



المركز الوطني لأبحاث النخيل و التمور بالأحساء
Al Hassa National Date Palm Research Center
Project UTFN/SAU015/SAU



Research of new strategies of date palm multiplication (*Phoenix dactylifera* L.)

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**dioecious
species**

Heterozygote

Slow reproduction



**High eterogeneity
of pollinators**

DATE PALM

**Multiplication
by offshoots is
limited**

Need of new and rapid techniques of multiplication

2 methods of propagating date palm :

- 1- Asexual-vegetative propagation or offshoots propagation**
- 2- Recently approach using tissue culture techniques**

Plant material : 4 Tunisian female varieties

Allig - Deglet nour - Kentichi and Menakher

Tissue culture techniques

2 techniques were used for propagating tunisian date palm varieties



Offshoot



Apical Zone



**Somatic
Embryogenesis**



Organogenesis



SOMATIC EMBRYOGENESIS :

4 cultivars were used : Allig – Deglet Nour et Kentichi

1. Somatic Embryogenesis

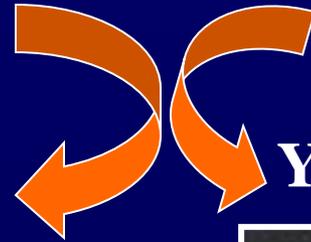
★ Media :

- Tisserat (1979)
- Daguin et letouzé (1988)
- Murashige & Sckoog (1962)

100 mg.l⁻¹ of **2,4D**, 3 mg .l⁻¹ of **IPA**
& 3 g .l⁻¹ activated charocal

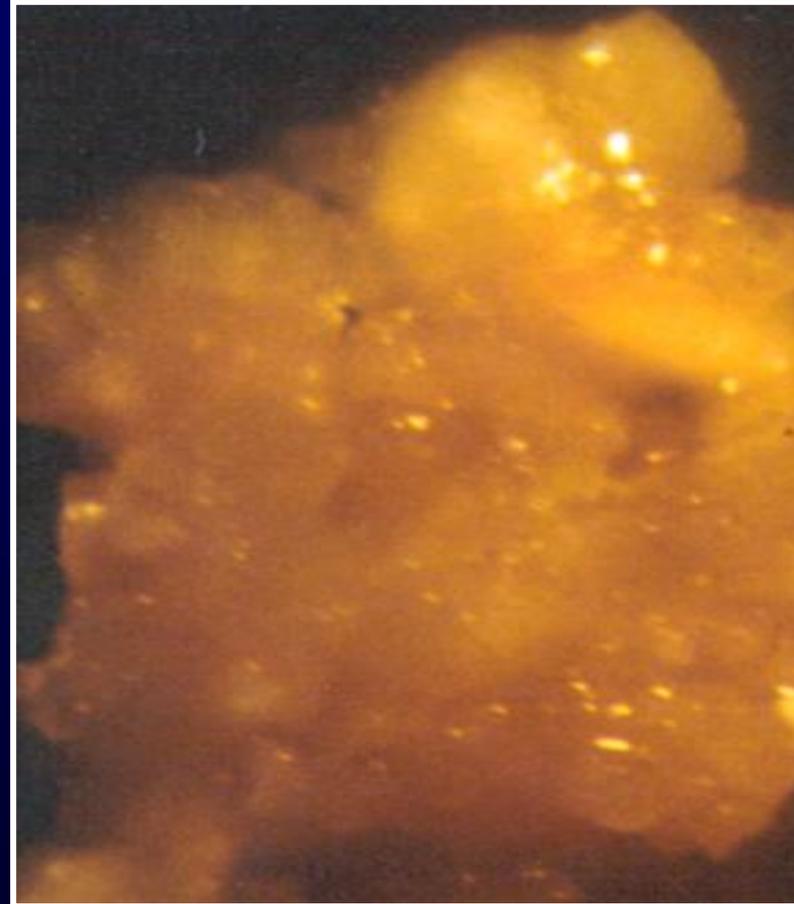


2 types of callus were obtained (cv. Allig)



Yellow callus

Embryogenic calls



6 months of culture

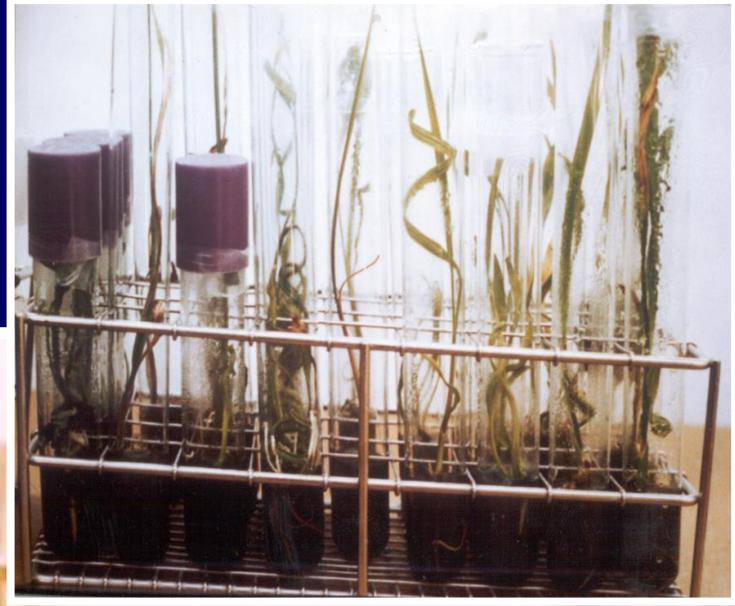
embryogenic Callus (cv Kentichi)



4 months and half (3 subcultures)



Germination & development of somatic embryos



★ **Effect of the period of introduction of the explant on the % of embryogenic callus**

Period	Number of explants in culture		% of embryogenic callus	
	Allig	Kentichi	Allig	Kentichi
avril	36	36	22,2	8,3
juillet	35	35	45,7	40
septembre	36	34	22,2	14,7
décembre	33	32	66,6	71,8

Explants introduced during the winter and the summer were better for the obtention of embryogenic callus

Our work concludes to the best adequacy so far of somatic embryogenesis for multiplication of our varieties compared with organogenesis



Pollination with irradiated (γ Cobalt 60) pollen

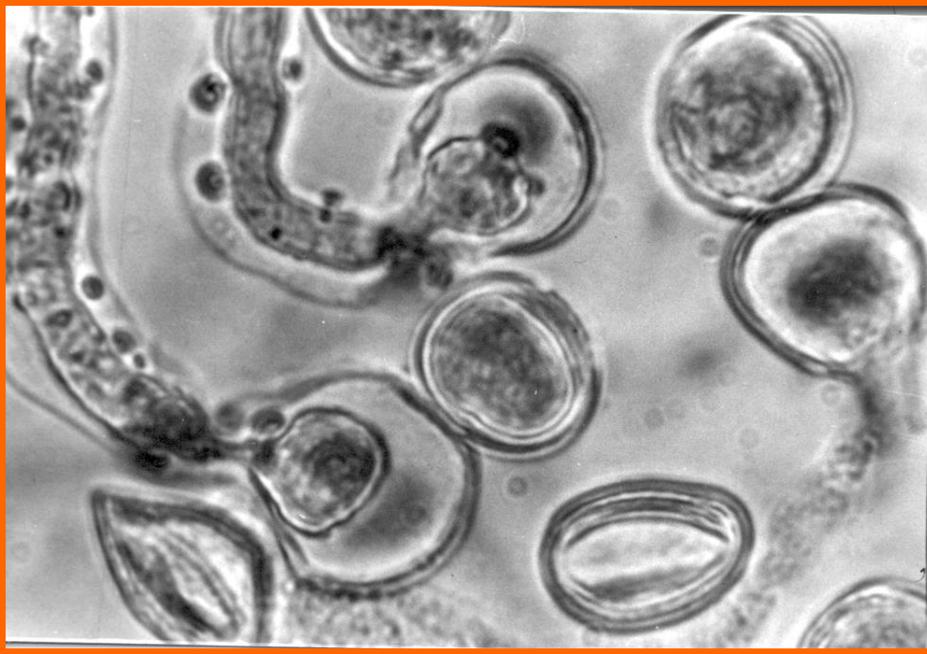
The possibility of in situ parthenogenesis induced by irradiated pollen was explored for date palm

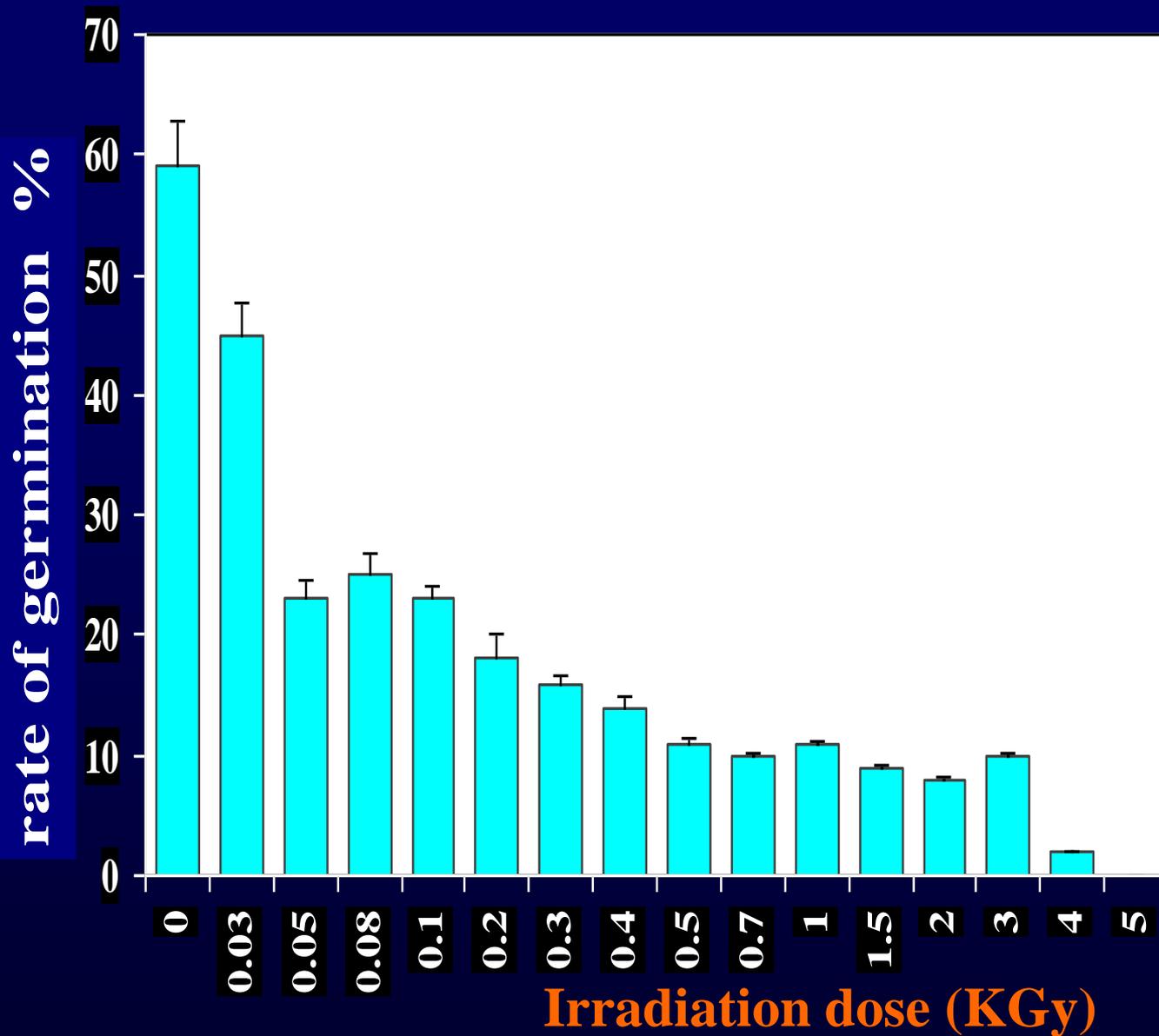
The experimentation was performed with two male genotypes (T106 and T23) and four female cultivars (Deglet Nour, Allig , kentichi and Menakher) in Tozeur

different doses of γ radiations used to irradiate the pollen of two date palm male genotypes T₁₀₆ and T₂₃ during three campaigns of experimentation.

Season	1	2	3
GY	•100-200-500	•25-50-75-100- 200-300-400-500- 700-1000-1500- 2000-3000	•25-50-100-200- 300-400-500-700- 1000-1500-2000- 3000-4000-5000

Viability analysis of irradiated pollen





Effect of γ Cobalt 60 irradiation on the germinative faculty of date palm pollen (pollinator T106)

**Effect Gamma Cobalt 60 radiation (doses : 700 & 1000 Gy)
on the number of kernels per fruit {cultivar “Kentichi”} .**



Regenerated Plants *in vitro* from embryos resulting after using irradiated pollen with various doses

Cultivar	Deglet Nour	Kentichi	Allig	Menakher
Embryos in culture	563	483	522	534
Plants /callus	100	65	57	73
Plants /cytom-Flux	11	15		
Level of ploidy	2n	2n, 3n, 4n		
Emb-plant	73	57	59	54
Plants /cytom-Flux	16	12		
Level of ploidy	2n	2n		
Total plants <i>in vitro</i>	173	122	116	127



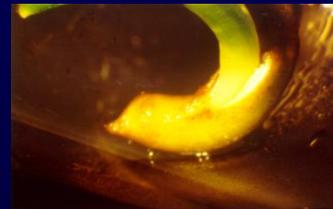
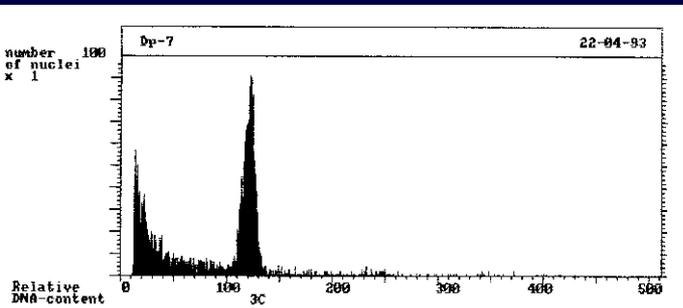
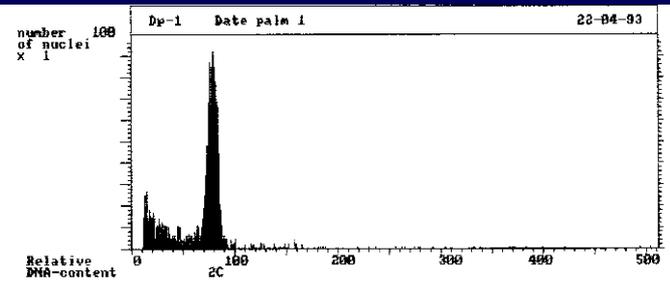
POLLINATION WITH IRRADIATED POLLEN

**Fruit set
Harvesting of the fruits samples**

IMMATURE EMBRYO



IN VITRO - CULTURE



CAL EMBRYOGENE

**GERMINATION:
EMBRYO - PLANT**

**Analysis of ploidy of 54 plants by
CYTOMETRIC FLUX**

**3 Plants : 3n et 4n
51 plants are 2n**

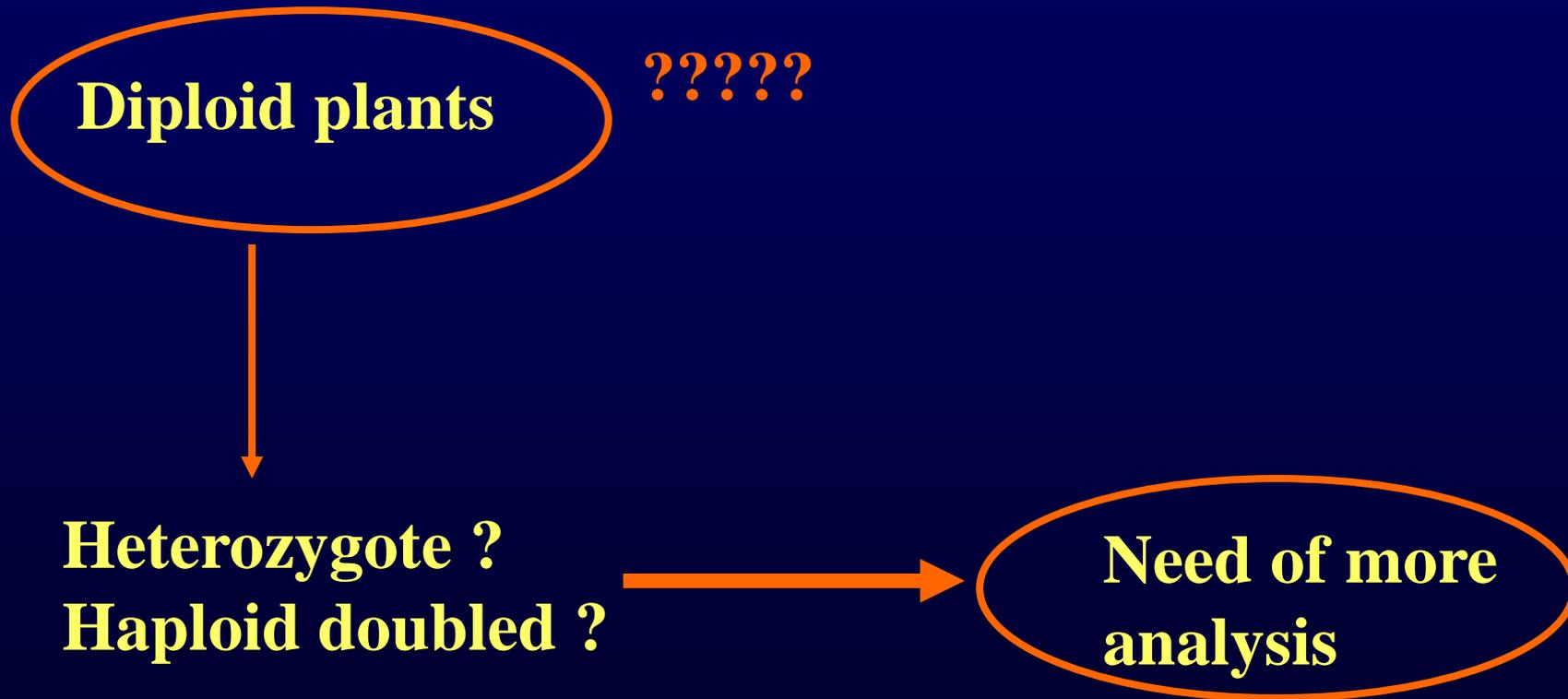


ACCLIMATATION

**PLANTS
REGENERATION**

**REGENERATION
Problems**

- No haploid plants
- 3 plants were $3n$ and $4n$
- All the rest of plants analysed (51) were diploid



Production of date palm fruits and diploids embryos following gibberellic acid treatment of unpollinated female inflorescences

GA3

5-10-30-60-100-200 mg/l



unpollinated female
inflorescences

At spathe opening
and one week
after

**T1 : Unpollinated inflor.
With no GA3 treatment
covered until fruit set**

**4 female cultivars
One palm and eight
inflorescences / Cultivar**

**T2: inflorescence
pollinated with T23
pollen**

GA3 induces apomictic seeds when applied to date palm female inflorescences

(Ben Abdallah & P. Lepoivre, 2000)



+ GA3 (e.g. 30 mg . l⁻¹)



Removal of the protection bags



**Fruit set rate –
Fruits
characteristics**



**Germination
tests / seeds**

**Germinated plants
Flow cytometry
analysis of the ploidy**



GA3 - induced apomixis produces normal embryos.

Pollination	GA3 treatment	Cultivar			
		Menakher	Kentichi	Deglet Nour	Allig
		% of normal embryos			
+	-	88	75	86	59
-	+	29	18	29	25
-	-	0	0	0	0

Origin of the fruits produced following GA treatments and without pollination ????

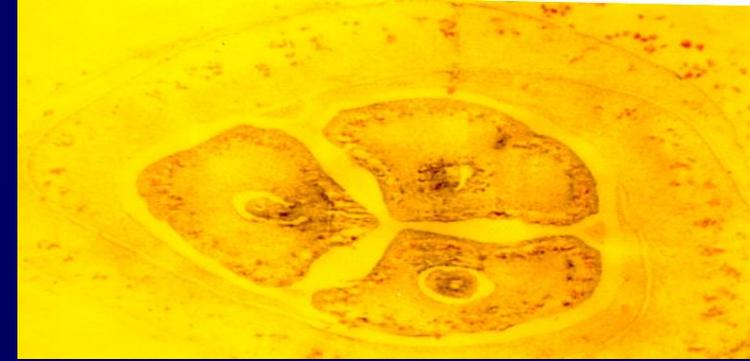
1st Hypothesis

- Self pollination resulting from the development of staminodes

Histological Study of the fruit development after GA treatment



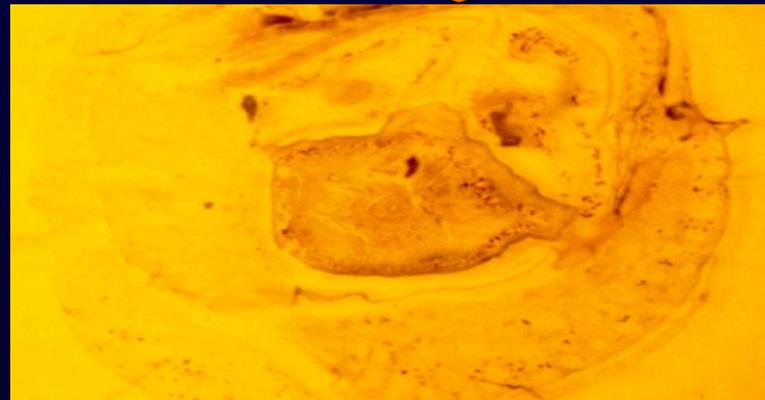
Inflorescence (female flowers) treated with GA



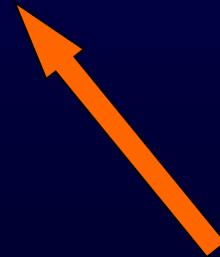
Female flower 3 carpels (c_1 , c_2 et c_3) before the fruit set (x20)



Female flower 2 carpels & staminods development (x20)

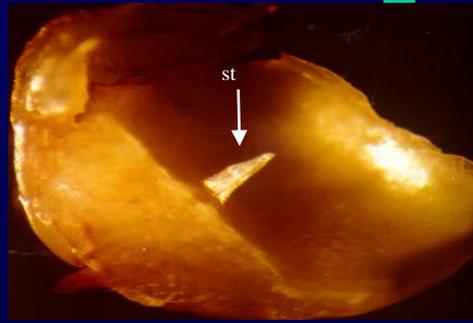


2 carpels stop developing and only one carpel will develop producing the fruit (x20).



st

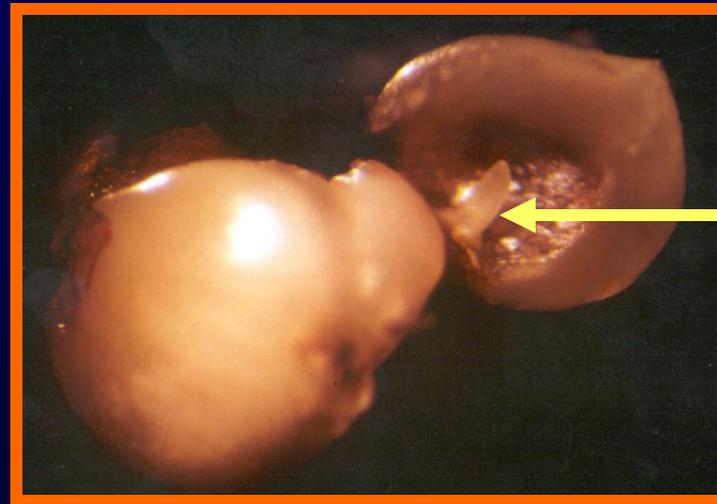
st



Development of Staminods (st) without pollen after the development of the embryos (x1,5).



The Self pollination resulting from the development of staminodes Can not explain the obtention of the fruits because the embryos is already developed when the staminodes are still developing



staminode

Origin of the fruits produced following GA treatments and without pollination ????

Second Hypothesis

- Induction of Apomixis ?

Apomixis is the production of viable seeds without fertilization.

Ovular tissues

Megasporocyte (2n)

MEIOSIS ↓

Megaspore (n)

Embryo sac (n)

Oosphere (n)

Zygote (2n)

Amphimixis

Embryo sac (2n)

Oosphere (2n)

Zygote (2n)

Gametophytic apomixis

Diplospory

Embryo sac (2n)

Oosphere (2n)

Zygote (2n)

Apospory

Zygote (2n)

Sporophytic apomixis

**Aqueous
solution of
GA3**

**frequency of fruit set 18 to 29%
compared to 9 % of the control**



**Cytometric
flow analysis**

**All the
plants are
diploid 2n**

Genotyping of date palm seed progenies obtained by induced apomixis – monitoring of the genotypic conformity

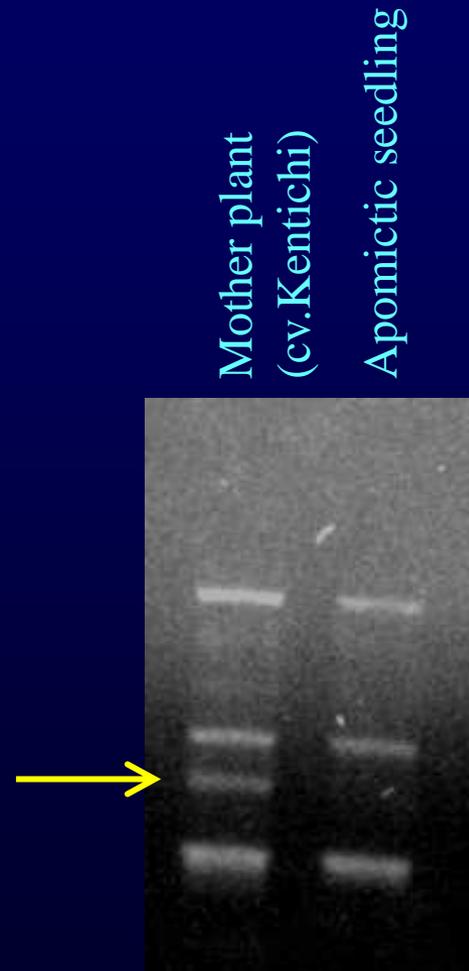
- **Plant material :**

Apomictic seedlings from cv. Menakher,
Kentichi, Deglet Nour, Allig
(Ben Abdallah et Lepoivre, 2000)

- **The question :**

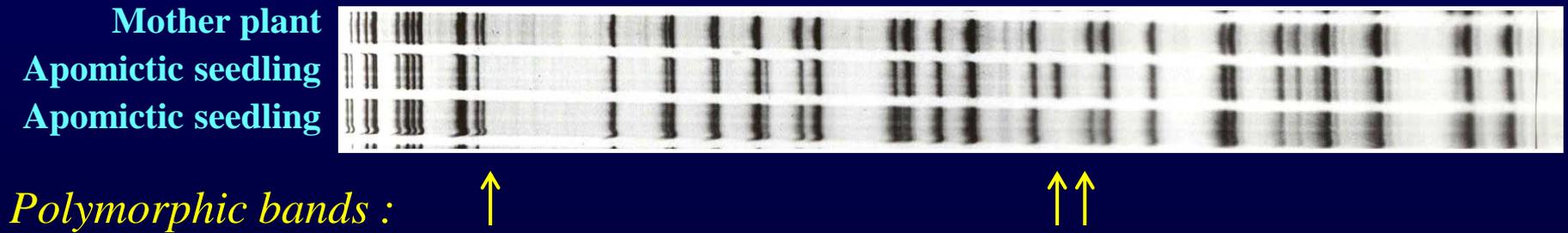
Is the apomictic offspring true-to-type ?

RAPD profiling of apomictic seedlings



Primer OPA16

AFLP identifies DNA polymorphism between apomictic seedlings and their parental cultivars.

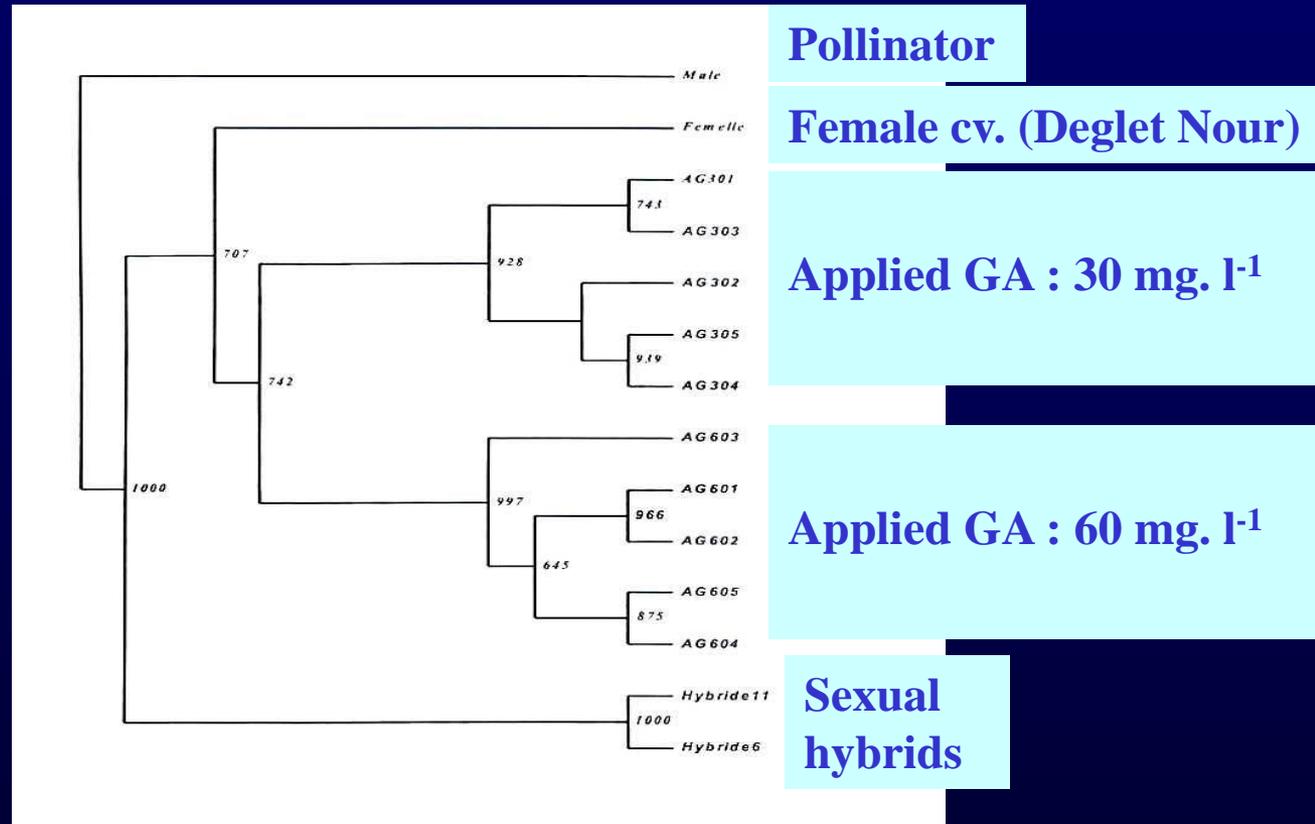


Autoradiograph showing the amplification products obtained with primer combination AGG/CAA



**Our Results excluding
true to type multiplication**

Doses of applied GA seems to have an impact on the genetic changes observed in the apomictic progeny.

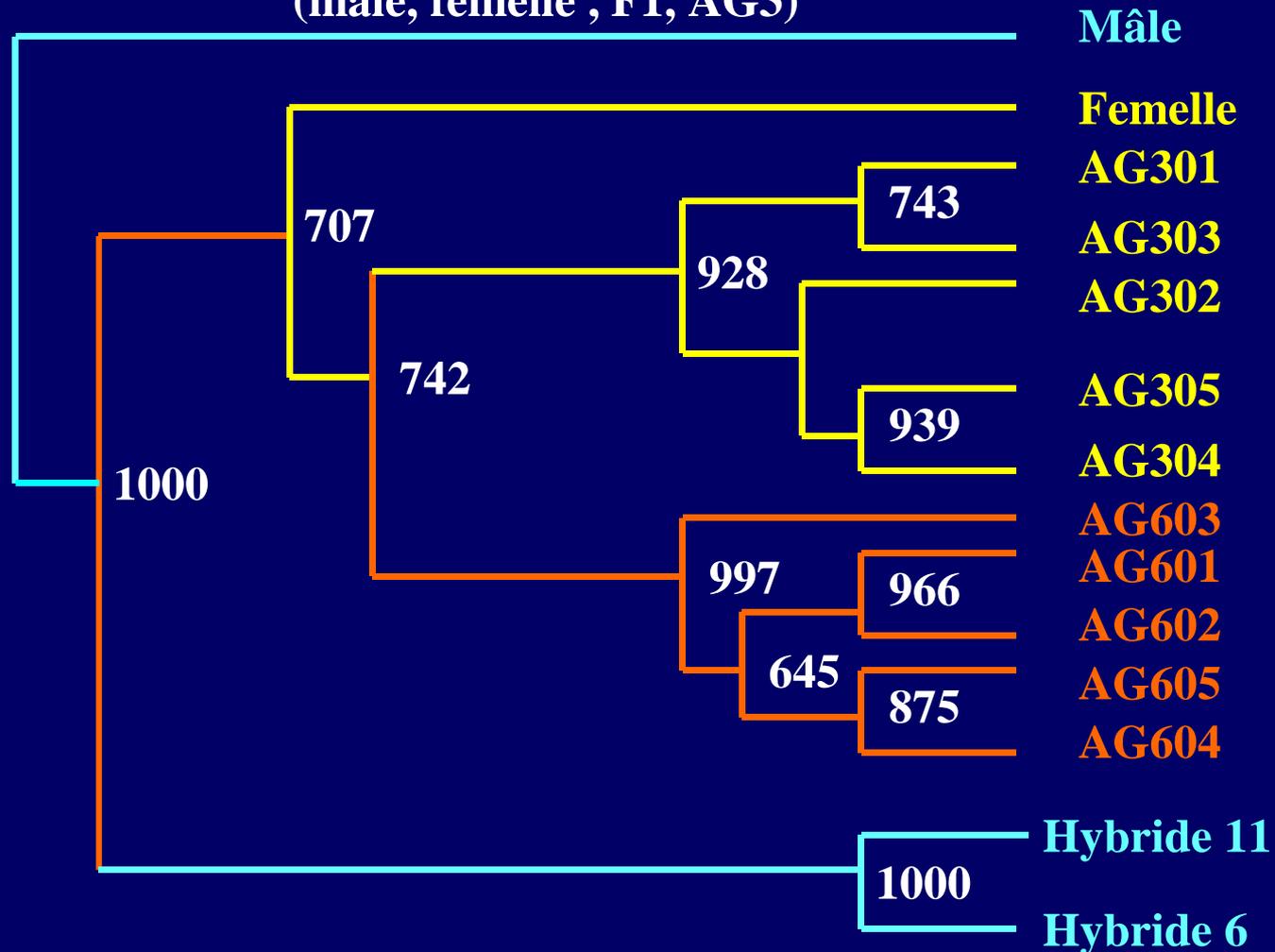


Methods :

- 513 bands generated with 9 primer pairs
- 248 (48 %) bands polymorphic between ♂ and ♀ cvs, hybrids and apomicts
- UPGMA grouping based on Nei genetic distances

Doses of applied GA3 seems to have an impact on the genetic changes observed in the apomictic progeny.

**AFLP : Arbre UPGMA / Distances génétiques de Nei
(mâle, femelle , F1, AG3)**



Methods :

- 513 bands generated with 9 primer pairs
- 248 (48 %) bands polymorphic between ♂ and ♀ cvs, hybrids and apomicts
- UPGMA grouping based on Nei genetic distances

Apomixis in date palm : working hypothesis and prospect

- Apomixis in date palm is efficient but not clonal.
- The apomictic pathway is probably diplosporic (partial meiosis is expected).

Ovular tissues

Megasporocyte (2n)

MEIOSIS ↓

Megaspore (n)

Partial meiosis
may occur !

Embryo sac (n)

Embryo sac (2n)

Embryo sac (2n)

Oosphere (n)

Oosphere (2n)

Oosphere (2n)

(+ ♂ gamete) ↓

Zygote (2n)

Zygote (2n)

Zygote (2n)

Zygote (2n)

Amphimixis

Gametophytic apomixis

Sporophytic apomixis

Diplospory

Apospory

Apomixis in date palm : working hypothesis and prospect

- Apomixis in date palm is efficient but not clonal.
- The apomictic pathway is probably diplosporic (partial meiosis is expected).
- The genetic variability needs to be quantified and compared with the existing variation within offshoots.

Evaluation of the Tunisian Deglet nour Within - cultivar polymorphism

- Plant material :

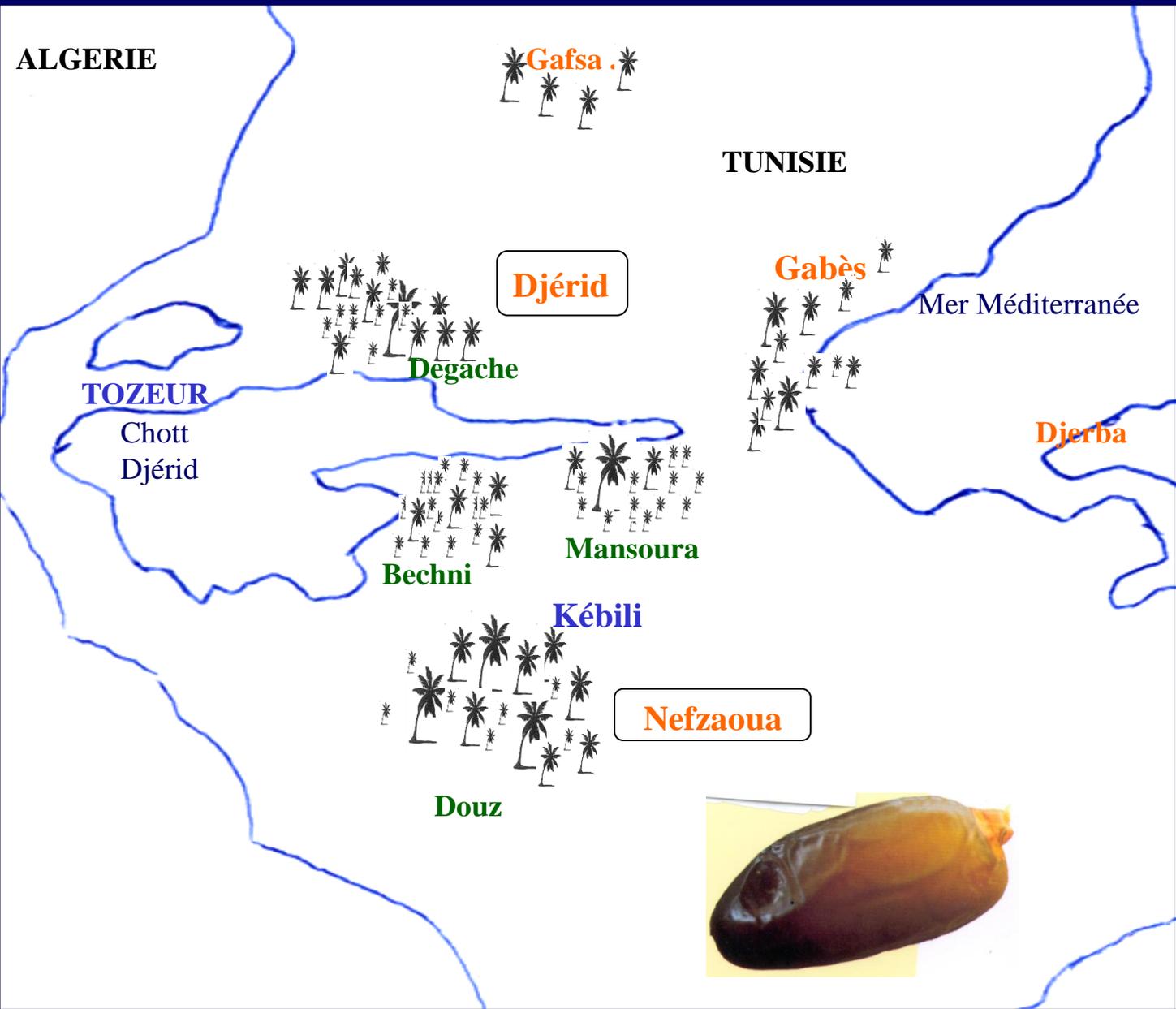
Deglet nour cultivars from 4 different sites:

Kébili for Degache oasis

Bechni, Douz and Mansoura for Nefzaoua oasis

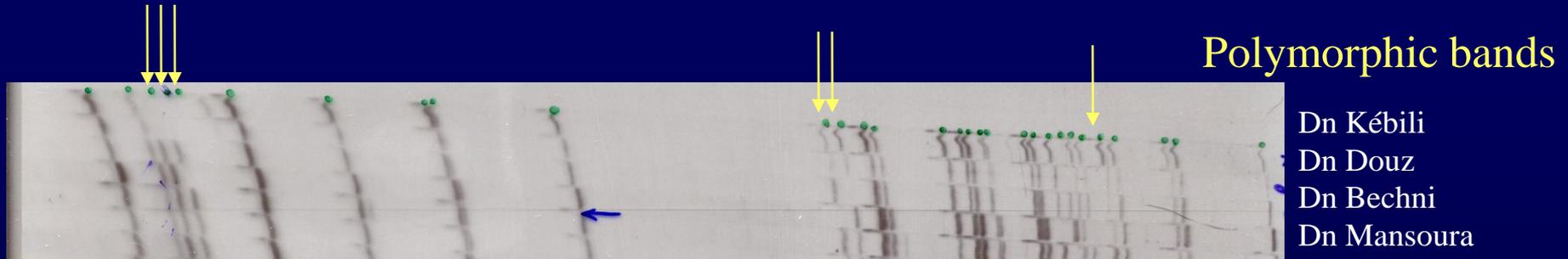
- The question :

Is there any within-cultivar variability relative to Deglet nour ?



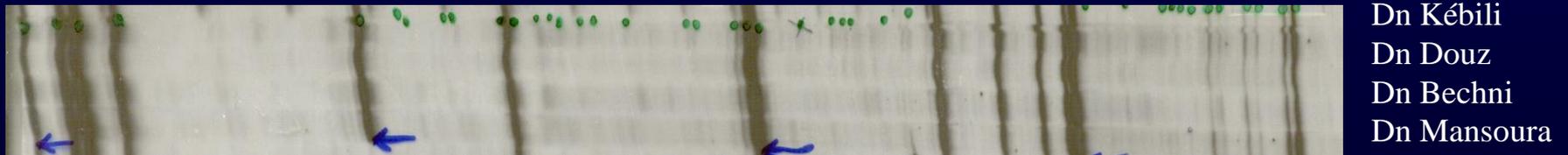
Date palm groves in the Tunisian south, different oasis

AFLP identifies DNA polymorphism between Deglet nour cultivars collecting from different palm groves.



Primer combination AGG/CAA

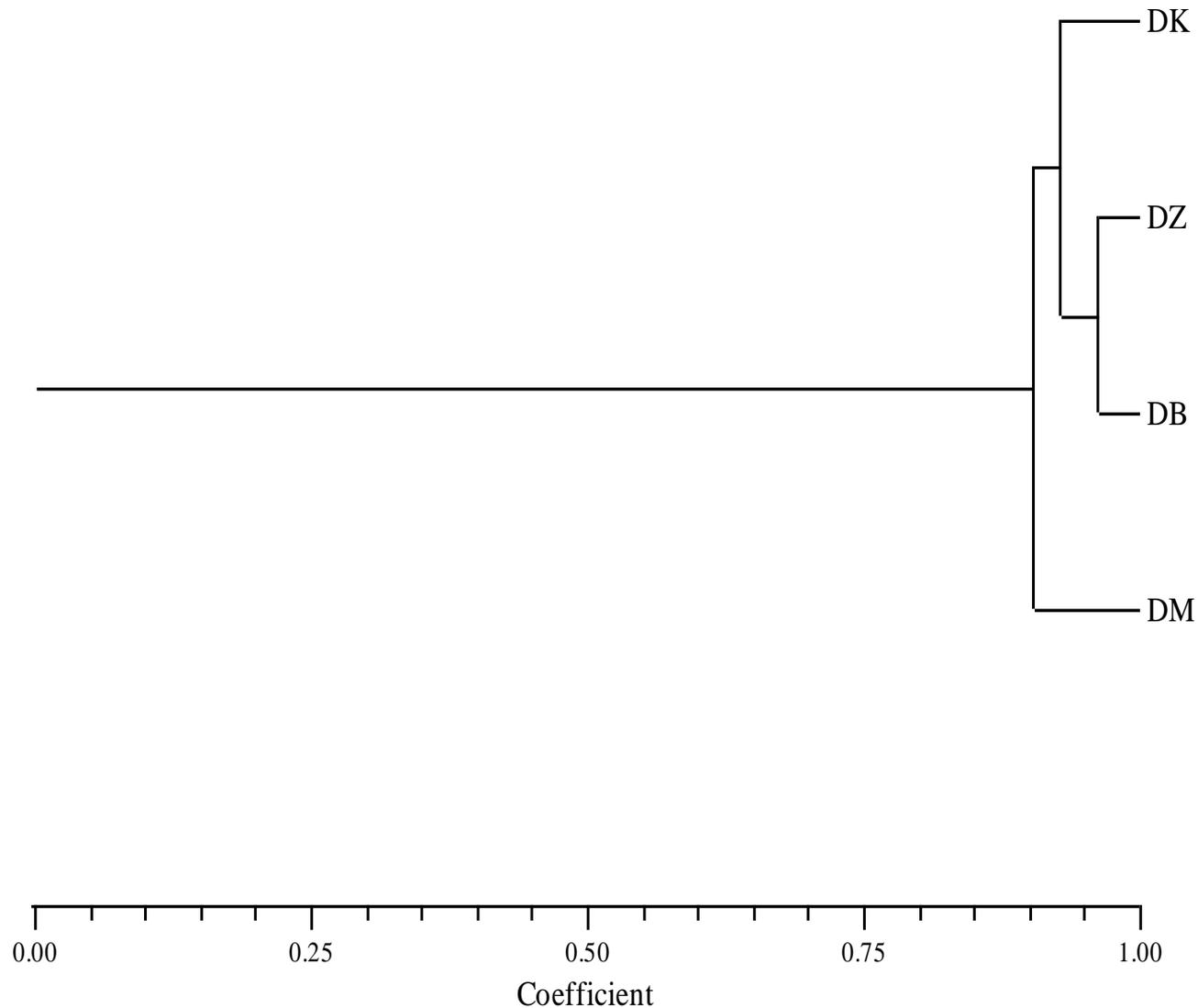
No Polymorphic bands detected



Primer combination AGG/CTA

Methods:

- 304 bands generated with 6 primers pairs
- 60 (19,74%) polymorphic bands between Deglet nou CVs
- UPGMA grouping based on Dice genetic similarities

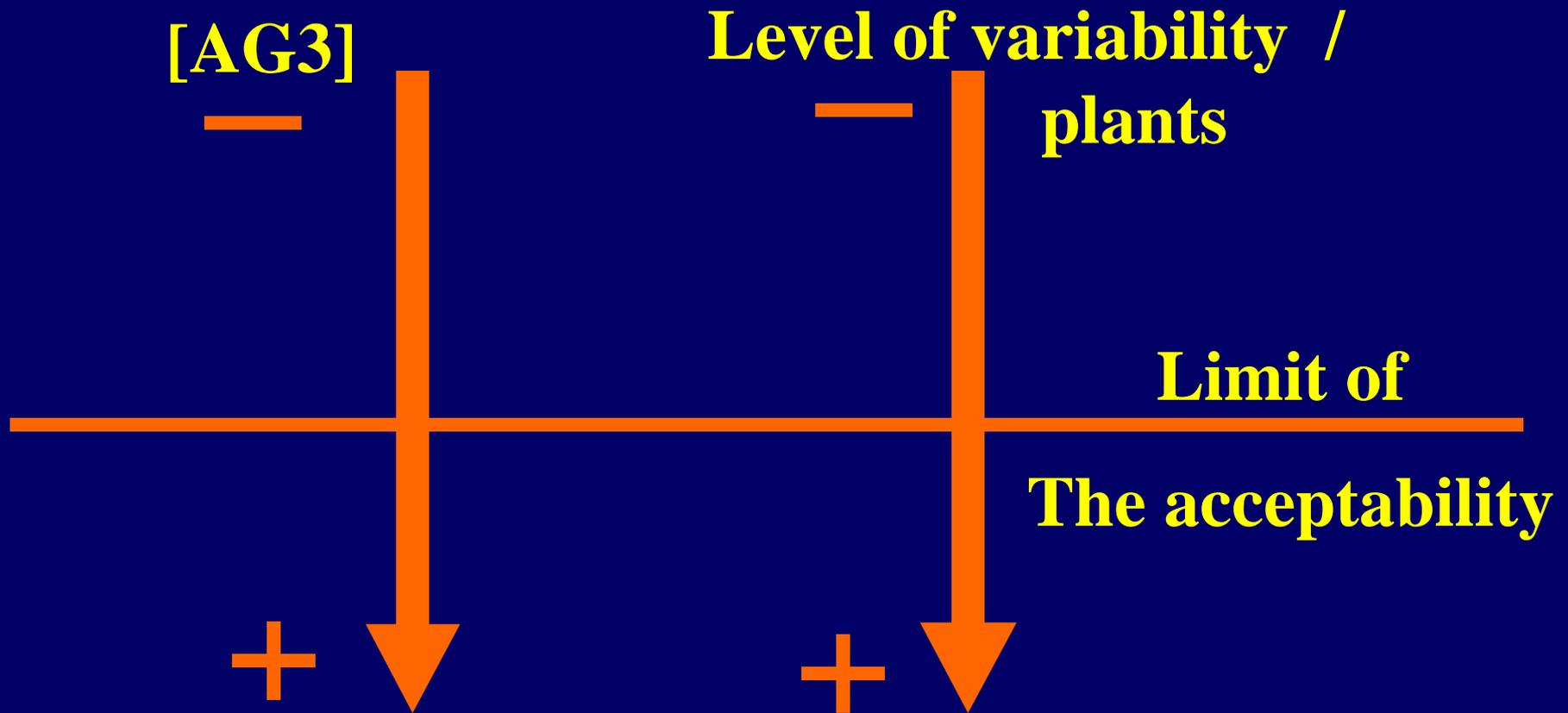


Dendrogram generated after UPGMA using AFLP- based genetic similarity. Numbers on the scale are coefficient Dice values obtained

Deglet nour within – cultivar polymorphism : working hypothesis and prospect

- * A Deglet nour within – cultivar polymorphism was identified (Nefzaoua)
- * Genetic similarities between cultivars were high (89,5% - 96,2%) , **No 100% of similarity**
- * We cannot speak about an **uncompromising conformity** between Deglet nour cultivars

Effect of GA3 concentration on the conformity level of the plants compared with the mother variety



Our work concludes to the best adequacy so far of somatic embryogenesis to meet the immediate imperatives for rapid multiplication of date palm

In the long run, production of seed without sexual hybridization opens original perspectives, so much for the multiplication than for the genetic improvement of date palm

Ben Abdallah A., Lepoivre P. 2000. Production of date palm (*Phoenix dactylifera L.*) fruit and diploid embryos following gibberellic acid treatment of unpollinated female inflorescences *Cahiers Agricultures N°9 : 467-473 ed. John Libbey Eurotext – France*

Thank you شكرا

