DOI : 10.5958/0974-0112.2018.00042.7

Indian J. Hort. 75(2), June 2018: 245-251

Comparative evaluation of hybrid seed production of bitter gourd in rainy and spring-summer season

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ABSTRACT

A study was conducted with the parental lines of two popular bitter gourd hybrids; Pusa Hybrid-1 and Pusa Hybrid-2 to assess the effect of season on pollination behaviour, fruit setting, fruit yield and seed quality with respect to hybrid seed production. The hybrid seed production was carried out by hand pollination for four weeks in spring summer and rainy season. The results showed high pollen viability (R: 97.63-100%, S-S: 90-95%) and stigma receptivity in both the seasons however higher fruit yield, fruit traits (weight, length, width) were achieved in rainy season whereas the fruit setting percentage (80-90%) and seed quality were superior in the spring-summer season. Among the weeks of pollination compared, first and second weeks of pollination i.e. last week of April to first week of May in spring summer, third and fourth week of September in rainy season were found to be better for hybrid seed production than later weeks of pollination in both the seasons.

Key words: *Momordica charantia*, pollen viability, fruit setting, seed yield, seed quality.

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is an important cucurbitaceous fruit vegetable crop rich in nutritional and medicinal value. In spite of its importance, bitter gourd area and production are low in India (Area-93,000 ha, Production-1046 TMT; NHB 2015-16) mainly because, large area of cultivation is under open pollinated varieties due to non availability of hybrid seed at an affordable cost. To increase the production and productivity of bitter gourd, more area has to be covered under hybrids, which calls for localization of seed production area and standardization of hybrid seed production technology. Although, the crop can be successfully grown in both rainy and spring-summer season, seed production during rainy season is affected by high incidence of fruit fly which not only affects seed yield but also quality. Spring-summer season has shorter duration of pollination and crop encounters high temperature during pollination, which affects the seed setting, yield and quality. So, the knowledge of pollen viability and stigma receptivity is very important in hybrid seed production particularly in crops like bitter gourd where pistillate flower remains viable only for one day. This will enable the seed producers to know when to pollinate and for how long pollination can be continued. Bitter gourd hybrid seed production is mainly undertaken in Southern India and hybrid seed is transported to North India where the major area of cultivation under the crop is there. Thus diversification of seed production area will ensure timely availability of hybrid seed at an affordable cost and there is also a need to identify optimum season for hybrid seed production of bitter gourd under North Indian conditions. Hence, keeping the importance of these points in the mind, crop growth, duration of pollination, fruit setting, seed yield and quality was studied in the female parental lines of Pusa Hybrid-1 and Pusa Hybrid-2 for assessing the feasibility of hybrid seed production of bitter gourd under North Indian conditions.

MATERIALS AND METHODS

Field studies were conducted at Indian Agricultural Research Institute (IARI), New Delhi, India (Lat. 28° 38'23" N; Long. 77° 09'27" E; Elevation 228.61m) for two seasons spring-summer (November-May) and rainy season (July-November) of 2012 to determine the effect of season on hybrid seed production of bitter gourd. The experiment was carried out with the parental lines of two bitter gourd hybrids; Pusa Hybrid-1 and Pusa Hybrid-2. The field experimental design was randomized block design (RBD) with three replications. The seeds were scarified, soaked overnight and treated with bavistin (0.1%) followed by sowing in plug trays. Twenty five day old seedlings (2-4 leaf stage) were transplanted in spring-summer and rainy season. Soil was ploughed twice and harrowed once before transplanting. Seedlings were transplanted on both the edges of raised flat bed (1.5 m width) at a spacing of 90 cm (between plants). The irrigation was given as per the requirement of the crop. Manual weeding was done 3-4 times to keep the field free from weeds.

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Pollen viability was studied by acetocarmine staining. Four male buds were collected from three plants of male parent between 9-10 am. Using dissecting forceps, scalpel and a needle, anthers were opened to allow extraction and subsequent transfer of pollen dust on to a microscopic glass slide in a drop of acetocarmine stain. Cover slips were gently placed on to slides and slides were then observed under a light microscope. Observations were recorded under three randomly selected microscopic fields and pollen viability was expressed in percentage (McKellar *et al*., 11).

To standardize the time of pollination for hybrid seed production, unopened female flower buds of seed parent which will be opening next day morning are covered with butter paper bag having five to six tiny holes to facilitate the ventilation and to avoid the build-up of high temperature inside the butter paper bag. Male buds in the pollen parent were also covered with non-absorbent cotton in the previous evening between 4.00 to 5.00 pm. Next day morning, the male buds were collected and their pollen was dusted on the stigma of female flowers followed by tagging and covering with butter paper bag. The pollination was performed with five timings, viz. 7.00 am, 9.00 am, 11.00 am, 1.00 pm and 3.00 pm with 2 replications regularly up to 40 days after initiation of flowering with 3 days interval. Five plants from each replication were selected at random and tagged for recording observations on fruit setting, yield and quality parameters. Based on these observations, pollination time between 7.00 to 9.00 am was chosen to ascertain stigma receptivity as described above. For stigma receptivity studies, the crossing was carried out three times in a week for the period of four weeks from 10 days after opening of first female flower i.e. between 26th April to 22nd May in spring-summer and 17th Sept to 14th October in rainy season. The buds and flowers that appeared subsequently after the completion of crossing programme were manually removed to facilitate better development of the crossed fruits and to avoid the selfed seeds in the hybrid. Stigma receptivity was estimated based on setting percentage by counting number of fruit set seven days after hand pollination. Fruit setting was expressed in percentage.

To study the crop growth, vine length (cm) was measured using a flexible tape from the base to tip of the plant at 15 days interval from 15 days after transplanting up to end of the flowering in a vine. The total number of female and male flowers borne in a vine was visually counted from the beginning of first male and female flower opening till end of the flowering period in both the seasons and used to calculate sex ratio. Calculation was done according to the following formula; Sex ratio= Number of female / Number of male flowers (Marie and Mohamed, 10). The observations on fruit setting percentage, fruit length, weight and width, number and weight of filled seeds per fruit were recorded. The length of the fruit was measured using centimetre ruler while their width was assessed by using a vernier caliper at successive harvesting intervals. Seed quality was assessed by seed germination test according to ISTA recommendations (ISTA, 7) and other quality parameters like root length (cm), shoot length (cm), seedling dry weight were also recorded and utilized for vigour index calculation. Seedling vigour index I and II were calculated as per Abdul Baki and Anderson, (1) formula as follows; Seedling vigour index I = Seed germination % × (Root length + Shoot length)
Seedling vigour index II = Seed germination % × Seedling dry weight

Percentage data such as pollen viability, stigma receptivity and fruit set were transformed via arcsine before analysis (Gomez and Gomez, 6). The data was analyzed statistically for testing heterogeneity of means, adopting two and three factorial analysis with OPSTAT package developed by CCS Haryana Agricultural University, Hisar, India (http://www.hau.ernet.in/opstat.html). Mean value separations were performed using a least significant difference (LSD) test at a 5% significance level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Hybrid seed production of bitter gourd involves hand pollination and final seed yield depends on all the crop growth stages, thus determination of optimum season and time is important for minimizing labour cost. During hybrid seed production of bitter gourd, environmental conditions viz., temperature, relative humidity and photoperiod plays an important role in all the crop growth stages especially the early vegetative growth (vine length). The results of the present study showed that season had a significant effect on the vine length. The vine length in both the parental lines was longer during rainy season (222 cm female parent; 223.5 cm male parent) as compared to spring-summer season (156.2 cm female parent; 159 cm male parent) and increase in vine length was significantly higher in first two fortnight of crop growth (96.4 cm, 30-60 DAT) as compared with later stages (44 cm, 60-90 DAT) (Fig 1a). Longer vine length in rainy season was due to longer crop duration (133 days) with moderate temperature (16.2-31.4^{o}C) and RH (61.0-90.5%) during crop growth which is the optimum temperature for photosynthesis of bitter gourd (23 to 34^{o}C) as compared to the spring summer season (temperature: 17.8-35.5^{o}C, RH: 31.5-63.5%) (Table 1) (Bairwa *et al*., 3).

Fruit and seed yield mainly depends on the number of pistillate flowers borne per vine. In bitter gourd,

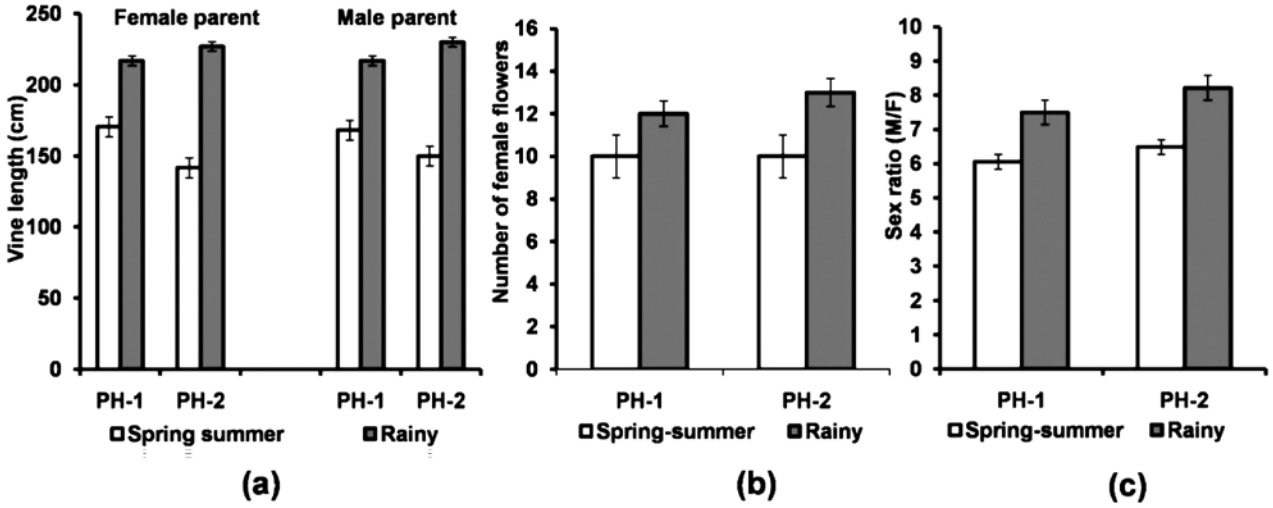

Fig. 1. Effect of season on vine length (a) number of female flowers (b) and sex ratio (c) in parental lines of bitter gourd hybrids.

average ratio of staminate to pistillate flowers varies from 50:1 (Rasco and Castillo, 12) to 9:1 (Dey *et al*., 4). In the present study, number of female flowers per vine was more (11.00) with higher sex ratio (F: M) (6.96-8.62) in rainy as compared to spring-summer season respectively (10.00; 6.06-6.49) (Fig.1b and 1c). This higher female sex expression may be due to low temperature, short photoperiod and high moisture availability which are conditions that encourage the build up of carbohydrates. These environmental factors also influence the levels of endogenous hormones (especially ethylene, auxin and gibberellic acid, chemical composition), which in turn influence sex expression by suppressing the staminate flowers and promoting more number of pistillate flowers (Robinson and Walters, 14; Agbaje *et al*., 2; Sandra *et al*., 15). In our present study higher number of female flowers in rainy season might be due to high rainfall and low temperatures during the August and September months which are in contrary to the results of Agbaje *et al*., (2) who reported more number of female flowers in the early season (May to August) than late season (August to November) in pumpkin (*Cucurbita pepo*).

Pollen viability studies in male parents of Pusa Hybrid-1 and Pusa Hybrid-2 showed high viability throughout the pollination period (90.0-100%) in both the seasons. However, pollen viability was higher in rainy season (97.0 to 100%) compared to spring-summer season (90-94%), but there was no significant difference in pollen viability between the

Table 1. Meteorological data during crop growth in spring-summer and rainy season

Date/Season	Temperature (^{0}C)			RH (%)			Rainfall (mm)
Period of pollination	Max.	Min.	Mean	Max.	Min.	Mean	Total
Spring-Summer							
23-29th April (1W)*	40.4	22.4	31.4	58	27	42.5	0.0
30th April-6th May (2W)*	38.9	25.0	31.9	60	36	48.0	8.8
7-13th May (3W)*	38.8	24.0	31.4	57	36	46.5	0.0
14-20th May (4W)*	43.8	27.0	35.4	42	25	33.5	0.0
Rainy							
17-23rd September (1W)**	28.8	22.9	25.9	97	82	89.5	141.8
24-30th September (2W)**	31.6	21.7	26.7	93	59	76.0	0.0
1-7th October (3W)**	33.3	20.7	27.0	96	55	75.5	0.0
8-14th October (4W)**	33.5	19.6	26.6	82	46	64.0	0.0

*Week of pollination during spring summer season
**Week of pollination during rainy season

parents (Table 2). The results revealed that pollen viability was not a constraint in hybrid seed production of bitter gourd.

Studies on standardization of time of pollination showed a significant difference in fruit setting percentage, seed yield and quality parameters for various pollination timings (7.00 am, 9.00 am, 11.00 am, 1.00 pm and 3pm). These all parameters were significantly higher in 7.00 am to 9.00 am pollination compared to other pollination timings (Table 3). The higher fruit setting percentage might be due to availability of viable pollen which resulted in better fertilization and high stigma receptivity during early part of the day and pollination periods. The plants will be more active physiologically at this stage. So, this 7.00 am to 9.00 am pollination was chosen for stigma receptivity studies.

To ascertain the receptivity of stigma, fruit setting percentage was taken as an index. Maximum fruit setting percentage was observed in first and second weeks of pollination and thereafter it decreased (Table 4). The fruit set values of the flowers pollinated on third and fourth week of pollination found non-significant. Between the seasons fruit setting percentage was higher in spring summer season (89.37 and 80.12) compared to rainy season (73.75 and 78.75) (Table 4). The high fruit setting in the beginning of pollination period could be attributed to adequate availability of

Table 2. Effect of season on pollen viability in male parental lines of bitter gourd hybrids Pusa Hybrid-1 and Pusa Hybrid-2.

Parental lines/ character	Pollen viability (%)									
	Spring-Summer					Rainy				
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Male parent of PH-1	91.34 (72.84)	92.15 (73.68)	90.46 (72.05)	90.21 (71.76)	91.04	97.63 (81.33)	99.00 (85.46)	99.08 (86.84)	99.33 (86.73)	98.76
Male parent of PH-2	93.67 (75.46)	92.49 (74.00)	91.64 (73.15)	90.87 (72.44)	92.16	98.33 (82.66)	98.83 (85.06)	98.50 (83.15)	99.16 (85.77)	98.70
C.D. (0.05): 0.108; SE(m): 0.036										

Values in parenthesis are arc sine converted values.

Table 3: Effect of time of pollination on fruit setting, fruit yield, seed yield and seed quality in the female parental lines of bitter gourd hybrids Pusa Hybrid-1 and Pusa Hybrid-2.

Time of pollination	Fruit Setting %	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Number of filled seeds	Weight of filled seeds (g)	Germination (%)	Seedling length (cm)	Seedling dry weight (g)	Seedling vigour index I	Seedling vigour index II
Female parent of PH-1											
7.00 am	81.00	137.80	15.11	4.65	24.50	3.60	70.20	22.58	0.18	1577.14	12.67
9.00 am	78.00	140.20	15.72	4.90	22.80	3.38	72.10	22.86	0.18	1655.27	13.32
11.00 am	78.00	140.50	14.89	4.85	21.50	3.74	66.50	22.81	0.18	1476.90	11.99
1.00 pm	55.00	118.08	14.31	4.13	17.50	2.76	61.10	19.62	0.17	1175.94	10.73
3.00 pm	48.00	91.50	13.31	3.95	16.60	2.18	60.40	17.59	0.17	1078.74	10.34
Mean	68.00	125.61	13.97	4.49	20.58	2.99	66.26	21.17	0.17	1392.79	11.81
Female parent of PH-2											
7.00 am	86.00	147.80	14.52	5.88	25.70	3.79	67.30	21.46	0.17	1452.22	11.79
9.00 am	98.00	157.50	14.23	5.89	23.70	3.64	67.00	23.26	0.18	1514.60	10.92
11.00 am	92.00	157.00	13.03	6.27	22.70	3.55	62.90	22.89	0.17	1372.55	11.07
1.00 pm	62.00	122.00	13.06	5.30	17.30	2.59	57.40	20.97	0.16	1120.52	9.64
3.00 pm	38.00	113.90	10.48	5.38	17.50	1.44	57.60	18.04	0.16	981.65	9.28
Mean	75.20	139.64	12.22	5.70	21.39	3.00	62.44	21.32	0.17	1288.30	10.54
C.D. (0.05)	2.92	2.17	1.154	0.142	1.502	0.031	2.53	2.15	NS	3.56	NS
SE(m)	1.157	1.052	0.151	0.012	0.12	0.007	0.81	1.06	0.32	1.21	0.49

pollen grains, congenial weather conditions, high vigour of the crop and better fruit bearing potential of a vine. Low fruit setting percentage in later weeks of pollination might be due to prevailing higher temperatures during spring summer and very low temperatures in rainy period during crop growth period. Similar results were reported in tomato (*Lycopersicon esculentum)*, brinjal (*Solanum melongena)* and bitter gourd (*Momordica charantia)* (Rahman *et al*., 13).

Fruit traits i.e. fruit weight, length were higher during first two weeks of pollination as compared to later weeks of pollination in both the parents (Table 4). Between the two seasons, these parameters were higher in rainy season (133.39-145.82 gm, 14.2- 16.2 cm) compared to spring-summer season (53.03-65.71 gm, 12.7-13.5 cm) but fruit width was found to be non significant between two seasons. This may be attributed to longer crop duration (4.5 m), moderate temperature (16.2-26.6^{0}C) and RH (61.0-89.5%) which favoured better source sink relation leading to better translocation of photosynthetates to the developing fruits as compared to spring-summer season (3.5 m, temperature:30.5-35.5^{0}C and RH: 32.5-48.5%). The results are in accordance with Sundriyal *et al.* (17) in bitter gourd, and Ganar *et al*. (5) in ash gourd in brinjal.

Marked difference in seed yield and its attributes like seed weight per fruit, number of seeds per fruit were recorded. These were found higher in spring-summer season compared to rainy season (Table 5). In the present study maximum seed yield might be due to large sized fruits, higher fruit weight, longer duration for fruit development after pollination leading to better filling (Rainy: 40-50 days and spring summer season: 30-35 days). Reduced seed weight in later weeks of pollination specifically in rainy season was due to cool and very low temperatures which were lower than the optimum resulted in impaired biomass accumulation. These results are in conformity with findings of Stephenson *et al*. (16) in *Cucurbita pepo*, Venangamudi and Palaniswamy (18), Sundriyal *et al*. (17) in bitter gourd, and Kortse *et al*. (9) in *Citrullus lanatus*.

Higher seed quality parameters like germination percentage and seedling vigour index were recorded in spring-summer season (92.90-94.04%, 3613.8-3788.3) compared to rainy season (63.80-68.37%, 1361.91-1480.5) in both the parental lines (Table 6). Similarly, first two weeks pollination achieved seeds with higher germination as compared following weeks of pollination. Some reports showed that temperature

Table 4. Effect of seasons on fruit setting, fruit weight, length and width in female parental lines of bitter gourd hybrids Pusa Hybrid-1 and Pusa Hybrid-2.

Parental lines	Spring-Summer					Rainy				
	Fruit setting percentage									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	97.50	95.00	82.50	82.50	89.37	84.00	85.00	70.00	56.00	73.75
Female of PH-2	93.00	85.50	85.00	77.50	80.12	86.00	83.00	77.00	69.00	78.75
C.D. (0.05): 3.796; SE(m): 1.259										
	Fruit weight (g)/plant									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	72.85	67.85	65.00	57.14	65.71	146.70	146.30	125.20	115.38	133.39
Female of PH-2	61.42	52.85	51.42	46.42	53.03	152.50	147.80	154.00	129.00	145.82
C.D. (0.05): 3.170; SE(m): 1.052										
	Fruit length (cm)/plant									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	15.71	13.62	13.33	11.40	13.5	19.92	17.90	15.92	11.40	16.2
Female of PH-2	14.81	13.51	11.71	10.93	12.7	17.34	15.63	12.30	11.85	14.2
C.D. (0.05): 0.154; SE(m): 0.051										
	Fruit width (cm)/plant									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	5.01	4.87	4.25	4.31	4.61	6.10	5.50	4.90	4.17	5.16
Female of PH-2	4.32	4.41	3.85	3.91	4.12	6.97	7.45	5.72	5.28	6.36
C.D.(0.05): 0.024; SE(m): 0.007										

Table 5. Effect of seasons on number and weight of filled seeds per fruit in female parental lines of bitter gourd hybrids Pusa Hybrid-1 and Pusa Hybrid-2.

Parental lines	Spring-Summer					Rainy				
	Number of filled seeds									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	30.00	28.71	21.85	15.57	24.03	27.40	25.70	23.10	15.70	22.97
Female of PH-2	22.57	28.57	19.57	17.42	22.03	28.86	26.70	24.70	16.80	24.26
C.D. (0.05): 1.502; SE(m): 0.012										
	Weight of filled seeds (g)									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	4.93	3.37	3.39	3.49	3.79	4.06	3.74	3.84	2.24	3.47
Female of PH-2	5.02	3.21	3.15	3.28	3.67	3.85	3.71	3.46	2.30	3.33
C.D. (0.05): 0.021; SEM(m): 0.007										

during seed filling may affect seed weight and vigour in various crops (Jansen, 8). As bitter gourd is a summer crop, air temperature must be more than 25ºC for its better growth and development which will hamper below 20ºC. Better seed quality of spring-summer season produce in this study could be attributed to favourable environmental conditions i.e. more or less optimum temperature throughout the whole growing period as compared with rainy season where there were mild temperature (temp: 16.2-23.0ºC and RH: 61.0-76.0%), intermittent rains and the fruit matured in November and December when the winter has set in. An increase in seed quality parameters such as seed germination percentage and seedling vigour index might be attributed to the higher seed weight components which provided more reserve food material for the vigorous growth of seedling. From the above discussion, it can be concluded that the pollination on the early weeks was found to be more ideal for higher fruit set, seed yield and better seed quality parameters such as germination and seedling vigour index as compared to later weeks of pollination.

Table 6: Effect of season and week of pollination on seed quality parameters in female parental lines of bitter gourd hybrids Pusa Hybrid-1 and Pusa Hybrid-2.

Parental lines	Spring-Summer					Rainy				
	Germination %									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	100.00 (90.00)	95.00 (80.78)	91.66 (73.28)	89.50 (71.09)	94.04	70.90 (57.42)	70.70 (57.31)	67.50 (55.27)	64.40 (53.46)	68.37
Female of PH-2	93.10 (74.99)	95.00 (80.78)	95.00 (80.78)	88.00 (69.73)	92.90	67.20 (55.11)	66.20 (54.49)	62.10 (52.05)	59.70 (50.62)	63.80
C.D. (0.05): 2.723; SE(m): 0.903										
	Seedling vigour index-I									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	3993.0	4180.0	3570.2	3409.9	3788.3	1513.7	1595.7	1445.7	1366.8	1480.5
Female of PH-2	3565.7	4082.6	3537.8	3269.2	3613.8	1463.9	1431.6	1363.2	1189.4	1361.9
C.D. (0.05): 3.962; SE(m): 1.314										
	Seedling vigour index-II									
	1W	2W	3W	4W	Mean	1W	2W	3W	4W	Mean
Female of PH-1	27.00	27.55	21.99	19.69	24.05	12.69	12.83	12.25	11.47	12.31
Female of PH-2	24.18	25.78	21.85	18.63	22.61	12.02	11.60	10.88	9.03	10.88
C.D. (0.05): NS; SE(m): 0.5										

From the results of the present study, it is evident that duration of pollination had a significant effect with respect to setting percentage, fruit and seed yield and quality attributes. Under North Indian conditions, best time of pollination for achieving higher fruit setting, seed yield and quality are first two weeks i.e. 26th April to 6th May during spring-summer and 17th September to 30th September in rainy season. In conclusion, rainy season growing delays the maximum maturation stage of bitter gourd seeds in turn seed quality. However highest quality of seed can be obtained in spring-summer growing season. Although seed production is feasible in both spring-summer and rainy season, the time of occurrence of maximum fruit setting and seed quality, affecting seedling growth in this work may depend on the environmental conditions.

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Received : July, 2017; Revised : April, 2018;
Accepted : May, 2018