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Effect of plant growth regulators on sex expression, fruit setting, seed yield and quality in the parental lines for hybrid seed production in bitter gourd (*Momordica charantia*)

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ABSTRACT

Effect of plant growth regulators on vegetative growth, sex expression,fruit setting, seed yield and quality was studied for hybrid seed production in the parental lines of bitter gourd (*Momordica charantia* L.) hybrids,Pusa hybrid 1 and Pusa hybrid 2 in rainy and spring-summer season. Plant growth regulators namely GA_3 @ 50 ppm, NAA @ 200 ppm, maleic hydrazide @ 100 ppm, etherel @ 50 ppm were sprayed at three leaf and tendril initiation stage. The results showed that application of GA_3 significantly enhanced vine length, number of branches and nodes/vine, fruiting, seed yield and quality in the parental lines in both the seasons.The plants sprayed with growth regulators showed induction of female flowers at lower nodes with 3-5 more pistillate flowers per vine and higher sex ratio as compared to unsprayed control. In manually pollinated flowers, plants sprayed with GA_3@ 50 ppm had higher fruit and seed setting, fruit weight and hybrid seed yield. All the growth regulators had positive influence on vegetative, flowering and fruit traits in both the seasons but effect of growth regulators were more evident in rainy than spring-summer season. GA_3 @ 50 ppm, NAA @ 200 ppm, etherel @ 50 ppm were effective for enhancement in vegetative growth, fruit and seed yield and modification of sex expression but GA_3 @ 50 ppm sprayed twice at three leaf and tendril initiation stage was most effective for hybrid seed production of bitter gourd.

Key words: Fruit setting, Fruit yield, Growth regulators, Hybrid seed production, Sex expression, Seed yield, Seed quality

Bitter gourd (*Momordica charantia* L.) is monoecious in nature, and bears staminate and pistillate flowers separately where the proportion of staminate flowers is very high as compared to pistillate flowers. The average ratio of staminate to pistillate flowers in monoecious lines varies from 50:1 (Rasco and Castillo 1990) to 9:1 (Dey *et al*. 2005). To achieve higher yield, synchronized flowering and high sex ratio (number of female : male flower in vine) is desirable (Mia *et al*. 2014). The principle of sex modification in cucurbits lies in altering the sequence of flowering and sex ratio. Besides the environmental factors, endogenous levels of auxins, gibberellins, ethylene and abscisic acid at the seat of ontogeny determines the sex ratio and sequence of flowering. Plant growth regulators (PGR) (GA_3, IBA, MH, ethephon, NAA, GABA) play an important role in plant morphology, growth and development. They are reported to influence the vegetative growth, flowering, sex expression, fruit and seed yield in cucurbitaceous crops, but for best results their stage and concentration of application needs to be optimized (Ghosh and Basu 1983, Shantappa 2004, Birader and Navalagatti 2008). The plant growth regulators are commonly applied at two to four leaf stage but their effectiveness increases when applied twice or thrice at different stages (Basu unpublised).

In India, hybrid seed production is feasible both in rainy (July-October) and spring-summer (February - May) season but primarily it is undertaken in rainy season. To meet the increasing demand of quality seed, area under hybrids have to be increased which calls for development and standardization of hybrid seed production technology in bitter gourd. The present study was undertaken, to evaluate the effect of PGRs on the crop growth, sex modification, fruiting, seed yield and quality in female parental lines of bitter gourd hybrids, Pusa Hybrid 1 and Pusa Hybrid 2 for identification of potential PGR and season for successful hybrid seed production under north Indian conditions.

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MATERIALS AND METHODS

The research was carried out in the experimental fields of Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi in spring summer and rainy season of 2010. The seeds of the parental lines of bitter gourd hybrid, Pusa Hybrid-1(PH-1) and Pusa Hybrid 2 (PH 2) were procured from Division of Vegetable Science, ICAR-IARI, New Delhi. The seeds were soaked in Bavistin @ 2.5g/kg of seed/17hr/25^{o}C for better sprouting and seedling growth. Presoaked seeds were sown in the plug trays placed in glass house maintained at 25^{o}C. 25 days old seedlings (2-4 leaf stage) were transplanted on raised bed (10m × 1.5m wide) on 22 February 2010 in spring-summer season and on 30 July 2010 in the rainy season. A spacing of 0.9m was maintained between plants for facilitating proper growth and data observation. During final field preparation, farmyard manure @ 25 tonnes/ha, nitrogen @100 kg/ha, phosphorous @ 60 kg/ha and potassium @ 60 kg/ha was applied as basal dose. 50 kg N/ha was applied as top dressing at 30 and 50 days after transplanting. Trellies made from bamboo were erected to facilitate vine climbing, pollination, harvesting and fruit protection from rotting. The experiment was laid down in randomized block design (RBD) with three replications.

Preliminary field studies were conducted for standardization of PGR dose and stage of application on parental lines, i.e. Gibberellic acid (GA_3) @ 25, 50ppm, naphthalene acetic acid (NAA) @200, 300ppm; maleic hydrazide (MH) @ 100ppm, etherel @ 50, 100ppm were sprayed at 3-4 leaf stage (T_1), 3-4 leaf stage + tendril initiation (T_2), 3-4 leaf stage + tendril initiation stage + bud initiation stage (T_3) on the female lines of Pusa Hybrid 1 and Pusa Hybrid 2 in spring summer and rainy season of 2009. Based on the results of this study, the GA_3 @ 50ppm, NAA @ 200ppm, MH @ 100ppm, etherel @ 50ppm were selected along with distilled water spray as control. The PGR sprays were given to female parental lines at three leaf (4-5 days after transplanting) and tendril initiation stage (20-25 days after first spraying)(T_2). For each treatment, observations were recorded on vine length, total number of branches, node of induction of first male and female flowers in vine, total node number/vine, number of female and male flowers, number of fruits/vine (under open pollination), fruit weight, length and width, number of seeds, seed weight/fruit were recorded on 30 plants (10/replication). Fruit traits were observed on matured orange ripe fruits. For identification of potential growth regulator for hybrid seed production, 10 plants per treatment were hand pollinated. The pistillate buds in female parent of PH 1 were covered with butter paper in the evening and male buds in the male parent were covered with cotton. Next morning female buds were hand pollinated (between 9.00-9.30 AM) with male buds from male line and hybrid fruit setting, fruit size, weight, hybrid seed yield was measured in different treatments. Seeds were extracted from ripened fruit manually by maceration of ripe seeds with sand followed by shade drying. Seed of fruits harvested from each plants were pooled to generate data on number of seeds/fruit, seed weight/fruit and quality. The seed germination was tested as per the ISTA procedure (ISTA 2008). Seedling vigour index was calculated as per Abdul Baki and Anderson (1973). Analysis of variance of the data from each attribute computed using the OPSTAT statistical package.

RESULTS AND DISCUSSION

Vegetative growth

Growth regulators are reported to have stimulatory effect on vegetative growth in bitter gourd (Hilli *et al*. 2010). In the study, vine length increased significantly with the application of GA_3, NAA and etherel (228.1, 221.4, 207.1cm) over control (193.8cm). Plants treated with 50ppm GA_3 attained maximum plant height, followed by NAA@ 200 ppm and ethereal @ 50 ppm. Application of MH @ 100 ppm had no significant effect on vine length. Among seasons, vine length, primary and secondary branches were significantly higher in rainy season due to longer crop duration as compared to spring summer season. Application of growth regulators produced more branches and nodes per plant as compared to control. Among the growth regulators, GA_3 @ 50 ppm, NAA @ 200 ppm and etherel @ 50 ppm significantly increased the number of primary, secondary branches (26.0, 25.5, 25.0) and number of nodes (59.7-61.2; 58.5-58.8; 54.5-55.2) at final harvest as compared to control (20.2) (47-47.5). Application of GA_3@50ppm resulted in higher vine length and vegetative growth due to greater stem elongation, a principle property of gibberellins. The increase in vine length and branches in vines may be attributed to enhanced nutrient uptake, higher photosynthetic activity and translocation efficiency resulting in rapid cell division, elongation and vegetative growth (Mangal *et al*. 1981).

Sex expression

The plant growth regulators are reported to influence flowering and modification of sex expression especially sex ratio. In the study, PGR sprayed plants bore male and female flowers at lower nodes with more female flowers/vine favouring higher female to male flower, i.e. sex ratio (8.2-10.1) compared to control (6.8-7.4) (Table 1). Among growth regulators, application of etherel @ 50 ppm resulted in appearance of male and female flower at lower node (8^{th} node, 13^{th} node) followed by GA_3 at 50 ppm (9^{th} and 14^{th} node) (Table 1).The highest number of female flowers/vine (15.00) was recorded with 200 ppm NAA followed by 14 and 13 flowers respectively in etherel and GA_3 sprayed plants as compared to control (10.0) (Fig 1, Table 1). In rainy season, due to better vegetative growth and longer crop duration male and female flowers were borne at marginally higher node but number of female flowers were more with better sex ratio than spring-summer season. Baruah and Das (1997) reported that application of NAA

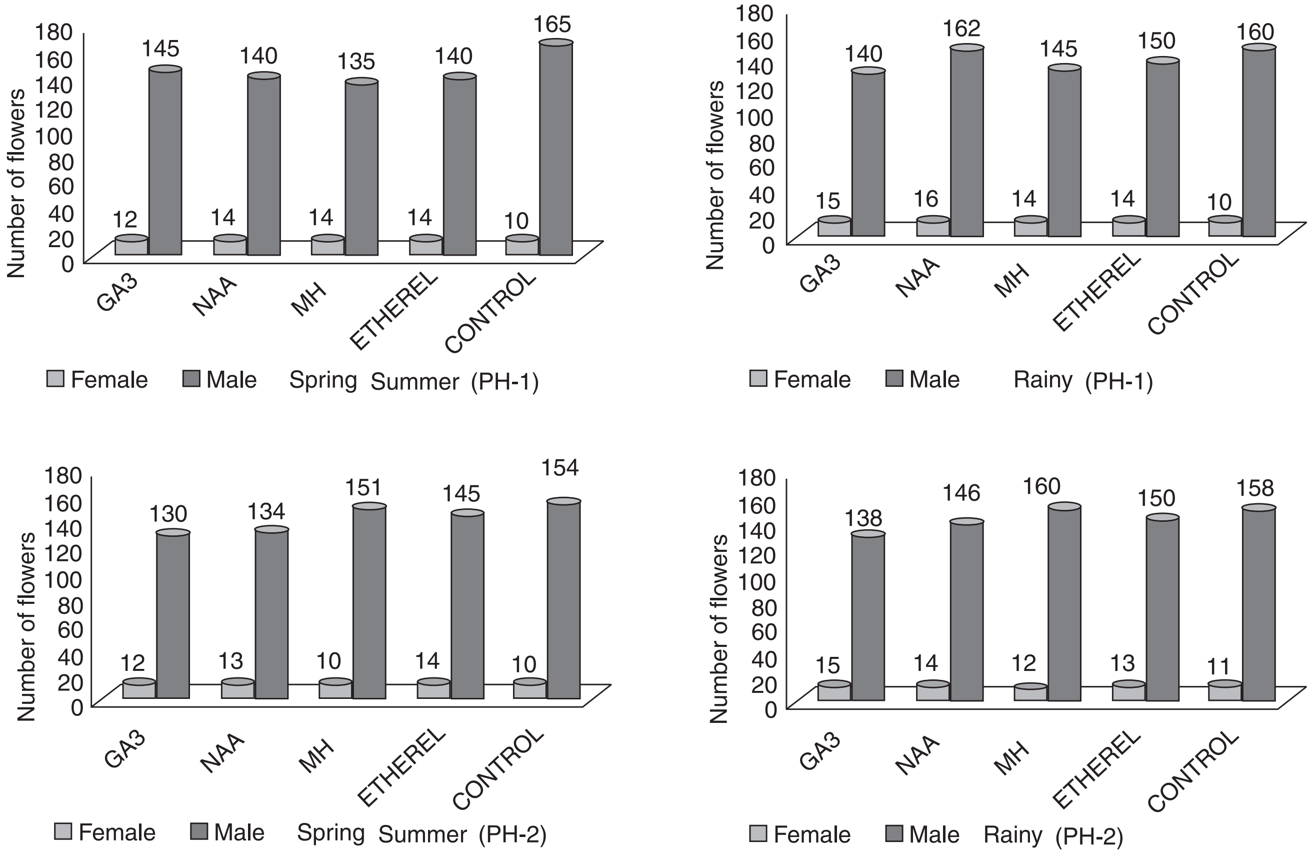

Fig 1 Effect of growth regulators on flowering behaviour of the female parental lines of PH 1and PH 2 in spring summer and rainy season

increased the endogenous auxin level in the plant which hastened the female flower induction ultimately narrowing the sex ratio in bottle gourd. The results of the present study are in agreement with Gedam *et al*. (1998) regarding flowering behavior and sex ratio in bitter gourd.

Fruit traits

Total fruit yield depends on number of fruits/vine and fruit weight. In the study, application of plant growth regulators increased the fruits plant significantly as compared to control in both the seasons. Among seasons, significantly higher fruits were borne in rainy season (11.8 and 11.9) as compared to spring summer season (9.8 and 9.0) (Table 2). Among the growth regulators, GA_3 @50ppm recorded higher fruit set (12.0-12.4) and fruit weight (90.4-109.9g) followed closely by etherel @ 50ppm (11-11.3: fruits/ plant,93.1-94.9g : fruit weight) (Fig 2). All growth regulators increased the fruit length and width as compared to control. Among the growth regulators, etherel @ 50 ppm had maximum fruit length (15.71 and 13.23 cm) and width (4.20 and 5.66 cm) (Table 2). The fruit length was also significantly higher in rainy season (14.73 and 13.50 cm) as

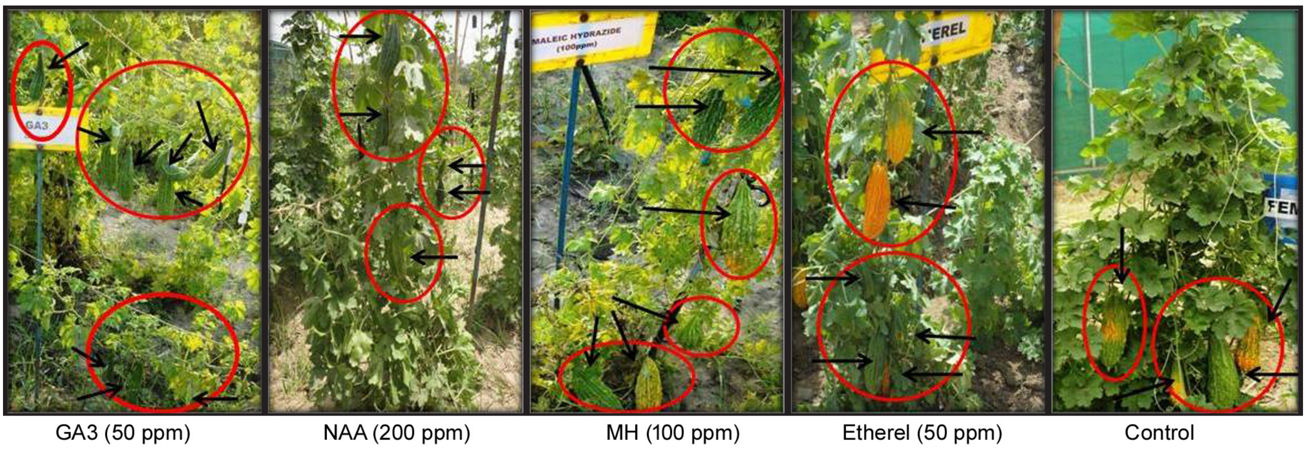

Fig 2 Effect of plant growth regulators on number of fruits/vine in female parental lines of Pusa Hybrid 1 and Pusa Hybrid 2

Table 1 Effect of growth regulators on sex expression of the female parental lines of bitter gourd

Treatment	Node bearing first female flower (Induction of female flower)			Node bearing first male flower			Number of female flowers			Sex ratio		
	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean
Female of PH 1												
GA_3 @ 50 ppm	13.5	15.0	14.2	8.0	10.0	9.0	12.0	15.0	13.5	8.3	10.7	9.5
NAA @ 200 ppm	15.0	17.0	16.0	7.5	8.0	7.8	14.0	16.0	15.0	10.0	9.9	9.95
MH @ 100 ppm	17.0	19.0	18.0	9.0	10.0	9.8	14.0	14.0	14.0	10.4	9.7	10.1
Etherel @ 50 ppm	13.0	14.5	13.7	7.0	9.0	8.0	14.0	14.0	14.0	10.0	9.3	9.7
Control	17.5	19.5	18.5	9.5	11.0	10.8	10.0	10.0	10.0	6.1	8.6	7.4
Mean	15.2	17.0	16.1	8.6	9.7	9.2	12.8	13.8	13.3	8.9	9.6	9.3
Female of PH 2												
GA_3 @ 50 ppm	16.5	17.0	16.7	10.0	12.0	11.0	12.0	14.0	13.0	9.2	10.1	9.7
NAA @ 200 ppm	15.5	16.0	15.7	8.5	7.5	8.0	13.0	14.0	13.5	8.2	8.9	8.6
MH @ 100 ppm	23.5	25.5	24.5	11.0	13.5	12.3	10.0	12.0	11.0	6.6	7.5	7.1
Etherel @ 50 ppm	14.0	15.5	14.7	8.5	7.5	8.0	11.0	13.0	12.0	7.6	8.7	8.2
Control	16.5	20.0	18.2	10.5	11.0	10.8	10.0	11.0	10.5	6.5	7.0	6.8
Mean	17.2	18.8	18.0	9.7	10.3	10.0	10.8	12.8	12.0	7.6	8.4	8.0
CD (P=0.05)												
Genotype (A)		NS			NS			0.9			0.291	
Seasons (B)		1.0			0.07			0.9			0.291	
Growth regulators (C)		2.3			2.0			1.4		0.551		
A × B		NS			NS			NS			0.460	
A × C		NS			NS			2.0			0.651	
B × C		NS			NS			NS			0.651	
A × B × C		NS			NS			NS			0.921	

Table 2 Effect of growth regulators on fruit characters in the female parental lines of bitter gourd

Treatment	Number of fruits/plant			Fruit weight (g)			Fruit length (cm)			Fruit width (cm)		
	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean
Female of PH 1												
GA_3 @ 50 ppm	11.0	13.5	12.4	95.8	124.0	109.9	13.7	15.2	14.5	3.2	3.8	3.5
NAA @ 200 ppm	10.0	10.0	10.0	100.0	110.5	105.3	13.3	17.0	15.1	3.9	4.4	4.2
MH @ 100 ppm	10.0	11.5	10.8	57.6	102.5	95.0	11.3	13.3	12.3	3.8	4.1	3.9
Etherel @50 ppm	9.5	12.5	11.0	85.7	104.0	94.9	15.6	15.8	15.7	4.1	4.3	4.2
Control	7.5	9.5	8.5	67.5	95.0	81.3	10.4	12.4	11.4	3.7	3.9	3.8
Mean	9.8	11.8	10.8	87.3	107.2	97.3	12.9	14.7	13.8	3.7	4.1	3.9
Female of PH 2												
GA_3 @ 50 ppm	10.0	14.0	12.0	86.1	94.8	90.4	12.2	13.6	12.9	5.2	5.8	5.5
NAA @ 200 ppm	10.5	12.5	11.5	80.9	90.6	85.8	10.2	14.0	12.1	5.1	5.6	5.3
MH @ 100 ppm	8.0	12.0	10.0	86.7	86.0	86.3	11.3	12.3	11.8	5.1	5.6	5.3
Etherel @ 50 ppm	10.0	12.5	11.3	91.3	95.0	93.1	11.6	14.9	13.2	5.4	5.9	5.7
Control	6.5	8.5	7.5	72.5	80.3	76.4	10.5	12.8	11.6	4.8	4.8	4.8
Mean	9.0	11.9	10.5	83.5	89.3	86.3	11.1	13.5	12.3	5.1	5.5	5.3
CD (P=0.05)												
Genotype (A)		1.2			3.6			1.0			0.4	
Seasons (B)		0.6			3.6			0.0			0.4	
Growth regulators (C)		1.0			5.8			1.6			0.4	
A × B		NS			5.1			NS			NS	
A × C		NS			8.1			NS			NS	
B × C		NS			NS			NS			NS	
A × B × C		NS			NS			NS			NS	

compared to spring summer season (12.87 and 11.13 cm) in both the parental lines (Table 2). Similar beneficial effect of exogenous application of etherel on fruit traits have been reported by Papadopoulou *et al*. (2005) in squash and Ghani *et al*. (2013) in bitter gourd.

Seed yield

Application of plant growth regulators enhanced the seed yield significantly as compared to control. In the present study, maximum number of seeds/fruit were recorded in rainy season (25.5 and 24.0) compared to spring-summer season (21.8 and 20.2). GA_3 @ 50 ppm and etherel @ 50 ppm significantly increased the total number of seeds/ fruit in both the parents (Table 3). Plants treated with growth regulators showed higher vegetative growth, better photosynthesis and greater accumulation of food reserves in seed resulting in higher seed yield. Growth regulators, GA_3 @ 50 ppm, NAA @ 200 ppm and etherel @ 50 ppm significantly increased the number and weight of filled seeds as compared to control (Table 3). Among seasons, seed yield was higher in rainy season due to better vegetative growth, longer crop duration and days from pollination to maturity resulting in greater accumulation of photosynthetates in the seed. Similar results were reported by Arora and Partap (1988) in bitter gourd.

Seed quality

Seed germination and vigour was significantly higher in seeds harvested from PGR treated plants (GA_3 @ 50 ppm, NAA @ 200 ppm and etherel @ 50 ppm) as compared to control due to better transportation and accumulation of photosynthates to developing fruits resulting in development of filled and bolder seeds (Table 4). Similar results were reported by Gedam *et al*. (1996) and Dostogir *et al*. (2006) in bitter gourd. Seed quality in terms of germination and vigour was better in spring-summer season produce than rainy season (Table 4) due to prevailing hot (>40^{o}C) and dry conditions (<60% RH) (May-June) during fruit development, seed maturity and seed extraction as compared to rainy season (30-35^{o}C, >70% RH) (Oct- Nov).

Hybrid seed yield

Among the plants sprayed with GA_3 @ 50 ppm, NAA @ 200 ppm, maleic hydrazide @ 100 ppm, etherel @ 50 ppm at three leaf and tendril initiation stage,10 plants per treatment were hand pollinated to validate effect of PGRs on hybrid seed production. The results showed that GA_3@50ppm sprayed plants showed higher fruit setting (>98%) as compared with 92.0% in control. The fruit and seed traits were also highest in GA_3 treated plants (Table 5) which could be due to better enzyme induction and endogenous synthesis of growth regulators (Akter and Rahman 2010, Hilli *et al*. 2010, Sure *et al*. 2012).

The results of the study showed that, exogenous application of GA_3 @ 50ppm sprayed at three leaf + tendril initiation stage significantly improved vegetative growth,

Table 3 Effect of growth regulators on seed traits in the parental lines of bitter gourd

Treatment	Total number of seeds/fruit			Total number of seeds/plant			Number of filled seeds			Weight of filled seeds		
	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean
Female of PH 1												
GA_3 @ 50 ppm	24.5	29.5	27.0	269.5	398.3	333.9	24.5	29.5	27.0	2.8	4.7	3.8
NAA @ 200 ppm	20.0	28.0	24.0	200	280	240	20.0	28.0	25.3	2.2	4.7	3.5
MH @ 100 ppm	18.0	25.5	21.8	180	293.3	236.7	17.0	20.0	18.5	2.5	3.4	2.9
Etherel @50 ppm	24.0	28.5	26.3	228	356.3	292.2	29.0	29.0	29.0	3.3	3.0	3.2
Control	19.0	23.0	21.0	142.5	218.5	180.5	18.5	21.0	19.8	2.5	3.3	2.9
Mean	21.1	26.9	24.0	204	309.3	256.7	21.8	25.5	23.9	2.7	3.8	3.3
Female of PH 2												
GA_3 @ 50 ppm	23.0	28.0	25.5	230	392	311	22.5	27.5	25.0	2.6	3.6	3.2
NAA @ 200 ppm	22.0	26.0	24.0	231	325	278	22.0	27.0	24.5	2.6	3.4	3.0
MH @ 100 ppm	19.5	24.5	22.0	156	294	225	19.0	22.0	20.5	2.6	3.8	3.0
Etherel @ 50 ppm	21.5	26.5	24.0	215	331.3	273.2	21.5	25.5	23.5	2.9	2.8	2.9
Control	18.5	23.5	21.0	120.3	199.8	160.1	16.0	18.0	17.0	2.4	2.8	2.7
Mean	20.3	25.7	23.3	190.5	308.4	249.5	20.2	24.0	22.1	2.7	3.3	2.9
CD (P=0.05)												
Genotype (A)		0.7			2.894			NS			NS	
Seasons (B)		2.89			2.894			1.03			0.67	
Growth regulators (C)		1.98			4.092			4.81			0.21	
A × B		NS			4.575			NS			NS	
A × C		NS			6.470			NS			NS	
B × C		NS			6.470			NS			NS	
A × B × C		NS			9.151			NS			NS	

Table 4 Effect of growth regulators on seed quality traits in the parental lines of bitter gourd

Treatment	Germination (%)			Seedling vigour index I [Germination (%) Seedling length (cm)]			Seedling vigour index II [Germination (%) Seedling dry weight (g)]		
	SS	Rainy	Mean	SS	Rainy	Mean	SS	Rainy	Mean
Female of PH 1									
GA_3 @ 50 ppm	93.9 (75.7)	81.0 (64.2)	87.4 (69.9)	2993.4	2352.0	2672.7	19.9	14.9	17.4
NAA @ 200 ppm	88.3 (69.9)	75.9 (60.6)	82.1 (65.3)	2718.2	2092.0	2405.1	17.9	12.9	15.4
MH @ 100 ppm	89.5 (73.4)	69.3 (56.3)	79.4 (64.9)	2203.7	1559.7	1881.7	16.9	12.1	14.5
Etherel @50 ppm	98.0 (81.9)	80.0 (56.8)	64.0 (69.4)	3026.2	1960.8	2493.5	24.0	13.6	18.8
Control	85.5 (68.0)	68.9 (56.2)	77.3 (62.1)	2449.7	1454.3	1952.0	17.6	11.9	14.7
Mean	91.1 (73.8)	73.0 (58.8)	82.0 (66.3)	2678.2	1883.7	2280.9	19.3	13.1	16.2
Female of PH 2									
GA_3 @ 50 ppm	93.4 (75.7)	71.4 (57.7)	82.4 (66.7)	2995.9	1703.9	2349.9	22.3	13.4	17.8
NAA @ 200 ppm	92.5 (74.3)	61.0 (51.4)	76.7 (62.8)	2686.1	1352.4	2019.2	21.7	11.9	16.8
MH @ 100 ppm	91.9 (73.7)	62.7 (52.4)	77.3 (63.1)	2516.3	1392.5	1954.4	19.2	10.9	15.0
Etherel @ 50 ppm	87.2 (69.3)	62.0 (51.9)	74.6 (60.6)	2968.6	1477.2	2222.9	21.2	12.6	16.8
Control	87.9 (70.1)	65.2 (53.8)	76.5 (61.9)	2199.8	1324.6	1762.2	18.7	10.9	14.8
Mean	90.6 (72.6)	64.4 (53.4)	77.5 (63.0)	2673.3	1450.1	2061.7	20.6	11.9	16.3
CD (P=0.05)									
Genotype (A)		(3.40)			NS			1.20	
Seasons (B)		(3.37)			122.95			1.15	
Growth regulators(C)		(3.05)			194.40			1.83	
A × B		NS			173.87			1.63	
A × C		NS			NS			NS	
B × C		NS			NS			NS	
A × B × C		NS			NS			NS	

Values in parenthesis are arc sine converted values

Table 5 Effect of growth regulators on fruit traits in the female parent of PH 1

Treatment	Fruit setting (%)	Fruit weight (g)	Length (cm)	Width (cm)	Hybrid seed/ fruit	Seed weight/ fruit (g)
GA3 @50 ppm	98.25	141.2	18.3	4.75	28	4.38
NAA @200 ppm	96.75	140.7	17.7	4.57	25	4.00
MH @100 ppm	91.25	139.2	15.8	4.60	23	4.22
Etherel @50 ppm	98.50	145.4	18.1	4.85	26	4.28
Control	92.00	134.9	14.9	4.45	23	3.95
CD (P=0.05)	2.8	3.26	1.08	0.24	0.9	0.39

sex ratio, fruit setting, seed yield and quality of bitter gourd. This could be recommended for hybrid seed production of bitter gourd under north Indian conditions.

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