Seed Germination Enhancement in *Solanum stramonifolium* and *Solanum torvum*

Nur Eva Hayati[1], Sutevee Sukprakarn[1] and Sunanta Juntakool[2]

ABSTRACT

Seeds of *Solanum stramonifolium* Jacq. (SM 293) and *Solanum torvum* Sw. (SM 287 and SM 259) were harvested at 70 days after anthesis and kept in dry storage at 20°C and 30°C for five months to determine the seed dormancy periods. The results showed that seed dormancy of these accessions was broken after three months of storage at 20°C or five months at 30°C. Under both storage regimes, the dormancy of *S. stramonifolium* was broken faster than *S. torvum*. When newly harvested seeds of these three accessions were treated with various methods for breaking dormancy, it was found that dormancy could also be overcome by the following treatments: 12 hour soaking, 30 minute pre-washing, one-day pre-heating at 50°C, pre-chilling at 5°C for one day (SM 293 and SM 287) and five days (SM 259), 0.1% KNO_3 or 0.01% GA_3.

Key words: Solanum, dormancy breaking

INTRODUCTION

Solanum constitutes the largest and most complex genera of the Solanaceae family. It is composed of more than 1500 species many of which are economically important such as eggplant, *Solanum melongena* L. (Edmonds and Chweya, 1997). The other species of concern are hairy-fruited pea-eggplant (*Solanum stramonifolium* Jacq) and turkey berry, also known as Thai eggplant (*Solanum torvum* Sw).

Although Solanum species are grown in all agricultural areas, the greatest concentrations of the species are in the tropical and warm temperate regions. The greatest centers of diversity occur in South America, Australia and Africa while relatively less diverse species are found in Europe and Asia (Edmonds and Chweya, 1997).

Solanum species are mainly propagated by seed. However, Solanum seeds have a certain period of time with reduced ability to germinate. For example, buried seed of *S. nigrum* remained dormant for at least 39 years in Britain (Edmonds and Chweya, 1997). Joshua (1978) noted that one of the germination problems in *S. incanum* seeds is slow germination due to the hard seed coat. Abdoulaye (1992) reported that embryo dormancy is a major constraint in African eggplant (*S. ethiopicum* L.) and their new seeds can germinate only after 4–5 months under suitable conditions. In addition, primary dormancy was also a problem in freshly harvested *Solanum nigrum* (Bithell *et al*., 2003) and "Kopek" *S. melongena* seeds (Caecilia, 1990).

Seed dormancy in cultivated plants not only causes problems in actual agricultural

production but also complicates assessment of seed quality (Geneve, 1998). Hence, this problem deserves greatest consideration in areas of seed production technology where study of seed dormancy in many agricultural seeds is an important input.

This research was proposed in the view that information regarding overcoming this trait will enable us to advise the producers to have uniform seedlings and good crop stand at seedling stage. Therefore, the objectives of this study were to determine seed dormancy periods at different storage temperatures and effectiveness of various treatments in overcoming dormancy in Solanum seeds.

MATERIALS AND METHODS

The experiments were conducted at the Seed Quality Testing Laboratory of Tropical Vegetable Research Centre (TVRC), Kasetsart University, Kamphaeng Saen Campus from June 2003 to December 2004. One accession of *Solanum stramonifolium* (SM 293) and two accessions of *S. torvum* (SM 287 and SM 259) obtained from TVRC were used in this study.

Fruits of the three accessions were harvested at 70 days after anthesis (DAA) and the seeds were extracted directly after harvesting. The extraction was done by hand to minimize mechanical damage and mixture. Seeds were air-dried under shade for three days to lower the seed moisture content, and cleaned by using a seed blower and placed in a drying room at 25°C and 30% RH until the seeds reached about 6% moisture content. Only pure seeds were used for germination test.

The seeds were packed in aluminum foil bags and kept at either 20°C or 30°C. Germination tests were conducted at monthly intervals for five successive months using 50 seeds per treatment/ accession and replicated four times. The seeds were placed on the top of moistened paper towels (top of paper method) and kept at 20°/30°C (16/8

hours). First and final counts were taken at 7 and 14 days, respectively (ISTA,2003) .

Different dormancy breaking treatments used were soaking in water (12, 24, 36 and 48 hours), pre-washing under running water at room temperature (30, 60, 90 and 120 minutes), pre-heating at 35°, 40°, 45°, 50° and 55°C (1, 3, 5 and 7 days), pre-chilling at 5°C (1, 3, 5 and 7 days), potassium nitrate (KNO_3) (0.1, 0.2, 0.3 and 0.4%), and gibberellic acid (GA_3) (0.01, 0.02, 0.03, 0.04 and 0.05%). The method of germination test after dormancy breaking was carried out as described earlier.

Data on germination percentages were subjected to arcsine transformation for statistical analysis. Results were analysed to a factorial in complete randomized design and means were tested by the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

1. Determination of seed dormancy periods

After the seeds of *S. stramonifolium* (SM 293) and *S. torvum* (SM 259 and SM 287) harvested at 70 DAA were kept at 20° and 30°C, it was found that dormancy of the three accessions could be overcome within 3 months at 20°C and 5 months at 30°C (Figure 1). Leubner-Metzger (2003) noted that the level and sensitivity of ABA decreased but GA-responsive system increased during the periods of storage.

The dormancy of *S. stramonifolium* (SM 293) was broken faster than *S. torvum* (SM 287 and SM 259). It indicated that these species have after-ripening periods that differ among accessions. Besides, it seemed that low storage temperature had a tendency to overcome the problems of these species. In Thai cucumber cv. 'Puang', the GA contents of the seed increased after 4-month storage under cold conditions (Aroonrungsikul, 2001).

Based on these results, low storage temperature seemed to overcome the dormancy problems of both *Solanum* species but it may not

be suitable to apply for breaking dormancy of these accessions due to the long treatment periods.

**II**. **Methods of breaking seed dormancy**

Seeds soaked in water at room temperature were permeable to water. This indicated that the cause of dormancy of these accessions might not be attributed to the seed coat as a physical barrier to water absorption. The germination percentages of *S. stramonifolium* (SM 293) seeds for all times of soaking were lower (below 90%) than those of the two accessions of *S. torvum* (SM 259 and SM 287) whose dormancy was broken after a 12-hour soaking (Figure 2).

It seems that soaking treatment may promote the leaching of germination inhibitors on the seed coat (Xia and Kermode, 2000). Beside that, soaking may remove hardseededness of the seed coats (ISTA, 2003). Leubner-Metzger (2002) pointed out that in endospermic seed such as tobacco, the contributions of both testa and the endosperm layers have to be considered as a physical constraint to radicle protrusion. On the other hand, Watkins and Cantliffe (1983) noted that mechanical restriction to embryo growth could occur due to the presence of a hard internal part of seed including endosperm, which usually occurs in seeds that do not have hard seed coats. Thus, the weakening of the endosperm lowers its resistance to radicle penetration when combined with an increase in the growth potential, allows the seed to germinate (De Miguel and Sánchez, 1992; Sánchez and de Miguel, 1997). Nomaguchi e*t al.* (1995) also found that the physical strength of the endosperm was a mechanical barrier for embryo growth in tomato and pepper seeds.
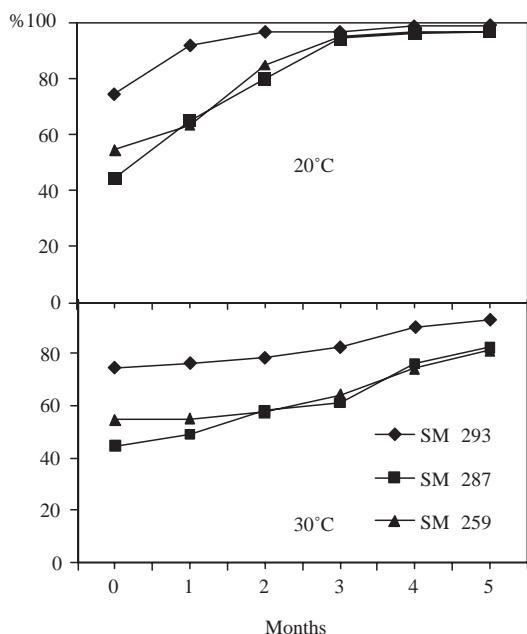
Figure 1   Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds kept at 20° and 30 °C for 5 months.
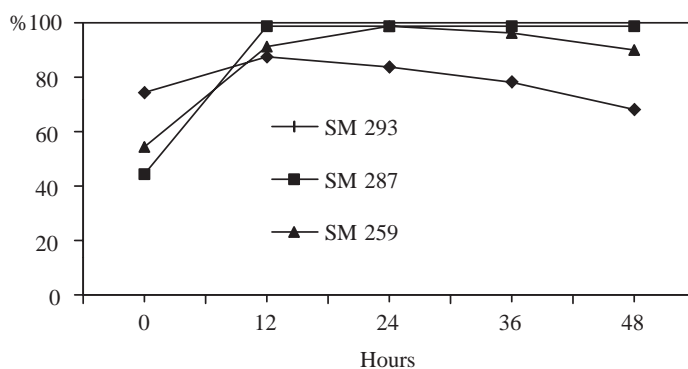
Figure 2   Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds after soaking for 12-48 hours.

It was noticed that longer soaking tended to decrease germination especially in SM 293. It may be due to water trapped in tissue between the embryo and seed coat creating an oxygen barrier as in the case of *Datura ferox* and *D. stramonium* seeds (Reisman-Berman *et al.,* 1989). In addition, the moistened seed coat may also limit the leaching of inhibitors from the embryo (Porter and Wareing, 1974). In this study, long soaking tended to enhance the destruction of embryo (rotten). Norton (1986) concluded that anoxia caused by prolonged soaking of seeds may result in irreversible injury due to accumulation of toxic metabolites.

Pre-washing in running water for 30 minutes was able to increase germination of all dormant accessions to more than 90% (Figure 3). Pre-washing treatment was able to overcome the dormancy of the accessions, which may be due to the presence of inhibitors on the seed coat. Moreover, Geneve (1998) has also summarized that germination inhibitors have been found commonly in seeds of fleshy fruit of *Solanum* sp.

Pre-heating at 50°C for only one day was able to break dormancy of these seeds (Figure 4). Bewley and Black (1982) noted that high temperature frequently enhanced the degradation of the seed tissues. As a result, the energy supply to embryonic axis may increase and diffusion into and out of the seeds by such things as water,

oxygen, inhibitors and carbon dioxide, may be easier, and hence, it promotes germination.

It was noticed that lower temperatures needed longer times to increase germination of the dormant accessions. On the other hand, exposure of these accessions to higher temperature (55°C) tended to increase the number of fresh ungerminated seeds. It may cause seeds to go into secondary dormancy. Induction of the secondary dormancy is a process highly dependent on temperature and is among other things, characterized by a closing of favourable germination temperature ranges (Hilhorst and Karssen, 1992). In addition, they have also reported that the seeds entering secondary dormancy decreased responsiveness to GAs and had physical changes.

The effects of pre-chilling varied with accession and time of pre-chilling. In one *S. torvum* (SM 259), pre-chilling for 5 days was able to break dormancy while the other *S. torvum* (SM 287) and *S. stramonifolium* (SM 293) needed only one day to break dormancy (Figure 5). Duration of moist-chilling to release embryo dormancy is influenced by factors such as covering structures and inhibitors (Khan, 1997). In the case of SM 293 and SM 287 the constraints may be lighter than those of SM 259. Therefore, these two accessions needed only a shorter time compared with SM 259.
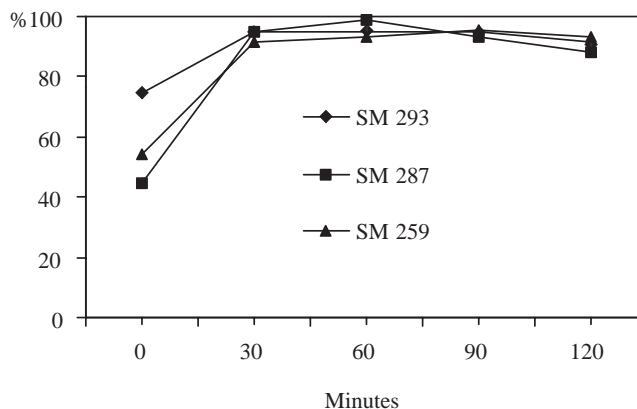
Figure 3 Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds after pre-washing for 30-120 minutes.
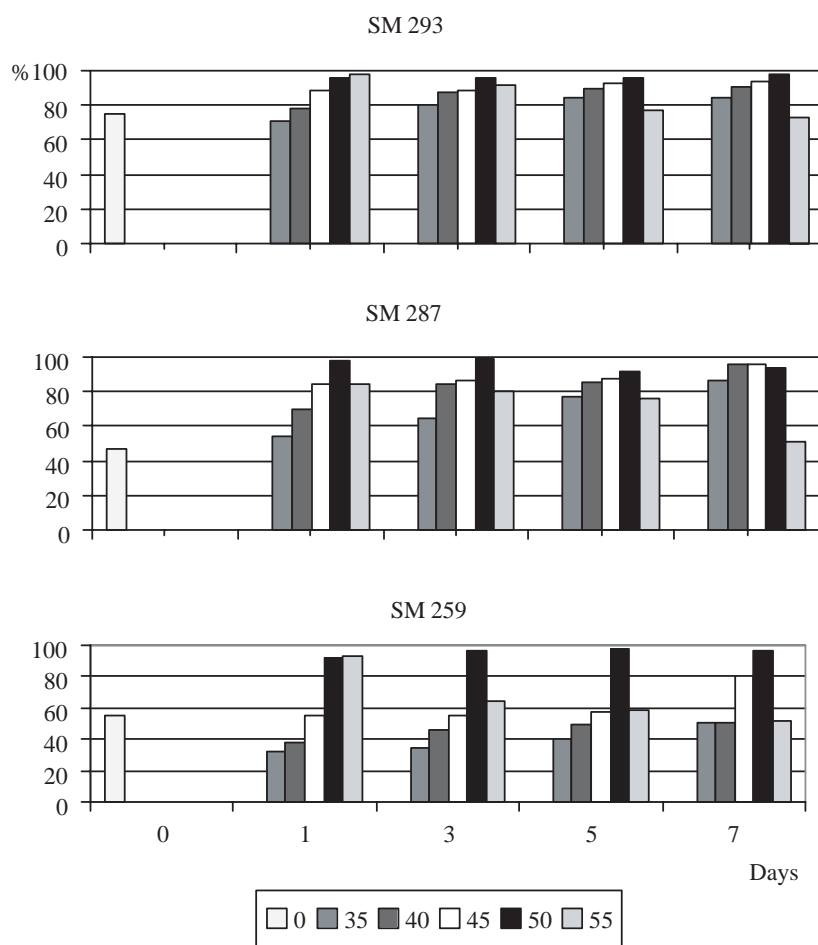
Figure 4 Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds
after pre-heating at 35° - 55 °C for 1-7 days.
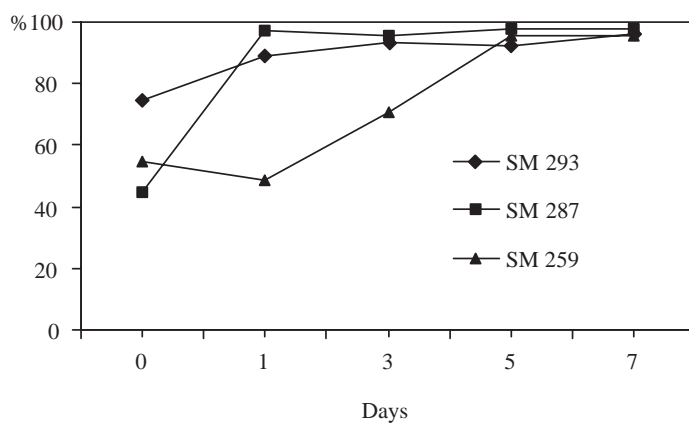
Figure 5 Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds
after pre-chilling treatment at 5°C for 1-7 days.

The ability of pre-chilling to release dormancy probably due to various metabolisms that occur during these treatments such as increasing the level and responsiveness of endogenous gibberellins (Hilhorst and Karssen, 1992) but substantial decreasing in ABA level (Bewley and Black, 1982).

When seeds were germinated with KNO_3 (as moistening agent) at various concentrations it was found that the germination percentages significantly decreased with increased "KNO_3 concentration. In general, KNO_3 treatment at 0.1% was able to overcome the donnarcy" of all accessions while concentration of 0.2% was still effective to break dormancy of SM 293 and SM 287 (Figure 6). It has been hypothesized that application of KNO_3 may accelerate water and oxygen uptake as well as improve seed nutritional status such as amino acids (McIntyre *et al.,* 1996).

In this study, however, the application of low concentration of KNO_3 (0.1%) as moistening agent gave the highest germination and the lowest in fresh ungerminated seeds, which indicated that KNO_3 was very effective at low concentration for breaking dormancy. Xia and Kermode (2000) also noted that low nitrate concentration was more effective in breaking dormancy. In contrast, they also pointed out that positive effects of this chemical are not always observed due to the excess of salt on substrates. Consequently, the osmotic potential of the substrates was greater than that of the seeds so that water would not enter into the seeds. In the case of this study, the higher concentration of KNO_3 caused stunted seedlings.

In general, GA_3 treatment at 0.01% was able to increase germination of all dormant accessions to almost 100% (Figure 7). Therefore, based on the gibberellin concentrations applied, the dormant accessions seemed to have a weak physiological dormancy (ISTA, 2003).

It was hypothesized that gibberellins induce a large increase in the activity of hydrolytic enzymes, supporting (galacto) mannan hydrolysis required to decrease the mechanical resistance of the endosperm and permit embryo growth (Groot *et al.*, 1988). In addition, gibberellins perhaps also facilitate water uptake due to alteration of cell wall extensibility (Hilhorst, 1995) and enhance the action of low phytochrome far-red (Pfr) (Bewley and Black, 1982). Watkins and Cantliffe (1983) have shown that the endosperm layers of pepper and tomato seeds are also weakened by application of GA_3. Thus, it is likely that germination of Solanaceous plants is mediated by endosperm degradation (Hilhorst, 1995; Leubner-Metzger 2003). Hilhorst and Karssen (1992) confirmed that the majority of angiosperm families that retain endosperms in mature seeds seem to have high
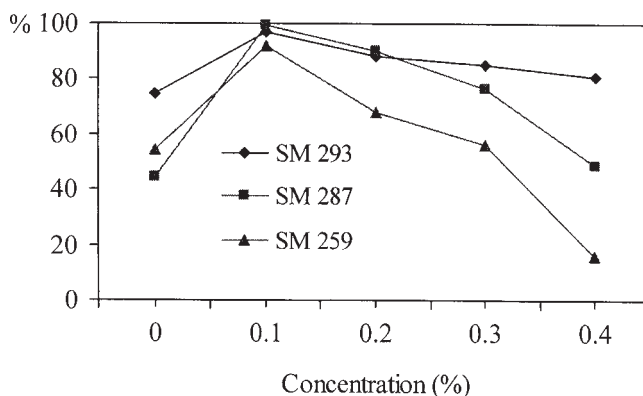
Figure 6 Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds treated with KNO_3.
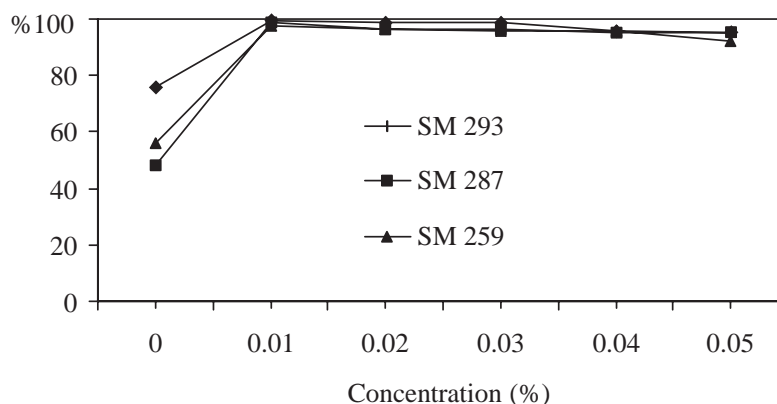
Figure 7 Germination (%) of *S. stramonifolium* (SM 293) and *S. torvum* (SM 287 and SM 259) seeds treated with GA$_3$ at 0.01–0.05%.

contents of (galacto) mannans in their endosperm cell wall.

Of all the breaking dormancy methods tested, it is suggested that 12-hour soaking, 30-minute pre-washing, 0.1% of KNO$_3$ or a one-day pre-heating at 50°C were effective and efficient to break seed dormancy in some *Solanum* sp. (*S. stramonifolium* and *S. torvum*) in the laboratory, but pre-heating treatment was more appropriate for commercial purposes. However, seed companies usually keep a large stock of seeds, therefore, further studies on storability of pre-heated seeds are necessary, especially for commercial varieties.

In addition, on the basis of the responses to the breaking dormancy methods tested, it was indicated that the dormancy mechanisms involved in *S. stramonifolium* and *S. torvum* may be due to the mechanical resistance of endosperm, presence of inhibitors on the seed coat and physiological state of the embryo. Thus, all dormant accessions, according to Baskin and Baskin (2004), may belong to the physiological dormancy class of non-deep level which is usually found throughout the Angiospermae, including *Solanum*. For clarification, however, it still needs other methods such as endogenous hormone analysis or removal of the seed coat.

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