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Ethyloxanilates as specific male gametocides for wheat (*Triticum aestivum* L.)

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Abstract

Induction of male sterility by deployment of male gametocides holds immense potential in heterosis breeding of wheat. The efficacy of a new class of male gametocide for wheat (*Triticum aestivum* L.) is described: ethyloxanilates, the most active example of this class being ethyl 4-fluorooxanilate (E4FO). E4FO induces male sterility, specifically, without detectable effects on various agronomic features and female fertility. The plants sprayed once with 0.15% E4FO exhibited 100% pollen and floret sterility without causing a significant reduction in total yield. E4FO was screened on 29 genotypes of wheat at 0.15% test concentration and was observed to induce 99.76 ± 0.37% male sterility. Thirteen F_1-hybrids of wheat were produced using the gametocide in Winter 2000–2001 and were evaluated for their agronomic performance in Winter 2001–2002. The cross combinations viz., lines WR 544 × HW 2046 and HW 2044 × WR 956 have outperformed their respective better parents by 48.17% and 23.42% in grain yield/plant and thus have potential as hybrids.

Key words: *Triticum aestivum* — male sterility — male gametocide — ethyl 4-fluoro oxanilate (E4FO) — F_1-hybrids

Exploitation of heterosis at a commercial level depends on the availability of either cytoplasmic-genetic male sterility (CGMS) or through certain chemicals called male gametocides. The success of hybrid wheat programmes, other than by using chemicals, depends upon two key elements viz., development of stable CGMS lines and a perfect restorer system in a three-line approach. But this methodology is beset with many obstacles and limitations such as non-availability of breeding stocks containing CGMS and restorer systems, their instability and the laborious method of heterosis breeding. Besides being tedious and time-consuming, this technique sometimes becomes untenable because of the lack of a consistent restorer system for the genetic restoration of fertility. The alternative approach using male gametocides requires neither restoration nor conversion of parental lines to a CGMS background (Colhoun and Steer 1983). Gametocides facilitate cross breeding in plant species with perfect flowers by selectively sterilizing male sex cells or by interrupting microsporogenesis to prevent self-pollination and to promote fertilization by an outside pollen source and thus offers opportunities to develop hybrids (Collantes et al. 1999).

In self-pollinated crops like wheat, wherein the male and female organs are in the same flower, selective sterilization of a male organ (pollen) is of paramount importance in heterosis breeding, which can be achieved by the deployment of male gametocides facilitating a 'two-line' approach (McRae 1985). A number of such hybrids have been submitted for commercial registration in several European countries and have entered the marketplace in both Europe and the United States. All of these hybrids have been produced with gametocide technology. The last CGMS hybrid to be submitted for testing in uniform trials co-ordinated by USDA was in 1995. Using gametocides, one can develop a large pool of heterotic combinations with various traits including higher productivity (Cross and Ladyman 1991). This large pool could then provide an array of wheat hybrids. Lack of availability of safe and selective chemicals capable of inducing male sterility without causing any adverse effect on plant growth and development has been the major constraint in the pursuit of this approach. The current generation of gametocides was developed specifically for their male pollen-suppressing activity and they provide a much improved safety margin with significantly reduced phytotoxic effects. The Shell chemical WL 84811 and the Monsanto CHA GENESIS® are examples of this type of chemical. A group of gametocides viz., RH-531 applied at the rate of 0.125–10 lb/acre induced >90% male sterility in wheat (Carlson 1978) but was found to adversely affect the female fertility in barley (Hocket and Feltner 1978) and wheat (Jan et al. 1976), where it retarded the stigmatal growth. Gibberillic acid and surf-excel were reported to be an effective male gametocide for *Carthamus tinctorious* (Bayder and Gokmen 2003) and *Brassica juncea* (Singh and Chauhan 2003), respectively. Ethrel was reported to be an effective gametocide for wheat (Dotlacil and Apltauerova 1978), but was found to induce a very high degree of female sterility at the rates required for male sterility. A number of herbicide-CHA chimera and amino acid analogues were found to induce male sterility in wheat (Chakraborty and Devakumar 2005c). Ciha and Ruminski (1991) reported pyridine monocarboxylates as potential male gametocides for wheat.

Therefore, the present research programme has great scientific relevance and was planned with the objective of obtaining an ideal male gametocide to produce a wheat hybrid. A number of male gametocides have been reported for cereals viz., rice (Ali et al. 1999), wheat (Mizelle et al. 1989, Mogensen and Ladyman 1989, Guilford et al. 1992), and maize (Loussaert 2004). In a programme of design and development of male gametocides for crop plants, there have been earlier reports of the deployment of *N*-acylanilines in wheat (Chakraborty et al. 2003, Chakraborty and Devakumar 2005a,b; Chakraborty and Devakumar 2006a) and chickpea (Chakraborty et al. 2001, Chakraborty and Devakumar 2006b).

It was therefore of interest to expand the list of potential gametocides based upon the leads postulated and predicted in earlier studies, and at the same time validate the utility of one potent gametocide in F_1 seed production. The present study consisted of screening of potential molecules from a pool of 26 male gametocides belonging to the ethyloxanilates. Based upon the preliminary screening, a short-list of six potent gametocides (showing ≥98% of induction of male sterility in wheat) were tested for their selectivity in action, and technology has been standardized towards the development of potent molecules as well as systematic ranking of the gametocides based upon activity vs. selectivity. Using ethyl 4-fluoro oxanilate (E4FO), which has emerged as a superior male gametocide in the present study, a pilot-scale production and performance evaluation of F_1-hybrids of wheat has been carried out.

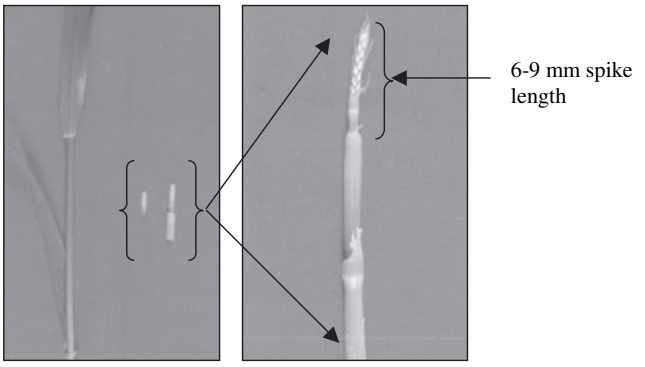

Fig. 1: Treatment with male gametocides at a premeiotic stage (6–9 mm spike length) of wheat

Materials and Methods

The chemicals evaluated as CHAs (Chemical Hybridizing Agents) belong to the ethyloxanilate class of chemicals (chemical nos. 1–26). A solution of substituted anilines (0.025 mol) and diethyl oxalate was heated to reflux for 30–45 min to furnish a residue, which was recrystallized in ethyl alcohol to yield ethyloxanilates as crystalline solids, with a purity of >98% and a yield of 70–95% (Chakraborty et al. 2003). The gametocides were subjected to field evaluation at five concentrations viz., 0.10%, 0.15%, 0.20%, 0.25% and 0.30% for induction of male sterility. Since gametocide concentrations >0.15% were found to adversely affect various plant growth and yield parameters, data were reported only at 0.10% and 0.15% test concentrations. Three high-yielding varieties (HYVs) of bread wheat (*Triticum aestivum* L.) viz., PBW 343, HW 2046, and HD 2733 recommended for timely sowing in the North Western Plain Zone (NWPZ) of India were chosen for evaluation of chemical induction of male sterility. The experiment was laid out following a randomized block design with three replicates. Seeds were sown by drilling, using 100 kg N, 60 kg P_2O_5, and 40 kg K_2O and a 100-kg/ha seed rate in the experimental research farm of Indian Agricultural Research Institute, New Delhi. Row to row distance was kept at 23 cm. Other optimum agronomic practices were also followed, which included timely weeding and other cultural operations. Five irrigations were given at different stages of crop growth viz., crown root initiation (CRI), late tillering, late jointing, flowering, and dough stage. CRI is the first critical period when crown root initiates from the point where stem and root joins together, which occurs about 21 days after sowing.

A liquid, homogenous preparation (emulsifiable concentrate) of test gametocides (1.5 g) in solvent cyclohexanone (7 ml) using Tween-80® (1 g) as an emulsifier was prepared as an emulsion after dilution in water. These products are cheaper to use for jobs requiring a large volume of field spray. Before spraying, a detailed experiment was conducted on the formulation to be sprayed on wheat. Different emulsifiers have been tried in combination with various solvents (anisol, ethyl cellosive, methyl ethyl ketone, xylene, cyclohexanone, and 1,1,1-trichloroethylene) to furnish emulsifiable concentrates, among which the cyclohexanone/Tween-80 combination worked out to be the best as far as solubility is concerned. Generally, Tween-80 gives higher dispersion of the gametocides when compared with any other emulsifiers. The higher water solubility of the gametocides in this study facilitated their fast dispersion in the emulsion. To ensure phytosafety of cyclohexanone, a blank solvent emulsion without gametocides was also sprayed on the crop well in advance. No serious phytotoxic symptoms were visible two to three days after the spray. From emulsifiable concentrate appropriate dilutions with water furnished spray emulsions of 0.10% and 0.15% test CHA solutions. The formulations were sprayed on the crop to run off on three replicate plots of 1 m length of two lines containing about 200 tillers, keeping the outermost two lines as pollinator lines (HW 2045). The test chemicals were sprayed at a premeiotic stage when the length of the spike emerging from the first node was about 6–9 mm in length (Fig. 1) (Zadoks et al. 1974). This occurred 60 days after sowing. As the degree of synchrony of flowering varied with the variety, care was taken to tag the treated tillers of the appropriate stage. At heading, individual ears were isolated with greaseproof paper bags. Anthers from three to four florets of the sprayed genotypes were smeared together on a glass slide over a drop of acetocarmine (1%) and/or KI-I_2 (2%) and examined under a light microscope. Pollen sterility was calculated in percentages. Ten bagged and ten unbagged spikes from each treatment, including one control, were harvested at maturity. The spike sterility was calculated three weeks after flowering. To study the floret sterility, the number of fertile (filled) and sterile (unfilled) grains was counted and the percent male sterility was computed as the percent inhibition of seed set in bagged spikes of treated plants. Similarly, data on parameters such as plant height, and spikelet number were recorded for each treatment and for the untreated controls. Analysis of variance (ANOVA) of a factorial randomized block design was performed with all treatments. Based on the significance of the treatments, least square differences (LSD) at the 5% level of significance ($P = 0.05$) were computed.

It is not sufficient that a chemical should exhibit a very high degree of induction of male sterility, but it should also not cause any adverse side effects on the plant. The most potent gametocides (ethyl 4-fluoro oxanilate, E4FO; ethyl 4-bromo oxanilate, E4BO; ethyl 2-trifluoromethyl oxanilate, E2TO; ethyl 4-trifluoromethyl oxanilate, E4TO; ethyl 3 -trifluoromethyl oxanilate, E3TO, and ethyl 4-cyanooxanilate, E4CNO) were tested for the adverse effects on growth and yield parameters of wheat viz., spikelet number and female fertility.

Production of F_1-hybrids was carried out using one of the most potent gametocides (E4FO) discovered in this study. In Winter 2000–2001, thirteen genetic stocks of wheat, *Triticum aestivum* L. were selected as female parents and were used in a crossing programme using diverse pollen parents. In Winter 2001–2002, 29 genetic stocks were selected as female parents and were used in a crossing programme using a uniform pollen parent (HW 2045). Five rows of 2 m length were taken as a plot in which the outermost two rows were treated as the pollen parents and the inner three rows as the female parents. Any foreign pollen was prevented from entering the plot by planting two border rows of oats (*Avena sativa* L.) on the surroundings of the experimental plot. E4FO was sprayed on the female lines 60 days after sowing. Crossing of the female parents was performed by generous dusting of the pollen from HW 2045, and bagging was performed immediately to prevent any further cross-pollination by any undesirable foreign pollen. To ensure cross-pollination, pollen from the pollinator was manually dusted on the female parents at an appropriate stage, where there was synchrony between the anthesis of the pollinator variety and reproductivity of the female parents. The tillers were then tagged. The F_1-hybrids were sown together with their parents in a layout consisting of two rows of each F_1-hybrid of 5 m

length at 30 cm apart with plant-to-plant distance of 10 cm. After maturity, plants from each replicate were harvested and the data on different parameters viz., plant height, spike length, number of spikelets/spike, thousand grain weight, number of seeds/spike, total spike weight, number of tillers/plant, and yield/plant were worked out for estimating the extent of heterosis. Using standard methods of estimation such as mid-parent heterosis (MPH%), better-parent heterosis (heterobeltiosis or BPH%), and heterosis over the standard check (standard heterosis) (SH%) the heterotic performance of the F_1-hybrids was evaluated.

Results

The potential male gametocides in the ethyloxanilate group of chemicals viz., E4FO (chemical no. 5), E4BO (chemical no. 6), E4TO (chemical no. 25), and E4CNO (chemical no. 22), respectively, were found to be the best, in that order, when considered across two test concentrations (0.10 and 0.15%) and three genotypes of wheat (PBW 343, HW 2046, and HD 2733). E2TO (chemical no. 23) and E2CNO (chemical no. 20) performed next best, inducing >82.5% spikelet sterility on HD 2733 at a 0.15% test concentration. CHA evaluation was carried out by the pollen sterility method using either the KI-I_2 (2%) or the acetocarmine (1%) staining method. Percent pollen sterility was found to have a high correlation ($r = 0.99$) with the spikelet sterility. Thus pollen sterility appears to be the major cause of spikelet sterility. From the staining tests, it was seen that the sterile grains were transparent, thereby confirming the disintegration of the cytoplasm and nucleus in the sterile pollen. In contrast, fertile pollen from control plots stained a uniform, deep red or blue colour in the acetocarmine and KI-I_2 stain tests, respectively, thus confirming the induction of male sterility in the various treatments (Fig. 2). In some cases the sterile pollen grains became shrivelled and the mass of cytoplasm and nucleus contracted inside the grain, keeping the external pollen wall (exine) intact. Pollen grains of sterile lines exhibited collapsed pollen morphology.

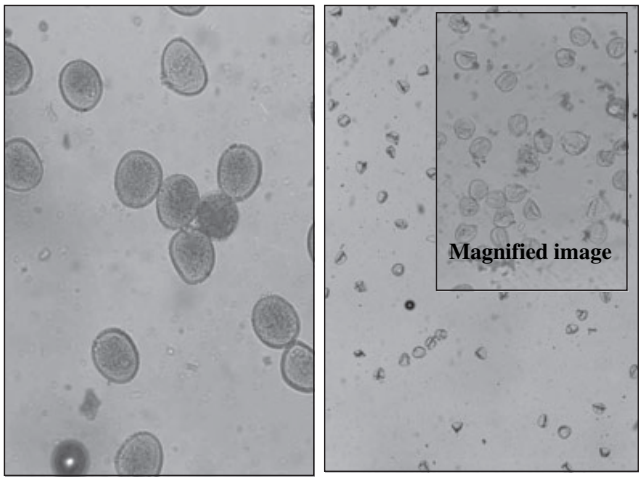

Fig. 2: Sterile pollen of wheat following treatment with ethyl 4-fluoro oxanilate (E4FO; chemical no. 5) *vis-à-vis* fertile pollen as revealed by the KI-I_2 stain test

The second generations of gametocides developed in the present study provide for improved seed quality and can be used on a wide array of genotypes. Besides high and selective induction of male sterility, the most potent male gametocides developed by this study (a shortlist of five gametocides from a pool of 26) showed neither any adverse effects on growth parameters such as plant height nor on yield parameters such as spikelet number and female fertility. E4TO (chemical no. 25) did not show any reduction in plant height with respect to the emulsion control (Table 1). Both E4FO (chemical no. 5) and E4BO (chemical no. 6) caused very moderate reductions in plant height, irrespective of the genotypes tested. The data pertaining to female fertility revealed that E3TO and E4TO exhibited no reduction in female fertility in all the genotypes tested. Both E4FO and E4BO showed a moderate, i.e. about 2% reduction in female fertility. E4CNO (chemical no. 22) had some detrimental effects on female fertility, showing a reduction of about 10%. In E4TO (chemical no. 25) and E4BO (chemical no. 6) treatments there was only a marginal or no reduction in germination percentage. The 4-cyano analogue of ethyloxanilate (E4CNO, chemical no. 22) showed some detrimental effects on germination (a reduction by about 6% when compared with the control values).

Using E4FO, CHA technology was optimized in terms of variation in genotype, number of sprays, types of formulation, and dose. Thirteen F_1-hybrids of wheat were produced at multigram levels using E4FO oxanilate as a male gametocide in Winter 2000–2001 and were evaluated for their agronomic performance in Winter 2001–2002. E4FO induced significant induction of male sterility (99.76 ± 0.37%) at the 0.15% test concentration over 29 diverse wheat genotypes (Table 2). As far as the side effects are concerned, there was an overall reduction in mean plant height of about 5 cm, but this also

Table 1: Performance indicators of the selected male gametocides (tested at 0.15%) on different agronomic traits on three genotypes of wheat ('PBW 343', 'HW 2046' and 'HD 2733')

	Plant height (cm)			Spikelet number			Female fertility (%)			Germination (%)		
Ch. code[1]	PBW 343	HW 2046	HD 2733	PBW 343	HW 2046	HD 2733	PBW 343	HW 2046	HD 2733	PBW 343	HW 2046	HD 2733
E4FO	66.22	66.43	64.88	19.24	14.96	20.91	96.52	95.87	96.30	97.93	97.72	97.09
E4BO	66.44	67.50	67.50	19.74	15.09	21.00	96.42	95.76	96.07	98.89	98.51	97.66
E4CNO	64.73	63.53	62.18	18.16	15.38	19.10	88.51	87.68	88.33	93.86	93.74	93.03
E2TO	68.75	66.39	63.50	21.19	20.43	20.17	97.01	95.19	96.43	89.14	88.21	90.57
E3TO	68.44	65.89	64.58	20.54	16.01	21.55	97.55	97.18	97.79	96.64	96.33	95.18
E4TO	69.25	67.10	66.97	20.85	16.39	21.90	96.81	96.54	96.91	98.89	98.70	97.30
Control	69.89	68.49	67.60	21.28	16.59	22.12	99.16	98.87	98.03	99.16	98.87	98.03
LSD (P = 0.05)	5.29	3.71	0.75	1.46	1.57	0.95	0.66	1.84	0.79	1.66	1.39	1.70

[1]Chemical name of the corresponding ch. code are mentioned under Table 5.

Table 2: Performance of E4FO for important traits of various genotypes of wheat at 0.15% test concentration

Genotypes	Plant height[1]	Male sterility (%)	Female fertility (%)	Spike length (cm)
T-1668	87.16 (93.32)	100	98.25	9.89 (10.53)
T-1744	84.39 (88.18)	100	97.11	11.28 (12.98)
T-1760	79.72 (83.63)	99.96	94.29	10.50 (10.59)
T-2134	89.65 (96.29)	99.72	98.28	10.53 (11.33)
HW-4022	86.41 (89.56)	100	97.39	10.07 (10.82)
T-1226	83.98 (88.47)	100	94.41	10.65 (11.00)
T-1355	82.39 (89.62)	100	98.28	10.06 (10.80)
T-1515	88.75 (91.35)	100	92.57	11.08 (13.62)
HD 2833	79.15 (83.46)	99.53	98.11	9.86 (10.54)
HD-2835	82.53 (88.59)	99.78	99.62	10.39 (11.29)
T-116	82.49 (84.20)	100	97.30	11.21 (11.43)
PBW 485	74.18 (75.37)	100	99.44	9.58 (10.08)
GW 326	79.44 (81.90)	99.77	96.59	10.38 (10.57)
NW 1014	73.16 (75.41)	99.82	94.33	10.23 (10.77)
HW 2015	78.72 (79.68)	98.75	98.25	9.24 (9.68)
T-109	83.46 (85.38)	99.65	96.85	10.29 (12.73)
HW 2041	75.33 (75.80)	100	99.14	9.76 (10.66)
HW 2044	78.62 (80.62)	100	94.62	10.24 (10.92)
HD 2643	77.38 (78.15)	99.39	98.29	9.03 (9.79)
HD 2790	82.10 (88.04)	99.85	95.15	10.27 (10.59)
PBW 373	75.69 (77.28)	100	94.37	11.14 (11.42)
PS 640	82.15 (84.96)	99.66	97.60	7.68 (9.98)
P 331–62	80.69 (86.26)	98.50	98.85	9.26 (11.53)
GW 326	76.32 (81.31)	99.37	95.15	10.36 (10.68)
HW 2045	74.69 (78.69)	100.00	97.15	11.53 (11.83)
HW 2044	69.59 (76.07)	100.00	97.39	10.73 (10.83)
HD 2687	71.15 (79.21)	99.76	98.70	10.75 (10.79)
WR 251	73.06 (80.38)	99.64	91.76	16.26 (17.05)
WR 544	75.92 (78.37)	100.00	97.45	15.47 (16.23)
Control	84.45 ± 5.66	–	–	11.01 ± 0.94
Mean	79.60 ± 5.20	99.76 ± 0.37	96.78 ± 2.07	10.61 ± 1.65
LSD (P = 0.05)	1.86	0.09	1.29	0.87

[1]Figures in parentheses indicate the control values.

applied to the blank emulsion. The treatments did not cause significant reductions in spike length or spikelet number of the female parents. Ovular sterility is a major undesirable effect in hybrid seed production technology, which leads to lower level of seed setting in the treated spikes. The chemical did induce very marginal female sterility (female fertility was found to be 96.78 ± 2.07%) among the wheat genotypes and the grains that formed ranged from normal, through moderately shrivelled to completely shrivelled.

Thirteen F_1-hybrids produced in Winter 2000–2001 were cultivated to study their hybrid vigour in comparison with the standard check (PBW 343), mid parent, and better parent. Various agronomic traits for parents, standard check, and F_1-hybrids are presented in Table 3. The mean plant height of parents ranged from 63.03 (WR 956) to 79.02 cm (HD 2687), the range for hybrids was from 72.41 (HD 2687 × PBW 343) to 88.58 cm (WR 251 × PBW 343) (Table 3). In other words, plant height increased because of heterosis. Positive significant standard heterosis over the best check (PBW 343) was exhibited by the crosses HW 2045 × NW 1014, HW 2044 × WR 956, WR 251 × HD 2733, and WR 544 × HW 2046. Positive significant MPH for number of seeds/spike was exhibited by the cross HW 2044 × WR 956. Mean yield/plant among the parents ranged from 7.24 g (HW 2015) to 13.05 g (PBW 343), while in hybrids it ranged from 8.21 (WR 544 × HW 2733) to 12.98 g (HW 2044 × WR 956). Positive significant MPH was exhibited by the crosses viz., HW 2044 × WR 956 (56.20%) and WR 544 × HW 2046 (46.44%; Table 4). The cross combination HW 2044 × WR 956 was found to almost the same as the standard check (PBW 343) as far as the standard heterosis is concerned.

Discussion

Among all the different test concentrations, 0.15% was found to be optimum as far as both activity and selectivity are concerned. It was observed that almost all the male gametocides showed very high phytotoxicity at a concentration >0.15%. Among ethyloxanilates, E4FO, E4BO, E4TO, and E4CO at a 0.15% test concentration were found to be very active and they induced >99.5% male sterility on PBW 343 (Table 5). Similar trends were also observed on other genotypes of wheat. From the staining tests, it was seen that the sterile grains were transparent, thereby confirming the disintegration of cytoplasm and nucleus in the sterile pollen. In contrast, fertile pollen from control plots stained a uniform deep red or blue colour in acetocarmine and KI-I_2 stain tests respectively, thus confirming the induction of male sterility in various treatments (Fig. 2). The negative colour in KI-I_2 stain test shown by sterile pollen was indicative of the absence of starch. The absence of any starch material in the sterile pollen grains, as shown by the KI-I_2 pollen stain test, could indicate that either the processes leading to starch depletion or its synthesis has been blocked. This could provide an important lead in unravelling the mode of action of the gametocides.

The first generation of chemicals to be tested as gametocides generally caused a high degree of phytotoxicity at rates required for effective sterility. This often resulted in poor female receptivity or fertility, or failed to produce adequate male sterility. The risks in commercial production or their utility in developing breeding populations are unacceptable with any of this class of chemicals. The second generation of chemicals was developed and tested specifically for male

Table 3: Mean performance of parents (used to produce F_1 hybrids) and F_1 hybrids (obtained from the crosses between various parents)

Source	Plant height (cm)	Spikelet no.	Spike length (cm)	1000 grain weight (g)	Seeds/spike	Total spike weight (g)	Tillers/plant	Yield/plant (g)
HW 2045	76.21	19.87	11.83	36.52	52.28	3.34	5.26	9.96
HW 2044	77.21	17.59	10.80	35.53	53.31	3.16	4.64	8.76
HD 2687	79.02	20.75	10.81	36.58	57.22	3.46	6.01	12.57
WR 251	78.07	17.01	12.46	36.41	47.40	3.22	4.57	7.88
WR 544	75.10	17.70	9.76	34.80	50.24	2.22	4.11	7.25
HW 2015	76.62	19.72	9.70	32.40	48.71	3.55	4.56	7.24
NW 1014	90.51	20.24	9.65	35.08	46.76	2.61	4.97	8.07
PBW 343	73.75	26.08	14.68	39.78	63.70	4.00	5.18	13.05
HD 2684	69.93	22.16	10.60	38.33	52.80	2.47	4.52	9.10
WR 956	63.03	18.32	10.71	35.50	50.55	2.93	4.34	7.86
HD 2329	72.51	19.43	11.75	34.81	53.73	2.21	5.20	9.56
WH 542	68.70	21.66	10.77	30.36	44.43	2.19	4.70	6.31
UP 2338	77.68	20.43	11.54	26.27	52.73	2.44	6.41	8.90
HD 2733	68.80	22.21	10.68	26.63	54.11	2.64	4.35	6.19
HW 2046	63.46	20.55	10.63	33.52	49.61	3.08	5.08	8.47
HW2045 × NW 1014	84.30	22.51	13.32	36.67	55.14	2.90	5.10	10.31
HW2015 × NW 1014	77.32	17.40	11.57	35.84	46.44	3.44	5.25	8.75
HW 2045 × PBW 343	74.11	22.46	11.22	37.72	49.91	3.74	6.18	11.64
HW 2045 × HD 2684	76.35	18.35	9.40	37.47	48.80	3.43	5.49	10.04
HW 2044 × WR 956	81.94	24.57	14.41	39.76	63.60	3.94	5.16	12.98
HD 2687 × HD 2329	72.84	17.61	11.70	35.63	56.42	3.02	5.25	10.56
HD 2687 × WG 542	79.15	20.55	10.74	35.80	51.03	3.41	5.95	10.87
HD 2687 × UP 2338	77.66	24.54	11.75	36.23	52.90	3.38	6.10	11.69
HD 2687 × PBW 343	72.41	22.58	10.50	39.43	57.61	3.94	5.49	12.46
WR 251 × HD 2733	85.55	18.46	12.57	37.20	54.07	2.61	4.20	8.46
WR 251 × PBW 343	88.58	25.15	15.57	38.14	58.54	3.95	5.78	12.91
WR 544 × HW 2733	75.30	23.35	11.53	35.89	53.02	2.52	4.32	8.21
WR 544 × HW 2046	77.27	19.88	11.09	36.58	53.88	3.19	5.83	11.51
LSD (P = 0.05)	5.19	1.28	1.50	1.09	5.36	0.55	1.01	1.90

Table 4: Extent of heterosis of the wheat hybrids based on mid parent heterosis (MPH)

Source	Plant height	Spikelet no.	Spike length	1000 grain weight	No. of seeds/spike	Total spike weight (%)	No. of tillers/plant	Yield/plant (%)
HW 2045 × NW 1014	1.13	12.24**	24.02**	2.43	11.35	−2.52	−0.29	14.36
HW 2015 × NW 1014	−7.47*	−12.91**	19.59*	6.22**	−2.71	12.05	10.18	14.30
HW 2045 × PBW 343	−0.93	−2.24	−15.35*	−1.13	−13.93**	1.91	18.39	1.17
HW 2045 × HD 2684	4.49	−12.68**	−16.18*	0.01	−7.12	18.07	12.27	5.35
HW 2044 × WR 956	16.86**	36.84**	37.61**	11.95**	22.47**	29.39**	14.2	56.20**
HD 2687 × HD 2329	−3.86	−12.34**	3.72	−0.018	1.70	6.53	−6.33	−4.56
HD 2687 × WG 542	7.16	−3.09	−0.46	6.96**	0.40	20.71	11.11	15.15
HD 2687 × UP 2338	−0.92	19.18**	5.15	15.29**	−3.77	14.58	−1.77	8.90
HD 2687 × PBW 343	−5.20	−3.57	−17.61*	3.27	−4.71	5.63	−1.88	−2.73
WR 251 × HD 2733	16.50**	−5.86	−9.21	18.02**	6.53	−10.92	−5.83	20.34
WR 251 × PBW 343	16.69**	16.73**	−1.74	0.12	5.38	9.45	18.56	23.42*
WR 544 × HW 2733	4.66	12.00**	−18.76**	16.85**	1.62	3.70	2.13	22.17
WR 544 × HW 2046	11.53*	−0.24	−21.71**	7.08**	7.92	20.38	26.88*	46.44**

*, **Significant at P = 0.05 and P = 0.01, respectively.

gametocide activity. These were generally found to work within a narrowly defined set of conditions. It was found out that E4FO, E4BO, E3TO, and E4TO were most effective as selective male gametocides for wheat. E4CNO although effective, was found to be wanting in selectivity. E4FO residues were non-detectable in mature grain as well as the husk, and thus it will not contaminate any crop products.

Among various potent male gametocides screened for their activity as well as their selectivity, E4FO (chemical no. 5) was selected for a hybrid experiment because of the ease of synthesis with very high yield, very high induction of male sterility (99.76 ± 0.37% at 0.15% concentration over 29 diverse wheat genotypes), less impact on agronomic features (plant height and spikelet number), and female fertility (female fertility was found to be 96.78 ± 2.07% in E4FO treated plants).

Using E4FO, the CHA technology was optimized in terms of variation in genotype, choice of CHA, stage of spray, numbers of spray, types of formulation, and dose. A premeiotic stage (6–8 mm of spike length) was found to be ideal and a single spray was adequate and safe. Oil in water emulsion was used. Cyclohexanone was found to be the best of the solvents tested. The cross HW 2044 × WR 956 showed positive significant MPH for different traits, viz., spike length, spikelet number, number of seeds/spike, total spike weight and the grain yield/plant. For plant height, this cross combination showed significant positive heterosis over the standard check PBW 343. Grain yield/plant is an important parameter in the

Table 5: Percent spikelet sterility in wheat genotypes 'PBW 343', 'HW 2046', and 'HD 2733' after treatment with various ethyloxanilates at 0.10% and 0.15% concentration

			PBW 343		HW 2046		HD 2733	
Ch. no.[1]	Ch. code	Chemical name of gametocides	1500 ppm	1000 ppm	1500 ppm	1000 ppm	1500 ppm	1000 ppm
1	EO	Ethyloxanilate	84.18	78.00	83.02	76.73	86.35	79.92
2	E2FO	Ethyl 2-fluoro oxanilate	68.13	48.85	64.37	48.67	70.09	47.6
3	E3FO	Ethyl 3-fluoro oxanilate	50.04	27.91	46.31	28.95	56.37	27.71
4	EFCO	Ethyl 3-chloro 4-fluoro oxanilate	77.91	64.85	80.29	64.39	81.58	64.21
5	E4FO	Ethyl 4-fluoro oxanilate	99.97	99.54	99.48	99.30	99.99	99.59
6	E4BO	Ethyl 4-bromo oxanilate	99.96	98.76	99.38	97.64	99.97	99.12
7	E2CO	Ethyl 2-chloro oxanilate	50.25	23.95	55.24	23.44	58.85	24.26
8	E3CO	Ethyl 3-chloro oxanilate	44.25	15.77	48.39	15.44	56.73	14.17
9	EDCO	Ethyl 2,4-dichloro oxanilate	41.56	20.23	39.77	20.72	45.68	21.21
10	E4CO	Ethyl 4-chloro oxanilate	72.09	44.00	75.58	45.18	76.13	43.29
11	E2MO	Ethyl 2-methoxy oxanilate	78.02	66.39	74.82	63.26	78.75	69.21
12	E3MO	Ethyl 3-methoxy oxanilate	39.07	14.16	42.11	14.93	49.35	11.95
13	E4MO	Ethyl 4-methoxy oxanilate	84.39	77.69	83.12	74.12	85.76	78.38
14	EDMO	Ethyl 2,4-dimethoxy oxanilate	63.43	55.34	62.39	54.14	68.49	56.49
15	E2NO	Ethyl 2-nitro oxanilate	61.00	34.26	65.37	34.27	70.27	32.06
16	E3NO	Ethyl 3-nitro oxanilate	32.13	11.28	40.57	4.65	48.16	11.71
17	E4NO	Ethyl 4-nitro oxanilate	79.06	70.41	70.85	61.91	81.57	70.85
18	EDNO	Ethyl 2,4-dinitro oxanilate	82.37	72.48	75.34	72.38	85.48	71.94
19	E3MTO	Ethyl 3-methyl oxanilate	23.29	1.92	19.38	1.85	31.89	2.28
20	E2CNO	Ethyl 2-cyano oxanilate	81.43	68.95	76.79	67.15	82.56	69.45
21	E3CNO	Ethyl 3-cyano oxanilate	71.74	57.13	66.36	55.42	72.71	62.28
22	E4CNO	Ethyl 4-cyano oxanilate	96.63	91.31	96.13	90.45	98.46	92.65
23	E2TO	Ethyl 2-trifluoromethyl oxanilate	84.59	76.40	83.62	74.90	93.03	77.19
24	E3TO	Ethyl 3-trifluoromethyl oxanilate	94.86	87.20	93.17	86.80	96.81	91.72
25	E4TO	Ethyl 4-trifluoromethyl oxanilate	99.57	98.44	98.83	97.33	99.98	99.47
26	E4EO	Ethyl 4-ethyl oxanilate	18.48	11.28	14.84	9.81	20.18	12.89
Emulsion control			0.46	0.26	0.33	0.22	0.49	0.34
LSD (P = 0.05)			1.42	0.98	1.83	1.55	1.39	2.68

[1] Chemical number.

performance of hybrids. The cross combinations WR 544 × HW 2046 and HW 2044 × WR 956 exhibited significant positive MPH in grain yield/plant and thus have potential as hybrids. The utility of E4FO as a CHA for the production of F_1-hybrids of wheat has been amply demonstrated in this study. It is significant that E4FO not only induces a very high degree of male sterility, but also modifies the reproductive biology in such a fashion to ensure cross-pollination in the cleistogamous wheat flowers and increase the probability of the development of hybrids. Wheat is a self-pollinated crop having closed florets. Florets of male-sterile wheat opened twice to facilitate cross-fertilization. First floret opening with the action of lodicules lasted only for a short period. Second floret opening started after the lodicules had collapsed, the carpels in the sterile floret continuing to move the palea and lemma apart. This second opening lasted for more time (5–6 days) and was normally sufficient for cross-pollination to take place from the pollen source. The extent of cross-pollination without cutting the palea and lemma is indicative of natural floret opening, stigma receptivity and out crossing percentage. To ensure cross-pollination there is no need to cut the palea and lemma, as the second floret opening is sufficient for cross-pollination to occur. The peak period for stigma receptivity lasted for about 3–4 days, which helped in planning for hybrid seed production.

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