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Evaluation of male gametocides causing male sterility in pigeonpea

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ABSTRACT

Five growth regulators were evaluated for their gametocidal properties on four genotypes of pigeonpea. Among the treatments, 1.0-1.5% Maleic hydrazide (MH) and 0.25% Naphthalene acetic acid (NAA) induced sterility. Genotype P 856 was the most responsive while 89-7-16 was the least responsive to different treatments. Spray just before the flower bud initiation was found to be the most vulnerable stage of crop growth for inducing high pollen sterility but phytotoxic effects were observed with ethrel at higher concentrations.

Key words : *Cajanus cajan*, Male gametocides, Male sterility

The basic problem of the cytoplasmic genetic male sterility system for exploitation of heterosis includes the time required for conversion of agronomically superior genotypes into male sterile lines, lack of efficient fertility restorers and their unstable expression over environments. A chemical with the ability to induce pollen sterility or to check pollen shedding will not only overcome these problems but also impart flexibility in the choice of genotype as a potential female parent to develop hybrids. Incidentally, very little is known about the gametocides, their specificity for different varieties and the optimum stage of application particularly in pigeonpea. Keeping this in view, the present study was undertaken to know the relative gametocidal potency of five plant growth regulators for induction of male sterility in pigeonpea.

MATERIALS AND METHODS

The experiment was laid out following split-split plot design with three replications involving four varieties, namely P 856, P 84, P 606, and 89-7-16 as main-plot treatments, three phenological stages (S_1= one spray before initiation of floral buds, S_2= one spray after the initiation of floral buds and S_3= one spray each before and after bud initiation) as sub-plot treatments and a factorial combination of five chemicals *i.e.*, Naphthalene acetic acid (NAA), Maleic hydrazide (MH), Ethrel, Dalapon and 2,4-Dichlorophenoxy acetic Acid (2,4-D) each with three concentrations *i.e.*, NAA at 0.05, 0.10 and 0.25%, MH at 0.5, 1.0 and 1.5%, Ethrel at 0.025, 0.05 and 0.10%, Dalapon at 0.10, 0.25 and 0.50%, and 2,4-D at 0.1, 0.2 and 0.3% concentrations along with one control (distilled water) corresponding to each variety as sub-sub plots. Utmost care was taken to avoid spray drift of the chemical to the neighbouring treatments by erecting polythene sheets of 4 m height in between and around the plots at the time of spray. Pollen sterility was studied in 20 flower buds selected randomly from five plants for each treatment by 1% Acetocarmine procedure as described by Kaul and Singh (1967). In all the genotypes, the flower buds which were likely to open in the next day were selected for fixation and killing processes. Pollen grains were counted in five foci on each slide for each of 20 flower buds in each treatment and the average of sterile pollen grains was expressed as per cent of pollen sterility.

Per cent pod set in each of the five randomly chosen plants per treatment was noted separately under both self pollination (bagged) and open pollination (unbagged) conditions and averaged to get mean per cent pod set per plant (female sterility). The mean values for pollen sterility and female fertility were used for statistical analysis as per the standard procedure (Panse and Sukhatme 1985).

RESULTS AND DISCUSSION

The analysis of variance for three characters indicated the varied levels of gametocide effects as evident from highly significant variances for varieties, stages, chemical treatments and their all possible interactions.

Pollen sterility: The differential response of genotypes to gametocide treatments indicated that P 856 was highly responsive while the genotype 89-7-16 was the least responsive to treatments over all the stages (Table 1). The effect of treatments over the varieties revealed the highest pollen sterility of 83.9% with 1.5% MH followed by 1.0% MH (79.9%) and 0.25% NAA (79.7%). The remaining gametocides induced partial male sterility in the range of 37.8-75.2%. Similar results were reported in cotton (Singh *et al.* 1989) and faba bean (Awasthi and Dubey 1983). The differential response of varieties to the chemicals could be attributed to some of the factors *viz.*, (a) doses tried may not be within the effective range for all the varieties, (b) differential ability of varieties to absorb applied chemicals, (c) possible changes in the potency of chemicals within the plant and (d) stage specificity of the chemicals used. Whatever may be the cause(s) for differential response of genotypes to gametocides, the study clearly showqed that in the use of chemical hybridization agents, it is the variety x treatment interaction which breeders should take advantage for commercial hybrid technology. This would imply that rather than looking for chemicals with wide range effect, variety specific chemicals should be identified and exploited. In the present study, the treatment 1.5% MH was identified as the most potential gametocide in inducing high pollen sterility in the range of 80-86.5% in all the varieties. However, the maximum pollen sterility was induced with 1.5%

Table 1. Mean pollen sterility for varieties, treatments and V x T interaction in pigeonpea

Variety (V)	Treatments (T) and concentrations (%)															Control	Mean
	NAA			MH			Ethrel			Dalapon			2,4-D				
	0.05	0.10	0.25	0.5	1.0	1.5	0.025	0.05	0.10	0.10	0.25	0.50	0.1	0.2	0.3		
P 856	58.2	65.9	78.4	65.1	75.6	80.1	49.5	78.5	63.8	52.9	64.2	65.3	61.3	72.4	70.0	0.8	62.6
	(49.8)	(54.6)	(63.0)	(54.3)	(62.2)	(65.3)	(44.6)	(63.3)	(53.9)	(46.8)	(57.4)	(54.7)	(52.8)	(58.4)	(57.3)	(5.1)	(52.7)
P 84	65.5	74.6	85.9	70.7	77.5	83.3	46.0	57.8	63.7	40.0	42.2	47.9	62.4	66.3	75.3	1.2	60.0
	(54.0)	(61.1)	(68.9)	(57.8)	(62.8)	(67.4)	(42.7)	(48.9)	(53.2)	(39.2)	(40.3)	(43.7)	(52.5)	(55.1)	(61.0)	(6.2)	(50.9)
P 606	66.6	70.8	74.4	81.7	86.1	86.5	31.2	50.5	57.9	32.6	50.2	57.4	78.8	46.8	53.9	1.7	57.9
	(55.0)	(57.9)	(60.4)	(65.0)	(68.2)	(68.7)	(33.3)	(45.3)	(49.8)	(34.6)	(45.3)	(49.2)	(62.7)	(43.2)	(47.2)	(7.5)	(49.6)
89-7-16	66.3	71.4	80.1	83.3	80.4	85.9	24.5	25.0	33.3	37.8	39.8	61.5	26.3	22.8	36.0	1.8	48.5
	(54.6)	(57.8)	(63.7)	(66.0)	(63.9)	(68.1)	(29.6)	(29.9)	(35.3)	(37.8)	(39.0)	(51.7)	(30.6)	(28.5)	(36.8)	(7.5)	(43.8)
Mean	64.2	70.7	79.7	75.2	79.9	83.9	37.8	53.0	54.7	40.8	49.1	58.0	57.2	52.1	58.8	4.1	57.3
	(53.4)	(57.8)	(64.0)	(64.3)	(64.3)	(67.4)	(37.6)	(46.8)	(48.0)	(39.6)	(45.5)	(49.8)	(49.6)	(46.3)	(50.6)	(6.0)	(49.3)

	SEm	CD (0.05)
Varieties (V)	0.08	0.27
Treatments (T)	0.44	1.23
Varieties x treatments (V x T)	0.86	2.40
Treatments x varieties (T x V)	0.88	2.45

Figures in parentheses denote transformed values.

MH (86.5%) followed by 1.0% MH (86.1%) in P 606. Similarly, 1.5% MH was also found effective to cause high pollen sterility in the genotype 89-7-16 (85.9%) while 0.25% NAA treatment induced highest pollen sterility in P 84 (85.9%).

In general, 'before flower bud initiation' stage was invariably more vulnerable in all the genotypes to the given treatments than 'after flower bud initiation' stage for pollen sterility induction (Table 2). Most of the chemicals gave better response either at pre-meiotic or meiotic stages. Comparable response of 'before flower bud initiation' (S_1) and 'before + after flower bud initiation' (S_3) stages suggest that whatever pollen sterility observed in the later was due to induction mostly at before flower bud initiation (S_1) stage and second spray at 'after flower bud initiation' (S_2) stage had only marginal increase in pollen sterility. This does not mean that subsequent sprays would not increase the level of sterility, as instances of second spray at later stages restricting anther dehiscence, pollen release and impairment to pollen viability are known in other crop plants (Kaul 1988). Higher female sterility was observed in combination treatment (S_3) as compared to S_1 stage as evident from per cent pod set on selfing and open pollination further lent support to the aforesaid possibility warranting more intensive and critical study for confirmation.

Female sterility: Female sterility (ovular sterility) is the major undesirable effect associated with the utilization of gametocides for selective induction of male sterility. The per cent pod set on selfing as well as on open pollination were drastically reduced in P 84 while it was least affected in 89-7-16 indicating that P 84 is more sensitive than other genotypes (Table 3). Among different gametocides, 1.5 and 1.0% MH, and 0.25% NAA caused higher female sterility. Two sprays of gametocides at 'before + after flower bud initiation' stages (S_3) exhibited more female sterility rather than 'before flower bud initiation' (S_1) and 'after flower bud initiation' (S_2) stages and could be attributed to the prolonged and continuous effect of treatments.

Table 2. Mean pollen sterility for stages, treatments and S x T interaction in pigeonpea

Stage (S)	Treatments (T) and concentrations (%)															Control	Mean
	NAA			MH			Ethrel			Dalapon			2,4-D				
	0.05	0.10	0.25	0.50	1.0	1.5	0.025	0.05	0.10	0.10	0.25	0.50	0.1	0.2	0.3		
Before flower bud initiation	72.8	78.3	85.1	78.9	84.2	90.6	35.0	54.0	55.8	36.7	49.4	58.5	57.9	50.2	61.6	1.5	59.4
	(58.7)	(62.5)	(67.6)	(63.0)	(66.9)	(72.2)	(35.8)	(47.5)	(48.4)	(37.2)	(44.9)	(49.9)	(50.3)	(45.2)	(52.3)	(6.6)	(5.6)
After flower bud initiation	51.3	56.2	65.5	63.7	67.8	72.8	29.5	46.6	44.0	39.5	33.6	51.7	49.0	42.1	48.0	1.3	47.7
	(45.7)	(48.8)	(54.1)	(53.5)	(56.2)	(59.2)	(32.6)	(42.8)	(41.5)	(38.8)	(38.1)	(46.4)	(44.7)	(40.4)	(43.9)	(6.5)	(43.3)
Before and after flower and initiation	68.4	77.5	88.5	83.1	87.7	88.4	49.0	58.3	64.3	46.3	64.3	63.9	64.7	63.9	67.0	1.4	64.8
	(55.7)	(62.2)	(70.3)	(65.9)	(69.8)	(70.7)	(44.2)	(50.3)	(54.1)	(42.8)	(53.6)	(53.2)	(53.9)	(53.4)	(55.6)	(6.7)	(53.9)
Mean	64.2	70.7	79.7	75.2	79.9	83.9	37.8	53.0	54.7	40.8	49.1	58.0	57.2	52.1	58.8	1.4	57.3
	(53.4)	(57.8)	(64.0)	(60.8)	(64.3)	(67.4)	(37.6)	(46.8)	(48.0)	(39.6)	(45.5)	(49.8)	(49.6)	(46.3)	(50.6)	(6.6)	(49.3)

	SEm	CD (0.05)
Stages (S)	0.40	1.21
Treatments (T)	0.44	1.23
Stages x treatments (S x T)	0.85	2.40
Treatments x stages (T x S)	0.77	2.13

Figures in parentheses denote transformed values.

Table 3. Effect of gametocides on mean per cent pod set (under bagged and unbagged conditions) in four varieties of pigeonpea

Gametocide	Concentration (%)	Mean (%) pod set							
		Bagged condition (selfing)				Unbagged condition (open pollination)			
		Varieties				Varieties			
		P 856	P 84	P 606	89-7-16	P 856	P 84	P 606	89-7-16
NAA	0.05	21.8	16.7	18.8	19.0	21.6	20.9	22.6	23.0
	0.10	22.8	13.4	17.2	17.2	19.9	18.2	20.6	20.5
	0.25	21.1	9.8	16.0	14.5	17.3	15.4	24.7	18.9
Maleic hydrazide	0.5	22.5	15.0	14.0	13.3	21.8	19.9	18.4	17.4
	1.0	20.9	12.8	12.2	14.3	18.2	17.2	15.7	20.0
	1.5	19.9	10.7	11.9	12.3	17.2	15.9	18.9	16.9
Ethrel	0.025	21.1	21.2	27.8	34.3	23.5	24.2	29.6	30.2
	0.05	20.4	18.6	23.5	30.0	16.7	21.3	26.3	30.5
	0.10	17.6	17.1	21.3	27.0	20.5	19.7	24.7	28.9
Dalapon	0.10	20.3	22.5	27.8	26.1	23.4	24.7	27.2	27.5
	0.25	20.3	21.9	22.8	25.7	20.4	22.9	26.3	25.5
	0.50	17.2	20.3	21.7	20.2	20.2	21.5	25.1	25.3
2,4-D	0.1	18.8	17.2	15.1	28.6	22.3	17.9	19.3	29.6
	0.2	15.4	16.0	23.5	29.2	19.5	17.2	25.4	30.5
	0.3	15.8	13.6	22.5	26.4	18.7	15.2	24.2	28.0
Control		29.5	29.5	34.6	33.9	31.4	29.9	35.5	34.1
	Mean	20.2	17.3	20.7	23.3	20.8	20.1	24.2	25.4
	CD (0.05)	3.13				0.59			

Higher female sterility on selfing was observed with 0.25% NAA and 1.5% MH in P 84 and 89-7-16. Similarly, 0.3% 2,4-D, 0.25% NAA in P 84 and 1.0% MH in P 606 reduced the pod set on open pollination. The decrease in the number of pods per plant and yield by the use of MH was reported in pigeonpea (Kaul and Singh 1967), with NAA in mungbean (Bhardwaj and Dubey 1977) and with Ethrel and 2,4-D in rice (Aswathanarayana and Mahadevappa 1992).

Among the five chemicals tested, maleic hydrazide (MH) at 1.5 and 1.0% concentrations was proved to be the most effective followed by 0.25% NAA for high pollen sterility induction. The remaining chemicals, Ethrel, Dalapon and 2,4-D were found to induce partial male sterility with high female sterility at higher concentrations. Hence, these chemicals were found to be ineffective for selective induction of male sterility and cannot be possible to utilize them in hybrid seed production as they cause phytotoxicity effects on the treated plants. The results of the present study warrant further confirmation regarding pollen sterility and female sterility of the identified gametocides, Maleic hydrazide and NAA. Further, it is suggested that chemically induced partial male sterility of the order of 80-90% and of about 10% pod set on open pollination achieved through gametocides could be successfully used for facilitating random mating for population improvement in pigeonpea.

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