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## Inheritance of andromonoecy in *Tibish*

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### Abstract

In melon, the presence of stamens in female flowers is controlled by the recessive allele *a*, while the absence of stamens in the female flowers is controlled by the allele *a*<sup>+</sup> (or *A*). *Tibish* group of melon is characterized by small, ovoid fruits which are harvested before maturity and consumed raw in salad, like cucumber. The plants have male and perfect flowers, characteristics of andromonoecy. The F<sub>1</sub> progenies between *Tibish* and monoecious cultivars belonging to the *flexuosus* group were andromonoecious. In F<sub>2</sub> and backcross progenies between *Tibish* and 'Védreantais' (andromonoecious cultivar belonging to the *cantalupensis* group), monoecious plants were observed indicating that a genomic region independent from the locus *a/a*<sup>+</sup> was involved. The segregations observed in F<sub>2</sub> progenies between *tibish* and 3 cultivars belonging to the *flexuosus* group could be explained by two linked loci. The symbols *A-2* and *A-3* are proposed.

### INTRODUCTION

Melon (*Cucumis melo*) is cultivated throughout the world for different kind of fruits. Two main groups correspond to the non-sweet types (for instance *conomon*, *chate*, *flexuosus*, *acidulus*) and the sweet types (for instance *cantalupensis*, *inodorus*, *ameri*). On a typical melon plants, there are more than 20 female flowers which will give only 4 or 5 mature fruits due to a very strong competition between fruits. In cultigroups with non-sweet fruits, the fruits are harvested before maturity and the competition between fruits is less severe. By increasing the female flower number it could be possible to increase the total yield. Breeding hermaphrodite cultivars, with perfect flowers at each node on the main stem and the lateral branches, belonging to cultigroups where fruits are harvested before maturity, as in *flexuosus* or *tibish* groups, could be a possibility to increase earliness and yield.

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## Characterization of gene expression and protein accumulation in melon ethylene receptors

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Melon cultivar 'Vedrantais' (*Cucumis melo* L. var. *cantalupensis*) is a typical climacteric fruit and the ripening phenomena dramatically proceed within two to three days after initiation of ripening. Ethylene has critical roles in ripening of climacteric fruit and ethylene receptor is an important factor located front line of the signal transduction pathway. It is known that ethylene receptor genes construct multigene family. Three ethylene receptor genes, CmETR1, CmETR2 and CmERS1 have been identified in melon. However their molecular characterization during fruit development is still unclear.

We analyzed melon ethylene receptors (CmETR1, CmETR2, CmERS1) for pattern of gene expression and protein accumulation of various tissues, fruit enlargement and ripening stages of the climacteric 'Vedrantais' melon. Additionally, the connection with ethylene was studied with 1-methylcyclopropen, a gaseous ethylene action inhibitor.

In this study, we suggest that ripening initiation is involved in the change of expression levels of each ethylene receptor before or after ripening ethylene production on the basis of gene expression analysis and that the expression levels of ethylene receptors are adjusted by both ethylene dependent and independent systems.

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Wild melons are monoecious and many modern cultivars are andromonoecious. A few accessions from China are hermaphrodite. From these last accessions, breeders have been able to develop gynoecious lines. Two main genes are involved in the genetic control of these phenotypes: locus *a* controls the presence (allele *a*) or absence (allele *a*<sup>+</sup> or *A*) of stamens in female flowers (Rosa 1928) and locus *g* controls the presence of one type (allele *g*) or two types of flowers (allele *g*<sup>+</sup> or *G*) on one plant (Poole and Grimball 1939). The combinations of these two independent loci generate four phenotypes: monoecious (*a*<sup>+</sup>- *g*<sup>+</sup>*g*<sup>+</sup>), andromonoecious (*aa g*<sup>+</sup>-), gynoecious (*a*<sup>+</sup>*a*<sup>+</sup> *gg*) and hermaphrodite (*aa gg*).

Four types of melons are cultivated in Sudan: sweet melons, snake melon (*C. melo* var. *flexuosus*), a salad melon known locally as 'Tibish' (*C. melo* var. *tibish*), and a melon type used for its edible seeds known locally as 'Seinat'. True wild melons known locally as 'Humaid' are present in central, northern and western parts of Sudan. The *tibish* type is characterized by small (300-500 g), ovoid smooth fruits, light green with dark green stripes, five placentas, white firm flesh, non sweet at maturity. It is andromonoecious.

To breed hermaphrodite *flexuosus* and *tibish* lines an hermaphrodite accession from China was crossed with cultivars belonging to both types. The observed segregations with *tibish* did not fit the expected ones. It prompted the study of the inheritance of sex expression in *tibish*.

## MATERIALS AND METHODS

### Plant material

Two accessions of *tibish* are used, namely Jebel Kordofan 4 and Khurtagat 15. Both were collected from Kordofan area in 2006 and were andromonoecious. They were crossed with three monoecious accessions of *flexuosus* melon, namely 'Alimin' and 'Silka', which are commercial cultivars of snakemelon in Sudan, and PI 222187 from the Afghanistan and with 'Védrantais', an andromonoecious Charentais line (obt. Vilmorin).

### Sex expression

Opening flowers were examined daily to observe the presence of pistillate flowers, with only female organs like stigma, or perfect flowers with both female organs and stamens. Segregation ratios in F<sub>2</sub> and BC populations were tested for goodness of fit to theoretical ratio with Chi square test.

## RESULTS

### Allelism of andromonoecy

The F<sub>1</sub> progeny of the cross between *tibish* Khurtagat 15 and 'Védrantais', both andromonoecious, was andromonoecious as expected (Table 1). Monoecious

plants were observed in the F<sub>2</sub> and BC progenies indicating that andromonoecy in *tibish* was not controlled by the locus *a/a*<sup>+</sup> and that Khurtagat 15 had the allele *a*<sup>+</sup>.

### Inheritance of andromonoecy in *tibish*

The F<sub>1</sub> progenies of the five crosses between both andromonoecious *tibish* accessions and the three monoecious *flexuosus* accessions were clearly andromonoecious (Table 1) indicating that andromonoecy is dominant. In the F<sub>2</sub> progenies, more andromonoecious plants were observed than monoecious in opposition with the dominance of monoecy controlled by the *a*<sup>+</sup> allele.

Taking in account that Khurtagat 15 had the allele *a*<sup>+</sup>, different hypothesis were tested, with the symbols *A-2* and *A-3* for the dominant alleles in *tibish*:

H1 = One dominant gene for andromonoecy in *tibish* independent from the locus *a* and epistatic on *a*<sup>+</sup> i.e. *tibish* [*a*<sup>+</sup>/*a*<sup>+</sup> *A-2/A-2*] and *flexuosus* [*a*<sup>+</sup>/*a*<sup>+</sup> *A-2<sup>+</sup>/A-2<sup>+</sup>*]. The observed segregation on the pooled F<sub>2</sub> (380 andromonoecious *versus* 199 monoecious) did not fit the expected 3 andromonoecious *versus* 1 monoecious ( $\chi^2 = 27.11$ , Prob <0.001).

H2 = Two independent dominant genes, each sufficient for andromonoecy, in *tibish* independent from the locus *a* and epistatic on *a*<sup>+</sup> i.e. *tibish* [*a*<sup>+</sup>/*a*<sup>+</sup> *A-2/A-2 A-3/A-3*] and *flexuosus* [*a*<sup>+</sup>/*a*<sup>+</sup> *A-2<sup>+</sup>/A-2<sup>+</sup> A-3<sup>+</sup>/A-3<sup>+</sup>*]. The observed segregations did not fit the expected 15 andromonoecious *versus* 1 monoecious ( $\chi^2 = 781.35$ , Prob <0.001).

H3 = Two independent dominant genes, both necessary for andromonoecy, in *tibish* independent from the locus *a* and epistatic on *a*<sup>+</sup>. The observed segregations did not fit the expected 9 andromonoecious *versus* 7 monoecious ( $\chi^2 = 20.703$ , Prob <0.001).

H4 = Two linked dominant genes, both necessary for andromonoecy, in *tibish* independent from the locus *a* and epistatic on *a*<sup>+</sup>. The recombination frequency between the two loci *A-2* and *A-3* was estimated by the maximum likelihood method and varied between 0.12 and 0.44 on the five F<sub>2</sub> and was estimated at 0.21 on the pooled F<sub>2</sub>. With this last value, the observed segregations of the five F<sub>2</sub> between *tibish* and *flexuosus* and also of the F<sub>2</sub> and BC between *tibish* and 'Védrantais' fitted the expected segregations.

### DISCUSSION AND CONCLUSIONS

Monoecy in *C. melo* has always been considered under a monogenic dominant control (Rosa 1928; Poole and Grimball 1939; Kubicki 1969; Kenigsbuch and Cohen 1990) with the recessive allele *a* for andromonoecy. This is the first work to study inheritance of andromonoecy using *tibish* genotypes. Andromonoecy is clearly dominant to monoecy and another genomic region than the locus *a* is involved. It seems that two linked dominant genes are necessary for this phenotype. We

propose the names *Andromonoecy-2* (symbol *A-2*) and *Andromonoecy-3* (symbol *A-3*) for these genes. The genotypes of the lines studied here could be 'Védraçais' [*aa A-2<sup>+</sup>A-2<sup>+</sup> A-3<sup>+</sup>A-3<sup>+</sup>*], *flexuosus* [*a<sup>+</sup>a<sup>+</sup> A-2<sup>+</sup>A-2<sup>+</sup> A-3<sup>+</sup>A-3<sup>+</sup>*], *tibish* [*a<sup>+</sup>a<sup>+</sup> A-2A-2 A-3A-3*]. Gene *a* corresponds to an ACC synthase (*CmACS-7*) inactive for the allele *a* and active for the allele *a<sup>+</sup>* (Boualem et al. 2008). Further study is needed to know if the dominant genes correspond also to enzymes involved in the ethylene pathway.

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Table 1. Phenotypes observed for sex expression (monoecy or andromonoecy) in melon in crosses between *tibish* (Khurtagat 15 or Kordofan 4) and monoecious accessions of *flexuosus* melon, namely 'Alimin' and 'Silka', which are commercial cultivar of snakemelon in Sudan, and PI 222187 from the Afghanistan and with 'Védrantais', an andromonoecious Charentais line (obt. Vilmorin).

Genotypes	Number <sup>z</sup>		H1 <sup>y</sup>		H2 <sup>y</sup>		H3 <sup>y</sup>		H4 <sup>y</sup>	
	Andro	Mono	Ratio <sup>x</sup>	Prob <sup>w</sup>	Ratio <sup>x</sup>	Prob <sup>w</sup>	Ratio <sup>x</sup>	Prob <sup>w</sup>	Rec <sup>v</sup>	Prob <sup>w</sup>
F <sub>1</sub> (Védrantais x Khurtagat 15)	15									
F <sub>2</sub> (Védrantais x Khurtagat 15) ⊕	17	7	3:1	63.7	61:3	<0.001	43:21	70.4		73.0
BC (Védrantais x Khurtagat 15) x Védrantais.	16	7	1:1	6.1	7:1	0.9	5:3	48.4		98.5
Silka		14								
Alimin		25								
PI 222187		27								
Kordofan 4	19									
Khurtagat 15	15									
F <sub>1</sub> (Silka x Kordofan 4)	17									
F <sub>1</sub> (Alimin x Khurtagat 15)	15									
F <sub>1</sub> (PI 222187 x Kordofan 4)	16									
F <sub>1</sub> (PI 222187 x Khurtagat 15)	13									
F <sub>1</sub> (Silka x Khurtagat 15)	16									
F <sub>2</sub> (Silka x Kordofan 4) ⊕	134	66	3:1	0.9	15:1	<0.001	9:7	0.22	0.18	67.7
F <sub>2</sub> (Alimin x Khurtagat 15) ⊕	45	20	3:1	28.0	15:1	<0.001	9:7	3.5	0.12	53.8
F <sub>2</sub> (PI 222187 x Kordofan 4) ⊕	55	40	3:1	0.01	15:1	<0.001	9:7	74.7	0.44	11.4
F <sub>2</sub> (PI 222187 x Khurtagat 15) ⊕	74	35	3:1	8.6	15:1	<0.001	9:7	1.4	0.15	61.5
F <sub>2</sub> (Silka x Khurtagat 15) ⊕	72	38	3:1	2.1	15:1	<0.001	9:7	5.2	0.21	97.4
F <sub>2</sub> pooled	380	199	3:1	<0.001	15:1	<0.001	9:7	<0.001	0.21	98.8

<sup>z</sup> Observed number of andromonoecious and monoecious plants in different progenies

<sup>y</sup> Various hypothesis for the genetic control of andromonoecy in *tibish*. H1 = one dominant gene;

H2 = two independent dominant genes, each sufficient; H3 = two independent dominant genes, both necessary; H4 = two linked dominant genes, both necessary.

<sup>x</sup> Expected ratio of segregation according to the hypothesis H1 to H3. <sup>w</sup> Probability of the  $\chi^2$  for the expected ratio

<sup>v</sup> Recombination fraction estimated by the maximum likelihood method.