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Response of Two Local Varieties of Onion to Chemically Induced Male Sterility

Osama H. Alabdalla¹; Rawaa M.M. Albabilie¹; Mouwafak N. Jbour¹, Roula G. Bathoush¹, Mostafa M. Alozon¹, Rasha R. Eid^{2*}

¹ The General Commission for Scientific Agricultural Research Administration of Horticulture Research, Damascus, Syria,

² Potato and Vegetatively Propagated Vegetables Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt

* Correspondence: rashaeid46@gmail.com

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Abstract

The research was carried out during two agricultural seasons 2020 and 2021 at the General Commission for Scientific Agricultural Research in Damascus. The objective was to study the response of two local varieties of onion plants to three chemical treatments, GA₃ (250, 500, and 1000 mg/l), clothes washing powder (5, 10, and 15 g/l), 2,4-D (0.1, 0.5, and 1 ml/l) to induce artificial male infertility. The experiment was designed as a randomized complete block design with four replicates. The results showed the efficiency of used chemicals in inducing male sterility in both varieties. The 1000 mg/L treatment of GA₃ was superior and significantly affected flowering parameters (inflorescence diameter, number of flowers per inflorescence, number of set flowers, percentage of flower setting) of both varieties Baladi red (10.03 cm, 1077.9 flower/inflorescence, 5 set flowers/inflorescence, and a percentage of 0.44%) and Salmon white (9.33 cm, 1084.2 flower/inflorescence, 5.1 set flowers/inflorescence, and a percentage of 0.47%), compared to the controls (7.67 cm, 695.1 flower/inflorescence, 495.78 set flower/inflorescence, 71.43%; 8.02 cm, 652.9 flower/ inflorescence, 545 set flower/inflorescence, and 82.76%, respectively). This was reflected positively in decreasing seed productivity per inflorescence to 0.009 g in Baladi red, 0.003 g in Salmoni white compared to the controls (8.86 g, 10.89 g). GA₃ can then be considered to have a selective effect on reducing or de-masculinize flowers, thus inducing male sterility and facilitating the production of F1 hybrid seeds without the need for back crossing within future breeding programs.

Keywords: *Onion, bulbs, flowers set, male sterility, seed productivity.*

Introduction

Onion (*Allium cepa* L.) belongs to the Alliaceae family (Chase *et al.*, 2009). It originated in Central Asia, then its cultivation spread to the Mediterranean basin, which is a secondary place of origin for it (Vavilov, 1956). The area cultivated with onions in Syria amounted to 6,089 hectares, with an average production of 76,700 tons (Annual agricultural statistical book, 2021). The dominant pattern of pollination in the onion crop is insect pollination. The flowers are hermaphrodite,

the anthers open and the pollen grains are dispersed before the maturity of the stigmas, so the onion flowers are early masculine (Boras, 1990). In general, the production of hybrid onion seeds in conventional breeding programs depends basically on male sterility. Jones and Clarke discovered male sterility in an onion plant in 1925, and they showed in 1943 that male sterility was the result of an interaction between a recessive genetic factor in the nucleus and a cytoplasmic factor (Hassan, 1994). Male sterility in vegetable crops is a never-ending process due to the rapid advancement in molecular technologies and their implementation, where significant progress has been made in understanding the mechanism of male sterility in vegetable crops on a global level (Hussain *et al.*, 2018). Thus, it is difficult to produce hybrids from onions using conventional methods, given that this requires the presence of female parents with male sterility to be used in the production of new hybrids, which are expensive and scarce.

Hand emasculation of onion flowers is inefficient, time consuming, labour intensive and costly process. Therefore, resorting to the use of some chemicals that may kill male gametes and contribute to the development of chemically induced male sterility is one of the options used in obtaining new hybrids of onions, and these substances have a selective effect on reducing or de-masculinize flowers, thus inducing male sterility and facilitating the production of F1 hybrid seeds without the need for back crossing within future breeding programs (Virmani, 1985), (Kaul, 1988), (Amma and colleagues, 1990), (Bushra and colleagues, 2002), (Yu and colleagues, 2006), (Cheng *et al.*, 2013).

Badino (1981) showed that spraying the inflorescences of onion plants with 0.2% of GA₃ for 10 time with 1-3 days intervals, starting from commencement of flowering stalks, caused pollen sterility (1 seed/100 flowers was produced). Prayaga and Anjani (2002) demonstrated that treating sunflower inflorescence with 0.01 to 0.03% of GA₃ gave sterile pollen grains with a percentage ranging from (80 to 95%). Manjula and Ibrahim, (1999) concluded that GA₃ was effective in inducing male sterility; as it stimulated the formation of sterile pollen in many plants including onions, safflowers, and rice (Baydar 2002).

Chauhan and Singh (2002) sprayed the flowers of mustard plants (*Brassica juncea* L.) with 6% of washing powder and found that the three application times gave 100% sterile pollen, and treated plants failed to produce seeds. Singh and Chauhan (2003) reported that spraying rapeseed flowers with a concentration of 2% of washing powder resulted in decreasing the number of seeds per fruit, and the weight of 1000 seeds compared to the control plants, and it was associated with 100% pollen sterility. Agnihotri *et al.*, (2007) demonstrated that when spraying radish flowers with three concentrations of washing powder for several times, the lowest concentration of 0.5% with 3 spraying times gave 100% sterile pollen grains with the least decrease in productivity.

Kaymak and Gökalp Muranlı (2005) concluded that when soaking onion roots in 0.1, 0.2, 0.3, and 0.4% of Avenoxan (the commercial compound of the herbicide 2,4-D) and for several periods (6-12-24 hours), the chromosomal

abnormalities increased with the increment of concentration and duration of treatment, and consequently the proportion of sterile pollen grains increased.

Tripathi and Singh (2008) showed that washing powder succeeded in producing male sterility in several plant species when treated with low concentrations, while high concentrations caused a great abnormality. This is also consistent with Singh (2005) findings. Khatib *et al.*, (2016) mentioned that the treatment of lettuce flowers with gibberellic acid (200 ppm) gave a higher percentage of hybrid seeds 85% and the highest percentage of sterile pollen 80%.

In view of the difficulty of hand emasculation in onions, which requires intensive effort and long time, and because artificial induction of male sterility using some chemicals could open the way for local production of F1 hybrid seeds using male sterile strains as female parents when crossing between pure strains, and due to the scarcity of local studies and research in the field of chemically induced male sterility of local onion varieties, this study was conducted with the following objectives:

1-Studying the effect of using some chemicals in inducing male sterility in two local varieties of onions (Baladi red and Salmoni white).

2-Determining the optimal concentration(s) of studied chemicals in inducing male sterility, to be adopted later in the crossing process to produce new hybrids.

Material and Methods

Plant material

Two varieties of onions (Baladi red and Salmon white) were used. Healthy, free of diseases and mechanical damage bulbs, of similar shape and size (5-6 cm in diameter) from the General Commission for Scientific Agricultural Research were planted to produce the seeds.

Implementing date and experimental site

The research was carried out during the first week of January of the two agricultural seasons of 2020 and 2021 at Ghouta research station affiliated with the General Commission for Scientific Agricultural Research. The station is situated 15 km southeast of Damascus, at an altitude of 610 meters above sea level. It is characterized by cold and humid winters and hot and dry summers. The soil is heavy clay, good in organic matter, and rich in phosphorus and potassium content (Table 1).

Table 1. Soil mechanical composition and chemical content

Sand%	Silt%	Clay%	Available potassium mg/kg	Available phosphorus mg/kg	Total Nitrogen%	Organic matter%	EC dS/m	pH
48	22	30	1600	160.42	0.26	5.22	1.37	8

Preparation of soil bed and planting

The soil was first plowed and planned. Then, the mother bulbs were planted in experimental plots comprising 3 lines and 24 bulbs/plot. Planting took place in

the middle of the rows with 20 cm between bulbs. Surface irrigation was immediately applied after planting. Thereafter, irrigation frequency took place every 7 days on average during the growth period, according to the prevailing weather conditions; and every 5 days during flowering and ripening phase as low humidity affects the percentage of flower set and seed viability. Manual weeding took place twice during the season, and no pesticides were applied.

Studied chemical treatments

Nine treatments were applied using three different concentrations of each of following three chemicals (Table 2) in addition to non-treated control.

Dichlorophenoxy acetic acid 2,4-D: it is a kind of herbicides organic compound and a transparent brown liquid whose formula is $C_8H_6Cl_2O_3$. Three concentrations (0.1, 0.5 and 1 ml/l) were prepared using distilled water. The basal plates of the bulbs were soaked in them for a period of 12 hours/ just before planting. Gibberellic acid (GA_3): a plant regulator that is a white crystalline powder and has the following molecular formula $C_{19}H_{22}O_6$. Three quantities of 250, 500, and 1000 mg were first solved in 1 ml of ethyl alcohol 99% for each, and then the solutions were supplemented separately with distilled water and several drops of a dispenser to a one-liter volume for each. Spraying started with commencement of flowering stalks, 3 months after planting, and repeated every two days up to 10 times for each concentration. Spraying took place in the early morning and till the inflorescences were completely soaked with the solution. The main flowering stalk and a secondary one were kept on each plant. Plots were isolated using a green tulle mesh (Fig 1).

Clothes washing powder, the commercial washing powder (Persil) was used. It mainly contains sulfonic acid, sodium silicate, sodium carbonate, sodium bicarbonate). Studied concentrations were prepared by dissolving each of the following quantities 5, 10, and 15 g of the washing powder in one liter of distilled water. Spraying was carried out in the same methodology described with Gibberellic acid.

Table 2. Chemical treatments applied to induce onion male sterility

Ser.	Treatments	Used chemical	Concentration
1	D1	2,4-D	0.1 ml/l
2	D2	2,4-D	0.5 ml/l
3	D3	2,4-D	1 ml/l
4	G1	GA_3	250 mg/l
5	G2	GA_3	500 mg/l
6	G3	GA_3	1000 mg/l
7	S1	Washing powder	5 g/l
8	S2	Washing powder	10 g/l
9	S3	Washing powder	15 g/l



Fig 1. Spraying onion inflorescence under the green tulle mesh

Inflorescences harvesting

they were harvested when matured during June and were collected in morning in the presence of dew to avoid seeds scattering. Harvested inflorescences of each plot were placed separately in bags of soft tulle in a shaded and ventilated place until they were completely dry. Then the seeds of each inflorescence were manually extracted, and the necessary readings were registered.

Experimental design and statistical analysis

the experiment was designed as a randomized complete block design with four replications for each treatment. GenStat 12th was used to conduct ANOVA, and to compare means of treatments using Fisher's least significant difference (LSD) at 5% significant level (Roger *et al.*, 2009).

Studied parameters:

The following readings were recorded using 10 plants per each replicate and for each treatment of both varieties:

- Average inflorescence diameter/cm: measured by Vernier at full blooming.
- Average total number of flowers per inflorescence.
- Average number of set flowers per inflorescence.
- Average percentage of flower setting %: calculated as the following:
- Percentage of flower setting % = $\left(\frac{\text{number of set flowers}}{\text{total number of flowers}} \right) \times 100$
- Average number of seeds per capsule.
- Average number of seeds per inflorescence.
- Average seeds weight per inflorescence (g).

Results

The effects of chemical treatments on flowering parameters in *Baladi red variety*

The results showed clearly that all gibberellic acid treatments significantly increased inflorescence size and consequently their number of flowers compared to control plants. The treatments of the other two chemicals increased the inflorescence diameter, but 2,4-D treatments decreased the number of flowers per inflorescence. The diameter of inflorescence of the G3 treatment (10.03 cm) significantly outperformed that of D1, D2, D3, S1 and the control (8.00, 8.50, 8.67, 8.28, and 7.67 cm, respectively), whereas it had insignificant differences with the treatments (G1, G2, S2, S3). The G3 treatment was significantly superior in terms of the total number of flowers per inflorescence (1077.9) over the rest of the treatments and the control (695.1), except the two treatments (G2, S1) where differences were not significant (Table 3).

Table (3) shows that all chemical treatments had a desirable negative effect on the number and percentage of flower setting. G3 had the lowest number of set flowers (5.00) with significant differences with most of the studied treatments and the control which gave the largest number of set flowers (495.78). No significant differences were observed between G3 and G1, G2, S3. In a similar manner, all treatments gave significantly lower flower setting percentage than control plants which gave the highest percentage (71.43%). The G3 treatment had the lowest percentage of flower setting. No significant differences were observed between G3 (G1, G2, S2, and S3) (0.44, 3.83, 1.42, 6.00, and 4.20%, respectively).

Table 3. Effect of studied chemical treatments on flowering parameters of Baladi red onion plants.

Treatment	Average inflorescence diameter/cm	Average total number of flowers/ inflorescence	Average number of set flowers/ inflorescence	Average percentage of flower setting %
G 1	9.67 ^a	879.6 ^{bc}	25.89 ^{ab}	3.83 ^{ab}
G2	9.78 ^a	920.1 ^{ab}	12.78 ^{ab}	1.42 ^a
G3	10.03 ^a	1077.9 ^a	5.00 ^a	0.44 ^a
S 1	8.28 ^{bcd}	918.3 ^{ab}	77.22 ^{cd}	8.56 ^{bcd}
S 2	9.78 ^a	824.0 ^{bcd}	45.00 ^{bc}	6.00 ^{abc}
S 3	9.56 ^a	783.4 ^{bcd}	32.33 ^{ab}	4.20 ^{ab}
D 1	8.00 ^{cd}	622.2 ^e	99.89 ^d	17.48 ^e
D 2	8.50 ^{bc}	728.4 ^{cde}	88.44 ^d	12.23 ^{de}
D 3	8.67 ^b	677.8 ^{de}	75.00 ^{cd}	10.31 ^{cd}
control	7.67 ^d	695.1 ^{de}	495.78 ^e	71.43 ^f
LSD _{0.05}	0.656	179.8	36.06	5.785
CV%	7.9	15.55	16.02	18.90

Different Latin letters mean that there are significant differences between means at 5% significance level (P<0.05).

The effect of chemical treatments on flowering parameters in *Salmoni white variety*

The data clearly shows that all gibberellic acid treatments resulted in a significant increase in inflorescence diameter, and average flowers number per inflorescence over control plants. However, only S3 of the washing powder treatments had a significant increase (8.94 cm, 841.8 flower) over the control (8.02 cm, 652.9 flowers/inflorescence). The effect of the 2,4-D treatments was insignificant and variable (Table 4). All chemical treatments significantly and clearly decreased the number of set flowers and, as a result, flower setting percentage compared to control. This shows that these treatments are potential male sterility inducers. The most effective treatment among them was G3 (5.1 set flowers/inflorescence, 0.47% flower setting percentage) compared to control plants (545, 82.76%, respectively). No significant differences related to the previous two parameters were registered among G3 and each of G2, G1, S3, S2, and D3.

Table 4. Effect of studied chemical treatments on flowering parameters of *Salmoni white onion plants*.

Treatment	Average inflorescence diameter/cm	Average total number of flowers/ inflorescence	Average number of set flowers/ inflorescence	Average percentage of flower setting %
G 1	9.33 ^a	875.0 ^{bc}	15.7 ^a	1.98 ^a
G2	9.28 ^a	932.8 ^{ab}	13.1 ^a	1.64 ^a
G3	9.33 ^a	1084.2 ^a	5.1 ^a	0.47 ^a
S 1	8.16 ^{cd}	707.1 ^{def}	88.6 ^b	11.93 ^{bc}
S 2	8.50 ^{bc}	755.7 ^{cdef}	56.7 ^{ab}	8.27 ^{abc}
S 3	8.94 ^{ab}	841.8 ^{bcd}	42.4 ^{ab}	5.03 ^{ab}
D 1	7.78 ^d	636.1 ^f	96.2 ^b	15.01 ^c
D 2	8.11 ^{cd}	761.1 ^{cdef}	82.2 ^b	11.13 ^{bc}
D 3	8.37 ^c	799.3 ^{bcde}	63.0 ^{ab}	7.75 ^{abc}
control	8.02 ^{cd}	652.9 ^{ef}	545.0 ^c	82.76 ^d
LSD _{0.05}	0.536	161.8	60.97	7.926
CV%	6.7	18.4	10.12	15.6

Different Latin letters mean that there are significant differences between means at 5% significance level (P<0.05).

The effects of chemical treatments on seed production in *Baladi red variety*

All chemical treatments significantly reduced the average number of seeds per capsule compared to the control. The most effective treatments were G3 and G2 (0.65, and 0.71 seed/capsule, respectively), followed by G1 and S3. Generally, 2,4-D was the least effective chemical in this experiment. As for the inflorescence, all treatments also led to a significant and clear decrease in the average number of seeds produced, which ranged between 1.9-197.3 seeds/inflorescence, in comparison with the control (1171.3). The most effective treatments, without significant differences among them, were G3, G2, G1, and S3, while the least effective treatments were D1 and D2 (241.9, and 197.3 seeds/inflorescence, respectively). The previous indicators had a direct impact on the average weight of seeds produced per inflorescence, which was significantly decreased in all studied treatments. The most effective treatments were G3 and G2 (Table 5).

Table 5. Effect of studied chemical treatments on seed production parameters of Baladi red onion plants.

Treatments	Average number of seeds /capsule	Average number of seeds /capsule	Average seed weight g/inflorescence
G 1	1.56 ^b	39.2 ^{abc}	0.199 ^{ab}
G2	0.71 ^a	18.2 ^{ab}	0.100 ^a
G3	0.65 ^a	1.9 ^a	0.009 ^a
S 1	2.67 ^c	154.2 ^{de}	1.247 ^e
S 2	2.77 ^{cd}	76.0 ^{bc}	0.987 ^{de}
S 3	1.64 ^b	53.7 ^{abc}	0.583 ^{bc}
D 1	3.31 ^d	241.9 ^f	1.797 ^f
D 2	2.97 ^{cd}	197.3 ^{ef}	0.808 ^{cd}
D 3	3.30 ^d	95.3 ^{cd}	0.380 ^{ab}
control	4.00 ^e	1771.3 ^g	8.856 ^g
LSD _{0.05}	0.595	72.91	0.3861
CV %	16.66	15.33	13.25

Different Latin letters mean that there are significant differences between means at 5% significance level (P<0.05).

The effect of chemical treatments on seed production in *Salmoni white variety*

The results of this variety were like those of the previous one (Table 6), as all chemical treatments led to a significant reduction in the average number of seeds per capsule. G3 had the most important significant effect (0.83 seeds/capsule) compared to the control (3.93). G2 came in second place with an average of (1.31 seeds/capsule), while the 2,4-D treatments were the least effective. The average number of seeds per inflorescence ranged between 1.1-148 in the applied treatments, which is much lower than the value recorded in the control case (1877.8 seed/inflorescence). As a result, the average seed weight per inflorescence decreased from 10.89 in control plants to 0.003 g/inflorescence in case of G3, which was the most effective chemical treatment. No significant differences were recorded with this regard between G3, G2, G1, S3, and S2.

Table 6. Effect of studied chemical treatments on seed production parameters of Salmoni white onion plants

Treatments	Average number of seeds /capsule	Average number of seeds /capsule	Average seed weight g/inflorescence
G 1	1.63 ^{bc}	22.6 ^{ab}	0.092 ^a
G 2	1.31 ^{ab}	23.6 ^{ab}	0.107 ^a
G 3	0.83 ^a	1.1 ^a	0.003 ^a
S 1	2.35 ^d	122.2 ^{abc}	0.904 ^b
S 2	2.11 ^{cd}	92.9 ^{ab}	0.604 ^{ab}
S 3	2.16 ^{cd}	65.9 ^{ab}	0.350 ^{ab}
D 1	3.12 ^e	234.9 ^c	2.767 ^d
D 2	2.51 ^{de}	148.0 ^{bc}	1.687 ^c
D 3	2.34 ^d	129.6 ^{bc}	0.633 ^{ab}
control	3.93 ^f	1877.8 ^d	10.890 ^e
LSD _{0.05}	0.635	127.4	0.7143
CV %	18.9	16.4	20.5

Different Latin letters mean that there are significant differences between means at 5% significance level (P<0.05).

Discussion

According to the foregoing results, it can be concluded that there is a positive effect of gibberellic acid spraying treatments, especially the concentration of 1000 mg/l, in reducing the number and percentage of set flowers, which reflected positively on reducing the productivity of seeds. Spraying treatments with washing powder and 2,4-D in high concentrations also contributed to reducing productivity of Seeds. The effect of these substances can be attributed to their effect that kill gametes (preventing microsporogenesis and stopping the opening of anthers without affecting female fertility), and when they applied with certain doses at the stage of anthers development, they prevent fertile pollen development and gives deformed pollen that is incapable of growth or fertility (Cross and Ladyman, 1991). Previous findings are consistent with those of Chauhan and Singh (2002), Singh and Chauhan (2003), Kaymak and Gökcalp Muranlı (2005), Badino (1981), Khatib *et al.*, (2016), and Singh *et al.*, (2018). Agnihotri *et al.*, (2007) showed that GA₃, the herbicide Dichlorophenoxy acetic acid 2,4-D, and washing powder at high concentrations caused sterility in pollen on different plants.

Usually, pollen grains are formed within the pollen sac, and each pollen sac is covered from the outside by a layer that is the epidermis, and inside it there is a layer of fibrous cells and then the tapetum (a layer of cells located along the inner wall of the pollen sac). The mechanism of abortion and death of pollen grains in artificially male sterile plants is due to the abnormal behavior of the tapetum (Naohiko, 2006). Such behavior prevents small spores from feeding, causing their death (Awasthi and Dubey, 1985). Therefore, the substances used to kill male gametes mainly target the cells of this layer, bearing in mind that there is no scientific explanation for the abnormal development of these cells in sterile plants (Naohiko, 2006), which contributes to the possibility of obtaining superior new hybrids within future genetic breeding programs (Tinna, 2019).

Conclusion

The studied chemicals (Gibberellin, herbicide 2-4 D, washing powder) were effective in inducing male sterility in the flowers of onion plants of local *Baladi red* and *Salamoni white varieties*.

The treatment of gibberellic acid 1000 mg/l was the most efficient treatment in inducing male sterility in the two local onion cultivars, which opens new horizons for potentially producing new superior hybrids of onions.

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استجابة نباتات صنفين من البصل المحلي للمعاملة ببعض المواد الكيميائية بهدف استحداث العقم الذكري الاصطناعي

أسامة حسين العبد الله¹، روعة محمد مروان البيبي¹، موفق ناهي جبور¹، رولا غسان بطحوش¹، مصطفى محمد الأذن¹، رشا رمضان عيد^{2*}

¹ الهيئة العامة للبحوث العلمية الزراعية، إدارة بحوث البستنة، دمشق، سوريا.
² قسم بحوث البطاطس والخضر خضريه التكاثر، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

نفذ البحث في الهيئة العامة للبحوث العلمية الزراعية /دمشق، سورية خلال الموسمين الزراعيين 2021/2020 بهدف دراسة استجابة نباتات صنفين من البصل المحلي (الأحمر البلدي والسلموني الأبيض) للمعاملة ببعض المواد الكيميائية بثلاث تراكيز مختلفة، GA₃ (250-500-1000 ملغ/ل)، مسحوق الصابون الصناعي (0.5-1-1.5%)، 2,4-D (0.1-0.5-1 مل/ل) لاستحداث العقم الذكري الاصطناعي، صممت التجربة وفق تصميم القطاعات العشوائية الكاملة بأربعة مكررات لكل معاملة.

بينت النتائج المتحصل عليها فعالية استخدام المواد المطبقة في استحداث العقم الذكري الاصطناعي لدى نباتات صنفى البصل المدروسين، فقد تفوقت معاملة GA₃ تركيز 1000 ملغ/ل معنوياً في مؤشرات الأزهار (قطر النورة، عدد الأزهار العاقدة، النسبة المئوية للعقد) عند كلا الصنفين الأحمر البلدي والسلموني الأبيض على التوالي (10.03 سم، 5.00 زهرة/النورة، 0.44%، 9.33 سم، 5.1 زهرة/ النورة، 0.47%) مقارنة مع الشاهد (8.67 سم، 495.78 زهرة/نورة، 71.43%، 8.02 سم، 545 زهرة/النورة، 82.76%)، والذي انعكس ايجاباً في انخفاض الإنتاجية من البذور على النورة (0.009 غ، 0.003 غ) مقارنة مع الشاهد (8.86 غ، 10.98 غ)، ويمكن اعتبار مادة GA₃ عندها انتقائية لإبطال ونقص الذكورة من الأزهار وبالتالي تيسر إنتاج بذور الهجين F1 بدون الحاجة إلى التلقيح الرجعي ضمن برامج التحسين الوراثي المستقبلية.

الكلمات المفتاحية: الأبدال، أزهار عاقدة، إنتاجية بذور، عقم ذكري.