Selcuk Journal of Agriculture and Food Sciences

https://dergipark.org.tr/tr/pub/selcukjafsci

Research Article

(2024) 38 (1), 41-52 DOI:10.15316/SJAFS.2024.004 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

The Stimulation of Chemical Male Sterility for F1 Hybrid Lettuce (*Lactuca Sativa* Var. Longifolia) Production

Arif Selim Arici[1], Ertan Sait Kurtar[2,*]

[1] Selçuk University, The Graduate School of Natural and Applied Sciences, Konya, Türkiye
[2*]Selçuk University, Horticulture Department of Agriculture Faculty, Konya, Türkiye

HIGHLIGHTS

- Male sterility is utilized in the production of commercial F1 hybrid seeds of some vegetables.
- Türkiye seed market has not a native F1 hybrid lettuce variety.
- Procedures are not required to the detecting sterile and restorer lines and ensure the continuity of these lines in gametocyte-originated sterility.

Abstract

Male sterility is a unique application in the production of F1 hybrid seeds of some important species. Today, while F1 hybrid lettuce varieties of abroad origin take their place in the Türkiye seed market, unfortunately, we do not have a native F1 hybrid variety. Besides, F1 hybrid seed production has become a prestige for multi-national companies regardless of the type of vegetable. Within this perspective, the effects of some chemical hybridizing agents (CHAs) such as Ethyl 2-(4-fluoroanilino)-2-oxoacetate E4FO, 2-chloroethyl phosphonic acid (Ethrel) and GA_3 on male sterilizing activity in lettuce cultivars (Maylight352 F1, Presidential and Yedikule) were evaluated. Therefore, pollen presence in the early bud stage, seed formation and seed viability (germination) were examined. The applications had different effects on pollen presence, seed formation and seed germination, and thus male sterility. Ethrel was not effective at low doses, but at high doses, it caused flower bud deformation and growth retardation. E4FO is partially effective, but the application doses are low. Therefore, E4FO should be used at higher than 1500 ppm and 2000 ppm. GA_3 applications produced the best results in stimulating male sterility, and full sterility (% 100) was achieved from 200, 250 and 300 ppm in all cultivars. As a result, 200 ppm GA_3 was determined as the recommendable dose in the production of F_1 hybrid lettuce.

Key Words: Male Sterility, Gametocytes (CHAs), Lettuce

1. Introduction

Lettuce (*Lactuca sativa* L.) is at the forefront of the vegetable species (Eşiyok 2012) and its origin is considered to be Anatolia, Caucasus, Iran and Turkistan (Balkaya and Özgen 2019). They are grouped as curly leaves (*L. sativa* var. crispa), head (*L. sativa* var. capitata) and cos lettuce (*L. sativa* var. longifolia) concerning the leaf characteristics (Şalk et al. 2008). Curly lettuces show great diversity in

Citation: Arıcı SA, Kurtar ES (2024). The Stimulation of Chemical Male Sterility for F1 Hybrıd Lettuce (Lactuca Sativa Var. Longifolia) Production. *Selcuk Journal of Agriculture and Food Sciences*, 38 (1), 41-52. https://doi.org/10. 15316/SJAFS.2024.004
Corresponding Author E-mail: ertansaitkurtar@selcuk.edu.tr
Received date: 14/06/2023
Accepted date: 21/11/2023

terms of leaf size, shape, leaf color and texture (Karaağaç and Balkaya 2019). Lettuce can be grown all over the world due to its short vegetation period and the continuity of the lettuce's presence in the market is ensured by planting more than once a year.

Lettuce product shares vary considerably according to regions and segment groups. Availability of genetic material with variation is needed to achieve success in breeding efforts. Successful lettuce breeding requires observation, evaluation, selection and seeding stages by adjusting the conditions suitable for the plant's growing demands in a limited time. Breeding studies in salad group species are carried out in the world with classical methods (Prohens and Nuez 2008). In lettuce, inflorescences shoot bear 12-20 flowers and hermaphrodite flowering lettuces are obligate self-pollinated (Feráková 1977).

Due to the flower structure, the style is covered with pollen during elongation between the anthers and the external pollination rate remains very low (1%) (Thompson 1958). Lettuce pollen is heavy and sticky, making it difficult to transport by wind; the use of insects is unsuccessful and hand pollination is not feasible in large quantities. Thus, F1 hybrid seed production needs the support of some applications (Ryder 1999). The emasculation process for classical hybridization is based on the principle of washing the pollen by spraying water when the stigma of the female flower reaches the "V" shape (Nagata 1992).

Male sterility can be of cytoplasmic, genetic, and cytoplasmic-genetic origin, and sources are showing their use in lettuce breeding (Curtis et al. 1996; Goubara and Takasaki 2004; Takada et al. 2007; Hayashi et al. 2011; Ananthi et al. 2013; Michel and Soussin 2014). However, the materials containing the inducer ms genes are patented, and gametocyte applications are preferred for breeders who cannot obtain these materials (Eenink and Vereijken 1978). In addition, in gametocyte-originated sterility, procedures are not required to the detecting sterile and restorer lines and ensure the continuity of these lines.

Gametocytes are generally known as chemicals that cause deformations in pollen formation (in the meiosis), leading to the formation of pollen with lost germination ability and therefore inability to fertilize, and have been used successfully in F1 hybrid breeding in many species (Collantes et al. 1999; Colombo and Galmarini 2017; Hussain et al. 2018; Tinna 2019). A suitable gametocyte should not be mutagenic and non-toxic, does not limit the application dose and time, is environmentally friendly, has no negative effects on F1 hybrid seed, does not cause problems in seed setting, and is also applied cheaply and easily. In particular, some chemical hybridizing agents (CHAs) such as maleic hydrazide, gibberellins, dalapon, mendok, ethephone and ethyl oxanylates are used as male sterility-stimulating chemicals in vegetables. The first gametocyte applications to stimulate male sterility in lettuce were carried out by Eenink (1977) obtained male sterile lettuce plants with GA_3 applications in the early bud period in lettuce.

Most of the varieties cultivated are open-pollinated (OP) varieties in current lettuce production. Because F1 hybrid seed production is not possible with classical methods due to the flower structure, and it is imperative to benefit from the male sterility method used in species such as onion, carrot, cabbage and maize (Billore 2015). Although GMS (Genetically Male Sterility) in lettuce has been known since the 1960s, with the development of gene transfer techniques, GMS-derived F1 hybrid lettuce varieties have been put in the world seed market since 2002.

In this study, it was aimed to determine the effective chemical substance and dose, which reveals high male sterility (> 95%) and does not adversely affect plant and flower development (does not reduce seed setting and seed viability) by applying gametocytes at the beginning of flowering and early bud period. The objectives of the study were to find the appropriate concentration of E4FO, Ethrel and GA_3 to (1) the presence of pollen, (2) the seed setting, (3) the germination rate of the seeds (4) induce male sterility in lettuce.

2. Materials and Methods

The study was carried out in the Department of Horticulture, Faculty of Agriculture, Selcuk University, between April – August 2021. Field studies of the research were carried out in the greenhouse of Selçuk University.

2.1. Material

Mylight352 F1, Presidential and Yedikule cultivars with cos lettuce types were used in terms of leaf types (Figure 1).

Figure 1. The cultivars (Original)

2.2. Methods

2.2.1. Cultivation of Plants

Seeds of the cultivars were sown in plastic containers containing peat moss in the growth chamber, and the seedlings, which reached a certain size, were staggered into 45-mesh vials filled with peat moss-perlite (1:1 volume). Seedlings at the 3-4 leaves stage were planted at 0.7 x 0.4 m spacing and distances in the greenhouse, and then sap water was applied with the drip irrigation system. According to the soil analysis, the plants were fertilized by a drip irrigation system using Ammonium Sulphate (10.5 kg da^{-1}) and Potassium Sulphate (8.5 kg da^{-1}) fertilizers twice a week during their development period. Cultural practices such as weeding, hoeing, disease and pest management were implemented regularly. "Movento SC 100" was used against whitefly periodically, and "Flo-Captan 50 WP" was applied against mildew (*Bremia lactucae*). The plants were covered with a shade net to prevent early bolting of the lettuce at high light intensity and temperatures.

2.2.2. Determination of application time of gametocytes

For male sterility in lettuce, gametocytes must be applied in the early bud period. The optimum bud size was determined as 3-4 mm. Because this period is the first stage of meiosis and microspore mother cell formation and is accepted as the stage in which transformation into mature pollen can be prevented (Eenink and Vereijken 1978).

The buds in the blue and red areas are unsuitable and they were removed from the plants before gametocyte application. The buds in the yellow area are the most convenient size (Figure 2).

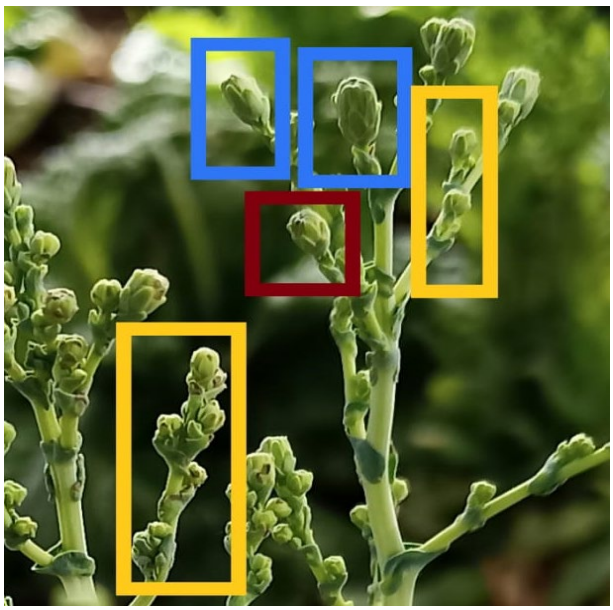

Figure 2. Flower buds (cv. Presidential) at different developmental stages

2.2.3. Gametocytes and applications

GA_3 (Gibberellic acid) was used in the commercial Berelex form, Ethrel (Ethephon) was applied in the commercial Maysal form, and E4FO [Ethyl 2-(4-fluoroanilino)-2-oxoacetate] was purchased from the ChemCruz company (Table 1).

Table 1. Gametocytes, chemical formulas and application doses.

Gametocyte	Formula	Dose (ppm)
GA_3 (Gibberellic acid)	$C_{19}H_{22}O_6$	50, 100, 150, 200, 250, 300
Ethyl 2-(4-fluoroanilino)-2-oxoacetate	$C_{10}H_{10}FNO_3$	1500, 2000
Ethrel (Ethephon)	$C_2H_6ClO_3P$	1000, 1500, 2000, 3000, 4000

Before the application, stock solutions of the chemicals were prepared and the final concentrations were created by taking appropriate amounts of these stock solutions before each application. Stock solutions were stored in the refrigerator at -18 ^oC.

Gametocyte applications to flower buds were made in the early morning at daylight or just after light. Applications were started in the cool and early times, as applied during the noon or hot hours would reduce the activity of the gametocyte. Before the application, inappropriate buds were discarded, and then gametocytes were applied to inflorescences.

To be able to apply in equal doses, each inflorescence was sprayed 3 times from the prepared gametocytes, and the inflorescences were closed from the back so that the applications would not reach other buds. After the first application, other applications were carried out 3 times at 3 days intervals to transmit the chemical to the flowers that may occur later in the inflorescence. After the first application, the buds were isolated with net sacs to avoid external pollen contamination, then labeled and recorded (Figure 3). Distilled water was only sprayed in control lots.

Figure 3. Gametocyte applications

2.2.4. Pollen and Seed Formation

After the applications, 5 flowers were gently removed from the inflorescences that bloomed 4 or 5 days after application. Then the flowers were cut longitudinally and the presence of pollen in the anthers was visualized under a light microscope at 10 x 10 magnifications. For the seed formation in inflorescences, 5 flower capsules were gently collected for each application, and the presence of seeds was examined under a light microscope at 10 x 10 magnifications.

Mature flower buds were carefully removed with the isolation sacs, and dried at room temperature for 2 weeks. The seeds were gently collected from dried capsules, cleaned from all other plant parts and 100 seed weight (g) was determined in randomly selected seeds for each genotype and each application

2.2.5. Germination tests

The seeds were subjected to a germination test in petri dishes with a diameter of 90 x 15 mm at 20 ± 1 ^{o}C. A double layer of Whatman No.1 filter paper had placed on the surface of Petri dishes, and filter papers had been moistened with a 5 ml carbendazole mixture (0.75 g lt^{-1}) to avoid fungal contamination. The germination tests were carried out in 3 replications and 100 seeds in each replication except for the Ethephon 1000 ppm treatment, due to the limited number of seeds could be obtained. The germination test was realized for 14 days and percentage germination were calculated at the end of the 14th day (ISTA 2009).

2.2.6. Data evaluation

The study was carried out with 3 replications for each application. However, in some applications, as no data could be obtained (at high doses of Ethephon), statistical analysis was not performed, and only standard deviations were presented.

3. Results and Discussion

3.1. Pollen Formation

Flower deformations were determined and pollen formation could not be examined at doses of 1500 ppm and above in Ethephon (Table 1). The plant growth inhibitory effect of Ethephon was reported in a male sterility study, and a shortening of the plant length was observed in 3 barley cultivars (Ma and Smith 1992). At the 1000 ppm Ethephon, a small number of flowers were obtained, but all of the flowers produced the pollen grains. These flowers then produced mature buds and seeds. The results revealed that Ethephon doses between 1000-1500 ppm should be examined or the range of applications should be arranged in further studies.

Table 1. Rate of pollen-containing flower (%).

Application	Dose (ppm)	Cultivar	PR (%)	Average
CONTROL	-	P	100	100
		Y	100	
		M	100	
ETHEPHON	1000	P	100	100
		Y	100	
		M	100	
	1500 - 4000	Could not evaluate due to floral deformation		
GA_3	50	P	100	100
		Y	100	
		M	100	
	100	P	100	100
		Y	100	
		M	100	
	150	P	93.3	91.1
		Y	86.7	
		M	93.3	
	200	P	93.3	91.1
		Y	93.3	
		M	86.7	
	250	P	20.0	24.5
		Y	26.7	
		M	26.7	
	300	P	0	0
		Y	0	
		M	0	
E4FO	1500	P	100	100
		Y	100	
		M	100	
	2000	P	66.7	71.1
		Y	73.3	
		M	73.3	

P: Presidential; **Y:** Yedikule; **M:** Maylight352
PR: Pollen-Containing Flower Rate

While pollen formation was observed in all the flowers of the cultivars at 50 and 100 ppm GA_3, pollen formation was in 91% of the flowers at 150 and 200 ppm, 24% at 250 ppm, and all the flowers were found to be sterile in the 300 ppm (Figure 4). Eenink and Vereijken (1978) stated that high doses of GA_3 did not produce pollen grains.

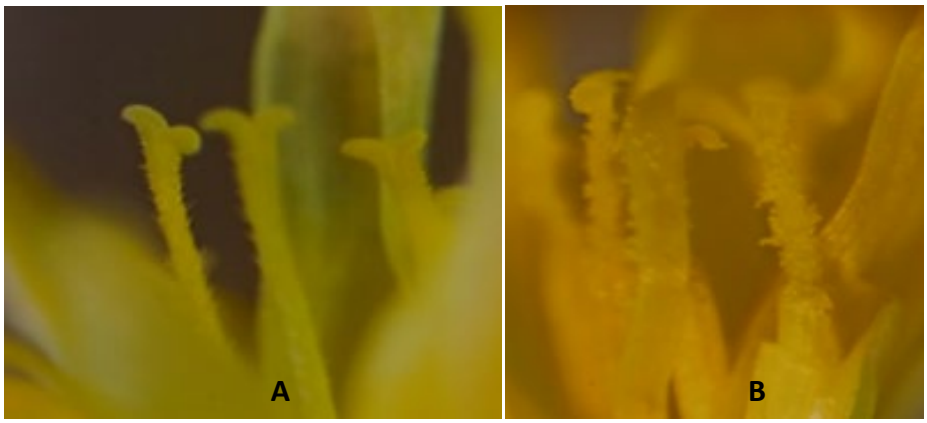

Figure 4. Sterile flower (A), and fertile flower with pollen grains (B).

Pollen was detected at 1500 ppm E4FO, and it was 71% in the 2000 ppm application. The results revealed that the doses of E4FO are also low and higher doses should be examined in further studies. Conversely, 1500 ppm E4FO induced 99.7% or 100% male sterility in rice (Ali et al. 1999) and wheat (Devakumar 2006). 2 mg l^{-1} E4FO provided complete pollen sterility (97% - 100%) and it had no adverse effects on female activity in sorghum (Amelework et al. 2016). In a study investigating the effects of Ethrel, acetic acid, E4FO and promaline (1.8% GA 47 – gibberellins A 4 +A 7 and 1.8% 6-BA-benzyl adenine) on male sterility in *Eragrostis*, 99.50% pollen sterility was achieved in the 1500 to 3000 ppm E4FO and 5000 ppm Ethrel (Ghebrehiwot et al. 2015).

All cultivars had similar responses to the applications, and no genotype differences were observed. This situation revealed that the recommended chemicals and doses for the induction of male sterility in lettuce will reveal sterility at a similar rate in all genotypes and it can be used safely.

3.2. Seed Formation

The number of seeds and 100 seed weight (g) were differentiated with CHAs and application doses (Table 2). Due to the deformation of the buds caused by Ethephon applications, either no seeds or very few seeds were detected. Only a limited number of buds were harvested in 1000 ppm Ethephon and a limited number of seeds were harvested compared to the control. While Ethrel was an effective gametocyte for wheat (Dotlacil and Apltauerová 1978), it also induced very high female sterility at the rates required for male sterility (Chakraborty and Devakumar 2006).

1500 and 2000 ppm E4FO and GA_3 up to 150 ppm produced similar results with the control lots and there was no negative effect on seed formation. Conversely, there was a noticeable decrease in seed weight at higher doses. It is suggested that even if seed formation occurs at 200 ppm and above GA_3 doses, these seeds may be relatively empty and nonviable (Figure 5). Treated once with 0.15% E4FO provided 99.8% male sterility without a significant reduction in total yield in wheat (Chakraborty and Devakumar 2006).

Although there is no statistical data, 200 ppm and above GA_3 applications cause elongation in flower stems. Likewise, GA_3 increased stem elongation in lettuce (Yılmaz et al. 2002) and transgenic tobacco plants treated with GA_3 had male sterility but developed undersized (Huang et al. 2003).

Since chemically induced male sterility was first introduced by Nelson and Rossman (1958) in maize, gibberellins have been successfully used in safflower (Baydar 2000), tobacco (Huang et al. 2003), sunflower (Duca et al. 2008; Yılmaz 2010) in barley (Altındal 2019).

Table 2. Number of seeds per 100 buds and 100 seeds weight (g)

Application	Dose (ppm)	Cultivar	SN	100 SW (g)
CONTROL	-	P	1238.0 ± 51.0	0.094 ± 0.008
		Y	1989.4 ± 57.6	0.076 ± 0.019
		M	1830.0 ± 45.2	0.050 ± 0.015
ETHEPHON	1000	P	142.0 ± 64.9	0.065 ± 0.009
		Y	642.4 ± 122.5	0.056 ± 0.044
		M	121.4 ± 98.0	0.042 ± 0.006
	1500 - 4000	Could not evaluate due to floral deformation		
GA_3	50	P	1348.6 ± 82.7	0.060 ± 0.018
		Y	1292.6 ± 76.3	0.054 ± 0.024
		M	1011.6 ± 61.1	0.049 ± 0.033
	100	P	1271.6 ± 80,0	0.037 ± 0.012
		Y	1198.2 ± 65,7	0.056 ± 0.023
		M	1003,5 ± 83,4	0.051 ± 0.029
	150	P	1102.4 ± 58.7	0.069 ± 0.047
		Y	1073.2 ± 76,3	0.071 ± 0.034
		M	971,9 ± 82,5	0.050 ± 0.042
	200	P	1189.4 ± 72.1	0.035 ± 0.014
		Y	1201.7 ± 86.6	0.039 ± 0.022
		M	1061.1 ± 49.4	0.032 ± 0.034
	250	P	1067.0 ± 61.1	0.031 ± 0.039
		Y	1101.6 ± 63.3	0.033 ± 0.031
		M	989.5 ± 42.2	0.027 ± 0.019
	300	P	1115.8 ± 54.8	0.034 ± 0.011
		Y	1001.1 ± 88.3	0.031 ± 0.027
		M	865.4 ± 55.5	0.029 ± 0.051
E4FO	1500	P	961.4 ± 79.7	0.078 ± 0.036
		Y	1014.1 ± 48.3	0.079 ± 0.025
		M	909.4 ± 75.3	0.064 ± 0.030
	2000	P	953.3 ± 30.7	0.070 ± 0.029
		Y	869.0 ± 72.9	0.065 ± 0.022
		M	963.0 ± 38.5	0.077 ± 0.034

P: Presidential; **Y:** Yedikule; **M:** Maylight352; **SN:** Seed Number; **SW:** Seed Weight

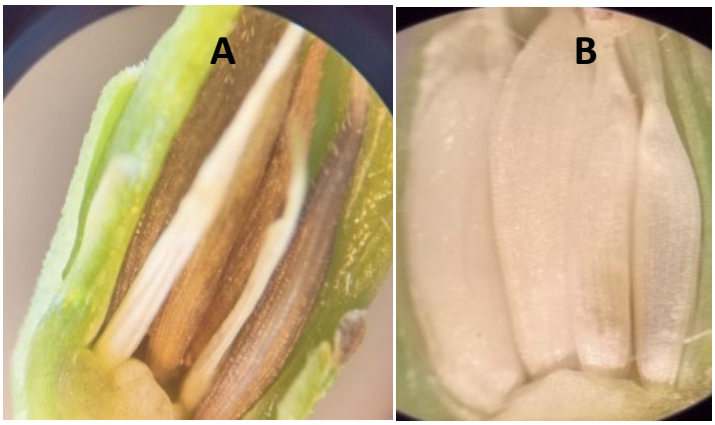

Figure 5. The buds with full seeds (A) and empty seeds (B).

3.3. Seed Germination

Due to excessive bud deformations, the seeds could not be obtained and therefore germination tests could not be evaluated at Ethephon doses higher than 1000 ppm (Table 3). 1000 ppm Ethephon had not any effect on sterility and the seeds produced similar germination with the control lots. Similar effects were also determined at 1500 and 2000 ppm E4FO. This revealed that between 1000-1500 ppm of Ethephon should be examined or the range of applications should be arranged and doses of E4FO above 2000 ppm should be evaluated in lettuce.

Seed germination decreased below 50% in 50 ppm, and it was 10% and 3% in 100 ppm and 150 ppm GA_3, respectively. The germinating seeds could not be detected and complete sterility was achieved in 200, 250 and 300 ppm applications. Our results are similar to the Khatib et al. (2016) findings and the 200 ppm GA_3 was the recommendable dose.

Table 3. Effects of CHAs on seed germination (%) of lettuce cultivars

Treatments	Dose (ppm)	Cultivar	G (%)
CONTROL	-	P	95.7 ± 1.2
		Y	97.4 ± 0.6
		M	98.0 ± 2.0
ETHEPHON	1000	P	96.0 ± 2.1
		Y	95.4 ± 1.2
		M	97.2 ± 2.7
	1500 - 4000	Could not evaluate due to floral deformation	
GA_3	50	P	48.3 ± 15.2
		Y	44.1 ± 11.7
		M	46.9 ± 9.6
	100	P	13.0 ± 4.2
		Y	15.5 ± 6.7
		M	12.0 ± 4.2
	150	P	2.7 ± 0.4
		Y	3.5 ± 1.1
		M	2.3 ± 0.9
	200	P	0.0
		Y	0.0
		M	0.0
	250	P	0.0
		Y	0.0
		M	0.0
	300	P	0.0
		Y	0.0
		M	0.0
E4FO	1500	P	95.7 ± 2.6
		Y	95.0 ± 3.0
		M	96.0 ± 2.0
	2000	P	95.4 ± 2.4
		Y	94.7 ± 7.7
		M	96.0 ± 1.0

P: Presidential; **Y:** Yedikule; **M:** Maylight352
G: Germination

4. Conclusion

It is imperative to produce male sterile lines, especially in species where emasculation is impractical due to their flower structure and morphology and it is imperative to utilize the male sterility mechanism to develop new F1 hybrid varieties. Our findings indicated that;

1) Gibberellic acid did not show any effect at low doses, and the most effective dose was 200 ppm. All cultivars produced similar results.

2) Flower and bud formation was occurred at 1000 ppm Ethephon, while 1500 ppm and upper doses caused deformations in the inflorescences. Thus, Ethephon dosses between 1000-1500 ppm should be examined.

3) The 1500 and 2000 ppm E4FO were also not effective on male sterility and produced similar results with the control. It would be beneficial to study higher E4FO doses in further lettuce breeding programs.

5. Acknowledgment

This research was funded by the Scientific Research Projects Coordination Office of Selcuk University, Turkey (Project No.20201079).

Conflicts of Interest: The authors declare no conflict of interest.

References

Ali AJ, Devakumar C, Zaman F, Siddiq E (1999). Identification of potent gametocides for selectiveinduction of male sterility in rice. *Indian Journal of Genetics and Plant Breeding*, 59 (04): 429-436.

Altındal D (2019). Effect of gibberellic acid (GA_3) application on male sterility in barley (*Hordeum vulgare* L.). *Pakistan Journal of Botany*, 51(6): DOI: http://dx.doi.org/10.30848/PJB2019-6(40).

Amelework A, Laing M, Shimelis H (2016). Evaluation of effective gametocides for selective induction of male sterility in sorghum. *Czech Journal of Genetics and Plant Breeding*, 52 (4): 163-170.

Ananthi M, Selvaraju P, Srimathi P (2013). Transgenic male sterility for hybrid seed production in vegetables-A review. *Weekly Sci. Res. Journal* 1 (16): 1-10.

Balkaya A, Özgen R (2019). The place and economic importance of lettuce cultivation in agricultural production in Turkey. T*arım Gündem Dergisi,* [13.05.2022].

Baydar H (2000). Effect of gibberellic acid on male sterility, seed yield, and fat and fatty acid synthesis in safflower (*Carthamus tinctorius* L.). *Turkish Journal of Biology* 24: 159-168.

Billore M (2015). Male-sterility and its exploitation. *Genetic Improvement of Field Crops*, 254.

Chakraborty K, Devakumar C (2005). N-acylanilines, herbicide− CHA chimera, and amino acid analogues as novel chemical hybridizing agents for wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry*, 53 (20): 7899-7907.

Chakraborty K, Devakumar C (2006). Ethyloxanilates as specific male gametocides for wheat (*Triticum aestivum* L.). *Plant Breeding* 125 (5): 441-447.

Collantes ER, Xing L, Miller PC, Welsh WJ, Profeta S (1999). Comparative molecular field analysis as a tool to evaluate mode of action of chemical hybridization agents. *Journal of Agricultural and Food Chemistry*, 47 (12): 5245-5251.

Colombo N, Galmarini CR (2017). The use of genetic, manual and chemical methods to control pollination in vegetable hybrid seed production: a review. *Plant Breeding*, 136 (3): 287-299.

Curtis IS, He C, Scott R, Power JB, Davey MR (1996). Genomic male sterility in lettuce, a baseline for the production of F1 hybrids. *Plant Science*, 113 (1): 113-119.

Devakumar C (2006). Evaluation of chemical compounds for induction of male sterility in wheat (*Triticum aestivum* L.). *Euphytica*, 147 (3): 329-335.

Dotlacil L, Apltauerová M (1978). Pollen sterility induced by ethrel and its utilization in hybridization of wheat. *Euphytica*, 27 (2): 353-360.

Duca M, Port A, Orozco-Cardenas M, Lovatt C (2008). Gibberellin-induced gene expression associated with cytoplasmic male sterility in sunflower. *Biotechnology & Biotechnological Equipment*, 22 (2): 691-698.

Eenink A (1977). Induction of male sterility in lettuce (*Lactuca sativa* L.) by application of gibberellic acid: a technical note. *Euphytica*, 26(1): 31-32.

Eenink A, Vereijken A (1978). Anatomical changes in flower buds of lettuce (*Lactuca sativa* L.) treated with a GA3-solution for induction of male sterility. *Acta Botanica Neerlandica*, 27 (3/4): 199-204.

Eşiyok D (2012). Winter and summer vegetable cultivation. (In Turkish) Ege University, Agriculture Faculty, *Horticulture Department*, 410 p.

Feráková V (1977). Genus *Lactuca* L. in Europe. *Univerzita Komenského*, p. 88-94.

Ghebrehiwot H, Burgdorf R, Hussein S, Laing M (2015). The efficacy of four gametocides for induction of pollen sterility in *Eragrostis tef* (Zucc.) Trotter. *African Journal of Biotechnology*, 14 (9): 774-780.

Goubara M, Takasaki T (2004). Pollination effects of the sweat bee *Lasioglossum villosulum trichopse* (Hymenoptera: Halictidae) on genic male-sterile lettuce. *Applied Entomology and Zoology*, 39 (1): 163-169.

Hayashi M, Ujiie A, Serizawa H, Sassa H, Kakui H, Oda T, Koba T (2011). Development of SCAR and CAPS markers linked to a recessive male sterility gene in lettuce (*Lactuca sativa* L.). *Euphytica*, 180 (3): 429-436.

Huang S, Cerny RE, Qi Y, Bhat D, Aydt CM, Hanson DD, Malloy KP, Ness LA (2003). Transgenic studies on the involvement of cytokinin and gibberellin in male development. *Plant Physiology*, 131 (3): 1270-1282.

Hussain SM, Hussain K, Farwah S, Rizvi S, Rashid M, Saleem S, Andrabi N, Rashid H (2018). Male sterility in vegetable crops. *Journal of Pharmacognosy and Phytochemistry*, 7 (3): 3390-3393.

ISTA (2009). International Rules for Seed Testing. *The International Seed Testing Association (ISTA), Zurichstr*. 50, CH-8303, Bassersdorf, Switzerland.

Karaağaç O, Balkaya A (2019). Varieties used in today's production and their properties. *Tarım Gündem Dergisi*, [13.05.2022].

Khatib P, Olfati CJA, Hamidoghli Y (2016). Efficiency of different lettuce (*Lactuca sativa* L.) emasculation methods for hybrid seed production. *Seed and Plant Journal*, 32(1):141-146.

Ma BL, Smith DL (1992). Post-anthesis ethephon effects on yield of spring barley. *American Society of Agronomy*, 84: 370-374.

Michel H, Soussin T (2014). Production of hybrid *Lactuca sativa* seeds. *US Patent*, Patent No: US 8,716,551 B2.

Nagata R (1992). Clip-and-wash method of emasculation for lettuce. *HortScience*, 27 (8): 907-908.

Nelson PM, Rossman E (1958). Chemical induction of male sterility in inbred maize by use of gibberellins. *Science*, 127 (3313): 1500-1501.

Prohens J, Nuez F (2008). Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae. *Handbook of Plant Breeding*, DOI: https://doi.org/10.1007/978-0-387-30443-4.

Ryder EJ (1999). Lettuce, endive and chicory. *USDA-ARS, Salinas*, CA 93905, USA. ISBN: 9780851992853.

Şalk A, Arın L, Deveci M, Polat S (2008). Special vegetable book. (In Turkish). Tekirdağ: Namık Kemal University Agriculture Faculty, *Horticulture Department*, 488 p.

Takada K, Watanabe S, Sano T, Ma B, Kamada H, Ezura H (2007). Heterologous expression of the mutated melon ethylene receptor gene Cm-ERS1/H70A produces stable sterility in transgenic lettuce (*Lactuca sativa*). *Journal of Plant Physiology*, 164 (4): 514-520.

Thompson RC (1958). Natural cross-pollination in lettuce. Proc. Am. *Soc. Hort. Sci*., 403-409.

Tinna D (2019). Chemically induced male sterility in hybrid breeding of vegetables: A review. *Journal of Pharmacognosy and Phytochemistry*, 8: 430-434.

Yılmaz Mİ (2010). Investigation of male sterility effects of some growth regulators on hybrid seed production in sunflower (*Helianthus annuus* L.). *MSc Thesis, Namık Kemal University*, Turkey.

Yılmaz C, Yetişir H, Sarı N (2002). The effects of some chemical treatments on head formation in lital lettuce (*Lactuca sativa* L.). *Alatarım*, 1 (2): 36-41.