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Herbicide Influence on Germination Response of Salt Stressed *Abelmoschus esculentus* L. Okra Seeds

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ABSTRACT

In order to understand adaptation mechanism of okra to abiotic and biotic stress, our work has studied okra germination stage under salt and fluridone combined interaction. (Fluridone is an inhibitor of abscisic acid biosynthesis (ABA)).

Abelmoschus esculentus L. germination was conducted varying concentrations of NaCl (25meq.l^{-1} and 50meq.l^{-1}) solution, and a fluridone (10µM and 20 µM) solution, or both constraints at room temperature.

Results confirm that fluridone induces a very rapid seed germination progress compared to control. On the other hand, fluridone treatment seems to reduce delay effect of salt with tested concentrations. Fluridone improves okra seeds germination from the very first day with a 99.83 to 100% rate.

Analyzing some biochemical parameters related to okra seeds germination (soluble sugar and phenolic compounds) we recorded that stressed plants have a weak total sugar rate compared to control plants. This is true except for plants treated with 50meq.l^{-1} salt solution added with 20µM fluridone recording a 2.79% sugar accumulation apex. Same rate is recorded with phenolic compounds having a 50meq.l^{-1} salt treatment either associated or non-associated to fluridone. On the other hand, compared to control sample, compounds rate increases with seeds treated with 25meq.l^{-1} associated or non-associated to fluridone.

Observing results, we can assess that salt stress helps in reducing *Abelmoschus esculentus* L. total sugar rate and increases phenolic compounds with certain treatments.

Given these results, we can conclude that fluridone inhibits salt negative impact on germination and improves studied biochemical parameters.

KEY WORDS: *Abelmoschus esculentus* L., fluridone, germination, salt stress, NaCl, soluble sugar, phenolic compounds.

1. INTRODUCTION

Analyzing consequences of climate change we can conclude that many plant species having aptitude to resist or tolerate natural constraints are starting to loose such aptitude. Plant biodiversity is thus exposed to a high level ecological threat.

According to latest research, response or tolerance to salt depends of species variety, salt concentration, culture conditions and plant development stage [1, 2]. Plant improvement towards tolerance to environmental conditions requires a better understanding of stress-resistant plants adaptation response.

In this case, scientists from all disciplines should gather their efforts together in their laboratories and should focus their research towards a better understanding of new mechanisms shown by organisms facing new environmental conditions[3].

Many studies assessed that salinity has a depressive effect on seeds germination and production[4,5,6,7,8,]. Nevertheless, depressive effect varies according to stress intensity and plant health. Among constraining factors during plant life, hormones have an important function. Abscisic acid (ABA) is one of plant hormones inhibiting embryo and dormancy break. Recent research assessed those new substances such as fluridone, with herbicide properties, contributes to dormancy break mechanisms by inhibiting ABA.[9,10,11] .The main purpose of such researches is to insure a better growth and optimal production of seeds in order to replant damaged areas. In this context, our work aims to analyze okra (*Abelmoschus esculentus* L.) seeds behavior during germination stage while submitted to salt constraints associated or non-associated to fluridone.

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2. MATERIAL AND METHODS

2.1. Origin of plant material

Ripe fruits used in germination tests were harvested during July 2010 on natural plants from Nechmaya region south of Annaba, Algeria. Fruits were preserved in appropriate conditions until germination tests in May 2012. When fruits are ripe okra capsule becomes yellow and contains about 90 seeds that are easy to collect through slits.

2.2. Preparing seeds for germination tests

Seeds were first disinfected with a 0.8 % sodium hypochlorite solution during three minutes. Seeds were then rinsed out several times in distilled water and dried out on sterile filter paper. Germination tests were processed as follows: 15 sterile petri boxes with 10 seeds each on two Wattman filter paper layers humidified with 7ml distilled sterile water. Same procedure was followed with seeds treated with different fluridone solutions (10µM and 20µM) and NaCl solutions (25meq.l^{-1} and 50meq.l^{-1}). Boxes were incubated at optimal germination temperature (25 °C).

2.3. Germination tests:

Seed germination tests under salt and fluridone treatment were conducted in order to better understand:

- ✓ Okra seeds germination capacity under fluridone treatment with a 25°C temperature using 10µM and 20 µM concentrations.
- ✓ Okra seeds germination capacity under salt stress with a 25°C temperature using 25 meq.l^{-1} and 50 meq.l^{-1} NaCl concentrations.
- ✓ Okra seeds germination capacity under salt stress with a 25°C temperature using 25 meq.l^{-1} and 50 meq.l^{-1} NaCl concentrations combined with two fluridone concentrations (10µM and 20 µM).
- ✓ Dosing of main biochemical parameters sensitive to abiotic (sugar and phenolic compounds) stress during germination stage.

Observations were recorded every day to evaluate first germinations as soon as radical appeared. We have considered that seeds were germinated when radical had come through seed coat, showing 1mm out of seed tegument sand visible at the naked eye according to [12] definition. When germination rate became stable, observations were completed.

2.4. Estimating germination rate

Basing ourselves on total seeds number (TN), we have calculated percentage of germinated seeds (GS) as follows: GR = GS × 100 / TN(GR: germination rate)

2.5. Germination kinetics:

Germination kinetics often represents germination evolution percentages cumulated over time. Kinetics is settled from germinated seeds cumulated rates, i.e. germination rate variation according to time expressed in days.

2.6. Germination speed

Germination speed means over time germination rate variation as soon as radicle until germination becomes steady.It can be assessed by:

- Time to obtain 50% of germination.
- Speed coefficient (SC) as proposed by [13] with an average germination time (GT).

$SC = (N_1 + N_2 + N_3 + \ldots + N_n / N_1T_1 + N_2T_2 + N_3T_3 + \ldots + N_nT_n) \times 100$

$GT = N_1T_1 + N_2T_2 + N_3T_3 + \ldots + N_nT_n / N_1 + N_2 + N_3 + \ldots + N_n$

N_1: Number of seeds germinated at T_1 time

N_2: Number of seeds germinated at T_1 and T_2 time

N_3..... N_n: Number of seeds germinated from T_3 to T_n time

2.7. Taking and preparing plant material for biochemical analyses:

As germination is assessed (5 days after beginning treatment), seeds are wrapped one by one in aluminum paper, numbered and weighed to be dried at 80°C during 48 hours. Samples are then weighed again before being ground in a mortar. Powder is preserved in pill organizers closed hermetically and kept frozen until tests.

Fresh and dry weighs are recorded with an OHUS type precision scale.

2.8. Determining total sugar and phenolic compounds content:

Total sugar is carried out as described by [14]. Phenolic compounds are carried out as described by [15], the Prussian blue method.

3. RESULTS AND DISCUSSION

3.1. Germination precocity

Germination precocity is assessed by first germinated seeds rate corresponding to time interval between seedling and first germinated seeds. Table 1 records first germinated seeds variation rate according to different treatments.

Table 1: germination precocity of treated okra seeds.

	T	T+F1	T+F2	25 meq.l^{-1}	25meq.l^{-1}+F1	25meq.l^{-1}+F2	50meq.l^{-1}	50 meq.l^{-1}+ F1	50 meq.l^{-1}+F2
GP%	99.33±0	100±0	100±0	96.83±1	100±0	99.83±0.34	96.5±2.57	96±1.34	98±0.94

GP%: germination precocity

T: seeds treated with distilled water.

T+F$_1$, T+F$_2$: seeds treated with 10 µM and 20 µM fluridone.

25meq.l^{-1}, 50meq.l^{-1}: seeds stressed with 25meq.l^{-1} and 50meq.l^{-1} NaCl.

25meq.l^{-1} + F$_1$, 25meq.l^{-1} + F$_2$: seeds stressed with 25meq.l^{-1} NaCl plus 10µM, 20µM fluridone.

50meq.l^{-1}+F$_1$, 50meq.l^{-1}+F$_2$: seeds stressed with 50meq.l^{-1} NaCl plus 10µM, 20µM fluridone.

First germinated seeds response is similar in samples tested with 10µM and 20µM fluridone, germination starts the 1st day after seedling with a 100% estimated maximum rate, namely a 0.67% increase compared to control sample. On the other hand, 25 meq.l^{-1} salt treatment caused a notable germination decrease compared to control sample. 10µM and 20µM fluridone treatment seems to reduce salt delay effect assessed by respectively 100% and 99.83% estimated germination rates.

With a 50 meq.l^{-1} salt treatment either associated or non-associated to fluridone, we recorded a discernable germination rate decrease. With 50 meq.l^{-1} NaCl and 50 meq.l^{-1} NaCl +10µM fluridone treatment, first germinated seeds do appear from the very first day after seedling with respectively a 2.83% and a3.33% weak germination rate compared to control sample. Rate is improved when fluridone concentration equals to 20µM estimated to 98% the very first day after seedling.

3.2. Germination kinetics

Germination kinetics mostly represents evolution of germination percentages cumulated over to time (expressed in days).

Table2: Germination kinetics of treated okra seeds.

	T	T+F1	T+F2	25meq	25meq+F1	25meq+F2	50meq	50meq+ F1	50meq+F2
1st day	99.33±2.58	100±0	100±0	95.33±9.15	100±0	99.33±2.58	92.67±9.61**s**	94±7.37**s**	96.67±6.17
2nd day	99.33±2.58	100±0	100±0	97.33±5.94	100±0	100±0	97.33±5.94	96.67±4.88**s**	98±4.14
3rdday	99.33±2.58	100±0	100±0	97.33±5.94	100±0	100±0	98±4.14	96.67±4.88s	98.67±3.52
4thday	99.33±2.58	100±0	100±0	97.33±5.94	100±0	100±0	98±4.14	96.67±4.88s	98.67±3.52

S: significant effect compared to control sample

Table records cumulated germination rates of okra seeds with different treatments. Results assess that 10µM and 20 µM concentration treatments cause a germination evolution compared to control sample with a 100% estimated cumulated rate from the very first day after seedling, namely a 0.67% increase.

Seeds germination with 25 meq.l^{-1} NaCl treatment goes from 95.33% to 97.33%, namely a2 to4% difference compared to control sample and a 3.67% to 5.67% difference compared to seeds treated with 25 meq.l^{-1} NaCl associated to10µM and 20µM fluridone.

Seeds germination treated with 25meq.l^{-1} + F$_1$ and 25meq.l^{-1} + F$_2$ progresses over time. 25 meq.l^{-1} + F$_2$ treatment causes a 99.33% germination the 1st day and reaches a 100% from the 2nd day.

With 50 meq.l^{-1}treated seeds, germination starts the 1st day after seedling with a 92.67% rate, namely a7.06% decrease compared to control sample and 2.27% to 4.40% compared to 50 meq.l^{-1}+F$_1$and 50 meq.l^{-1}+F$_2$treatment respectively. Rate becomes steady with98%the 3rdday after seedling.

On the other hand, 50 meq.l^{-1}+F$_1$ treatment causes a slow growth compared to control sample and to other treatments (50 meq.l^{-1}, 50 meq.l^{-1}+F$_2$).Growth has a 94% cumulated rate from the first day after seedling, namely a 5.33% decrease compared to control sample. Growth becomes steady after the 2nd with a 96.67% cumulated rate. With 20µM fluridone concentration seeds germination rate immediately increases faster compared to 50 meq.l^{-1}+F$_1$ treatment with a 96.67% cumulated rate from the first day to a 98.67% cumulated rate the 3rd day.

Finally, it is notable that cumulated rate growth of germinated seeds treated with 50meq.l^{-1} either associated or non-associated to fluridone remains slower than control sample.

3.3. Germination speed

Germination speed is considered as being the time left between seedling and germination for seeds to germinate (Lang, 1965). For a better understanding of factors acting on okra seeds germination, we have adapted two simple formulas: speed coefficient (SC) and average germination time (GT) as proposed by Kotowski (1926).

3.3.1. Germination speed (speed coefficient (SC) and average germination time (GT)) :

Results (table 3) assess that 25 meq.l^{-1} salt treatment decreases germination speed and extends germination average time compared to control sample. Nevertheless, adding 25 meq salt solution with au 10µM fluridone increases again germination speed with a shorter germination average time. Then, germination speed of 25 meq+F_2 treated seeds gradually slows down with a longer germination average time compared to control sample and to 25meq+F_1 treated seeds.

Table3: germination speed (speed coefficient. average time) of okra treated seeds.

	T	T+F1	T+F2	25meq	25meq+F1	25meq+F2	50meq	50meq+ F1	50meq+F2
SC%	100±0	100±0	100±0	98.15±5	100±0	99.39±2.35	95.03±7.94 **s**	97.19±6.53	94.92±8.11**s**
GT day	1±0	1±0	1±0	1.02±0.06	1±0	1.01±0.03	1.06±0.1 **s**	1.04±0.08	1.03±0.06

S: significant effect compared to control sample

50 meq stress either associated or non-associated to fluridone induces a germination speed decrease and a longer germination average time compared to control sample.

3.4. Sugar and phenolic compounds rate of *Abelmoschus esculentus* L. seeds under salt stress either associated or non-associated to fluridone

Results shown in table 4 assess that sugar rates fluctuate almost in a similar way whatever the fluridone concentration treatment is (10 or 20 µM). We have indeed recorded respectively a 0.62% to 0.66% decrease compared to control sample. With 25 meq.l^{-1} salt treatment, we have recorded a 1.29% sugar rate decrease compared to control sample. As salt solution is added to fluridone, compounds content increases as fluridone concentration does. Indeed, sugar rate increase from 0.30% to 0.78% compared to 25 meq.l^{-1} treatment.

Table 4: Sugar rate (SR%) and phenolic compounds rate (PC%) in *Abelmoscus esculentum* L. seeds under NaCl and fluridone stress.

Lot	SR%	PC%
T	2.15±0.78	0.445±0.01
T+F1	1.49±0.2	0.42±0.01
T+F2	1.53±0.57	0.715±0.01
25meq NaCl	0.86±0.32 s	0.525±0.01
25meq+flu1	1.16±0.26 s	0.63±0.01
25meq+flu2	1.64±0.2	0.605±0.005
50meq NaCl	0.84±0.41 s	0.365±0.004
50meq+flu1	1.51±0.13	0.365±0.01
50meq+flu2	2.79±2.7	0.375±0.18

S: significant effect compared to control sample

Thus, 50 meq.l^{-1} salt treatment induces a notable 1.31% sugar rate decrease compared to control sample. As soon as salt solution is added with fluridone, sugar rate increases constantly to reach a 2.79% apex with a 50 meq.l^{-1} + F_2 treatment, namely being a 0.66% increase compared to control sample.

Phenolic compounds rates recorded with fluridone F_1 treated seeds are quite similar to control sample rates, namely a 0.025% slight decrease. On the other hand, F_2 concentration causes a 0.27% increase compared to control sample.

Indeed, a 0.08% and a 0.19% increase were respectively recorded with 25 meq.l^{-1} and 25 meq.l^{-1}+ F_1 treatment compared to control sample. Then, a slight decrease was recorded with 25 meql^{-1}+F_2 treatment, namely a 0.003% decrease compared to 25 meq.l^{-1}+F_1 treatment.

Phenolic compounds rates of seeds treated with 50 meq.l^{-1}and 50 meq.l^{-1}+F_1have an almost steady fluctuation. With50 meql^{-1}+F_2, a slight decrease of phenolic compounds is recorded, estimated from 0.08% to 0.09% compared to control sample.

Several studies assessed that new substances having herbicide proprieties such as fluridone contribute to breaking dormancy mechanism by inhibiting ABA.[16] confirmed such mechanism by assessing that fluridone easily breaks induced seeds dormancy. [17] works confirmed the same hypothesis in 2004, assessing that

fluridone has a very efficient function in deleting secondary dormancy development of seeds having a high potential dormancy (HPD) from *Brassica napus*plant species.

Moreover, according to [9] study on abscisic acid effectin controlling embryo development and germination, ABA settling is not ably delayed by spraying a fluridone solution onto young *Helianthus annuus* fruits.

4. CONCLUSION

In order to better understand resistance mechanisms of salt-stressed plants, our study is based on fluridone and/or salt influence on plant response.

To conclude our work, *Abelmoscus esculentus* L. seeds germination follow-up assessed that germination rate and speed fluctuate according to treatment processed. According to results, we can point out the following essential topics:

- With 10µM and 20µM fluridone tested samples, germination starts the 1st day after seedling, treatment inducing indeed a cumulated rate with a fast progression compared to control sample and estimated to 100%.
- Salt stress associated to 20µM fluridone seams to reduce salt delay effect helping okra seeds germination appearing from the first day.
- Speed germination, expressed in speed coefficient de (Cv) varies in a reverse way compared to salt concentration.
- Biochemical analysis of seeds sugar and phenolic compounds assesses a variation in compounds accumulation. Generally, a sugar accumulation apex is recorded when seeds are treated with 50 meq.l^{-1}salt solution added with fluridone F_2.
- With phenolic compounds, the highest rate is recorded when medium is added with fluridone F_2 solution.

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