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Effect of seed priming on physiological parameters of aged and non-aged seeds of bitter gourd, *Momordica charantia* L

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ABSTRACT

Two seed lots viz V_1 (high vigour seeds) and V_2 (72 hours accelerated aged seeds/low vigour seeds) of bitter gourd (*Momordica charantia* L) cultivar Solan Hara were subjected to osmopriming, halopriming, solid matrix priming and seed soaking in water (farmers' common practice) to find out the ability of priming treatments in improving seed germination vigour through initiation of metabolic processes and repair of deterioration sustained during ageing. Seed quality parameters like germination percentage, seedling length, dry weight and vigour index I and vigour index II were measured using paper towel and sand method. Overall accelerated ageing reduced the above mentioned quality parameters. However seed priming improved germination in both the vigour groups of seeds through initiation of cell cycle, metabolic repair and reduction in the deleterious effects of ageing. Among all the seed priming treatments solid matrix priming with perlite proved superior for increasing germination and improving other quality attributes followed by solid matrix priming with cocopeat and vermiculite.

Keywords: Bitter gourd; vigour; accelerated ageing; solid matrix priming

INTRODUCTION

Bitter gourd, *Momordica charantia* L is a vegetable widely grown for its edible fruit and is popular due to its medicinal properties. In India during the year 2012-13 bitter gourd covered an area of 83.22 million hectares with average annual production of 940.15 million tons (Anon 2012-13). In Himachal Pradesh to get crop early in the season the seeds need to be sown in the month of March-April. Similarly in north western Indian plains bitter gourd is generally sown early in the month of December or January so that the crop gets ready for harvesting in March-April. The major problem during this period is poor and slow emergence of bitter gourd seedlings due to prevailing sub-optimal temperatures. The germination of bitter gourd seed is adversely hampered at sub-optimal temperatures ie below 18^0C. In addition to this thick seed coat enclosing the embryo affects germination by imposing mechanical restriction on embryo growth (Pandita and Nagarajan 2004). Moreover the carry over viable seeds in the market have unsynchronized and low emergence rate due to differential rate of deterioration experienced by seed lots under different storage environments. This problem of poor and slow seed germination can be solved through many techniques and one of them is seed priming.

MATERIAL and METHODS

The present investigations were carried out in the laboratory of Department of Seed Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP during the year 2012 on Solan Hara cultivar of bitter gourd. The experiment was performed using paper towel (replicated four times with 100 seeds per replication) and sand method (replicated three times with 20 seeds per replication) in completely randomized design (CRD). Two vigour groups of seeds viz V_1 (high vigour seeds) and V_2 (72 hours accelerated aged seeds/low vigour seeds) and nine seed priming treatments viz P_1 (osmopriming with PEG 6000- 1.0 MPa), P_2 (halopriming with KNO_3 @ 1%), P_3 (halopriming with KH_2PO_4 @ 1%), P_4 (halopriming with $CuSO_4$ @ 100 ppm), P_5 (solid matrix priming with cocopeat), P_6 (solid matrix priming with perlite), P_7 (solid matrix priming with vermiculite), P_8 (seed soaking in water), P_9 (control -without treatment) were used.

Accelerated ageing

The seeds were subjected to controlled deterioration (accelerated aging) by equilibrating the seeds in about 90 per cent relative humidity at 40 $\pm$ 2°C for 72 h in dessicator. Thereafter the seeds were dried back at room temperature to reach 80 per cent moisture content.

Standardization of seed priming

Bitter gourd seeds (100 g) were placed in water for 85 h at 20°C. After every 1, 2, 3, 6, 12, 20, 24, 35, 45, 55, 64, 75 and 85 h per cent water imbibed (wet seed weight minus initial seed weight) was determined to estimate the maximum water absorption capacity of the seeds. Values of the per cent water absorption by seeds were 23, 28, 32, 40, 46, 50, 51, 54, 55, 56, 57, 60 and 70 per cent respectively for the given durations. The per cent water absorption from 23 to 50 was considered as phase I of the germination where rapid water uptake occurred. It was followed by a plateau phase (phase II) with little change in water content from 51 to 57 per cent. A subsequent increase in water content coinciding with radical emergence and resumption of growth with water absorption from 60 to 70 per cent was considered phase III of the germination. Here germination strictly refers to phase I and phase II of this process during which imbibed seeds maintained their desiccation tolerance. The per cent water absorption of phase I and II which ranged from 23 to 57 per cent with maximum water soaking time of 72 h was included in the study (Fig 1).

Method of seed priming

For osmopriming and halopriming samples of 100 g of bitter gourd seeds from each two seed lots of different vigour groups per treatment were soaked in double the volume of osmoticum in the petriplates lined with filter paper for about 72 h at 20^{o}C and 90 per cent relative humidity in germinator. The seeds were then washed 3-4 times with tap water. In case of seed soaking 100 g seeds from two seed lots of different vigour groups per treatment were simply soaked in water for 24 h at 20^{o}C and 90 per cent relative humidity in germinator. Care was taken that entire seed surface did not get completely immersed in water to avoid the build up of anaerobic condition.

For solid matrix priming sample of 100 g of seeds from two seed lots of different vigour groups per treatment were put in separate containers containing carrier. Seeds/solid matrix/water were mixed in the ratio of 1:2:1 for each treatment and shaked well so that seeds and priming media were mixed properly. Seed priming was done for 72 h at 20^{o}C and 90 per cent relative humidity in germinator. The containers were shaked manually after every 3-4 h for proper aeration. After seed priming for 72 h the solid matrix carrier was separated from the seeds through sieving and seeds were washed 3-4 times with tap water.

The primed seeds were then dried completely at room temperature to 8 per cent moisture content. Unprimed seeds served as control.

Method of data collection

After priming the seeds were subjected to germination test using paper towel and sand method following the ISTA procedure (Anon 2001). The first and final count was taken on 4th and 14th day of the test respectively. Germination percentage was worked out using the following formula:

$$\text{Germination (\%)} = \frac{\text{\# Normal seedlings germinated}}{\text{\# Seeds kept for germination}} \times 100$$

On the first count of the germination test ie 4th day ten normal seedlings were selected at random from both paper towel and sand method to work out other quality parameters like seedling length, dry weight, vigour index I and vigour index II. Seedling length was worked out by taking the total length of each seedling from the tip of the primary leaf to the tip of primary root with the help of scale and expressing the mean value in centimetre (cm). The same 10 seedlings were placed in butter paper

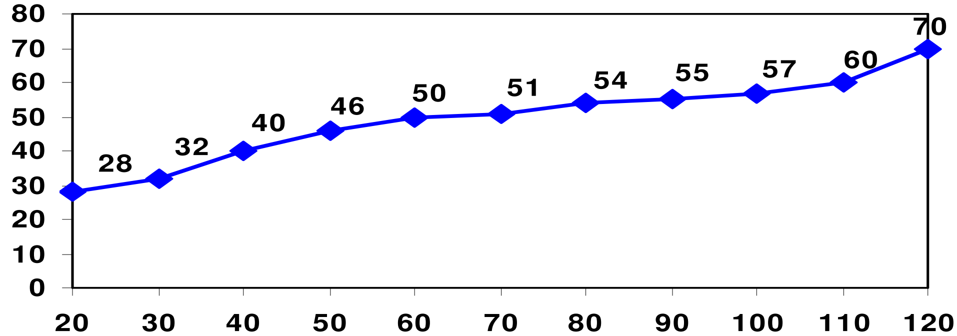

Fig 1. A graph representing the per cent water absorption

pocket and kept in oven at 50^{o}C for 48 hours to work out the dry weight. The seedling dry weight was recorded using the electronic weighing balance and the mean value was expressed in milligrams (mg). The seedling vigour index I was calculated as the product of germination and seedling length whereas seedling vigour index II was calculated as the product of germination and seedling dry weight (Abdul-Baki and Anderson 1973). The statistical analysis was done as per design of the experiment as suggested by Gomez and Gomez (1984).

RESULTS and DISCUSSION

The mean germination percentage, seedling length, dry weight, vigour index I and vigour index II differed significantly among the seed vigour levels. Maximum seed quality attributes were noticed in high vigour seeds V_1 and lowest were observed in low vigour seeds V_2 irrespective of priming treatments in both paper towels (Table 1) and sand method (Table 2). Sung and Jeng (1994) reported enhanced lipid peroxidation and increased peroxide accumulation in the axis and cotyledons of aged seed that inhibited seed germination and seedling growth of peanut. Similar findings were also reported by Amritaphale and Singh (1994) in soybean seeds. They concluded that seedling dry matter and vigour index decreased due to ageing accompanied by an increase in the leakage of solutes. Kapoor et al (2011) concluded that ageing led to deterioration of both germinability and seed viability in rice.

The main effect of seed priming treatments differed significantly for seed germination percentage, seedling length, dry weight, vigour index I and vigour II (Table 1 & 2). Seeds primed with perlite (P_6) recorded highest above mentioned parameters in both paper towel (83.63%, 18.49 cm, 133.85 mg, 1554.74 and 11225.61) and sand method (80.25%, 18.05 cm, 253.88 mg, 1438.97 and

20422.20) followed by cocopeat (P_5) and vermiculite (P_7). The possible reason why solid matrix carriers proved good compared to other priming treatments could be that these priming treatments mimic the behavior of the planting substrate ie soil. The porosity or the friable nature with adequate aeration might have further added for better results compared to other treatments. Unprimed seeds (P_9) stood the poorest of all the treatments with respect to traits under consideration. The increased germination and seedling vigour was due to the repair of deteriorated seed parts during the hydration phase of the priming process (Karssen and Haigh 1989, Saha et al 1990). Pandita and Nagarajan (2000) reported that priming treatments result in increased seedling vigour and growth in tomato. Seed soaking in water treatment (P_8) recorded germination of 76.13 per cent, 16.16 cm, 124.78 mg, 1233.84 and 9523.23 values for the quality parameters.

The interaction due to seed vigour levels and seed priming treatments also differed significantly. The treatment perlite stood the best among all the treatments followed by cocopeat and vermiculite wrt both the vigour groups of seeds and methods viz paper towel and sand method. Study of interaction effects between seed vigour levels and priming treatments revealed the significant improvement in germination percentage, seedling vigour index I and II over control in both high vigour and low vigour levels (Table 1 & 2). High vigour seeds primed with perlite (V_1P_6) recorded an increase in germination, seedling vigour index I and II of 17.25 per cent, 735.15 and 3327.97 in paper towel method and 17.00 per cent, 585.88 and 8350.5 in sand method over respective controls (V_1P_9). On the other hand low vigour seeds primed with perlite recorded an improvement of 15.00 per cent, 615.15 and 3343.1 in paper towel method and 14.75 per cent, 726.83 and 9269.97 in sand method wrt characters studied over respective controls (V_2P_9). Interaction effect due to seed soaking in water also depicted improvement over respective controls wrt concerned parameters in both the vigour groups of seeds under study. The possible reason for enhanced germination lies in the fact that during seed priming there is a rapid initial water uptake leading to the initiation of metabolic processes which gives a primed seed a head start over the un-primed seed. Many proteins and enzymes involved in cell metabolism are synthesized to a level intermediary between the dry seed and the seed imbibed directly in water while a few are synthesized to the same extent as the germinating seed during priming. Serge et al (2011) concluded that priming induced catalase synthesis which is involved in seed recovery during priming. Another ameliorative effect of priming is the repair of damaged DNA, early onset of RNA, protein synthesis and polyribosome formation (Varier et al 2010). All these factors together were effective in improving seed performance.

Table 1. Effect of seed priming on germination percentage, seedling length, dry weight, vigour index I and vigour index II among different vigour levels of bitter gourd seeds (paper towel method)

Parameter	Seed priming									
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	Mean (V)
Germination (%)										
V_1	85.50	84.00	83.50	75.50	90.00	91.25	87.50	82.00	74.00	83.69
	(67.70)	(66.45)	(66.05)	(60.32)	(71.62)	(72.87)	(69.31)	(64.98)	(59.33)	(66.51)
V_2	74.00	71.50	71.25	69.00	75.00	76.00	73.50	70.25	61.00	71.28
	(59.32)	(57.71)	(57.56)	(56.16)	(59.98)	(60.65)	(58.99)	(56.93)	(51.34)	(57.63)
Mean (P)	79.75	77.75	77.38	72.25	82.50	83.63	80.50	76.13	67.50	
	(63.51)	(62.08)	(61.80)	(58.24)	(65.80)	(66.76)	(64.15)	(60.95)	(55.33)	
$CD_{0.05}$ (V): 0.81, $CD_{0.05}$ (P): 1.71, $CD_{0.05}$ (V x P): 2.42										
Seedling length (cm)										
V_1	17.47	16.94	16.92	15.15	18.48	19.58	18.14	16.88	14.21	17.08
V_2	16.05	15.56	15.47	15.39	16.93	19.58	16.65	15.44	11.61	15.61
Mean (P)	16.76	16.25	16.19	15.27	17.70	18.49	17.39	16.16	12.91	
$CD_{0.05}$ (V): 0.19, $CD_{0.05}$ (P): 0.41, $CD_{0.05}$ (V x P): 0.58										
Seedling dry weight (mg)										
V_1	134.18	131.21	130.00	123.83	137.61	138.20	136.40	129.15	125.38	131.77
V_2	123.73	123.45	122.18	116.35	127.53	129.50	126.10	120.40	106.53	121.75
Mean (P)	128.95	127.33	126.09	120.09	132.57	133.85	131.25	124.78	115.95	
$CD_{0.05}$ (V): 0.89, $CD_{0.05}$ (P): 1.91, $CD_{0.05}$ (V x P): 2.69										

Seedling vigour index I										
V_1	1494.26	1424.21	1412.77	1143.17	1662.88	1786.57	1586.94	1383.17	1051.42	1438.37
V_2	1187.85	1112.48	1102.17	1061.91	1269.67	1322.92	1224.00	1084.52	707.77	1119.25
Mean (P)	1341.06	1268.34	1257.47	1102.54	1466.27	1554.74	1405.47	1233.84	879.59	
$CD_{0.05}$ (V): 24.98, $CD_{0.05}$ (P): 52.99, $CD_{0.05}$ (V x P): 74.94										
Seedling vigour index II										
V_1	11469.90	11023.67	10856.45	9349.69	12384.35	12609.17	11934.15	10586.95	9281.20	11055.06
V_2	9155.15	8826.38	8705.88	8024.50	9564.69	9842.05	9268.15	8459.50	6498.95	8705.03
Mean (P)	10312.53	9925.03	9781.16	8687.10	10974.52	11225.61	10601.15	9523.23	7890.08	
$CD_{0.05}$ (V): 148.84, $CD_{0.05}$ (P): 315.74, $CD_{0.05}$ (V x P): 446.53										

Figures in the parentheses are angular transformed values

Table 2. Effect of seed priming on germination percentage, seedling length, dry weight, vigour index I and vigour index II among different vigour levels of bitter gourd seeds (sand method)

Parameter	Seed priming									
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	Mean (V)
Germination (%)										
V_1	80.00	78.50	75.63	71.25	85.00	86.00	84.00	74.38	69.00	78.19
	(63.41)	(62.36)	(60.39)	(57.68)	(67.21)	(68.02)	(66.41)	(59.57)	(56.15)	(62.34)
V_2	72.13	71.13	69.75	66.75	72.25	74.50	71.63	68.50	59.75	69.59
	(58.12)	(57.48)	(56.62)	(54.77)	(58.19)	(59.65)	(57.79)	(55.84)	(50.60)	(56.56)
Mean (P)	76.06	74.81	72.69	69.00	78.63	80.25	77.81	71.44	64.38	
	(60.76)	(59.92)	(58.51)	(56.16)	(62.70)	(63.84)	(62.10)	(57.71)	(53.38)	
$CD_{0.05}$ (V): 0.46, $CD_{0.05}$ (P): 0.98, $CD_{0.05}$ (V x P): 1.38										
Seedling length (cm)										
V_1	16.98	16.69	16.61	16.01	17.79	18.12	17.61	17.35	14.08	16.80

V_2	16.33	16.30	15.67	13.53	17.73	17.99	17.34	14.88	9.93	15.52
Mean (P)	16.66	16.49	16.14	14.77	17.76	18.05	17.47	16.11	12.01	
$CD_{0.05}$ (V): 0.35, $CD_{0.05}$ (P): 0.74, $CD_{0.05}$ (V x P): 1.05										
Seedling dry weight (mg)										
V_1	235.60	222.00	221.28	207.60	250.33	262.88	241.60	218.68	206.60	229.62
V_2	210.80	210.00	202.88	187.40	231.60	244.88	226.20	191.83	150.08	206.18
Mean (P)	223.20	216.00	212.08	197.50	240.96	253.88	233.90	205.25	178.34	
$CD_{0.05}$ (V): 1.79, $CD_{0.05}$ (P): 3.79, $CD_{0.05}$ (V x P): 5.36										
Seedling vigour index I										
V_1	1358.81	1310.36	1255.40	1139.87	1511.91	1557.75	1478.73	1290.39	971.87	1319.45
V_2	1178.29	1159.42	1092.35	902.36	1300.60	1320.19	1242.05	1018.97	593.36	1089.73
Mean (P)	1268.55	1234.89	1173.87	1021.12	1406.26	1438.97	1360.39	1154.68	782.61	
$CD_{0.05}$ (V): 26.37, $CD_{0.05}$ (P): 55.94, $CD_{0.05}$ (V x P): 79.11										
Seedling vigour index II										
V_1	18848.95	17425.60	16735.25	14788.80	21272.22	22603.45	20290.95	16265.13	14252.95	18053.70
V_2	15205.85	14927.85	14149.60	12506.25	16731.40	18240.95	16201.25	13141.50	8970.98	14452.85
Mean (P)	17027.40	16176.72	15442.43	13647.53	19001.81	20422.20	18246.10	14703.31	11611.96	
$CD_{0.05}$ (V): 152.97, $CD_{0.05}$ (P): 324.49, $CD_{0.05}$ (V x P): 458.90										

Figures in the parentheses are angular transformed values

Seed priming improved germination and other seed quality parameters among both the vigour groups of seeds. Solid matrix carrier perlite proved its potential over the rest of the treatments in both paper towel and sand method followed by cocopeat and vermiculite wrt germination, seedling length, dry weight, vigour index I and vigour index II.

In case where market contains carry over seeds or aged seeds priming can prove a useful venture in repairing the deteriorative effects due to ageing. Farmers can use seed soaking as means to enhance the germination for uniform emergence and optimum field establishment if any type of osmoticum is not available to them.

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