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Studies on emasculation and pollination time in bell pepper's hybrid seed production (*Capsicum annuum* L. var *grossum* Sendt.)

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Abstract
Bell pepper's hybrid seed production is done by hand emasculation and pollination. Hence, it needs a lot of skilled labour as a result seed production has become costly and the availability of quality seeds to the farmers at a reasonable price is the need of hour. Keeping in mind, this investigation has been planned to evaluate the most appropriate post-emasculation pollination time to enhance hybrid seed yield with better quality and reduced costs. In field RCBD design was used with two varieties *viz*. Solan Bharpur and California Wonder and CRD in the laboratory with factorial analysis. The reciprocal cross was attempted between Solan Bharpur and California Wonder during Kharif, 2018 and 2019 by pollinating them at different times *viz*. pollination immediately after morning emasculation (P_1), pollination immediately after evening emasculation (P_2), pollination 12 hours after evening emasculation (P_3), pollination 18 hours after evening emasculation (P_4) and pollination 24 hours after evening emasculation (P_5). In addition, the effect of different times of pollination, two crosses, and their interaction was studied on various growth, seed quality and quantity parameters. ANOVA exhibited significant differences among the treatment combinations for all the characters studied. C_2P_5 (California Wonder x Solan Bharpur i.e., pollinated after 24 hours of evening emasculation) was found significantly superior when compared with other treatments for growth, seed yield, and seed quality characters. Individually treated P_5 (pollination 24 hours after evening emasculation) was found significantly better having maximum standards for growth, seed yield, and quality parameters. In the case of crosses, C_2 (California Wonder x Solan Bharpur) was found significantly superior for getting higher yields of quality bell pepper seeds.

Keywords: Hybrid bell pepper, seed quality, seed yield, emasculation, pollination time

1. Introduction
Bell pepper (*Capsicum annuum* L. var *grossum* Sendt.) is a three to four-lobed non-pungent solanaceous vegetable commonly known as sweet pepper/capsicum and most popularly Shimla mirch with the fact of its introduction in India by the Britishers during 19th century at Shimla hills. Besides this, it is native to Mexico with the secondary center of origin in Guatemala (Heiser and Smith, 1953) [10]. It is rich in Vitamin C (180 IU), Vitamin A, and the B-complex group of vitamins including Vitamin B1 (thiamine) and Vitamin B-6 (pyridoxine). It is also rich in minerals like iron, potassium, calcium, magnesium, phosphorus, sodium, and selenium (Agarwal *et al.*, 2007) [2]. In India, Bell pepper occupies an area of 37.15 thousand hectares and production of 562.97 thousand metric tons (Anonymous, 2020) [5]. In Himachal Pradesh, it holds a very prominent position as leading off-season vegetable generating cash revenue for the farmers by selling the produce in the neighboring states and metropolitan cities. In Himachal Pradesh, it is extensively grown as a cash crop (June-October) in mid-hills in the open environment. Major capsicum-producing districts in Himachal Pradesh are Solan, Sirmaur, and Mandi, it covers a total area of 2850 ha with a production of 48,860 MT (Anonymous, 2018) [4].

On a commercial scale, Bell pepper's hybrid seed production is done through hand emasculation and pollination. Hence, it requires a lot of skilled labour as a result of which, seed production has become costly. The availability of quality seeds to the farmers at a reasonable price is the need of the hour since the labour cost cannot be reduced until alternate hand emasculation and pollination techniques are developed. There is a need to standardize the techniques like most appropriate emasculation and pollination time with proper stigma receptivity and pollen viability as knowledge of the genetic architecture of genotypes is necessary to formulate an efficient breeding method.

In Bell pepper majority of the flowers open at 5.00 am. The stigma receptivity is dependent on temperature and showed variation during and after anthesis. From a day earlier to anthesis stigma is receptive and it continues for about two days after anthesis. On the day of anthesis, it is highest when anthers are fully developed but are still indehiscent whereas the corollas are ready to open. Considering all these facts, this research has been planned to find out the most suitable post-emasculation pollination time to enhance hybrid seed yield with reduce production costs and better quality.

2. Material and Methods

The experiment was laid out in a Randomized Complete Block Design (RCBD) with 3 replications and two-parent varieties Solan Bharpur and California Wonder at 60 cm X 45 cm. The parent variety California Wonder was transplanted on 25th April 2018 and Solan Bharpur was transplanted on 6th May 2018 in the plots having the size of 1.5m X 1.2m i.e., 1.8m^2 each. Solan Bharpur x California Wonder (C_1) during Kharif, 2018 and California Wonder x Solan Bharpur (C_2) during Kharif, 2019 were two reciprocal crosses attempted by pollinating them at different times i.e., pollination immediately after morning emasculation (P_1), pollination immediately after evening emasculation (P_2), pollination 12 hours after evening emasculation (P_3), pollination 18 hours after evening emasculation (P_4) and pollination 24 hours after evening emasculation (P_5).

Fertilization and other crop-raising practices were done as per the package of practices of vegetable crops, Directorate of Extension Education, Dr. Y.S. Parmar UHF, Nauni, Solan (HP). Crosses were made and 10 fruits were retained on each plant. The observations were recorded on randomly selected five fruits in each treatment for 3 plant growth, 4 seed quantity and 5 seed quality parameters (Days from pollination to fruit set, days from fruit set to ripe fruit maturity, ripe fruit weight in g, number of seeds per crossed fruit, 1000 seed weight, seed yield in g/plant, seed yield in kg/ha, Percent germination, seedling length in cm, seedling dry weight in mg, seedling vigour index-length and seedling vigour index-mass etc.

Results obtained were statistically analyzed for their significance by using Randomized Complete Block Design (RCBD), Completely Randomized Design (CRD), and Factorial Experiment as per Gomez and Gomez (1984) [9]. 100 seeds from all replications of each treatment were used for conducting the germination test as per ISTA (Anonymous, 1985) [3]. Seedling vigour index-Length (SVI-L) was calculated as per the formula given by Khare and Bhale (2000) [13] while to calculate seedling vigour index-Mass (SVI-M) methodology by Abdul-Baki and Anderson (1973) [1] was used.

3. Results and Discussion

Considering different pollination times, minimum days (4.05) to fruit set was observed in treatment P_5 (pollination 24 hours after evening emasculation) which had a significant difference over other pollination times (Table 1). Maximum days (5.85) to fruit set was observed in treatment P_1 (pollination immediately after morning emasculation) which was at par with P_2 (pollination immediately after evening emasculation, 5.72). The individual effect of crosses revealed that cross C_2 (California Wonder x Solan Bharpur) took minimum days (4.79) from pollination to fruit set whereas cross C_1 (Solan Bharpur x California Wonder) took maximum days (5.26) from pollination to fruit set. These findings are in agreement with the reports of Yogeesha *et al*. (1999) [18], Jolli (2004) [12] and Himral (2006) [11] in tomatoes and Kivadasannavar (2008) [14] in chilli who noticed maximum stigma receptivity next day after emasculation. This suggests that stigma receptivity is at its peak one day after emasculation. Himral (2006) [11] also noticed considerably lower fruit set and more days to fruit set when pollination was done just after emasculation. This indicates that at time of emasculation stigma is not very receptive or if it is capable of receiving pollen, all the ovules may not be ripe enough resulting in extended days to fruit set.

In the case of interaction (Table 1), minimum days (3.60) from pollination to fruit set was recorded in treatment combination C_2P_5 i.e., California Wonder x Solan Bharpur having pollination 24 hours after evening emasculation whereas, maximum days (6.10) from pollination to fruit set was observed in treatment combination C_1P_1 (Solan Bharpur x California Wonder pollinated immediately after morning emasculation) which was at par with treatment combination C_1P_2 (Solan Bharpur x California Wonder pollinated immediately after evening emasculation i.e., 5.83).

Table 1 about the number of days from fruit set to ripe fruit maturity revealed that treatment P_1 (pollination immediately after morning emasculation) took maximum of days (65.17) to harvestable maturity after fruit set, whereas treatment P_5 i.e., pollination done 24 hours after evening emasculation was earliest (56.33). Cross C_1 (Solan Bharpur x California Wonder) took the maximum number of days (65.60) to attain harvestable maturity whereas cross C_2 (California Wonder x Solan Bharpur) was the earliest for harvestable maturity (56.60). In case of treatment combinations, C_1P_1 took maximum days (69.00) to harvestable maturity after fruit set which was statistically at par with C_1P_2 (67.67 days) and C_1P_4 (67.33 days) whereas, C_2P_5 was the earliest to attain harvestable maturity (50.67 days).

Data for different times of pollination revealed that maximum ripe fruit weight (69.48 g) was obtained in treatment P_5 which had a significant difference over other times of pollination (Table 1). Minimum ripe fruit weight (46.37 g) was noted in treatment P_1 which was at par with treatment P_2 (48.5 g). These findings are corroborated by the studies of Yadav and Malabasari (2014) [17], who reported significantly higher fruit set percentage, fruit weight, seed weight per fruit and seed yield per plant when pollination was done on the day of flower opening. Kivadasannavar (2008) [14] also found that fruit set percentage and ripe fruit weight per plant were highest when pollination was done the next day immediately after emasculation compared to pollination on the same day of emasculation. The reduction in fruit weight may be attributed to the fact that on the day of emasculation stigma receptivity is not high and ovules may not be mature enough resulting in partial fertilization. Similar results have been reported by Patta *et al*., 2015 [15].

Among the crosses, the maximum ripe fruit weight (61.93 g) was found in cross C_2 (Table 1) which was significantly higher than in cross C_1 (54.58 g). In the case of interactions, combination C_2P_5 showed maximum ripe fruit weight (73.42 g) whereas, minimum ripe fruit weight (41.68 g) was observed in the treatment combination C_1P_1 which was at par with the treatment combination C_1P_2 (43.22 g).

In case of different times of pollination, the maximum number of seeds (135.17) per crossed fruit was obtained in treatment

P_5 which was significantly higher over other times of pollination (Table 1). Whereas, the minimum number of seeds per crossed fruit (103.50) was found in treatment P_1. These results are in accordance with the reports of Auerswald (1978) [6], Yogeesha *et al*. (1999) [18] and Jolli (2004) [12] in tomatoes who noticed the maximum stigma receptivity next day after emasculation of flowers as exhibited by higher fruit set, seed set and seed weight per fruit. Himral (2006) [11] stated that on the day of anthesis the number of ovules ready to be fertilized are maximum resulting in higher number of seeds per crossed fruit. Whereas, Dev (1998) [8] observed considerably lower fruit set and less number of seeds per fruit when pollination was done just after emasculation. They emphasized that stigma was capable of receiving pollens but all the ovules were not ripe thereby, resulted in less number of seeds. Among the crosses, the maximum number of seeds (120.53) per crossed fruit was noted in cross C_1 which was significantly higher than in cross C_2 (115.67). fruit. In case of interactions, maximum number of seeds per crossed fruit (140.33) was obtained in treatment combination C_1P_5 whereas, minimum number of seeds per crossed fruit (101.67) was observed in treatment combination C_2P_1.

For 1000 seed weight at different times of pollination (Table 1), maximum 1000 seed weight (6.38 g) was observed in treatment P_5 which had a significant difference over other times of pollination. Minimum 1000 seed weight (4.44 g) was found in treatment P_1 which was at par with treatment P_2 (4.57 g). These findings are in consonance with the findings of Kivadasavnnavar (2008) in chilli, Singh *et al*. (2010) [16] in okra and Himral (2006) [11] in tomato. This is mainly attributable to the fact that one day after emasculation, stigma is highly receptive, as it coincides with the flower anthesis and leads to effective fertilization, which results in higher fruit set, seed set and seed weight leading to bolder seeds. Among the crosses, maximum 1000 seed weight (5.50 g) was noted in cross C_2 which was significantly higher than cross C_1 (5.01 g). In case of interaction, maximum 1000 seed weight (6.81 g) was observed in treatment combination C_2P_5 whereas, minimum 1000 seed weight (4.23 g) was found in C_1P_1 which was statistically at par with treatment combination C_1P_2 (4.35 g).

Data on Table 1 pertaining to seed yield (g per plant) revealed that maximum seed yield per plant (4.39 g) was obtained in treatment which was significantly higher than other times of pollination. The minimum seed yield per plant (2.27 g) was obtained in treatment P_1. Seed yield depends upon various factors like 1000 seed weight, number of seeds per fruit, number of fruits per plant, and other environmental factors. The observations recorded here is supported by the fact that pollination 24 hours after emasculation resulted in higher fruit percentage, higher number of seeds per fruit and higher 1000 seed weight which resulted in higher seed yield per plant. This is because stigma receptivity is at its peak one day after emasculation or at the time of anthesis. Reduced fruit set due to drying of stigmatic surface was observed in delayed pollination resulted in lower seed yield. Similar results were obtained by Patta *et al*. (2015) [15], Deshi (2013) [7], Kivadasannavar (2008) [14] and Yogeesha *et al*. (1999) [18]. Similarly, Dev (1998) [8] noticed considerably lower fruit set and less number of seeds per fruit when pollination was done just after emasculation. Therefore, resulting in lower seed yield. This may be due to the incapability of the stigma to receive pollen.

In case of crosses, the maximum seed yield per plant (3.26 g) was found in cross C_2 (Table 1) having a statistically significant difference over cross C_1 (3.06 g). This is because the cross between California Wonder and Solan Bharpur leads to higher ripe fruit weight. Therefore, resulting in higher seed yield. In C_2P_5 maximum seed yield per plant (4.59 g) whereas, minimum seed yield per plant (2.23 g) was found in the treatment combination C_1P_1 which was at par with the treatment combination C_2P_1 (2.31 g).

The maximum seed yield (97.48 kg/ha) was observed in treatment P_5 which was significantly higher than at other times of pollination (Table 1). Minimum seed yield (51.18 kg/ha) was found in treatment P_1. Among the crosses, the maximum seed yield per hectare (72.64 kg/ha) was obtained in cross C_2 which was significantly higher than cross C_1 (67.97 kg/ha). The interaction effect between the time of pollination and crosses on seed yield was also significantly different, where maximum seed yield (102.08 kg/ha) was noted in the treatment combination C_2P_5 whereas, minimum seed yield (49.48 kg/ha) was observed in C_1P_1.

Maximum germination (93.00%) was recorded in treatment P_5 and minimum germination (67.83%) was observed in treatment P_1 (Table 1) which was statistically at par with treatment P_2 (68.50%). Yogeesha *et al*. (1999) [18] and Kivadasavnnavar (2008) in chilli also found the same results. This parameter increased due to higher seed weight and bold seeds recorded in this treatment. Patta *et al*. (2015) [15] reported that the seed germination and other seed quality parameters recorded were better when pollinated 24 hours after emasculation as compared to other times of pollination because at that time stigma is at the most appropriate stage for effective fertilization and hence results in the proper development of embryo in seeds produced.

In case of different crosses (Table 1), cross C_2 (California Wonder x Solan Bharpur) showed maximum germination (78.47%) whereas cross C_1 (Solan Bharpur x California Wonder) showed minimum germination (77.13%). Among different treatment combinations, maximum germination (93.33%) was recorded in C_2P_5 i.e., California Wonder x Solan Bharpur pollinated 24 hours after evening emasculation, having at par with C_1P_5 (Solan Bharpur x California Wonder when pollinated 24 hours after evening emasculation) (92.67%). Minimum germination (66%) was observed in the treatment combination C_1P_1 (Solan Bharpur x California Wonder pollinated immediately after morning emasculation).

Data pertaining to seedling length (Table 1) revealed that among different times of pollination, minimum seedling length (7.26 cm) was noted in treatment P_1 which was statistically at par with treatment P_2 (7.63 cm), whereas treatment P_5 resulted in maximum seedling length (9.38 cm). Deshi (2013) [7] reported that seed produced from pollination next day after emasculation noted significantly higher seed germination, root length, shoot length, 1000 seed weight, seedling dry weight and seedling vigour index. This parameter increased due to higher seed weight and bold seeds recorded in this treatment. The same results were reported earlier by Yogeesha *et al*. (1999) [18], Dev (1998) [8] and Kivadasavnnavar (2008) in chilli. In case of crosses, C_2 (California Wonder x Solan Bharpur) had maximum seedling length (8.39 cm) whereas cross C_1 (Solan Bharpur x California Wonder) had minimum seedling length (of 7.82 cm). Among interactions, treatment combination C_2P_5

resulted in maximum seedling length (9.89 cm) whereas the treatment combination C_1P_1 resulted in a minimum seedling length (6.67 cm).

Maximum seedling dry weight (2.55 mg) was observed in treatment P_5 (Table 1) which was significantly higher than other times of pollinations. Minimum seedling dry weight (1.67 mg) was recorded in treatment P_1. These results were found in equality with the results of Yogeesha *et al*. (1999) [18], Kivadasavnnavar (2008) in chilli and Singh *et al*. (2010) [16] in orka. Deshi (2013) [7] and Patta *et al*. (2015) [15] reported that an increase was attributed to higher seed weight and bold seeds being recorded at appropriate times of pollination, which results in higher seed quality. In case of different crosses, C_2 showed maximum seedling dry weight (2.17 mg) whereas, cross C_1 showed minimum seedling dry weight (2.00 mg). Among the different treatment combinations, the maximum seedling dry weight (2.62 mg) was found in C_2P_5 having at par in performance with C_1P_5 (2.48 mg). Minimum seedling dry weight (1.48 mg) was observed in treatment combination C_1P_1.

Maximum seed vigour index (237.35) was found in treatment P_5 which had a significant difference over other times of pollination (Table 1). Minimum seed vigour index (113.49) was observed in treatment P_1. These findings matched the results of Yogeesha *et al*. (1999) [18], Jolli (2004) [12], Kivadasavnnavar (2008) in chilli, and Singh *et al*. (2010) [16] in okra. Himral (2006) [11] also reported that maximum seed vigour index was obtained when pollination was done 24 hours after emasculation. This may be attributed to the fact that pollination at appropriate times when pollens and ovules are at maximum physiological maturity resulted in the formation of seeds with high vigour. Among crosses, maximum seed vigour index (174.43) was noted in the cross C_2 which was significantly higher than cross C1 (157.64). In case of interactions, maximum seed vigour index (244.89) was observed in treatment combination C_2P_5 whereas, minimum seed vigour index (97.41) was found in treatment combination C_1P_1.

The data recorded on seed vigour index-II (Table 1) revealed that among different times of pollination, the maximum seed vigour index (872.00) was recorded in treatment P_5 which was significantly higher than other times of pollination. A minimum seed vigour index (493.61) was found in treatment C_2 which was at par with treatment P_2 (523.08). The increase in seedling vigour can also be attributed to his gear per cent of germination and root-shoot length. Similar results were also reported by Yogeesha *et al*. (1999) [18] and Jolli (2004) [12] in tomato hybrid seeds, who noticed higher seed quality attributes in terms of seed germinability, seedling vigour and dry weight when pollination was done one day after emasculation. Maximum seed vigour index (665.37) was found in cross C_2 which was significantly higher than cross C_1 (610.82). In case of interactions, the maximum seed vigour index (923.07) was noted in treatment combination C_2P_5 whereas, the minimum seed vigour index (439.97) was found in C_1P_1.

Table 1: Effect of duration of emasculation and pollination time on growth, seed quality, and quantity of Bell pepper's hybrid seed production

Traits	Pollination to fruit set			Fruit set to ripe fruit maturity			Ripe fruit weight (g)			Number of seeds per crossed fruit			1000 seed weight (g)			Seed yield (g per plant)		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	6.10	5.60	5.85	69.00	61.33	65.17	41.68	51.06	46.37	105.33	101.67	103.50	4.23	4.64	4.44	2.23	2.31	2.27
P_2	5.83	5.60	5.72	67.67	59.00	63.33	43.22	53.78	48.50	111.00	108.67	109.83	4.35	4.78	4.57	2.42	2.65	2.53
P_3	4.73	4.13	4.43	62.00	54.00	58.00	63.13	67.57	65.35	129.00	123.00	126.00	5.52	5.95	5.74	3.55	3.66	3.60
P_4	5.13	5.00	5.07	67.33	58.00	62.67	59.34	63.80	61.57	117.00	115.00	116.00	4.97	5.31	5.14	2.92	3.06	2.99
P_5	4.50	3.60	4.05	62.00	50.67	56.33	65.54	73.42	69.48	140.33	130.00	135.17	5.96	6.81	6.38	4.18	4.59	4.39
Mean	5.26	4.79	Error: 0.05*	65.60	56.60	Error: 0.96*	54.58	61.93	Error: 3.55*	120.53	115.67	Error: 2.18*	5.01	5.50	Error: 0.02*	3.06	3.26	Error:0.008*
CD0.05	P: 0.26	C: 0.17	C X P: 0.37	P: 1.19	C: 0.75	C X P: 1.68	P: 2.28	C: 1.44	C X P: 3.23	P: 1.79	C: 1.13	C X P: 2.23	P: 0.15	C: 0.10	C X P: 0.22	P: 0.11	C: 0.07	C X P: 0.15
	Seed yield (kg per hectare)			Germination percentage			Seedling length (cm)			Seedling dry weight (mg)			Seed vigour index-1			Seed vigour index-1I		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
P_1	49.8	52.89	51.18	66.0 (54.34)	69.67 (56.58)	67.83 (55.46)	6.67	7.85	7.26	1.48	1.86	1.67	97.41	129.57	113.49	439.97	547.25	493.61
P_2	53.70	58.89	56.30	68.33 (55.76)	68.67 (55.96)	68.50 (55.86)	7.60	7.67	7.63	1.85	1.89	1.87	126.40	129.58	127.99	519.31	526.84	523.08
P_3	78.89	81.25	80.07	89.33 (70.94)	90.33 (71.89)	89.83 (71.42)	8.27	8.34	8.31	2.22	2.44	2.33	198.60	224.62	211.61	739.19	753.35	746.27
P_4	64.88	68.07	66.48	69.33 (56.38)	70.33 (57.00)	69.83 (56.69)	7.71	8.19	7.95	1.96	2.04	2.00	136.01	143.49	139.75	534.71	576.33	555.52
P_5	92.89	102.08	97.48	92.67 (74.30)	93.33 (75.05)	93.00 (74.67)	8.86	9.89	9.38	2.48	2.62	2.55	229.80	244.89	237.35	820.92	923.07	872.00
Mean	67.97	72.64	Error: 3.39*	77.13 (62.34)	78.47 (63.30)	Error:0.43*	7.82	8.39	Error:0.12	2.00	2.17	Error: 0.01	157.64	174.43	Error: 772.20*	610.82	665.37	Error:46.37*
CD0.05	P: 2.23	C: 1.41	C X P: 3.16	P: 0.79	C: 0.50	C X P: 1.12	P: 0.42	C: 0.27	C X P: 0.59	P: 0.11	C: 0.07	C X P: 0.16	P: 8.20	C: 5.19	C X P: 11.60	P: 33.47	C: 21.17	C X P: 47.33

4. Conclusion

C_2P_5 was found significantly superior over all other treatments for seed yield seed quality and growth characteristics. Individually, treatment P_5 was found better in terms of performance for the majority of growth, seed yield and seed quality traits. In the case of crosses, C2 was found significantly superior to C_1 for getting higher yields and quality of bell pepper seeds. Thus, investigation revealed that comprehensive knowledge is required to study all the aspects of bell pepper hybrid seed production in relation to improve quality and yield by giving priority to the optimum time of pollination after emasculation

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