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Solid matrix priming and bio-agents improve seed emergence in okra [*Abelmoschus esculentus* (L.) Moench]

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Abstract
Rapid and uniform seedling emergence is a pre-requisite to better stand establishment and subsequent plant growth and yield in vegetable crops. One of the major problems of okra growers is difficulty in obtaining proper seedling emergence under low temperature. Since environmental factors often cannot be controlled, modification of the seed by either biological, chemical, or physiological techniques should be evaluated to improve the vigour of the seedling and uniformity of stand. Solid matrix seed priming was performed by mixing seed with moist vermiculite for 6, 12, 18, 24, 30, 36 and 48 h incubation at 20 and 25 °C. Based on seed germination, speed of germination and seedling vigour parameters, SMP for 24 h duration at 20 °C was optimum for improving seed quality in okra cv. Pusa A-4. Solid matrix priming followed by coating with *Trichoderma viridae* has improved laboratory germination by 8.3% over unprimed seeds. Coating seeds with bio-agents like *Trichoderma viridae* and *T. harzianum* after SMP significantly improved speed of germination and seedling vigour. Solid matrix priming followed by coating of *Trichoderma viridae* has improved field emergence of okra seeds by 33% over unprimed control. Field emergence index and 25 days old seedling growth and vigour was significantly higher in solid matrix primed seeds coated with bio-agents. The results indicated that solid matrix priming along with coating of bio-agents like *Trichoderma viridae* and *T. harzianum* improves field emergence and seedling growth in okra. Solid matrix priming can be effectively employed to prime large amount of okra seed at one time. It is also cost effective as the medium can be re-used many times.

Keywords: Okra, priming, germination, field emergence and bio-agents

Introduction
Rapid and uniform seedling emergence is a pre-requisite to better stand establishment and subsequent plant growth and yield in vegetable crops. One of the major problems of the okra growers is the possible difficulty in obtaining proper seedling emergence under field conditions. Okra seed germinates at soil temperature in the range of 21 to 35 °C with an optimal temperature requirement of 29 °C [1]. The presence of hard seed coat is another major reason for delayed and erratic germination in okra [2, 3, 4]. Environmental factors such as inadequate and excessive soil moisture, low soil temperature and pre-emergence damping off caused by *Pythium* spp. also contribute to erratic germination of okra seed [5].
Various pre-sowing treatments have been used to increase the rate and uniformity of emergence in many vegetable and flower species [6, 7, 8, 9]. Among different priming methods, the solid matrix priming (SMP) technique developed by Eastin [10] can be used to promote rate and uniformity of germination in several crops [11, 12, 9]. It is a process that used solid matrix materials, water and seed in combination to control water, oxygen and temperature effects on germination. This process controls the hydration of seed to a level that allows 'pre-germination' activity but prevents radicle emergence. Seed water uptake is regulated by the matric potential of the seed. The characteristics of the solid matrix system minimizes aeration problem and facilitates the incorporation of fungicides or biological agents. The main objective of this experiment was to standardize SMP for okra and test the effectiveness of best solid matrix priming protocol along with bio-agents and fungicides under field conditions.

Materials and methods
There are many different variables that affect the outcome of the priming treatments, regardless of the method used. The temperature at which seed priming is done influence their subsequent germination. The temperature of priming solution can affect the length of priming [6]. The lengths of time the seeds are primed also have significant effect on the success of the treatment. For solid matrix priming, 100 g seeds per replicate were mixed with 200 g vermiculite to which 250 ml of distilled water was added. The vermiculite and seeds were

mixed thoroughly, sealed in a plastic bag and incubated at 20 and 25 ^{0}C for 6, 12, 18, 24, 30, 36 and 48 h. for standardization of SMP treatment. After this incubation period, seeds were sieved out and dried to the original moisture content. Germination was performed as ISTA [13] procedures. One hundred seeds per replicate were used. Seeds were incubated in growth cabinets maintained at 25 ^{0}C. Daily germination counts were performed until no further germination occurred for three consecutive days, when percentage and speed of germination were calculated. High speed of germination is an indication of vigourous seed lot. Numbers of germinated seeds were counted every day from the first day and the cumulative index was made by the formula Maguire [14]. The vigour indices were computed adopting the method of Abdul-Baki and Anderson [15].

The best solid matrix treatment was used for further field performance studies with bio-agents and fungicides. In one set of treatments seeds were first primed and then coated with the formulation of *Trichoderma viridae* and *T. harzianum* isolates. In other set of treatments, seeds were first treated with formulation of *Trichoderma viridae* and *T. harzianum* isolates and then primed**.** The following treatments were subjected to analysis of variance (ANOVA) tests in a RCBD with three replications.

S. No.	Treatments	Remarks
1.	SMP	Solid matrix priming at 20°C for 24 hrs
2.	SMP+TV	Dry dressing with *T. viridae* @ 2g/kg seed after SMP
3.	TV+SMP	Dry dressing with *T. viridae* @ 2g/kg seed before SMP
4.	SMP+TH	Dry dressing with *T. harzianum* @ 2g/kg seed after SMP
5.	TH+SMP	Dry dressing with *T. harzianum* @ 2g/kg seed before SMP
6.	SMP+T	Dry dressing with Thiram 75%WS @ 1.0 g/kg seed
7.	SMP+C	Captan 75% WS @ 2.5 g/kg of seed
8.	Control	Dry seed

Field emergence was estimated by sowing 50 seeds in three replications in the field. Observations were recorded on each day till 25th day of sowing. The emergence was expressed as percentage of seedling emergence. Field emergence index (FEI) was calculated based on the procedure used by Egli and Tekrony [16].

Results and discussion

Standardization of solid matrix priming treatments

There are many different variables that affect the outcome of the priming treatments, regardless of the method used. The temperature at which seed priming is done influence their subsequent germination. The temperature of priming solution can affect the length of priming [6]. The lengths of time the seeds are primed also have significant effect on the success of the treatment. Therefore, we have to standardize solid matrix priming duration and temperature for okra that allows development of a simple priming protocol. The present investigation revealed that among the different priming duration and temperature interactions tested, maximum seed germination (73.8%) was noticed at 24 h priming duration (Table 1).

Table 1: Effect of solid matrix priming duration and temperature on germination and vigour in okra seeds.

Treatments	Germination (%)	Speed of germination	Vigour index I	Vigour index II
Priming duration (hours)				
6	70.8 ^{b}	48.4 ^{d}	1817.6 ^{c}	10.2 ^{a}
12	71.8 ^{b}	49.4 ^{cd}	1842.7 ^{c}	10.4 ^{a}
18	72.1 ^{ab}	50.5 ^{c}	1914.1 ^{b}	10.4 ^{a}
24	73.8 ^{a}	66.8 ^{a}	1970.0 ^{a}	10.5 ^{a}
30	66.9 ^{c}	54.5 ^{b}	1619.1 ^{d}	9.4 ^{b}
36	65.3 ^{c}	54.4 ^{b}	1528.1 ^{e}	9.0 ^{b}
48	63.0 ^{d}	53.7 ^{b}	1268.9 ^{g}	8.5 ^{c}
Control	65.5 ^{c}	34.5 ^{e}	1439.4 ^{f}	9.1 ^{b}
C.D (p=0.05)	1.83	1.63	43.6	0.32
Temperature				
20 °C	69.5 ^{A}	49.4 ^{A}	1840.3 ^{A}	9.9 ^{A}
25 °C	67.8 ^{B}	53.6 ^{B}	1509.7 ^{B}	9.5 ^{B}
C.D (p=0.05)	0.19	0.81	21.8	0.16
Interaction C.D (p=0.05)	2.59	2.31	61.7	0.46

Means followed by the same letters are not significantly different. Separation by Duncan's Multiple Range Test (DMRT) at 5% level.

Longer priming duration usually resulted in lower germination as a result minimum germination (63.0%) was observed at 48 h priming duration. Among temperature effects, 20 °C temperature was found to be optimum for conducting solid matrix priming. Solid matrix primed seeds showed higher speed of germination (66.8) as compared to unprimed seeds (34.5). Higher shoot length (20.3 cm) was noticed in 24 h priming duration and 20 °C temperature treated seeds. Unprimed seeds and 48 h duration priming showed lowest shoot length. The effect of duration of priming on pepper seed performance was demonstrated by Cantliffe *et al.* [17]. They reported that seeds osmoprimed for six days had faster germination, but they produced 60% abnormal seedlings compared to 14% or 0% of seeds primed for 5 or 4 days. Similarly, Heydecker *et al.* [18] reported different optimum temperatures during priming according to the species: 10 °C for onion, 15 °C for beet, and 20 °C for carrot. These results indicated that increasing solid matrix priming duration beyond 24 h results in poor seed quality. Pandita *et al.* [9] also reported that SMP is the best and effective method to prime large quantity of pepper seeds. Solid matrix priming improved germination 16% at 15 °C and 10% at 20 °C and 25 °C in pepper.

Harris [19] highlighted just how difficult it was for farmers in marginal areas to get their crops established effectively but he demonstrated that simply soaking seeds in water before sowing could increase the speed of germination and emergence, leading to better crop stands and make seedlings grow much more vigorously. Higher vigour index I (1970) and vigour index II (10.5) was also recorded at 24 h priming duration. Better vigour of seedlings was obtained at 20 °C as compared to 25 °C temperature. Seed priming has been reported to improve germination under adverse conditions. Nerson *et al.* [20] showed that higher germination at low temperature could be obtained in watermelons by seed coat splitting. Seed coat removal in melon seeds improved germination at low water potential [21] and low temperature [22]. Seed priming has been used extensively to improve germination of many species. Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination but permits pre-germinative physiological and biochemical changes to occur [23, 11, 24]. Upon rehydration, primed seeds may exhibit faster rates of germination, more uniform emergence, greater tolerance to environmental stress, and reduced dormancy in many species [11].

Seed quality enhancements

In the present investigation, best solid matrix priming treatment was used was combined with bio-agents *Trichoderma viridae* and *T. harzianum* and fungicides for improving field performance of okra cv. Pusa A-4. Solid matrix priming followed by coating with *Trichoderma viridae* has improved laboratory germination by 18.7 percent over unprimed seeds. Coating seeds with bio-agents like *Trichoderma viridae* and *T. harzianum* after SMP significantly improved speed of germination (Table 2).

Table 2: Effect of solid matrix priming in combination with bio-agents and fungicides on laboratory germination and field performance in okra seeds.

Treatment	Germination (%)	Speed of germination	Field emergence (%)	Field emergence index	Seedling ht (cm)	Seedling fresh wt (g)	Seedling dry wt (g)
SMP	76.3 b	62.4 c	65.0 b	85.1 a	6.5 c	3.48 c	0.50 c
SMP+TV	85.3 a	79.8 a	73.0 a	85.6 a	6.9 b	3.67 a	0.62 a
TV+SMP	70.3 d	52.2 d	50.6 d	72.0 bc	7.6 a	3.42 d	0.41 e
SMP+TH	83.0 a	71.0 b	64.0 bc	77.0 ab	5.8 d	3.59 b	0.59 b
TH+SMP	74.3 bc	54.8 d	48.6 d	65.4 cd	5.9 d	3.42 d	0.46 d
SMP+T	74.3 c	63.1 c	58.0 c	77.9 ab	5.9 d	2.73 e	0.24 f
SMP+C	72.3 cd	60.1 c	48.6 d	67.3 cd	5.4 e	1.74 f	0.24 f
Control	66.6 e	34.1 e	40.0 e	60.0 d	5.4 e	1.59 g	0.20 g
C.D (p=0.05)	3.01	4.49	6.42	8.08	0.118	0.013	0.009

Means followed by the same letters are not significantly different. Separation by Duncan's Multiple Range Test (DMRT) at 5% level.

Maximum speed of germination (79.8) was obtained in solid matrix primed seed that were coated with *Trichoderma viridae* and minimum was recorded in unprimed control (34.1). Since environmental factors often cannot be controlled, modification of the seeds by either biological, chemical, or physiological techniques should be evaluated to improve the vigour of the seedling and uniformity of stand. Solid matrix priming is a technique developed by Eastin [10]. In SMP, a solid matrix is used to regulate water imbibition by seeds. Solid matrix priming alone [25] or in combination with fungicides [26, 11] or biological agents [27, 28] improved the rate and uniformity of emergence of vegetable seeds and reduced damping-off disease. Solid matrix priming followed by coating of *Trichoderma viridae* has improved field emergence of okra seed by 82.5% over unprimed control. Field emergence index and 25 days old seedling growth and vigour was significantly higher in solid matrix primed seeds coated with bio-agents. Solid matrix priming markedly enhanced the ability of the *Trichoderma* strains to control *Pythium* spp. [29]. Biological agents like *Trichoderma* colonizes roots, increases root mass and health, and consequently, frequently provide yield increase, which chemical fungicides applied at reasonable rates cannot do. Solid matrix chemoprimed seeds of okra had more uniform and faster emergence compared with untreated seeds in field. Harman and Taylor [28] combined solid matrix priming with *Trichoderma* strains to control seed and soil borne diseases in cucumber and tomato. Germination rate was increased and post-emergence damping-off was reduced in both species. Copeland *et al.* [30] reported that both laboratory and field performance were generally improved by seed treatment over untreated seeds in soybean.

Many attempts have been made to relate standard germination to field emergence in several crops. A close association between two characters was reported in corn and soybean by Sherif [31] and Anthow *et al.* [32] while other studies have shown that standard germination consistently overestimates field emergence due to variation in field condition. Inadequate field emergence result in poor establishment of plant in chickpea due to moisture stress, low germination and vigour of seed, damping-off seed and seedling, effect of seed size and agronomic practice followed. Saxena [33] stated that inadequate plant stand may be a constraint in realization of real genetic potential of a cultivar. Further, stated that at least 33 plant per square meter form optimum plant population in chickpea [34]. In soybean, measure of seed vigour provides a better relationship to field emergence than standard germination [35]. High quality seed expected to produce better field emergence in a wide range of seed bed condition as compared to seed of low quality (germination and vigour). The results obtained in the present studies also indicated better laboratory and field performance of solid matrix primed okra seeds.

Conclusion

Coating SMP seeds with bio-agents had synergetic effect on field performance of okra cv. Pusa A-4. SMP can be effectively employed to prime large amount of okra seeds at one time. It is also cost effective as the medium can be re-used many times.

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