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Spin drying of imbibed seeds enhances the vigour potential of hydroprimed seeds by influencing the anatomical and molecular changes of seeds

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Abstract
Seed vigour determines productivity of crops by influencing germination and vigour of seedlings. Seed vigour can be improved by several quality enhancement procedures including priming which improves emergence and establishment of seedlings. Several seed priming methods viz., Osmopriming, hydropriming, halopriming, biopriming etc., have been developed to meet diverse requirements based on seed quality, size and viability. However, drying of large quantity of primed seeds is laborious and time consuming process. In high value, low volume crops, small quantity of seeds are required for sowing an acre of land by farmers where manual soaking and drying is a tedious process. Spin drying helps to dry the seeds quickly by removing the free water, without any damage to the seeds and thereby enhances seed germination in a wide range of crops. Percent increase in seed germination of spin dried seeds over control was found to be 40.6 (onion), 29.5 (chilli), 41.6 (beetroot), 18.6 (muskmelon), 17.4 (brinjal), 40.9 (tomato), 28.0 (carrot), 29.0 (bitter gourd), 65.9 (bhendi), 45.09 (snak egourd), 11.3 (radish) and 16.2 (ridge gourd).

Keywords: Dioxomalybdenum, *Synthetic Tetraza*, macrocyclic ligands, acetylacetonate

1. Introduction
Seed vigour is the first and foremost factor which decides the productivity potential of the seeds by way influencing two aspects *viz.*, seed germination and seedling vigour. The physiological vigour of seeds could be improved by various seed enhancement techniques. Among the techniques, priming is the foremost technique which improves the emergence and establishment of the seedling under wide range of environmental condition.

Based on various attempts by Ellis (1963) and other workers, Heydecker (1973) developed a new seed invigouration technique termed as “Seed priming”. It is physiologically complex yet simple in concept. Heydecker (1975) described the technology in his article entitled “Invigouration of seeds” in “Seed Science and Technology”. He used chemically inert PEG 6000, which has osmotic properties. Seeds in contact with an aqueous solution of this chemical begin to take up water in the normal way but cease to do so once they have reached equilibrium with the osmotic potential of the solution. It permits the seed to go through all the essential preparatory processes of germination but prevents cell elongation and in consequence radicle emergence, even after weeks of contact between seeds and osmotic solution. Moreover, during the waiting period at the osmotic barrier the “Slower” seeds tend to catch up with the Faster” ones so that the subsequent germination is more uniform, especially at lower germination temperatures, than that of untreated seeds.

Thus it can be defined that seed priming is a pre-sowing treatment that involves the controlled hydration of seeds sufficient to allow pre-germinative metabolic events to take place but insufficient to allow radicle protrusion through the seed coat (Heydecker *et al.*, 1977). Subsequently since different media have been experimented to bring about controlled pre-germintive metabolism in seeds, soaking of seed in different solution has been referred to respective priming treatment.

2. Types of Priming
1. Osmopriming
Soaking the seeds in osmotic solutions. Polyethylene glycol (PEG) is also commonly used as a priming osmoticum. This process is known as osmopriming. PEG is advantageous because it is chemically inert and does not have adverse effects on the embryo (Cantliffe, 1983).

The large size of the PEG molecules prevent its penetration into seed tissues (Brocklehurst and Dearman, 1984), thus reducing toxic side effects on seeds (Heydecker and Coolbear, 1977). The disadvantages of using PEG are its relatively high cost, problems with removing it from the seed coat after priming, obtaining uniform aeration during priming, and environmental hazards with PEG disposal.

2. Hydropriming

Soaking in water for the predetermined period of time, followed by drying back to original moisture content. In hydropriming, the water availability is not limited to the seeds. Therefore, the process must be arrested at a specific time to prevent the onset of phase III of germination.

3. Halopriming

Halopriming involves soaking of seed in salt solutions. Compared to PEG, salts are easier to aerate and to remove from the seed after treatment, they are less costly and may also provide a nutritional effect. The seed size, structure, biochemical constitution, position of seed protecting layers, the type of salt and soaking time are the principal factors influencing ion penetration into the embryo. High ion concentration in the embryo may affect germination adversely when inorganic salts are used as osmoticum in the seed priming process.

4. Solid matrix priming

This consists of mixing seeds with an organic or inorganic carrier and water for a period of time. The moisture content of the matric is brought to a level just below what is required for radicle protrusion. Seed water potential is regulated by the matric potential of the seed and during priming the water is largely held by the carrier.

5. Biopriming

It is nothing but soaking the seeds in solution made up of beneficial microorganisms such as *Pseudomonas fluorescens*, so as to facilitate advancement of pregerminative metabolism as well as seed inoculation with beneficial microbes.

3. Standardization of seed priming technique

Optimization of the best methodology of seed priming is the key to the success of the priming treatment, which includes standardization of priming method, osmotic potential of the priming media and duration of the treatment. As the germination and growth proceeds the resistance to drying of the embryo decreases. On the other hand the degree of the effect of treatment is claimed to be high, when the embryo is in more advanced stage of germination at the time of drying. The optimum duration of treatment should be a compromise between the two conflicting tendencies (Bewley and Black, 1978). According to Frett *et al.*, (1991) optimum priming conditions (priming agent, treatment duration, water potential of the priming solution etc.) should be assayed for each cultivar.

In order to standardize the optimum priming methodology for tomato, egg plant and chilli, Venkatasubramaniam and Umarani (2007) [13] conducted a study involving four different priming methods, five durations; two concentrations of priming media in osmopriming (PEG 1000) and four concentrations of halopriming (KNO_3 and NaCl). The comparison of optimum duration of each priming method revealed that for tomato seeds was hydropriming for 48 h (in double the volume of water) where as, for chilli and egg plant seeds, sand matricpriming at 80 % water holding capacity of sand for three days were adjudged best in terms of rate and uniformity of germination. (Fig. 1 & 2).

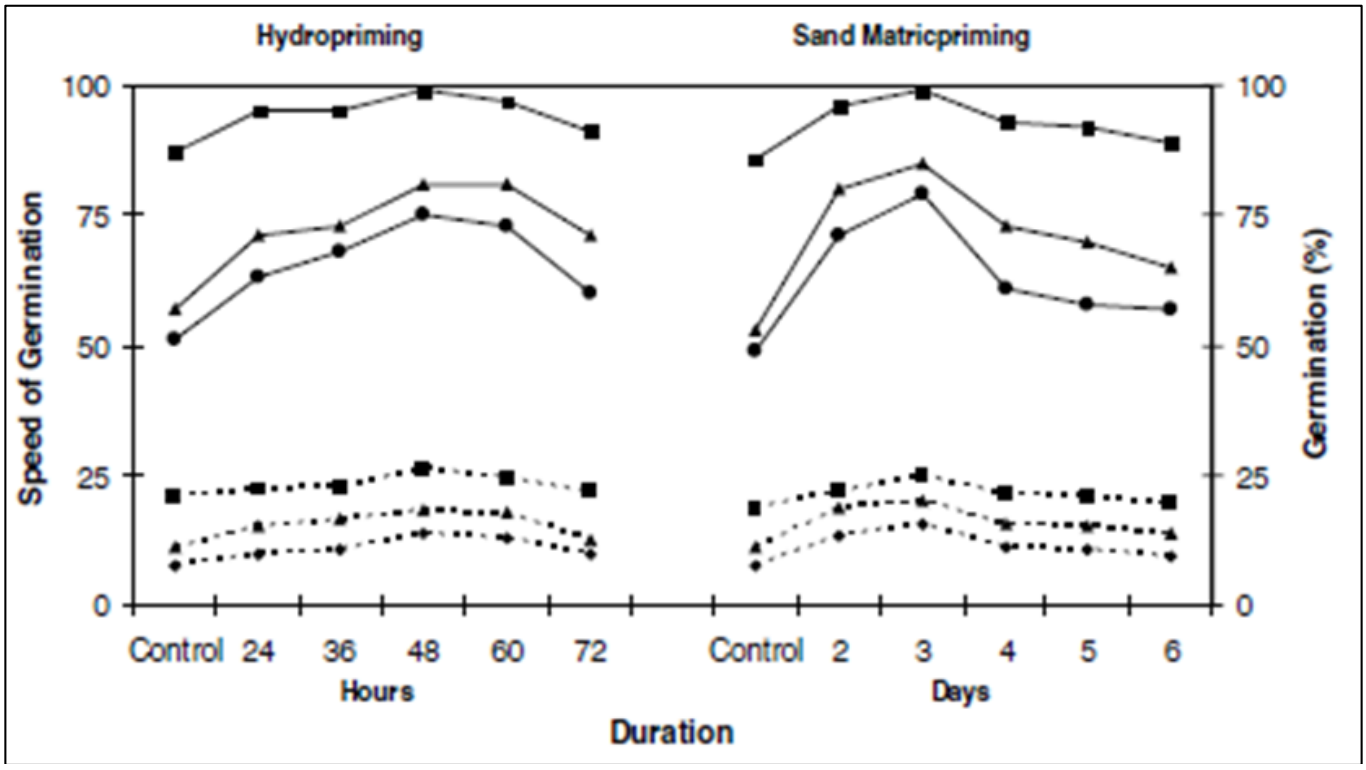


Fig 1: Effect of different methodologies of hydropriming, sand matricpriming and osmopriming on speed of germination and germination (%) of tomato, eggplant and chilli. (Venkatasubramaniam and Umarani, 2007) [13]

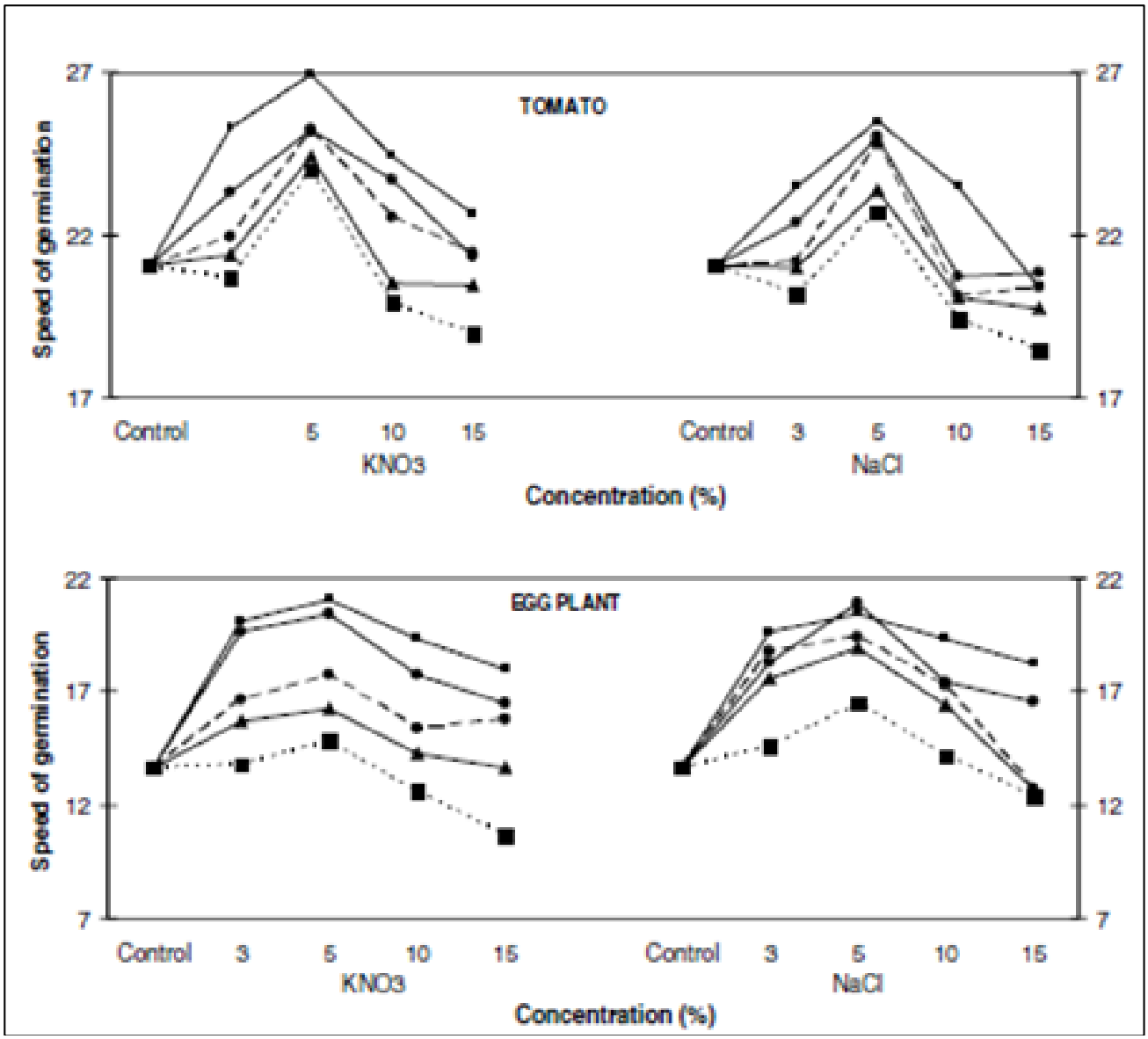


Fig 2: Effect of different durations and concentrations of KNO_3 and NaCl (halopriming) on speed of germination and germination (%) of tomato, eggplant and chilli. (Venkatasubramaniam and Umarani, 2007) [13]

The results established that accurate choice of priming duration and method and are important to attain maximum benefits from seed priming techniques. Similarly, Nirmala and Umarani (2007) [13] reported that for okra, sand matric priming (3 h in 60 % WHC of sand) was found to be the best, while for beetroot, hydropriming (12 h in water at double the volume of seed) was optimum.

4. Physiological basis of seed priming

i. Pre-germinative metabolism and seed priming

In orthodox seeds the dry seed, ready for germination, exhibits a triphasic pattern of water uptake (*Fig. 3*) (Bewley and Black, 1978). Phase I is the rapid water uptake that is largely a consequence of the matric forces exerted by the seed. During this phase, DNA and mitochondria are repaired and proteins are synthesized using existing messenger ribonucleic acid (mRNA) (McDonald, 2000). Phase II is a lag phase, in which seed water potential is in balance with that of the environment. In this phase the major metabolic changes preparing the embryo for germination occur, including the synthesis of mitochondria and proteins by new mRNA. Thus, phase II is also called activation phase. In phase II the germination process is completed *stricto sensu*; however, only in phase III the radicle emerges and the so called *visible germination* can be assessed (Bewley and Black, 1978; Bradford, 1995). In phase III, a second rapid uptake of water occurs. Phase I and II represent the most delicate phases for the process of germination and are crucial for a successful seed priming (Bewley, 1997).

The triphasic model has deep implications for seed viability. The seed tolerates a return to the initial moisture necessary for storage, a process know as drying-back or re-drying, when it is in phase I or II, whereas phase III is too advanced to allow a drying-back without seed damage (Taylor *et al.*, 1998) [10]. According to the triphasic model, the start of germination is associated with a rapid synthesis of RNA and proteins, to carry out the repairing processes before the beginning of DNA replication (Osborne, 1983). Seed priming typically involves an extension of phase II, which in turn permits the completion of more repair processes (Bray, 1995), and allows the drying-back, which is necessary when the final sowing is postponed (industrial seed production).Differences in the imbibition phases of a normal germination process compared to priming followed by drying-back and subsequent germination are shown in *Figure 4* (Bradford and Bewley, 2002).

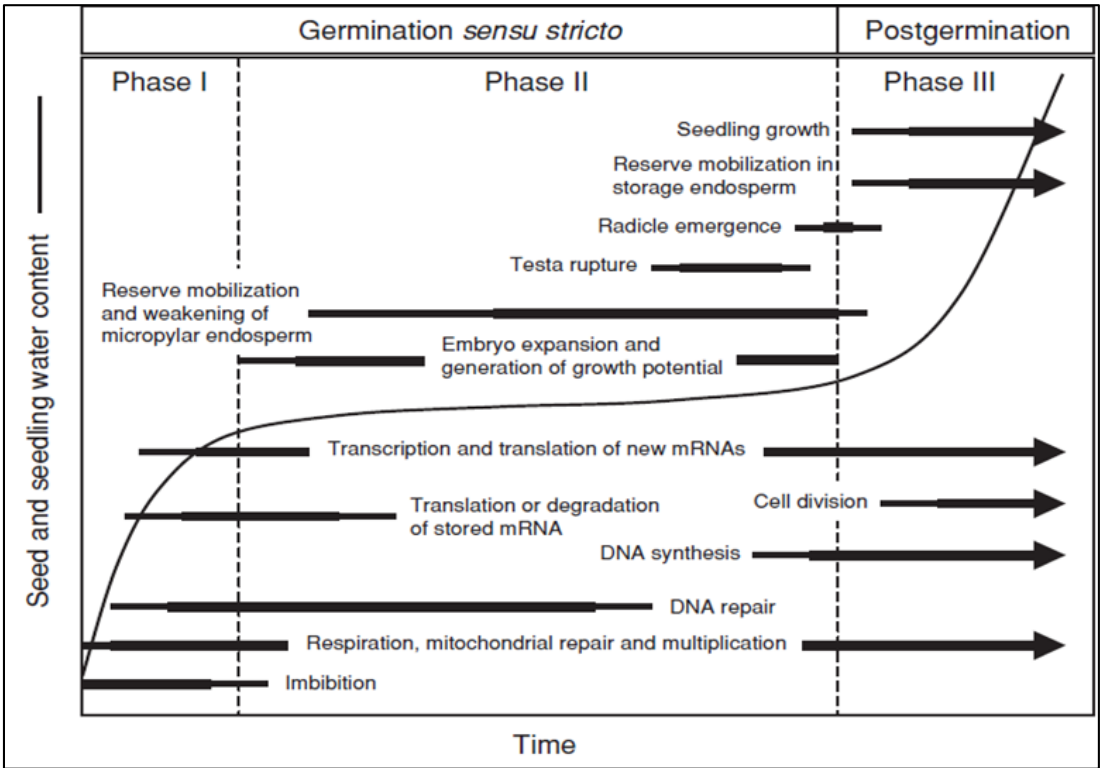


Fig 3: Events associated with germination and post-germination phases. The time for events to be completed varies from several hours to many weeks, depending on plant species, germinating conditions and seed lot quality. From: Bewley (1997), Seed germination and dormancy. Plant Cell. 9:1055-1066; www.plantcell.org. Copyright America Society of Plant Biologists.(©Copyright G. Di Girolamo and L. Barbanti, 2012Licensee PAGE Press, Italy Italian Journal of Agronomy 2012; 7:e25 doi:10.4081/ija.2012.25)

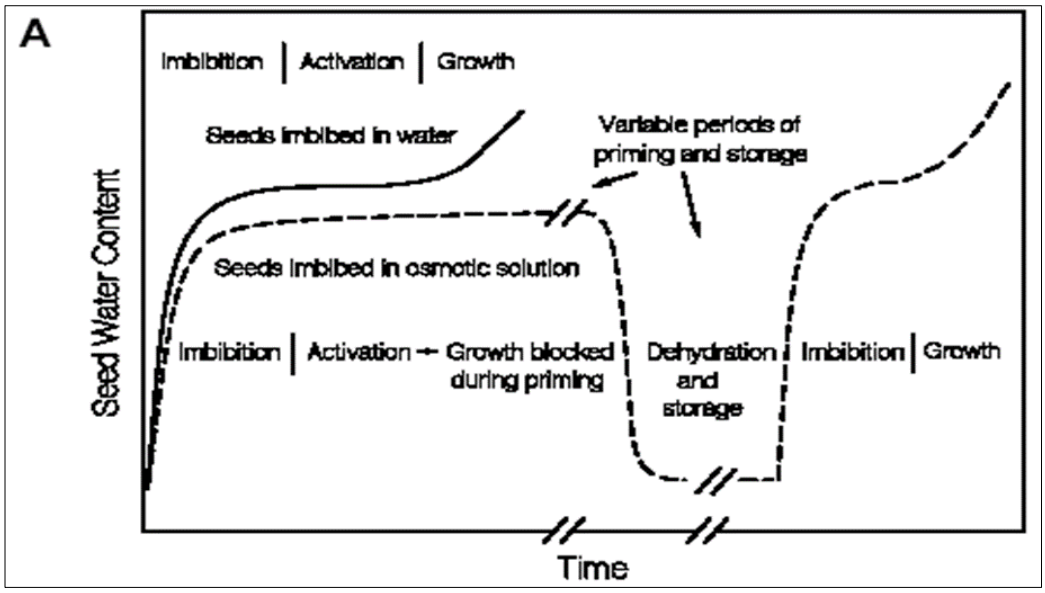


Fig. 4: Time course of a standard germination process compared to a germination process including seed priming, dehydration and storage. Seed soaking in water determines a normal course of imbibition, activation and radicle protrusion (phase I, II and III of the germination process, respectively). Priming with an osmotic solution extends the activation phase (phase II) for a certain time, without leading to radicle protrusion (phase III). Seed dehydration after priming (drying-back) allows to store the seed for a variable time without losing the advantages achieved with priming. At sowing, the seed must be able to soak and develop the radicle. From: Bradford and Bewley (2002), Seeds: Biology, Technology and Role in Agriculture. (©Copyright G. Di Girolamo and L. Barbanti, 2012Licensee PAGE Press, Italy Italian Journal of Agronomy 2012; 7:e25 doi:10.4081/ija.2012.25)

Under favourable conditions, the postponement of phase III involved by priming plus re-drying results in a better seed performance.

ii. Molecular changes during seed priming

A critical aspect of ongoing metabolism is cell cycling. The cell cycle describes the nuclear DNA content as 2C in cells that are not preparing for nuclear division, and as 4C in cells in which DNA replication has occurred, where the constant, C, denotes the DNA content of the haploid condition. During the cell cycle four distinct phases can be identified, *viz.*, the G1 phase (2C), which is followed by the S phase, during which DNA replication occurs; after this the cells enter the

G2 phase, during which the amount of DNA remains doubled (i.e. 4C) as a result of events in the S phase, and this is followed by the phase known as G2M, when mitosis reduces the DNA content to the 2C level typical of somatic cells in the next G1 phase (*Fig. 5*).

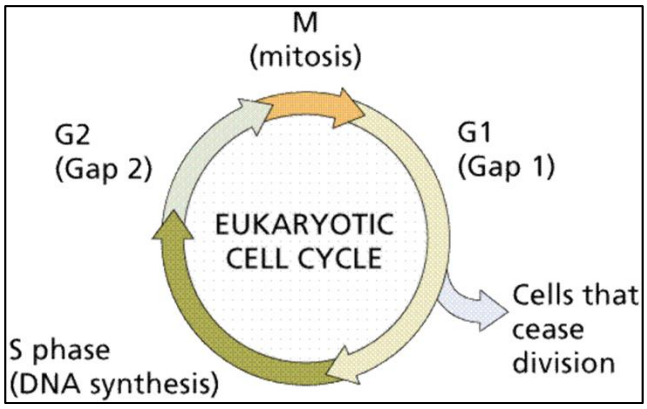


Fig 5: Cell cycle

Gurusinghe *et al*., (1999) studied the cell cycle activity during seed priming of tomato. They reported advancement of radicle meristem cells into the S and G_2 phases of the cell cycle, increasing the percentage of nuclei having a 4 C DNA content, has been reported to occur during priming. But the extent of cell cycle activity varied among different treatments and seed lots. In some lots, the percentage of 4 C nuclei in the radicle meristems prior to emergence increased in proportion to accumulated hydrothermal priming time, while in other lots, no increase in nuclear DNA content was detected. All, lots however, demonstrated rapid radicle emergence following priming. Thus, replicative DNA synthesis in radicle meristem nuclei often occurred during seed priming, but an increase in the percentage of 4C nuclei was not essential for germination advancement. It is perhaps not surprising that cell cycle activity in the meristem is not essential for rapid germination rates, since initial radicle protrusion is dependent upon cell expansion, not cell division (Haigh, 1988; Go´rnik *et al*., 1997).

4. Storability of primed seeds

The popular idea is that primed seeds which are hydrated for a longer time activating initial physiological, biochemical events of germination may have two disadvantages i) even though the seed subsequent redried, it is at a more advance physiological point than before priming and more prove to deterioration ii) the benefits of priming are lost relatively rapidly during seed storage.

On the contrary, Probert *et al.*, (1991) [9] has presented evidence that priming results in fundamental change in the resistance to ageing. However, the decrease in storability may be a feature of extending the pre hydration period too long (Tarquis *et al.*, 1992) Brocklehurst *et al.*, 1984) further asserts that successful storage of primed seed depends on both careful drying and well regulated storage condition. Better performance of primed seeds of tomato, brinjal and chilli (Venkatasubramaniam and Umarani, 2010) [12], beetroot and okra (*Fig. 6*) (Nirmala and Umarani, 2014) [7] have been reported upto six months of storage. These results not only prove that seed priming does not reduce the storage life of seeds but also underscores that the effects of priming are retained by the seed even after six months of storage.

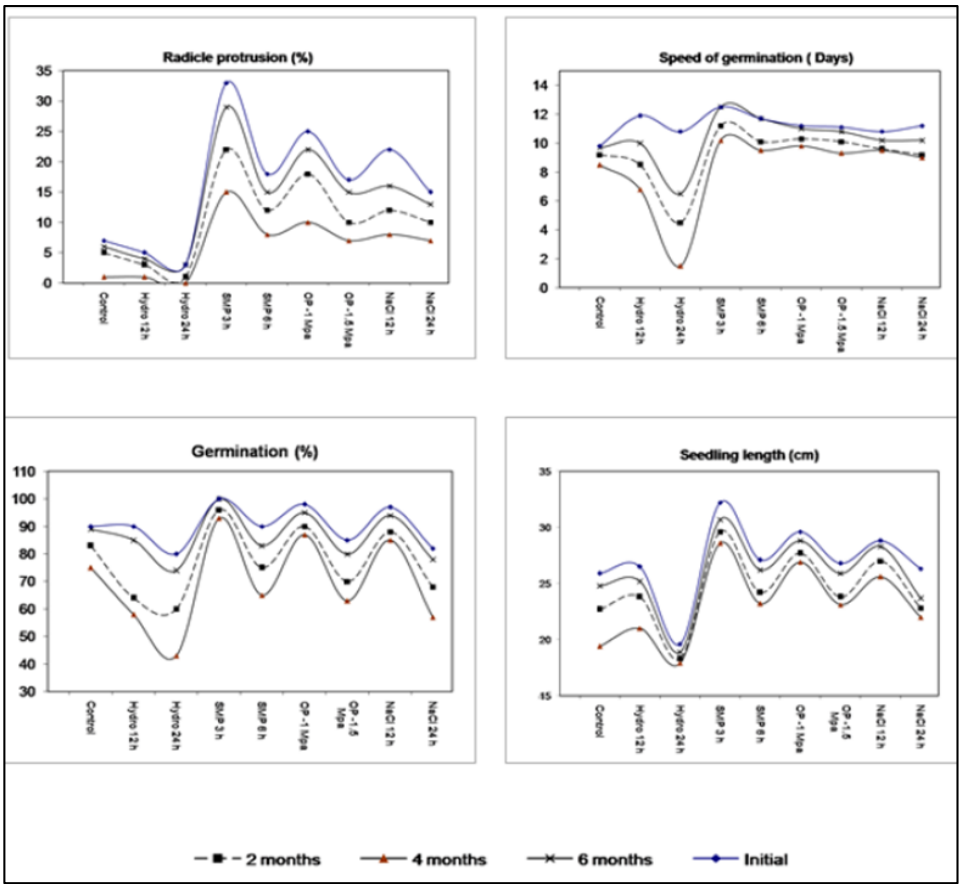


Fig 6: Effect of different methodologies of hydropriming, sand matricpriming and osmopriming on speed of germination and germination (%) of tomato, egg plant and chilli. (Nirmala and Umarani, 2014) [7]

5. Spin Priming

In the last two decades, seed priming, an effective seed invigoration method, has become a common seed technique to increase the rate and uniformity of emergence and crop establishment in most crops especially in advanced countries. However, drying of large quantity of primed seeds (imbibed condition) is labourious and time consuming process. The spin drying helps the seeds to dry quickly to a particular level by removing the free water, without any damage to the seeds. Practically, in high value low volume crops, small quantity of seeds are required for sowing an acre of land by farmers or in the case of seed industry also manual soaking and drying is a tedious process.

In Department of Seed Science and Technology of Tamil Nadu Agricultural University, seed priming studies were conducted by using a 'Seed Spin Priming cabinet' by Chandan (2017) [3]. The cabinet consists of 'priming drum' with holes for draining water. The seeds to be primed are tied very loosely in a cloth bag and placed in the drum filled with water. The drum can also be turned clock wise and anticlock wise to agitate the seeds during the soaking period of providing aeration during the seed priming process.

On completion of the soaking period, the 'SPIN' button has to be pressed by setting the duration of spin drying. Immediately, the solution in seed priming drum gets automatically drained and the drum starts to 'spin' centrifugally at 600 rpm, leading to partial drying of seeds, due to removal of surface moisture. The seeds are removed from the drum and subjected to shade drying so as to reach the original moisture content. The major advantage of the machine is automatic draining of the priming solution and 'hands free' partial drying of the seeds, which enables hassle free handling of seeds for further drying to original moisture content.

The seeds were subjected to conventional hydropriming which involves manual soaking, draining and shade drying and it was compared with mechanized seed priming and spin drying for 1, 2, 3, 4 and 5 min. Hydropriming and spin drying for 1 min. was standardized for onion, chilli and beetroot; 2 min. for muskmelon, brinjal, tomato and carrot; 3 min for bittergourd; 4 min for bhendi, snakegourd and radish; 5 min. for ridgegourd.

Significant increase was observed in seed germination of all the crop seeds experimented. The percent increase in seed germination recorded over control was found to vary based to the crop species and initial seed germination of the crop seeds. The percent increase in seed germination of spin dried seeds over control was found to be 40.6 (onion), 29.5 (chilli), 41.6 (beetroot), 18.6 (muskmelon), 17.4 (brinjal), 40.9 (tomato), 28.0 (carrot), 29.0 (bittergourd), 65.9 (bhendi), 45.09 (snakegourd), 11.3 (radish) and 16.2 (ridgegourd). (Plates 1- 3). There was also concomitant increase in seedling vigour, interms of speed and uniformity of seed germination, seedling growth, dry matter production as well as vigour index. The results envisage that the low and medium vigour seed lots which do not pass the "Indian Minimum Seed Certification Standards" can be well subjected to the TNAU Spin Priming method so as to enable improvement in seed germination percentage and eventual marketing.

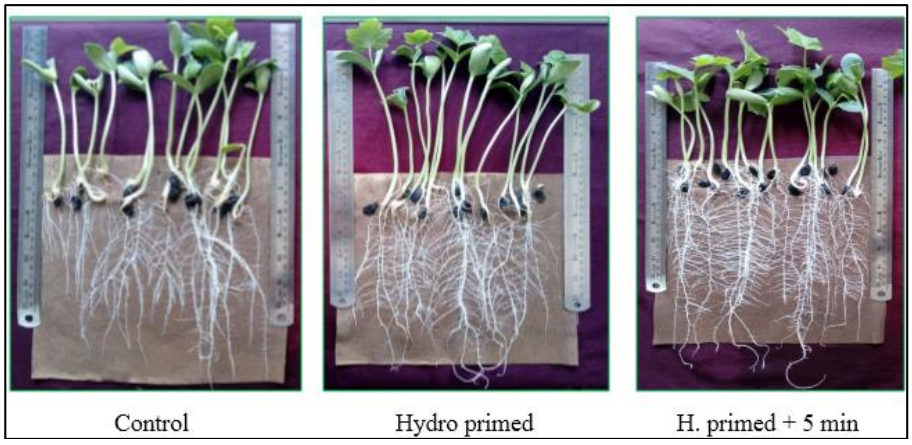


Plate 1: Effect of 'hydro priming and spin drying' on seed germination and seedling growth of ridge gourd

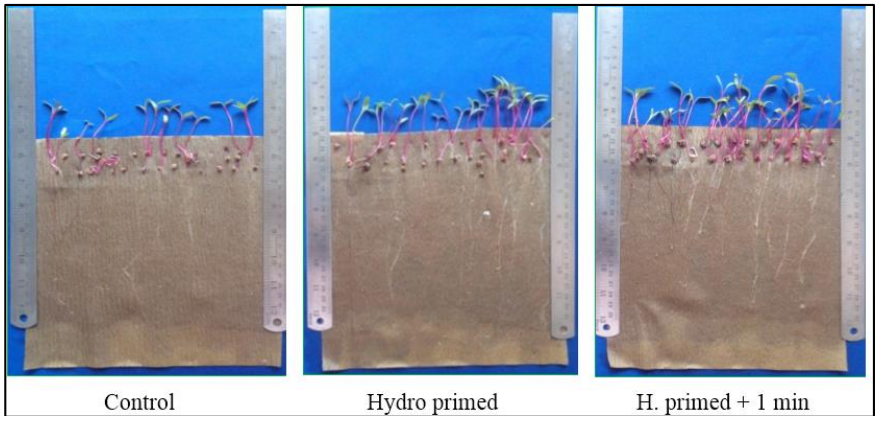


Plate 2: Effect of 'hydro priming and spin drying' on seed germination and seedling growth of beetroot

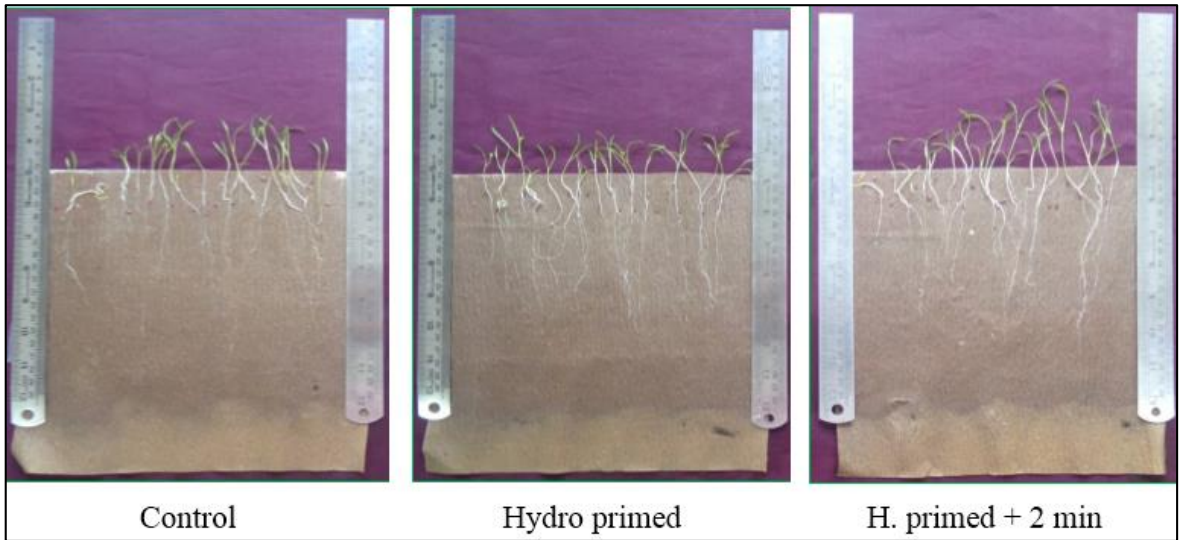


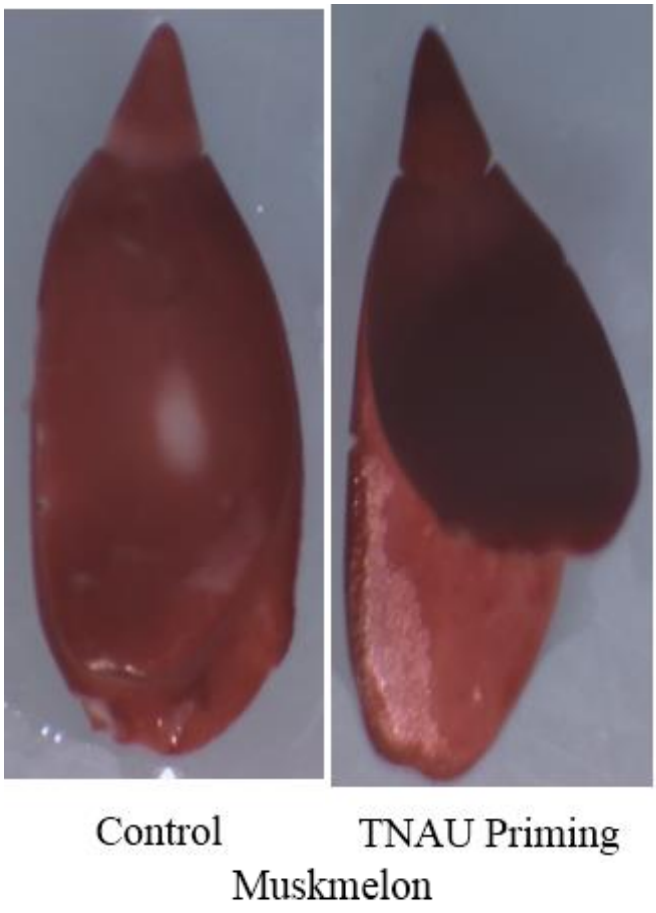
Plate 3: Effect of 'hydro priming and spin drying' on seed germination and seedling growth of carrot

Table 1: Seed Priming Protocol to enhance the anatomical and molecular potential of seeds

S. No	Crop	Duration	
		Hydropriming (h)	Spinning (min)
Vegetables			
1.	Tomato	48	2
2.	Brinjal	48	2
3.	Chilli	48	1
4.	Carrot	36	2
5.	Onion	24	1
6.	Bittergourd	24	3
7.	Snakegourd	24	4
8.	Ridgegourd	24	5
9.	Muskmelon	24	2
10.	Radish	12	4
11.	Bhendi	12	4
12.	Beetroot	12	1

Anatomical changes

Anatomical study by using Euromex Holland's Image Version 1.0 was conducted for the hydroprimed and spin dried seeds along with hydroprimed + shade drying and control seeds were subjected to The results indicated that high seed viability per cent was observed in hydroprimed + spin dried seeds for 1 min., which accounted 39.3, 30.7 and 39.7 per cent in onion, chilli and beetroot; hydroprimed and spin dried seeds for 2 min. registered 17.7, 19.3, 76.0 and 41.1 per cent in muskmelon, brinjal, tomato and carrot; likewise 3 min. in bittergourd registered 31.3 per cent; 4 min. in bhendi, snakegourd and radish registered 50.7, 26.7 and 22.1 per cent; 5 min. in ridgegourd registered 10.3 per cent over non primed seeds. Apart this, Spin dried seeds of tomato showed 116.7 and 22.4 per cent increase in length of radicle and plumule + hypocotyl length, respectively over non primed seeds. Brinjal spin dried seeds showed hike in radicle length and plumule + hypocotyl length of 35.8 and 95.3 per cent respectively over non primed seeds. In chilli, spin dried seeds radicle length and plumule + hypocotyl length was increased 42.1 and 3.2 per cent, respectively over non primed seeds. The average length of cotyledon was increased in spin dried seeds of onion (89.0 per cent) over nonprimed seeds. Radicle length and cotyledon length of spin dried seeds of bhendi was increased about 95.1 and 4.7 per cent respectively over nonprimed seeds. Muskmelon spin dried seeds recorded 28.7 and 9.3 per cent increase in length of radicle and width of cotyledon over non primed seeds. Ridgegourd spin dried seeds revealed 86.2 and 45.5 per cent increase in length of radicle and width of cotyledon over non primed seeds. Bittergourd spin dried seeds showed 43.6 and 17.3 per cent increase in length of radicle and width of cotyledon over non primed seeds. Snake gourd spin dried seeds revealed 75.2 and 17.7 per cent increase in length of radicle and width of cotyledon, respectively over non primed seeds. Spin dried seeds of radish showed 43.3 and 59.0 per cent increase in length of radicle and width of cotyledon respectively over non primed seeds. Beetroot spin dried seeds recorded 282.7 per cent increase in average length of embryo over nonprimed seeds. Spin dried seeds of carrot recorded 117.9 per cent increase in width of the cotyledon.



Control TNAU Priming

Ridge gourd

Molecular Changes

Protein profiling is a useful tool to ascertain the advanced state of molecular transition during priming. Variations in the protein profile of nonprimed, conventionally primed and spin primed seeds were observed crops studied. In bhendi, hydroprimed + 4 min spin dried sample showed the protein band (10 and 11) of size 25.1 and 22.3 kDa was expressed twice the quantity than the control and hydroprimed + shade dried samples. In bittergourd the control sample protein band of size 26.3 kDa expressed at half the intensity of the hydroprimed + spin dried sample. In ridgegourd, the protein of size 33.8 kDa, was appeared in hydroprimed + spin dried (3 min.) and hydroprimed + shade dried sample. In muskmelon, the protein size of 30.2 kDa observed in hydroprimed + shade dried sample was disappeared in control and the protein band with the size of 22.9 kDa was disappeared in hydroprimed + spin dried sample. In snakegourd, protein band (3, 4 and 5) of size 28.1 kDa, 26.9 kDa and 21.3 kDa expressed approximately thrice the quantity with condensed staining in hydroprimed + spin dried samples than the hydroprimed + shade dried sample and control.

The TNAU Spin Priming technology, has been proven to increase the seed germination as well as vigour of the seeds. The anatomical and molecular basis of such improvement has also been proved with help of modern tools such as high magnification microscope and protein profiling. The technology has immense scope to be practiced at farmers level as well as in seed companies to invigourate the seeds which inturn will be effective in increasing the productivity of the crop.

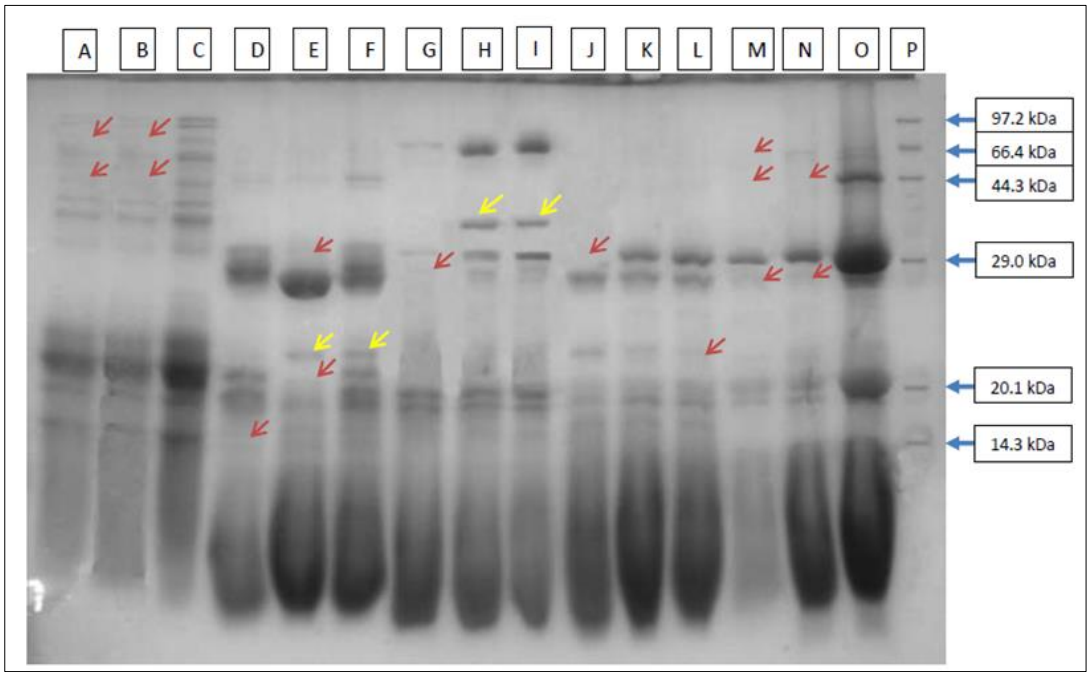


Plate 4: SDS-PAGE profile of the seeds subjected to priming treatments

The importance of seed priming process has been traditionally based on the seed imbibitions period and process. However, the studies on the effect of spin drying of seeds have provided sufficient data to underscore that seed dehydration is more critical to achieve the maximum seed invigouration effects in seeds subjected to seed priming. Therefore, in addition to the crucial effects of progression of seed imbibitions from Phase I to Phase II, progression of dehydration also has an immense effect on metabolic processes associated with invigouration process, which has showed corresponding improvements in seed germination characteristics. In future, further experiments have to be taken up to study the effect of dehydration on other metabolic events such as enzyme synthesis, breakdown of stored food reserves and protein synthesis etc., to ascertain the criticality of dehydration of seeds during the process of seed priming.

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