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# Effect of seed priming on squash (*Cucurbita maxima* Duch.) seed germination and seedling characteristics under salt stress

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# Introduction

## The importance of Squash germplasm in Tunisia


NGBTUN 745, 747,1004 (Batati Green, Galaoui large and smoll seed)

NGBTUN 748, 750,1006,1008 (Karkoubi, Batati white,Green)

NGBTUN 746,749,751,752,753,

NGBTUN 745,1009,1007(Batati, Bejaoui)

# *Morphological variability of squash landraces*

Monastir

Siliana

Sousse

Ariana

Sidi bouzid

# What is Seed Priming?

Seed priming is a simple pre-germination strategy to improve seed performance and alleviate the negative effects of abiotic stress

# What are Seed Priming techniques ?

Various techniques are applied for improving the seed emergence:

- Hydro-priming (sterilized water)
- Halopriming ($KNO_3$, NaCl)
- Osmopriming (PEG)
- Hormonal priming ($GA_3$)

# How salinity hamper plant growth ?

- Salinity causes unfavorable environment and hydrological situation that restrict normal crop production,
- Salinity reduces seed germination and lengthens the time needed for germination

# Importance of seed priming

- Rapid and uniform seedling emergence,
- Primed seeds produced higher germination rate and greater germination percentage,
- The primed seeds increased the total sugar and α amylase activity and exhibited earlier initiation of protein, RNA and DNA synthesis.

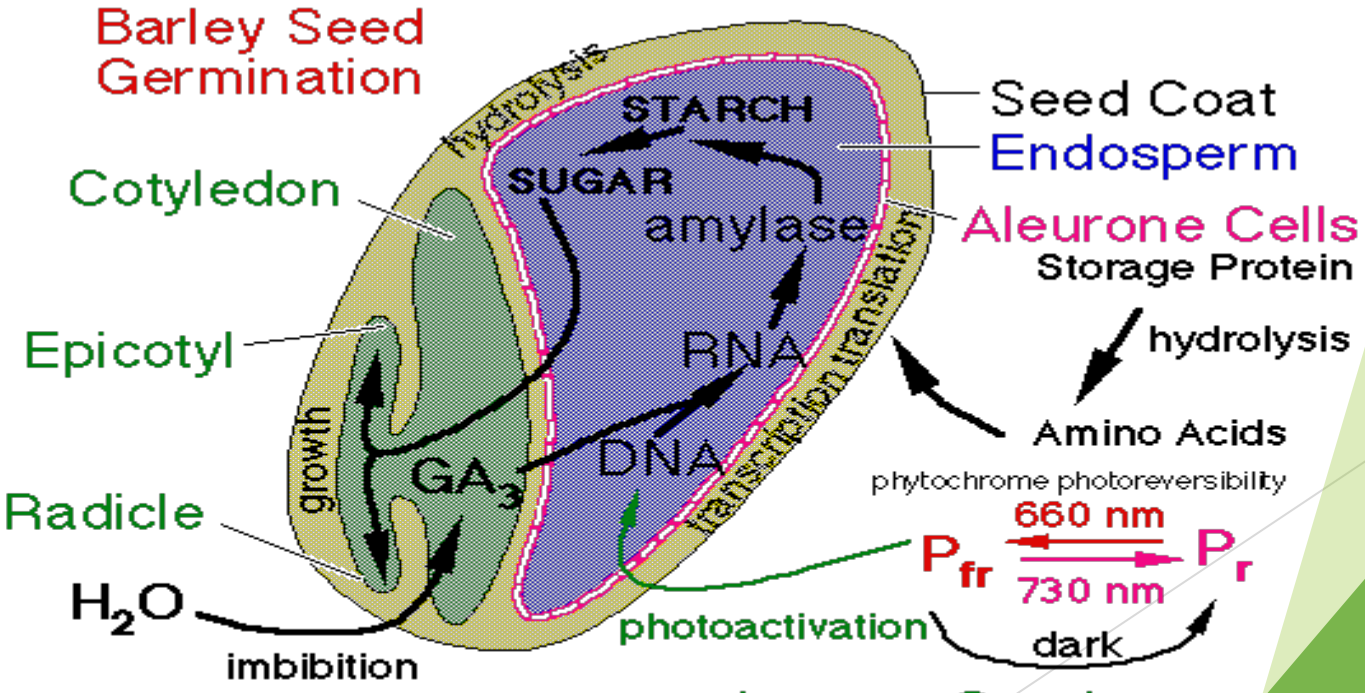
# SEED GERMINATION = radicle emergence

Seeds Lacking Dormancy | Moisture | Warmth

Dormant Seeds need more than this:

- Thick Seed Coat — Scarification
- Thin Seed Coat — Light or Darkness
- Insufficient Development — After-ripening with Fungi
- Inhibitors
  - Abscisic Acid — Vernalization
  - Phenolic — Repeated Leaching

## Barley Seed Germination




## Lettuce Seed Germination

# Physiological changes induced by priming

- Priming improves the swelling of the embryo and speed up germination by faciliting water absorption,
- Seed priming stimulates the imbibition processes and make the seed ready for radicle emergance

# Introduction

- Breeding for abiotic stress tolerance is seriously hampered by:
  - Trait's complex inheritance
  - Wide environmental variation, and
  - Lack of suitable selection methods
- The routinely employed screening methodologies are based on estimating yield reduction under stress conditions, usually applied at plant's most critical growth stages

# Introduction

- Given that vegetable crops are particularly susceptible to abiotic stresses at early growth stages the determination of seed germination potential and seedling growth under stress conditions will be employed as a method to select tolerant genotypes

- Screening for abiotic stress tolerance at early growth stages is a short-cut screening approach, which allows a time- and cost-efficient selection of suitable germplasm material to be exploited in relative breeding programs

# Aims of this study

1. Study of the local genetic heritage of Cucurbita spp

2. Evaluate squash local accessions with respect to abiotic stresses and their adaptation to organic and conventional cultivation within the framework of a PRIMA project

# Materials and Methods

## ❖ *Genetic material*

**Table 1:** Description of the Tunisian squash landraces employed in this study

| Landrace inventory number | Local name | Origin | Longitude | Short description |
|---|---|---|---|---|
| NGBTUN1008 | Batati Green | Monastir (Teboulba) | 10°42'39"E | globular fruit, flat stem end, green skin, light green flesh |
| NGBTUN1004 | Galaoui large seeds | Siliana (Sidi Hmada) | 10°11'7"E | turbinate interior fruit with basal tip, green skin, white green flesh |
| NGBTUN1005 | Galaoui small seeds | Ariana (Kalaat Andalous) | 10°11'7"E | Raised fruit with basal tip, green skin, green flesh |

# Materials and Methods

**Seed preparation**

Seeds surface-sterilised for 5 min in 20% hypochlorite/H2O solution supplemented with Tween-20 and then washed four times with sterile water

**Priming techniques and salt stress**

**Imbibition :GA3 0,1% and 0,2%, 24 h**
**KNO3 0,3 and 0,4%, 24 h (0,100,200mM NaCl)**
**Differents combinaisons were applied**

**Growth conditions**

Germination was conducted under seed germinator (25°C +-2°C) Plants were grown under controlled conditions (25°C, 18/6-h light-dark) for 15days

**Experimental Design**

*Completely random design (CRD)
* Four replications, each consisting of 50 seeds(germination stage) and 15 plants (growth stage)

## Materials and Methods

## Evaluation of germination traits

1. GP (%) = (TNGS /TNS) × 100

Where: GP is the germination percentage (%) at the end of experiment, TNGS: the total number of germinated seeds and TNS:the total number of seeds tested.

2. MGT = GtDt/G (Partheeban et al., 2017)

Where MGT: the mean germination time (day); Gt:the number of total germinated seeds on day t; Dt: the day t; and G is germinated seeds

3. Seed vigor index (SVI) = (Root length + Shoot length) * GP % (Ashraf et al., 2021)

# Materials and Methods

## Evaluation of Physiological traits

- chlorophyll fluorescence (Fo, Fv, Fm, Fv/Fm) and

- Determination of chlorophylls and carotenoids

(**Adhikari et al., 2022**)

## Results

**Table1: effect of Priming using GA3 and KNO3 on the germination percentage of 3 squash local accessions**

| Accession | Control | 0,1 % $GA_3$ | 0,2 % $GA_3$ | 0,3 % $KNO_3$ | 0,4 % $KNO_3$ |
|---|---|---|---|---|---|
| Galaoui large seeds | 88,66 ± 2,13 | **92,26** ± 2,63 | 49,44 ± 4,63 | 100 ± 0,00 | 100 ± 0,00 |
| Galaoui small seeds | 77,57 ± 2,33 | **87,22** ± 5,06 | 63,33 ± 4,33 | 100 ± 0,00 | 100 ± 0,00 |
| Batati Green | 68,73 ± 3,24 | **73,20** ± 2,63 | 42,44 ± 2,63 | 82,22 ± 2,62 | 100 ± 0,00 |

## Results

**Table 2:** Effect of priming with GA3 and KNO3 on the initiation (Days) of cotyledons in three accessions studied

| Accessions | Control | 0,1 % $GA_3$ | 0,2 % $GA_3$ | 0,3 % $KNO_3$ | 0,4 % $KNO_3$ |
|---|---|---|---|---|---|
| Galaoui large seeds | $9,61^{a}$ | $6,30^{bc}$ | $6,00^{bc}$ | $7,12^{b}$ | $6,81^{b}$ |
| Galaoui small seeds | $10,60^{a}$ | $9,51^{b}$ | $9,01^{b}$ | $8,91^{b}$ | $4,76^{c}$ |
| Batati Green | $8,40^{a}$ | $7,30^{b}$ | $6,61^{c}$ | $7,00^{b}$ | $6,51^{b}$ |

## Results

**Table 3.** Mean effect of priming with GA3 and KNO3 on the hypocotyl length of young plants in three accessions studied

| Acc | Control | 0,1 % $GA_3$ | 0,2 % $GA_3$ | 0,3 % $KNO_3$ | 0,4 % $KNO_3$ |
|---|---|---|---|---|---|
| Galaoui large seeds | $5,25^{a}$ | $4,83^{b}$ | $5,01^{b}$ | $5,55^{a}$ | $5,31^{a}$ |
| Galaoui small seeds | $4,75^{a}$ | $4,52^{c}$ | $4,41^{d}$ | $4,66^{b}$ | $4,81^{a}$ |
| Batati Green | $4,35^{c}$ | $4,52^{c}$ | $4,35^{c}$ | $5,37^{b}$ | $5,55^{a}$ |

## Results

**Table 4:** Effect of priming with GA3 and KNO3 on the vigor index (Root length + Shoot length) x GP %

| Acc | Témoin | 0,1 % $GA_3$ | 0,2 % $GA_3$ | 0,3 % $KNO_3$ | 0,4 % $KNO_3$ |
|---|---|---|---|---|---|
| Galaoui large seeds | 517,96$^{c}$ | 445,42$^{d}$ | 247,69$^{e}$ | 555$^{a}$ | 531$^{b}$ |
| Galaoui small seeds | 368,88$^{d}$ | 394,23$^{c}$ | 279,28$^{e}$ | 466$^{b}$ | 481$^{a}$ |
| Batati Green | 298,97$^{d}$ | 348,94$^{c}$ | 183,56$^{e}$ | 441,52$^{b}$ | 555$^{a}$ |

**Table 5:** Effect of priming with GA3 and KNO3 on the Germination percentage (means ± SD, n=50)

| Accessions | Traitement | GP (%) |
|---|---|---|
| Galaoui large seeds | Control | 88,66 ± 2,17 |
| | 100 mM NaCl | 92,22 ± 2,63 |
| | 200 mM NaCl | 49,44 ± 4,63 |
| | **100 mM + 0,1 % GA3** | **100,00 ± 0,00** |
| | **100 mM + 0,2 % GA3** | **100,00 ± 0,00** |
| | **100 mM + 0,3 % KNO3** | **100,00 ± 0,00** |
| | **100 mM + 0,4 % KNO3** | **100,00 ± 0,00** |
| | 200 mM + 0,1 % GA3 | 66,11 ±2,93 |
| | 200 mM + 0,2 % GA3 | 62,22 ± 2,63 |
| | 200 mM + 0,3 % KNO3 | 53,88 ± 3,33 |
| | **200 mM + 0,4 % KNO3** | **66,11 ± 4,85** |
| Galaoui small seeds | Control | 90,00 ± 0,00 |
| | 100 mM NaCl | 87,22 ± 5,07 |
| | 200 mM NaCl | 63,33 ± 4,33 |
| | 100 mM + 0,1 % GA3 | 81,66 ± 2,50 |
| | 100 mM + 0,2 % GA3 | 75,55 ± 2,90 |
| | 100 mM + 0,3 % KNO3 | 85,55 ± 3,90 |
| | 100 mM + 0,4 % KNO3 | 100,00 ± 0,00 |
| | 200 mM + 0,1 % GA3 | 72,77 ± 2,63 |
| | **200 mM + 0,2 % GA3** | **100,00 ± 0,00** |
| | **200 mM + 0,3 % KNO3** | **100,00 ±0,00** |
| | **200 mM + 0,4 % KNO3** | **100,00 ±0,00** |

## Results

**Table 5:** Effect of priming with GA3 and KNO3 on the Germination percentage (Continued)

| | | |
|---|---|---|
| Batatit Green | **Control** | **87.55 ± 2,20** |
| | 100 mM NaCl | 77,22 ± 2,63 |
| | 200 mM NaCl | 42,22 ± 1,63 |
| | **100 mM + 0,1 % GA3** | **100,00 ± 0,00** |
| | **100 mM + 0,2 % GA3** | **81,66 ± 2,50** |
| | **100 mM + 0,3 % KNO3** | **82,77 ± 2,83** |
| | **100 mM + 0,4 % KNO3** | **87,77 ± 2,88** |
| | 200 mM + 0,1 % GA3 | 45,00 ± 1,73 |
| | 200 mM + 0,2 % GA3 | 45,00 ± 1,73 |
| | 200 mM + 0,3 % KNO3 | 62,22 ± 2,44 |
| | 200 mM + 0,4 % KNO3 | 65,00 ± 2,48 |

| TREATMENT | Chl. a | Chl. b | car | Fv/Fm |
|---|---|---|---|---|
| Control | 13.02 d | 5.10 abc | 0.83 de | 0.80 cd |
| 0.1% GA3 | 12.93 d | 4.42 cd | 0.76 g | 0.80 cd |
| 0.2%GA3 | 14.38 b | 5.77 abc | 0.86 c | 0.80 cd |
| 0.3% KNO3 | 14.55 a | 6.47 a | 0.95 a | 0.81 c |
| 0.4%KNO3 | 14.18 c | 6.37 ab | 0.85 cd | 0.81 c |
| 100mM NaCl | 11.11 h | 4.55 bcd | 0.76 g | 0.81 c |
| 200mM NaCl | 8.73 j | 3.27 d | 0.59 i | 0.80 cd |
| 100mM NaCl+ 0.1% GA3 | 11.12 h | 4.28 cd | 0.75 g | 0.77 f |
| 100mM NaCl+ 0.2% GA3 | 11.96 e | 4.85 bcd | 0.82 e | 0.81 c |
| 100mM NaCl+ 0.3% KNO3 | 11.69 f | 4.53 bcd | 0.80 f | 0.83 b |
| 100mM NaCl+ 0.4% KNO3 | 12.97 d | 5.44 abc | 0.89 b | 0.81 c |
| 200mM NaCl+ 0.1% GA3 | 8.85 j | 3.38 d | 0.65 h | 0.80 cd |
| 200mM NaCl+ 0.2% GA3 | 9.73 i | 6.10 abc | 0.66 h | 0.84 a |
| 200mM NaCl+ 0.3% KNO3 | 9.64 i | 4.22 cd | 0.64 h | 0.77 f |
| 200mM NaCl+ 0.4% KNO3 | 11.44 g | 4.59 bcd | 0.78 fg | 0.83 b |

# Conclusion

- The results show that upon stress, the priming with 0.2% GA3 solution and especially the different levels of KNO3 largely improved the percentage and the rate of the germination compared to the control,
- Squash seeds from three local landraces (Galaoui large seed, Galaoui small seed, and Batati green), tolerated and overcame the effects of different concentrations of NaCl by adding KNO3 ( germination percentage)
- The priming reduced the germination time by 2-3 days compared to the control
- In combination priming and salt stress, the mentioned treatments alleviated this stress with Fv/Fm ratio outside the range of 0.79-0.81 and gave the tested Landraces a best salt tolerance by improving photosynthesis activity

**The significant variation of the responses of different landraces depending on the stress induced, indicates an intra-genotype pattern and points to the combination of salt and water stresses by using priming technique for a more efficient selection**




# THANK YOU FOR YOUR ATTENTION

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