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Investigating the Possibility of Producing Double Haploid Lines in Some Selected Cultivars of Greenhouse Cucumber Through Induction of Parthenogenesis

Sibgol Khoshkam[1], Davood Samsampour[2*], Mehran Enayati Shariat Panahi[3] and Behnam Naserian Khiabani[4]

[1]Department of Plant Breeding and Biotechnology in Horticultural Products, University of Hormozgan, Bandar Abbas, Iran
[2]Department of Horticulture, Faculty of Agriculture, University of Hormozgan, Bandar Abbas, Iran
[3]Associate Professor, Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII)
[4]Department of Plant Breeding, Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute (NSTRI)
*Corresponding author: Davood Samsampour

Abstract

Cucumber with the scientific name Cucumis sativus L. is an annual plant of the Cucurbitaceae family. The genus Cucumis has approximately 30 species, two of which, namely cucumber (C. sativus L., 2n=2x=14) and melon (C. melo L., 2n=2x=24) have high economic importance. Cucumber is among the top 10 summer vegetables produced in the world. Its origin is the warm regions of northeastern India. Ensuring the food security of a country is considered the authority of that country. The hybrid seed production industry is growing in the large industrial world and has a high turnover, providing suitable and timely seeds is considered an important structure to maintain the capacity and ability to supply food. Haploid and double haploid technology through gametophytic embryogenesis and changing the expression of genes involved in the emergence of important agricultural traits provides the possibility of exploiting the genetic potential of genotypes and is a suitable approach for hybrid seed production. Product productivity can be increased to a great extent by using hybrid F1 varieties resulting from the crossing of pure lines with desirable traits. Pure lines are very valuable in breeding programs and genetic research. In this regard, a research was conducted in the research greenhouses of the Southern Agricultural and Natural Resources Research Center of Kerman province as a factorial experiment in the frame of a completely randomized design in 3 replications. Genotypes at 5 levels were considered as the first factor and radiation treatment at two levels of 300 and 350 Gy for the sterilization of greenhouse cucumber pollen seeds were considered as the second factors. The results showed that the ploidy level distribution in double haploid seedlings was the most haploid induced by the treatment of 300 Gy gamma ray radiation. The produced plants had half the number of haploid chromosomes and a combination of normal chromosomes and half chromosomes (mixoploid). The number of chromosomes in haploid plants was doubled by the laboratory treatment of colchicine with a concentration of 500 mg/liter for 24 hours. The results of this research showed that this method can be used to produce homozygous lines in breeding programs and produce parental lines in different genotypes of greenhouse cucumbers by removing the obstacles of seedling regeneration.

Keywords: induction of parthenogenesis, parental line, chromosome counting, cucurbits, treated pollen.

Introduction

The seed production industry on par with the pharmaceutical industry in the world, is one of the intensive research and development industries in the world, which in turn shows the rapid pace of technological progress. 70% of the yield increase in the product is related to the type of seed used, and the rest is related to the protection of products and fertilizers. The use of biotechnology technologies, with the aim of continuously increasing the production of agricultural products to meet the needs of the growing population of the world, improving product quality. To ensure a long and healthy life, addressing the problems of global warming and environmental pollution, along with the challenges of developing new resources in plant breeding is of great importance. In the last half century, a large part of the significant increase in the yield of agricultural products has been the result of breeding programs and the improvement of management techniques. Haploid and double haploid technology can effectively help to select superior plants. Haploids are plants with a gametophytic chromosome number, and double haploids, whose chromosomes have been doubled, enable the one-step development of complete homozygous lines from heterozygous parents through gametophytic embryogenesis. Today, with the help of powerful biotechnology tools for strategic plant breeding programs and the use of haploid and double haploid induction techniques, it is possible to produce 100% homozygous lines within 1-2 years, during which each double haploid line has a distinct genotypic and phenotypic class. with a unique combination of homozygous alleles. Every year, new numbers of this product are offered by the production companies as the most efficient and attractive technology of seed production, which have this advantage exclusively. So far, many researches have been done on the production of double haploid lines in the Cacarbitaceae family. The first report of haploid plant production was reported in Datura innoxia in 1992 by Bailey and colleagues, and subsequently, haploids were investigated in many other species (Tian et al. 2020)., The production of new cultivars that have advantages in terms of performance, quality, resistance to diseases and durability, through traditional methods, requires 6 to 8 years of time (Ertan et al. 2018)., The performance of parental lines is often influenced by the environment. Cucumber resistance to pathogens is regulated by recessive genes and makes the production process difficult (Gémes-Juhász et al. 2002). In this method, double haploid lines can be inbred within 1-2 years using Obtained from ovarian cultures. This approach is potentially very valuable for discovering new genetic recombinations (Isidre et.al. 2020). In addition, the doubling of haploids is used for genetic analysis and gene function analysis in cucumber (Jahidul.et.al.2020). According to the research, the production of the first haploid from the Cucurbitaceae family in summer squash through unfertilized ovary culture It was reported (Tomas. et al. 1986). Also, in vitro induction of haploid in summer squash was studied by the method of in vitro culture of unfertilized anthers and ovaries, and both obtained haploid plants (Juhász and Jakše, 2005). Induction of reproductive haploids by in vitro culture of unfertilized ovule or ovary or flower buds has been transformed in inbredline production in different species (Diao et al. 2009; Shalaby. 2007).

Aliabadi et al. (2012) studied the heritability of traits affecting fruit flavor and introduced the best index for its improvement. They studied 15 local cucumber genotypes as parents and reported that in most of the traits, little incremental variance was observed. These researchers also reported that general heritability was high for most of the traits, which indicated the great genetic diversity of these traits in the germplasm used. In another study, in order to estimate the strength of general combinability for cucumber fruit texture firmness trait, a cross between five lines as paternal parent and four inbred lines (female parent) was investigated and it was found that the contribution of incremental variance for fruit tissue firmness trait was high. Nevertheless, some dominance for this trait was observed in the f1 generation. The heritability of fruit tissue hardness in mesocarp included additive effects without maternal effects. (Ene et al. 2018) using four parental lines and six hybrids obtained from them, they reported significant general and specific combining power for all investigated traits in cucumber and identified They showed that additive and non-additive effects were involved in the occurrence of genes for all traits. Elfati et al., compatibility and heterosis of quality fruit traits of Cucumis cucumber lines. sativus L. were investigated in the form of 6x6 incomplete dialle test to determine the mode of action of genes. The results of variance analysis showed a great difference in the F1 generation and the value of each parent in the hybrids was investigated. The values of public and private composability also showed a significant difference. The color of the fruit is controlled by the additive action of genes due to the high general compatibility. The shape of the fruit and the general appearance of the fruit are controlled additively and non-additively according to the high general

combinability of this trait. Lines 605 and 118 are desirable and recommended for the production of hybrid cucumber seeds due to their high public and private compatibility. The main objective of this study was to evaluate the commercial value and parthenogenetic capacity of selected greenhouse cucumber genotypes to be used to obtain double haploid lines as parental lines for breeding programs.

Materials and methods:

This research was conducted in 1402 in the research greenhouses of the Southern Agricultural and Natural Resources Research Center of Kerman province as a factorial experiment in the form of a completely randomized design with 3 replications, in which the genotype was at 5 levels (Evergreen, Royal 189, Negin, Saba, 547). And the second factor of radiation treatment at two levels of 350 and 300 Gy gamma rays was used for pollen grain sterilization and in that parthenogenetic capacity of selected genotypes was evaluated and optimized. To apply pollen grain sterilization treatments, after the plant reaches the flowering stage, one day before mating, female flowers were isolated with paper envelopes, and male flowers were collected and used to apply radiation treatment with gamma rays. Early the next morning, isolated female flowers were crossed with treated male flowers and they were again isolated using paper bags to prevent unwanted pollination and to control temperature and humidity. Three days after pollination, the isolation bags were removed and the female flowers were examined to assess their development into fruit or abscission. In addition, germination test and pollen tube growth were also evaluated. The fruit obtained from the crosses was harvested within 3 to 5 weeks after pollination and transferred to the laboratory. The seeds containing parthenogenetic embryos were extracted with the help of sharp tipped forceps and scalpel blade on open sterile filter paper and cultured in solid E20A medium. In order to obtain more number of plants in laboratory conditions, each of the rescued embryos were propagated at the stage of 4 to 6 small leaves to obtain the desired number of clones from each plant for adaptation, determination of ploidy level and doubling. Flow cytometric analysis was performed to determine the amount of genomic DNA and the ploidy level of seedlings. To double the chromosomes, the samples were exposed to 500 mg/L colchicine for 24 hours in laboratory conditions. After 24 hours, the micro-samples were washed three times with sterile distilled water and the surface moisture of the seedlings was taken on a sterile filter paper and cultivated in MS solid culture medium and entered the adaptation process at the 4-6 leaf stage. For adaptation, the lid of the culture containers was opened over time and the plants were slowly removed and placed in plastic cups containing sterile peat soil and covered with thin plastic bags to maintain relative humidity (approximately 85-95%). After acclimatization in the afternoon (temperature adjustment), they were cultivated in the greenhouse and grew until flowering, and after inducing the production of male flowers (GA3), they were self-pollinated, and after about 5-6 weeks, the resulting seeds were extracted.

Results:

Figure (1)**View of the greenhouse where the experiment was conducted.**

Figure (2): Preparation of male flowers for gamma ray irradiation

Table 1- ANOVA of genotype and ploid levels on studied traits of haploid induction test in selected cultivars of greenhouse cucumber

Sources of variance	Pollinated flowers	Developed flowers	Number of formed fruits	Number of parthenocarpic embryos	Number of cultured embryos	Losses (%)	Survival of embryos (%)
Genotype	125/900*	311/067 **	4/067 ns	139/43 **	54/233 **	748/53 ns	14/233**
Haploid level	105/800 ns	164/356 *	0/556 ns	69/68 **	33/800 **	254/08 ns	6/422 *
Genotype × haploid level	36/078 *	37/911 *	1.333 ns	20/96 *	6/552 *	223/91 ns	1/589 *
Error	60/900	36/867	3/467	9/633	2/700	656/193	1/567
(COV)	8/59	14/41	29/14	11/74	9/54	26/89	12/23

Table 2- The number of parthenogenesis embryos of cucumber genotypes during temperature treatments and gamma radiation doses.

Genotype	1	2	3	4	5
300gry	8.67^{ab}	10.67^{a}	5.00^{cd}	4.00^{cde}	4.67^{cd}
350gry	6.33^{bc}	4.00^{cde}	5.33^{cd}	1.33^{e}	3.00^{de}

Table (3): The effect of gamma irradiation dose on ploidy of the parthenogenetically induced regenerated Genotypes of cucumber(Cucumis sativus L.)

Irradiation dose	Genot[pe	Total number of regenerated of plants	Total number of Haploid plants	Total number of diploid/mixoploid plants
Control	G1	17	0(n)	17(2n)
	G2	19	0(n)	19(2n)
Total	G3	7	1(n)	6(2n)
Percentage of	G4	21	0(n)	21(2n)
plants respons	G5	30	1(n)	29(2n)

	5 100%	94 100%	2(n) 2.12	92(2n) 97/87
300Gy **Total** **Percentage of** **plants respons**	G1 G2 G3 G4 G5 5 100%	15 7 5 19 27 73 100%	7(n) 3(n) 2(n) 7(n) 9(n) 28 38/35	8(2n) 4(2n) 3(2n) 12(2n) 18(2n) 45 61.64
350Gy **Total** **Percentage of** **plants respons**	G1 G2 G3 G4 G5 5 100	14 8 3 11 24 60 100	4(n) 4(n) 3(n) 3(n) 5(n) 19(n) 31.66	10(2n) 3(2n) 0(2n) 8(2n) 18(2n) 39(2n) 62(2n)

Figure (3): Production of parthenocarp fruits containing parthenogenesis embryos (A), detection of parthenocarp embryos (B), removal of the shell and isolation of haploid embryos (C), cultivation of parthenocarp embryos (D), compatibility of double haploid seedlings (E).

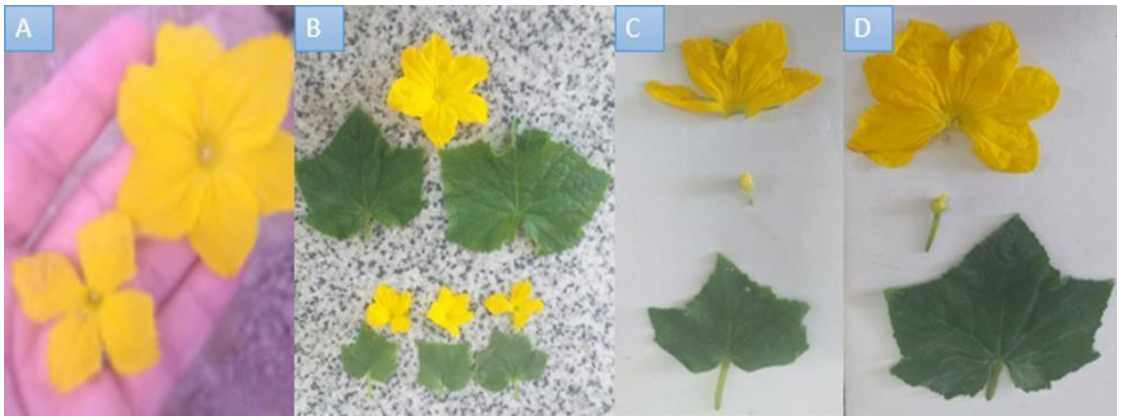

Figure (4): phenotypic difference of flower, stem and leaf of haploid and double haploid greenhouse cucumber (A, B, C, D).

In this research, donors were selected genotypes of greenhouse cucumber that were evaluated for haploid induction. Their parthenogenetic potential was evaluated by radiation treatment, parthenogenetic embryo rescue, seedling performance in laboratory conditions and chromosome doubling. About one-third of the harvested fruits from each treatment had at least 1-7 embryos, some fruits had empty seeds. The ratio of embryos per fruit was different in different treatments and genotypes. Based on the results of the analysis of variance table, in all genotypes, a significant difference was observed between the traits of survival percentage, embryo loss, the number of cultivated embryos, the number of fruits formed, the number of pollinated and developed flowers. (Table 1). The work of parthenocarpy induction of selected genotypes of greenhouse cucumber based on the data from table (4) indicates that the highest number of parthenocarp embryos is related to 300 Gy radiation treatment. This means that the dose of 300 Gy was more effective in inducing parthenocarp embryos than the dose of 350 Gy. 300 and 350 Gy radiation treatments did not have a significant difference in the survival of the embryos, this means that after the separation of the induced embryos, there was no significant difference in their survival depending on which treatment was obtained, considering that the genotype of the donor plants is a key factor for The success of haploid studies, the number of haploid embryos was different in different genotypes, the highest number of embryos obtained in genotypes 1 and 2 were 8.67 and 103.67, respectively. The haploid induction response was closely related to the genetic structure of the genotypes. Some genotypes had a good parthenocarpic response, while others were poor. In various researches, the difference in ploidy level has been mentioned related to diploid anther wall tissue, spontaneous diploid and irregular meiosis (Tian et al., 2023).

Based on the results of Table 3, the highest number of fruits produced from double haploid lines was obtained from genotype 5 and the lowest from genotype 3. Phenotypic examination of male flowers in plants resulting from self-pollination of the plant in cultivar 2 resulted in small size and limited pollen amount, which is consistent with the results of similar research.

Discussion and conclusion:

The results of data analysis showed that the parthenogenetic response of the genotypes was different in different treatments and changed in growing conditions and seasons. The best time of pollination in the south of Kerman was November and December with strict control of the conditions in the greenhouse and laboratory, an average of 8 fruits were produced from each treatment, and by analyzing 5 genotypes, a significant difference ($P < 0.01$) was obtained between the genotypes. There was a great variation in the measured parameters among the 5 evaluated genotypes. The highest number of fruits produced from double haploid lines was obtained from genotype number 5 with 7 fruits and the lowest number was obtained from genotype number 3 with two fruits. The phenotypic investigation of male flowers in the self-pollinated plants in cultivar number 2 was obtained with a small size and a limited amount of pollen, which was consistent with the results of similar research. The conditions of light, temperature, humidity, pests and diseases governing the greenhouse had a great effect on the success rate of the parthenogenesis process, which was consistent with the research conducted by Salim et al. (Salehiam., 2023). Parthenogenetic embryo efficiency was expressed as the number of embryos in each treatment. The highest number of parthenocarp embryos was obtained in genotypes 2 and 1 at the rate of 10.67 and 8.67%, respectively, and the lowest in genotype number 5 (Table 2). Colchicine was successfully used to induce diploidy at a concentration of 500 mg/L in vitro for 24 hours. Ploidy levels had an effect on the morphology of leaves, flowers and fruit morphological parameters. In the present research, comparing the shape and size of leaves and flowers showed an obvious difference between haploids and double haploid plants (Figure 3). In this study, the production of double haploid lines from 5 greenhouse cucumber genotypes was evaluated and produced. Of course, further optimization using different changes should be measured in order to produce more double haploid lines and can be used in cucumber breeding programs. Keeping irradiated male flowers in plastic bags in the dark for one to two days was successful in maintaining pollen viability (researcher's experience). Using more than this time led to a decrease in pollen viability and the inability to form cucumber fruit (Hogost et al., 2020).

Parthenogenetic embryo efficiency was expressed as the number of embryos in each treatment. The process of parthenogenetic embryo diagnosis was laborious and required the researcher's experience Considering that currently, the fastest way to obtain cucumber parental lines is to produce haploid plants and multiply their chromosomes, using haploid and double haploid induction technology is a suitable approach in breeding programs

to save time and produce pure parental lines. which is necessary for large-scale hybrid seed production and provides many advantages to hybrid seed producers (Glazka et al., 2013). This is considered as an advantage, especially in countries like Iran, which is still dependent on other countries for the production of vegetable seeds. Identifying optimal environmental conditions for growth, pollination time, hormonal treatments, embryo rescue and doubling method can further improve the efficiency of double haploid production.

Findings:

- Induction of double haploidy was made possible by using gamma irradiation treatment as a fast method to develop pure lines in one year (researcher mastery, time, facilities and laboratory and greenhouse conditions were very effective)
- The difference between haploid and diploid plants in terms of the phenotype of leaves and flowers was clearly evident in the greenhouse. Colchicine at a concentration of 500 mg/L was successfully used for doubling.
- Examination of one-by-one seeds under laminar light was successful for searching, identifying and isolating haploid embryos.

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