synthesis. In tomato seeds, GAs are involved in the enzymatic degradation of the endosperm prior to radicle protrusion (Groot *et al.* 1987). It is concluded that the absence of endosperm weakening opposite the embryo radicle limits cell growth and inhibits cell cycle activities in *gib-1* seeds. Indeed, when the endosperm and testa of the *gib-1* seeds are removed, 4C DNA signals are initiated and radicles protrude, even without the addition of GAs (Liu *et al.* 1997).

Gib-1 seeds can be considered dormant, and only when the dormancy is broken by the application of GAs are germination processes activated. A state of (secondary) dormancy can also be induced in wild-type seeds by treatment with far-red light. Far-red irradiated seeds do not germinate in the dark, and even after 5 days imbibition in water no change occurs in the 4C/2C DNA ratio compared to dry control seeds. Only when dormancy is broken by imbibition in white light do 4C/2C DNA ratios increase and seeds germinate (Table 1). This type of dormancy can be broken by removal of

Table 1. 4C/2C ratios of radicle tip cells from mature tomato seeds under different conditions. Data from Groot *et al.* (1997) and Lanteri *et al.* (1994). Data on temperature effect are original

Treatment	4C/2C ratio
Dry seed	0·07
Far-red + 5 d imbibition in light	0·92
Far-red + 5 d imbibition in dark	0·18
7 d imbibition in −1·0 MPa PEG	0·25
7 d imbibition in −1·5 MPa PEG	0·11
7 d imbibition in −2·0 MPa PEG	0·07
8 d imbibition at 10°C	0·10
8 d imbibition at 15°C	0·51

the testa and endosperm seed layers, which further indicates the regulating role of these tissues in tomato seed germination.

Seed functioning is accompanied by programmed transitions from cell proliferation to quiescence upon maturation and from quiescence to re-initiation of cellular metabolism upon imbibition. In tomato, the transitions are accompanied by modifications in nuclear replication stages which can be monitored through changes in 4C/2C ratios of the radicle tip cells of the embryo. In this way the amounts of DNA can be used as a marker for the advancement of seed germination processes. Tomato seed germination is affected by the composition of the germination medium and various other external factors. Changes in 4C/2C ratios can be used to test the response of tomato seeds to these conditions. DNA replication in seeds of tomato is retarded if water uptake is limited by low water potentials (Table 1), or when seeds are imbibed at low temperatures (Table 1) or at low oxygen concentrations (N. Ozbingöl, personal communication). The relation between DNA replication and germination of seeds of tomato and pepper (*Capsicum annuum*) at low water potential has been studied thoroughly (Bino *et al.* 1993; Lanteri *et al.* 1993; Saracco *et al.* 1995; Baker & Bradford 1995). Seed imbibition at low water potentials revealed a negative relation between the induction of 4C signals and the concentration of PEG in the medium, even beyond the PEG osmotic potential at which radicle protrusion is inhibited (−0·5 to −1·0 MPa). Apparently, during imbibition at low water potentials, seeds can still take up sufficient water for the induction of replicative DNA synthetic processes but not enough for radicle protrusion. The responses of germination to the osmolarity of the germination medium have been quantitatively characterized using a population threshold model (Ni & Bradford 1992). The model explains that the progress of pre-germinative processes is proportional to the prevailing water potential of the imbibition medium. Thus, in tomato this progress can be analysed through the change in 4C/2C ratio. Apart from the imbibition conditions, the increase in 4C/2C ratio depends on the seed lot quality. During imbibition, low quality seed lots show a reduced increase in 4C/2C ratios compared to high quality seed lots.

After imbibition at low water potentials ('priming'), non-germinated seeds can be dried back to their original water content, stored and germinated without affecting the

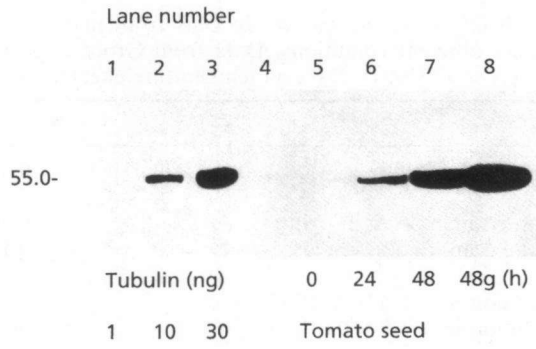

Fig. 3. Immunoblot showing the 55 kD β-tubulin accumulation pattern in dry and imbibing tomato seeds (0, 24 and 48 h). The gel was loaded with 70 μg of proteins extracted from whole seeds. Lanes 1–3: pure tubulin; lanes 5–8, MODIL extracted radicles according to De Castro *et al.* (1998).

4C/2C ratio that was attained during priming. Seeds with an enhanced 4C/2C ratio will germinate more rapidly and with improved uniformity than non-primed seeds or seeds with a lower 4C/2C ratio. Apparently, during the priming period, pre-germinative processes, including DNA replication, are activated which facilitate germination. Upon imbibition, the first events which are initiated are protein, DNA and RNA synthesis (Coolbear *et al.* 1990; Bewley & Black 1994). The major component of the early DNA synthesis comprises DNA repair activities, which are operative during the first hours of imbibition (Osborne 1983). Hence, before DNA synthesis can start, both DNA repair and other cell cycle-related mechanisms must operate. These processes can be activated at lower (more negative) osmotic potentials than those required for DNA replication and enable the seeds to improve their efficiency in synthesizing DNA during a subsequent period of imbibition in water. Lanteri *et al.* (1993) observed that pre-conditioning treatments which do not induce DNA replication may accelerate and increase the amount of DNA synthesis during a subsequent period of imbibition in water. The level of 4C signals can therefore be used as a parameter for the advancement of late pre-germinative processes. For applied reasons this is important as the 4C/2C ratio can be used both to determine the response of a seed lot to the pre-conditioning treatments and to predict the germination performance. Also, the 4C/2C ratio gives an indication of the storability of the pre-conditioned seed lot as the induction of G_2 phase in radicle cells of tomato and pepper seeds are correlated with an increased sensitivity to deteriorating conditions (Baker & Bradford 1995; Saracco *et al.* 1995). This increased sensitivity can be related to the fact that cells in G_2 phase are more sensitive to stress factors than cells in G_1 (Sybenga 1972).

The cell cycle involves many regulatory and structural proteins. One of these proteins is β-tubulin, a component of the microtubular cytoskeleton. Studies using antibodies against tubulin have shown that the progression through the cell cycle is associated with changes in the specific organization of the microtubules (Hussey *et al.* 1990; Traas *et al.* 1992). In roots of maize (*Zea mays*), it was demonstrated that the progression of the cell cycle through G_1 phase is dependent on the turnover of the microtubular cytoskeleton (Baluska & Barlow 1993). However, the relation between synthesis of tubulins and cell cycle activity is not yet fully elucidated. In seeds of tomato, β-tubulin levels accumulate during imbibition in water (Fig. 3) (De Castro *et al.* 1995, 1998). As

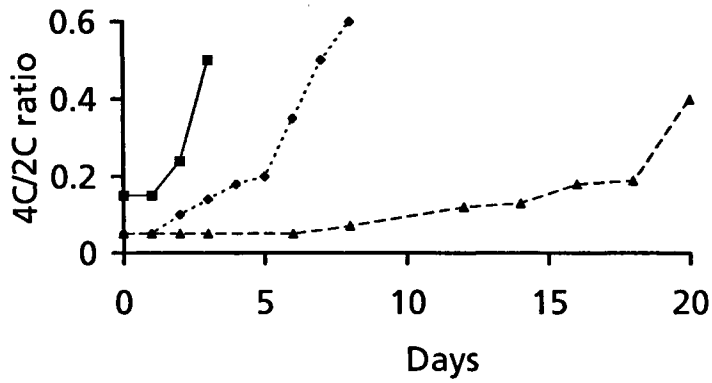

Fig. 4. Increase in 4C/2C ratio in radicle tip cells of tomato seeds during imbibition in water. Non-treated control seeds, seeds pre-imbibed (primed) for 7 d at −1.0 MPa PEG, and seeds that underwent controlled deterioration for 4 d at 45% relative humidity at 60°C. Redrawn from De Castro *et al.* (1995) and original data. ■, primed; ◆, control; ▲, aged.

with nuclear DNA replication, β-tubulin accumulation was strongest in the embryonic root tip compared to the other seed tissues. The increase in the β-tubulin signal relates to the progression of the cell cycle through G_2 phase towards mitosis and cell division. During these processes microtubules undergo continuous assembly and rearrangements into new configurations for interphase cortical, pre-prophase, spindle and phragmoplast arrays (Fosket & Morejohn 1992; Goddard *et al.* 1994).

In various ways, β-tubulin signals have been correlated with nuclear replication stages during tomato seed imbibition. Far-red irradiated seeds, possessing secondary dormancy, did not accumulate β-tubulin upon imbibition in water in the dark. Relief of dormancy by white light induced β-tubulin accumulation and initiated germination. The induction of 4C signals and the accumulation of β-tubulin follow similar patterns. When the time course of DNA replication and β-tubulin accumulation are compared with the germination data, it is obvious that in all cases activation of both cell cycle-related events precede visible germination. On the other hand, controlled deterioration of seeds considerably delayed DNA cell cycle-related events (Fig. 4), as well as germination (not shown) (De Castro *et al.* 1995). Apparently, the cell cycle plays an important role in tomato seed germination and changes in both nuclear replication stages and β-tubulin accumulation can be used as a parameter for monitoring processes that are activated during imbibition.

The water relations of tomato seed germination

Water uptake during imbibition and subsequent radicle protrusion is usually triphasic (Bewley & Black 1994). During phase I there is a rapid uptake of water due to the large water potential (ψ) gradient between the seed and its environment. This is caused by the very low matrix potential of the dry seed. In phase II there is hardly any change in water content. The length of phase II may vary in duration, ranging from minutes to years, in deeply dormant seeds. Apparently, during this plateau phase of water uptake the ψ_{seed} equals that of the environment (Bradford 1986). In the case of pure water $\psi_{seed}=0$. Indeed, Liptay & Schopfer (1983) showed that tomato seed ψ was 0

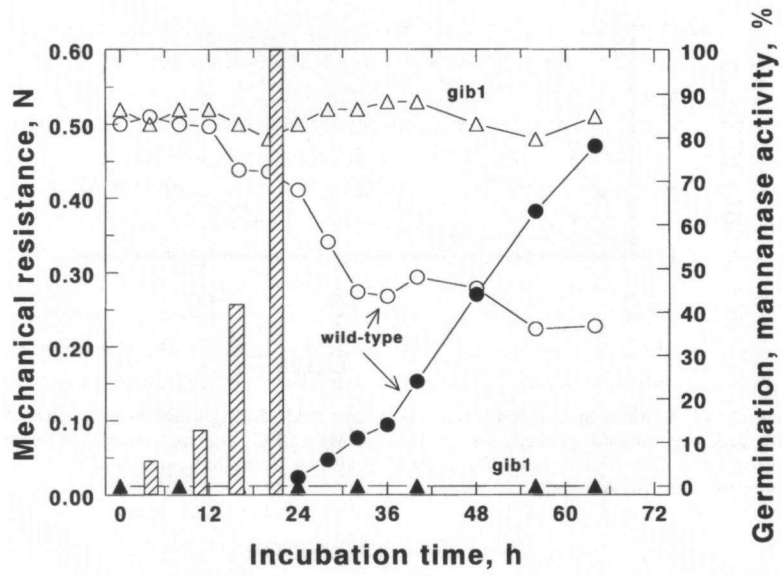

Fig. 5. Germination (closed symbols) and mechanical resistance of endosperm caps (open symbols) in imbibing wild-type and *gib-1* tomato seeds. Bars represent pre-emergent mannanase activity in wild-type seeds relative to t(20 h) = 100%. No mannanase detectable in *gib-1* seeds. Redrawn from Groot & Karssen (1987) and Groot *et al.* (1988).

MPa prior to radicle protrusion. This was confirmed by a study of the water relations of tomato seed germination by Haigh & Barlow (1987). However, they also showed that the embryo ψ was not in equilibrium with the imbibitional solution during phase II. They measured a ψ_{embryo} of $-1{\cdot}5$ MPa which increased to $-0{\cdot}3$ MPa during radicle protrusion. Surprisingly, there was no evidence of lowering of the embryo osmotic potential (ψ_π), nor was there a build-up of turgor (ψ_p) before radicle protrusion. These results were explained by assuming that the embryo-surrounding layers (endosperm and testa) imposed a mechanical restraint on the expansive force of the embryo (Haigh & Barlow 1987). Similar results were reported for muskmelon seed germination (Welbaum & Bradford 1990). In these seeds the ψ_{embryo} was approximately $-0{\cdot}6$ MPa prior to radicle protrusion. Also in embryos of this species there was no build-up of turgor or lowering of ψ_π. It was concluded that perisperm and testa restricted embryo expansion. In conclusion, radicle protrusion of tomato and muskmelon and, possibly, more endosperm retaining seeds, is governed by a weakening of the constraint imposed by the endosperm.

Endosperm weakening and germination

In tomato seeds endosperm weakening occurs prior to radicle protrusion (Groot & Karssen 1987; Fig. 5). This was shown by measuring the force required to puncture the endosperm and testa layers opposite the radicle tip. Tissue weakening prior to radicle protrusion was also demonstrated in endosperm of seeds of *Daturo ferox* (De Miguel & Sanchez 1992) and pepper (Watkins & Cantliffe 1983), in megagametophyte tissue of white spruce (Downie *et al.* 1997) and in the perisperm of muskmelon seeds (Welbaum *et al.* 1995).

Mannose polymers are the major constituents of the hemicellulose cell wall component of the tomato seed endosperm, as well as of several other species. Prior to visible germination, and concomitant with endosperm weakening, an increase of endo-β-mannanase (EC 3.2.1.78) activity in endosperm tissue was shown (Groot *et al.* 1988). This led to the hypothesis that hydrolysis of the mannose polymers would result in a weakening of the endosperm cell walls. The weakening process appeared to be regulated by GA. Seeds of the gibberellin-deficient tomato mutant do not germinate in the absence of exogenous GAs, there is no endosperm weakening and mannanase activity is absent (Fig. 5). Both endosperm weakening and mannanase activity are induced by the application of GAs (Groot & Karssen, 1987; Groot *et al.* 1988). It was hypothesized that GAs are synthesized in the embryo and secreted to the endosperm cap to induce mannanase activity, leading to cell wall hydrolysis and endosperm weakening. Nomaguchi *et al.* (1995) demonstrated that the mannanase activity prior to visible germination was located exclusively in the micropylar part of the endosperm. Only after radicle protrusion mannanase activity increased in the rest of the endosperm, possibly to mobilize the cell walls as reserve food for energy supply to the growing embryo. Under normal conditions the lateral endosperm remains attached to the growing cotyledons for several days after radicle protrusion. Employing scanning electron microscopy, Sanchez *et al.* (1990) presented evidence that the internal surface of the micropylar endosperm of *Datura ferox* and *D. stramonium* became eroded prior to germination. In the endosperm cap of tomato seed the walls show porosity which coincides with the increase in mannanase activity and decrease of endosperm resistance (P. E. Toorop, unpublished results).

These results strongly suggest a keyrole for endo-β-mannanase in the germination of several species. However, so far evidence is strictly correlative. There are several indications that the enzyme may be essential but not limiting for germination and, in addition, may have other functions in seed germination. For example, when tomato seed germination is completely inhibited by ABA, mannanase activity nevertheless develops normally (Toorop *et al.* 1996). Furthermore, a quantitative relationship between mannanase and germination is lacking. Using a single seed assay, Still & Bradford (1997) and Still *et al.* (1997) showed that there is a 100–10 000-fold range of mannanase activity among individual seeds. Generally, there was some relationship between high mannanase activity and germination but, again, when germination was inhibited by ABA or osmoticum the initial increase in mannanase activity was unaffected. Upon imbibition, the rise in endo-β-mannanase activity in the endosperm cap is paralleled by an increase in endo-β-mannanase activity in the radicle tip (Toorop *et al.* 1996). The role of the enzyme in this part of the seed remains to be elucidated, but may be associated with cell wall modification for (elongation) growth. These possible differing roles of the enzyme may be related to the occurrence of several isozymes. In tomato seed different isozymes are expressed in the different tissues, such as embryo, micropylar and lateral endosperm (Toorop *et al.* 1996; Voigt & Bewley, 1996). There is also evidence that the enzyme is ubiquitous throughout the plant kingdom and may be present in all plant organs, displaying highly variable isozyme patterns (Dirk *et al.* 1995). Recently, the mannanase gene has been cloned from germinated tomato seeds (Bewley *et al.* 1997). Apparently, the enzyme is derived from a family of about four genes. This finding may provide the tools to establish how essential the enzyme is for seed germination.

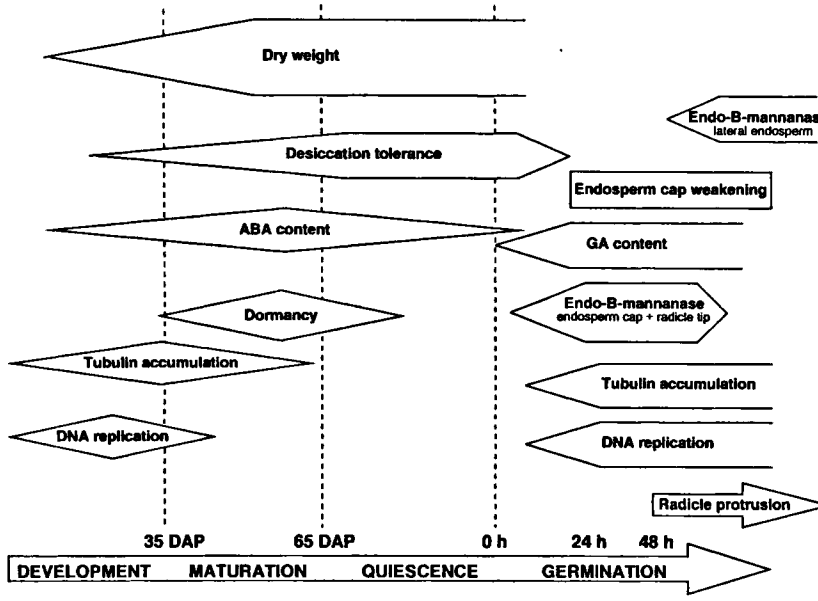

Fig. 6. Events identified at different stages of developing, maturing, quiescent and germinating tomato seeds. Diverging lines indicate increasing and converging lines decreasing activities.

CONCLUSIONS

Over the past 10 years a number of processes have been identified that are intimately linked to performance of the seed, such as DNA replication and other cell cycle-related events, endosperm weakening and hydrolytic enzyme activity, and water relations during seed development and germination. From this a descriptive model, including the known steps leading to radicle protrusion and their (hormonal) regulation, can be drawn (Fig. 6). However, it is important to recognize that most evidence obtained so far is strictly correlative. The development of the hormone-deficient *gib-1* and *sit*^{w} tomato mutants has led to major steps in understanding the control of dormancy and germination. Because of the rapid development of molecular techniques it is now possible to pursue lines of research that may provide conclusive evidence for the involvement of the different processes in seed performance. For example, the role of endo-β-mannanase in endosperm weakening and the role of cell cycle proteins in the initiation of embryo growth can be established by an antisense approach, since many of the genes of these proteins have been cloned. It is of eminent importance to test the validity of the current model for tomato seed development and germination for other species. As shown, a number of other endosperm-retaining species appear to possess similar characteristics to tomato. Other species to be tested should also include wild species. Through intensive breeding, many of our culture crops have lost several unwanted characteristics, such as (deep) dormancy or the responsiveness to natural environmental factors, such as light and nitrate. In order to improve crop seed quality further the study of wild species may prove beneficial, for example to enhance seed developmental processes and seed performance under stress conditions or to improve storability.

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