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Effect of hot water treatment of seeds on seed quality parameters and seedling growth parameters in bell pepper (*Capsicum annuum*)

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ABSTRACT

The experiment was undertaken to extrapolate the effect of hot water seed treatment comprised of different ranges of temperature (47-49, 50-52 and 53-55°C) and different duration of time (30, 45 and 60 min.) on seed quality parameters and seedling growth parameters in bell pepper (*Capsicum annuum* L.) cv. Solan Bharpur. The aim was to improve seed quality parameters and seedling growth parameters in nursery. Results showed that under *in vitro* conditions, the hot water treated seed with temperature 50-52 °C for 30 min. showed maximum germination, seedling length, seedling dry weight, seed vigour index-I and seed vigour index-II as compared to untreated seeds (control). In nursery conditions, same treatment, viz. temperature 50-52 °C for 30 min. showed maximum values *w.r.t.* total emergence, seedling height, seedling dry weight, seed vigour index-I and seed vigour index-II as compared to control.

Key words: Bell pepper, Hot water treatment, Seed quality, Seedling growth

Bell pepper (*Capsicum annuum* L.), a member of Solanaceae family, also known as sweet pepper, capsicum or *Shimla mirch*, is a high value vegetable and an important cash crop cultivated in temperate and tropical areas of the world whose fruits are appreciated worldwide for its flavour, aroma and colour. Seed is the primary and essential starting point of majority of vegetables but delayed, erratic germination and emergence, poor stand, slow early seedling growth rate and non-uniform maturity often limit crop production even under optimum environmental conditions (Cavero *et al.* 1995). In gaseous exchange and seed germination seed coat plays inhibitory role. Hot water pre-treatment promotes germination by influencing factors such as permeability of the seed coat to water and gases, and the release of germination inhibitors (Sharma *et al.* 2008). Speedy germination and vigorous seedlings results into higher crop yield. According to Harris *et al.* (2000) crop has a critical soaking period of water and it must not be exceeded. Also, Mcdonnell *et al*. (2012) stated that dipping seeds in hot water at different temperatures affect the seed germination as hot water seed treatment of 70-80°C showed better growth compared to hot water seed treatment with 80°C. Due to sensitivity of seeds to high temperature, hot water treatments must be applied in strictly controlled conditions with ensured uniform treatment for all seeds. It also become an alternative method for conventional farming especially in case of failure of chemicals permitted for seed treatment. Therefore, the experiment was designed to study the effect of different temperature and time combinations of hot water seed treatment on seed quality parameters and seedling growth parameters in bell pepper.

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MATERIALS AND METHODS

The present experiment was carried out at Experimental Farm and Laboratory of Department of Seed Science and Technology, Dr Y S Parmar University of Horticulture and Forestry, Solan. The experimental farm of Department of Seed Science and Technology is located at an altitude of 1183 m above mean sea level with latitude of 30.51ºN and longitude of 77.09ºE the mid-hill zone of Himachal Pradesh, India observed with GARMIN'S GPS 12 Personal Navigator. The soil texture of polyhouse was loam to clay loam having pH ranging from 6.85-7.05. The healthy, disease free, bold and uniform seeds of bell pepper cv. Solan Bharpur, were obtained and treated with hot water in automatically controlled hot water bath tub at different temperature range for discrete time period (Table 1). The *in vitro* experiment was laid in completely randomized block design with four replications as per ISTA recommendation. The experiment in nursery condition was laid in randomized block design with 10 treatments replicated thrice taking 30 seedlings per replication.

Hot water bath tub works automatically controlling temperature with time. Seeds were soaked in normal

Table 1 Treatment details

Treatment	Temperature (ºC)	Duration of treatment (min.)
T_1D_1	47-49	30
T_1D_2	47-49	45
T_1D_3	47-49	60
T_2D_1	50-52	30
T_2D_2	50-52	45
T_2D_3	50-52	60
T_3D_1	53-55	30
T_3D_2	53-55	45
T_3D_3	53-55	60
T_0 (control)	Untreated seeds	

water for 15 min. wrapped in muslin cloth. Then, poured about 3 l water in the device and it was connected with electricity. With the help of heater coil, the device was heated with desired time and thermostat bulb the device was regulated to the desired temperatures, i.e. 47- 49, 50-52 and 53-55°C. With the thermometer, the desired temperature was denoted. After the desired temperature was obtained, the seeds were dipped in water for a fixed period of time. At the end of the treatment, seeds were taken out of the hot water bath and spread on blotter paper. Blotter paper with seeds was placed in shade for drying of seeds and the seeds were used for evaluating various seed quality and nursery growth parameters.

Germination percentage was worked out according to standard germination procedure (ISTA 2015). This was carried out by using between paper method in the seed germinator at 25°C. The germination was counted on 14th day. Germination percentage was calculated by using the formula given below:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds used}} \times 100$$

Ten seedlings selected for measuring seedling length were used to work out seedling dry weight (mg). Seedlings were kept in oven at 80°C for 48 hr and weights were measured then mean value was expressed in mg. Seedling vigour index-I (SVI-I) and Seedling vigour index -II (SVI-II) were calculated as per the formula given by Abdul-Baki and Anderson (1973). In nursery, SVI-I and SVI- II were measured at weakly interval.

SVI-I = Germination (%) × Seedling length (cm)

SVI- II = Germination (%) × Seedling dry weight (mg)

The length of seedlings (cm) was measured on 14th day of germination test. Ten normal seedlings selected at random from between paper method were used to work out the seedling length. Total seedling length was worked out by taking the total length of seedlings form the tip of the primary leaf to the tip of primary root with the help of scale and expressing the mean value in cm.

Total emergence (%) per treatment studied in nursery was calculated as follows:

$$\text{Emergence (\%)} = \frac{\text{Number of seedlings emerged}}{\text{Total number of seeds used}} \times 100$$

Seedling height was measured from the base of the plant to the top of the main axis and mean height was expressed in cm and was recorded at weekly interval.

The statistical analysis of the data generated was done as per design of the experiment as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Highest seed germination percentage (90.75%) was observed in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by seeds soaked at 47-49 °C for 60 min. (T_1D_3) with 87.25% germination. The germination (%) declined when seeds were soaked for more than 30 min. at temperature higher 50-52 °C**.** The lowest germination (70.00%) was recorded in seeds soaked at 53-55 °C for 60 min. (T_3D_3) followed by in seeds soaked at 53-55 °C for 45 min. (77 %), i.e. T_3D_2 .The values under these treatments of seeds were significantly lower than control as well as other treatments. Similar trend was observed in seedling length with highest seedling length (10.98 cm) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by 10.86 cm in seeds soaked at 47-49 °C for 60 min. (T_1D_3), however, these treatment were statistically at par with each other. Whereas lowest seedling length (8.76 cm) was observed when seeds were soaked at 53-55 °C for 60 min. (T_3D_3). Control demonstrated a seedling length of 10.16 cm. Furthermore, the data also revealed a significant difference in seedling dry weight in different treatments. Seeds soaked at 50-52 °C for 30 min. (T_2D_1) produced highest seedling dry weight (2.48 mg) followed by T_1D_3. However, these treatments were statistically at par with each other, while seeds soaked at 53-55 °C for 60 min. (T_3D_3) produced lowest seedling dry weight (2.01 mg). The control treatment demonstrated an intermediate seedling dry weight (2.32 mg). Seedling vigour index-I was recorded highest (996.43) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by 947.56 in seeds soaked at 47-49 °C for 60 min. (T_1D_3). However both the treatments differ significantly with each other and lowest (613.52) in seeds soaked at 53-55 °C for 60 min. (T_3D_3). Control group demonstrated intermediate seedling vigour index-I (881.12). The trend was similar *w.r.t.* the effect of different hot water treatments on Seedling vigour index-II. Seedling vigour index-II was recorded highest (225.29) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by 211.59 in seeds soaked at 47-49 °C for 60 min. (T_1D_3). However both the treatments differed significantly with each other. Lowest (140.91) seed vigour-II was observed in seeds soaked at 53-55 °C for 60 min. (T_3D_3). Control demonstrated intermediate seedling vigour index-II of 201.04 (Table 2). High temperatures denatured certain enzymes and possibly DNA in exposed seeds (Bewley and

Black 2000). The medium temperature range of hot water is known to result in increased imbibition and this process stimulated germination related activities such as gibbrellic acid synthesis, RNA synthesis, protein synthesis and DNA replication. Finally weakening of the endosperm and thereby increasing germination (Groot and Karssen 1989, Bewley and Black 2000). Hot water treatments of bell pepper seed prior to storage has been observed to affect the seed quality parameters significantly (Musazura and Bertling 2013). The increase of temperature range from 45-53 °C decreased seed viability with no significant effect on seed vigour. Under present investigation also, the seed germination reduced when the temperature was raised higher than 53-55°C. However, the seed germination was more when the seed were treated with a lower range of temperature, i.e. 50-52 °C. Miller and Ivey (2004) had reported that pepper seeds are more sensitive to hot water treatment. The soaking of pepper seeds in hot water at appropriate temperature improved their germination rate. Ramamoorthy *et al*. (1989) had reported similar effects of hot water seed treatment on improved speed and percentage of germination in other plant species like *Pinus pinnata*. Pasiecznik *et al.* (1998) also reported that treated seeds of *Prosopis* species by boiling water increased germination.

The decrease in the number of days to germination in treated seeds by hot water was attributed to the increasing of permeability of seed coat to water. According to Mwase and Mvula (2011) soaking in boiled water made the seed coats permeable to water and the seeds imbibed and swell as the water cools. Various studies have reported that immersion in hot water was the best method for breaking the dormancy of hard coat seeds (Azad *et al.* 2010, 2011).

There is meagre information available on the effect of hot water seed treatment on seedling length. However, Alamgir and Hossain (2005) found the higher seedling growth by hot water treatment in *Albizia saman*. The differences in the seedling length were observed due to the difference of temperature and the boiling time and also due to the variation of seed coat thickness (Azad *et al.* 2011). The enhanced seed vigour index-I and seed vigour index-II upon hot water treatment of 50-52 °C was observed due to the reduced incidence of microflora in the seeds of okra (Begum and Lokesh 2012).

Hot water treatment of seeds has a significant effect on field emergence percentage and number of days to first emergence in bell-pepper nursery. Number of days to first emergence varied significantly from 14.00 in control to lowest 12.00 in seeds soaked at 50-52 °C for 30 min. (T_2D_1) which was followed by 13.00 in seeds soaked at 47-49 °C for 60 min. (T_1D_3). However these treatments were found statistically at par with each other. Highest days taken for first emergence were observed in T_3D_3 (17.33 days). Total emergence (%) varied from 70.00 in control to highest (87.78) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed 82.22 % in T_1D_3. However, both the treatments differ significantly with each other, and lowest emergence percentage (58.89) was in nursery wherein seeds were soaked at 53-55 °C for 60 min. (T_3D_3). High temperature range, i.e. 53-55°C reduced emergence sharply (Table 3). Probable reason for reduced seedling emergence might be attributed to the fact that increased temperature affected the physiological activities of the embryo of seeds. The temperature 50-52°C increased the germination (%) of seed. Increased seed germination (%) and seed vigour might have resulted in increased seedling emergence as evident from the present investigation. The data on days to first emergence peculiarly indicated the superiority of this treatment upon all other treatments. However, there are meagre reports documented in literature on the increased field emergence of bell pepper after seed treatment at this temperature. Hence, present study might be considered as one of first attempts in this field. However, Missanjo *et al.* (2014) had observed increased field emergence upon hot water seed treatment in *Acacia polyacantha* and quoted the

Table 2 Effect of hot water seed treatment on seed quality parameters of bell pepper cv. Solan Bharpur

Treatment	Seed quality parameters				
	Germination (%)*	Seedling length (cm)	Seedling dry weight (mg)	Seedling vigour index-I	Seedling vigour index-II
T_1D_1	86.00(9.33)	10.41	2.31	895.12	198.83
T_1D_2	86.50(9.35)	10.46	2.32	904.48	200.68
T_1D_3	87.25(9.39)	10.86	2.42	947.56	211.59
T_2D_1	90.75(9.58)	10.98	2.48	996.43	225.29
T_2D_2	86.25(9.34)	10.44	2.32	900.41	200.31
T_2D_3	79.75(8.99)	10.55	2.27	841.51	181.02
T_3D_1	81.50(9.08)	9.59	2.29	781.42	187.05
T_3D_2	77.00(8.83)	9.21	2.09	709.89	161.01
T_3D_3	70.00(8.43)	8.76	2.01	613.52	140.91
T_0	86.75(9.37)	10.16	2.32	881.12	201.04
CD (P=0.05)	0.08	0.37	0.07	34.12	6.76

*The values in parentheses are square root transformed values

Table 3 Effect of hot water seed treatment on total emergence in bell pepper cv. Solan Bharpur under nursery conditions

Treatment	Total emergence (%)**	Days to emergence
T_1D_1	68.89(56.08)	14.00
T_1D_2	74.44(59.62)	14.00
T_1D_3	82.22(65.13)	13.00
T_2D_1	87.78(69.55)	12.00
T_2D_2	76.67(61.13)	14.00
T_2D_3	72.23(58.23)	14.00
T_3D_1	73.33(58.91)	15.00
T_3D_2	64.45(53.39)	16.00
T_3D_3	58.89(50.11)	17.00
T_0	70.00(56.79)	14.00
CD (P=0.05)	3.65	1.04

**The values in parentheses are angular transformed values

Table 4 Effect of hot water seed treatment on seedling height of bell pepper cv. Solan Bharpur under nursery conditions

Treatments	Seedling height (cm) after days of sowing			Seedling dry weight (mg) after days of sowing		
	28	35	42	28	35	42
T_1D_1	4.36	6.40	9.20	35.00	88.33	151.00
T_1D_2	4.36	6.58	9.78	38.67	104.33	168.00
T_1D_3	4.46	7.26	10.69	50.33	119.33	189.33
T_2D_1	4.68	7.63	11.16	57.00	130.67	204.00
T_2D_2	4.39	7.22	10.08	41.67	111.00	179.67
T_2D_3	4.24	6.68	9.65	35.33	92.67	155.67
T_3D_1	4.31	6.75	9.90	35.00	95.67	156.33
T_3D_2	3.17	5.20	8.84	26.33	80.67	142.67
T_3D_3	2.52	4.48	6.84	15.67	49.67	87.87
T_0	4.24	6.60	9.96	38.33	102.17	163.67
CD (P=0.05)	0.30	0.47	0.58	7.25	9.60	13.16

same physiological reasons for increased field emergence.

Height of seedling at different time intervals also varied with respect to treatment. At all the time intervals, i.e. 28, 35 and 42 days after sowing, the seedling heights were highest (4.68, 7.63 and 11.16 cm, respectively) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by T_1D_3 (4.46, 7.26 and 10.69 cm, respectively). The seedling height of both the treatments was statistically at par with each other. Lowest seedling height of 2.52, 4.48 and 6.84 cm, respectively was recorded in seeds soaked at 53-55 °C for 60 min. (T_3D_3). In control, the seedlings achieved intermediate heights of 4.24 cm at day 28, 6.60 cm at day 35, and 9.96 cm at day 42 after sowing. The dry weights of seedling at different time intervals varied *w.r.t.* treatment. Like height of seedlings, the dry weight of seedlings at different time intervals, i.e. day 28, 35 and 42, were highest (57.00, 130.67 and 204.00 mg, respectively) in seeds soaked at 50-52 °C for 30 min. (T_2D_1) followed by T_1D_3 (50.33, 119.33 and 189.33 mg, respectively). The values of seedling dry weight in both the treatments were statistically at par only on 28 DAS. Lowest dry weight 15.67, 49.67 and 87.87 mg, respectively was observed in seeds soaked at 53-55 °C for 60 min. (T_3D_3). Seedlings kept as control achieved intermediate dry weights of 38.33 mg at day 28, 102.17 mg at day 35, and 163.67 cm at day 42 after sowing (Table 4).

Analysis of variance revealed a significant effect of hot water treatment on seed vigour index-I and seed vigour index-II of bell pepper cv. Solan Bharpur under nursery conditions (Table 5). The seedling vigour index-I was highest (410.90, 669.94 and 979.99 at days 28, 35 and 42 post sowing, respectively) in seeds soaked at 50-52 °C for 30 min. (T_2D_1), and lowest (149.44, 264.77 and 404. 17 at days 28, 35 and 42 post sowing, respectively) in seeds soaked at 53-55 °C for 60 min. (T_3D_3). Seedlings kept as control achieved intermediate seed vigour index-I of 296.17 at day 28, 461.14 at day 35, and 696.83 at day 42 after sowing. Seed vigour index-II followed similar pattern as that of seed vigour index-I. The seedling vigour index-II was highest (5000.00, 11465.56 and 17900.00 at days 28, 35 and 42 post sowing, respectively) in seeds soaked at 50-52°C for 30 min. (T_2D_1), and lowest (930.00, 2932.22 and 5180.89 at days 28, 35 and 42 post sowing, respectively) in seeds soaked at 53-55 °C for 60 min. (T_3D_3). Seedlings kept as control achieved intermediate seed vigour index-I of 296.17 at day 28, 461.14 at day 35, and 696.83 at day 42 after sowing.

The reasons behind the increased seedling growth parameters after hot water treatment at 50-52 °C for 30 min. than control and higher as well as lower temperature treatment might be similar as discussed under the *in vitro* studies in earlier experiment. This temperature range of hot water might have resulted in increased imbibition and stimulated germination related activities such as gibberellic acid synthesis, RNA synthesis, protein synthesis and DNA replication and finally weakening of the endosperm and thereby increasing germination (Groot and Karssen 1989, Bewley and Black 2000). The increased germination (%) as well as vigour index might have increased field emergence and growth pararmeters of treated seedlings under nursery conditions under present investigations. The reduction in seed microflora upon hot water treatment as discussed earlier might have also added in the improvement of field emergence and growth parameters under present investigations. There existed no such reference on the effect of hot water seed treatment on field emergence and growth parameters under nursery conditions in bell pepper. Hence, present is the first attempt in this regard. However, hot water treatment was found one of the best pre-treatment seed method for the increased growth of *Acacia polyacantha* seedlings by Missanjo *et al.* (2014). The hot water treatment was also found to reduce the incidence of micoflora associated with the seeds and there by increased the vigour index of the

Table 5 Effect of hot water seed treatment on Seed vigour index-I and Seed vigour index-II of bell pepper cv. Solan Bharpur under nursery conditions

Treatment	SVI-I after days of sowing			SVI-II after days of sowing		
	28	35	42	28	35	42
T_1D_1	300.17	440.84	634.21	2413.33	6086.67	10404.44
T_1D_2	324.37	489.73	727.82	2880.00	7754.45	12486.67
T_1D_3	366.71	596.29	878.56	4137.78	9802.22	15560.00
T_2D_1	410.90	669.94	979.77	5000.00	11465.56	17900.00
T_2D_2	336.89	553.13	772.85	3197.78	8502.22	13765.56
T_2D_3	306.91	482.57	697.66	2561.11	6702.22	11250.00
T_3D_1	315.92	494.11	725.82	2555.56	7001.11	11448.89
T_3D_2	204.47	335.85	569.82	1700.00	5204.44	9195.56
T_3D_3	149.44	264.77	404.17	930.00	2932.22	5180.89
T_0	296.17	461.14	696.83	2678.89	7137.78	11435.56
CD (P=0.05)	30.50	44.11	69.28	601.87	736.70	1104.68

okra seedlings (Begum and Lokesh 2012).

Conclusion

The hot water treated seed with temperature 50-52 °C for 30 min. showed better seed quality characters as well as seedling growth parameters as compared to untreated seeds (control). However, further studies are needed to standardize the required temperature for producing seeds and seedlings of high quality and to extend the wide use of hot water seed treatment for production of healthy crop of bell pepper.

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