RESEARCH ON THE POSSIBILITY OF INDUCING HAPLOID EMBRYOS THROUGH IRRADIATED POLLEN TECHNIQUE IN COTTON

Dr. Şaire R. TÜRKOĞLU

A. DOLANÇAY, H. KAYA, F. TOKLU, P. GÖK GÜLER, R. HATİPOĞLU, Z. NASIRCI, S.SÜLÜ, B.S. ÖZBEK

Çukurova Agricultural Research Institute, Adana/TURKEY

saire_t@yahoo.com

Introduction

- The most common breeding procedure for producing new cultivars in cotton
  - hybridization
  - pedigree selection during the segregation generations
- According to the this procedure, it is needed intensive labor for 5-7 years for purification of the lines after hybridization.

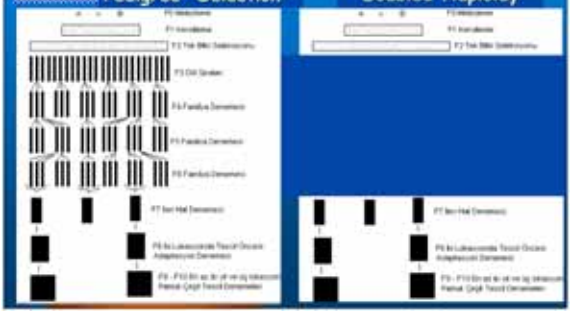
Introduction

- With the doubled haploid technique,
  - it can be possible to obtain pure lines in a single generation
  - the time required a cultivar developing process can be shortened importantly.
- The important advantage of this method is to be able to obtain 100% homozygous plants in a single generation.

Hybridization followed by a pedigree selection and advantage of doubled haploid technique

Pedigree Selection

Doubled Haploidy



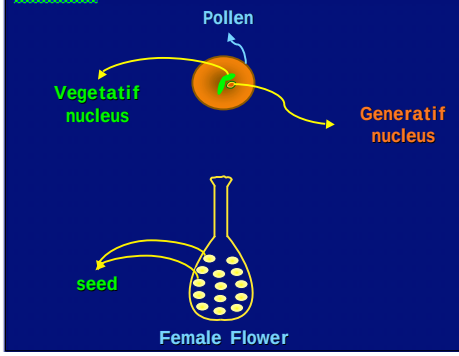
Doubled Haploid Technique

Haploidization techniques have been applied as

- *in vitro* androgenesis
  - anther culture and
  - microspore culture
- *in vitro* gynogenesis and partenosis
  - unpolinated ovule-ovary culture,
  - chromosome elimination,
  - pollination with incomplete pollen.

Irradiated Polen Technique

Normal Fertilization



Normal Fertilization

Normal Fertilization

Normal plant with 2n chromosome number

Irradiated Pollen Tecnique

Polen Irradiation

It is expected:

- Generative nucleus is inactivated (incomplete pollen)
- Vegetative nucleus is activated for formation of pollination tube and stimulating the embryo sac.

Irradiated Pollen Tecnique

Haploid Embryo Induction

Embryo formation from the haploid cells in embryo sac. (antipod, synergid and especially egg cell)

Fruit/Boll Formation

Rescue Haploid Embryos

In vitro culture of the embryos at an early stage

Haploid Plant Formation

Doubling Chromosomes

Homozygous, fertil plant with 2n chromozom number

Irradiated Pollen Technique

Successful results were obtained by the induced partenogenesis through irradiated pollen technique on mostly

- Cucurbitaceae species, (watermelon, cucumber, snake cucumber, squash)
- the others (sugar beet, strawberry, kiwi, petunia, carrot, cabbage and sunflower)

Haploidy on Cotton

- There are some previous studies on anther culture, microspore culture, partenogenesis, and semigamy to obtain haploids in cotton.

Haploidy on Cotton

- Although semigamy has been already applicable method, it has some disadvantages being unstable and to be restricted to the two tetraploid species

(Zhang and Stewart, 2004, Bajaj and Gill, 1988).

Haploidy on Cotton

- As a result, there hasn't been any effective method for shortening to obtain the pure lines in cotton breeding programs yet.

Aim of this study

- In this study, we aimed to investigate the possibility of inducing haploid embryos and plants through irradiated pollen technique as a new approach to produce doubled haploids in cotton breeding programs.

Material and Method

- Çukurova 1518 cotton variety was used as the plant material.
- Laboratory studies were carried out at Çukurova University in Adana.
- Pollen irradiation treatments were made at the Turkish Atomic Energy Authority in Ankara.

Material and Method

- The flowers were emasculated one day before anthesis and isolated with paper bags.
- At the same day the flowers were collected from the male plants were sent to Ankara for irradiation.
- Pollens of them were irradiated by a reactor that supplied gamma rays from 60Co.

Material and Method

- After pollination the female plants were shaded with polypropylene shading cover.
- The bolls on the plants were harvested and the immature seeds were investigated under the binocular and cultured E20A medium.
- In the study depending on the gamma ray doses, normal, small and abortive seeds formation rates and embryo induction rates at different ages and stages (point, globular, arrow tips, heart, torpedo) were recorded.

Investigated Traits

- Number of bolls
- Number of Seeds
  - Normal
  - Small
  - Abortive
  - Total

Normal ve Abortive Seeds

Investigated Traits

Embryo Traits Cultured *In vitro*

- Nokta
- Globular
- Arrow Tips
- Heard
- Torpedo
- Amorphous
- Diploid

Be able to haploid embryos

Investigated Traits

Shapes and Development Stages of Embryos Cultured *In vitro*

These types of embryos can be haploid embryos resulting of gamma ray stimulating.

RESULTS AND DISCUSSION

The number and the formation rates of normal, small and abortive immature seeds depending on gamma ray doses

Gamma Ray Doses	Total Number	Total Number of Immature Seeds	The Rate of Immature Seeds (%)		
			Normal	Small	Abortive
		260	88,5	0,00	11,5
		613	28,4	7,7	63,9
		453	14,6	4,6	80,8
		363	10,7	4,4	84,8
		182	9,3	6,0	84,6
300	10	274	6,9	6,2	86,9
Top/Ort	83	2145			

Normal seed formation was reduced and abortive seed formation was increased with increasing of gamma ray doses.

RESULTS AND DISCUSSION

The number of different development stages embryos and embryo induction rates depending on gamma ray doses

Doses (Gray)	Embryo Traits							Number of Total Embryos	Number of Total Seeds	Rate of Embryo Induction %
	Point	Glob.	Arrow Tips	Heart	Torpedo	Amorp	Diploid			
0 (K)	0	0	0	0	0	0	215	215	260	82,7
100	8	10							613	4,6
150	7	2							485	2,6
200	5	1							363	1,7
250	0	0							182	1,6
300	0	0							274	0,7
Total	20	13	12	1	1	4	215	266	2145	

It is indicated that haploid embryo induction rate was reduced with the increasing the level of gamma ray doses.

RESULTS AND DISCUSSION

The number of different development stage embryos and embryo induction rates obtained from the bolls harvested at the different ages

Ages of Boll (Day)	Embryo Traits							Number of Total Embryo	Number of Total Seeds	Rate of Embryo Induction (%)
	Point	Globi	Arrow Tips	Heart	Torp	Anor	Diploid			
									149	0,0
									201	2,0
									159	4,4
									479	3,5
									147	3,4
									750	2,4
									1885	2,7

- The highest embryo formation rate was observed from the **21 days old** bolls.
- Embryo formation rates of **30 and 35 days** seeds were not very low but they were necrotic and didn't grow.
- **18, 21 and 28 days old** embryos were showed better growing in culture compared with the others.

CONCLUSION

- Although our results indicated a low frequency of haploid embryos and
- we didn't succeed to obtain haploids completely converted into plants,
- this study is the first beginning for inducing haploid embryos through irradiated pollen technique in cotton.
- It is needed much more studies for increasing the effectiveness of the method.

CONCLUSION

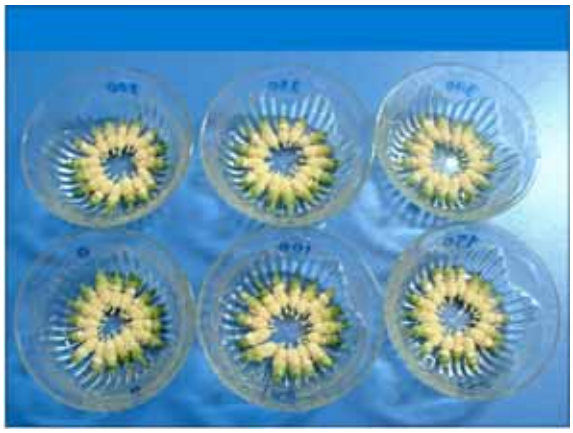
According to the results of the study, we can recommend that;

- improving culturing conditions of female plants,
- using lower gamma ray doses,
- trying different embryo rescue mediums and
- culturing 18-28 days old embryos

Emasculation of female flower

Preparation of male flowers for irradiation

Preparation of male flowers for irradiation




Irradiation of pollens
(Turkish Atomic Energy Authority- Ankara


Irradiation of pollens
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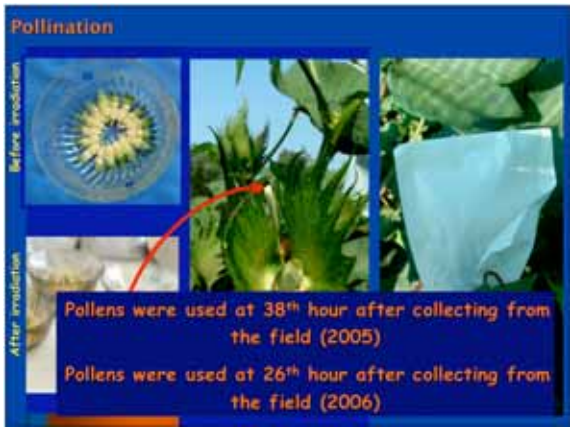

Pollination
Before irradiation
After irradiation
Pollens were used at 38th hour after collecting from the field (2005)
Pollens were used at 26th hour after collecting from the field (2006)


Shading of female plants

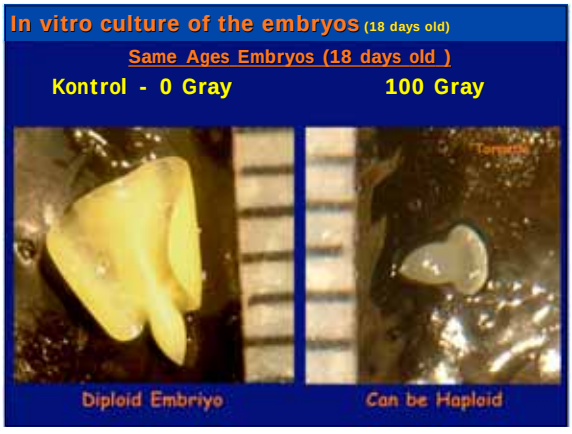

Harvested bolls


Surface Sterilization of the bolls

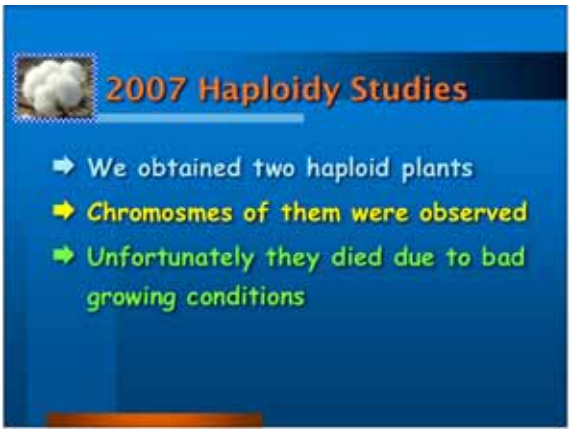

In vitro culture of the embryos (18 days old)
18. Gün
Control
0 Gray
Diploid Embriyo


In vitro culture of the embryos (18 days old)
18. Gün
Kontrol
0 Gray
Diploid Embriyo


In vitro culture of the embryos (18 days old)
Same Ages Embryos (18 days old)
Kontrol - 0 Gray
100 Gray
Diploid Embriyo
Can be Haploid


In vitro culture of the embryos (18 days old)
Same Ages Embryos (18 days old)
Kontrol - 0 Gray
100 Gray
Diploid Embriyo
Can be Haploid

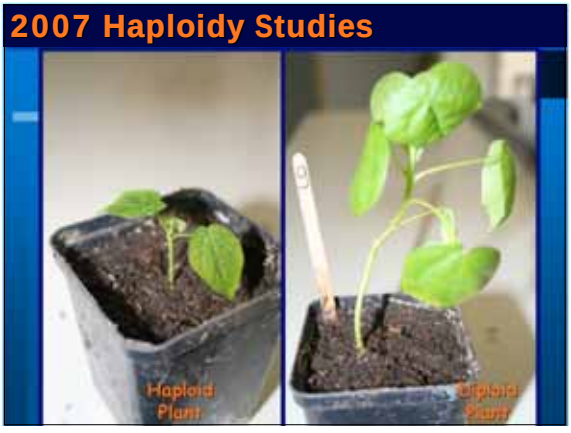

100 Gray
Can be haploid
Control - 0 Gray
Diploid
18 days old

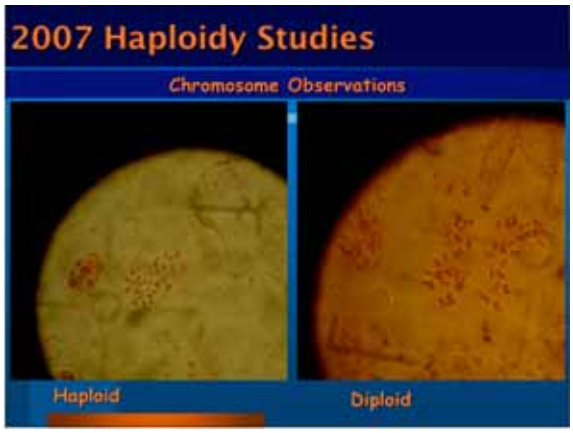

2007 Haploidy Studies
We obtained two haploid plants
Chromosmes of them were observed
Unfortunately they died due to bad growing conditions


2007 Haploidy Studies
Torpede (during in vitro culture)
cotyledon (Subculture)


2007 Haploidy Studies
Acclimatization


2007 Haploidy Studies
Acclimatization


2007 Haploidy Studies
Haploid Plant
Diploid Plant


2007 Haploidy Studies
Chromosome Observations
Haploid
Diploid


THANKS