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Egyptian Faba Bean Tolerance to Drought and Heat Stresses: Varietal Susceptibility and SRAP Functional Molecular Markers

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INTRODUCTION

- Faba bean (*Vicia faba* L.) is a worldwide important legume crop, ranking as the fourth most important grain legume
- Belongs to the family of *Fabaceae* (Leguminosae), subfamily of *Papilionoideae*, tribe of *Viceae*
- There are other names for faba bean such as Broad bean, Fava bean, Field bean, Bell bean, English bean, Horse bean, Windsor bean, Pigeon bean and Tick bean
- Wild species contain 2n=14 chromosomes whereas cultivated faba bean has 2n =12 chromosomes

- The nutritional value of faba bean is high (rich of seed protein content) and it is the most important pulse crop cultivated in Egypt
- Faba bean is used as human food in developing countries and as animal feed
- It can be used as a vegetable, either in green or dried, fresh or canned.
- It is a common breakfast food in the Middle East, Mediterranean region (specially in Egypt), China and Ethiopia

Harvested area, production yield and consumption of faba bean in Egypt during 2000 to 2016 (FAO STAT, 2016)

Year	Harvested area ha	Production ton	Yield ton/ha	Import ton	Export ton	Consumption ton	% Import
2000	113670	353909	3.113	172446	8986	517369	33.33
2001	140178	439480	3.135	242524	6478	675526	35.9
2002	127190	400910	3.152	287869	4995	683784	42.1
2003	106070	336840	3.176	307668	5306	639202	48.13
2004	101160	330490	3.267	314003	6624	637869	49.23
2005	83240	281650	3.384	380433	9383	652700	58.29
2006	73650	247490	3.36	459258	18547	688201	66.73
2007	89030	301770	3.39	301433	14600	588603	51.21
2008	71445	246801	3.454	277875	7406	517270	53.72
2009	86519	297620	3.44	155554	20516	432658	35.95
2010	77149	233523	3.027	455831	11202	678152	67.22
2011	55200	174631	3.164	297333	4433	467531	63.6
2012	41121	140713	3.422	270251	15459	395505	68.33
2013	44065	157639	3.577	281197	16956	421880	66.65
2014	37677	134175	3.561	----	----	----	----
2015	34412	119849	3.483	----	----	----	----
2016	34314	119104	3.471	----	----	----	----

--- Not obtained

- This data demonstrates the presence of a big gap between production and consumption
- This gap is filled through importing causing a significant expenditure of hard currency
- Hence, this gap should be filled by increasing production (horizontal such as reclaiming new lands) and productivity (Vertical) of the crop.
- This calls for the development of improved cultivars with high yield and stress tolerance characteristics.

Environmental stress

Abiotic stress

Biotic stress (insect, fungi, bacteria, etc.)

Radiation

Temperature

Water

Floods

Drought

Chemical

Other: pressure
Wind, etc.

Drought stress

- Faba bean plants are more sensitive to drought than other grain legumes
- Drought in faba bean severely affects plant growth, grain yield and quality
- Reduction in faba bean yield was positively related to the amount of water reduction and reach up to 50% of seed yield
- Drought causes morphological, physiological, biochemical, and molecular changes in Faba bean plants.

Heat stress

- According to the fourth assessment of the Intergovernmental Panel on Climatic Change (IPCC), global average temperature will be increased between 1.8°C and 4° C in 2100
- Heat stress affects plant growth and yield of faba bean plants and induces significant morphological, physiological, biochemical, and molecular changes in Faba bean plants.
- Temperature rises of 3 - 4°C might cause 15–35% loss of crop yield in Africa and Asia, and 25 - 35% in the Middle East.
- The Combined effect of both heat and drought on yield of many crops is stronger than the effect of each stress alone

The Study Objectives

- Determine drought and/or heat tolerant faba bean genotypes and their sensitivity to the stresses.

- Study of genetic diversity of some important Egyptian faba bean varieties using SRAP markers.

Material and Methods

Field experiment

Plant material

No	Varity	Origin	Pedigree
1	Giza 843	FCRI	Cross 461 x cross561
2	Giza 716	EGYPT	(83/453/503x83/824/461)
3	Misr 1	FCRI	(123A/45/76XG.3)×(62/1570/66×G.2)×(Romi×Habashi)
4	Sakha1	EGYPT	(85/283/620x88/724/716)

FCRI- Field Crop Research Institute.

Four treatments

Control
- cultivated in November 15
- irrigated each two days.

The heat treatment
- cultivated in January15
- irrigated each two days.

The drought treatment
- cultivated in November 15
- irrigated once a week.

The drought with heat treatment
- cultivated in January15
- irrigated once a week.

- Grains of each variety were planted in six bags, each contained two grains, irrigation was done until soil saturation, six plants were used to measure morphological traits.

Morphological traits of faba bean varieties and their recording stage

No	Trait	Stage of recording
1	Leaves number (L)	At maturity
2	Leaflets number ((LL)	At maturity
3	LL/ L	At maturity
4	Leaflet width	At maturity
5	Days to flowering	Flowering stage
6	Total number of Flowers	Flowering stage
7	First pod height	Harvest stage
8	Pods number/ plant	Harvest stage
9	Grains number/pods	Harvest stage
10	Grains number/plant	Harvest stage
11	Grains weight/pod (g)	Harvest stage
12	Grains weight/plant (g)	Harvest stage
13	100 Grains weight (g)	Harvest stage
14	Number of branching	Harvest stage
15	Numbers of nodes	Harvest stage
16	Plant height (cm)	Harvest stage
17	Dry plant weight (g)	Harvest stage

Statistical analysis

- Susceptibility equation was used to calculate the sensitivity of the varieties to heat and drought stress as following:

$$\text{Susceptibility coefficient} = \sum (\text{Treatment}_{\text{mean}} - \text{Control}_{\text{mean}})$$

- The two-way multivariate hierarchical cluster analysis was made using JMP® 7.0 software.

Molecular experiment (SRAP Molecular marker)

- DNA isolated from leaves of all genotypes and their treatment using i-genomic Plant DNA Extraction Mini Kit
- Twelve SRAP primers combinations used to study genetic diversity of four faba bean genotypes under different heat and/or drought stress level.
- Specific bands characterized the sensitive, moderate sensitive and tolerant varieties were investigated.
- The total number and the number of polymorphic bands produced from SRAP analysis were calculated as well as the polymorphic information content (PIC).
- Phylogenetic dendrogram of SRAP data was constructed using the UPGMA method in NTSYSpc software.

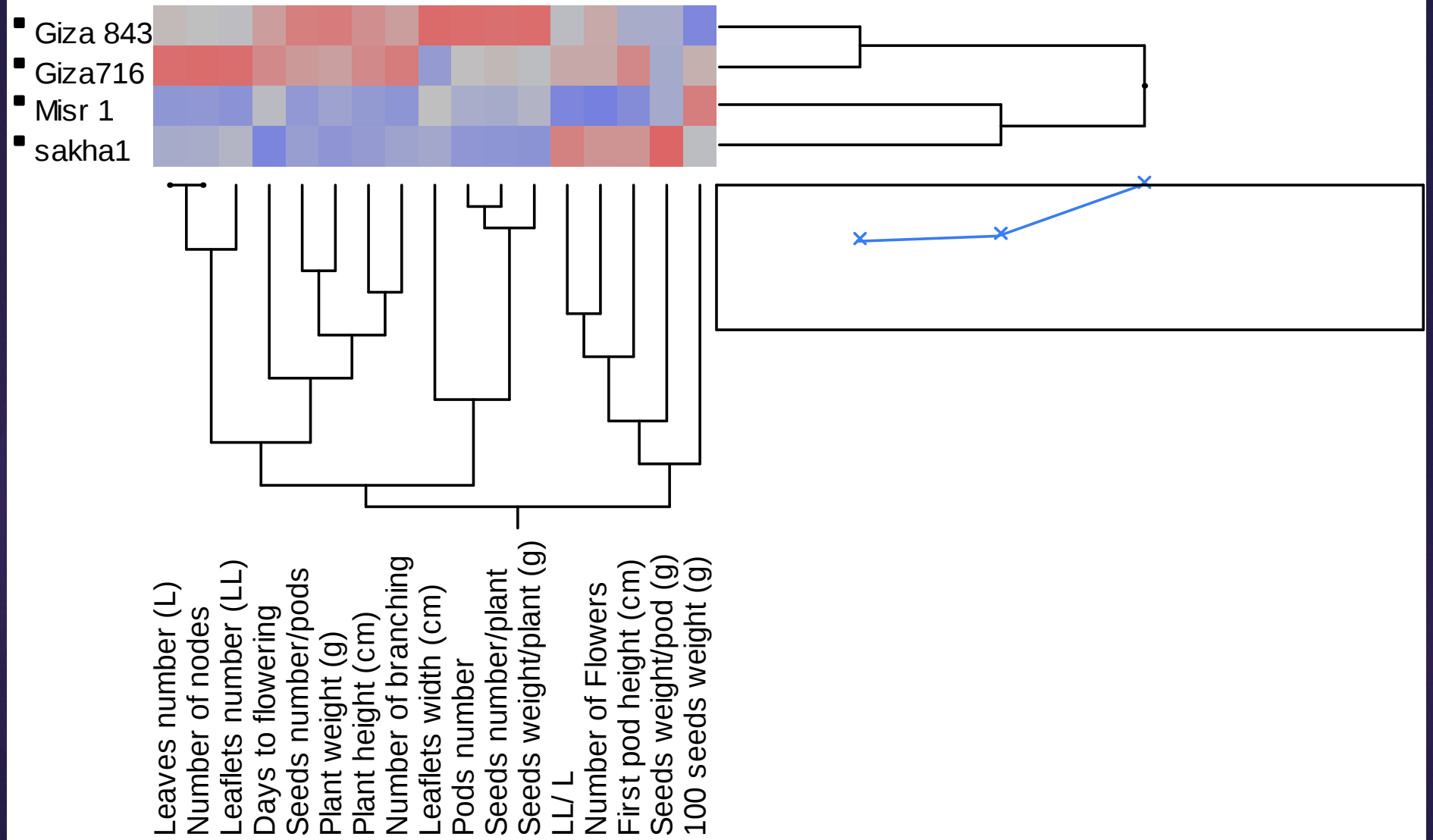
Results and Discussion

Estimation of sensitivity of four faba bean varieties under heat and/or drought stresses using morphological traits.

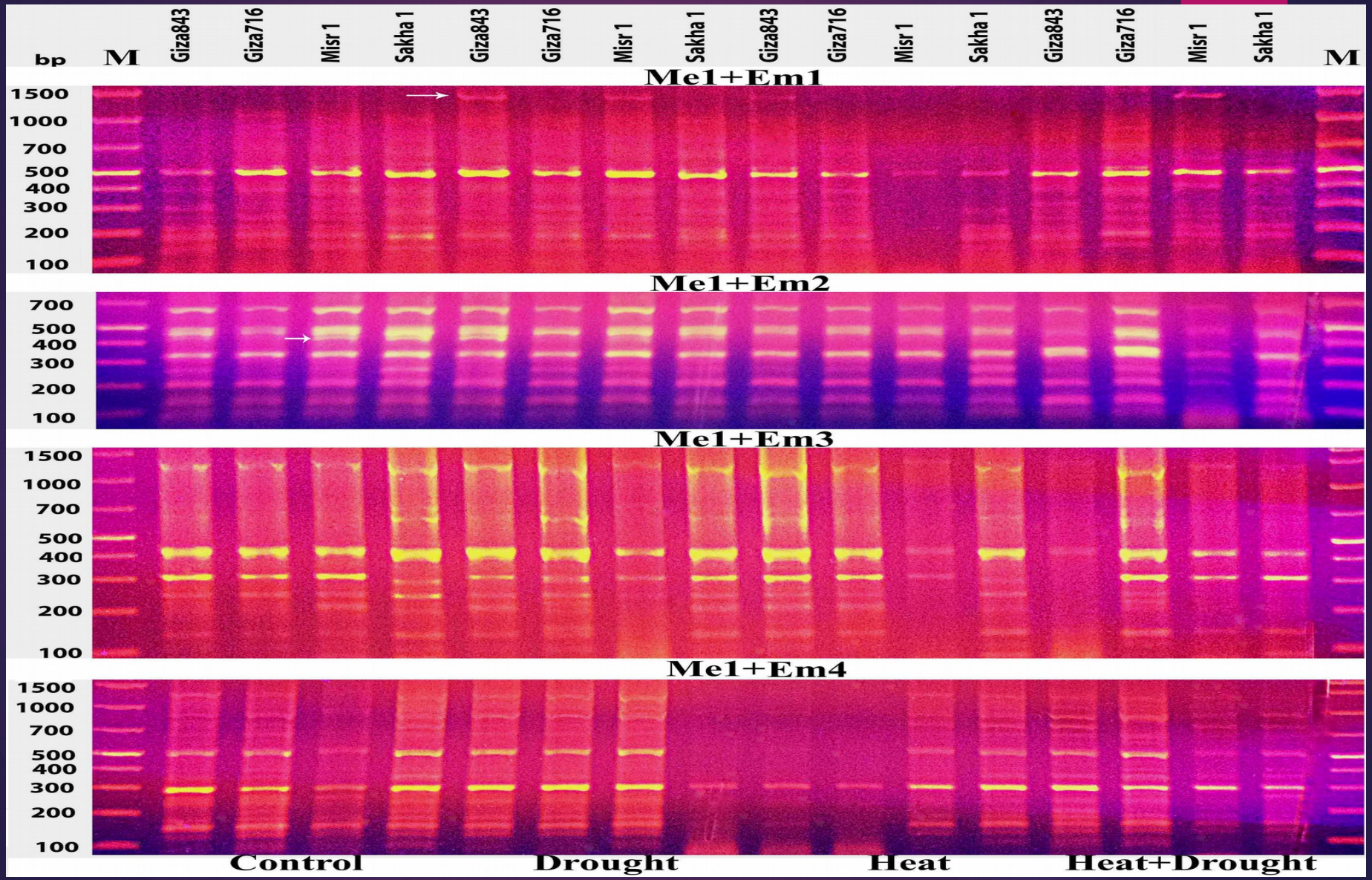
Genotype	Leave number (L)	Leaflet number (LL)	LL/ L	Leaflet width	Days to flowering	number of Flowers	First pod height	Pods number	Grains numbe r/pods
Giza843	-34A	-191.2A	-2.03B	3.22A	-7.8B	-99.69A	6.7A	-16C	-3.6B
Giza716	-60C	-375.8D	-2.93D	2.6B	-18.8D	-122B	16.8C	-12.4B	-3A
Misr1	-65D	-304.1C	-1.34A	1.31D	-9.79C	-139.5C	15.4B	-19D	-3.6B
Sakha1	-54.4B	-282.2B	-2.48C	1.54C	-5.39A	-175.9D	22.9D	-5A	-4.94C
Genotype	Grains number/ plant	Grains weight/ pod (g)	Grains weight /plant (g)	100grain s weight(g)	Number of branching	Number of nodes	Plant height (cm)	Dry plant weight (g)	
Giza843	-22.6A	-2.88A	-47.43C	-39A	-48B	-31.86C	-1.4C	-33.4A	
Giza716	-49.8C	-3.15B	-37.03B	-45B	-49.9C	-13.52A	-0.9A	-63.99D	
Misr1	-57D	-3.65C	-49.54D	-59C	-77.7D	-117.64D	-1.79D	-62C	
Sakha1	-28B	-4.82D	-24.38A	-75D	-45.7A	-30.25B	-1.3B	-50.6B	

Values connected with the same letter are not significantly different at 0.05 probability level

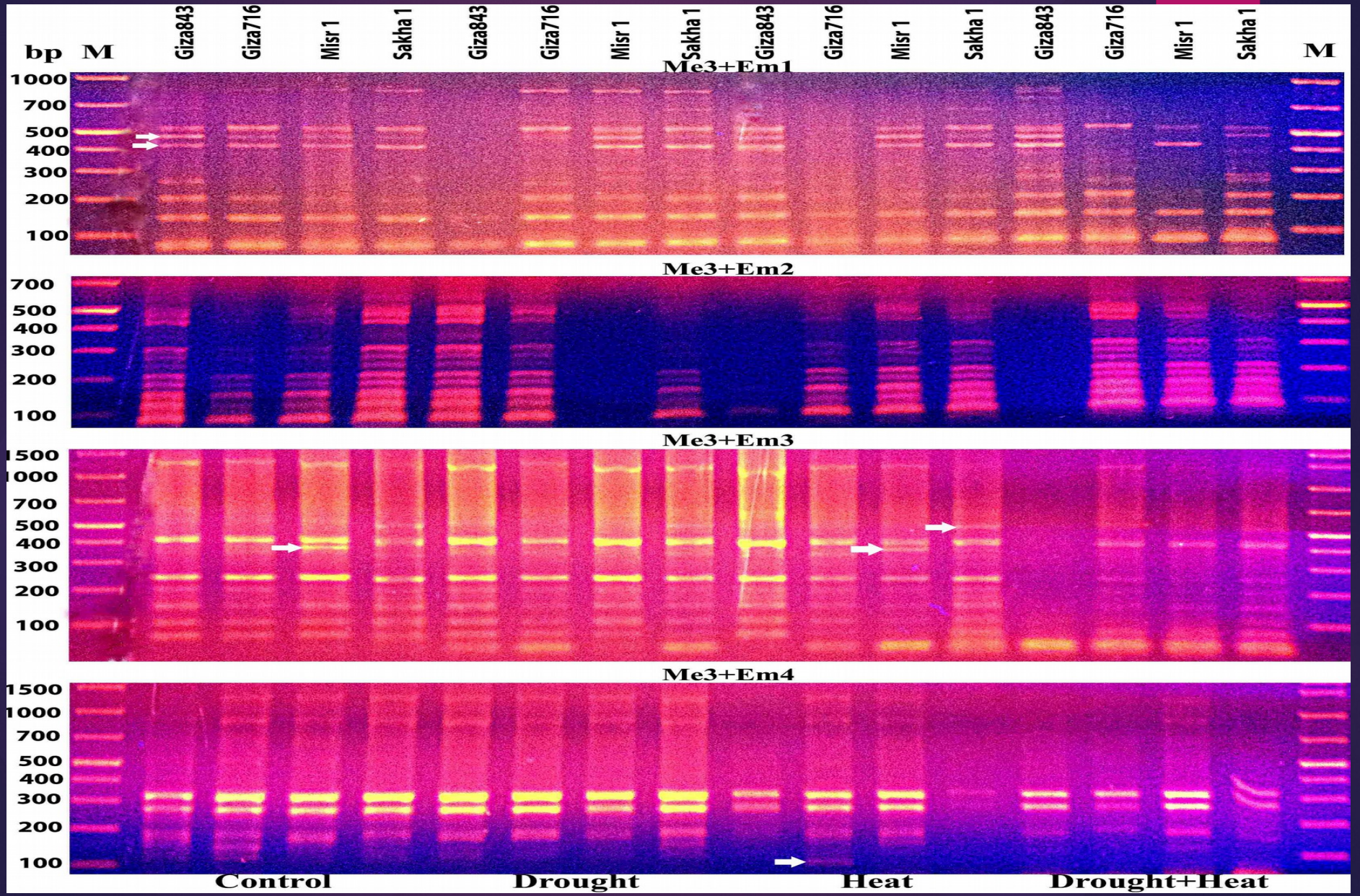
Morphological cluster analysis



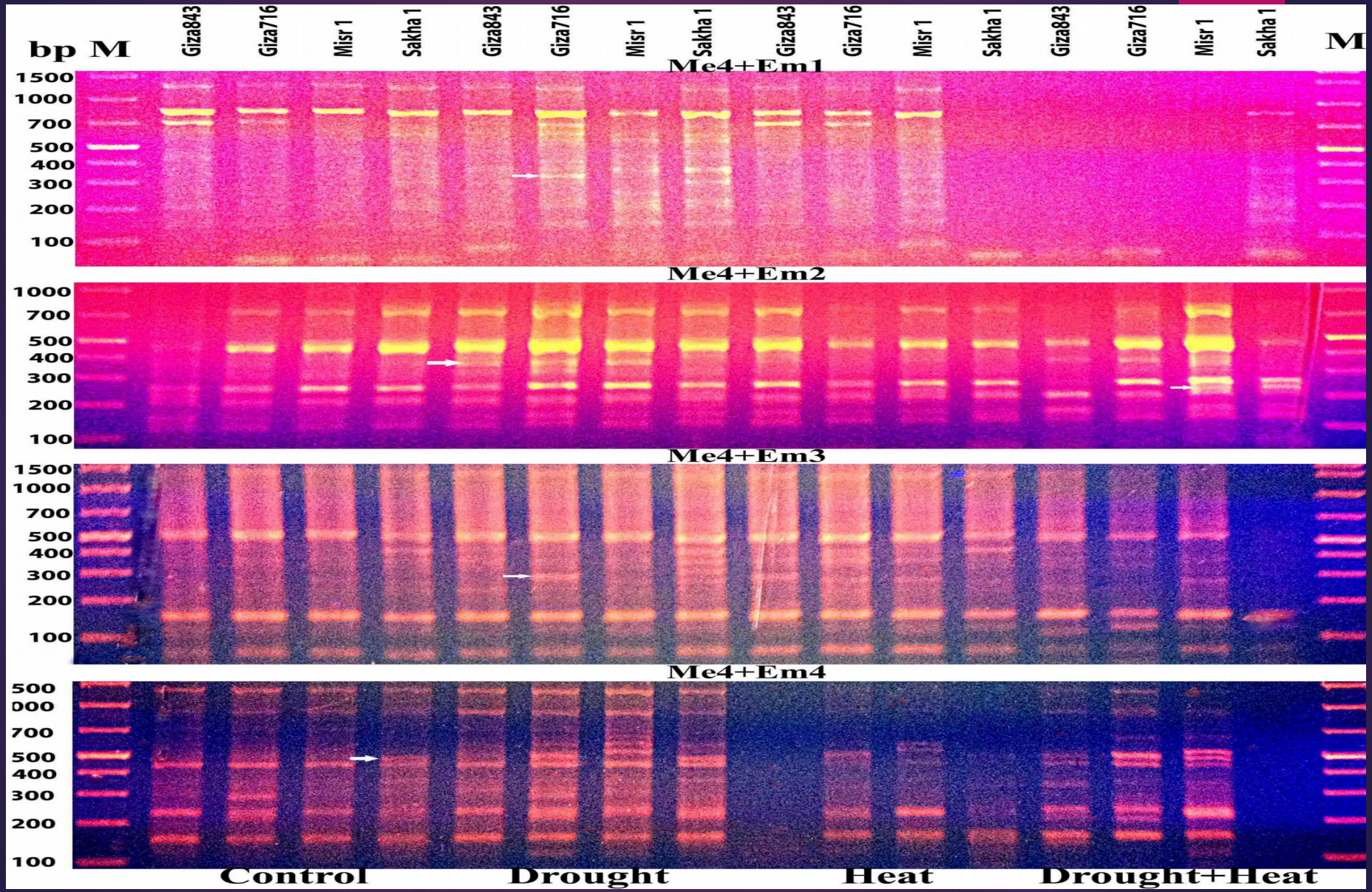
SRAP Molecular pattern



Molecular marker analysis



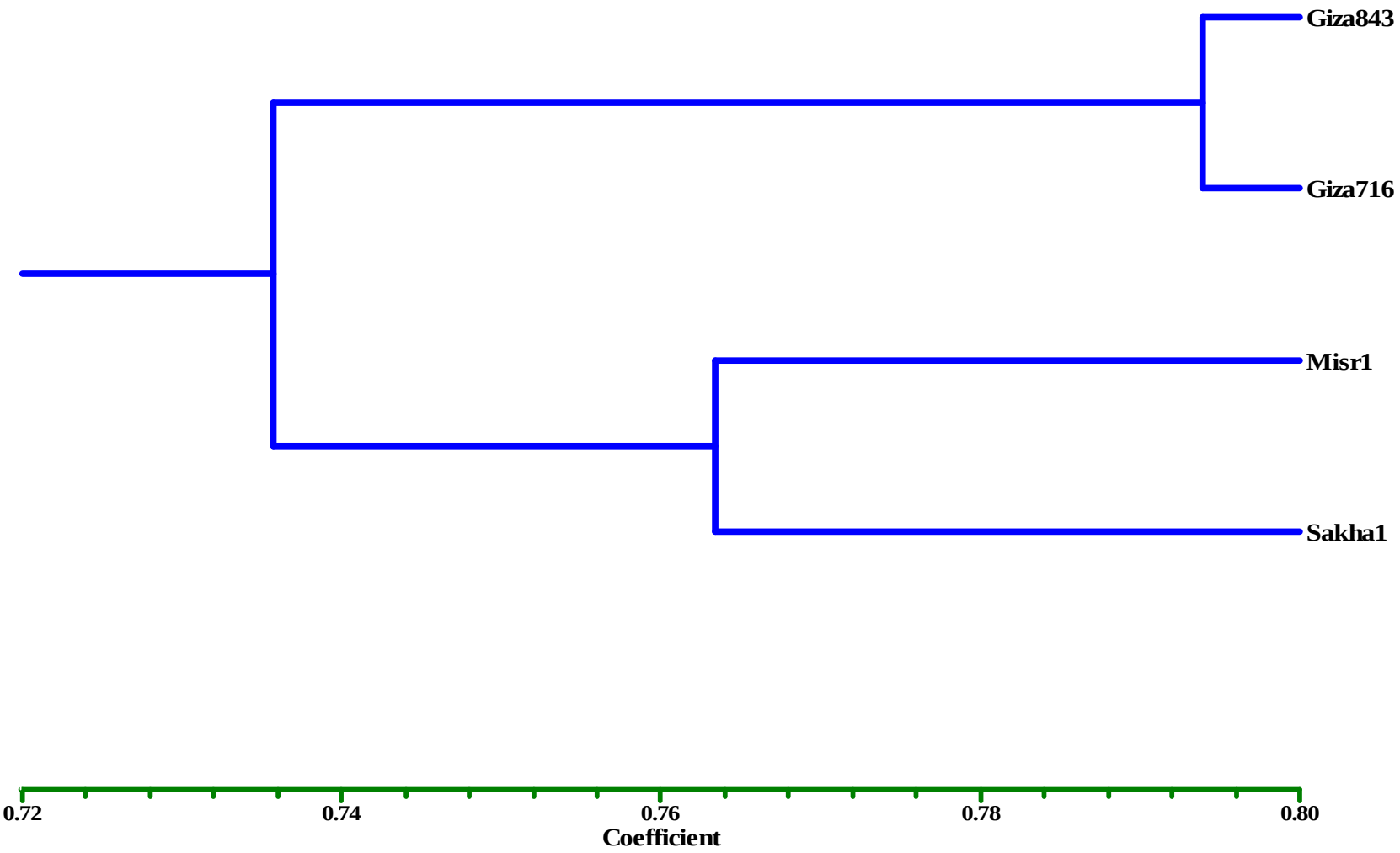
Molecular marker analysis



SRAP polymorphisms

Primer combination	Number of bands		%polymorphism	PIC (%)
	Total	Polymorphic		
Me1+Em1	13	12	92.3	85
Me1+ Em2	10	6	60	83
Me1+ Em3	10	9	90	84
Me1+ Em4	14	13	92.9	78.6
Me3+ Em1	10	9	90	90
Me3+ Em2	10	10	100	60
Me3+ Em3	11	11	100	56.6
Me3+ Em4	9	7	77.8	86.4
Me4+ Em1	14	14	100	89.8
Me4+ Em2	9	4	44.4	64.2
Me4+ Em3	9	6	66.7	81.5
Me4+ Em4	12	12	100	92.3

Cluster analysis for SRAP markers



Conclusions and Prospects

- The varieties"Giza716" and "Giza843" appeared to be tolerant but “Misr1” and “Sakha1” varieties appeared to be sensitive to drought and heat stress
- SRAP molecular markers could be useful for plant breeding programs in detecting the real differences among genotypes considering their genetic background in relation to the environmental stresses.
- Tolerant varieties "Giza716" and "Giza843” can be used in breeding programs as a source of drought and/or heat stresses to accumulate the QTL of these traits and produce super tolerant varieties to drought and/or heat stresses.

" وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ "

Σας ευχαριστώ πολύ για το ενδιαφέρον σας