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Evaluation of anatomical potential of primed and non-primed seeds of solanaceous vegetables

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Abstract
The experiments were conducted at the Department of Seed Science and Technology during 2017 on mechanization of seed priming by standardizing the spin drying duration of the Seed Priming cabinet, to enhance the seed viability per cent, embryonic growth of the solanaceous vegetables *viz*., brinjal, chilli and tomato seeds. 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 (48 hrs) and spin drying for 1 min. was standardised for chilli, likewise, 48 hrs + 2 min. for both brinjal and tomato. The hydroprimed and spin dried seeds along with hydroprimed + shade drying and control seeds were subjected to anatomical study by using Euromex Holland's Image Version 1.0. The results indicated that high seed viability per cent 19.3, 30.7 and 76.0 was observed in hydroprimed + spin dried seeds of brinjal, chilli and tomato respectively, over non primed seeds. Apart this, Spin dried seeds of all the above said crops showed significant difference in radicle and cotyledon length over nonprimed seeds. In which, 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.

Keywords: fenugreek, hematological, *O. niloticus*, serum

Introduction
Vegetables are considered as essential building blocks of any diet. They are good sources of vitamins, minerals and complex of carbohydrates with low fat and high fibre required for a healthy and active life. They constitute an important component of a balanced diet. Vegetables in addition to their vital role in health and nutritional security of human being play a major role in improving their national economy. In the last two decades, seed priming, an effective seed invigouration 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. The advantage of seed priming is increased seed germination percentage, better speed of emergence and improved tolerance to abiotic stress (Karimmojeni *et al.*, 2012) [1].

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. Therefore, mechanization of seed priming process is required to enable easy implementation of the "seed enhancement technique" both at farmer's level as well as by the seed industry. Top loading washing machine is household machine and that is easily available to farmers. The characteristics of this machine is automatic draining of priming solution as well as spin drying options, which are two basic functions that are found to be tedious to practice in a traditional manual priming methodology.

The basic need of adaptation involves standardization of automatic draining of priming solution as well as spin drying duration for the soaked seed. After the standardization of spinning duration efforts were taken to evaluate the anatomical changes of best spinning duration seeds over conventional priming and control seeds.

No reports have been published about spin priming of seeds previously in any of the crops. In the current study, soaking in water (hydro priming) is adopted with required adaptation in drying process amenable for mechanization of seed priming process. Therefore, the literatures relevant to image analysis is reviewed. Sahoo *et al.* (2000) [2] reported varietal discrimination of sunflower seeds using machine image approach. Senthil kumar (2003) [3] grouped the different varieties of lucerne seeds based on the image analysis of seed morphology. Thangavel (2003) [4] described machine vision system to discriminate the varieties by their seed morphological characters in sorghum. Eevera (2003) [5] in rice and Vimal (2003) [6] in groundnut also used the machine vision system to discriminate the seeds.

Materials and methods

Genetically pure fresh seeds of brinjal (CO 2), chilli (K 2), and tomato (Arkavikas) were used in the experiments. Seed priming cabinet was used for standardization of spin drying duration. 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 soaked seeds for further drying to original moisture content.

Study of changes in anatomical structure of primed seeds in comparison with non-primed seeds

In this experiment first treatment was considered to Mechanized priming which implies the best spin drying duration which was standardized earlier *viz.*, hydro priming (48 h) + 1 min. for chilli, hydro priming (48 h) + 2 min. for brinjal and tomato followed by shade drying for 12 h to facilitate to reach its original moisture content. Second treatment includes Conventional priming which implies the seeds of all test crops were soaked for the crop specific duration were drained and dried back to original moisture content. Last treatment was considered to Control which were un primed raw seeds.

Seven replicates of 10 seeds extracted from each of the treatment were subjected to overnight soaking and in the following day the seed coats were removed carefully to expose the embryo. The seeds were subjected to incubation for 4 h. in a solution of 0.25 % (w/v) 2, 3, 5 triphenyl tetrazolium chloride (TTC) prepared in phosphate buffer 0.05 M, at pH 7.3. After 4 h. seeds were washed and observed for staining pattern. Later they were subjected to microscopic analysis to study the anatomy of seeds by using Euromex Microscope Hollands IMAGE VERSION 1.0. With a different magnifications for different crops were detailed as follows. The Nexius Zoom stereo microscope of Euromex enabled to examine the specimen with high-performance precision and generate three-dimensional images allowing observation for highest demanding microscopy applications. This top level zoom microscope is perfect for analysing all kinds of material surfaces or to observe and prepare biological samples. The Nexius Zoom stereo microscope is supplied with a large choice of stands, with LED illumination. Ideal for a wide variety of educational and laboratories applications. With additional lenses and objectives magnifications up to 180x can be achieved. For the current study 17.5x magnification was used. All the specimens were subjected to measure Seed viability (%) and length of the radical (mm), average length of cotyledon / plumule + hypocotyl / embryo (mm) which was estimated by using Euromex Software which was calibrated according to the magnification of the images. The data analysis was done by using completely randomised block design.

Observations

Seed viability (%)

The seeds subjected to tetrazolium staining and washed with water were observed using a dissection microscope pattern of staining was observed and recorded. The seeds were categorized as viable and non-viable by using the following assessment.

S. No	Viable seeds	Non-viable seeds
1	Complete staining	Complete non-staining
2	Staining in more than ½ the area with complete staining of embryonic axis	Staining in less than ½ the area with complete staining of embryonic axis
3	-------------------	Embryonic axis fully unstained

Percentage of seed viability was calculated by using the following formula

$$\text{Seed viability (\%)} = \frac{\text{Number of seeds gets proper stained}}{\text{Total number of seeds subjected to stain}} \times 100$$

Average width of cotyledons/ plumule + hypocotyl/ embryo (mm)

Average diameter of the cotyledon (mm) was estimated by using Euromex Software which was calibrated according to the magnification of the images.

Length of radicle (mm)

Length of radicle (mm) was measured by using euromex software which was calibrated according to the magnification of the images.

Statistical analysis

The data obtained from various experiments were analysed for the 'F' test of significance adopting the procedure described by Panse and Sukhatme (1985) [7]. Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The critical difference (CD) was calculated at 5 per cent (P = 0.05) probability level and wherever 'F' value is non-significant it is denoted by 'NS'.

Results

Effect of 'hydropriming + spin drying' on seed anatomical parameters

Chilli (Table 1)

Seed viability (%)

The results on Seed viability percentage of hydro primed + spin dried seeds showed significant difference among the treatments. The seeds hydro primed + spin dried for 1 min. recorded significantly the higher seed viability percentage (72 %) followed by the seeds hydro primed (55 %). The control seed recorded lower seed viability (49 %). The improvement in seed viability percentage with best treatment over control was 75.7 per cent.

Average length of radicle (mm)

The average length of radicle was statistically significant among the hydro priming + spin drying treatments. The seeds hydro primed + spin dried for 1 min. recorded significantly the highest average length of radicle (3.41 mm) followed by the seeds hydro primed (3.09 mm) compared to control seed (2.40 mm). The improvement in average length of radicle noticed with best treatment was 42.1 per cent higher than control seeds.

Table 1: Effect of hydropriming and spin drying on seed anatomical parameters of chilli

Treatments	Chilli		
	Seed viability (%)	Average length of radicle (mm)	Average length of plumule + hypocotyl (mm)
Hydro priming & best spin duration (1 min.)	72 (58.05)	3.41	4.82
Hydro priming +shade drying (12 h)	55 (47.87)	3.09	4.76
Control	49 (44.42)	2.40	4.67
Mean	59 (50.11)	2.96	4.75
SEd	0.4543	0.0384	0.0553
CD(P = 0.05)	0.9545	0.0808	0.1161

(Figures in parentheses indicates arcsine values)

Average length of plumule + hypocotyl (mm)

Significant differences were observed for average length of plumule + hypocotyl due to hydropriming + spin drying treatments. Chilli seeds hydroprimed + spin dried for 1 min. recorded significantly the highest average plumule + hypocotyl length of 4.82 mm. It was closely followed by seeds hydro primed (4.76 mm) which remained on par with seeds hydro primed + spin dried for 1 min. The lowest value of 4.67 mm was recorded for control seeds. The best treatment of seeds hydro primed + spin dried for 1 min. recorded 3.21 per cent increased average length of plumule + hypocotyl over control seeds and 1.26 per cent over primed seed.

Brinjal (Table 2)

Seed viability (%)

Significant difference was observed in the seed viability percentage due to hydro primed + spin dried treatments when compared to control. Among the treatments, hydro priming + spin drying for 2 min. recorded the highest viability (99 %) followed by seeds hydro primed (93 %), while the lowest (89 %) was with control seeds.

Average length of radicle (mm)

Highly significant difference was observed in the average length of radicle. Among the different treatments, the maximum radicle length was recorded in hydro primed + spin dried seeds for 2 min. (4.40 mm), followed by hydro priming (3.57 mm). The minimum germination percentage was recorded in control (3.24 mm). The improvement in average lenth of the radicle with best treatment over control was 35.8 per cent.

Table 2: Effect of hydro priming and spin drying on seed anatomical parameters of brinjal

Treatments	Brinjal		
	Seed viability (%)	Average length of radicle (mm)	Average length of plumule + hypocotyl (mm)
Hydro priming & best spin duration (2 min.)	99 (84.26)	4.40	3.71
Hydro priming +shade drying (12 h)	93 (74.66)	3.57	2.50
Control	89 (70.63)	3.24	1.90
Mean	94 (76.51)	3.73	2.70
SEd	1.1898	0.0464	0.0338
CD(P = 0.05)	2.4997	0.0975	0.0711

(Figures in parentheses indicates arcsine values)

Average length of plumule + hypocotyl (mm)

Significant difference was observed for average length of plumule + hypocotyl due to hydro primed + spin dried treatments. Among the different treatments, the maximum average length of plumule + hypocotyl was observed in spin dry for 2 min. (3.71 mm) followed by hydro primed seeds (2.50 mm). The minimum average length of plumule + hypocotyl value was recorded in control (1.90 mm).

Tomato (Table 3)

Seed viability (%)

Significant difference was observed in the seed viability percentage due to hydro primed + spin dried treatments when compared to control. Among the treatments, hydro priming + spin drying for 2 min. recorded the highest viability (86 %) followed by seeds hydro primed (57 %), while the lowest (39 %) was with control seeds.

Average length of radicle (mm)

Highly significant difference was observed in the average length of radicle. Among the different treatments, the maximum radicle length was recorded in hydro primed + spin dried seeds for 2 min. (3.25 mm), followed by hydro priming (2.90 mm). The minimum germination percentage was recorded in control (1.50 mm).

Table 3: Effect of hydro priming and spin drying on seed anatomical parameters of tomato

Treatments	Tomato		
	Seed viability (%)	Average length of radicle (mm)	Average length of plumule + hypocotyl (mm)
Hydro priming & best spin duration (2 min.)	86 (68.02)	3.25	4.04
Hydro priming +shade drying (12 h)	57 (49.02)	2.90	3.60
Control	39 (38.64)	1.50	3.30
Mean	61 (51.89)	2.55	3.64
SEd	0.440	0.0381	0.0477
CD(P = 0.05)	0.9249	0.0799	0.1002

(Figures in parentheses indicates arcsine values)

Average length of plumule + hypocotyl (mm)

Significant difference was observed for average length of plumule + hypocotyl due to hydro primed + spin dried treatments. Among the different treatments, the maximum average length of plumule + hypocotyl was observed in spin dry for 2 min. (4.04 mm) followed by hydro primed seeds (3.60 mm). The minimum average length of plumule + hypocotyl value was recorded in control (3.30 mm).

Discussion

The anatomical potential of the seeds subjected to ‘hydro priming + best spin drying duration’ corresponding to each crop, conventional hydro priming and control seeds were subjected to seed incubation for prescribed duration. The incubated seeds were prepared as per ISTA rules and subjected to Topographical Tetrazolium Test. The embryos were then evaluated for staining pattern so as to categorize the seeds into viable or non-viable. Further the embryos were subjected to image analysis at magnification of 17.5 x for chilli, brinjal and tomato. In chilli, the ‘hydro priming + best spin drying 1 min' recorded 30.9 and 46.9 per cent higher seed viability over conventional hydro priming and control, respectively. The percentage increase obtained in average length of radicle (mm) was 10.3 and 57.1 per cent over conventional hydro priming and control, respectively (Plate 1).

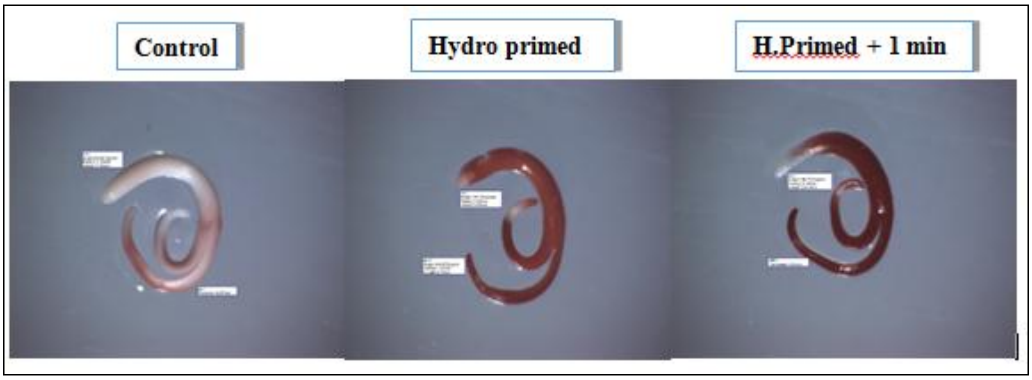

Plate 1: Effect of 'hydro priming and spin drying' on seed anatomical changes of chilli

In brinjal, the 'hydro priming + best spin drying 2 min.' recorded 6.4 and 11.2 per cent higher seed viability over conventional hydro priming and control, respectively. The percentage increase obtained in average length of radicle (mm) was 23.2 and 35.8 per cent over conventional hydro priming and control, respectively (Plate 2). In tomato, the 'hydro priming + best spin drying 2 min.' recorded 50.9 and 120.5 per cent higher seed viability over conventional hydro priming and control, respectively. The percentage increase obtained in average length of radicle (mm) was 12.0 and 116.6 per cent over conventional hydro priming and control, respectively (Plate 3). However, no reports have been made on anatomical basis of improvement in seed germination and seedling vigour. The increase in the size of the embryo, has been observed in seeds which have shown no symptoms of radicle protrusion. Therefore, it is inferred that prior to the cell division process, extensive growth in the embryonic plant takes place, probably due to cell elongation, ultimately resulting in increase in size of the embryo.

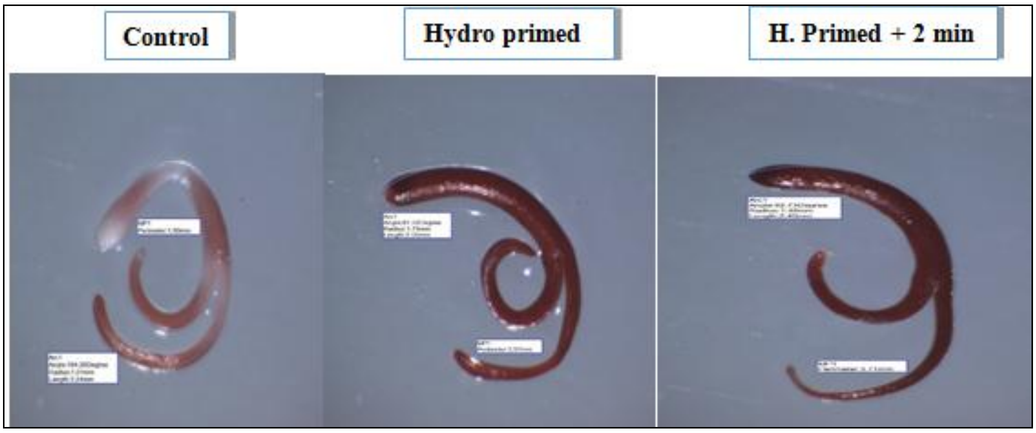

Plate 2: Effect of 'hydro priming and spin drying' on seed anatomical changes of brinjal

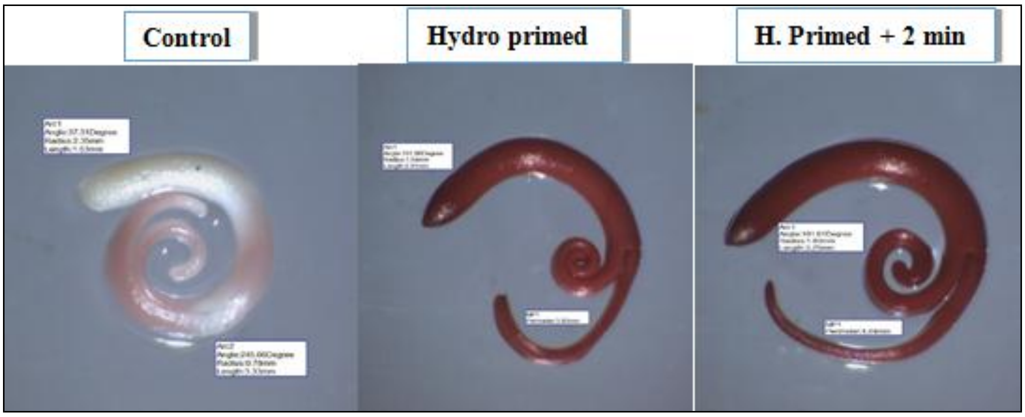

Plate 3: Effect of 'hydro priming and spin drying' on seed anatomical changes of tomato

Summary and Conclusion

Seed priming studies have extensively explored the biochemical basis of the improvement in seed performance. In this study, the observations made through Euromex Holland's Image Analysis System with different magnification specific to crop seeds, has clearly brought out that the seed priming enhances the viability of individual cells, leading to increase in the area of staining, which has in turn increased the viability per cent of a given seed lot. The important revelation is that the average length of the embryo has also shown remarkable increase in seeds subjected to priming when compared to control. In fact, cell elongation and increased size of the embryo is interpretable as the basis for increase in the speed of emergence as well as improvement in uniformity of emergence. The salient findings of the study as follows:

- The hydro primed seed subjected to spin drying recorded significant improvement in the seed viability percentage.
- Significant improvement in average length of the radicle and cotyledon.

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