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Evaluation of chemical compounds for induction of male sterility in wheat (*Triticum aestivum* L.)

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Summary

The short-list of eleven chemical hybridizing agents (CHAs) showing 98% or more induction of male sterility were identified following application at the pre-meiotic stage of wheat. Among ethyl oxanilates, 4-fluoro (CHA A1), 4-bromo (CHA A2), 4-trifluoromethyl (CHA A5), and 4-cyano (CHA A3) derivatives; and among pyridones, 4-chloro (CHA B3), 4-fluoro (CHA B1), 4-bromo (CHA B2), and 4-trifluoromethyl (CHA B6) derivatives were the most promising. These agents showed no adverse effects on plant growth and yield. Ethyl 4-fluoro oxanilate (CHA A1) was tested on 29 wheat genotypes at 1500 ppm and induced 99.76 ± 0.37% male sterility. Ethyl 4-fluoro oxanilate residues were non-detectable in grain and husk and thus appeared to have no lasting residue effects.

Introduction

Heterosis breeding offers a means to increase current yield levels in wheat. In self-pollinated crops like wheat with the male and female organs in the same flower, selective sterilization of pollen is of paramount importance for heterosis breeding. Chemical hybridizing agents (CHAs) are an option for this, thus facilitating a "two-line" approach to the production of hybrid seed (McRae, 1985; Guilford et al., 1992). CHAs can be used to develop a large pool of heterotic combinations eliciting various traits (Mogensen & Ladyman, 1989). Effective CHAs allow the sterilization of a large number of potential parents of wheat and permits the production of large numbers of hybrids. The lack of safe and selective chemicals capable of induction of male sterility without adverse effects on plant development has been the main constraint of this approach (Colhoun & Steer, 1983). The current generations of CHAs were developed specifically for their pollen-suppressing activity. They provide much improved safety with significantly reduced phytotoxic effects. The Shell compound 'WL 84811' and Monsanto 'CHA GENESIS' (Collantes et al., 1999) are examples of this class of chemistry. A number of CHAs have been reported for cereals viz, rice and wheat. The azetidine group of CHAs rendered wheat male sterile (Cross & Ladyman, 1991). Another group of CHAs, viz, 'RH-531', applied at 0.125 to 10 Kg/ha induced >90% male sterility in wheat (Carlson, 1978) but was found to adversely affect female fertility in barley (Hocket & Feltner, 1978) and wheat (Jan et al., 1976) where it retarded stigmatal growth. CHA 'RH 532' induced 100% male sterility in a broad spectrum of wheat genotypes at application rates of 1 to 3 Kg/ha (Miller & Lucken, 1977; Jan & Rowell, 1981). The fenridazon group of CHAs induced 95–100% male sterility in hexaploid winter and spring wheat genotypes at application rates of 0.2 to 2.0 Kg/ha (Mizelle et al., 1989). Ethrel is an effective CHA for wheat (Dotlacil & Apltauerova, 1978) but it induced high female sterility at application rates required for male sterility. Ciha & Ruminski (1991) reported pyridine monocarboxylates as potential CHAs for wheat.

The present research was planned with the objective of obtaining an ideal CHA. In a program of design and development of CHAs for crop plants, we earlier reported the deployment of *N*-acylanilines and anilides in rice (Ali et al., 1999), wheat (Chakraborty et al., 2003; Chakraborty & Devakumar, 2005a), and chickpea (Chakraborty et al., 2001) inducing >80% male sterility. It was therefore of interest to investigate a broader group of potential CHAs based upon the leads and predictions from the earlier studies (Chakraborty & Devakumar, 2005b, 2005c; Chakraborty et al., 2003). The present work trialed a wide range of related chemicals within the ethyl oxanilate and pyridone groups. The results of the most promising 11 potential CHAs are being reported here.

Materials and methods

Screening CHAs on wheat under field conditions

Experimental lay-out

The 52 chemicals selected for screening belonged to two classes, viz, ethyl oxanilates and pyridones. Eleven CHAs were chosen based on 97% or better sterility in the initial screen. These were used in field evaluations at rates of 1000 and 1500 ppm. Three high-yielding common wheat (*Triticum aestivum* L.) cultivars, viz, PBW 343, HW 2046, and HD 2733, recommended in the North Western Plain Zone of India were chosen for preliminary evaluations. The experiment was a randomized block design (RCBD) with three replicates. Seeds were sown at 100 Kg/ha at the Indian Agricultural Research Institute, New Delhi. Row to row distance was kept at 23 cm. Optimum agronomic practices were followed.

Field applications

The chemicals were prepared as emulsifiable concentrates (5 EC) in cyclohexanone using Tween-80® (1 g) as emulsifier. Prior trials showed that applications of cyclohexanone without CHAs gave no serious phytotoxic effect 2 to 3 days after application. From EC 5 stocks appropriate dilutions with water furnished spray emulsions of 1000 and 1500 ppm for trials. 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 female parents. Incoming pollen was reduced by two border rows of oats (*Avena sativa* L.) around the experimental plot. The pollinator cultivar HW 2045 was selected because tall height (80–82 cm), medium maturity, high yield components, disease resistance, and good seed yield. On the other hand, the female parents were shorter (70.36 ± 0.97 cm), high yielding, and narrow leafed.

The formulations were sprayed on the female lines keeping the outermost two lines as pollinator parent (HW 2045). The test compounds were sprayed at the premeiotic stage when the length of the spike was 6–9 mm. This occurred 60 days after sowing. Crossing of the female parents was done by generous dusting of the pollen from HW 2045. Pollen from the pollinator was manually dusted on twenty spikes of female parents and bagging was done immediately to prevent further cross-pollination. As the degree of synchrony of flowering varied with the cultivar, care was taken to tag the treated tillers of appropriate stage. Ethyl 4-fluoro oxanilate (CHA A1, Table 1) was sprayed on 29 genetic stocks of wheat as female parents, which were then pollinated with HW 2045.

Pollen and spikelet sterility

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 as a percentage. Ten bagged and unbagged spikes from each treatment, including one control, were harvested at maturity. To study the floret sterility, the numbers of fertile (filled) and sterile (unfilled) florets were counted and percent male sterility was computed as percent inhibition of seed set in bagged spikes of treated plants using the formula:

$$\text{Percent floret sterility} = \frac{(S_c - S_f)}{S_c} \times 100$$

where, S_c = seeds per spike in control plants; S_f = seeds per spike in bagged and treated plants.

Data on plant height, spike length, spikelet number, and 1000-grain weight were recorded for each treatment and the untreated controls. To test the viability of the seeds obtained form the treated spikes, germination tests were conducted on blotting paper. Analyses of variance (ANOVA) were performed for all treatments.

Screening of CHAs for their side effects

It is not adequate that a chemical induces a high degree of male sterility, but it also should not cause adverse side effects in the plant. The most potent CHAs (CHA A1, CHA A2, CHA A5, CHA A4, CHA A3, CHA B3, CHA B1, CHA B2, CHA B4, CHA B6, and CHA B5 (Table 1)) were assessed for effects on growth

Table 1. Percent spikelet sterility in wheat genotypes PBW 343, HW 2046, and HD 2733 after treatment with ethyl oxanilates (A1 to A5) and pyridones (B1 to B6) at 1000 and 1500 ppm

		PBW 343		HW 2046		HD 2733	
CHA No.	CHA name	1500 ppm	1000 ppm	1500 ppm	1000 ppm	1500 ppm	1000 ppm
A1	Ethyl 4-fluoro oxanilate	99.97	99.54	99.48	99.30	99.99	99.59
A2	Ethyl 4-bromo oxanilate	99.96	98.76	99.38	97.64	99.97	99.12
A3	Ethyl 4-cyano oxanilate	96.63	91.31	96.13	90.45	98.46	92.65
A4	Ethyl 3-trifluoromethyl oxanilate	94.86	87.20	93.17	86.80	96.81	91.72
A5	Ethyl 4-trifluoromethyl oxanilate	99.57	98.44	98.83	97.33	99.98	99.47
B1	4-Fluoro pyridone	98.61	94.92	97.06	92.67	98.81	95.41
B2	4-Bromo pyridone	98.44	95.02	97.63	93.96	96.73	93.69
B3	4-Chloro pyridone	98.88	95.07	98.02	93.77	99.35	96.39
B4	4-Cyano pyridone	89.06	81.77	85.74	79.94	90.06	82.78
B5	3-Trifluoromethyl pyridone	87.15	82.37	85.31	78.97	90.04	84.56
B6	4-Trifluoromethyl pyridone	96.22	91.11	94.38	87.04	96.54	92.23
	Emulsion control	0.46	0.26	0.33	0.22	0.49	0.34
	LSD ($p = 0.05$)	1.69	0.67	2.37	1.36	2.11	1.59

parameters. Plants treated with ethyl 4-fluoro oxanilate (CHA A1) was analyzed by reverse phase high performance liquid chromatography for residues at maturity in grains as well as husk.

Results and discussion

Spikelet sterility

The ethyl oxanilate group of CHAs viz, CHA A1, CHA A2, CHA A5, and CHA A3, respectively, were found to be the best in that order when considered across both test concentrations (1000 and 1500 ppm) and three wheat genotypes. Among pyridones, CHA B3 was the best, inducing 98.75±0.67% spikelet sterility at 1500 ppm. It was closely followed by CHA B1 and B2 (Table 1).

Pollen sterility

Pollen sterility was highly correlated ($r = 0.99$) with the spikelet sterility. Sterile pollen grains were

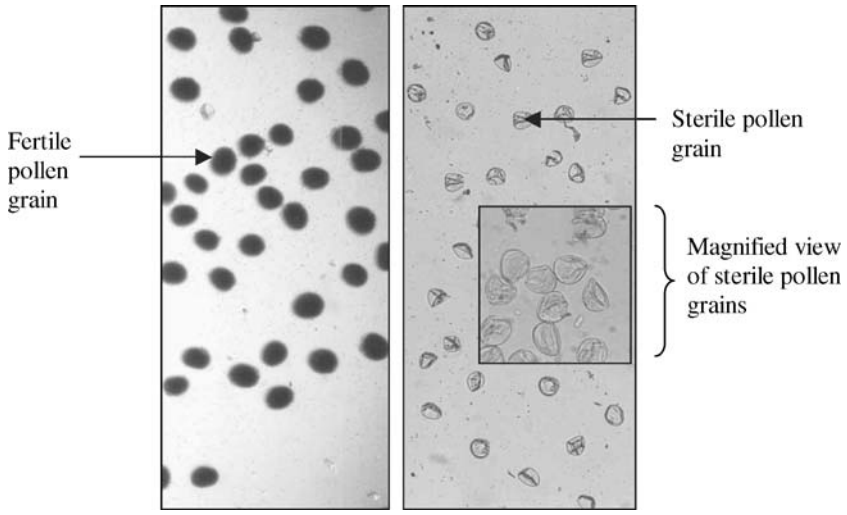

Figure 1. KI-I_2 – stained fertile and sterile pollen grains for the control and CHA – treated plants of cv. PBW 343 following treatment with CHA A1.

transparent and clearly distinguishable from fertile pollen (Figure 1).

Effect of CHAs on performance parameters

CHA A1, A2, A4, A5, B3, B5, and B6 were the most effective and selective CHAs for wheat. CHA A3 and B4, though effective were found to be lacking in selectivity. For example, ethyl 4-trifluoromethyl oxanilate (A5) did not cause a significant reduction in plant height in the wheat genotypes (Figure 2). Whereas both ethyl 4-fluoro (A1) and ethyl 4-bromo oxanilate (A2) induced very small (2.5–3.0 cm) reductions in plant height irrespective of the genotype tested. CHA B1 and

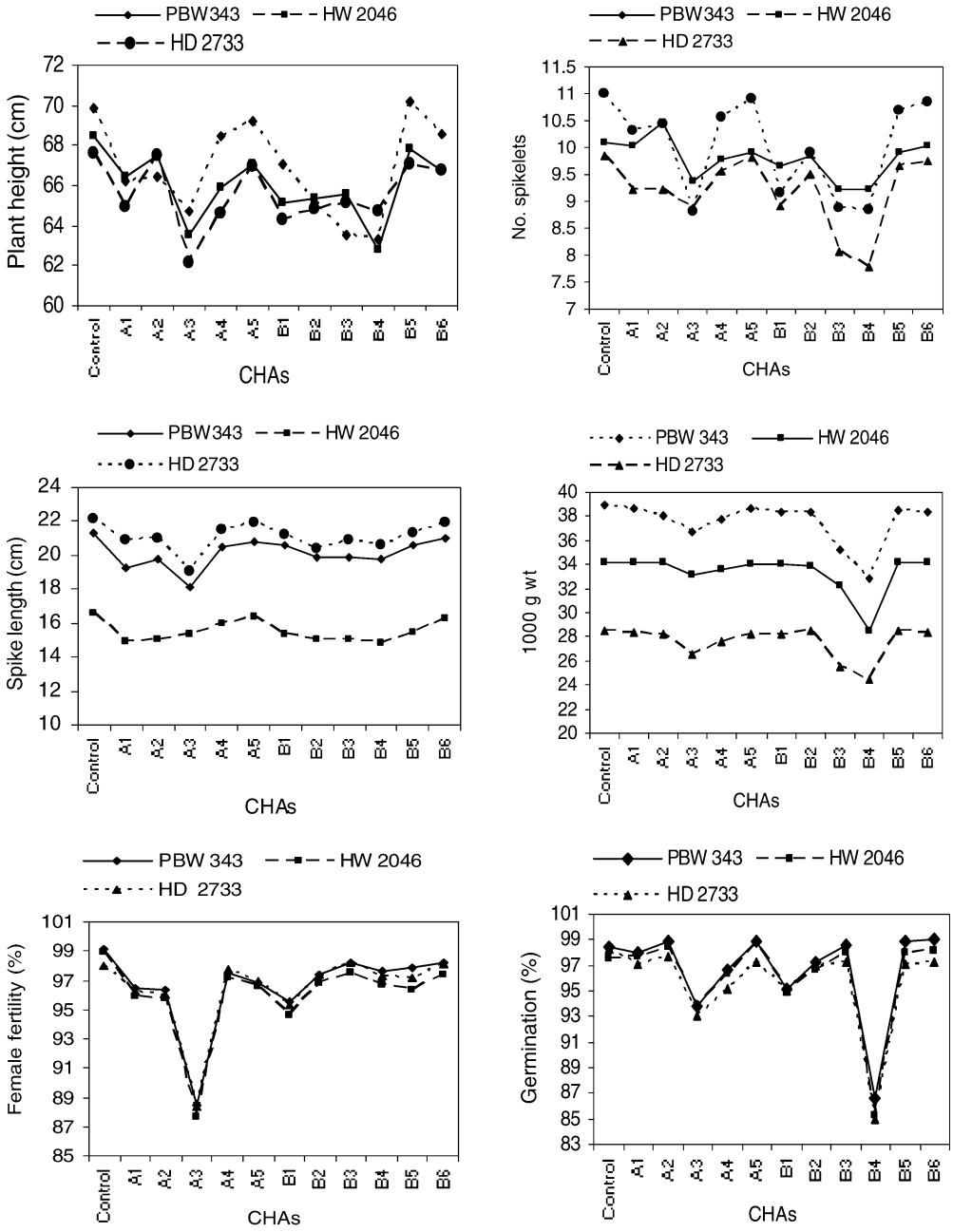

Figure 2. Effects of selected CHAs (tested at 1500 ppm) on various traits of three wheat genotypes (PBW 343, HW 2046 and HD 2733); CHAs (l. to r.): control and CHAs in same order as listed in Table 1.

B2 treatments showed plant heights of 65.49 ± 1.42 and 65.08 ± 1.28 cm, respectively. Both spike length and spikelet numbers were not significantly reduced from their respective control values. CHA A1, A2, A4, A5, B5, and B6 did not show significant reductions in one-thousand grain weight. CHA B3 showed a 2–3 g reduction in thousand grain weight, whereas B4 caused a reductions of 3–4 g. The data pertaining to female fertility revealed that CHA A5 and B6 showed marginal reductions in grain weight. CHA A1, A2, and A5 exhibited 96.23 ± 0.33, 96.08 ± 0.33, and 96.75 ± 0.17% female fertility, respectively, in the wheat genotypes. CHA B3 and B6 treatments similarly led to very high overall female fertilities (97.98 ± 0.43 and 97.89 ± 0.40%, respectively). CHA A3 had a detrimental effect on female fertility. CHA A5 and B6 exhibited marginal or no reduction in germination percentage (Figure 2). CHA A3 and B4 showed reductions of 6 and 13% germination compared to the emulsion control. 4-fluoro pyridone (B1) exhibited a reduction of 2.5 to 3.5% germination, whereas 4-bromo pyridone (B2) caused a 1–1.5%.

The chemical induction of male sterility through the suppression of pollen formation is a rapid and flexible method to prepare hybrids. The first generation

Table 2. Effects of ethyl 4-fluoro oxanilate (1500 ppm test concentration) on certain traits of 29 wheat cultivars

Genotype	Plant height[a]	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)	1100.00	97.15	11.53 (11.83)
HW 2046	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

[a]Figures in parentheses indicate control values.

of chemical compounds to be tested as CHAs 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 second generation of CHAs, viz, GENESIS®, was developed and tested specifically for CHA activity (Collantes et al., 1999). These generally worked within narrowly defined conditions. Phytotoxicity often occurred outside of these conditions, which generally included a narrow window for chemical application and a restricted array of genotypes. The second generation of CHAs developed in this study provide for improved seed quality and can be used on a wide array of genotypes. The most potent CHAs (a shortlist of 6 CHAs from a pool of 11) had less impact on agronomic traits (plant height and spike length) and female fertility.

The reason for the lower persistence of the chemicals in soil, water and tissues is high hydrophilicity (water solubility) of the CHAs. Unlike chemicals like DDT and HCH which are highly persistent in tissues due to lipid solubility, the present CHAs are excreted from the body due to their high polarity (water solubility). The solubility and log (octanol/water) value of CHA A1 were experimentally recorded to be 0.5332 g/l and 1.506 at 25 °C indicating very high water solubility. It can be concluded that there are no residue problems with these chemicals and they appear to be safe although detailed toxicological studies on birds and mammals have not been undertaken. The residues of CHA A1 were non-detectable in mature grain and husk.

CHA A1, identified as a potent CHA from our preliminary screening induced significant male sterility ($99.76 \pm 0.37\%$) at 1500 ppm over 29 wheat cultivars (Table 2). There was an overall reduction in mean plant height of about 5 cm and this was partly contributed by blank emulsion as well. The treatments did not cause significant reductions in spike length of the female parents. The chemical induced very marginal female sterility (overall female fertility was $96.78 \pm 2.07\%$).

The CHAs modified the reproductive biology to ensure cross-pollination in the cleistogamous wheat flowers (Figure 3). Wheat is a self-pollinated crop having closed florets. Florets of male sterile wheat opened twice to facilitate the cross-fertilization. The first floret opening with the action of lodicules lasted only for a shorter period. The second floret opening starts after the lodicules have collapsed and the carpel in the sterile floret forces the palea and lemma apart. This second opening lasted for 5–6 days and was more than adequate for cross-pollination to occur. The extent of cross-pollination without cutting the palia and lemma is indicative of natural floret opening, stigma receptivity and out – crossing percentage. The peak period for stigma receptivity lasted for 3–4 days, providing some flexibility for hybrid seed production. The most potent CHAs, especially CHA A1, should facilitate hybrid seed production without greatly affecting important agronomic traits and thus have potential for development of commercial wheat hybrids.

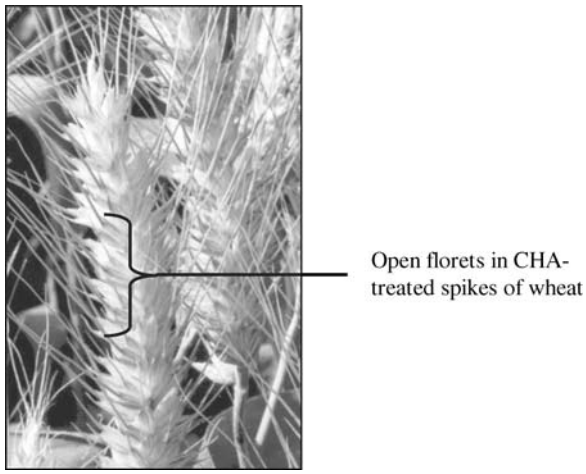

Figure 3. Ethyl 4-fluoro oxanilate – treated spikes of cv. PBW 343 showing open florets receptive to pollination.

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