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Chemically induced male sterility in hybrid breeding of vegetables: A review

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Abstract
Large scale hybrid seed production in vegetables remains handicapped because of high labour cost or unavailability of trained labour for manual emasculation. Wide range of genetic mechanism such as male sterility, use of sex regulators, self- incompatibility and use of chemically induced male sterility eliminate the need for manual emasculation. The chemicals such as Maleic hydrazide, Gibberellins, Dalapon, Mendok, Ethephon etc. disrupts the normal pollen development inducing male sterility without affecting female functionality are known as male gametocides or chemical hybridizing agents. An ideal hybridizing chemical must selectively sterilize male gametes, economical, non- mutagenic, environmentally safe, easy and flexible in use and time of application. Gametocides result in disruption of meiosis and exine formation, abnormal growth of tapetum, delayed dehiscence of developed anthers or non- germination of pollen on stigma, which ultimately cause male sterility. Optimum environmental conditions, synchronous flowering in male and female parents and cross pollination is required to maximize the hybrid seed production. They had been successfully used in many vegetables such as *Coriander sativum, Abelmoschus esculentum, Allium cepa, Capsicum annuum* and various cruciferous plants. Although male gametocides have relatively larger application in cereal crops but their use in vegetables is yet to be commercialized.

Keywords: Vegetables, male sterility, chemical hybridizing agents, gametocides, hybrid seed production

Introduction
Vegetables are the most extensively utilized plants for exploitation of heterosis. Large scale hybrid seed production sometimes remains handicapped because of high labour cost or unavailability of trained labour. Discovery of male sterility widens the basis of hybrid seed production (Dhall 2010) [8]. Male sterility in vegetables is commercially utilized to develop hybrids and substantial progress has made in understanding the mechanism of male sterility (Mopidevi *et al* 2017) [22]. Male sterility can be defined as inability to produce or release functional pollen, and is the result of failure of formation or development of functional stamens, microspores or male gametes. Male sterility can be classified into two major groups i.e. genetic (spontaneous or induced) and non-genetic (induced) male sterility (Kaul 1988) [12]. Under non- genetic type sterility, chemical induced male sterility is important.

The sterility which is non- genetic, non- heritable and induced with the help of chemicals that makes the male part abortive, known as chemical induced male sterility. The chemicals such as Maleic hydrazide, Gibberellins, Dalapon, Mendok, Ethephon etc. which disrupts the normal pollen development without affecting female functionality are known as gametocides (Kempe and Gils 2011) [14]. These are also known as chemical hybridizing agents (CHA), male sterilants, pollen suppressants, pollenocides, androcides etc. These gametocides sterile the male part of female parent so that female parents can be effectively utilized for hybrid seed production. Chemical induced male sterility have major application in cereal crops but in vegetables, its use has yet to be commercialized (Muthuvel 2003) [24].

In general scheme of producing a hybrid through chemical induce male sterility, firstly a gametocide or CHA must be sprayed on the fertile female parent. The gametocide abort the pollens and make the female parent male sterile. After that, the male sterile female parent is crossed with male fertile pollen parent. The resultant F_1 hybrid produced is male fertile (Kempe and Gils 2011) [14].

History

The first success in the chemical induction of male sterility was reported in 1950. Moore (1950) [23] and Naylor (1950) [25] demonstrated that male sterility could be obtained in *Zea mays* L. (maize) with maleic hydrazide. The same year, Laibach and Kribben (1950) [17] first published that α-naphthaleneacetic acid and β- indoleacetic acid could be used to increase the proportion of female flowers in *Cucumis sativus* L. (cucumber). In this case, male sterility is not required for hybrid seed production, but, it is only necessary to disrupt development of staminate flowers so that most of the functional flowers are pistillate. Therefore, the chemicals that are effective in altering sex expression cam be used as hybridizing agents also.

Need of chemical induced male sterility

Even we have various male sterility systems such as GMS, CMS and CGMS, then why, there is need of CIMS chemical induced male sterility. Firstly, to overcome the tedious process of emasculation, CIMS is needed. Dhaliwal (1986) [7] reported that about 40% of labour cost involved in emasculation process of hybrid seed production. Alike in other systems, there is no need to identify and maintain male sterile and restorer lines. Thus the lengthy time period is reduced. Beside these, in genetically controlled male sterility, there is problem of detecting, roguing and then restoration of male sterile lines. Such type of problem does not arise in chemical induced male sterility (McRae 1985) [18].

Characteristics of an ideal gametocide

To induce male sterility through chemicals, firstly an ideal gametocide or CHA is required. An ideal gametocide must possess properties like induces male sterility only and does not affect the female functionality. They must induce complete male sterility i.e. more than 90 % by inhibiting pollen development. It must be free from varied environmental conditions and genotypic differences. They have wide flexibility in rate and time of application i.e. not be stage specific. They are non- mutagenic and should not have any phytotoxic effects. They must be environmentally safe and do not have any carry over effects in seeds of F_1 hybrids. Seed set should not be effected by its application. It must be cost- effective because chronic sprays of gametocides makes the hybrid seed production costly (Adhikari 2012) [1].

Mode of action of gametocides

Rajendra and Bates (1981) [28] postulated three modes of gametocidal action. He postulated that gametocides cause pollen abnormalities by directly act on genetic process of meiosis. The gametocides could act directly on the gene (or) genes for pole determinants and (thus disrupt the meiotic spindle formation, which in turn would result in abnormal cytokinesis. Any disruption of pole and pole determinants would lead to unequal distribution of chromatin, resulting in polymorphic pollen and multiporate (or) sporate pollen. The gametocide also disturbs the gene expression for exine differentiation also. Secondly, they could be involved in carbohydrate metabolism, which somehow starves the tapetum and makes it persistent. As nutrients cannot be transferred to the developing pollen and thus pollen remains non- functional. But the pollen and meiotic study suggest that microspore degeneration is more likely due to meiotic abnormalities. The third potential mode of action could be directly on DNA synthesis prior to entry of cells into meiosis. The gametocides may act on some of the chromosomes before entry of cells into prophase-I which could delay the condensation of the chromosomes which in turn delays bivalent formation. The final result of this abnormality would be expressed as partial cleavages, aporateness, multiporateness and other pollen abnormalities.

Effect of gametocides

Most studies on the site and mode of action of CHA, have concentrated on observable micro and microscopic events that takes place in anthers. These actions can induce a range of specific effects initiated from early stages of sporogeneous cells to non- dehiscence of viable pollens. Some general effects includes: Disruption of meiosis at early stage lending of arrest of anther development and degeneration of pollen mother cells (PMC) or curly microspores, Disruption of exine formation resulting in thin walled, irregular and non-viable microspores, Decrease of starch deposition and appearance of abnormal vacuoles in the microspores making them non-viable, Persistence and abnormal growth of tapetal layers, indehiscence or delayed dehiscence of normally developed anthers within viable pollen and Non- germination of pollen on stigma or cessation of elongation of pollen tube resulting in non- fertilization (Kumar and Singh 2005) [16].

Histological studies of microtome section in okra, it was revealed that development of anther up to meiotic division in pollen mother cells alike in both male fertile and male sterile flowers. But in sterile anthers, tapetum persists for a longer period and sterile anther is vacuolated and crumpled. But in fertile anthers, pollen grains are turgid, fully developed and full of cytoplasm (Deepak *et al* 2007) [6].

Factors affecting cha technology

Our main objective is to produce a hybrid. The technique for making hybrids with CHA is identical to CMS method. The only difference is that functional male sterility in female parent is due to chemicals rather than by genetic manipulations. The utilization of CHA is dependent on the factors such as source of viable pollen from a male parent that can outcross the male sterile female parent, female planting configuration that can maximize out crossing, an pollinating agent such as wind or insects to transfer pollens from the male parent to the female parent, Synchronization of female and male parents i.e. pollen from the male parent must shed when the stigma of female parent is receptive, an abundance of pollen from the male parent and good flower opening characteristics in the female parent.

Method of application of gametocides

The most common and easy method of applying gametocides is in the form of aqueous sprays, before or at the time of floral bud initiation (Saimbhi and Brar 1978) [33]. Injection with a syringe is another method (Chopra *et al* 1960; Kaul and Singh 1967; Kumar, 1969) [3, 13, 15]. Meer and Bennekom (1973) [20] tried 3 different methods *viz*. dipping bulb bases in solution of GA_3, sprays with GA_3 and injection by a micro-syringe to the inflorescence stalk. The injection was rather complicated, ineffective and not suitable for practical application. Bajracharya (1977) [2] observed no difference between injection and foliar spray methods in onion, but injection increased the injury.

Stage of application of gametocides

There are three stages at which gametocides should be applied: Pre- meiotic, Meiotic and Post- meiotic phase. At pre- meiotic stage they affect normal development of anthers and PMC's by disturbance to plasmodesmata, which connects sporogeneous and tapetal cells. Once PMC's are formed, tapetum cause sterility by over growth in terms of either formation of double layer or clone action or tapetal cells prior to callus formation of PMC's leads to crushing of PMC's. A blockage in synthesis of calluses may also render the PMC's sterile and cause pre-meiotic disturbance. If the developmental process reaches meiotic phase, then gametocides effect in the form of varied cytological anomalies such as chromosome breakage, irregular pairing, orientation and disfunction of chromosomes, occurrence of laggards and uneven distribution of chromosomes, which affects normal development and viability of pollen. Post-meiotic disturbance starting from tetrad stage extend till dehiscence of anther and release of pollen. Hindrance to microspore separation during tetrad stage cause sterility is due to lack of callose formation. Chemicals specifically disrupting the callose synthesis induce sterility at this stage (Sharma and Sharma 2005) [34].

Naveen *et al.* (2017) [26] found that different development stages of microsporogenesis in okra were occur in 3- 5 mm bud size and almost complete before bud size reach 10 mm. After that, microspore undergo cell division to produce gamete from 10 mm to 45 mm bud size. But developed microspore remain non- viable till the bud size reaches 40 mm and thus do not stain in acetocarmine test. So, study on CHA's for induce sterility was implemented from 40- 50 mm size buds. Among the gametocides used for inducing sterility, Maleic hydrazide @ 450 ppm induce higher pollen sterility. Among the flower bud size, 45 mm size bud showed higher pollen sterility. The results were further confirmed with *in vitro* pollen germination test, which also showed higher pollen sterility in 45 mm flower bud size with the application of 450 ppm maleic hydrazide followed by application @ 500 ppm.

Hybridizing chemicals used in vegetables

There are various groups to which gametocides belongs such as auxins and anti- auxins (Maleic Hydrazide, Tri-iodobenzoic acid, Napthalene acetic acid, 2,4 -D etc.), halogenated aliphatic acids (Mendok and Dalapon), Gibberellins, Ethephon, Arsenical compounds etc. (Colombo and Galmarini 2017) [5].

Auxins and anti-auxins

They cause sterility by blocking nutrient transport to developing anther. As a result, tapetal cells enlarges atypically and becomes persistent. In this group, Maleic hydrazide (MH) is a mitotic poison as it affects the mitosis of microspore. It had been used in crops like okra, capsicum, onion, coriander, tomato, pumpkin. Other auxins like TIBA used in Watermelon and tomato; NAA in cucumber and summer squash; 2, 4 -D in coriander and brinjal.

Deepak *et al.* (2007) [6] conducted a study on chemical induction of male sterility in okra. Results revealed that application of maleic hydrazide induce higher pollen sterility (84.33 %) followed by gibberellic acid (82.20 %) and ethrel (82.00 %) with three sprays (20+30+40 days after sowing). These chemicals also induce ovular sterility, maximum with ethrel (14.83 %). Higher concentration of treatments resulted in reduction of number of fruit per plant, fruit set percentage, number of seeds per fruit and seed yield per plant by causing ovular sterility. Therefore, Maleic hydrazide is safe for hybrid seed production on okra at lower concentration (200 ppm).

Halogenated aliphatic acids

They affect mitochondrial protein by reducing efficiency of normal metabolic processes and cause sterility. Gametocide FW-450 (Mendok) had been used in various studies in various crops like brinjal, watermelon, tomato, radish, okra, spinach, bitter gourd, peas etc. Likewise, Dalapon interferes with mitotic activity of dividing meristem and cause sterility. But it also reduces vegetative and reproductive growth. It had been used in crops like okra, coriander, capsicum etc.

Salgare (1989) [32] studied the gametocidal effect of dalapon on chilli (*Capsicum frutescens*) cultivars Christmas Paper and Holiday cheer. Application of dalapon @ 100 ppm caused 36 % pollen sterility in Christmas Paper and 40 % male sterility in Holiday Cheer.

Gibberellins

They cause sterility by affecting sexual determination and floral development. It also promotes feminization. Nelson and Rossman (1950) induced 100% pollen sterility by using potassium gibberellate. It should be applied at optimum stage i.e. before completion of meiosis. If it is applied at too early stage, it cause inadequate male sterility and at later stages, it results in weakening of stems. It had been used in onion, lettuce, cole crops, capsicum etc.

Spraying of 2 % GA_{4+7} solution resulted in higher number of male sterile plants (26 permanently and 6 temporarily male sterile) in onion cultivar *Rijnsburger*. But spraying of Gibberellic acid also result in lower seed production. Therefore, it can be used as substitute for emasculation for making interspecific crosses, intervarietal crossed and recurrent back crosses. Because only moderate quantity of seed required in these programs (Meer and Bennekom 1973) [20].

In Brussels sprouts, period of complete male sterility was maximum (14 days) when spraying of 50 mg/L GA_{4+7} was done for first four weeks after beginning of flowering. In another experiments, they revealed that higher frequencies of spraying at higher concentration resulted in prolonged period of complete male sterility in Brussels sprout and Cauliflower (Dam 1979) [21].

Ethephon

It was reported as a gametocide in 1969 by McMurray and Miller and Robinson. in the same year. It cause sterility by inhibiting microspore development through disruption of filament and corolla growth. It is economically not feasible and also induces morphological anomalies. It had been studied in crops like cucumber, pumpkin, lettuce, spinach, squash and brinjal.

None of the gametocides (Gibberellic acid, 2, 4-D, Maleic Hydrazide, Ethrel and Surf Excel) found effective in inducing pollen sterility in coriander except 2, 4- D @ 50- 100 ppm and MH @ 125- 250 ppm. They did not have any significant effect on pollen germination. However, spraying of Maleic Hydrazide at 100 ppm from 25 DAS on wards until the cessation of flowering caused suppression of anther dehiscence during entire flowering period due to severe agglutination of the pollen. Therefore, maleic hydrazide was identified as potential male gametocide and an effective alternative for cumbersome hand emasculation in coriander. Successful crosses were obtained using Maleic Hydrazide as chemical emasculation agent (Kalidasu *et al* 2009) [11].

Others

Another gametocides used in vegetables for inducing male sterility were reported by different researches. For example- Dimethyl arsenic acid used in sugar beet (Hecker *et al* 1972) [9], Phosphon in onion (Cohan and Weigle 1966) [4], SADH (Succinic acid, 2,3- Tri- iodobenzoic acid) along with ethrel in muskmelon (Rudich *et al* 1969) [31] and cucumber (Rodriquez and Lambeth 1972) [30].

Muthuvel *et al* (2003) [24] exploring the possibilities of utilizing chemical induced male sterility in *Capsicum annuum* L. Application of higher dose of maleic hydrazide (0.8%) and gibberellic acid (1.0%) induce higher pollen sterility. Application of 1.0% maleic hydrazide resulted in severe scorching that no flowers were formed. Among various cultivars, Arka lohit exhibit maximum sterility. Besides pollen sterility, higher dose of gametocides resulted in lower pollen germination, fruit weight, fruit set percentage and number of seeds per fruit.

Among other gametocides, GS-1 used on cabbage (HongWei *et al* 2009) [10] and sulfonylurea herbicide tribenuron methyl on different species of cruciferous plants (Yu *et al* 2017) [35]. The percentage of male sterile plants reached to 100% with application of 5µg/ml of GS-1 in line MP01 and 20 µg/ml of GS-1 in line Y03. The hybridization rate of hybrid seeds of F_1 is 100%.

Application of tribenuron methyl induce 80-100 % male sterility with lower damage to pistil function. Higher level of male sterility induced by tribenuron methyl brings an opportunity for utilization of heterosis in cruciferous plants. It also suggests the possible use of tribenuron methyl as gametocide in hybrid seed production of these plants.

Hybrid seed production based on cha’s

Plant breeders have exploring effective chemicals and application methods to emasculate female parent pollens for hybrid seed production. In fact, they perform some preliminary works to start producing hybrid seeds of any crop even they used chemical emasculation. Firstly, they must identify or select suitable female and male parent. After selection, they must determine a particular chemical that sterilize pollen without lowering ovular fertility and seed setting potentiality of seed parent. Once they know the exact chemical, then optimum dose of that chemical must be determined for the crop of interest. The chemicals should be applied precisely and correctly on plants of female parent only. After that, there must be a need of pollinating agent such as honey bees or other insects or wind to transfer pollen grains from male parent to female parent. After seed setting or fruit maturity, the matured seeds are harvested very carefully without any seed contamination (Adhikari 2012) [1].

Conditions for hybrid seed production

For hybrid seed production, firstly proper environmental conditions such as adequate sunshine, optimum temperature and relative humidity must be required. There should be no rains during or after spraying gametocides. Besides these environmental conditions, there must be synchronization in flowering of male and female parent so that pollen shed when stigma becomes receptive. It can be achieved by sowing at different dates or by applying nutrients to induce vegetative and reproductive growth. The most important point is the effective chemical emasculation and cross pollination. It can be achieved by application of gametocide at precise stage. It was found that two sprays of a gametocide at the lower dose was more effective than single spray at higher dose. During spraying, care must be taken that no pollen rows were sprayed.

Advantages

There are various advantages of inducing male sterility through use of gametocides. Some of the most important advantages are flexible choice of parents, rapid method, no need of maintaining male sterile and maintainer line, two line based system, fully fertile F2 hybrids, unstable CMS lines also used. Besides these advantages, they are also exploited as breeding tools. They can be effectively substitute the tedious process of hand emasculation. Higher amount of hybrid seed can be produced with relative ease. They are also used in recurrent selection programmes.

Disadvantages

There are also various disadvantages of using chemical induced male sterility. Due to these disadvantages, their use in commercial seed production has been limited. Major disadvantages includes- Dose and duration specificity, depend on environmental factors, genotype specificity, tissue specificity, stage specific, carryover residual effects in F1 seeds, incomplete male sterility, chronic spray, higher cost of hybrid seed production, fertility impairment, phytotoxic, health hazards etc.

Conclusion

It is concluded that we can produce large quantities of hybrid seed with relative ease by effectively utilizing the gametocides. These gametocides are also valuable breeding tools which can be utilized for emasculation in hybrid breeding programmes and in recurrent selection programmes. Furthermore, there is no need of maintaining A, B and R lines in chemically induced male sterility. However, paucity of knowledge regarding dose and duration specificity, ovular sterility and genotypic variations, limit their use in commercial hybrid seed production. In the future, there must be scope for gametocides which possess stable and systemic action. They can also be utilized for chemical restoration of genetic male sterile line. Besides all these aspects, there is limited research on gametocides in vegetables. So there must be need to conduct research on development of gametocides and exploited in vegetable breeding.

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