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# Valorization of squash (*Cucurbita maxima* Duch) Biodiversity: approaches and main results

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# INTRODUCTION

- Biodiversity in Tunisia is quite important for plant and animal

species : more than 2200 indigenous plant species and 550

animal species were recently inventoried (NGB, 2014)

- These local varieties have better adaptability to climate change and contribute to sustainable agriculture development and ecosystem balance

- However, 10% of this biodiversity is in extinction phase due to several causes

- **For plant species, there are more than 1700 tree species and 250 vegetables species: fruit vegetables (pepper, melon, squash…) root vegetables (carrots) and leafy vegetables (parsley, celery)**

- **Squash (*Cucurbita maxima* Duch.) is a member of economically important Cucurbitaceae in Tunisia, more than 3800 ha are cultivated in three main regions: North, Center and Sahel, with an average yield of 70t/ha (Anonymous, 2020)**

- **Tunisia is one of the important diversity center for cultivated cucurbits (adaptation, natural selection and farmers selection); However, dispite the large genetic variability of squash local populations, a high heterogenity on agro-morphological characteristics is recorded ….**

- At the research level, the diversity of genetic ressources of squash has a low interest;

- In the current research program, the aims are:
  (i) Conduct the first collection and characterization of squash local populations,

  (ii) Conserve this local plant diversity in the NGB,

  (iii) Maintain by self pollination the main accessions and start a breeding program.

# Material and Methods

Fifteen Tunisian landraces of squash (*Cucurbita maxima* Duch., Tab.1) collected from different geographic regions of Tunisia during the period extending from 2018 to 2020 were used in the present study

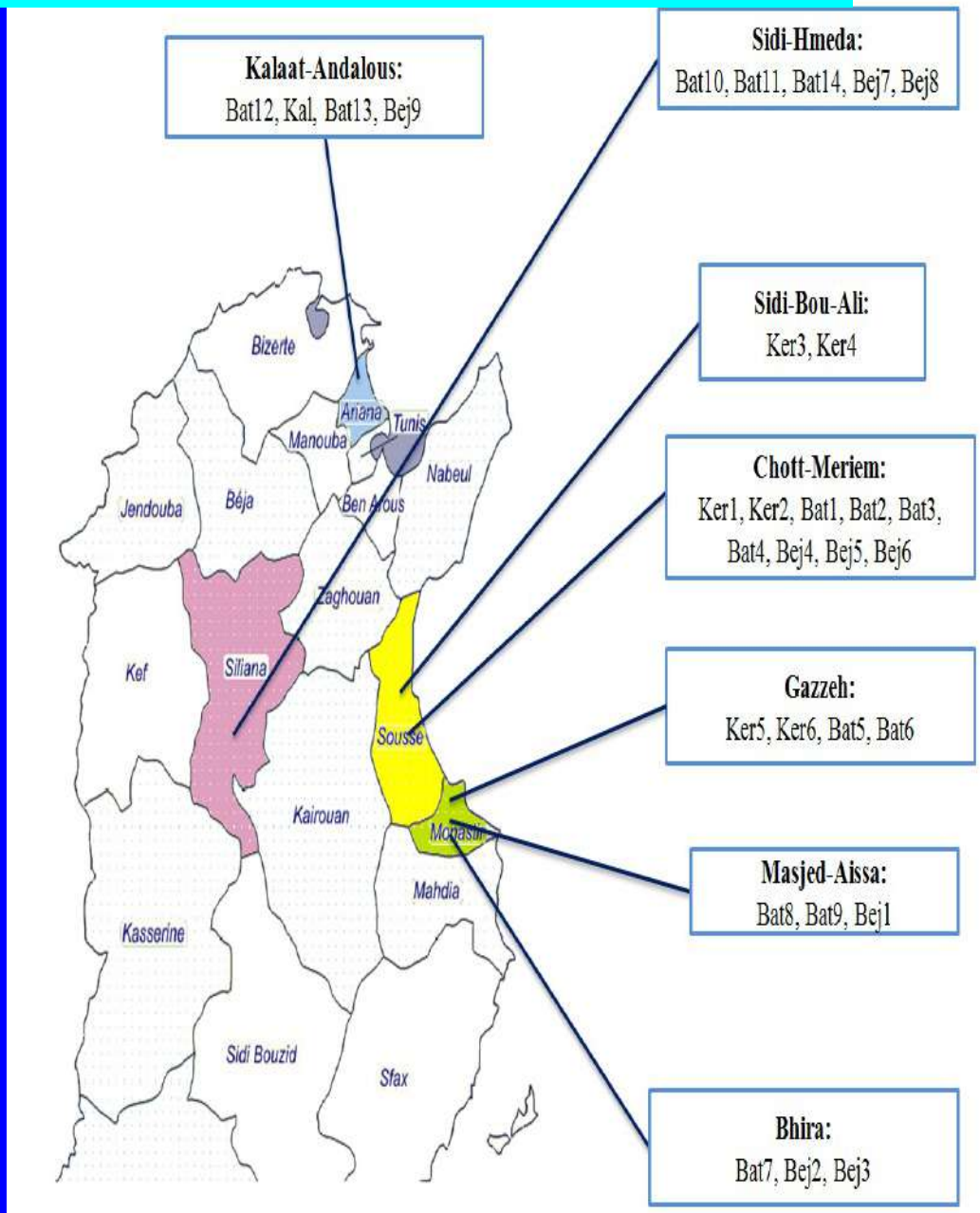



**Figure 1.** Geographic distribution of *Cucurbita maxima* collection used in this study. The names of provinces and locations are in bold.

# Table1. Description of Tunisian squash landraces used in the present study

| Landrace inventory number | Local name | Origin | Latitude | Longitude | Short description |
|---|---|---|---|---|---|
| NGBTUN745 | Batati Green | Ariana(Kalaat Andalous) | 37°033"N | 10°11'7"E | ±rounded fruit, light green skin, green flesh |
| NGBTUN746 | Batati orange | Siliana (SidiHamada) | 35°57‘28"N | 9°32'57"E | ±rounded fruit, orange skin, light orange flesh |
| NGBTUN747 | Galaoui | Ariana (Kalaa Andalous) | 37°033"N | 10°11'7"E | raised fruit with basal tip, green skin, green flesh |
| NGBTUN748 | Karkoubi orange | Sousse (Sidi Bouali) | 35°54'22.21"N | 10°32’47.81"E | Flattened fruit, dark yellow skin, yellow flesh |
| NGBTUN749 | Batati yellow spotted with white | Siliana (SidiHamada) | 35°57‘28"N | 9°32'57"E | ±globes fruit, orange skin spotted with white, orange flesh |
| NGBTUN750 | Batati white | Monastir (Sahline) | 35°45'05"N | 10°42'39"E | globes fruit, white skin, white flesh |
| NGBTUN751 | Bejaoui Green | Siliana (SidiHamada) | 35°57‘28"N | 9°32'57"E | Flattened fruit, dark green skin, light green flesh |
| NGBTUN752 | Batati yellow | Siliana (North) | 35°57‘28"N | 9°32'57"E | rounded fruit, yellow skin, light orange flesh |
| NGBTUN753 | Béjaoui Green | Siliana (South) | 35°57‘28"N | 9°32'57"E | Flattened fruit, dark green skin, light green flesh |

| | | | | | |
|---|---|---|---|---|---|
| NGBTUN1004 | Galaoui large seeds | Ariana(Kalaat Andalous) | 37°033"N | 10°11'7"E | raised fruit with basal tip, green skin, white green flesh |
| NGBTUN1005 | Galaoui smoll seeds | Ariana(Kalaat Andalous) | 37°033"N | 10°11'7"E | raised fruit with a big basal tip, green skin, white green flesh |
| NGBTUN1006 | Karkoubi orange | Monastir (Sahline) | 35°45'05"N | 10°42'39"E | Flattened fruit, dark yellow skin, yellow flesh |
| NGBTUN1007 | Batati Green | Siliana | 35°57‘28"N | 9°32'57"E | rounded fruit, green skin, green flesh |
| NGBTUN1008 | Batati Green | Monastir (Teboulba) | 35°45'05"N | 10°42'39"E | rounded fruit, flat stem end, green skin, light green flesh |
| NGBTUN1009 | Bejaoui spotted with yellow | Siliana (SidiHamada) | 35°57‘28"N | 9°32'57"E | rounded fruit with flat stem end, spotted with yellow light green skin, light green flesh |

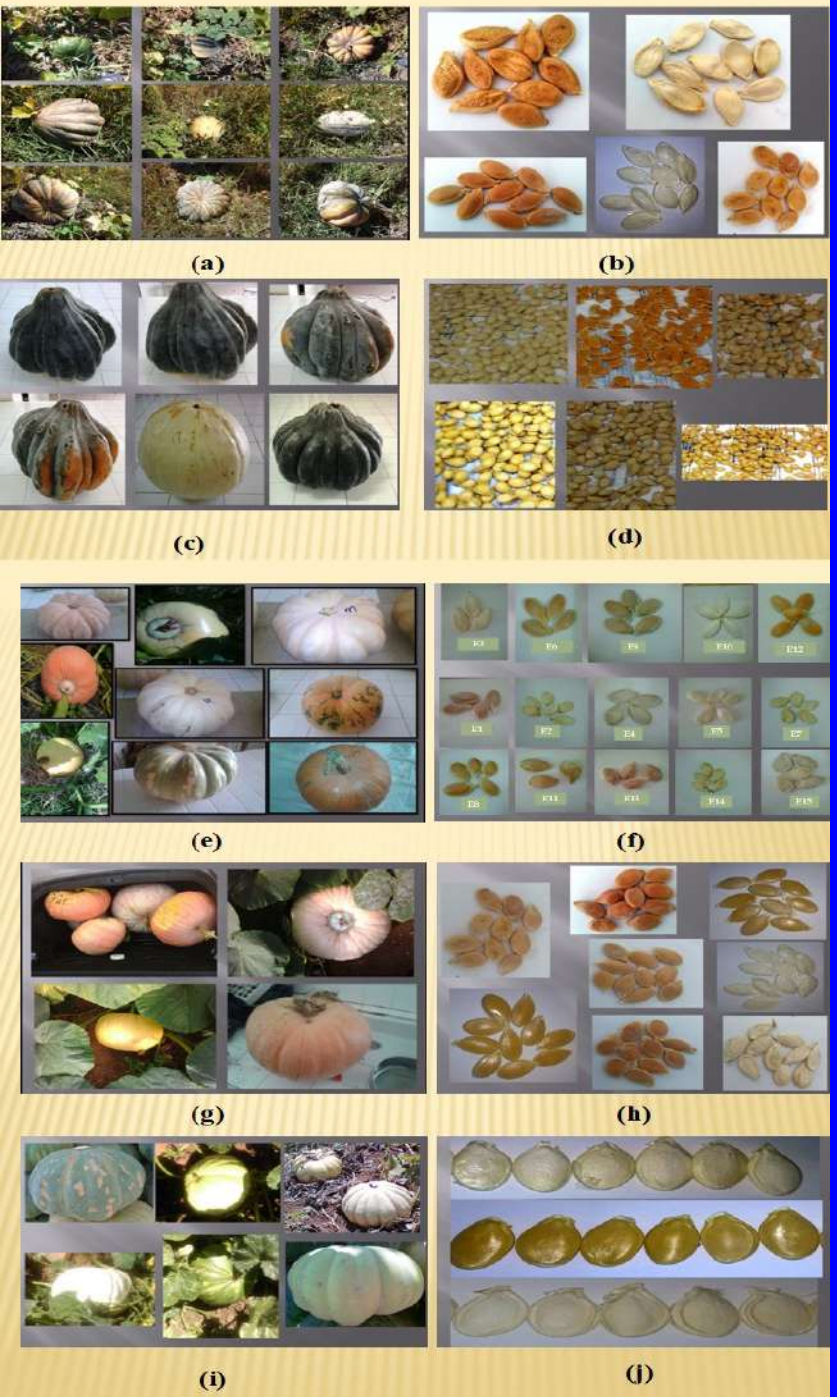




**Figure 2 .**Variability of fruits and seeds of different accessions collected from the different prospected zones: (a) and (b) from Sidi-Hmeda Siliana, (c) and (d) from Kalaat-Andalous, (e) and (f) of Chott-Meriem, (g) and (h) of Sidi-Bou-Ali and (i) and (j) of Sehline


The diversity of mature fruit size, shape and color for Squash *populations* which were collected from three regions (North, Center, Sahel)

- **30 plants for each ecotype (10 per replication) were evaluated for the morphological characteristics based on UPOV's squash descriptor list (UPOV, 2007)**
- **Quantitative traits of different parts (seeds, leaves, flowers and fruits) were evaluated per plant**
- **Fourteen morphological characteristics were assessed per plant, starting from the seed till the stage of mature fruit**
- **Chemical parameters ($Ca^{2+}$, $Mg^{2+}$ and $K^{+}$) were analyzed using an atomic absorption spectrophotometer (Kim et al., 2007)**
- **Total carotenoids content was determined following the method of Lucia et al.(2015).**
- **Molecular characterization using** 60 RAPD and 17 ISSR markers

- **Principal Component Analysis (ACP)was used to group different ecotypes into homogenous classes, while quantitative traits for all characteristics and chemical parameters were submitted to SAS (version 9.2) analysis; means were separated by Duncan's multiple range test at 5% level.**

# RESULTS

√ Establishment of a Tunisian map for squash population

√ Conservation of this national plant biodiversity at the National Gene Bank

√ Substantial genetic diversity was recorded for the majority of traits; morphological variation was most apparent in fruit characteristics. Most populations in this study had transverse broad elliptical fruit (75.6%) , a medium skin thickness (89.12%), a medium flesh thickness (57.89%) and a big cavity diameter (94.7%)



# Ecotype repartition

# Frequency distribution of main fruits characteristics (10/ landrace)

| Character | Description | Frequency (%) |
|---|---|---|
| Fruit weight (Kg) | Heavy > 10 | 21,2 |
| | Medium 5-10 | 75,8 |
| | Light < 5 | 3,0 |
| Fruit shape | Transverse elliptic | 16,20 |
| | Transverse broad ellip. | 75,61 |
| | Cylindrical | 8,19 |
| Peduncle length (cm) | Long > 10 | 3,75 |
| | Medium 5-10 | 89,12 |
| | Short <5 | 7,13 |
| Skin thickness (cm) | Thick >1 | - |
| | Medium 0,1- 1 | - |
| | Thin <0,1 | 100 |
| Flesh thickness (cm) | Thick >5 | 38,27 |
| | Medium 3- 5 | 57,89 |
| | Thin <3 | 3,84 |
| Cavity diameter (cm) | Big >20 | 94,7 |
| | Medium 15-20 | 5,2 |
| | Small <15 | 0,1 |
| Seed number/fruit | High >300 | 36,32 |
| | Medium 200-300 | 59,48 |

# 3. Flowers characteristics

## 3.1. Number of female flowers per plant (FF), male flowers (MF), FF/ MF and length of male flower (MFSL) and female flower (FFSL) sepals

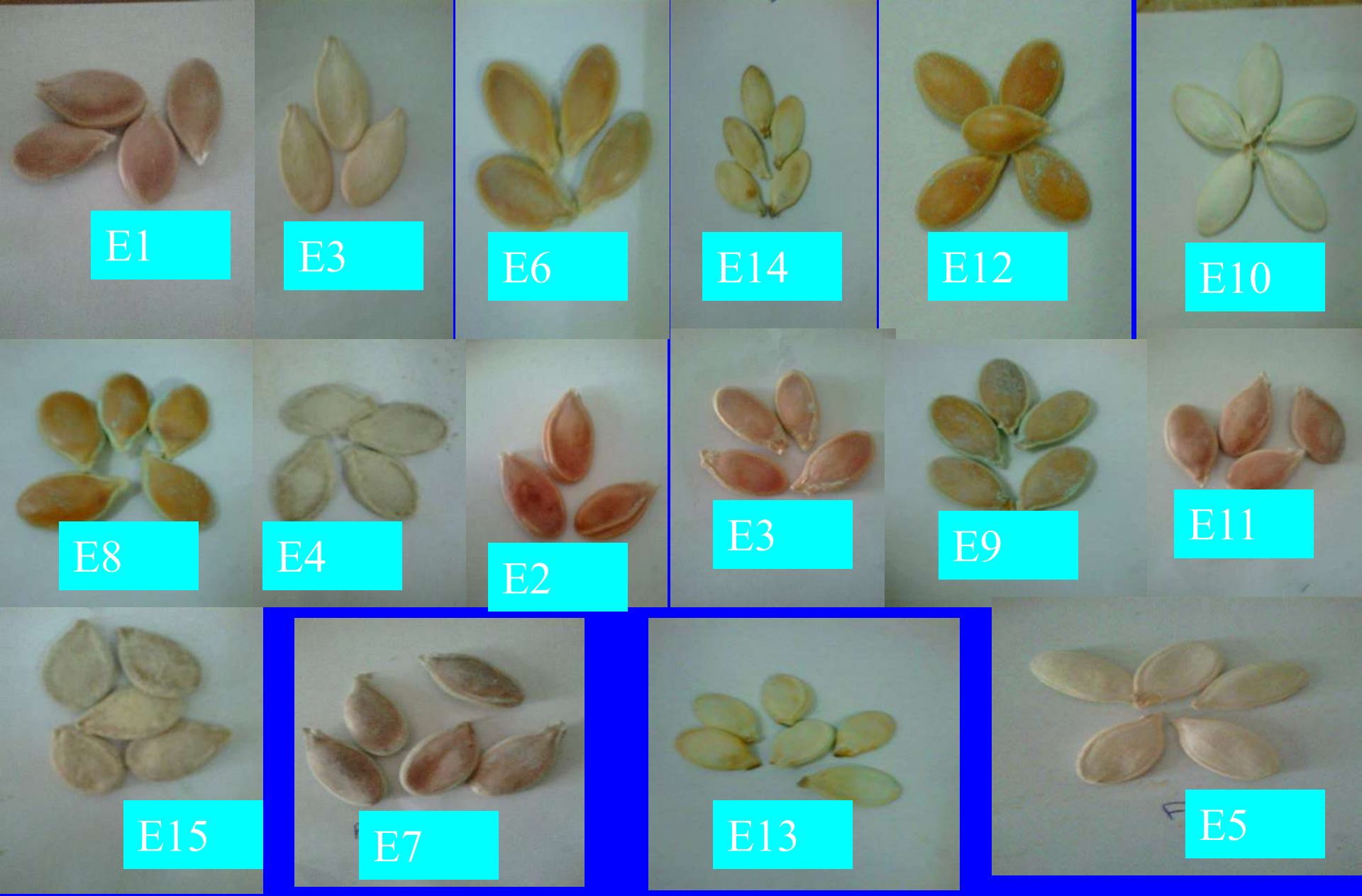
| Ecotype | FF | FM | FF/MF | MFSL (cm) | FFSL (cm) |
|---|---|---|---|---|---|
| E1 | 11,86 abc* | 97,23 ab | 0,13 abcd | 3,30 abcd* | 3,09 bc |
| E2 | 14 ,00ab | 100,97 ab | 0,14 abcd | 3,06 bcd | 2,77 c |
| E3 | 12,63 ab | 111,03 a | 0,12 bcd | 3,88 a | 2,94 c |
| E7 | 13,70 ab | 94,63 ab | 0,15 abc | 3,14 abcd | 2,67 cd |
| E8 | 11,33 abc | 82,17 abcd | 0,16 abc | 3,20 abcd | 3,10 bc |
| E10 | 9,57 bcd | 79,00 abcd | 0,14 abcd | 3,47 abc | 3,32 bc |
| E11 | 6,33 de | 77,33 abcd | 0,08 de | 3,24 abcd | 3,68 ab |
| E4 | 14.43 a | 90,63 abc | 0,18 ab | 3,13 abcd | 3,18 bc |
| E6 | 12,90 ab | 97,9 ab | 0,13 abcd | 2,97 cd | 2,78 c |
| E9 | 12 ,87 ab | 98,27 ab | 0,13 abcd | 3,30 abcd | 2,95 c |
| E12 | 13,33 ab | 78,42 abcd | 0,18 a | 3,19 abcd | 3,91 a |
| E13 | 6,33 de | 68 ,00bcde | 0,094 cd | 3,22 abcd | 2,89 c |
| E15 | 7,74 cd | 39,5 e | 0,03 e | 2,74 d | 1,90 e |
| E5 | 5 ,68 e | 59,18 dce | 0,03 e | 3,09 abcd | 2,94 c |
| E14 | 5 ,82 e | 72,93 bcde | 0,03 e | 3,69 ab | 2,18 ed |

*Means followed by the same letter are statistically different at 5% (Duncan test)

# 1. Seed characteristics

| Ecotype | Type | Length (cm) | Width (cm) | Colour | (L/W) | Weight of 100 seeds (g) |
|---|---|---|---|---|---|---|
| E1 | Batati | 2 ,41f* | 1,41abc | 3 | 1,71 e | 40 c |
| E2 | Batati | 2,57f | 1,43ab | 2 | 1,80de | 46 a |
| E3 | Batati | 1,80h | 1, 34de | 2 | 2,10 b | 43 b |
| E4 | Kerkoubi | 2,27g | 1 ,25gh | 2 | 1,81cde | 28 f |
| E5 | Bejaoui | 1,80h | 0 ,90j | 3 | 1,80ed | 14 h |
| E6 | Kerkoubi | 2 ,31g | 1 ,23gh | 2 | 2,08b | 30 e |
| E7 | Batati | 2,78a | 1 ,38bcd | 2 | 1,93c | 42 b |
| E8 | Batati | 2,30g | 1,21h | 2 | 1,85cd | 26 g |
| E9 | Kerkoubi | 2 ,78a | 1 ,28fg | 1 | 2,21 a | 42 b |
| E10 | Batati | 2 ,73ab | 1 ,37cde | 2 | 2,08b | 45 a |
| E11 | Batati | 2,45ef | 1,34de | 2 | 1,84cd | 42 b |
| E12 | Kerkoubi | 2,68ab | 1,32ef | 1 | 2,05b | 42 b |
| E13 | Kerkoubi | 2,66bc | 1,44a | 2 | 1,84cd | 46 a |
| E14 | Bejaoui | 1 ,76h | 1,10i | 1 | 1,59f | 15 h |
| E15 | Kerkoubi | 2,45ed | 1 ,30cde | 1 | 1,80ed | 37 d |

*Means followed by the same letter are statistically different at 5% (Duncan test)




The diversity of seed ( size, shape and color) for Squash *populations* which were collected from three regions (North, Center, Sahel)

# Agromorphological characterization

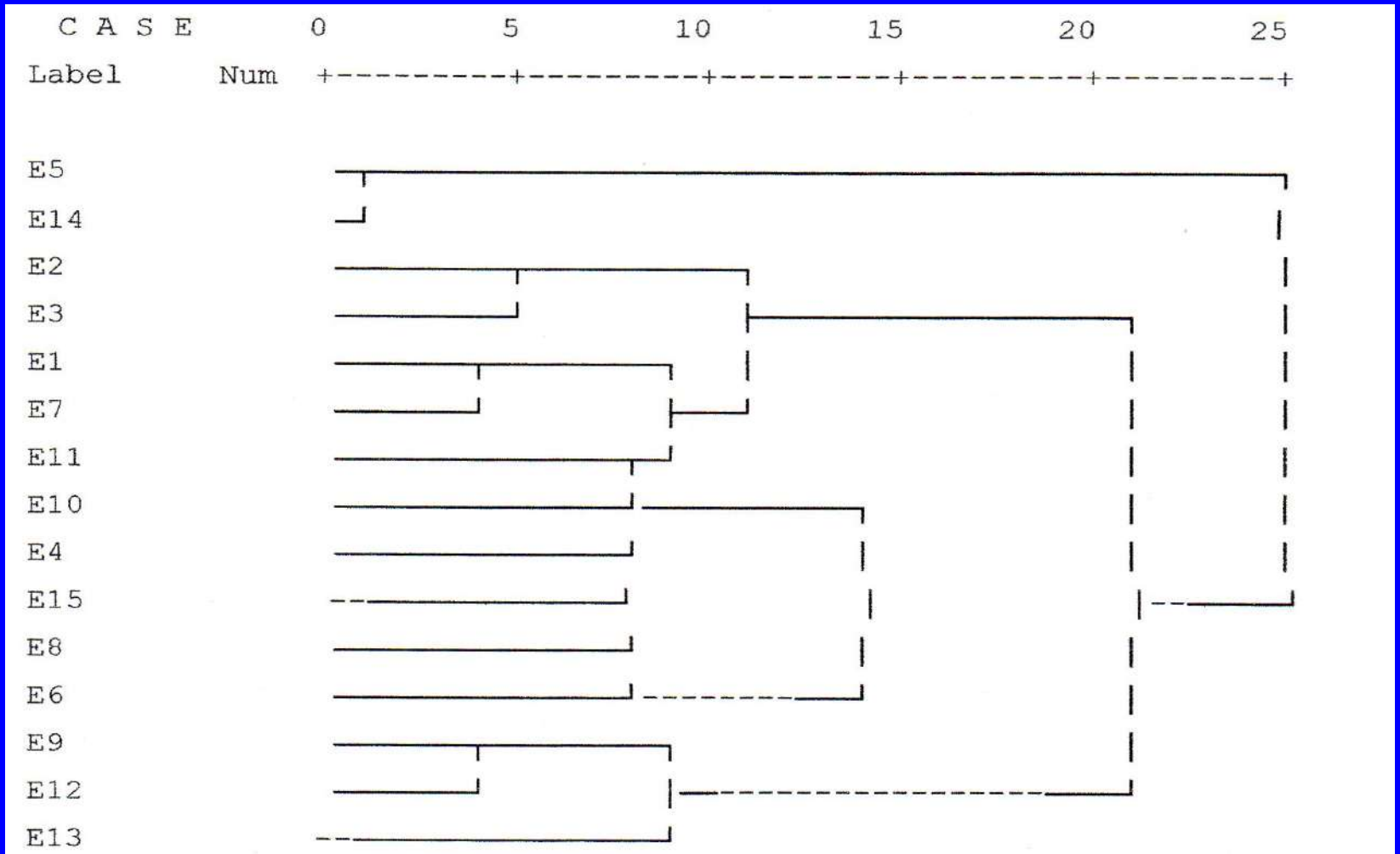
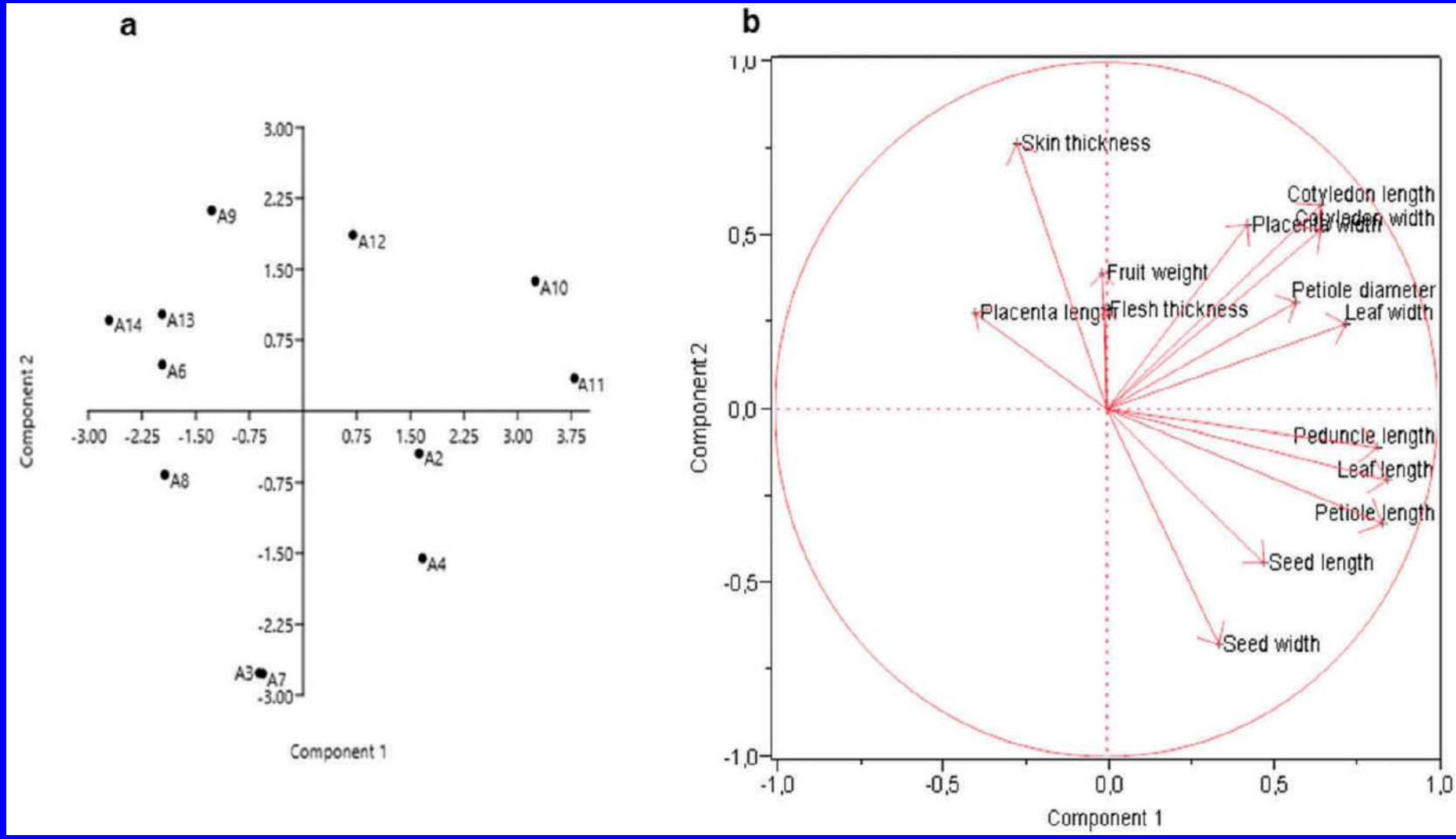



**Figure :** Dendrogramme of quantitative and qualitative traits for the fifteen local squash populations




**PCA analysis of Tunisian accessions of Cucurbita maxima based on morphological data: (a) Scatter diagram of the distribution of the accessions; (b) Biplot analysis of the eigenvalues**

# Biochemical characterization

| Ecotype | Carotenoid content (mg /gDM) | Total sugar (%) | K (%) | Mg (%) | Ca (%) |
|---|---|---|---|---|---|
| E1 | 161.26 b | 2.72 cd | 1.12 ab | 1.82 a | 0.74 bcd |
| E2 | 152.23 b | 2.67 cd | 1.48 ab | 1.63 a | 1.59 a |
| E3 | 161.26 b | 3.37 bc | 0.93 b | 1.13 a | 0.79 abcd |
| E4 | 286.56 a | 4.59 ab | 2.24 ab | 1.66 a | 0.89 abcd |
| E5 | 93.35 c | 1.41 b | 1.08 ab | 1.42 a | 0.13 b |
| E6 | 276.69 a | 5.38 a | 2.42 a | 0.78 a | 1.00 abc |
| E7 | 152.23 b | 2.52 cd | 1.48 ab | 1.36 a | 0.74 bcd |
| E8 | 156.08 b | 2.67 cd | 1.01 ab | 1.28 a | 1.59 a |
| E9 | 274.99 a | 4.88 a | 2.32 ab | 1.08 a | 1.37 ab |
| E10 | 157.45 b | 3.37 bc | 0.93 b | 1.41 a | 0.29 cd |
| E11 | 155.61 b | 2.33 cd | 1.64 ab | 1.67 a | 1.22 ab |
| E12 | 256.93 a | 4.88 a | 1.64 ab | 1.36 a | 0.63 bcd |
| E13 | 286.56 a | 5.68 a | 2.42 a | 1.28 a | 0.89 abcd |
| E14 | 89.99 c | 2.07 cd | 0.99 ab | 1.08 a | 0.32 cd |
| E15 | 256.93 a | 5.24 a | 2.42 a | 1.41 a | 0.89 abcd |
| CV (%) | 12.48 | 21.53 | 45.59 | 57.30 | 48.36 |

# Biochemical characterization

** Shelled seeds of all accessions were analysed for total phenolic contents (TPC), and total antioxidant capacity (TAC). The parameter values are showed in Table 1.

** Total phenolic content, expressed as gallic acid equivalent (GAE), ranged from 72.70 mg GAE / 100g FW (Kal2) to 228.86 (Ker5) mg GAE / 100g FW

** Antioxidant levels squash seeds ranged from 104.69 to 248.15 µmol Trolox eq / 100g FW; the highest value was found at the accession of Bejaoui from Kalaat-Andalous while the lowest content was recorded in Bejaoui vert from Siliana .

**Table 1. The total phenolic content and antioxydant capacity in squash seeds**

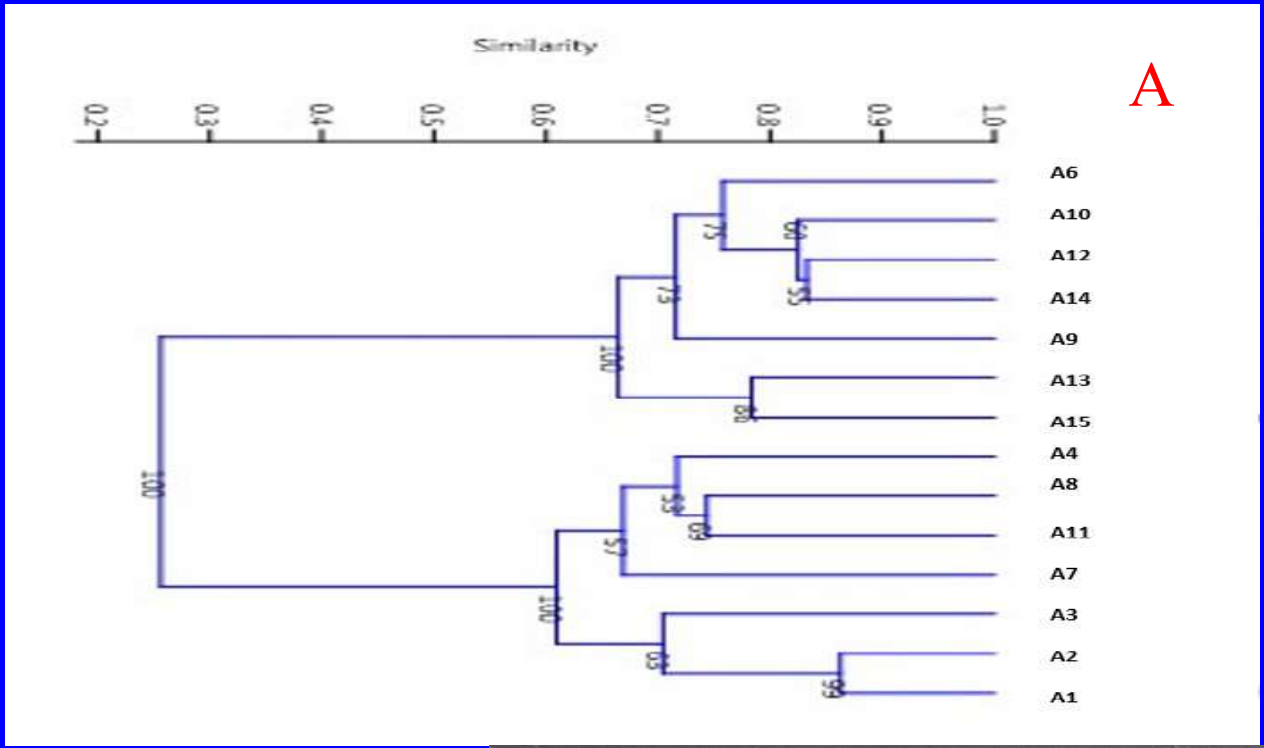
| Seeds | Minimum | Maximum | Mean | Ecart-type |
|---|---|---|---|---|
| TPC (mg GAE/100g FW) | 72,703 | 228,864 | 139,338 | 43,882 |
| ANTOX (µmol Trolox eq/100g FW) | 104,699 | 248,15 | 157,792 | 43,042 |

# Molecular characterization

60 RAPD markers, 17 ISSR markers and 25 different combination of SRAP markers, were used to characterize the best fourteen squash accessions ...

96% of genetic variation between the *cucurbita maxima* accessions were recorded. Then, 477 polymorphic bands were amplified (Fig.4a et b)

The PIC values for the three types of markers, ranged between 1.04 and 0.38 for our collection for which the RAPDs marker resulted the most informative.



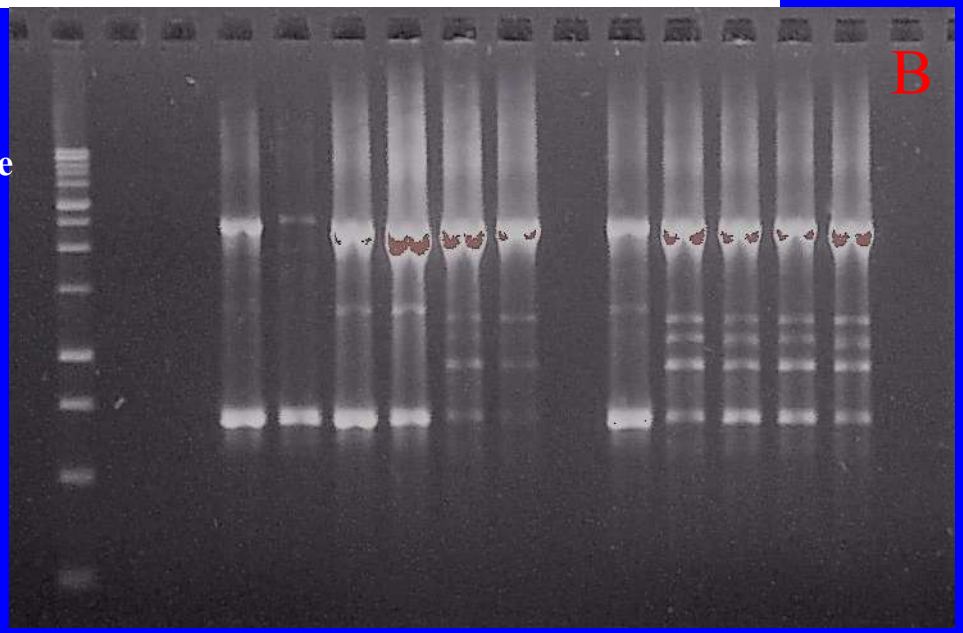




**Figure 4. A dendrogram showing the genetic divergence among fifteen *C. maxima* accessions using cluster analysis of RAPD, ISSR and SRAP data (A) and Evaluation of PCR-labeled probes by agars gel electrophoresis (B).**

# Conclusion & recommandations

- √ It should be pointed out that the current study revealed considerable variation in agro-morphological characters of Tunisian squash populations;

- √ The molecular characterization of the local genetic recourses of *cucurbita maxima* collection, revealed a large variability that will help breeders to a more efficient use of the genetic variation in breeding program;

- √ The results obtained highlighted the importance of the bioactive compounds, indicating the needs for further investigation to identify the phenolic profiles of *cucurbita maxima* seeds, that can be considered as functional foods for its nutritional and therapeutic value;

- **Such a research program consolidates the efforts of the NGB to conserve this genetic heritage against possible erosion**

- **Start a varietal improvement program for this species, which has so far remained represented by standard varieties**

- **This work was the basis of our contribution to a Mediterranean project 'Prima Pulping' in progress**




# Thank you for your attention