



Chemical hybridising agents V[#]: Evaluation of acylanilines in normal and late sown bread wheat (*Triticum aestivum* L.)

K. Chakraborty, C. Devakumar¹, S. M. S. Tomar² and S. Chowdhury²

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012

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Abstract

There is an urgent need for the introduction of hybrid wheat in India to meet the projected target of production of wheat i.e., 105 m. tonnes by 2020 A.D. In the absence of cytoplasmic-genetic male sterile (CGMS) system, use of chemical hybridising agents (CHAs) is another possibility already successful in some western countries. Keeping this approach in view, a total of 19 test CHAs comprising of 10 oxanilates, 5 acetoacetanilides and 4 malonanilates were synthesised and screened as CHAs on two genotypes of wheat (PBW 343 and HD 2687) at premeiotic stage and at different concentrations (1000-4000 ppm). The field trial was conducted following randomised block design (RBD) with three replicates. Fluoroanalogues (1, 5 and 10) gave the best performance in inducing male sterility in both the varieties. At 3000 ppm, they respectively induced 100, 90.33 and 88.54 per cent male sterility on HD 2687 (late sown). Similar trend of results was obtained on these cultivars sown at normal sowing dates. No other substituent could match fluoro in enhancing the property of male sterility induction. Neither modification of the side chain nor change in substituent position in the phenyl ring could improve the observed induction of male sterility.

Key words : Wheat, male sterility, anilates, acetoacetanilides, CHA

Introduction

The production of hybrid wheat (*Triticum aestivum* L.) offers an exciting opportunity for overcoming the stagnating yield plateau of wheat in India. Exploitation of heterosis at commercial level depends on the availability of stable male sterile lines. A viable and stable cytoplasmic-genetic male sterile (CGMS) system along with perfect restorer lines in wheat is not in place though considerable research efforts are underway. In view of this, the other option using chemical hybridising agents (CHAs) involving two-line hybrid breeding needs to be pursued intensively. The

CHAs facilitate cross breeding in plant species with perfect flowers by selectively sterilising male sex cells or by interrupting microsporogenesis to prevent self-pollination and to promote fertilisation by an outside pollen source. Thus they offer great opportunities to develop hybrids. France, Italy, USA and Australia are already ahead in commercialising wheat hybrids using CHA approach. Unlike CGMS system, the CHAs have unique advantages of saving precious time and labour [1] since no restorer-maintainer lines are required [2]. Also, any profitable heterotic combination is apparently enormous. Search for effective CHAs is being pursued by us as well as at Directorate of Wheat Research, Karnal [3]. In our earlier study, twenty oxanilates were evaluated on rice variety Pusa 150 and a few oxanilates were found to be very effective, inducing 95-100 per cent male sterility [4]. Pilot study on hybrid seed production revealed that ethyl 4'-fluorooxanilate applied as single spray at stamen-pistil primordia stage to be superior to sodium methyl arsenate with higher percentage seed set, test grain weight and seed yield [5].

Keeping this scenario in view, and encouraged by considerable success in the synthesis and screening of some oxanilates as CHAs for rice, we have expanded the scope of the chemicals and extended this study for wheat at different concentrations (1000- 4000 ppm) on two genotypes of wheat (PBW 343 and HD 2687) at premeiotic stage. In this study, a total of nineteen test CHAs comprising of 10 oxanilates, 5 acetoacetanilides and 4 malonanilates were synthesised and their ability to induce male sterility was evaluated.

Materials and methods

Ten oxanilates (Table 1) were prepared by the methods described earlier [4]. Similarly, four malonanilates and five acetoacetanilides were synthesised by the

[#]For Part IV see Chakraborty *et al.*, 2000; Contribution No. 699 from the Division of Agricultural Chemicals; ¹Author for correspondence, e.mail: cdevakumar@yahoo.com; ²Division of Genetics, I.A.R.I., New Delhi 110 012

condensation of diethyl malonate and ethyl acetoacetate respectively with different substituted anilines [6].

Field trials

Two high yielding cultivars of bread wheat namely, PBW 343 and HD 2687 recommended as timely sown varieties in North Western Plain Zone comprising Punjab, Haryana, Western U.P. and parts of Rajasthan and having high yield potential and high rust resistance were chosen for chemical induction of male sterility.

The experiment was laid out in a randomised complete block design with three replicates. In the first phase of evaluation, all the test CHAs were tested at two concentrations viz., 1000 and 2000 ppm on both the varieties. In the second phase of evaluation, ten (short-listed) test CHAs (4 oxanilates, 1 malonanilate and 5 acetoacetanilides) were further tested as CHAs on late sown HD 2687 at four concentrations (1000, 2000, 3000 and 4000 ppm).

Seeds of both of the varieties *i.e.*, PBW 343 and HD 2687 (normal sown) were sown in November, 1998 under drilling, using 100 kg N, 60 kg P_2O_5 and 40 kg K_2O following a 100 kg ha⁻¹ seed rate at IARI Farm, Block No. 11. Late sown HD 2687 was sown in December, 1998 under the same conditions. Row to row distance was kept at 23 cm. Four rows of 2 m length were taken as a plot. Other optimum agronomic practices were also followed.

The test compounds were sprayed at premeiotic stage when the length of the spike emerging out of the first node was about 7-8 mm in length [7]. This occurred 60 days after sowing. The test CHAs were sprayed as emulsion of appropriate concentrations of test CHAs (*i.e.*, 1000/2000/3000/4000 ppm) containing chlorobenzene and Tween 80. No serious phytotoxic symptoms were visible two to three days after spray of solvent emulsion. The formulations were sprayed on the crop to run off on three replicate plots of 1m length of two lines containing about 200 tillers, keeping the outermost two lines as pollinator lines. Five spikes of each treated plots were covered with bag at pre-emergence [7] when awns were just emerging. For study of pollen sterility, anthers from three to four florets were smeared together over a drop of acetocarmine (1%) and examined under a light microscope. In respect of floret sterility, five each of bagged and unbagged spikes including one control were harvested at maturity.

Numbers of fertile (filled) and sterile (unfilled) florets were counted and per cent male sterility was computed therefrom. Data on parameters such as plant height, spike length, spikelet number and thousand grain weight were recorded on twelve plants of each

treatment and untreated controls. Germination test of seeds was conducted by putting the water-soaked blotting paper containing seeds in a petri dish. The data were subjected to analysis of variance.

Results and discussion

Effect of CHAs on induction of male sterility (mst) on timely sown HD 2687 and PBW 343

In the first phase of evaluation, maximum mst was observed in plots treated with ethyl 4'-fluoro oxanilate (79.79 and 93.75 per cent mst at 1000 and 2000 ppm in HD 2687 and 88.70 and 95.37 per cent mst in PBW 343 in that order). It was followed by 4'-fluoroacetoacetanilide and ethyl 4'-fluoro malonanilate. The former induced 82.33 and 85.06 per cent mst at 2000 ppm in HD 2687 and PBW 343, respectively and the malonanilate analogue induced 78.13 and 83.15 per cent mst in that order. Ethyl 4'-chloro analogue of oxanilate induced 61.04 and 68.33 per cent mst at 2000 ppm test concentration in HD 2687 and PBW 343, respectively. Ethyl 2'-methoxy oxanilate induced about 60 and 70 per cent mst at 2000 ppm in that order. All other anilates found to cause low induction (< 50 per cent) of mst on both the wheat genotypes even at 2000 ppm test concentration (Table 1).

Table 1. Relative per cent male sterility (mst) induced by promising CHAs on HD 2687 and PBW 343

Compound No.	Compound name	Male sterility (%) [*] in genotypes			
		HD 2687		PBW 343	
		1000 ppm	2000 ppm	1000 ppm	2000 ppm
1	Ethyl 4'-fluoro oxanilate	79.79	93.75	88.70	95.37
2	Ethyl 4'-chloro oxanilate	20.49	61.04	41.48	68.33
3	Ethyl 2'-methoxy oxanilate	36.25	59.79	53.52	70.00
4	Ethyl 2'-nitro oxanilate	24.37	44.17	32.78	52.22
5	Ethyl 4'-fluoro acetoacetanilide	72.21	82.33	79.11	85.06
6	Ethyl 4'-chloro acetoacetanilide	12.28	35.12	19.29	47.92
7	Ethyl 2'-chloro acetoacetanilide	8.32	23.07	10.12	29.65
8	Ethyl 3'-chloro acetoacetanilide	6.53	8.98	8.20	9.76
9	Ethyl 3'-nitro acetoacetanilide	2.29	4.57	10.25	12.59
10	Ethyl 4'-fluoro malonanilate	66.25	78.13	77.22	83.15
CD (P = 0.05)		0.54		0.72	

^{*}Mean of three replicates

Effect of CHAs on induction of mst on late sown HD 2687

Encouraged by the trend of results that became available with timely sown varieties, ten test CHAs were shortlisted and subjected to extensive evaluation at four different concentrations in late sown HD 2687. The results are summarised in Table 2. At 3000 and 4000 ppm test concentrations, the induction of mst by compound 1 was found to be 100 per cent. Significantly high degree of mst was also observed in case of 4'-fluoro acetoacetanilide and ethyl 4'-fluoro malonanilate. 4'-fluoro acetoacetanilide induced 88.25 and 90.33 per cent mst at 3000 and 4000 ppm respectively (Table 2). None of the other CHAs showed any promise even at the highest test concentration.

Table 2. Relative per cent mst induced by 10-CHA formulations on late sown cultivar HD 2687

Compound No.	Compound name	Male sterility (%)*			
		1000 ppm	2000 ppm	3000 ppm	4000 ppm
1	Ethyl 4'-fluoro oxanilate	86.35	98.29	100.00	100.00
2	Ethyl 4'-chloro oxanilate	26.22	63.53	68.12	68.60
3	Ethyl 2'-methoxy oxanilate	44.36	65.75	70.81	79.55
4	Ethyl 2'-nitro oxanilate	30.27	51.32	58.15	62.06
5	Ethyl 4'-fluoro acetoacetanilide	75.24	84.67	88.25	90.33
6	Ethyl 4'-chloro acetoacetanilide	18.30	39.38	42.06	48.28
9	Ethyl 3'-nitro acetoacetanilide	18.38	29.38	36.22	42.35
10	Ethyl 4'-fluoro malonanilate	73.07	82.53	86.49	88.54
11	Ethyl 2'-methoxy acetoacetanilide	18.74	36.22	43.78	52.38
12	Ethyl 2'-nitro malonanilate	15.54	23.64	30.96	39.29
CD (P = 0.05)		0.47			

*Mean of three replicates.

Incidentally, significant levels of male sterility and decrease in female fertility (~ 3 per cent) were noticed in late sown wheat cultivar HD 2687 in comparison to timely sown cultivar HD 2687. Apparently, warmer temperature might be responsible for the deterioration in these characters.

In terms of structure-activity relationship, only fluoro substituent seemed to have an enhancing effect on induction of male sterility as seen in compounds 1, 5 and 10. Side chain modification of ethyl 4'-fluoro oxanilate through malonanilate to acetoacetanilide moieties, caused moderate reduction in the observed activity of mst induction.

Performance indicators of fluoro analogues on wheat

The three best CHAs viz., ethyl 4'-fluoro oxanilate, 4'-fluoro acetoacetanilide and ethyl 4'-fluoro malonanilate were further screened for their possible effects on growth characteristics of wheat. Their performance indicators on three cultivars viz., HD 2687 (both timely sown and late sown) and PBW 343 are given in Table 3. As it is evident, no significant adverse effect on any of the plant growth parameters was detectable in all the three CHA treatments. In addition, they had shown excellent selectivity in induction of male sterility without affecting female fertility. Further the germination of seeds obtained from treated plants under unbagged condition was again very high touching 95 per cent. Similar levels of performance were observed with the cultivar PBW 343 [8]. A comparison of published data [3] on the performance of CH 9832 on PBW 343 with that of our best CHA (1) on PBW 343 points to the superiority of the latter in containing adverse effects.

Table 3. Performance indicators of fluoro analogues on three cultivars of wheat sprayed at 7-8 mm spike length stage at 2000 ppm test concentration

Compound No.*	Variety	Plant growth parameters						
		Plant height (cm)	Spike length (cm)	Spike-let No.	Seed set in unbagged condition (%)	Test weight (g)	Germination (%)	Female fertility (%)
1	HD 2687	78.83	10.66	15.90	53.65	36.66	95.00	99.05
	PBW 343	76.37	10.63	18.23	53.12	37.64	95.00	99.88
	HD 2687 (late sown)	77.92	10.48	15.65	50.72	35.51	94.96	96.82
5	HD 2687	79.20	10.68	15.85	53.88	36.90	98.26	99.78
	PBW 343	77.29	10.72	18.26	53.10	37.86	98.10	99.87
	HD 2687 (late sown)	77.90	10.65	15.80	50.89	35.80	96.91	96.17
10	HD 2687	78.20	10.63	15.90	53.41	36.67	97.00	98.63
	PBW 343	76.33	10.60	18.13	52.81	37.00	96.85	98.13
	HD 2687 (late sown)	77.96	10.48	15.82	50.49	35.46	95.10	95.71
Emulsion control	HD 2687	79.62	10.86	16.07	54.16	36.98	99.23	100.0
	PBW 343	78.13	10.79	18.30	53.18	37.92	98.62	100.0
	HD 2687 (late sown)	79.60	10.78	15.93	51.63	35.82	98.20	97.62
CD (P = 0.05)		0.73	0.30	0.18	0.52	0.69	0.22	1.10

*Compound numbers are according to Table 1.

Conclusions

Fluoro analogues (compounds 1, 5 and 10) have shown very selective and high induction of male sterility in two cultivars of wheat and at the same time did not show any serious adverse effect on different wheat growth and yield parameters. Better results were obtained under late sown conditions. Ethyl 4'-fluoro oxanilate can serve as the most sought after CHA for the identification of useful heterotic combinations in wheat. Simultaneously, efforts are also in progress to identify better and more potent analogues as well as better formulations.

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