Effect of Pre-sowing Seed Treatments on Physiological Potential of Seed Germination in Okra

ABSTRACT

Aims: The present investigation was carried out at SKUAST-Kashmir during the year 2020 to improve the seed vigour and germination potential of okra through pre-sowing seed treatments.

Study design: The experiment was laid in completely randomized design (CRD) with three replications

Place and duration of study: The present investigation was carried out at the division of Basic Sciences and Humanities, SKUAST-Kashmir during the year 2020

Methodology: There were nine pre-sowing seed treatments comprised of simple seed *soaking* and seed *priming* with water (T_1 and T_5), -1.0MPa solution of PEG (T_2 and T_6), 100ppm solution of GA_3 (T_3 and T_7) and 1.5% solution of KH_2PO_4 (T_4 and T_8), respectively along with untreated control (T_0). Fifty seeds of each treatment were cultured in 14.0cm Petri dishes lined with 10 layers of moist filter paper. Observations on various germination parameters were recorded at (25±2°C). **Results:** Findings of the study revealed that seed *soaking* in water (T1) resulted in maximum FGP (93.3%) and GI (16.29) compared to other treatments. Discrete middling of different *soaking* (AvS) and *priming* (AvP) treatments indicated the novelty of seed *priming* over simple *soaking*. However, unlike FGP and GI, all other parameters *viz.,* CVG, MGT, MGR, T_{50} and Z-index were found superior when seeds were primed in water (T_5) with their recorded values of 87.34%day^{-1}, 1.15day^{-1}, 0.614 day^{-1}, 1.18 day, 0.790, respectively. Values of CVG, MGR and Z index with respect to separate means of simple seed *soaking*, AvS (69.5%day-^{1}, 0. 0.566day^{-1} and 0.53) and *priming*, AvP (75.6%day^{-1}, 0.573day^{-1} and 0.63) further confirmed the dominancy of seed *priming* technique over simple seed *soaking*. Pearson correlation coefficient analysis revealed strong relationship between different quantities of seed germination attributes.

Conclusion: Seed *priming* resulted in better germination attributes in okra compared to tradirional seed *soaking* and as such *priming* treatments should be practiced instead of traditional seed *soaking* in okra.

Key words: Okra, Seed germination, CVG, MGT, Z index

1. INTRODUCTION

Okra (*Abelmoschus esculantus* L.) is one of the most important fruit vegetable worldwide and cultivated for its immature fruits (seed pods) generally 3 - 10 inches long, tapering, usually with ribs down its length. It is a good source of vitamin, protein, carbohydrates, fats and minerals and also have medicinal values for many diseases [1]. India stands first in area and production with productivity of about 11.90 tonnes/h followed by Nigeria [2]. However, the highest productivity is reported from Egypt followed by Saudi Arabia and India. The area under okra cultivation in Jammu and Kashmir during 2017-18 was 3620 ha and with production of 14985 MT [2].

Okra prefers temperature between 22-35°C and susceptible to frost and temperatures below 12°C. The optimal temperature for seed germination, growth and fruit setting in okra is between 25 and 30°C while its seed do not germinate below 20°C. Okra often have poor seed germination and field establishment probably due to hard and impermeable seed coat [3,4], which prevents seed from absorbing water. One of the factors responsible for low okra yield is the poor stand establishment resulting from slow and uneven seed germination particularly in the early spring planting with commercial cultivars reaching only up to 66% initial germination [5].

Pre-sowing seed *soaking* and/or *priming* are among the known seed invigoration techniques [6,7]. Seed *soaking* allows seed to imbibe water before sowing but imbibition is uncontrolled. Generally seed *soaking* is practices for an overnight period and seeds are only visibly dried before sowing. However, in case of *priming,* important part is to dry the seed to original moisture content before sowing and imbibition of seed is controlled [6,8]. In this seeds are partially hydrated to a point where pre-germination metabolic activities start without actual germination by treating with different chemicals or growth regulators, and then re-dried until close to the original dry weight. However, information available with regard to comparative effectiveness of pre-sowing *soaking* and *priming* are scanty. Therefore, the present investigation was carried out to improve the seed germination potential through pre-sowing seed *soaking* and *priming* techniques and to determine the comparative efficacy of traditional seed *soaking* and *priming* techniques in improving the germination potential of okra.

2. MATERIALS AND METHODS

The present study was carried out at Division of Basic Sciences and Humanities (FoH), Sher-e-Kashmir University of Agricultural Sciences and technology of Kashmir (SKUAST-K) during the year 2020. There were two sets of pre-sowing seed treatments; one is traditional seed *soaking* for specified period of time and sowing – simple seed *soaking* (T_1-T_4) and other is seed *soaking* for specified period of time and drying back to original seed weight and then sowing – seed *priming* (T_5-T_8). Simple seed *soaking* as well as *priming* treatments were performed in water (T_1 and T_5) as well as aqueous solutions of -1.0MPa PEG6000 (T_2 and T_6), 100ppm GA_3 (T_3 and T_7) and 1.5%KH_2PO_4 (T_4 and T_8), respectively for 18 hours (except PEG) at 25±2°C. Pre-soaking of seeds in PEG6000 was done for 24 hours due to their slow movement into the seeds [4]. Weighed amounts of okra seeds cv. Pusa Sawani were soaked in water as well as in aqueous solutions of different chemicals. After specified period of *soaking* half of the seeds were used directly for germination test (T_1-T_4) while other half were dried back to original seed weight (*priming*) and then used for germination test (T_5-T_8).

Three replicates of 50 seeds were put in petri-dishes lined with 10 layers of filter paper and saturated with distilled water and placed in seed germinator (ScienoCraft) at 25°C±2°C. Germination was observed daily until a constant count was achieved. The final germination percentage (FGP), coefficient of velocity of germination (CVG), time to 50 percent seed germination (T_{50}), mean germination time

(MGT), mean germination rate (MGR), synchrony of germination (Z-index) and germination index (GI) were calculated using equation I [9], II [10], III [11], IV [12], V [13], VI [14] and VII [15], respectively.

$$\text{FGP (\%)} = \frac{\text{No. of normal seedlings}}{\text{No. of seeds set for the test}} \times 100 \qquad\qquad .... \text{ (I)}$$

$$\text{CVG} = \frac{\sum_{i=1}^{k} niti}{\sum_{i=1}^{k} ni} \times 100 \qquad\qquad .... \text{ (II)}$$

Where, 'n' is the number of seeds germinated on every day and 't' is the number of days from seeding corresponding to 'n'.

$$T_{50} = T_i + \frac{(\frac{N+1}{2} - N_i)\,(T_j - T_i)}{N_j - N_i} \qquad\qquad .... \text{ (III)}$$

Where, N is the final number of germinated seeds, N_i and N_j are the total number of seeds germinated in adjacent counts at time T_i and T_i, respectively, when $N_i < (N+1)/2 < N_j$.

$$\text{MGT} = \frac{\sum(n \times d)}{N} \qquad\qquad (.......... \text{ IV)}$$

Where, n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

$$\text{MGR (day}^{-1}\text{)} = \frac{\text{CV}}{100} = \frac{1}{\text{T}} \qquad\qquad (.......... \text{ V)}$$

Where, T is mean germination time and CV is coefficient of velocity of germination.

$$Z = \frac{\sum_{i=1}^{k} Cni,2}{\sum ni,2} \qquad\qquad (.......... \text{ VI)}$$

Where, Cni,2 is the combination of seeds germinated in the ith time, two by two and ni is the number of seeds germinated in the ith time.

$$\text{GI} = (10 \times n1) + (9 \times n2) + (8 \times n3) + (7 \times n4) + (6 \times n5) + (5 \times n6) + (4 \times n7) + (3 \times n8) + (2 \times n9) + (1 \times n1) \qquad (.......... \text{ VII)}$$

Where, n1, n2, n3,……, n10 are the number of germinated seeds on the 1st, 2nd and subsequent days until the 10th day.

The data collected on different aspects were analyzed at 5% significance level [16]. Pearson correlation coefficient analysis between different seed germination attributes were performed [17].

3. RESULTS AND DISCUSSION

FGP is an important physiological parameter which is used to assess the planting value of seeds. Present study revealed that pre-sowing seed treatments significantly ($p \leq 0.05$) alter the FGP in okra (Table 1). The maximum FGP (93.3%) was recorded with T_1 (seed *soaking* in water) followed by T_2 (seed *soaking* in PEG solution), T_4 (seed *soaking* in KH$_2$PO$_4$ solution) and T_7 (seed *priming* in GA$_3$ solution) with FGP value of 88.0-90.0% that were statistically at par with each other. Among all the pre-sowing seed treatments, seed *priming* with PEG solution (T_6) exhibited the minimum FGP value of 78.30% that was still significantly higher than the FGP value of 65.0% recorded with untreated control (T_0). Further, average values of the two sets of pre-sowing treatments separately *viz.*, seed *soaking* and *priming*

indicated that seed *soaking* techniques were superior to seed *priming* in improving the FGP of okra. A lesser FGP in primed seeds may be attributed to a sort of stress experienced by the seeds during re-drying phase of *priming*. Moreover, growing embryo being a sensitive structure may be adversely affected by different chemicals used as *priming* agents that is why pure water *soaking* performed better than other treatments. Higher osmotic concentration can also reduce the germination percentage by altering the rate of water uptake [18].

The coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination (Jones and Sanders, 1987). Theoretically, the highest CVG possible is 100 and this would occur if all seeds germinated on the first day. In the present study (Table 1) the maximum CVG value (87.343 % day^{-1}) was recorded with seed hydro*priming* (T_5) which was significantly followed by T_6 (seed *priming* with PEG), T_7 (seed *priming* with GA_3) and T_1 (seed *soaking* in water) with measured CVG values of 77.14, 76.18 and 75.69% day^{-1}, respectively while as T_3 (seed *soaking* in GA_3 solution) showed the least CVG value (59.47% day^{-1}) among the treated seeds. Again CVG of control remained as significantly lower (39.56% day^{-1}) compared to treated seeds. Means of the CVG values of two sets of treatments indicated that seed *priming* (AvP) was better pre-sowing seed treatment than seed *soaking* (AvS). Seed *priming* has been reported to enhance DNA replication and DNA repair and promote mobilization of reserved materials [19] which contribute to accelerate seed germination. Superiority of seed *priming* treatments over seed *soaking* may be attributed to the improved membrane integrity linked with enhanced antioxidant defense mechanism due to seed *priming* [7].

Mean germination time (MGT) is a quick and reliable test to measure seed vigor and predict the rate of emergence, final emergence and uniformity. It is the reciprocal of the rate of germination. However, it is not the real time to mean germination but just an index [20]. Perusal of the data presented in table 1 reveal that T_5 (seed hydro*priming*) was found as most effective treatment in reducing the MGT (1.15day^{-1}) followed by T_7 (seed *priming* with GA_3) and T_6 (seed *priming* with PEG) along with T_1 (seed *soaking* in water) with measured MGT values of 1.30 and 1.32 day^{-1}, respectively. Among all the treatments T_3 (seed *soaking* in GA_3 solution) took highest MGT (1.70day^{-1}) followed by T_8 - seed *priming* with KH_2PO_4 solution (1.64 day^{-1}). Like CVG, seed *priming* (AvP) was also established as more effective treatment in reducing the MGT compared to seed *soaking* (AvS). Comparable results have also been reported in soybean [21] and in okra [4].

Different treatments were also evaluated in terms of mean germination rate (MGR) (Table 2) wherein T_5 (seed hydro*priming*) again proved as most effective treatment with highest MGR (0.614 day^{-1}) followed by T_7 (seed *priming* with GA_3 solution), T_4 (seed *soaking* in KH_2PO_4 solution) and T_1 (seed *soaking* in water) with MGR values of 0.598, 0.597 and 0.588day^{-1}, respectively, while as T_3 as well as T_8 were found as least effective treatments with measured MGR values of 0.505 and 0.517day^{-1}. However, comparison of the mean values of the two sets of treatments *viz.*, pre-sowing seed *soaking* (AvS) and seed *priming* (AvP) clarified that these two sets of treatments did not differ significantly with each other.

Enhanced germination rate due to different pre-sowing seed treatments may be attributed to the fact that many biochemical processes are modified due to these treatments which are basically needed for starting germination process *viz.*, dormancy breaking, hydrolysis, enzyme creation, and seed imbibition[22]. A faster seedling growth may also be attributed to higher α-amylase activity and total soluble sugar contents in soaked or primed seeds and seedlings[23.

An estimate of the time taken (in days) to achieve a certain percentage of germination is described as germination index (GI) is an estimate of the time (in days) it takes a certain germination percentage to occur that explain the germination percentage/speed relationship. The GI of okra seeds ranged among the treatments from 12.91 in T_8 (seed *priming* with KH_2PO_4 solution) to 16.29 in T_1 (seed *soaking* in water) against the minimum GI value (6.05) achieved in control (T_0). Treatment T_2 (seed *soaking* in PEG-6000 solution) as well as T_7 (seed *priming* with GA_3 solution) were proved as the second best treatment with observed GI values of 15.42 and 15.44, respectively. These treatments (T_2 and T_7) were significantly followed by T_4 (seed *soaking* in KH_2PO_4 solution) and T_6 (seed *priming* with PEG-6000 solution) with their GI values of 14.36 and 14.03, respectively. Analysis of the data further clarified that T_1 was at par with T_7 while as T_4 was at par with T_6. However, average values of the two sets of pre-sowing seed treatments indicated that pre-sowing seed *soaking* (AvS) and seed *priming* (AvP) did not differ significantly with regard to GI. An improved GI in pre-soaked and primed seeds may be attributed to increased metabolic activities, DNA replication and DNA repair [18].

Time to 50 percent seed germination (T_{50}), also known as the median germination time is another index to describe the speed of seed germination. Table 2 indicated that T_5 (seed *priming* with pure water) was found as most effective treatment and resulted in the least estimated value of T_{50} (1.18 day) which was statistically at par with T_1 (seed *soaking* in water), T_2 (seed *soaking* in PEG-6000 solution) T_4 (seed *soaking* in KH_2PO_4 solution) T_6 (seed *priming* with PEG-6000 solution) and T_7 (seed *priming* with GA_3 solution) with their observed T_{50} values of of 1.21, 1.22, 1.22, 1.23 and 1.20 day, respectively. Untreated okra seeds (T_0) exhibited a T_{50} value of 1.95 day. However, among the treated seeds T8 (seed *priming* with KH_2PO_4 solution) recorded the highest estimated value (1.36 day) of T_{50} followed by T_3 (seed *soaking* in GA_3 solution) with measured T_{50} value of 1.35 day. However, mean values of the two sets of pre-sowing seed treatments indicated that there was no significant difference between the pre-sowing seed *soaking* and *priming* with respect to T_{50}. Corroborating results have also been reported by earlier workers [18].

The Z index tells about the dynamics of the germination process wherein higher the value of Z index, more the uniformity in germination. The maximum value of Z = 1 that means all the seeds germinated at the same time while Z = 0 means at least two seeds could germinate, one at each time. Data presented in table 2 clarified that the maximum value of Z index (0.790) obtained with T_5 (seed *priming* with pure water) of the present study that was significantly (p≤0.5) followed by T_6 (seed *priming* with PEG-6000 solution), T_1 (seed *soaking* in water), T_7 (seed *priming* with GA_3 solution), and T_2 (seed

soaking in PEG-6000 solution) with measured Z index value of 0.693, 0.610, 0.600, and 0.573 in that order. However, T_1, T_2 and T_7 were found statistically at par with each other. Among all the treatments T_3 (seed *soaking* in GA_3 solution) and T_8 (seed *priming* with KH_2PO_4 solution) showed the least Z index value of 0.433. Mean values of the two sets of pre-sowing seed treatments indicated that pre-sowing seed *priming* is superior over pre-sowing seed *soaking*. The synchronization and promotion of germination with seed *priming* may take place for several reasons, but osmoconditioning and changes in metabolite levels are important events during seed *priming* [24,25]. Higher α-amylase activity and total soluble sugar contents in soaked or primed seeds and seedlings [23] were associated with the better seed germination and synchrony.

Pearson correlation coefficient analysis between different seed germination attributes (Table 3) revealed that FGP in the present investigation was positively correlated with CVG (r=0.63[**]), MGR (r=0.80[**]) and GI (r=0.89[**]) while as a negative relationship was observed between FGP and MGT (r=-0.76[*]) and between FGP and T_{50} (r=-0.76[*]). Significant relationship of CVG has also been seen with MGT (r=-0.97[*]), MGR (r=0.95[**]), GI (r=0.91[**]), T_{50} (r=-0.91[*]) and Z index (r=0.96[**]). Z index has also been found to be negatively associated with MGT (r=-0.87[*]), MGR (r=0.84[**]) GI (r=0.76[**]) and T_{50} (r=-0.77[*]). Correlation analysis also indicated a relationship of MGT with MGR (r=-0.97[*]), GI (r=-97[*]) and T_{50} (r=98[**]). In addition, a significant but small relationship (r=-0.98[*]) was also existed between GI and T_{50} in the present study.

4. CONCLUSION

Different pre-sowing seed treatments *viz.*, traditional *soaking* or *priming* significantly improved the seed germination potential of okra cv. Pusa Sawani in terms of final germination percent, rapidity of germination and uniformity of germination. Seed *priming* of okra with water (hydro*priming*) was proved as superior pre-sowing treatment over all other treatments. Comparative analysis of traditional seed *soaking* in water as well as in different chemical solutions with *priming* seeds using same liquid clarified that *priming* treatments, in general were established as superior pre-sowing seed treatments for okra compared to traditional seed *soaking* treatments. As such seed *priming* treatments should be practiced instead of traditional seed *soaking* treatments to achieve the better germination in okra.

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Table 1: Effect of different pre-sowing seed treatments on final germination per cent (FGP), coefficient of velocity of germination (CVG) and mean germination time (MGT) in okra

Parameter / Treatment	FGP	CVG ($\%day^{-1}$)	MGT (day^{-1})
T_0 Control	65.0[e] (8.12)	39.56[h] (6.37)	2.55[a]
T_1 Seed *soaking* in water (18 hours)	93.3[a] (9.71)	75.69[c] (8.76)	1.32[ef]
T_2 Seed *soaking* in PEG-6000 solution @-1.0MPa(24 hours)	90.0[b] (9.54)	73.14[d] (8.61)	1.37[e]
T_3 Seed *soaking* in GA_3 solution @100ppm (18 hours)	85.0[c] (9.27)	59.47[g] (7.78)	1.70[b]
T_4 Seed *soaking* in KH_2PO_4 solution @1.5% (18hours)	90.0[b] (9.54)	69.79[e] (8.41)	1.44[d]
T_5 Seed *priming* with pure water (18 hours)	83.3[c] (9.18)	87.34[a] (9.39)	1.15[g]
T_6 Seed *priming* with PEG-6000 solution @-1MPa (24 hours)	78.3[d] (8.91)	77.14[b] (8.84)	1.32[ef]
T_7 Seed *priming* with GA_3 solution @ 100ppm (18 hours)	88.3[b] (9.45)	76.18[c] (8.78)	1.30[f]
T_8 Seed *priming* with KH_2PO_4 solution @1.5% (18hours)	83.3[c] (9.18)	61.59[f] (7.91)	1.64[c]
AvS Average effect of different *soaking* treatments	89.6 (9.50)	69.52 (8.39)	1.46
AvP Average effect of different *soaking* treatments	83.3 (9.18)	75.56 (8.71)	1.35
C.D. (p= 0.05)	**0.141**	**0.047**	**0.037**

Values given in parentheses are squire root transformed values; Treatments that do not have the same letters are significantly different (p≤ 0.05) as determined by Duncan's multiple range test.

Table 2: Effect of different pre-sowing seed treatments on mean germination rate (MGR), germination index (GI) and time to 50 per cent germination (T_{50}) in okra

Treatment \\ Parameter		MGR (day^{-1})	GI	T_{50} (day)	Z index
T_0:	Control	0.394[f]	6.05[e]	1.95[a]	0.300[f]
T_1:	Seed *soaking* in water (18 hours)	0.588[b]	16.29[a]	1.21[c]	0.610[c]
T_2:	Seed *soaking* in PEG-6000 solution @-1.0MPa(24 hours)	0.573[c]	15.42[b]	1.22[c]	0.573[c]
T_3:	Seed *soaking* in GA_3 solution @100ppm (18 hours)	0.505[e]	12.98[d]	1.35[b]	0.433[e]
T_4:	Seed *soaking* in KH_2PO_4 solution @1.5% (18hours)	0.597[b]	14.36[c]	1.22[c]	0.513[d]
T_5:	Seed *priming* with pure water (18 hours)	0.614[a]	15.55[b]	1.18[c]	0.790[a]
T_6:	Seed *priming* with PEG-6000 solution @-1MPa (24 hours)	0.564[c]	14.03[c]	1.23[c]	0.693[b]
T_7:	Seed *priming* with GA_3 solution @ 100ppm (18 hours)	0.598[b]	15.44[b]	1.20[c]	0.600[c]
T_8:	Seed *priming* with KH_2PO_4 solution @1.5% (18hours)	0.517[d]	12.91[d]	1.36[b]	0.433[e]
AvS	Average effect of different *soaking* treatments	0.566	14.76	1.25	0.53
Avp	Average effect of different *soaking* treatments	0.573	14.48	1.24	0.63
C.D (p= 0.05)		**0.011**	**0.63**	**0.058**	**0.037**

Treatments that do not have the same letters are significantly different (p≤ 0.05) as determined by Duncan's multiple range tests.

Table 3. Pearson **correlation coefficient between different seed germination attributes**

Parameters	r	Explanation
FGP × CVG	0.6319	Significant large positive relationship between FGP and CVG, (r(9) = .632, p = .037).
FGP × MGT	-0.7636	Significant very small negative relationship between FGP and MGT, (r(9) = .764, p =.006).
FGP × MGR	0.7965	Significant large positive relationship between FGP and MGR, (r(9) = .797, p = .003).
FGP × GI	0.8895	Significant large positive relationship between FGP and GI, (r(9) = .89, p < .001).
FGP × T_{50}	-0.8428	Significant very small negative relationship between FGP and T_{50}, (r(9) = .843, p =.001).
CVG x MGT	-0.9705	Results of the pearson correlation indicated that there is a significant very small negative relationship between CVG and MGT, (r(9) = .971, p < .001).
CVG x MGR	0.9504	Results of the pearson correlation indicated that there is a significant large positive relationship between CVG and MGR, (r(9) = .95, p < .001).
CVG x GI	0.9066	Significant large positive relationship between CVG andGI, (r(9) = .907, p < .001).
CVG x T_{50}	-0.9081	Significant very small negative relationship between CVG and T_{50}, (r(9) = .908, p < .001)
CVG × Z index	0.9588	Significant large positive relationship between CVG andZ index, (r(9) = .959, p < .001).
MGT × MGR	-0.9704	Significant very small negative relationship between MGT and MGR, (r(9) = .97, p < .001).
MGT × GI	-0.97	Significant very small negative relationship between MGT andGI, (r(9)= .97, p < .001).
MGT × T_{50}	0.9811	Significant large positive relationship between MGT andT_{50}, (r(9) = .981, p < .001).
MGT × Z index	-0.8713	Significant very small negative relationship between MGT andZ index, (r(9) = .871, p < .001).
MGR × GI	0.9524	Significant large positive relationship between MGRandGI, (r(9) = .952, p < .001).
MGR × T_{50}	-0.9529	Significant very small negative relationship between MGRandT_{50}, (r(9) = .953, p < .001).
MGR × Z index	0.8352	Significant large positive relationship between MGRandZ index, (r(9) = .835, p = .001).
GI × T_{50}	-0.98	Significant very small negative relationship between GI and T_{50}, (r(9) = .98, p < .001).
GI × Z index	0.7628	Significant large positive relationship between GI and Z index, (r(9) = .763, p = .006).
T_{50} × Z index	-0.7675	Significant very small negative relationship between T_{50} and Z index, (r(9) = .767, p = .006).