



Studies on plasma treatment and priming of seeds of bell pepper (*Capsicum annuum* L.)

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Abstract: The present investigation was carried out at Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan during the year 2014 -2015 to study the "Effect of cold plasma treatment and priming on bell pepper (*Capsicum annuum* L. cultivar California Wonder) for seed germination and seedling vigour. The seeds were exposed to various durations of oxygen plasma treatment using glow discharge technique at FCIPT, Institute for Plasma Research, Gandhinagar, Gujarat, India. Seeds were pre-treated with power of 100 W for treatment durations of 0, 3, 6, 9, 12, 15 minutes for 0, 4, 8 and 12 month durations. The changes in surface morphology of plasma treated seeds were studied by Scanning Electron Microscopy (SEM) and Contact Angle Goniometer. Along with plasma treatment, seeds were also treated with standard priming method i.e osmoprimng for comparison. Results showed that plasma treatment alone as well as in combination with osmoprimng up to 6 minutes duration had positive effects on seed germination and seedling vigour. Germination and vigour indices significantly increased by 21.75% and 90.71% respectively. Characteristics of germination percentage, speed of germination, seedling vigour index-I & II, significantly increased by 13.92%, 1.39 cm, 0.38 mg, 322.07 respectively, compared with control. And it was found that immediately after plasma exposure the germination (84.41%) and vigour (228.50) was highest and it was reduced to (73.54%) and (174.27) after 12 months of storage. These results indicated that cold plasma treatment might promote the growth and modify the speed of germination i.e. higher speed of germination was observed in seeds exposed to plasma treatment for 6 minutes (59.82%), whereas, lowest germination speed (40.76%) was observed in untreated control.

Keywords: Bell pepper, Plasma treatment, Priming, SEM, Seed germination, Vigour

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is a warm-season crop and performs well under an extended frost-free season with an optimum temperature range of 25-30°C with the potential of producing high yields of outstanding quality. An average yield of 135.55q/acre was obtained under open field condition. The area under capsicum cultivation in India is 29,80,000 hectares with an annual production of 166,88,000 metric tonnes with productivity of 5.75 metric tonnes per hectare (NHB, 2014). Mid-hills of Himachal Pradesh are the leading suppliers of bell pepper during off-season (Choudhary *et al.*, 2009). Hard seed coat causes delayed or less germination in capsicum. Low seed germination and rapid deterioration during storage of bell pepper seeds are the major problems that affect the seed quality (McDonald, 1999).

Different seed enhancement technologies like priming, coating, pelleting etc increases the moisture content of seed during treatment. Priming is a seed enhancement

method that might improve seed performance under drought, freshly harvested or aged seeds. The most cost effective method for bell pepper is hydropriming for 24 hours. Hydropriming is simple and cost effective strategy for improving germination and emergence of bell pepper (Uche *et al.*, 2016). On the other hand, pelleting is a process of enclosing a seed into a small quantity of inert matter just large enough to produce a globular unit of standard size to facilitate precision planting. Here, a dry seed treatment like plasma treatment is employed to increase the seed coat permeability without increasing the moisture content of seed. Plasma seed treatment method is a physico-chemical method which has potential to improve germination yields and kill fungal spores carried on seed coats (Zhou *et al.*, 2012). Plasma treatment has been successfully applied in agriculture for seed quality improvement, seed enhancement and pathogenic microorganisms inactivation (Filatova *et al.*, 2013). Crop yields are improved by treating the seeds in a low temperature plasma discharge generated between spaced

electrodes connected to a source of high frequency electrical power (Krapivina *et al.*, 1994). Cold plasma treatment is a cost effective method that has been effectively used in seed technology because it is fast economic and pollution free method to improve the seed performance, it decontaminates off the pathogens from seeds, no loss of seed quality and a quick treatment with no side effects. Hence, effect of plasma treatment on germination and vigour of bell pepper seeds was studied.

However, reports about the effects of cold plasma on bell pepper are limited. The aims of the study were (1) to study the effect of cold plasma treatment on seed germination and seedling vigour of bell pepper seeds and (2) to explore the mechanisms of the effects of plasma on promoting seed germination and seedling vigour of bell pepper.

MATERIALS AND METHODS

Bell pepper (*Capsicum annuum* L.) seeds were exposed to Glow Discharge plasma of oxygen gas was used at a base pressure of 0.05m bar, operating pressure of 0.2m bar, voltage of 500 V, current of 0.2 A and power of 100 W for treatment durations of 0, 3, 6, 9, 12 and 15 minutes for 0, 4, 8 and 12 months duration.

Along, with the plasma treatment seeds were also treated with osmopriming for comparison. In osmopriming the seeds are soaked in osmoticum i.e. PEG for about 72 hours at 15°C and 90% relative humidity and after that wash it with tap water 3-4 times. The seed germination and vigour experiments were conducted immediately after treatment (0 month), four months, eight months and twelve months of storage.

After exposure to plasma the seeds were imaged by high resolution Scanning Electron Microscopy and Contact Angle Goniometer. Seed Surface Topography

of plasma treated and untreated seeds were studied by Scanning Electron Microscopy (SEM) at 50 X and 100K X magnifications. And Seed Surface Morphology was studied by Contact Angle Goniometer. Water contact angle quantifies the wettability of a solid surface by a liquid. Control seeds were not exposed to any treatment.

Seed surface topography: When observed under the Scanning Electron Microscopy under the magnification of 50 X, there was no apparent difference in treated and untreated seed (Figure 1a & b). However, the scanning electron micrograph at 100 K X (Figure 1c & d) clearly showed that in the treated seed there was etching on the surface on the seed at nanoscale.

Water contact angle: Surface morphology of the plasma treated seeds was studied by Contact Angle Goniometer (Figure 2 a & b) which depicts that plasma treated seeds results in dramatic decrease in apparent contact angle. Not only the chemical structure but also the roughness of the surface is affected by the plasma treatment, which can change the wettability of the surface (Dubinov *et al.*, 2000).

Experimental design: The experiment was carried out at the Department of Seed Science and Technology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan from March 2014 to September 2015. Seeds were germinated in petriplates at 22-27°C. The germination percentage was recorded every 24 h for 7 and 14 days. Seedling length, seedling dry weight, speed of germination and seedling vigour index-I and II were measured after the 14th day. The experiment was planned as a completely randomized design with four replications.

RESULTS AND DISCUSSION

Seed germination: The effect of cold plasma treatment on germination of bell pepper was significantly

Table 1. Effect of plasma treatment, seed priming and their interactions on germination percentage after different periods of storage of bell pepper seeds. (Paper Towel Method).

Plasma Treatment	Osmoprimed					Non-primed					Plasma Treatment × Storage				
	Storage Duration (Months)				Mean	Storage Duration (Months)				Mean	Storage				Mean
	0	4	8	12		0	4	8	12		0	4	8	12	
Vacuum	78.75 (8.93)	76.75 (8.82)	73.75 (8.65)	70.75 (8.47)	75.00 (8.72)	76.25 (8.79)	73.75 (8.65)	70.25 (8.44)	68.50 (8.34)	72.19 (8.55)	77.50 (8.86)	75.25 (8.73)	72.00 (8.54)	69.63 (8.40)	73.59 (8.63)
3 Min	93.25 (9.71)	90.25 (9.55)	82.25 (9.12)	77.75 (8.87)	85.88 (9.32)	90.75 (9.58)	87.00 (9.38)	81.50 (9.08)	75.75 (8.76)	83.75 (9.20)	92.00 (9.64)	88.63 (9.47)	81.88 (9.10)	76.75 (8.82)	84.82 (9.26)
6 Min	95.50 (9.82)	92.00 (9.64)	80.50 (9.03)	85.75 (9.31)	88.44 (9.52)	93.50 (9.72)	89.00 (9.49)	82.75 (9.15)	77.25 (8.92)	85.63 (9.32)	94.50 (9.77)	90.50 (9.57)	81.63 (9.09)	81.50 (9.08)	87.03 (9.38)
9 Min	90.00 (9.54)	87.00 (9.38)	82.75 (9.15)	78.00 (8.89)	84.44 (9.24)	88.00 (9.43)	85.00 (9.27)	81.25 (9.07)	75.50 (8.75)	82.44 (9.13)	89.00 (9.49)	86.00 (9.33)	82.00 (9.11)	76.75 (8.82)	83.44 (9.19)
12 Min	86.50 (9.35)	84.00 (9.22)	79.75 (8.99)	75.75 (8.76)	81.50 (9.08)	83.50 (9.19)	80.75 (9.04)	78.00 (8.89)	74.00 (8.66)	79.06 (8.95)	85.00 (9.27)	82.38 (9.13)	78.88 (8.94)	74.88 (8.71)	80.28 (9.01)
15 Min	82.75 (9.15)	80.00 (9.00)	76.50 (8.80)	72.75 (8.59)	78.00 (8.89)	79.00 (8.94)	76.00 (8.78)	74.25 (8.68)	70.25 (8.44)	74.88 (8.71)	80.88 (9.05)	78.00 (8.89)	75.38 (8.74)	71.50 (8.15)	76.44 (8.80)
Control	73.75 (8.65)	70.75 (8.47)	68.25 (8.32)	65.00 (8.12)	69.44 (8.39)	70.25 (8.44)	68.00 (8.31)	66.00 (8.19)	64.50 (8.03)	66.69 (8.23)	72.00 (8.54)	69.38 (8.39)	67.13 (8.25)	63.75 (8.05)	68.06 (8.31)
Mean	85.79 (9.31)	82.96 (9.16)	77.68 (8.91)	75.11 (8.72)	80.38 (9.02)	83.04 (9.16)	79.93 (8.99)	76.29 (8.79)	71.96 (8.55)	77.81 (8.87)	84.41 (9.23)	81.45 (9.07)	76.98 (8.83)	73.54 (8.63)	

Figures in the parenthesis are square root transformed

Table 2. Effect of plasma treatment, seed priming and their interactions on germination speed after different periods of storage of bell pepper seeds. (Petri plate Method).

Plasma Treatment	Osmoprimered					Non-primed					Plasma Treatment × Storage				Mean
	Storage Duration (Months)				Mean	Storage Duration (Months)				Mean	0	4	8	12	
	0	4	8	12		0	4	8	12						
Vacuum	52.31	50.35	48.25	48.25	49.79	50.86	48.55	43.48	30.56	43.36	51.59	49.45	45.87	39.40	46.58
3 Min	66.79	64.71	61.51	54.46	61.87	62.18	62.08	55.54	51.30	57.78	64.48	3.40	58.52	52.88	59.82
6 Min	63.79	61.82	58.73	50.66	58.75	60.18	58.58	54.31	47.93	55.43	62.35	60.20	56.52	49.29	57.09
9 Min	60.69	57.84	57.26	46.26	55.51	56.56	55.23	52.42	42.84	51.76	58.62	56.54	54.84	44.55	53.64
12 Min	57.02	55.51	56.48	41.35	52.59	54.91	53.74	51.98	38.36	49.75	55.96	54.63	54.23	39.85	51.17
15 Min	54.23	53.52	54.85	37.09	49.92	52.90	52.38	45.68	34.83	46.45	53.56	52.95	50.26	35.96	48.18
Control	49.87	45.92	41.44	29.56	41.70	47.16	43.21	41.81	27.10	39.82	48.51	44.57	41.63	28.33	40.76
Mean	57.81	55.67	54.07	43.95	52.88	55.07	53.40	49.32	38.99	49.19	56.44	54.53	51.69	41.47	

Table 3. Effect of plasma treatment, seed priming and their interactions after different periods of storage on SVI-I of bell pepper seeds. (Paper Towel Method).

Plasma Treatment	Osmoprimered					Non-primed					Plasma Treatment × Storage				Mean
	Storage Duration (Months)				Mean	Storage Duration (Months)				Mean	0	4	8	12	
	0	4	8	12		0	4	8	12						
Vacuum	701.76	612.52	492.61	439.10	561.50	668.45	572.35	459.64	436.61	534.26	685.10	592.44	476.12	437.86	547.88
3 Min	922.57	866.02	640.13	581.82	752.63	871.36	823.90	637.98	555.35	722.15	896.96	844.96	639.05	568.59	737.39
6 Min	962.30	919.75	660.28	656.22	799.64	901.97	854.55	782.64	574.37	778.38	932.13	887.15	721.46	615.29	789.01
9 Min	869.37	814.56	628.21	566.36	719.63	831.69	759.48	601.90	540.34	683.35	850.53	787.02	615.06	553.35	701.49
12 Min	815.09	756.37	587.24	525.73	671.11	767.74	705.02	568.16	515.74	639.16	791.41	730.70	577.70	520.73	655.14
15 Min	762.89	695.17	528.72	465.43	613.05	707.00	642.27	507.84	471.56	582.17	734.95	668.72	518.28	468.49	597.61
Control	640.23	532.59	443.68	385.43	500.48	595.79	493.50	417.53	349.65	464.11	618.01	513.04	430.60	367.54	482.30
Mean	810.60	742.43	568.69	517.16	659.72	763.43	693.01	567.95	491.94	629.08	787.01	717.72	568.32	504.55	

higher in seeds treated for 6 minutes (87.03%) and the seeds exposed for 15 minutes to plasma showed significant lower seed germination (76.44%). Germination of bell pepper seeds was significantly higher in osmoprimered seed (80.38%) then that of non-primed seed (77.81%).

While comparing the storage duration, the germination was affected significantly with the increase in the storage duration. Osmoprimered seed showed highest germination (84.41%) prior to storage at 22.40° C and 57% relative humidity, which was reduced to (73.54%) after 12 months of storage at 14.60° C and 58% relative humidity. Interaction effects due to priming and storage duration were found to be significant. The maximum seed germination

(85.79%) was observed in osmoprimered seeds immediately after treatment (i.e. 0 months storage) duration and lowest seed germination (71.96%) were observed in non-primed seeds up to 12 months storage.

The interaction effect of priming, plasma exposure time and storage duration were found to be significant. The maximum seed germination (95.50%) was observed in osmoprimered, 6 minutes plasma treated seeds stored at 0 months storage at 22.40° C and 57% relative humidity and the lowest seed germination (64.50%) was observed in non-primed, untreated seeds stored at 12 months storage duration at 14.60° C and 58% relative humidity.

The higher germination in osmoprimered seed as compared to non-primed seed is due to the rapid initial water uptake leading to the initiation of metabolic processes which gives primed seeds start over the non-primed seeds as reported by (Varier *et al.*, 2010). The increase in germination may be due to the reason that plasma treatment removes effectively the very thin lipid layer that makes seeds water repellent, and probably reduces the length of biopolymer chain that makes up the seed coat, thus enabling better water uptake

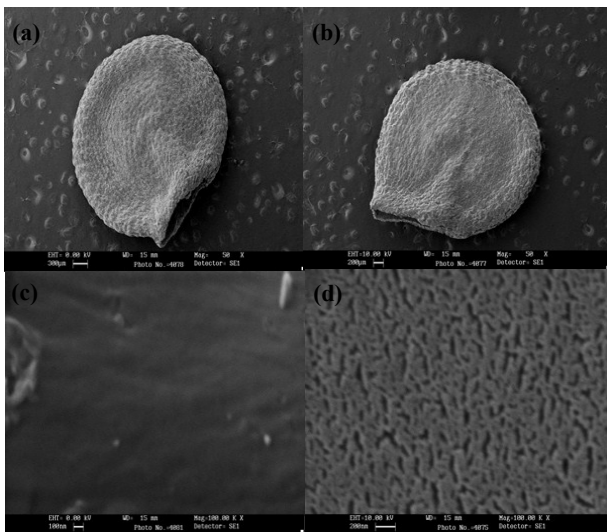


Fig. 1(a). Untreated seed, **(b).** Plasma treated seed, **(c).** Untreated seed, **(d).** Plasma treated seed.

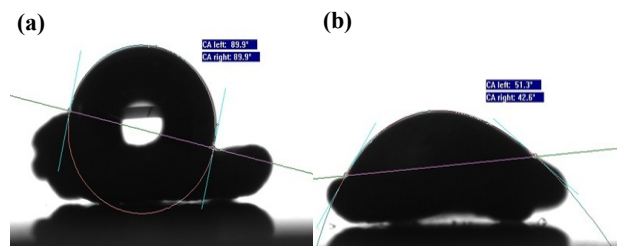


Fig. 2(a). Untreated bell pepper seed, **(b).** Plasma treated bell pepper seed.

Table 4. Effect of plasma treatment, seed priming and their interactions after different periods of storage on SVI- II bell pepper seeds. (Paper Towel Method).

Plasma Treatment	Osmoprimered					Non-primed					Plasma Treatment × Storage				
	Storage Duration (Months)				Mean	Storage Duration (Months)				Mean	× Storage				Mean
	0	4	8	12		0	4	8	12		0	4	8	12	
Vacuum	203.10	194.94	172.17	159.78	182.50	191.05	180.34	162.11	151.42	171.23	197.07	187.64	167.14	155.60	176.86
3 Min	264.14	247.04	212.47	194.07	229.43	254.96	236.28	218.79	185.60	223.90	259.55	241.66	215.63	189.83	226.67
6 Min	275.91	253.41	218.35	221.97	242.41	266.63	243.60	223.10	198.33	232.92	271.27	248.51	220.72	210.15	237.66
9 Min	251.92	234.36	207.30	189.88	220.86	241.40	227.38	201.47	181.62	212.97	246.66	230.87	204.39	185.75	216.92
12 Min	236.66	218.98	193.59	179.73	207.24	223.31	208.98	192.15	173.32	199.44	229.98	213.98	192.87	176.53	203.34
15 Min	221.09	206.61	180.97	166.63	193.04	209.82	194.45	172.82	159.32	184.10	215.46	200.53	176.89	162.97	188.96
Control	185.20	175.64	158.27	143.15	165.56	173.80	163.84	150.81	135.03	155.87	179.50	169.74	154.54	139.09	160.72
Mean	234.00	218.71	191.87	179.31	205.97	222.99	207.84	188.75	169.69	197.21	228.50	213.72	190.31	174.27	

through seed coat and improves germination (Tian *et al.*, 2002).

Speed of germination: Higher germination speed was, however, observed in the seeds exposed to plasma for 6 minutes (59.82) and is depicted in the Table 2. And the seed exposed for 15 minutes to plasma showed significant slower germination speed (48.18). The lowest germination speed (40.76) was, however, observed in untreated control.

The speed of germination of bell pepper seeds was significantly higher in osmoprimered seed (52.88) than that of non-primed seed (49.19). Speed of germination was found to be significant with the increase in the storage duration.

Osmoprimered seed showed highest germination speed (56.44) prior to storage (0 months), which was at par with seeds stored for 4 months (54.53) and was reduced to (41.47) after 12 months of storage.

Plasma treatment had a positive effect on speed of germination up to 6 minutes duration. This may be due to the changes of the wetting properties of seed and seed coat, due to oxidation of their surface which leads to faster germination (Bormashenko *et al.*, 2012).

Seedling vigour index-I: Higher seedling vigour index-I was, however, observed in the seeds exposed to plasma for 6 minutes (789.01). The lowest seedling vigour index-I (482.30) was recorded in untreated control seed. Data on seedling vigour index-I is presented in Table 3 which indicates that bell pepper seeds was significantly higher in osmoprimered seed (659.72) than that of non-primed seed (629.08).

While comparing the storage duration, the seedling vigour index-I was affected significantly with the increase in the storage duration. Osmoprimered seed showed highest seedling vigour index-I (787.01) prior to storage (0 months), which was reduced to (504.55) after 12 months of storage.

Interaction effects of plasma exposure and storage duration were found to be significant. The maximum seedling vigour index-I (932.13) was observed in 6 minutes plasma exposure immediately after treatment (i.e. 0 months) storage duration. And the lowest seedling vigour index-I (367.54) was observed in control at 12 months storage duration.

Interaction effects due to priming and storage duration

were found to be significant. The maximum seedling vigour index-I (810.60) was observed in osmoprimered seeds immediately after treatment (i.e. 0 months) storage duration and the lowest seedling vigour index-I (491.94) was observed in osmoprimered seeds at 12 months storage duration.

The interaction effect of priming, plasma treatment and storage duration were found to be significant for seedling vigour index-I. The maximum seedling vigour index-I (962.30) was observed in osmoprimered, 6 minutes plasma treated seeds stored at 0 months storage and the minimum seedling vigour index-I (349.65) was observed in non-primed, untreated seeds stored at 12 months storage duration.

Ling *et al.* (2014) reported that increase in the porosity makes the seed coat porous, as a result seed rapidly imbibes water and the germination process is accelerated. Spatenka *et al.* (2008) suggested that seeds with eroded seed coat would germinate faster than the healthy ones, accelerate water uptake resulting in increased seedling length. Sera *et al.* (2010) reported that seed immersed into air plasma is subjected by attack of O₂ radicals and bombardment by low energetic ions resulting in seed coat erosion, which significantly contributes to germination of *Chenopodium album*.

Seedling vigour index-II: Higher seedling vigour index-II (237.66) was, however, observed in the seeds exposed to plasma for 6 minutes. The lowest seedling vigour index-II (160.72) was recorded in untreated control. The effect of storage duration on seedling vigour index-II was affected significantly with the increase in the storage duration. Osmoprimered seeds showed highest seedling vigour index-II (228.50) prior to storage (0 month), which was reduced (174.27) after 12 months of storage.

Observations recorded for seedling vigour index-II of bell pepper seeds was significantly higher in osmoprimered seed (205.97) than that of non-primed seed (197.21) and is presented in Table 4.

Sera *et al.* (2010) reported that seed immersed into air plasma is subjected by attack of O₂ radicals and bombardment by low energetic ions resulting in seed coat erosion, which significantly contributes to germination of *Chenopodium album*. The extent of significance with different treatments presented within the p value

observed in ANOVA is <0.05 .

From the seed storage studies, it is evident that the beneficial effects of plasma treatment were better than the control in the 12 months of storage. Thereafter, control i.e. non-primed, untreated seeds recorded lowest value for seed quality attributes. However, when we compared plasma treated, osmoprimed seed to its respective control, higher values for seed quality parameters were obtained from 6 minutes plasma treated seeds which were osmoprimed throughout the storage period.

Conclusion

As a result of osmopriming, germination test in the laboratory depicted 21% increase in germination percentage and 90% in vigour over control prior to storage and 15% increase in germination and 78% in vigour over control during 12 months storage.

Comparison of plasma treatment with other duration: Plasma treatment up to 6 minutes duration proved its potential over other treatment durations i.e. 3, 9 and 12 minutes and control (untreated seeds) for various seed quality parameters, growth and yield, etc. Higher germination in osmoprimed seeds as compared to non-primed seed is due to rapid water uptake leading to initiation of metabolic processes which gives primed seeds start over non-primed seeds. Similarly, plasma treatment has a positive effect on growth parameters up to 6 minutes duration. This may be due to changes of the wetting properties of seed and seed coat due to oxidation of their surface which leads to faster germination. The deterioration of seeds with increase in storage duration depresses the germination capacity and growth of seedlings and ultimately reduces the dry matter and vigour of seeds during storage. The test depicted 23% increase in germination percentage and 92% in vigour over control during 0 months storage and 12% increase in germination and 63% in vigour over control during 12 months storage by treating seeds with plasma for 6 minutes. Hence, plasma treatment with 6 minutes duration can be used as a beneficial pre-sowing treatment to enhance the physiological, growth and yield characteristics in bell pepper in both osmopriming and non-priming. Germination and vigour of plasma treated seeds decreased with increase in storage time. Hence, the plasma treated seeds of bell pepper can be stored for 12 months.

Contribution of plasma treatment at a commercial scale is to enhance germination resulting in significant acceleration in germination percentage over the control seed. This method modifies the seed germination characteristics in agricultural as well as horticultural plant species. Plasma treatment results in enhanced speed of germination, extended growth of plants as well as sterilization of seeds. Seed storage of plasma treatment treated seeds is also found to have a beneficial effect. Plasma treated seeds can be stored for a longer duration i.e. 12 months as compared to control. Whereas, in

case of control the deterioration of seeds with increase in storage duration depresses the germination capacity and growth of seedlings.

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