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Hormonal Changes in Response to Different Chemicals Induced Flowering and Improved Fruit Yield in Pomegranate cv. Bhagwa

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Abstract: At the ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru, a field study was carried out to elucidate the impact of different propagules, chemicals, and their interactions on induction of flowering, improving fruit yield, and endogenous phytohormones (Gibberellins and Indole 3 Acetic Acid) in different propagules (tissue culture plants, grafted plants, and air layer plants) of pomegranate (*Punica granatum*). The effects of three chemicals, methyl jasmonate (100 ppm, 150 ppm, and 200 ppm plant^{-1}), nitrobenzene (1.0, 1.5, and 2.0 ml plant^{-1}), and paclobutrazol (0.375 g a.i. m^{-1} canopy diameter), applied 30, 45, and 60 days after withholding irrigation (bahar treatment) on all three propagules, were studied. During *ambe* and *hastha bahar*, tissue culture plants (P_1) produced the most hermaphrodite flowers (261.6, 233.2) and fruit yield (32.88 kg plant^{-1}, 35.57 kg plant^{-1}) among the propagules. In the instance of *ambe bahar*, soil drenching of paclobutrazol 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) resulted in the most hermaphrodite flowers (267.3) and fruit yield (30.54 kg plant^{-1}). In the instance of *hastha bahar*, foliar spraying with nitrobenzene 2.0 ml L^{-1} plant^{-1} (T6) resulted in a high number of hermaphrodite flowers (253.8) and fruit yield (48.19 kg plant^{-1}). In the case of *ambe bahar*, tissue culture plants (P_1) produced considerably more hermaphrodite flowers (335.3) and fruit production (30.54 kg plant^{-1}) than other interactions. Application of nitrobenzene 2.0 ml L^{-1} plant^{-1} (T6) to tissue culture plants (P_1) during *hastha bahar* resulted in the formation of 275.2 hermaphrodite flowers and the highest fruit yield (51.44 kg plant^{-1}). The leaves of tissue culture plants (P_1) showed decreased endogenous gibberellin levels and greater Indole 3 Acetic Acid levels during the blooming and fruit set stages of *ambe bahar* and *hastha bahar*. Paclobutrazol 0.375 g a.i. m^{-1} canopy diameter soil drenching 60 days after bahar treatment (T_9) resulted with reduced endogenous gibberellin levels and greater Indole 3 Acetic Acid levels among the chemicals.

Key words: Bahar treatment, Bhagwa, nitrobenzene, methyl jasmonate, paclobutrazol, air layer plants, tissue culture plants, grafted plants, tissue culture plants, pomegranate.

Pomegranate (*Punica granatum* L.) is a resilient fruit crop that may be produced in tropical and subtropical climates (Lal and Ahmed, 2012). The plant originated in Iran circa 2000 BC and was originally grown there **(**Supe and Saitwal, 2016**)**. With 2.86 million tons of fruit produced on 0.24 million hectares of land, India is the world's top pomegranate producer (National Horticulture Board, 2018). The cv. 'Bhagwa' is the most popular pomegranate cultivar among the several available due to its gorgeous red skin, rich crimson arils, soft seeds (mellowness), and export demand (Dhinesh *et al*., 2017).

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Physiological processes and endogenous hormone levels in horticulture crops are claimed to be influenced by plant growth regulators or chemicals, resulting in quantitative and qualitative benefits **(**Phawa *et al*., 2017**)**. Plant growth regulators or compounds like methyl jasmonate (MeJA), nitrobenzene (NB), and paclobutrazol (PBZ) influence flowering and yield in a variety of fruit crops at different doses **(**Bhujbal *et al*., 2013), and are particularly effective in pomegranate farming **(**Chaudhari and Desai, 1993**)**.

Pomegranates are grown in a variety of techniques, including air layering, hard wood cuttings, grafting, and tissue culture. Tissue culture is the most popular method

for establishing commercial orchards. The flowering behavior of trees developed through tissue culture or grafting, on the other hand, has not been widely studied. Furthermore, despite their commercial importance, production approaches for improving blooming in large-scale commercial pomegranate cultivation have gotten little attention.

In view of this, we looked at the effects of three plant growth regulators, methyl jasmonate, nitrobenzene, and Paclobutrazol, at various concentrations, on flowering and yield, as well as their effect on endogenous phytohormones, in pomegranate propagules like tissue culture plants (P1), grafts (P2), and air layers plants (P3). We chose 'Bhagwa,' a common commercial pomegranate cultivar, for the study.

Materials and Methods

The current study was undertaken at the ICAR-Indian Institute of Horticultural Research (IIHR) farm in Hesaraghatta, near Bengaluru (130 7'N, 770 29'E) during the *ambe bahar* (January-February) and *hastha bahar* (September-October) seasons of 2016 and 2017. During *ambe bahar*, the average maximum and minimum temperatures were 33.08°C and 20.43°C, respectively, with relative humidity and rainfall of 75.04% and 74.95 mm, respectively, and during *hastha bahar*, the average maximum and minimum temperatures were 26.13°C and 18.94°C, respectively, with relative humidity and rainfall of 59.06% and 12.10 mm, respectively. Irrigation for the ambe bahar and hastha bahar crops was ceased two months before bahar treatment. The soil in the plant basins was slightly scraped out to reveal the roots. The plants were lightly pruned to assist defoliation after being sprayed with ethrel (2 ml L^{-1}) mixed with 5 g of Diammonium Phosphate L^{-1}, which stressed the plants. Before and after pruning, the plants were sprayed with 1% Bordeaux mixture to prevent Bacterial blight. Sprouting occurs ten days after defoliation on the shoots. When the coppery green leaves began to turn dark green, the chemicals Methyl Jasmonate and Nitrobenzene were sprayed at pre-determined doses on the plants, then paclobutrazol (1.5 ml L^{-1}) was drenched in the soil. The field study used a Factorial Randomized Block Design with ten treatments replicated three times, with propagules as the first factor and chemicals as the second. The following treatments were applied to tissue cultured plants, grafts, and air layers:

T_1: Methyl Jasmonate 100 ppm $plant^{-1}$

T_2: Methyl Jasmonate 150 ppm $plant^{-1}$

T_3: Methyl Jasmonate 200 ppm $plant^{-1}$

T_4: Nitrobenzene 1.0 ml $plant^{-1}$

T_5: Nitrobenzene 1.5 ml $plant^{-1}$

T_6: Nitrobenzene 2.0 ml $plant^{-1}$

T_7: Paclobutrazol 0.375 g a.i. m^{-1} canopy diameter 30 days after bahar treatment

T_8: Paclobutrazol 0.375g a.i. m^{-1} canopy diameter 45 days after bahar treatment

T_9: Paclobutrazol 0.375g a.i. m^{-1} canopy diameter 60 days after bahar treatment

T_{10}: Control

The T_{10} control group's plants were also given the same treatment. Number of hermaphrodite flowers, percent of fruit set, fruit production (kg $plant^{-1}$), gibberellic acid (ng g^{-1}), and indole acetic acid content (ng g^{-1}) were all recorded, and the data was evaluated statistically using Gomez and Gomez's methodology (1984).

Results and Discussion

Number of hermaphrodite flowers

Tissue culture plants had the maximum number of hermaphrodite flowers (261.6) during *ambe bahar* (P_1). Among the chemicals, paclobutrazol 0.375 g a.i. m^{-1} canopy diameter soil drenching 60 days after bahar treatment (T_9) produced the most hermaphrodite flowers (267.3). For hermaphrodite flower formation, the interaction effect of the chemicals throughout the propagules revealed substantial variances. When soil was saturated with paclobutrazol 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9), tissue culture plants (P_1) produced considerably more hermaphrodite flowers (335.3). (Table 1). Tissue culture plants (P_1) produced the most hermaphrodite flowers (233.2) during *hastha bahar*, which differed considerably from the rest of the propagules. The use of nitrobenzene as a foliar spray 2.0 ml L^{-1} $plant^{-1}$ (T_6) was linked to a high number of hermaphrodite flowers $plant^{-1}$ (253.8), which was considerably different from the other chemicals tested. In terms of the effect of

Table 1. Flowering pattern and fruit set in pomegranate as influenced by planting material and plant growth regulators

Propagule	Number of hermaphrodite flowers per plant							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	226.66	154.66	158.66	180.00	204.58	185.14	207.07	198.93
T_2	230.00	176.00	242.00	216.00	206.84	200.75	209.73	205.77
T_3	231.33	182.66	164.66	192.88	198.3	192.91	215.86	202.35
T_4	248.66	212.66	188.66	216.66	236.1	226.25	224.47	228.94
T_5	268.66	223.33	196.66	229.55	253.93	238.36	232.33	241.54
T_6	274.00	240.00	199.33	237.77	275.22	246	240.09	253.77
T_7	282.00	166.66	202.00	216.88	245.05	214.64	228.63	229.44
T_8	296.00	196.66	205.33	232.66	258.95	224.46	221.99	235.13
T_9	335.33	208.66	258.00	267.33	269.82	233.33	227.8	243.65
T_{10}	223.33	145.33	144.00	170.88	182.77	165.18	193.22	180.39
Mean	261.60	190.66	195.93		233.16	212.7	220.12	
	P	T	P×T		P	T	P×T	
SE(m)	2.83	5.17	8.96		1.22	2.24	3.88	
CD (5%)	8.04	14.69	25.45		3.48	6.36	11.02	

Propagule	Fruit set (%)							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	58.29	48.44	51.01	52.58	46.63	40.71	51.39	48.19
T_2	59.06	53.42	65.76	59.41	49.62	46.64	46.72	48.20
T_3	60.32	57.59	53.70	57.20	45.80	42.12	39.64	42.52
T_4	63.58	60.1	53.17	58.95	43.07	48.27	52.95	47.55
T_5	64.21	62.91	54.41	60.51	47.94	49.53	51.31	48.49
T_6	66.33	64.42	56.84	62.53	49.76	52.85	53.30	50.86
T_7	67.00	52.08	62.40	60.49	42.87	46.23	46.66	44.31
T_8	68.01	59.54	63.05	63.53	44.98	46.57	52.04	45.91
T_9	70.36	59.68	66.98	65.67	46.88	43.41	48.08	44.14
T_{10}	57.01	46.79	48.50	50.76	46.47	37.47	43.89	47.74
Mean	63.41	56.5	57.58		46.40	45.38	48.60	
	P	T	P×T		P	T	P×T	
SE(m)	0.72	1.32	2.30		0.54	1.00	1.73	
CD (5%)	2.06	3.77	6.53		1.55	2.84	4.92	

P_1: tissue-cultured; P_2: grafted plants; P_3: plants raised from air layers.
T_1: Methyl jasmonate (MeJA) 100 ppm; T_2: MeJA 150 ppm; T_3: MeJA 200 ppm; T_4: Nitrobenzene (NB) 1.0 ml L^{-1}; T_5: NB 1.5 ml L^{-1}; T_6: NB 2.0 ml L^{-1}; T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy length applied 30 days after the 'bahar' treatment; T_8: Same as T_7 except applied 45 days after the treatment; T_9: same as T_7 except applied 60 days after the treatment; T_{10}: Control; sprayed with water after the 'bahar' treatment.

chemicals across the propagules, tissue culture plants (P_1) treated with nitrobenzene 2.0 ml L^{-1} plant^{-1} (T_6) produced more hermaphrodite flowers (275.2), which was comparable to soil drenching with paclobutrazol 0.375g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) (269.8).

Percentage of fruit set

Tissue culture plants (P_1) had the highest fruit set percentage (63.41%) during *ambe bahar*, which differed considerably from other propagules such as grafted (P_2) and air layer plants (P_3). Among the compounds, paclobutrazol soil drenching @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) has been linked to improved fruit set (65.67%). When it came to the interaction of chemicals throughout the propagules and the percentage of fruit set, there were significant differences. When soil drenched with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9), high fruit set percentage (70.36%) was reported in tissue culture plants (P_1).

Table 2. Yield and its attributes in pomegranate as influenced by different types of planting material and chemicals

Propagule	Number of fruits per plant							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	145.33	92.00	120.00	119.11	95.15	86.17	106.32	95.88
T_2	146.66	93.66	128.33	122.88	102.37	96.69	97.82	98.96
T_3	154.66	97.00	137.00	129.55	90.62	81.20	85.58	85.80
T_4	169.00	108.00	139.00	138.66	101.57	105.35	118.75	108.56
T_5	170.66	125.66	140.66	145.66	121.73	109.98	119.35	117.02
T_6	181.00	143.33	153.00	159.11	136.81	121.14	127.88	128.61
T_7	195.66	89.33	163.66	149.55	105.05	92.77	106.71	101.51
T_8	198.33	95.00	168.66	154.00	116.41	91.31	115.49	107.73
T_9	199.66	97.66	172.66	156.66	126.32	87.44	109.66	107.81
T_{10}	130.33	88.66	114.00	111.00	85.02	86.98	84.82	85.60
Mean	169.13	103.03	143.70		108.10	95.90	107.24	
	P	T	P×T		P	T	P×T	
SE(m)	1.80	3.30	5.71		1.11	2.02	3.51	
CD (5%)	5.13	9.36	16.22		3.15	5.75	9.96	

Propagule	Fruit yield per plant (kg)							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	25.95	16.54	17.92	20.13	28.27	26.92	30.98	28.72
T_2	27.18	17.01	29.45	24.54	31.01	32.52	28.72	30.75
T_3	29.13	17.75	21.65	22.84	27.60	26.33	25.49	26.47
T_4	33.16	21.30	22.80	25.75	36.05	40.76	40.70	39.17
T_5	33.83	24.73	23.48	27.34	43.95	43.79	41.56	43.1
T_6	35.95	28.61	26.05	30.20	49.42	51.44	43.71	48.19
T_7	39.20	16.81	28.07	28.03	36.36	34.93	35.84	35.71
T_8	40.46	18.17	29.30	29.31	38.98	34.11	37.26	36.78
T_9	41.20	18.87	31.55	30.54	40.49	35.70	34.27	36.82
T_{10}	22.78	14.62	16.69	18.03	23.58	25.63	22.91	24.04
Mean	32.88	19.44	24.70		35.57	35.21	34.14	
	P	T	P×T		P	T	P×T	
SE(m)	0.68	1.25	2.17		0.42	0.78	1.35	
CD (5%)	1.94	3.55	6.16		N.S	2.22	3.84	

P_1: tissue-cultured; P_2: grafted plants; P_3: plants raised from air layers.
T_1: Methyl jasmonate (MeJA) 100 ppm; T_2: MeJA 150 ppm; T_3: MeJA 200 ppm; T_4: Nitrobenzene (NB) 1.0 ml L^{-1}; T_5: NB 1.5 ml L^{-1}; T_6: NB 2.0 ml L^{-1}; T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy length applied 30 days after the 'bahar' treatment; T_8: Same as T_7 except applied 45 days after the treatment; T_9: same as T_7 except applied 60 days after the treatment; T_{10}: Control; sprayed with water after the 'bahar' treatment.
N.S. – Non significant.

Air layer plants (P_3) had the highest fruit set percentage (48.60%) in *hastha bahar*, which differed considerably from other propagules such as grafted (P_2) and tissue culture plants (P_1). Among the compounds, foliar spraying with 2.0 ml L^{-1} $plant^{-1}$ nitrobenzene (T_6) resulted in the highest fruit set percentage (50.86%). When it came to the interaction of chemicals throughout the propagules and the percentage of fruit set, there were significant differences. Foliar treatment of nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ (T_6) has been linked to an increase in the percentage of fruit set in air layer plants (P_3) (53.30%). Due to soil drenching with paclobutrazol, tissue culture plants (P_1) had the highest fruit set % during *ambe bahar*, whereas foliar spraying with nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ increased fruit set percentage in both air layer (P_3) and tissue culture plants during hastha bahar (P_1). It can be recommended for tissue culture or air layer plant seeding based on the above results.

Table 3. Effect of different plant growth regulators on levels (ng g^{-1} fresh leaves) of endogenous gibberellins in pomegranate raised from three types of planting material

Propagule	Flowering stage							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	245.00	394.00	441.00	360.00	272.00	193.33	385.00	283.44
T_2	237.43	306.00	167.50	298.66	237.00	250.66	422.00	303.22
T_3	235.00	229.00	385.00	297.77	243.33	154.33	487.00	294.88
T_4	204.00	197.50	422.00	298.66	197.33	277.00	257.00	243.77
T_5	193.33	194.95	385.00	282.38	190.00	258.66	246.33	231.66
T_6	188.33	145.50	335.50	187.77	174.33	240.33	206.66	207.11
T_7	175.66	396.00	306.00	280.83	175.66	269.66	275.50	240.27
T_8	171.33	223.50	275.50	280.83	166.00	226.66	267.00	219.88
T_9	158.33	208.50	126.50	160.77	183.33	204.00	126.50	171.27
T_{10}	263.00	495.00	487.00	365.27	245.33	295.33	335.50	292.05
Mean	207.13	278.95	335.85		208.43	237.00	300.85	
	P	T	P×T		P	T	P×T	
SE(m)	1.93	3.53	6.11		2.68	4.90	8.49	
C.D (5%)	5.49	10.02	17.36		7.62	13.91	24.1	

Propagule	Fruit set stage							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	166.50	491.00	281.50	303.00	320.00	297.00	224.66	280.55
T_2	165.00	474.00	279.00	293.66	391.33	323.33	267.33	327.33
T_3	155.50	422.00	251.50	302.00	375.66	299.33	265.00	313.33
T_4	143.50	306.00	209.50	283.00	224.66	273.00	252.33	250.00
T_5	142.50	256.50	205.50	250.66	306.00	258.00	233.66	265.88
T_6	125.50	224.50	202.00	220.00	269.66	222.33	226.33	239.44
T_7	123.00	512.50	292.00	246.83	274.66	265.66	250.33	263.55
T_8	101.00	415.50	216.00	227.50	286.66	279.66	282.66	283.00
T_9	95.50	376.00	213.50	194.00	280.33	267.33	278.00	275.22
T_{10}	201.50	554.00	331.50	323.00	436.00	319.33	314.00	356.44
Mean	141.95	403.20	248.20		316.50	280.50	259.43	
	P	T	P×T		P	T	P×T	
SE(m)	2.77	5.07	8.78		3.12	5.70	9.87	1.93
C.D (5%)	7.88	14.40	24.94		8.86	16.18	28.03	5.49

P_1: tissue-cultured; P_2: grafted plants; P_3: plants raised from air layers.
T_1: Methyl jasmonate (MeJA) 100 ppm; T_2: MeJA 150 ppm; T_3: MeJA 200 ppm; T_4: Nitrobenzene (NB) 1.0 ml L^{-1}; T_5: NB 1.5 ml L^{-1}; T_6: NB 2.0 ml L^{-1}; T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy length applied 30 days after the 'bahar' treatment; T_8: Same as T_7 except applied 45 days after the treatment; T_9: same as T_7 except applied 60 days after the treatment; T_{10}: Control; sprayed with water after the 'bahar' treatment.

Number of fruits per plant

The tissue culture plants (P_1) produced significantly more fruits than the ambe bahar plants (169.13). Nitrobenzene, when applied as a foliar spray at 2.0 ml L^{-1} plant^{-1} (T6), produced the most fruits of all the chemicals (159.11). Chemical effects varied widely throughout the propagules for fruit number plant^{-1}. Tissue culture plants (P_1) had the most fruits plant^{-1} due to soil drenching with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T9) (199.66) (Table 2).

During *hastha bahar*, tissue culture plants (P_1) produced much more fruits plant^{-1} (108.10) than other propagules. Nitrobenzene, when applied as a foliar spray at 2.0 ml L^{-1} plant^{-1} (T_6),

Table 4. Effect of different plant growth regulators on levels (ng g^{-1} fresh leaves) of endogenous indole acetic acid in pomegranate raised from three types of planting material

Propagule	Flowering stage							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	441.66	194.50	237.00	327.72	441.66	302.33	334.66	359.55
T_2	845.66	208.50	643.00	445.83	501.00	310.00	475.66	428.88
T_3	855.66	223.50	264.00	418.05	774.33	245.33	552.33	524.00
T_4	872.00	394.00	333.00	523.33	753.00	306.00	587.33	548.77
T_5	877.00	396.00	392.50	525.22	846.33	377.33	628.00	617.22
T_6	915.66	495.00	464.50	630.88	873.66	394.00	644.66	637.44
T_7	919.66	197.50	552.50	539.72	658.00	343.66	502.33	501.33
T_8	925.66	229.00	573.50	599.72	643.66	329.33	458.00	477.00
T_9	928.00	306.00	761.50	728.16	845.66	371.00	264.00	493.55
T_{10}	107.00	145.50	211.00	231.33	287.66	257.33	237.00	260.66
Mean	768.80	278.95	443.25		662.50	323.63	468.40	
	P	T	P×T		P	T	P×T	
SE(m)	8.51	15.55	26.93		6.53	11.93	20.66	
C.D (5%)	24.17	44.14	76.45		18.55	33.89	58.65	

Propagule	Fruit set stage							
	Ambe bahar				*Hastha bahar*			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
T_1	248.50	165.50	253.00	224.94	283.33	236.66	257.66	259.22
T_2	263.50	179.00	396.33	261.33	271.66	248.66	264.66	261.66
T_3	264.50	186.00	278.00	257.72	285.33	276.66	295.00	285.66
T_4	284.50	224.00	290.33	265.50	349.66	246.00	267.00	287.55
T_5	367.00	224.50	309.33	267.83	374.33	286.66	300.66	320.55
T_6	375.00	242.50	322.66	319.77	509.00	348.00	356.00	404.33
T_7	375.50	176.00	326.00	279.16	286.66	319.33	275.33	293.77
T_8	385.00	188.00	343.33	303.11	268.66	286.33	266.00	273.66
T_9	556.00	205.00	556.00	445.33	317.66	265.33	262.66	281.88
T_{10}	205.50	144.50	202.33	220.94	246.33	225.66	235.33	235.77
Mean	332.50	193.50	327.70		319.26	273.93	278.03	
	P	T	P×T		P	T	P×T	
SE(m)	5.94	10.85	18.80		3.73	6.82	11.82	
C.D (5%)	16.87	30.80	53.35		10.61	19.37	33.56	

P_1: tissue-cultured; P_2: grafted plants; P_3: plants raised from air layers.
T_1: Methyl jasmonate (MeJA) 100 ppm; T_2: MeJA 150 ppm; T_3: MeJA 200 ppm; T_4: Nitrobenzene (NB) 1.0 ml L^{-1}; T_5: NB 1.5 ml L^{-1}; T_6: NB 2.0 ml L^{-1}; T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy length applied 30 days after the 'bahar' treatment; T_8: Same as T_7 except applied 45 days after the treatment; T_9: same as T_7 except applied 60 days after the treatment; T_{10}: Control; sprayed with water after the 'bahar' treatment.

produced the most fruits of all the chemicals (128.61). Chemical influence on the quantity of fruits produced by plant^{-1} varied substantially across the propagules. The application of nitrobenzene as a foliar spray (T_6) to tissue culture plants (P_1) resulted in the highest fruit number plant^{-1} (136.81), followed by the same chemical to air layer plants (P_3) (127.88).

Fruit yield

In the instance of *ambe bahar*, there were significant differences in fruit yield plant^{-1} (kg) across the propagules, with tissue culture plants yielding the highest fruit yield plant^{-1} (32.88 kg plant^{-1}) (P1). Paclobutrazol 0.375 g a.i. m^{-1} canopy diameter soil drenching 60 days after treatment (T_9) produced the highest fruit

output of the compounds (30.54 kg plant^{-1}). Tissue culture plants (P_1) achieved 41.20 kg plant^{-1} fruit yield 60 days after bahar treatment due to soil drenching with paclobutrazol 0.375 g a.i. m^{-1} canopy diameter (T9) (Table 2).

During *hastha bahar*, there were no significant variations in fruit output plant^{-1} (kg) between the propagules. Regardless, tissue culture plants (P_1) yielded the greatest fruit (35.57 kg plant^{-1}). When compared to the other chemicals used, nitrobenzene 2.0 ml L^{-1} plant^{-1} (T_6) was connected to a significant increase in fruit output (48.19 kg plant^{-1}). There were also significant differences in the impact of chemicals throughout the propagules, which was discovered. When nitrobenzene 2.0 ml L^{-1} plant^{-1} was administered as a foliar spray (T_6) to grafted plants (P_2), the highest yield was attained (51.44 kg plant^{-1}).

Endogenous phytohormones

Gibberellic acid

Endogenous GA_3 levels varied significantly among the propagules during the flowering stage of *ambe bahar*. The tissue culture plants (P_1) were found to have a considerably lower GA_3 concentration (207.13 ng g^{-1} FW) than the rest of the propagules. The soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) had lower GA_3 concentration (160.77 ng g^{-1} FW), which was significantly different from the other chemicals used. The GA_3 content of air layer plants (P_3) was reduced by soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after treatment (T_9) (126.50 ng g^{-1} FW) (Table 3).

In the instance of *ambe bahar*, significant variations in GA_3 levels were observed at the fruit set stage throughout the propagules. The tissue culture plants (P_1) had significantly lower GA_3 content in their leaves (141.95 ng g^{-1} FW) than the remainder of the propagules. Among the chemicals, soil drenching with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) resulted in significantly lower GA_3 content (194.00 ng g^{-1} FW) than the other chemicals. 60 days after bahar treatment, soil drenching with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter (T_9) was linked to lower GA_3 concentration (95.50 ng g^{-1} FW) in tissue culture plants (P_1) (Table 3).

During *hastha bahar*, significant variations were apparent across the propagules for endogenous GA_3 content. The tissue culture plants (P_1) implicated in recording lesser GA_3 content (208.43 ng g^{-1} FW) which was significantly differed from the rest of the propagules. Across the chemicals, soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T9) registered less GA_3 content (171.27 ng g^{-1} FW) which differed significantly from the rest of the chemicals applied. Soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after treatment (T9) to air layer plants (P3) lowered the GA_3 content (126.50 ng g^{-1} FW).

During fruit set stage of *hastha bahar*, significant differences were evident across the propagules in GA_3 content at fruit set stage. The grafted plants (P_2) registered lesser GA_3 content in the leaves (280.50 ng g^{-1} FW), and it differed significantly from the rest of the propagules. Among the chemicals, foliar spray of nitrobenzene @ 2.0 ml L^{-1} plant^{-1} (T_6) implicated in lowering GA_3 content in the leaves (239.44 ng g^{-1} FW) which differed significantly from the other applied chemicals. Application of nitrobenzene @ 2.0 ml L^{-1} plant^{-1} (T_6) as foliar spray to grafted plants resulted in registering less GA_3 content (222.33 ng g^{-1} FW).

Indole acetic acid

During flowering stage of *ambe bahar*, all the propagules (Tissue culture, Grafted and Air layer plants) were significantly influenced with respect to IAA content during flowering stage. The tissue culture plants (P_1) implicated in recording high IAA content (768.80 ng g^{-1} FW) which differed significantly from the remaining propagules. Among the chemicals, soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) enhanced IAA content (728.17 ng g^{-1} FW) which varied significantly from the rest of the chemicals applied. Tissue culture plants (P_1) registered maximum IAA content (928.00 ng g^{-1} FW) following soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) (Table 4).

At fruit set stage of *ambe bahar*, all the propagules were influenced significantly for IAA content during fruit set stage and the tissue culture plants (P_1) registered high IAA content (332.50 ng g^{-1} FW) which varied significantly

from the rest of the propagules. Among the chemicals, soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) implicated in enhancing IAA content (445.33 ng g^{-1} FW) which differed significantly from the other chemicals applied. The interaction of chemicals with propagules altered significantly for IAA content during fruit stage. The tissue culture plants (P_1) registered highest IAA content (556.00 ng g^{-1} FW) due to soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment (T_9) (Table 4).

During flowering stage in *hastha bahar*, all the propagules (Tissue culture, Grafted and Air layer plants) were significantly influenced with respect to IAA content during flowering stage. The tissue culture plants (P1) implicated in recording high IAA content (662.50 ng g^{-1} FW) which differed significantly from the remaining propagules. Among the chemicals, application of nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ (T_6) enhanced IAA content (637.44 ng g^{-1} FW) which varied significantly from the rest of the chemicals applied. Tissue culture plants (P_1) registered maximum IAA content (837.66 ng g^{-1} FW) following application of nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ (T_6).

During fruit set stage of *hastha bahar*, all the propagules were influenced significantly for IAA content during fruit set stage and the tissue culture plants (P_1) registered high IAA content (319.26 ng g^{-1} FW) which varied significantly from the rest of the propagules. Among the chemicals, application of nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ as foliar spray (T_6) implicated in enhancing IAA content (404.33 ng g^{-1} FW) which differed significantly from the other chemicals applied. The interaction of chemicals with propagules altered significantly for IAA content during fruit stage. The tissue culture plants (P_1) registered highest IAA content (509.00 ng g^{-1} FW) due to application of nitrobenzene @ 2.0 ml L^{-1} $plant^{-1}$ as foliar spray (T_6).

Flower induction and yield

Because of its vast spectrum of medical characteristics and nutritional worth, as well as its high antioxidant potential, pomegranate fruit is in high demand (Ali *et al*., 2014). In India's tropical environment, this crop is evergreen and blooms throughout the year. The production is reduced and the fruit quality is poor due to the depletion of plant reserves induced by non-synchronized maturity (Ram *et al*., 2010). Given the high commercial demand for pomegranates, it is vital to improve fruit quality in order to maximise market returns, and continual efforts are being made in this area. Crop synchronisation, sometimes referred to as blossom control, is a great strategy to ensure a plentiful harvest (Madhuri 2017). Plant growth regulators/chemicals serve a key function in controlling flowering and fruiting in many perennial fruit crops. These plant growth regulators boost output by enhancing the internal physiology of developing fruits, which enhances fruit set and corrects different physiological abnormalities, resulting in higher quality and yield (Chaudhari and Desai, 1993; Aseri *et al*., 2008 and Bhujbal *et al*., 2013). This internal physiology also includes the carefully regulated activity of endogenous hormones. We looked at how varied doses of paclobutrazol (PBZ) affected flowering pattern, fruit set, yield, and endogenous phytohormones in different propagules (tissue culture plants, grafted plants, and air layer plants).

Among the several propagules studied, tissue culture plants had the most hermaphrodite flowers, and when tested in combination, tissue culture plants with soil saturated with paclobutrazol had a significantly larger number of hermaphrodite flowers. Other research have demonstrated that paclobutrazol boosts flower output, and our findings are consistent with that (Upreti *et al*., 2013; Protacio, 2000). An increase in the levels of the florigenic promoter (FP) and a decrease in the levels of the vegetative promoter (VP), particularly gibberellins, causes flower induction (Davenport, 2007). When the biosynthesis of gibberellins is suppressed by paclobutrazol, cell division occurs, but newly produced cells do not elongate, inhibiting vegetative growth (Dalziel and Lawrence, 1984). This suggests that the increased number of hermaphrodite flowers in the group may be owing to decreased VP levels, resulting in a higher FP: VP ratio, which promotes flowering (Adil *et al*., 2011; Iglesias *et al*., 2007; Voon *et al*., 1991; Yeshitela *et al*., 2004). The capacity of PBZ to boost blooming by increasing floral stimulus synthesis in an inductive loop could be another reason for increased flower quantity. The floral promoter effects of paclobutrazol could potentially be attributable to its effects

on hormones other than gibberellins (Murti and Upreti, 2000). PBZ, according to Kurian and Iyer (1992), may raise the total phenolic content of terminal buds and alter the phloem: xylem ratio in the stem, both of which are necessary for vegetative growth restriction. By modifying assimilate partitioning and nutrient delivery patterns to new growth in mango, this increases blooming. It is clear that pomegranate plants exposed to stress accumulated proline, which worked as an endogenous signal to increase blooming (Neale *et al*., 1990). Furthermore, the amount of proline in the leaves was found to be related to the quantity of hermaphrodite flowers produced (Powerwanto and Inoue, 1990). Defoliating pomegranates with ethrel at a high dosage (2 ml L^{-1}) also controls flowering (Saroj *et al*., 2017). When administered topically, ethrel induces increased gene production of cell–wall degrading enzymes such cellulase and polygalacturonase. In some plant species, ethrel perception has been revealed to be implicated in the arrest of stamen development by induction of DNA damage, which favors hermaphrodite flower formation (Xie *et al*., 2015). The results of the current experiment back up this theory.

When another plant growth regulator, nitrobenzene, was added, the number of blooms increased considerably. Nitrobenzene is a plant energizer and flowering stimulant that changes gibberellin, cytokinin, and ethylene ratios to support flowering, resulting in yield increases of 40-50% (Agrawal *et al*., 2009). Nitrobenzene is easily absorbed by plants and has been shown in research similar to ours to boost tomato flowering and fruit yield (Mithila ***et al***., 2012). The higher flower count in the study could be attributed to nitrobenzene's characteristics. Because both paclobutrazol and nitrobenzene promoted hermaphrodite flower production equally well, nitrobenzene would be the superior choice for large-scale cultivation because it is less expensive and less hazardous to the soil environment than paclobutrazol.

Endogenous phytohormones

Plants grown using all three propagative methods exhibited a significant decrease in GA3 concentration during the blooming and fruit set stages in plants treated with paclobutrazol in our current investigation. The GA inhibitor paclobutrazol inhibited both shoot and internode length elongation, demonstrating its morphological effects. The growth retarding impact of paclobutrazol has been described in apple, peach, and sweet cherry, which is consistent with our findings (Edgerton, 1986). Paclobutrazol, as previously indicated, hindered the oxidation of ent-kaurene to ent-kaurenoic acid by inactivating cytochrome P450-dependent monooxygenases, preventing gibberellic acid synthesis while boosting cytokinin synthesis (Kundy *et al*., 2013). Arun *et al*. (2016) discovered a comparable drop in GA3 content in olive 'Pendulino' leaves after paclobutrazol treatment, and Upreti *et al*. (2013) discovered a similar decline in mango 'Totapuri' leaves after paclobutrazol treatment. Paclobutrazol treatment resulted in an increase in IAA levels. Fletcher and Hofstra (1990) discovered that paclobutrazol aided the formation of Indole – 3 – acetic acid in the leaves of tomato plants in a study comparable to ours.

Conclusion

The goal of this study was to determine how plant growth regulators affected floral induction and fruit yield in various pomegranate propagules during the ambe and hastha bahar seasons. We explored how intrinsic characteristics such as endogenous hormones are changed by external application of plant growth regulators, and whether these factors have a role in blooming and fruiting, in addition to floral induction and fruit yield. PBZ was discovered to be the most effective plant growth regulator, improving a variety of parameters including the quantity of hermaphrodite flowers, fruit set, and fruit production. This could be owing to PBZ's inhibitory action on gibberellins, which leads in a greater floral to vegetative growth ratio. The source-sink relationship has also been demonstrated to be altered by PBZ, shifting the balance toward reproductive growth and improving fruit yield. Overall, the data show that plant growth regulators, particularly PBZ, play a key role in pomegranate flowering and fruiting by affecting endogenous hormones and the plant's internal physiology, consequently increasing the crop's commercial value.

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