weight range of 24–45 kDa also occurs in response to auxin treatment, accompanying caulonema differentiation in the auxin-sensitive mutant. This indicates that probably these chloroplast polypeptides accumulate during auxin-induced protonema differentiation. The fact that two of these polypeptides (Figure 2 *e*; lane 4, shown with arrowhead) also appear in untreated cultures at a later stage of growth, is in agreement with some degree of caulonema differentiation at this stage[15], indicating that these chloroplast proteins are caulonema-specific and their accumulation is promoted by auxin treatment. Likewise, certain high molecular weight proteins expressed in chloroplast preparation of the auxin-insensitive mutant (marked with arrowhead) indicate their preferential localization in the chloroplasts. Thus, some of the auxin-regulated polypeptides in the whole tissue extracts seem to be of chloroplast origin. Auxins have been found to induce the synthesis of new specific proteins in many higher plant tissues. Transcriptional regulation of auxin-responsive genes is well established for soybean genes, whereby activated as well as restricted genes are found with respect to specific tissues or organs[3,16]. Therefore, it can be expected that in moss protonema, which has a very specific auxin-regulated protonema development, differentiation-specific proteins are detected.

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Screening chemical hybridizing agents for development of hybrid wheat

The wheat (*Triticum* spp.) production during 1998–99 was around 73.2 million tons from 26.2 million hectare. The north-western plain zone (NWPZ) that sustains the food security system accounts for 36 per cent of cropping area, i.e. 9.5 million hectare. Here the per hectare productivity has become static and genetic gain of just 1% yield per year is being achieved by conventional plant breeding efforts. Therefore a need has arisen to look into alternate plant breeding approaches to break the present deadlock of stagnant per hectare productivity. The heterotic advantage that is being harnessed in maize, sunflower, sorghum and rice offers an exciting opportunity to increase wheat yield further. Wheat being a self-pollinated crop, an allo-hexaploid and a natural hybrid has several inherent limitations in heterosis breeding. But the new chemical hybridizing agent (CHA) molecules offer opportunities to induce selective male sterility, promote out-crossing and develop viable hybrids.

Efforts to induce male sterility in wheat with chemicals were initiated in the early fifties with little success. In the early seventies, a number of private concerns developed and tested chemicals like Dalpon, DPR 3778, Estrone, Gibberellic acid, Hybrex, LY 195259, Mendok, RH series, WL 84811, etc.[1]. These chemicals can be classified as growth regulators, growth retardants and analogues and exhibited partial to near-complete male sterility along with variable damage to plant vegetative growth and flowering. The discovery of CHA or gametocides provided a new way for inducing male sterility, thereby enhancing the process of selecting parental lines leading to commercial heterosis. In the last 10 years significant efforts have been made for commercial exploitation of hybrid wheat through the use of gametocide. In India research efforts on hybrid wheat were initiated in the mid-sixties primarily using a three-line approach; however, no significant results were obtained. Since 1995, we have re-addressed the issue of hybrid wheat by synthesizing 41 different molecules that were considered as potential CHA candidates. The objective was to identify a suitable CHA to induce perfect male sterility at a suitable dose and crop growth stage.

Two spring wheat varieties, viz. WH 542 (JUP/BJY//URES) and PBW 343 (ND/ VG 7944//KAL/BB/3/YACO'S'/4/VEE # 5'S') were grown in a plot in four rows of two metres each at the Directorate of Wheat Research, Karnal. Twenty-three molecules at different doses were evaluated over WH 542 and PBW 343 for their efficacy to cause selective male sterility.

Each formulation (100 ml) was sprayed once on the middle two rows of wheat while the outer rows were used as pollen source. A control plot was also maintained by spraying plain water without any CHA molecules.

CHA application was done using a fine spray pump that deposits a highly reproducible and uniform volume/unit area. The chemical along with 'Tween 80' as spreading agent was sprayed once when the length of spike on the first tiller in the plot was 7–8 mm (stage 22)[2]. In each plot the main tiller were chosen by stretching the representative plants along with leaves and picking up the longest leaf. At this point the plants measured 25–35 cm and were 40–45 days old.

Seven spikes in each plot were bagged at pre-emergence or decimal growth stage 50 (ref. 2) when awns were just emerging. Percentage of male and female sterility induced by the CHA[3] and the extent of seed shrivelling (scale 1–5) after storing the seed for one month, percentage of good seeds, and percentage of seeds that are shrivelled/plump and their germination[4] were recorded. In a scale of 1–5 seed shrivelling, '1' indicates normal seed and '5', a completely shrivelled seed. Data were also recorded for morphological characters like plant height.

Among the 23 molecules of CHAs synthesized and evaluated, only 6, viz. CH 9701, CH 9702, CH 9708, CH 9714, CH 9831 and CH 9832 exhibited acceptable levels of male sterility at different doses (Table 1). The effective chemicals can be broadly grouped on the basis of presence of single or double halogen. Group I with CH 9701, CH 9702 and CH 9708 has a single halogen while CH 9714 has double halogen. The biological efficacy of CH 9714 was further improvized as CH 9831 and CH 9832 (group II). These new molecules induced similar levels of male sterility as CH 9714 even at lower doses (Table 2) on WH 542 and did not interfere with female fertility. A number of molecules like Croisor[5], Hybrex[5], SC 2053 (refs 6, 7), Jin Ao Lin[8], WL 84811 (refs 9–12) (azetidine- 3-carboxylic acid), MON 8164 (ref. 13), EK[14] and ES[14] are known to be effective CHAs.

Ideally for effective commercialization of hybrid wheat, the CHA should be equally effective over a range of genotypes preferably at a single dose. Group II was uniformly effective on both WH 542 and PBW 343, permitted good seed set and did not interfere with seed germination. There was no visible deformity/change in the plant at the time of flowering (Table 2). At acceptable levels of male sterility, the female sterility was lowest with CH 9832 at 500 ppm (10.7%) followed by CH 9702 at 400 ppm (29.4%) in WH 542 (Table 2). The maximum female sterility (96.4%) was observed with CH 9831 in PBW 343. At suitable spraying time (primodia formation to connective state) SC 2053 (ref. 6) showed a relative male sterility over five varieties ranging from 89.3 to 100% (ref. 6). Also the relative male sterility on variety Jinmai No. 2 increased from 72.7 to 99.9% as the dose of SC 2053 increased from 0.3 to 0.9 kg/ha (ref. 6). In our experiments group II molecules were equally effective as group I in causing male sterility, seed setting and for other related characters.

Table 1. Doses of effective molecules in wheat

CHA	Doses (ppm)
CH 9701	50 100 125 150 175 200 250 500
CH 9702	100 150 200 225 250 275 400 500
CH 9708	50 100 150 200 250 300 350 400 500 700
CH 9714	1000 1500 2000
CH 9831	100 200 250 300 500 1000
CH 9832	100 200 250 300 500 1000

Table 2. Performance of chemical hybridizing agent exhibiting perfect male sterility at 7–8 mm of spike length for important characters in wheat

CHA	Variety	Effective dose (ppm)	Female sterility (%)	Good seed (%)	Shrivel seed (scale 1–5)	Germination (%)	Plant height (cm)
Group I (Single halogen salt)							
CH 9701	WH 542	250	90.7	85	2	85	78
	PBW 343	500	88.3	75	3	95	69
CH 9702	WH 542	400	29.4	85	3	55	63
	PBW 343	275	79.3	90	3	70	84
CH 9708	WH 542	400	51.8	75	3	60	72
	PBW 343	250	77.8	95	1	75	79
Group II (Double halogen salt)							
CH 9831	WH 542	1000	69.7	80	2	50	66
	PBW 343	1000	96.4	30	1	60	70
CH 9832	WH 542	500	10.7	75	2	65	75
	PBW 343	500	40.2	75	2	90	77
CH 9714	WH 542	1000	30.8	75	3	60	78
Check							
Untreated	WH 542	0	0	100	1	100	78
	PBW 343	0	0	100	1	100	81
Mean			51.2	80	2.08	74.2	74.6
SE ±			9.3	4.7	0.23	4.7	1.6

CH 9832 @ 500 ppm had lowest female sterility in both the varieties. The per cent seed set, seed shrivelling and seed germination were also comparable. In fact spraying CH 9832 hardly had any effect on the height of the plant or its development. This molecule therefore is a potential candidate for inducing male sterility in wheat and offers an opportunity for the development of 'hybrid wheat'. Further researches in this direction are on to develop a commercially viable hybrid.

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