CHROMOSOME MANIPULATION IN DISTANT HYBRIDIZATION

INTRODUCTION

- **Chromosome manipulation** - The term chromosome manipulation or chromosome engineering describes process or technologies in which chromosomes are manipulated to change their mode of genetic inheritance.
- **Distant hybridization** - a cross of two individuals belonging to different species (interspecific hybridization) or genera , it is called as intergeneric hybridization

TECHIQUES OF DISTANT HYBRIDIZATION

- Embryo Rescue
- Alien Addition lines
- Alien substitution lines

EMBRYO RESCUE :

- When embryos fails to develop due to endosperm degeneration, embryo culture is used to recover hybrid plants

TYPES OF CHROMOSOMES MANIPULATION

- Incorporation of a single or fragments of chromosome from a wild into the existing crops to enhance the genetic diversity
- Incorporation of an alien chromosome by chromosomes doubling in order to produce amphidiploids
- Elimination of an alien chromosome in order to induce haploids.

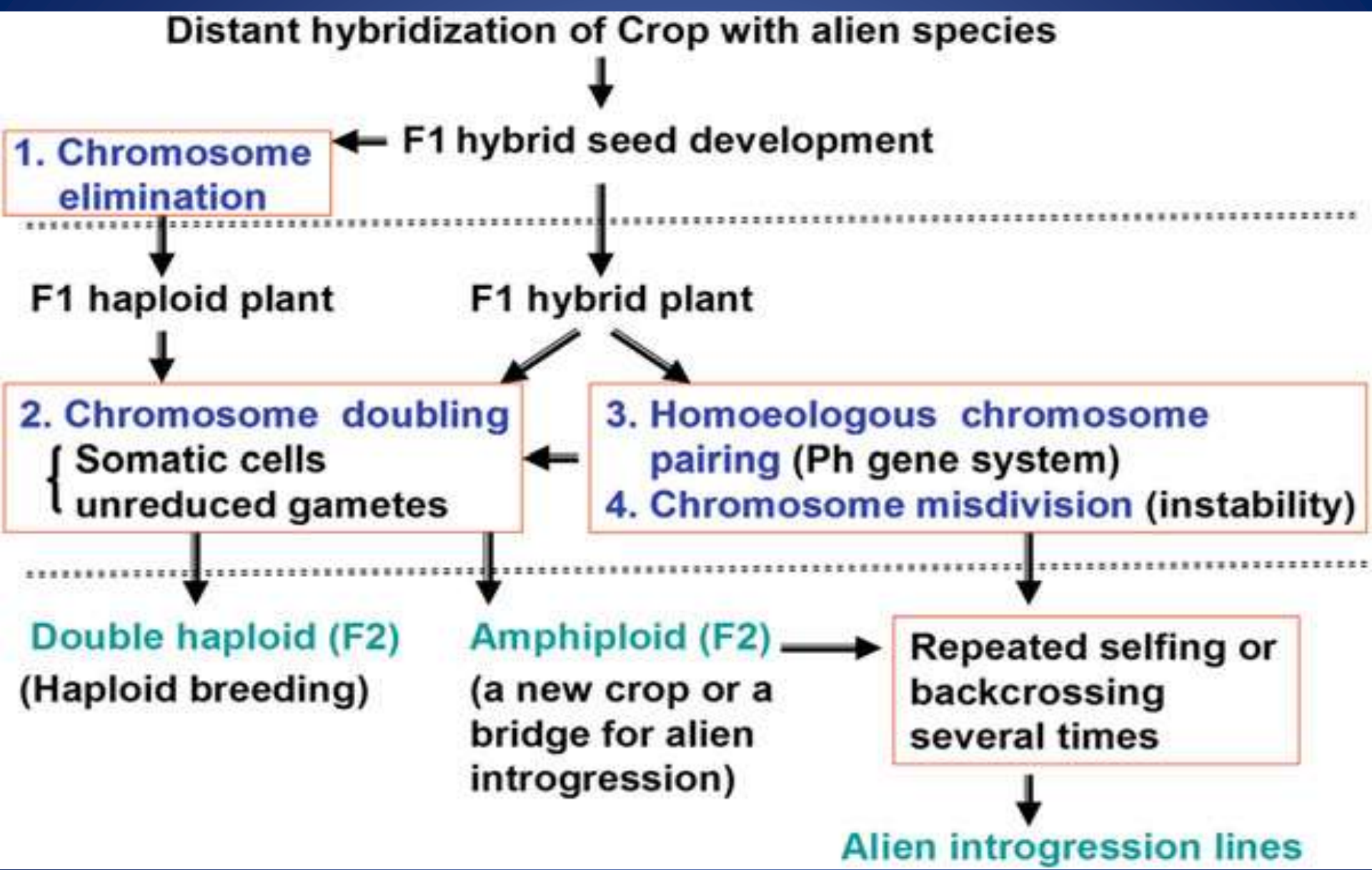


Fig. 1. Chromosome manipulation based on chromosome behaviors in F 1 hybrids.

CHROMOSOMES MANIPULATION IN CROP IMPROVEMENT

➢ Chromosomes elimination and haploid crops

- Chromosome elimination is the degeration of one parental chromosomes in F1 hybrid due to inactivation of kinetochore function.
- production of doubled haploids

➢ Chromosomes doubling and amphidiploids

- Chromosome doubling can be carried out through the treatment with antimicrotubule drugs, Colchicine (originally extracted from autumn crocus (*Colchicum autumnale*).

- Amphihaploid chromosomes of interspecific and intergeneric hybridizzation can be doubled by colchicine treatment.

➢ Homeologous chromosomes pairing

- The manipulation of *Ph1* gene can relieve the restriction of homoeologous chromosome pairing and thus improve the efficiency of alien translocation development.

Advantages of chromosomes manipulation

- Increase genetic diversity
- Produce variability and varieties
- Production of gene introgression lines
- Overcoming barriers of distant hybridization

Limitation

- Limited knowledge on process of gene expression and intremediary metabolism
- Complex nature of DNA

Table 1: Seed development percentages (embryo formation in parenthesis) in bread wheat, durum wheat and hexaploid triticale crossed with pearlmillet and maize

Genotypes	pearlmillet			Maize		Mean
	LGD-1-10-B	H-77/833-2P5	843B	NEC-7006	CML-246XCML-242	
Bread wheat						
Norin 61	72.8(0.3)	82.4(9.0)	77.6(18.0)	73.2(29.6)	72.5(17.0)	75.9a(20.6a)
Attila	3.0(0.7)	83.9(0.6)	94.5(28.2)	85.6(10.9)	81.0(14.9)	83.6a(11.0b)
Durum wheat						
Sora/plata-12	3.0(0.7)	48.9(14.1)	16.7(8.5)	20.0(4.5)	29.794.3)	23.7b(6.4bc)
Altar-84	0.0(0.0)	25.6(8.1)	2.7(0.4)	7.6(2.0)	0.3(0.0)	7.2c(2.1de)

Cont..

Genotype	pearlmillet			Maize		Mean
	LGD-1-10-B	H-77/833-2P5	843B	NEC-7006	CML-246XCML-242	
Hexaploid triticale						
Anoas-3/ tatu-4	18.4(0.0)	41.0(1.4)	10.3(5.5)	18.0(5.2)	15.5(1.2)	20.6b(2.7cd)
Jilotecpec-96	0.0(0.0)	1.4(0.4)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.3c(0.1e)
Mean	28.0(5.1b)	47.2a(5.6b)	33.6ab(10a)	34.1a(8.7a)	33.2a(6.2ab)	

Ref: Inagaki and Hash; Plant Breeding 117, 485—487 (1998)

Table 2. Frequencies of haploid formation following crossing between wheat x job`s tears and maize

Cross	No. of crossed embryo	No. of formed embryo (%)	No. of haploids plants (%)
Wheat x Job`s tear	218	23	6 (28)
Wheat x Maize	1196	125	33(28)

- The generated F1 plants from the cross of wheat x job`s tears carries 21 chromosomes indicating the complete elimination of chromosomes of job`s tears .
- Colchicine treated F1 plants showed to carries 42 chromosomes i.e. the normal chromosomes of common wheat.
- Job`s tears can be used as a suitable pollen donar plant for haploid wheat production.

(**Ref.**: K. Mochida and H. Tsujimoto. Production of wheat double haploid by pollination with job`s tears. Journal of heredity 2001.**92**(1)-82)

Fig. 1: Steps involved in wheat x maize system to develop haploid lines in wheat :

a) Spikes collected from the field **b)**Caryopses removed from the collected spikes **c)** Cultured embryos on half strength MS media (without growth regulator).

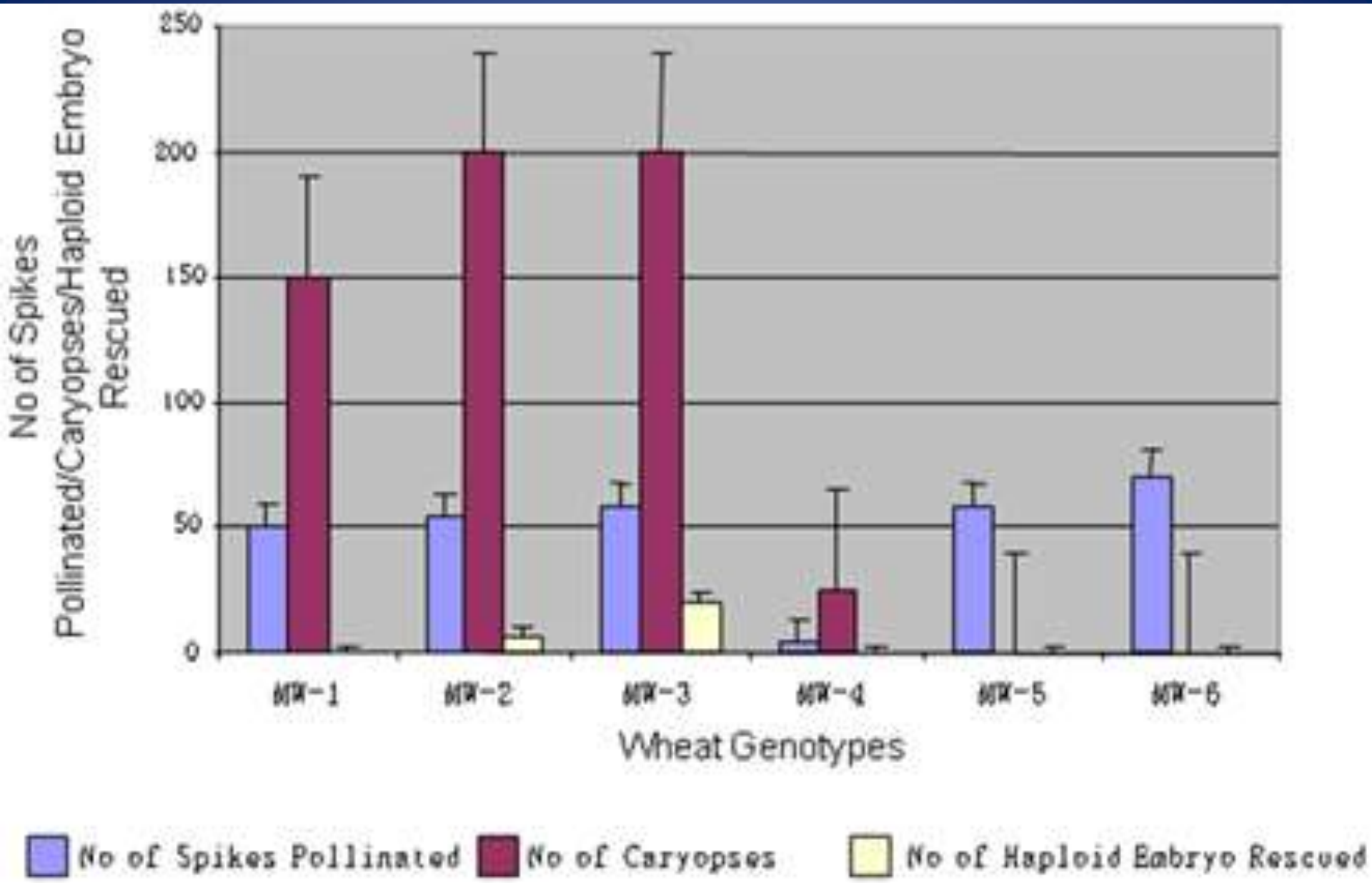
a. b. c.

Ref. : Bhattacharya *et al.* An insight into wheat haploid production using wheat x maize hybridization. J. App.Biol. Biotech.**3** (05): 2015: 45

Table 3: Details of wheat x maize wide hybridization technique used to develop haploid lines in wheat

Wheat Genotype	No. of Spikes (Pollination)	No. of Caryopsis obtained	No. of Haploid
MW-1	51	150	-
MW-2	54	200	07 (3.5%)
MW-3	58	200	20 (10.0%)
MW-4	4	25	-
MW-5	58	-	-
MW-6	71	-	-
Total	296	575	27 (4.69%)
Average	49.33	143.75	13.5
SEM	9.49	40.00	3.30

Source : Bhattacharya *et al.* An insight into wheat haploid production using wheat x maize hybridization. J. App. Biol. Biotech.**3** (05):2015:45



Ref. : Bhattacharya *et al.* J. App. Biol. Biotech.**3** (05);2015:046

Inference

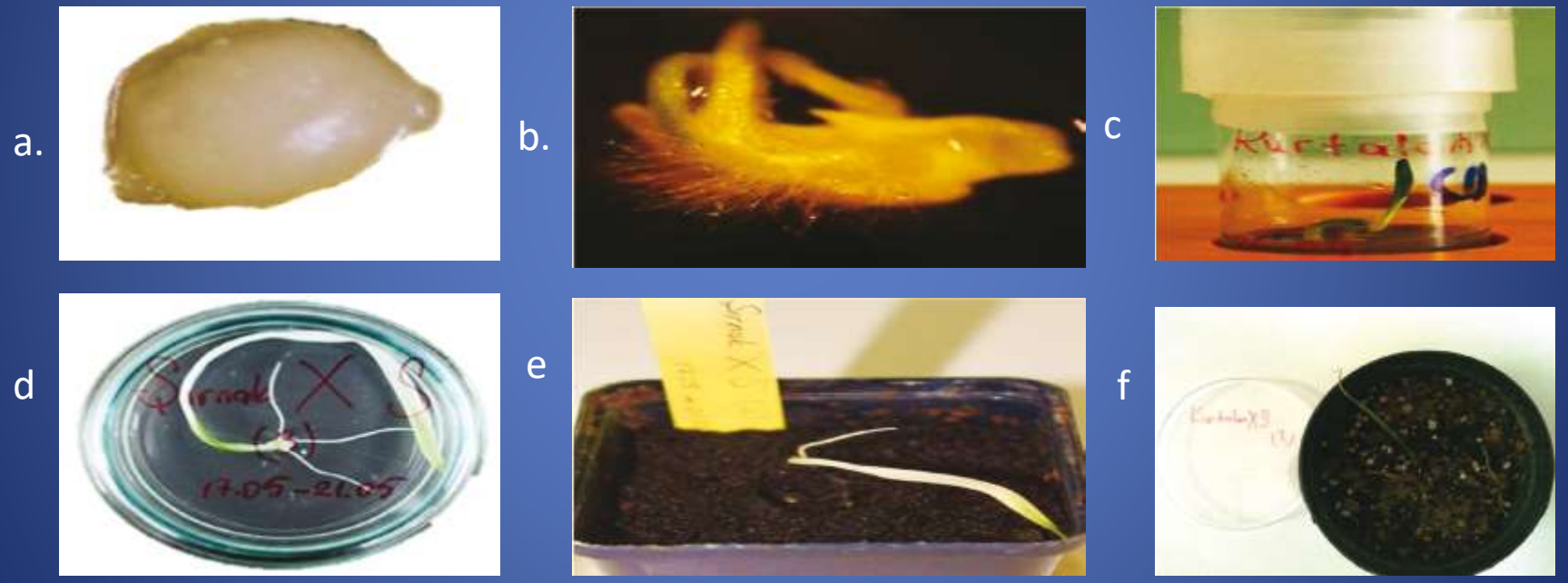
- Higher percentage of embryo formation (3.5-10.0%) was observed on half strength MS media without growth regulators.

- This technology may be used to reduce the time for release of new wheat varieties.

- Wheat x maize system of double haploid development could be the method of choice for wheat breeding programs.

(Bhattacharya *et al.* J. App. Biol. Biotech. **3** (05): 2015)

Fig. 2. Durum wheat × *I. cylindrica* derived dihaploid embryo formation and plant regeneration:
a) isolated embryo 14–16 days after hybridization; **b)** dihaploid embryo regeneration in MS medium (20 days after incubation);
c, d) the rooted regenerants (30 days after incubation);
e, f) the plantlets in acclimatization.



Ref. Celiktas *et al*. Production of dihaploids in durum wheat using *Imperata cylindrica* L. mediated chromosome elimination. Turk. *J.* Agric. For (2015) **39**: pg.50

Table 4. The efficiency of *I. cylindrica* mediated chromosome elimination technique with respect to seed setting % in crosses with durum wheat.

wheat genotypes	Seed setting %		
	Alahan	Antakya	samandag
Sirnak	**14.6**	0.0	20.0
Havrani	2.5	0.0	2.7
Karadere	4.5	0.0	11.1
Kurtalan	7.1	0.0	**31.1**
Menceki	4.4	**8.6**	3.4
Devedesi	6.8	0.0	8.6
Minnaret	8.3	0.0	12.6
Hacihalil	**13.4**	0.0	8.2
Bagicak	3.2	0.0	7.6
Karakilcik	4.6	**6.8**	10.0
Diyabakir-81	12.7	0.0	**24.3**
Mean	7.5	1.4	12.7

Celiktas et al. / Turk J Agric For(2015): **39** pg. 51

Table 5. The efficiency of *I. cylindrica* mediated chromosome elimination technique with respect to embryo formation % in crosses with durum wheat

wheat genotypes	Embryo formation %		
	Alahan	Antakya	samandag
Sirnak	**9.1**	0.0	**15.5**
Havrani	3.8	0.0	1.1
Karadere	4.9	0.0	8.6
Kurtalan	4.2	0.0	**13.1**
Menceki	2.3	**6.3**	2.7
Devedesi	4.2	0.0	4.1
Minnaret	**6.8**	0.0	5.2
Hacihalil	4.7	0.0	5.8
Bagicak	3.8	0.0	3.7
Karakilcik	1.5	**3.2**	7.7
Diyabakir-81	**6.9**	0.0	**13.3**
Mean	4.7	0.9	7.3

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Table 6. The efficiency of *I. cylindrica* mediated chromosome elimination technique with respect to regeneration % in crosses with durum wheat genotypes.

wheat genotypes	No. pollinated florret	Regeneration %		
		Alahan	Antakya	Samandag
Sirnak	**276**	0.0	0.0	**6.1**
Havrani	96	0.0	0.0	0.0
Karadere	139	0.0	0.0	2.5
Kurtalan	**338**	**4.6**	0.0	**11.5**
Menceki	142	0.0	0.0	4.6
Devedesi	64	0.0	0.0	0.0
Minnaret	148	0.0	0.0	0.0
Hacihalil	64	0.0	0.0	0.0
Bagicak	102	0.0	0.0	0.0
Karakilcik	112	0.0	0.0	0.0
Diyabakir-81	**248**	**4.6**	0.0	3.1
Mean		0.8	0.0	2.5

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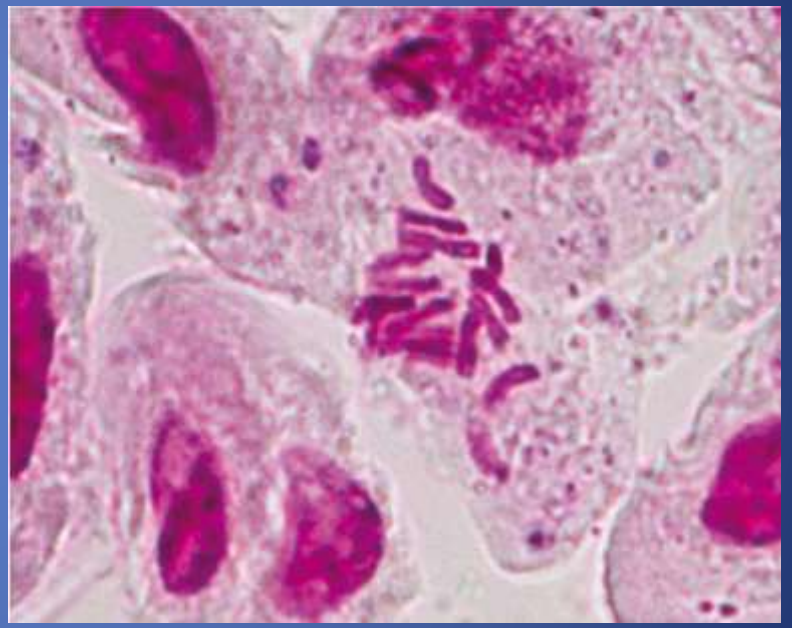
Fig. 3. Cytological investigations of the maternal tetraploid durum wheat (genotype Kurtalan) (2n = 4x = 28) **(a)** and regenerated doubled haploid plantlets (genotype Kurtalan) (2n = 2x =14) **(b)** obtained from the interspecific hybridization of wheat × *I. cylindrica* derived embryos.

a.

b.



Ref.: Celiktas *et al*. Production of dihaploids in durum wheat using *Imperata cylindrica* L. mediated chromosome elimination. Turk. *J.* Agric. For (2015) **39**: pg.52

Inference

- Chromosomes elimination mediated techniques are the method choice for haploids production in cereals.
- Interspecific hybridization with *Imperata cylindrica* results in production of maternal haploid(complete loss of parental chromosomes).
- Effective in the production of wheat haploids for large scale production and for gene mapping and other breeding studies.

(Celiktas *et al*. Turk. *J.* Agric. For (2015) **39**: 48-54)

CONCLUSION

- Chromosomes manipulation techniques manipulated the chromosomes change their mode of genetic inheritance.
- It is useful in the development of various varieties and lines of crops with desireable traits (transfer from wild to cultivated crops).
- It is an important technique to create genetic diversity in crops species(which is the basic for crop improvement and breeding).

THANK YOU