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Combined seed enhancement techniques involving seed priming and coating for improvised anatomical potential and vigour of okra (*Abelmoschus esculentus* L.) seeds

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ABSTRACT

The study was conducted to standardize the methodology of humid priming for improving the seed germination and vigour of okra seeds and to compare the efficacy of different enhancement techniques such as hydro priming, spin priming, seed coating and the combination of 'humid priming + seed coating' in improving the seed vigour characteristics of okra (*Abelmoschus esculentus* L.). In order to standardize the humid priming technique, okra seeds were subjected to humid priming for four different duration's *viz*., 2, 4, 6 and 8 h, shade dried to original moisture content and subjected to evaluation of seed germination under shade net conditions in raised beds. The results revealed that 4 h of humid priming was statistically significant to the rest of the durations, with respect seed germination and seedling growth. With respect to anatomical potential, the radicle length (3.39mm), cotyledon length (6.69mm) and cotyledon width (6.30mm), recorded by humid priming (4 h) was 62.2 %, 6.4 % and 2.6 % higher than control seeds. The second experiment conducted to compare the efficacy of hydro priming, spin priming, polymer coating and combination of humid priming + seed coating. The study revealed that seeds subjected to combination of humid priming (4 h) + seed coating (3g kg^{-1}) recorded highest physiological potential in terms of germination percentage (87 %), seedling length (20.2 cm), dry matter production (421.3 mg), vigour index (1765), root volume (3.5 cm^3) and root sheath (2.560 g) while the control recorded only 70 %, 15.1 cm, 328.7 mg, 1055, 2.0 cm^3 and 1.532 g, respectively.

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INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is cultivated throughout the tropical and warm temperate regions of the world [1]. Okra is highly nutritious with its fibrous fruit containing carbohydrate (5.4 %), protein (4 %) and total fat (0.5 %). In India, the crop is grown in an area of 514 ha, production of 6126 MT, productivity of 12 t/ha [2].

Using of high quality seeds and adopting adequate management strategies are the most popular ways to increase crop production [3]. The seed quality means structural soundness, high vigor and uniform field stand besides its genetic and physical purity [4]. Seed invigoration treatments are physiological treatments that enable an improvement in physiological status of seed, thereby achieving high germination percentage [5]. Seed priming is an important way of seed invigoration and can be done in different methods [6-8]. In the last two decades, extensive research has been conducted to perfect the art of seed priming so as to enable the farmer to harness maximum benefit in terms of increased seed germination and seedling vigour. Many priming techniques have been developed for priming the seeds like Osmopriming, Halopriming, Biopriming, Solid matric priming and Spin priming are commonly practiced [9]. In the present research, another concept of priming termed as 'humid priming' has been tested for okra. The humid priming technique is the process of soaking the seeds in a loosely tied cloth bag for predetermined duration of time, followed by placing the bags in a closed container on an elevated platform that allows to provide humid dark conditions that facilitate the invigouration process [10]. Seeds that are subjected to >80 % RH during the priming process improves the physiological parameters of seed like germination percentage, speed of emergence, vigour index and also biochemical parameters like decrease in seed leachates, higher α - amylase activity and increased sugar content [11, 12]. Role of RH on growth hormones have been reported by few authors in plants like avocado [13], papaya [14], walnut [15], aglaonema [16, 17], lily [18], etc. High humidity promotes pollen germination [19]. Relative humidity on pea nut plant growth was found to be intense [20]. Among the hormones tested (Auxins, Ethylene and Gibberellins) ethylene production was significantly affected by relative humidity. Humidification of seeds have been reported to improve the seed germination potential of cockscomb [21], *B. juncea* and *B. campestris* [22], sesame [23]. When incubating *Chenopodium album* seeds with GA_3 under dark conditions resulted in germination improvement from 2% to 22% [24] and in *Echinacea purpurea* (L.) *Moench* seeds [25].

In the present study, an attempt was made to standardize the seed priming technology involving exposure of soaked seeds to humid conditions (humid priming) for okra. Further, the efficacy of proven seed enhancement techniques was compared with the newly developed humid priming technique to evaluate the improvement obtained in physiological potential of seeds including seed germination and seedling vigour.

MATERIALS AND METHODS

The experiment was conducted with genetically pure, fresh seeds of okra cv. CO_2 at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, India.

Experiment I. Standardization of humid priming methodology

The first part of the experiment consisted of standardization of protocol for humid priming of okra seeds. Initially, the okra seeds were soaked in water for 12 h [26]. The soaked seeds were tied in a cloth bags and placed in a container, over an elevated platform so to allow excess water to drain. The container was then closed tightly and placed in dark conditions [10] to allow the seeds to be exposed to dark humid conditions. Seed samples in cloth bags were drawn after 2, 4, 6 and 8 h and shade dried for 12 h to attain original moisture content. The seeds were subjected to germination test along with the control seeds and observations were made on speed of emergence, seed vigour parameters. Anatomical parameters like length of radicle, length and width of cotyledons were measured under stereo microscope (Euromex Microscope Hollands IMAGE VERSION 1.0) with magnification 12.5x after subjecting the seeds to staining with 2,3,5 - triphenyl tetrazolium chloride and reported the results in mm.

Experiment II. Comparison of efficacy of different seed enhancement treatments

The main objective of the study was to compare the efficacy of different methods of seed enhancement. Therefore, okra seeds were submitted to seed priming protocols *viz*., (i) hydropriming (12 h) [26] (ii) spin priming (12 h soaking + 4 min spinning), [27] (iii) humid priming (12 h soaking + 4 h humid incubation) (iv) seed coating' (TNAU formulation @ 3 g kg^{-1}) [28] and humid priming (12 h soaking + 4 h humid incubation) + seed coating' (TNAU formulation @ 3g kg^{-1}). TNAU seed coating formulation developed in the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU) consists of a hydrophilic polymer (79.25 %), pigment (20.00 %), carboxy methyl cellulose (0.50 %) and gibberellic acid (0.25 %) [28]. The seeds submitted to different treatments were subjected to seed germination test.

The germination test was conducted in shade net under raised bed conditions with 100 seeds, in four replications for every treatment. At the final count, the number of normal seedlings were counted and the mean was expressed as germination per cent. Speed of emergence was recorded daily and calculated by using the formula proposed by Maguire [29]. Length of root and shoot were measured and showed in cm. After measuring the root and shoot length the seedlings were placed in a paper cover, shade dried for 24 hours and then dried in a hot air oven at 80 ± 2°C for 24 hours. Later, the samples were cooled in a desiccator, weighed and expressed as milli gram per 10 seedlings. Root volume was measured based on displacement of water. The roots separated from 10 seedlings were washed carefully to remove the soil particles, but not to damage the root parts. The roots were soaked in a 100 ml measuring cylinder, filled with 50 ml of water. The increase in the volume of water was measured and recorded as ml $seedlings^{-10}$. Root sheath denotes the volume of soil adhering to the roots uprooted from the soil. In order to record the root sheath, roots of 10 seedlings were removed carefully from soil and fresh weight was recorded. Immediately, the roots were carefully washed, the residual moisture was wiped with blotter paper and the roots were weighed once again.

Root sheath (g $seedlings^{-10}$) = Wt. of roots with soil – Wt. of roots without soil

Vigour index values were computed using the formula suggested by Abdul-Baki and Anderson [30] and the mean values were expressed as whole numbers.

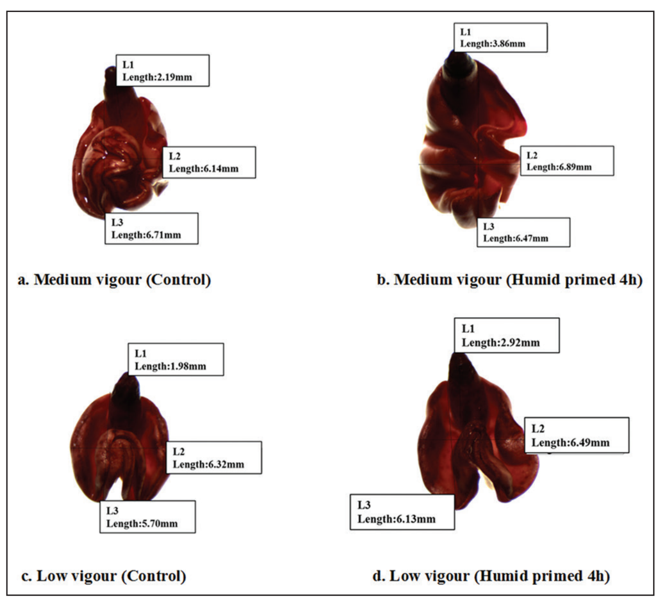

Plate 1: Effect of humid priming on anatomical potential of medium and low vigour lots of okra

Data were analyzed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at the 0.05 or 0.01 probability level. Values in percent data were arcsine transformed before analysis.

RESULTS AND DISCUSSIONS

In the present study, okra seeds soaked in water for 12 h were subjected to humid priming for four different durations *viz*., 2, 4, 6, 8 h. Statistically significant difference was observed among the different durations of humid priming with respect to seed germination, seedling vigour and changes in embryonic parameters *viz*., radicle length, length and width of cotyledon which was significantly greater than control seeds. Among the durations of humid priming, the speed of emergence of seedlings was highest in seeds subjected to 4 h of humid priming in both medium vigour (5.21) and low vigour (3.72) lots compared to respective control seeds which recorded a speed of germination of 3.03 and 4.34 (Table 1). The seed germination of medium vigour lot was found to reach the highest value of 93 per cent even with humid priming (12 h soaking + 2 h humid incubation), which was maintained even in humid priming (12 h soaking + 4 h humid incubation). However, the low vigour lot could attain the highest value of 77 per cent only with humid priming (12 h soaking + 4 h humid incubation). In both medium and low vigour lots, the germination percent as well as speed of germination showed a reduction with increased durations *viz*., 6 h and 8 h incubation. The data underscored that for the medium vigour lot with 81 per cent germination, 2 h of humid incubation was sufficient to bring about the cellular repair process including membrane reorganization, repair and synthesis of mitochondria and DNA, which is quintessential for invigoration of seeds. However, for the low vigour seeds with 58 per cent germination, a longer incubation duration of 4 h was necessary to maximum seed invigouration. Significant improvement in root length, dry matter production and vigour index, in both medium and low vigour lots was plausible due to humid priming (12 h soaking + 4 h humid incubation). The improvement in shoot length was found to reach the highest in humid priming (12 h soaking + 2 h humid incubation) in medium vigour lot, while it required humid priming (4 h) for low vigour lots (Table 2, 3).

Irrespective of duration of humid priming treatments and vigour status of seed lots, the anatomical potential of seeds, measured in terms of as radicle length, cotyledon width and cotyledon length was observed to be significantly higher in humid primed seeds than control seeds, in both medium and low vigour lot. The humid priming (12 h soaking + 4 h humid incubation) recorded the maximum radicle length (3.86 mm), cotyledon width (6.47 mm), cotyledon length (6.89 mm) in medium vigour lot as well as low vigour lot by recording 2.92 mm 6.13 mm 6.49 mm, respectively (Table 4) (Plate 1). This improvement in anatomical potential of embryo could have resulted in highest level of improvement in seed germination and seedling growth with humid priming (4 h). Therefore, irrespective of the vigour of the okra seed lot, the effective technique to achieve significantly higher improvement in seed germination and seedling vigour is determined to humid priming (12 h soaking + 4 h humid incubation). The obtained results were in accordance with earlier reports of seed priming in sesame [23].

Humidification of cockscomb (*Celosia argentea* var. cristata L.) improved the seed vigour and was attributed to repair of membrane

Table 1: Effect of duration of humid priming on speed of emergence and germination (%) of medium and low vigour lots of okra

Duration (h)	Speed of emergence		Mean	Germination (%)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	4.34	3.03	3.69	81 (64.15)	58 (49.60)	70 (56.79)
2	5.17	3.49	4.33	93 (74.66)	74 (59.34)	84 (66.42)
4	5.21	3.72	4.47	93 (74.66)	77 (61.34)	85 (67.21)
6	4.96	3.52	4.24	89 (70.63)	73 (58.69)	81 (64.15)
8	4.85	3.41	4.13	86 (68.02)	70 (56.79)	78 (62.02)
Mean	4.91	3.43	4.17	88 (69.73)	70 (56.79)	79 (62.72)
	T	L	T X L	T	L	T X L
SEd	0.037	0.023	0.052	0.725	0.458	1.025
CD (P=0.05)	0.076	0.048	0.107	1.480	0.936	2.094

(Figures in parentheses indicates arcsine values)

Table 2: Effect of duration of humid priming on root length (cm) and shoot length (cm) of medium and low vigour lots of okra

Duration (h)	Root length (cm)		Mean	Shoot length (cm)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	6.9	6.5	6.7	8.9	8.4	8.7
2	8.9	7.4	8.2	12.1	9.4	10.8
4	9.0	7.8	8.4	12.1	10.1	11.1
6	8.5	7.3	7.9	11.7	9.7	10.7
8	8.1	6.8	7.5	10.9	9.4	10.2
Mean	8.3	7.2	7.7	11.1	9.4	10.3
	T	L	T X L	T	L	T X L
SEd	0.088	0.055	0.124	0.064	0.041	0.091
CD (P=0.05)	0.179	0.113	0.253	0.131	0.083	0.186

systems as witnessed by reduction in solute leakage [21]. Our findings are also in accordance who reported that with 12 h aerated hydration of *B. juncea* and *B. campestris*, seeds registered significant improvement in germination rate and vigour [22].

The faster germination rate and germination improvement in pre-hydrated seeds was probably due to enlargement of embryo [31]. Humidification of aged pea seeds at varying periods for 3 to 7 days significantly improved the seed germination by reducing the percentage of abnormal seedling which is attributable to due to reduction in all the categories of chromosomal aberrations when compared with non-humidified seeds [32]. Wheat seeds hydrated for the duration of 24 h at 5°C improved the germination percentage and reduced the time to 50 % radicle emergence, increased dry weight of seedlings and decreased electrical conductivity of seed leachates which is a consequence of membrane reorganization and DNA repair [12]. The effect of humid priming (12 h soaking + 4 h humid incubation) of okra seeds as observed through the improvement in seed germination, seedling growth and embryo enlargement has emphasized the potential of humid priming as a potential seed invigouration technique for okra.

In the second experiment, the humid priming (12 h soaking + 4 h humid incubation) standardized in the previous experiment was compared with other seed quality enhancement techniques such as hydropriming (12 h), spin priming (12 h soaking + 4 min spinning), seed coating (TNAU formulation @ 3g kg^{-1}) as well as 'humid priming (12 h soaking + 4 h humid incubation) + seed coating' (TNAU formulation @ 3g kg^{-1}). Among the treatments, combined effect of humid priming (4 h) + seed coating (TNAU formulation @ 3g kg^{-1}) recorded remarkable improvement in speed of emergence in both low vigour (5.54) and low vigour seed lots (3.79) when compared with control seeds which recorded 4.42 and 2.94, respectively (Table 5). In germination among the various treatments humid priming (4 h) and humid priming + seed coating (TNAU formulation) showed significant increase in both the medium vigour (94%) and low vigour lot (80%) over the control seeds (Table 5). The seedling vigour also was found to be highest in 'humid priming + seed coating (TNAU formulation)' treatment (Table 6). Dry matter production recorded with humid priming (4 h) and seed coating (TNAU formulation) (3g kg^{-1}) was highest in both medium vigour (486.1) and low vigour lots (356.5) when compared with the control (371.0) and (286.4) (Table 7). The root volume and root sheath are important parameters to assess the growth and productivity potential of a plant. The root volume observed in seeds subjected to 'humid priming (12 h soaking + 4 h humid incubation) + seed coating (TNAU formulation @ 3g kg^{-1})' was significantly higher in both medium vigour (3.6 ml) and low vigour (3.3 ml) lots, while the values recorded by respective controls were only 2.1 ml and 1.9 ml. Similarly, the root sheath also was found to be highest seeds subjected to 'humid priming (12 h soaking + 4 h humid incubation) + seed coating (TNAU formulation @ 3g kg^{-1})' in both medium vigour (2.808 g) and in low vigour (2.312 g) than compared to respective control *viz.*, 1.678 g and 1.386 g (Table 8).

The results obtained in the present study has endorsed that the combined treatment consisting of 'humid priming (12 h soaking + 4 h humid incubation) + seed coating' (TNAU formulation @ 3g kg^{-1}) demonstrated the highest potential to enhance the seed germination and seedling vigour of okra. This is in concordance with the findings made on effect of seed coating on cotton seeds (40 g kg^{-1}) [33], which had improved all the physiological parameters like germination percentage, speed of germination, seedling length,

Table 3: Effect of duration of humid priming on dry matter production (mg seedlings^{-10}) and vigour index of medium and low vigour lots of okra

Duration (h)	Dry matter production (mg seedlings^{-10})		Mean	Vigour index		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	383.2	291.3	337.3	1280	864	1072
2	453.6	326.3	390.0	1953	1234	1594
4	459.0	343.9	401.5	1962	1378	1670
6	422.4	319.6	371.0	1798	1241	1520
8	407.6	306.2	356.9	1634	1134	1384
Mean	425.2	317.5	371.3	1725	1170	1448
	T	L	T X L	T	L	T X L
SEd	4.671	2.954	6.606	11.192	7.078	15.827
CD (P=0.05)	9.540	6.034	13.492	22.857	14.456	32.324

Table 4: Effect of humid priming on anatomical potential of medium and low vigour lots of okra

Duration (h)	Radicle length (mm)		Mean	Cotyledon width (mm)		Mean	Cotyledon length (mm)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	2.19	1.98	2.09	6.14	5.70	5.92	6.71	6.32	6.52
2	3.83	2.76	3.30	6.40	5.99	6.20	6.85	6.40	6.63
4	3.86	2.92	3.39	6.47	6.13	6.30	6.89	6.49	6.69
6	3.50	2.70	3.10	6.36	6.02	6.19	6.79	6.36	6.58
8	3.03	2.59	2.81	6.24	5.80	6.02	6.60	6.23	6.42
Mean	3.28	2.59	2.94	6.32	5.93	6.13	6.77	6.36	6.56
	T	L	T X L	T	L	T X L	T	L	T X L
SEd	0.029	0.019	0.042	0.054	0.034	0.076	0.044	0.028	0.062
CD (P=0.05)	0.061	0.038	0.086	0.110	0.070	0.155	0.089	0.056	0.126

Table 5: Comparison of effect of various seed enhancement techniques on speed of emergence and seed germination (%) of okra seeds

Treatments	Speed of emergence		Mean	Germination (%)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	4.42	2.94	3.68	81 (64.15)	58 (49.60)	70 (56.79)
Hydropriming	5.19	3.46	4.33	91 (72.54)	73 (58.69)	82 (64.89)
Spin priming	5.30	3.64	4.47	93 (74.66)	76 (60.66)	85 (67.21)
Humid priming	5.30	3.68	4.49	93 (74.66)	77 (61.34)	85 (67.21)
Seed coating	4.94	3.39	4.17	87 (68.86)	68 (55.55)	78 (62.02)
Humid priming+seed coating	5.54	3.79	4.67	94 (75.82)	80 (63.43)	87 (68.86)
Mean	5.12	3.48	4.30	90 (71.56)	72 (58.05)	81 (64.15)
	T	L	T X L	T	L	T X L
SEd	0.051	0.029	0.072	0.544	0.314	0.769
CD (P=0.05)	0.103	0.059	0.145	1.103	0.637	1.560

(Figures in parentheses indicates arcsine values)

Table 6: Comparison of effect of various seed enhancement techniques on root length (cm) and shoot length (cm) of okra seeds

Treatments	Root length (cm)		Mean	Shoot length (cm)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	6.7	6.3	6.5	8.8	8.4	8.6
Hydropriming	8.2	7.3	7.8	11.0	9.5	10.3
Spin priming	8.7	7.6	8.2	11.8	10.1	11.0
Humid priming	8.9	7.7	8.3	12.0	10.1	11.1
Seed coating	7.9	7.0	7.5	10.9	9.2	10.1
Humid priming+seed coating	9.4	8.1	8.8	12.5	10.3	11.4
Mean	8.3	7.3	7.8	11.2	9.6	10.4
	T	L	T X L	T	L	T X L
SEd	0.090	0.052	0.128	0.103	0.059	0.145
CD (P=0.05)	0.183	0.106	0.259	0.208	0.120	0.295

Table 7: Comparison of effect of various seed enhancement techniques on dry matter production (mg $seedlings^{-10}$) and vigour index of okra seeds

Treatments	Dry matter production (mg $seedlings^{-10}$)		Mean	Vigour index		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	371.0	286.4	328.7	1240	870	1055
Hydropriming	444.2	328.1	386.2	1747	1226	1487
Spin priming	455.4	339.0	397.2	1907	1345	1626
Humid priming	457.8	343.4	400.6	1943	1371	1657
Seed coating	420.0	314.6	367.3	1635	1101	1368
Humid priming+seed coating	486.1	356.5	421.3	2058	1472	1765
Mean	439.1	328.0	383.5	1755	1231	1493
	T	L	T X L	T	L	T X L
SEd	3.341	1.929	4.725	12.776	7.737	18.067
CD (P=0.05)	6.776	3.912	9.583	25.913	14.961	36.647

Table 8: Comparison of effect of various seed enhancement techniques on root volume (cm^3) and root sheath (g) of okra seeds

Treatments	Root volume (cm^3)		Mean	Root sheath (g)		Mean
	Medium vigour lot	Low vigour lot		Medium vigour lot	Low vigour lot	
Control	2.1	1.9	2.0	1.678	1.386	1.532
Hydropriming	3.2	2.6	2.9	2.312	2.111	2.212
Spin priming	3.4	3.0	3.2	2.508	2.241	2.375
Humid priming	3.5	3.1	3.3	2.536	2.256	2.396
Seed coating	2.9	2.4	2.7	2.201	1.984	2.093
Humid priming+seed coating	3.6	3.3	3.5	2.808	2.312	2.560
Mean	3.1	2.7	2.9	2.341	2.048	2.194
	T	L	T X L	T	L	T X L
SEd	0.035	0.020	0.049	0.024	0.014	0.033
CD (P=0.05)	0.071	0.041	0.100	0.048	0.028	0.068

vigour index and root volume than compared with control seeds. In the present study, the significantly higher improvement in seed germination and seedling vigour in the combination treatment involving 'humid priming (12 h soaking + 4 h humid incubation)

+ seed coating' (TNAU formulation @ 3g kg^{-1}) may attributed to effective accomplishment seed invigouration related biochemical processes mainly membrane reorganization, mitochondrial repair and synthesis, protein synthesis and DNA repair synthesis which is integral to any seed priming process. The seed coating of the humid primed seeds with TNAU seed coating formulation (3g kg^{-1}) could have augmented the improvement in seed vigour by way of influencing the imbibition process and other physiological manifestations since the formulation which consists of hydrophilic polymer and gibberellic acid. Gibberellin is an effective hormone that promotes seed germination [34]. It is widely used as a stimulant for germination [35]. Gibberellic acid (GA_3), can increase seed germination due to impacts on mobilization of reserves since it exerts control over hydrolysis reserve tissues to supply energy for the embryo [36].

The results obtained in the present study establish that submitting the okra seeds to a treatment combination of 'humid priming (12 h soaking + 4 h humid incubation) + seed coating' (TNAU formulation @ 3g kg^{-1}) is effective in bringing about increasing the anatomical potential of seed by initiating the membrane reorganization, repair and synthesis of DNA quintessential of seed priming process and by augmenting the seed imbibition and growth potential through the coating of seeds with TNAU seed coating formulation. Eventually the okra seeds demonstrate a better growth potential as visualized through higher root volume, root sheath and seedling vigour which is a forerunner for higher plant productivity.

AUTHOR'S CONTRIBUTIONS

Anilkumar. L designed the experiment, interpreted the data and writing an original draft. Malarkodi. K participated in the review and validation of manuscript.

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