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K Stephen
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

FA Khan
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

SA Bhat
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

S Narayan
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

SA Mir
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, Srinagar, India

MS Mir
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Khursheedhussain
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, Srinagar, India

Mansha Gul
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Asmakhurshid
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Irtizasiddiqi
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, Srinagar, India

Syed Mazahir Hussain
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Sujat Ahmad Lone
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Correspondence
K Stephen
Sher-E-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

Optimizing priming concentration and duration of various priming agents for improved seed germination in chilli (*Capsicum annum* L.)

K Stephen, FA Khan, SA Bhat, S Narayan, SA Mir, MS Mir, Khursheed Hussain, Mansha Gul, ASMA Khurshid, Irtiza Siddiqi, Syed Mazahir Hussain and Sujat Ahmad Lone

Abstract
The present investigation was carried out on chilli cv. Kashmir long –I during the year 2017 at the Division of Basic Sciences and Humanities, SKUAST-K to standardize the priming concentration and duration of different priming agents for improved seed germination potential. Uniform seeds were soaked in different concentrations of PEG 6000 solution – osmopriming (-0.5, -1.0, and -1.5MPa), vermiwash, vermin priming (4, 6 and 8× dilution) and melatonin – hormonal priming (5, 10 and 15ppm), kept in an incubator for 18, 24 and 30 hours. Seeds were also soaked in distilled water – hydropriming. After a specified period of time seeds were removed from the solutions and air dried at room temperature. 50 primed seeds were sown in petri-dishes lined with ten layers of water saturated blotting papers and kept in germinator for germination studies. Observations on various germination parameters were recorded at (25±2°C). Priming of chilli seeds for with -0.5 MPa solution of PEG (24 hours), 8× dilution of vermin wash (30 hours) and 5ppm solution of melatonin (30 hours) gave the best results in terms of various germination attributes. Among various hydro priming durations 30 hours was proved as best treatment.

Keywords: germination, chilli, hydropriming, osmopriming, vermipriming, melatonin priming

1. Introduction
Chilli (*Capsicum annum* L.) is an important solanaceous vegetable crop grown for its unripe-green and ripe-red fruit which, in whole or powder form is an indispensable condiment, digestive stimulant as well as flavouring and colouring agent in sauces, chutnies, pickles and other forms of food. Seed germination is an important and vulnerable stage in the life cycle of terrestrial plants as it determines the crop yield through per cent emergence and time from sowing to emergence. It has been reported that chilli seed germination is slow and non-uniform under normal as well as abiotic stress conditions including moisture and cold stresses (Yadav *et al*., 2011) [1]. Seed priming can increase the rate and extent of emergence, improve seedling vigour, advance flowering and maturity along with an increase in yield in most cases. It is a recognized technique to improve the seed vigour over a range of environmental conditions with quick and synchronized germination and emergence. Owing to its low cost, seed priming represents a good insurance for risk-averse, resource-poor farmers (Harris, 2006) [2]. It is an approach of pre-sowing seed treatment for influencing the seed germination and seedling development by tempering pre-germination metabolic activities, proceeding to emergence of the radicle and normally enhances germination rate and plant performance (Bradford, 1986) [3]. In the process of priming, seeds are partially hydrated so that pre-germinative metabolic activities proceed, while radicle protrusion is prevented; thereafter they are dried back to the original moisture level (Mc Donald, 2000) [4]. However, response of seed germination to priming varies from crop to crop, variety to variety, and success of this technique depends. On priming agents used, its concentration and priming duration. Numerous priming techniques have been found to be valuable pre-sowing seed treatments that upsurge the speed of germination and seedling development, as well as advance the pre-metabolic activities to withstand the field stress conditions such as deficiency of water or hostile temperatures. In the present study, in addition to traditional priming techniques of hydropriming and osmopriming we have standardized some new priming agents like vermiwash and melatonin. Hydropriming introduces liquid water to seeds in controlled and precise amounts to achieve a desired level of hydration (Mc Donald, 1999) [5]. Osmopriming, also known as osmoconditioning is described as a pre-sowing seed treatment in osmotic solution of low water potential (generally PEG). Vermipriming is an innovative addition to

already existing priming techniques which involves the soaking of seeds in diluted solutions of vermiwash for a certain period of time and then drying the seeds up to its original weight (Khan *et al*., 2015; Khan *et al*., 2017) [6, 7]. Seed priming with optimal concentrations of plant growth regulators (PGRs) to improve germination, stand establishment, plant growth and yield of crop plants is known as hormonal priming but melatonin (recently established as phytohormone) has shown enormous potential of seed invigoration but has not been exploited fully.

2. Materials and Methods

The current study was carried out at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar. The study was conducted on chilli cultivar Kashmir Long-1.The seeds were soaked for three different durations i.e. 18, 24 and 30 hours. Hydropriming was done with distilled water while as osmopriming, vermipriming and hormonal priming were done with PEG-6000 (-0.5 MPa, -1.0 MPa and -1.5 MPa), vermiwash (4×, 6× and 8×) and melatonin (5, 10 and 15ppm). Thus there were total nine treatment combinations each for osmopriming, vermipriming and melatonin priming and three treatments for hydropriming. Each treatment was replicated three times. Each replication had 50 seeds placed in petridishes lined with 10 layers of blotting paper. The petridishes were placed in a seed germinator with temperature maintained at 25 ± 2ºC and humidity at 70% and given adequate amount of water for germination. The parameters evaluated for determining the best treatment combination were:

Rate of germination - It was calculated as the number of seeds germinated per day. Daily germination count was taken till germination ceased. The rate of germination was calculated as given by Esechie (1994) [8],

Rate of germination = (Final germination percent / Days of completion of germination)

Final germination percent-It was calculated using the formula,

Final germination percent = (Number of seeds germinated / Total number of seeds kept for germination) × 100

Radicle length: The radicle length was measured from collar region of seedling upto the apex after 20 days from the date of sowing.

Plumule length: The plumule length was measured from the collar region of seedling upto the apex after 20 days from the date of sowing.

Germination Index: Germination index (GI) was calculated according to Bench *et al*. (1991) [9] using the following formula,

GI = (10×n1) +(9×n2) +(8×n3) + (7×n4) +(6×n5)+(5×n6) + (4×n7)+ (3×n8)+ (2×n9)+(1×n1)

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

Seedling vigour index-1 - Seedling vigour index – 1 was calculated as per the formula given by Abdul-Baki and Anderson (1973) [10],

Seedling vigour index -1 = Germination (%) × Seedling length

Statistical Analysis - Experimental data was subjected to the statistical analysis following procedures as described by Gomez and Gomez (1984) [11]. Level of significance used for F and t – tests were $p \leq 0.05$ from the table given by Fisher (1970) [12] and the data collected was subjected to statistical analysis using statistical software "SPSS."

3. Results

The results of the investigation are presented in the following Tables:

Table 1: Effect of different hydropriming durations on seed germination and seedling growth attributes of chilli seedlings

Hydropriming Durations (H)	Final germination percent (%)	Rate of germination (no./day)	Germination index	Radicle length (cm)	Plumule length (cm)	Seedling vigour index-1
18 hours (T_1)	96.00 (9.79)	6.00	1267.33	4.16	4.26	808.64
24 hours (T_2)	96.67 (9.83)	6.04	1417	4.36	4.3	837.4
30 hours (T_3)	99.33 (9.96)	6.20	1814	4.86	4.60	940.66
Mean	97.33 (9.86)	6.08	1509.44	4.46	4.38	862.23
C.D. ($P \leq 0.05$)	0.04	0.04	73.24	0.07	0.08	12.08

(Values in parenthesis are square root transformed)

Table 2: Effect of different concentrations of PEG 6000 solution and priming durations on seed germination and seedling growth attributes of chilli seedlings

Osmopriming Treatments	Final germination percent (%)	Rate of germination (no./day)	Germination index	Radicle length (cm)	Plumule length (cm)	Seedling vigour index-1
O_1T_1	98.67 (9.93)	6.16	1725.33	4.89	4.88	964.99
O_1T_2	97.33 (9.86)	6.08	1419.33	4.91	4.63	928.52
O_1T_3	91.33 (9.55)	5.70	1346.33	4.79	4.93	887.72
O_2T_1	96.00 (9.79)	6	1137.67	4.63	4.63	888.96
O_2T_2	97.33 (9.86)	6.08	1168	4.79	4.83	936.46
O_2T_3	95.33 (9.76)	5.95	1076	4.66	4.44	868.29
O_3T_1	96.00 (9.79)	6	958.67	4.75	4.69	906.88
O_3T_2	94.67 (9.72)	5.91	1207.67	4.74	4.08	835.84
O_3T_3	96.67 (9.83)	6.04	961	4.12	4.53	837.48
Mean	95.92 (9.79)	5.99	1222.44	4.69	4.62	895.01
C.D. ($P \leq 0.05$)	0.05	0.06	62.88	0.05	0.07	20.59

O1, O2 and O3 are PEG 6000 solutions with water potential of -0.5, -1.0 and -1.5 MPa, respectively; T1, T2 and T3 are priming durations of 18, 24 and 30 hours, respectively.

(Values in parenthesis are square root transformed)

Table 3: Effect of different dilutions of vermiwash and priming durations on seed germination and seedling growth attributes of chilli seedlings

Vermipriming Treatments	Final germination percent (%)	Rate of germination (no./day)	Germination index	Radicle length (cm)	Plumule length (cm)	Seedling vigour index-1
V_1T_1	88.67 (9.41)	8.86	1605.33	4.07	3.61	681.37
V_1T_2	96.67 (9.83)	9.66	2181.00	4.91	4.05	867.12
V_1T_2	94.67 (9.72)	9.46	2335.67	4.68	3.81	804.77
V_2T_1	97.33 (9.86)	9.73	1859.67	4.38	3.84	800.87
V_2T_2	97.33 (9.86)	9.73	2099.67	4.70	4.28	874.76
V_2T_3	96.67 (9.83)	9.66	2043.00	4.85	4.23	878.2
V_3T_1	97.33 (9.86)	9.73	1743.00	4.35	3.79	792.61
V_3T_2	97.33 (9.86)	9.73	2187.33	4.86	4.26	888.06
V_3T_3	100.00 (10)	10.00	2704.00	5.00	4.42	942.33
Mean	96.22 (9.80)	8.50	2014.37	4.64	4.03	836.67
C.D. (P≤0.05)	0.04	0.06	61.49	0.06	0.04	19.72

V1, V2 and V3 are 4.0, 6.0 and 8.0 × dilution of vermiwash, respectively; T1, T2 and T3 are priming durations of 18, 24 and 30 hours, respectively.
(Values in parenthesis are square root transformed)

Table 4: Effect of different concentrations of melatonin and priming durations on seed germination and seedling growth attributes of chilli seedlings

Melatonin Priming Treatments	Final germination percent (%)	Rate of germination (no./day)	Germination index	Radicle length (cm)	Plumule length (cm)	Seedling vigour index-1
M_1T_1	91.33 (9.55)	7.02	1454	4.39	4.11	777.01
M_1T_2	94.67 (9.72)	7.28	1434.33	5.09	4.23	882.32
M_1T_3	96.67 (9.83)	7.43	2202.33	5.02	4.94	962.82
M_2T_1	91.33 (9.55)	7.02	1426.67	4.48	4.50	821.30
M_2T_2	89.33 (9.45)	6.87	1043.67	4.18	4.14	744.06
M_2T_3	92.67 (9.62)	7.12	2314.13	4.67	4.73	871.46
M_3T_1	94.67 (9.72)	7.28	1497	4.37	4.54	843.41
M_3T_2	96.00 (9.79)	7.38	1725	4.45	4.6	868.8
M_3T_3	92.67 (9.62)	7.12	1711	3.98	4.83	816.95
Mean	93.25 (9.65)	7.16	1645.34	4.51	4.51	843.12
C.D. (P≤0.05)	0.04	0.04	47.28	0.09	0.11	25.94

M1, M2 and M3 are melatonin solution with 5.0, 10.0 and 15.0 ppm, respectively; T1, T2 and T3 are priming durations of 18, 24 and 30 hours, respectively.
(Values in parenthesis are square root transformed)

Hydropriming Treatments

Information with regard to optimization of priming concentrations and/ or priming duration of various priming agents for improved seed germination in chilli are presented in table 1. Hydropriming of seeds for 18, 24 and 30 hours at 25ºC results in significant variations with respect to different seed germination and seedling growth attributes. Hydropriming of chilli seeds for 30 hours resulted in highest seed germination (99.33%), rate of seed germination (6.20 no./day) and germination index (1814) which is also accompanied by highest seedling growth in terms of radical length (4.86 cm), plumule length (4.60 cm) and seedling vigour index-I (940.66) followed by 24 and 18 hours duration of seed priming.

Osmopriming Treatments

So far as different osmopriming concentrations and durations are concerned (Table 2) the highest values of germination speed in terms of final germination percent (98.67%), rate of germination (6.16 no./day), germination index (1725.33) and SVI-I (964.99) were recorded when seed priming was done in PEG 6000 solution of -0.5 MPa for 18 hours (O_1T_1). However, highest values in relation to seedling growth particularly radical length (4.91 cm) and plumule length (4.93 cm) were recorded in O_1T_2 (-0.5 MPa of PEG 6000 for 24 hours) and O_1T_3 (-0.5 MPa of PEG 6000 for 30 hours), respectively. Anyway, the highest values of radical length and plumule length recorded in O_1T_2 and O_1T_3 were statistically at par with O_1T_1. Hence, treatment O_1T_1*i.e.* seed priming in -0.5 MPa solution of PEG 6000 for 18 hours may be considered as best treatment among the different osmopriming treatments.

Vermi priming Treatments

Results pertaining to effect of seed priming treatments with different dilutions of vermin wash for a range of priming durations are presented in table 3. Data indicate that V_3T_3*i.e.* seed priming in 8× dilution of vermin wash for 30 hours produced highest values of seed germination rate (10.0 no./day) as well as seed germination index (2704). This particular treatment also resulted in maximum percentage of seed germination (100.00%), radical length (5.00 cm) and plumule length (4.42 cm) which caused the highest value (942.3) of seedling vigour-I. As such V_3T_3 was proved as the best priming treatment when we talk about different vermin priming concentrations and duration.

Melatonin Priming Treatments

Seed priming with different concentration of melatonin (recently established as phytohormome) for varying durations of time resulted in significant variations in different seed germination and seedling growth attributes (Table 4). Treatment M_1T_3 (seed priming with 5.0 ppm of melatonin solution for 30 hours) resulted in highest percentage of seed germination (96.67%) with fastest rate (7.43 no./day) accompanied by maximum plumule length (4.94 cm) and SVI-I (934.8). However, the highest values of seed germination index (2314.13) and radical length (5.09 cm) were recorded in M_2T_3 (seed priming with 10.0 ppm of

melatonin solution for 30 hours) and M_1T_2 (seed priming with 5.0 ppm of melatonin solution with 24 hours of priming duration) which were statistically at par with the values of seed germination index (2202.33) and radical length (5.02 cm) obtained under M_1T_3 treatment. Therefore, treatment M_1T_3 (seed priming with 5.0 ppm of melatonin solution for 30 hours) can be taken as best treatment among different melatonin priming treatments.

4. Discussion

Kang *et al*. (2000) [13] studied the effect of hydropriming to enhance the germination of bitter gourd seeds. The results revealed that compared with priming using chemicals (calcium nitrate, potassium hydroxide or polyethylene glycol), hydro priming was best in promoting germination (91.7%), and the optimum condition for hydropriming was at 25^0C for 2 days. Mosavian and Esmailzade-Moridani (2016) [14] conducted a laboratory experiment to study the effects of hydro priming on rapeseed. They found that hydro priming improved germination percentage and speed of germination. The highest germination percentage and speed was observed in the treatment of 24 hours duration. Generally, the increase in duration of priming improved the performance of seeds and seedlings of rapeseed. From these observations we see that the duration of hydro priming varies between crops depending on their rate of germination metabolic activities. Thus we see that for chilli the optimum duration of hydro priming is 30 hours. Fu and Fu (1990) [15] reported improved germination rate and uniform emergence both in laboratory and field conditions of two groundnut seed lots when treated with 20 to 25 percent PEG at 15^0C for two days. Murray *et al*. (1992) [16] conducted an experiment and found that seeds took 10 to 12 percent less time for 50 percent germination both in field and laboratory when onion seeds were primed with PEG-6000 (300g/1000 ml H_2O) for seven days. Cotton seed lots primed with PEG-6000 at -15 bars for 72 hours recorded maximum germination percentage, field emergence, seedling vigour index and low electrical conductivity compared to control (Ramegowda *et al*., 2006) [17]. We observe that each crop varies in respect to concentration and soaking duration for improved germination characteristics. In the present investigation we conclude that chilli shows best germination characteristics when primed with PEG-6000 at a concentration of -0.5 MPa and 18 hours soaking duration.

Although found to be cost effective and highly beneficial due to its increased amount of micronutrients, vermin priming is yet to find widespread application as a priming agent in agricultural crops. Khan *et al*. (2016) [18] conducted seed priming studies with vermiwash on Shalimar rice-I and reported that significant values of final germination percentage (FGP), time taken to 50% germination (T50), energy of germination (GE) and timson's index of germination velocity were recorded with vermipriming. Vermipriming treatment also indexed maximum root and shoot dry weight and length as well as seedling vigour index – I & II. Kamal *et al*. (2016) [19] conducted a field experiment and reported that okra seeds primed with vermiwash @ 20% and 50% showed improved seed germination, seedling length, dry weight, vigour index –I and II over unprimed control in both the varieties evaluated. Among all, vermiwash @ 50% showed significantly superior values over rest of the treatments. In our present investigation, we have found that priming of chilli seed with vermiwash solution diluted 8 times for a duration of 30 hours is best for improving the germination and establishment parameters. Jiang *et al*. (2016) [20] studied the effects of seed priming with melatonin in maize under salinity stress and found that seed priming with 0.8 mM melatonin significantly improved germination energy, germination percentage, seedling vigour index, shoot and root lengths, seedling fresh and dry weights, K+ content, relative water content, proline and total phenolic contents, superoxide dismutase, catalase and phenylalanin ammonia lyase activities; and significantly decreased mean emergence time, Na+ content, electrolyte leakage and malondialdehyde content compared with untreated seeds. Korkmaz *et al*. (2015) [21] conducted an experiment to study the effect of seed treatment with melatonin on germination and emergence performance of pepper seeds under chilling stress. They found that treatment of seeds with melatonin especially in 1 or 5 µM concentrations significantly improved germination and emergence percentage. Melatonin application also reduced the malondialdehyde (MDA) and hydrogen peroxide (H2O2) contents and elevated SOD and CAT enzyme activities. Among all the melatonin priming treatments under the present study we find that priming with melatonin solution at a concentration of 5 ppm soaked for 30 hours duration is best for chilli germination.

5. Conclusion

From the present study we conclude that the following treatments are the best from each of the priming agents- Hydropriming with distilled water for a duration of 30 hours, osmopriming with PEG-6000 at aconcentration of -0.5 Mpa for a duration of 18 hours, vermipriming with vermiwash solution diluted 8 times and soaked for a duration of 30 hours and melatonin priming with melatonin solution at concentration of 5 ppm and soaking duration of 30 hours.

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