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Induction of male sterility in lentil

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Abstract
The objective of this experiment was to evaluated the male sterilizing activity induced by the chemical hybridizing agent (CHA) on lentil (*Lens culinaris* L.).
Male sterility is defined as an absence or non –functional of pollen grain in plant in capability of plants to produce or release functional pollen grain. The use of male sterility in hybrid seed production has great importance as it eliminate the process of mechanical Emasculation. Chemical hybridizing agents (CHAs) can be used on a large scale for commercial production of hybrid seed. Most CHAS are applied to plants only at a critical stage of male gametophyte development. The aim of this study was to investigate an induction male sterility has been developed by surf excel in lentil. *Lens culinaris* L. surf excel a detergent known to induce male sterility in some crops and pollen sterility in lentil. Foliar application of surf excel brought about complete pollen sterility. This pollen sterility was long lasting. The effect of surf excel treated plants caused almost complete sterility ranging between 91.33-99.00%.

Keywords: lentil plant growing in garden, surf excel TTC solution FCR solution, fluorescence microscope, light microscope

Introduction
Chemical induction of male sterility in plant was first demonstrated by moore (1950) [14] and Naylor (1950) [16], various terms such as male sterilant, selective male sterilant polled suppressant, pollenocide, androcide and chemical gametogide, have been used but such terms may not embrace all conceivable modes or site of action. The terms chemical hybridizing agent (CHAs) was suggested as. Appropriate (Mc. Rae, 1985) [15]. These chemicals caused a range of effects including ferminization of male florets and inhibition of early anther development. Interference with to Tepetal functions and microscore development and defective germination of the mature pollen. The molecular and physiological mechanisms of these effects are unknown. The heaviest research investment has favoured discovery of chemical hybridizing agents for field crops that naturally reproduce by self pollination but lack the profit margin per seed to justify hand emasculation. (Mc. Rae, 1985) [15].
There are atlest four classes of chemical agents in current literature:

1. Plant growth regulators and substances that disrupt floral development
2. Metabolic inhibitors.
3. Inhibitor of microspore development.
4. Inhibitor of pollen fertility.

Chemical hybridizing agents (CHAs) can be used on a large scale for commercial production of hybrid seed.
Most of the CHAs are associated to varying degree of male sterility with other forms of growth regulating activity or sites of phytotoxicity, at least when Overdoses or applied at other times during the life cycle (Mc Rae, 1985) [15]. Stunting, Chlorosis or the leaves and reduced seed set are encountered with some regularity. A highly selective and successful
CHAs should not be excessively expensive it can be applied effectively in the field and it must be perfect for environment and safe for workers and consumers (Mc Rae, 1985) [15].
Certain early, CHAs had adversed effects on seed yield or seed quality from the treated plants. The Agronomic performance of hybrid seed production with leading chemical pollen suppressants is not inferior to equivalents crosses produced by genetic methods.
(Mc Rae, 1985, Oury *et al*. 1990) [15, 17]. Azetidine - 3 Carboxylate (A3C) effectively induce male sterility in small grains, particularly wheat (Cross and Ladyman, 1991, Kofoid, 1991) [8, 11].
Baydar and Gakmen (2003) [1] from their studies on GA3 induced male sterility in safflower reported that there successive sprays (75,82, and 89 days after sowing) with 100 ppm of GA3 safflower buds of less than 0.5 cm dia meter at a pre meiotic interphase stage resulted in reduced pollen viability from 81.6 to 6.7 percent compared with the control. The GA3 treatment did not significant affect the production of viable achenes. Khulbe *et al*. (2003) [10] induced male sterility in wild and releted species of sunflower (*Helianthus annus* L). using GA3 at different concentration (50,100, 200, and 500 ppm and different bud sizes (0.7, 0.8, 0.9 and 1.0 cm) in case of H. deblis and 1.5,2.0 and 2.5cm in H. argophyllus). representing different growth stages. Sreedhar (2003) [21] studied the induction of male sterility in niger using GA3 (100 and 150 ppm)and detergent solution (1and 2 per cent) during primary bud star bud stag. He concluded that GA3 is the most protent gametocide for induction for male sterility to the extent of 80.21 percent when sprayed for three times at a concentration of 150 ppm compared to detergent solution (surf excel). Lal *et al*. (2004) [13] studied use of gametocide for

emasculation in soyabean, Ethyl 4 fluoroxanilate at 1000 ppm was used to induce male sterility in Soyabean pollen sterility varied from 20.03 to 55.55 percent in the treated samples compared with 1.37 to 2.63 percent in the control, indicating the effectiveness of the gametocide in inducing male sterility.

Yu *et al*. (2006) [22] studied induction of male sterility in brassica napus. A sulphonylurea herbicide, tribenurn methyl, methyl-2[4-methoxy-6 methyl-123 triazinyl methylaminenes carbonyl amino] Sulphonyl benzolate] was used to induce male sterility in rapeseed. Application of 0.2 micro of tribenuren methyl per plant at the bolting stage with the longest floral bud < 2 mm and repeated 15 days after words. resulted in 94-5-100 percent plants being male sterile in different breeding lines, but combined with low phytotoxicity.

Chemical induction of male sterility has been developed primarily for the production of hybrid corps. The highly competitive hybrid seed market has narrowed the field to a few active agents currently under commercial developments. Because prior knowledge of their Bio-Chemical action was not required for commercialization their mechanisms are largely unknown.

There are large number of chemicals which inhibit the gamete development and they are known as gametocidal compound or also called as chemical hybridizing agent (CHAs). These chemical posses selective gametocidal properties, when applied or sprayed on the plant at the appropriate growth stage, they induce male sterility. Recently, male sterility have also been induced in plants by introducing chimaeras ribonuclases genes, chemical induction of male sterility has been considered desirable because it has the unique potential to directly out of elite germplanm, without the time and efforts required to regressively backcross male sterility genes and fertility restoration system. In several species, the available male sterile genes or the known restoration systems have not been sufficiently effective or reliable for commercial production of hybrid seed. There has been hope that chemical induction of male sterility would solve these problems (Mc. Rae, 1985) [15].

Pulse are a very important source of protein in India diets as majority of population is vegetarian. Sharma and Sharma (1978) [18] have induced male sterility in lentil var. L-235, Following mutagenesis with gamma rays and NMU. The Male sterile plant had reduced inter node length, profuse branching and matured later than the male fertile plants. They remained green part the usual maturity and had dark green thick leaves.

Materials and Methods

Experiment was conducted on lentil *(Lens clulinaris* L.)

In present experiment following chemical hybridizing agent was used.

Surf excel

Synthetic detergent powder consists of surface active agents, builders, phosphates and filters ‑ phosphate are added to detergents as builders for removing the hardness of water in addition they have additives like antideposition agents optical brightners etc.

Preparation of solutions

Surf excel the aqueous solution of different concentrations (0.5, 1. 0, 1.5%) was made by dissolving it with the distilled water. Number of sprayes. Three sprays of the both the chemicals Amount of chemicals -25 ml. of each chemical was used in each spray.

Spray of Chemicals

T1 = Single spray at pre floral bud initiation

T2 = Two sprays one at pre floral bud initiation stage and second at post floral bud initiation stage.

T3 = Three sprays one at pre floral bud initiation stage, second at post floral bud initiation stage and third at the time of anthesis.

The control plants were sprayed with distilled water.

Pollen Fertility

Pollen fertility is the ability of the pollen to fertilize. There are two tests for pollen fertility.

A. Stainability Test: (After staining pollen acquire colour)

1. TTC Test: 1% Tetrazolium chloride solution in 0.15 –Tris HCL buffer at 7.8 ph was used for testing the pollen viability of treated and untreated plants. Thissgive pink / red colour with viable pollen grain and non viable pollen grain are colourless.

2. FCR Test: (fluorochromatic Reaction) Heslop-harrion *et al.*; (1984).

- Take 2-5 ml of sucrose solution in a small glass tube, add drops of stock solution of FAD until the resulting mixture shows persistent turbidity.
- The mixture should be used within 30 min. from preparation.
- Take drop of sucrose-FAD mixture on a microslide.
- Suspend sufficient amount of pollen grains in the drops and ensure uniform distribution of the pollen in preparation.
- Incubate the preparation in a humidity chamber (>90RH) for 5-10 min.
- At the end of the incubation period, lower a coverglass and observe the preparation the fluorescence microscope with suitable filters.

B. Germination Test: (pollen tube germination on artificial culture medium.)

In vitro: Studies by hanging drop technique using Brew –Brew and Kwack's medium (1963).

Composition

Sucrose	10gm/l
Calcium nitrate	300 gm / l
Potassium Nitrate	100gm/l
Magnesium Sulphate	200gm/l
Borate	100gm/l

C. Light Microscopic Studies

Result and Conclusion

Pollen Fertility

Surf excel used in the present investigation have been found to be effective in inducing complete pollen sterility. The results obtained by surf excel has been discussed in following:-

Surf Excel

Surf excel was found to be very effective in inducing

complete pollen sterility.Thrice treatments of 0.5,1.0,%surf excel and all the treatments of 1.5% surf excel also brought about complete pollen sterility. Induction of complete male sterility by the surf excel have been recorded in *Brassica juncea* (Chauhan and Singh, 2002) [4] in *Gossypium arboreum* (Chaudhary, 2002) [7] and in *Vicia faba* (Chauhan and chauhan, 2003) [6].

Male sterility induced by the detergents may be occur due to presences of sodium carbonate and phosphate in the detergent.This is supported by the fact that, another synthetic detergent, Nirma has also been found to be effective in inducing male sterility in rice (Singh,1999) [20].

All the treatments of 1.5% surf excel and three sprays of 0.5% and 1.0% surf excel induce 100% Pollen sterility.

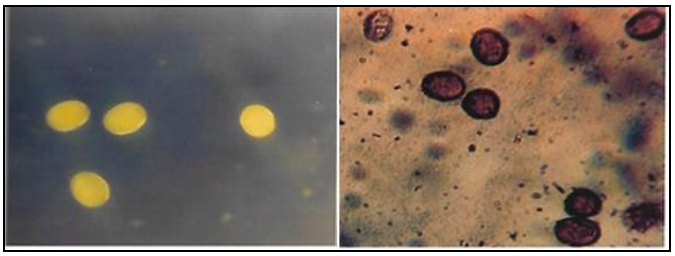

Fig 1: (FCR)

Fig 2: (TTC)

Viable pollen Grain of Control Plant as Checked by FCR & TTC Test

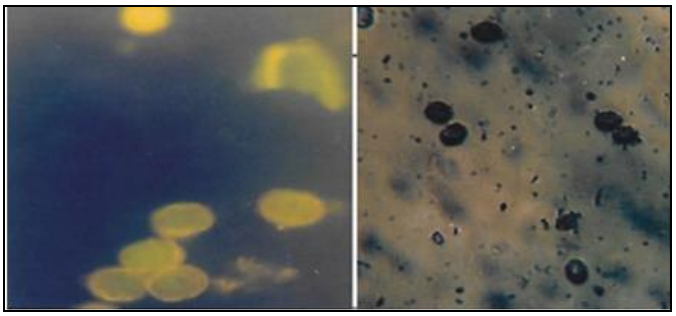

Fig 3: (FCR)

Fig 4: (TTC)

Non-Viable Pollen Grains in Plant Treated Thrice With 1.5% Surf Excel Checked By FCR & TTC Test

Light microscopic studies

Tapetum

The observation on another development in surf excel induced male sterile plants along with their fertile counterparts show that pollen abortion in these male sterile lines is associated with varying degree of abnormal tapetal behaviour.

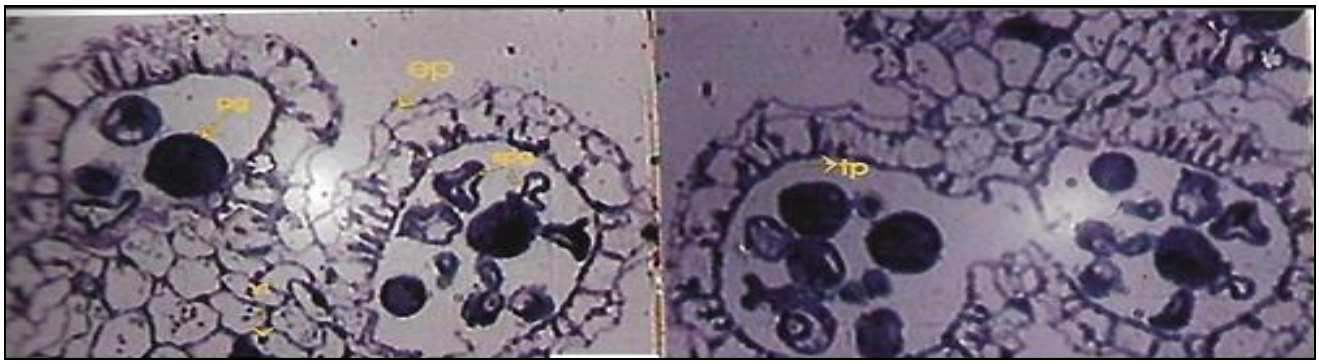

Fig 5: Deformed Aborted Pollen granins (spg)

Fig 6: narrow band of tapetum (TP)

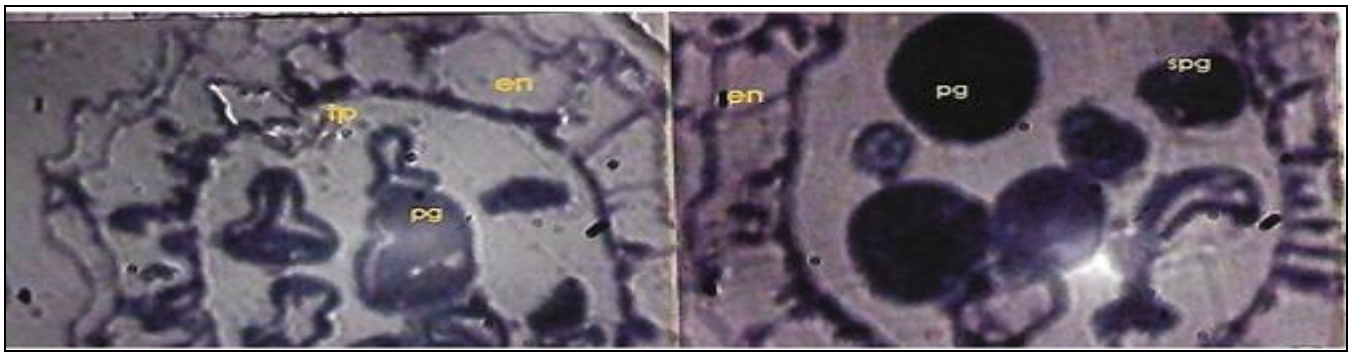

Fig 7: Thick Walled Endothecium (en)

Fig 8: Shriveled (spg) and comma Shaped Pollen Grains

Delayed degeneration of tapetum

Delayed degeneration of tapetum is found in anthers of surf excel treated plants. Delayed degeneration of tapetum has also been reported in male sterile lines of various plants by a number of investigater (Kaul, 1988; Shivanna and Johari, 1985; Chauhan and Kinoshita, 1982) [12, 9, 3].

This type of abnormal behaviour of tapetum was also recorded in *Brassica juncea* and *Gossypium arboreum*.treated with different CHAs (Chauhan *et al*. 2003) [6].

The tapetal calls degenerate to provide nutrition to pollen grains in subsequent stages.

The normal growth of the pollen grains is hampered by short

delay in tapetal degeneration and they fail to endure and abort resulting in to complete pollen sterility.

Non-viable pollen grains acquired irregular shape and became so compressed that they appear as rod shaped. The vascular supply was also poorly differentiated these treated plants.

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