Effect of salicylic acid on germination of *Ocimum gratissimum* seeds induced into dormancy by chlormequat

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Abstract— *The present work is to study the influence of salicylic acid in concentrations ranging from 0 to 1 mM on the germination of seeds of Ocimum gratissimum. This work is performed on non-dormant seeds and seeds induced into dormancy by chlormequat at a concentration of 550 mg / L. The study shows that salicylic seems to play an important role in acid stimulation of germination of non-dormant seeds that role varies with the concentration of salicylic acid: low concentrations lead to a small inhibition of germination whereas higher concentrations (greater than 0.5 mM) lead to stimulation of the germination. Furthermore, we found that the SA causes a dormancy dormant seeds. This dormancy is partial and slow at low concentrations (0.05 and 0.25 mM) and becomes complete when the concentration of SA increases. In this case, dormancy is very fast since the germination rate becomes important from the 3rd day.*

Keywords— chlormequat, dormancy, Ocimum gratissimum, salicylic acid.

I. INTRODUCTION

Germination, transitional stage between the dry seed stage and the emergence of the radicle[1] is possible when the physiological and environmental conditions are favorable (no primary dormancy, oxygen availability, adequate temperature ...). Dormancy can be defined as blocking the germination of intact and viable seed despite favorable environmental conditions[2].

Changes in these factors influence the levels of endogenous plant growth regulators (PGR)[3]. Many chemically substances can be used to break seed dormancy. It has been suggested that at an acid pH, substances like cyanide, nitrate, salicyl hydroxamic acid, and aliphatic monocarboxylic acid, are physiologically active as dormancy-breaking compounds[4 ,5]. The positive relationship between lipophilicity and dormancy-breaking capacity of compounds like alcohols, aldehydes, esters and ketones has been demonstrated [6].

It has also been suggested that dormancy is due to decreased oxygen diffusion to embryos, and activation of the oxidative pentose phosphate pathway has been proposed to be the main step leading to germination [7]. However, convincing evidence supporting this hypothesis is missing [8].

Abscisic acid (ABA) plays an important role in induction and maintenance of seed dormancy [9,10,11,12]. During germination, gibberellin (GA), induces embryo growth and, therefore, stimulates the germination process. At the beginning of germination, ABA and GA act in an antagonistic manner [13,7]. Removal of the effects of ABA is essential for GA to function during germination [12]. In barley seeds, It was previously proposed that dormancy is determined by at least three factors, synthesis of the plant hormone abscisic acid (ABA) following imbibitions, (n) the lack of breakdown and/or removal of ABA, and (m) changes in sensitivity to ABA [12]. If this hypothesis is correct, various chemically dissimilar compounds that break dormancy might interfere with at least one of the aforementioned factors.

Several authors have indicated that the salicylic acid which is an endogenous growth regulators;has a stimulatory effect on germination[14], and that this effect depends on its concentration [15,16].

Furthermore, chlormequat is a plant growth regulator (PGR) which is applied to change the physiological processes of the plant [17],in particular to induce germination inhibition [18].

The present work was to evaluate the effect of AS on the dormancy of seeds induced secondary dormancy by chlormequat taking as model the seeds of *Ocimum gratissimum.*

II. MATERIAL AND METHODS

Seeds of *Ocimum gratissimum* were disinfected with bleach and rinsed several times with distilled water.

A first test is performed by germinating in Petri dishes seeds of *Ocimum gratissimum* (50 seeds per dish) in the presence of salicylic acid at concentrations of 0; 0.05; 0.25; 0.5 and 1 mM. Germination is carried out in an incubator at 25 °C.

A second test is carried out on seeds induced in dormancy by a pre-soak in chlormequat at 550 mg/l. This concentration was chosen from tests on this species trying different concentrations of chlormequat 50, 100, 200, 300, 400, 550 mg/l. Dormant seeds are then allowed to germinate in Petri dishes (50 seeds per dish) in an incubator at 25 °C in the presence of salicylic acid (0, 0.05, 0.25, 0.5 and 1 mM).

3 repetitions are performed for each test.

Daily germination rate is determined by the percentage of germination expressed as the ratio of the number of germinated seeds on total number of tested seeds [19].

Germination rate = number of germinated seeds/total number of tested seeds×100

2.1 Statistical analysis

One-way analysis of variance was carried out for each parameter studied. Tukey's post hoc multiple mean comparison test was used to test for significant differences between treatments (at $p \leq 0.05$). Univariate analysis was used to test significant differences in treatments, and their interaction for an individual parameter. All statistical analyses were performed with IBM.SPSS statistics, Version 19.The results of each experiment were repeated three times.

III. RESULTS

3.1 Influence of salicylic acid on the seed germination rate of *Ocimum gratissimum*.

Figure 1 shows that the germination of seeds of *Ocimum gratissimum* starts from the 2nd day. The germination percentage increases regularly to 88.5% at 6th day. Furthermore, it is found that the application of the SA in the medium acts on the germination rate in function of the concentration and duration of treatment: at low concentrations (0.05 mM) a slight reduction in germination rate is observed and this reduction remained significant throughout the period of treatment. The concentration of 0.25 mM shows no significant differences from control. Contrariwise, beyond this concentration, there is an increase in germination rate from the 4th day to the concentration of 0.5 mM and from the 2nd day the concentration of 1 mM (72 against 62% for the control) and reach 98.6 and 100% on the 6th day.

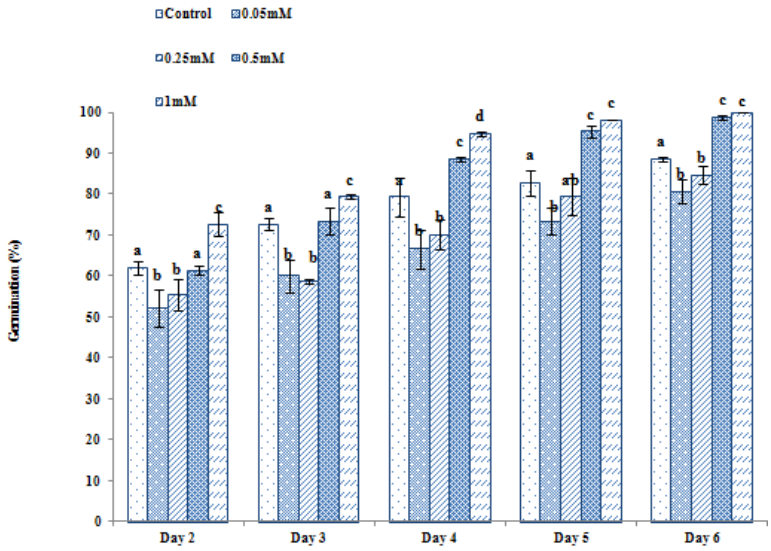

FIGURE 1: INFLUENCE OF SALICYLIC ACID ON THE SEED GERMINATION RATE OF *OCIMUM GRATISSIMUM*. THE VALUES INDICATED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P = 0.05)

3.2 Influence of salicylic acid on rate germination of *Ocimum gratissimum* seeds induced into dormancy by chlormequat.

Figure 2 shows that the germination of seeds of *Ocimum gratissimum* is completely inhibited by the application chlormequat to 550 mg / l. This inhibition is independent of the time since, after six days the germination rate remains zero.

The application of SA leads by against a breaking of dormancy of seeds and the rate depends on the concentration of SA. This rate is even higher than the concentration of SA and germination time increased; at low concentrations (0.05 to 0.25mM), there is a slight recovery in germination from the 2nd day. On day 4, a low concentration allows to restore significant germination rates compared to the control. High concentrations of SA (1 mM) also allow remedying significantly to the inhibitory effect of chlormequat from the 2nd day and the 6th day; the germination rate reached 90%.

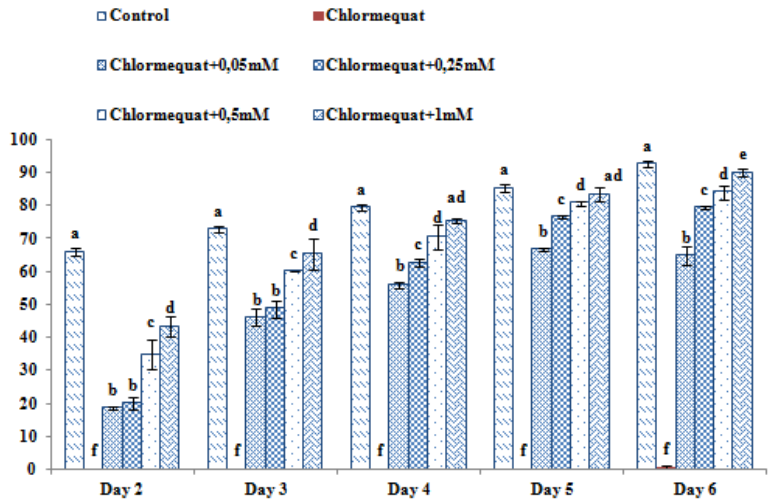

FIGURE 2: INFLUENCE OF SALICYLIC ACID ON RATE GERMINATION OF *OCIMUM GRATISSIMUM* SEEDS INDUCED INTO DORMANCY BY CHLORMEQUAT. THE VALUES INDICATED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P = 0.05)

IV. DISCUSSION

We found that chlormequat to 550mg / l causes the entry into secondary dormancy basil seeds. This inhibition is sustainable because after 6 days, the germination percentage remains zero. These results were confirmed by several studies in this area; [20] found that germination of seeds *Barbarea stricta* and *B. vulgaris* is inhibited by chlormequat to 20-200 mg / l and that these seeds remain dormant for over a year [21] showed that chlormequat causes a reduction in the percentage of germination of sorghum seeds [22] found that the application of Chlormequat to 6400 mg / 1 prevents the germination of seeds of *Brassica oleracea* L [23] also demonstrated that seeds of *Dioscorea batatas* Decne soaked in a solution of chlormequat to 1000 mg / l for 24 hours inhibited germination.

This inhibition of germination by chlormequat seems to be associated with inhibition of the synthesis of gibberellin. Indeed, gibberellic acid is a plant hormone generally involved in germination in many species [9]. It is recognized that chlormequat is considered an antagonist of gibberellin, a portion of the chlormequat action appears to be related to its inhibition of the biosynthesis of GA [24, 25]. It interferes with the early stages of biosynthesis of gibberellin mainly by blocking the activity of synthase ent-kaurene[26].

Furthermore, the influence of the salicylic acid on the germination of non-dormant seeds depends on its concentration. At low concentrations (up to 0.25 mM), there is a decrease in germination rate that lasted until the sixth day. At higher concentrations, there is a stimulation of germination in the early days mainly when the concentration reaches 1 mM. In this case, the germination rate goes from 62% to 72% in the 2nd day and from 88% to 100% on the 6th day. These results confirm those of [27] which showed that the salicylic acid effect depends on the applied dose [28] showed that the germination of seeds of some varieties of groundnut (W-55, TAG-11 and SB) is inhibited by low concentrations of SA, while higher concentrations permit contrariwise increased germination rate compared to the control.

[29] Showed that the seed germination *Simaru baglauca* DC is stimulated by the application of SA to 100ppm especially when these seeds are mechanically broken. Other studies also confirm our observations, [30] on tomato seeds, and[31] on the seeds melons, [16] on the cucumber seeds.

Several workers reported that stimulating effects of SA on germination are concentration dependent [32, 15,16]. SA significantly stimulated the activities of enzymes involved in germination such as transkelolase, enolase, malate dehydrogenase, phosphoglycerate kinase, glyceraldehyde 3-phosphate, dehydrogenase, fructose 1,6-diphosphatase, and pyruvate decarboxylase.

The SA stimulatory effect is also found on the basil seeds whose dormancy was induced by chlormequat. It is found that the germination rate is even higher than the concentration of SA and germination time increased. High concentrations of SA (1 mM) can remedy a major way to the inhibitory effect of chlormequat from the 2nd day and the 6th day, the germination rate reached 90%. These results confirm those of [32] which showed that the application of SA is effective in breaking dormancy *Simarou baglauca*. [33,34,14] showed that the barley seed germination rate increases with the application of SA [35] observed that the pretreatment of hybrid seeds of Alstro emeria with 100 mg / l SAallows breaking of dormancy.

Our results are also consistent with the conclusions of [36,37] in the gladiolus, [38] in tuberose.

This stimulation of the germination of dormant seeds appears to be associated with an increase of the synthesis of Gibberellins which is normally required to induce germination of seeds. This is confirmed by [39] have shown that treatment of bean dormant seeds by SA shows an improvement in germination percentage, and content in indole acetic acid (IAA) and GA increases in response to the action of SA [40] found that the effect of SA can be attributed to the effect of this acid in reducing the levels of ABA in the seed, ABA being the main endogenous factor dormancy of seeds. They reported that SA at 150 ppm can stop the dormant bulbs with a high percentage of germination. Furthermore, [41] attribute the effect of salicylic acid to the increased production of H2O2 which is a favorable factor in improving the germination of dormant seeds. In the same way [15], hypothesized that detoxification mechanism in germinating seeds counteract by exogenous SA treatments.

V. CONCLUSION

According to this work, we can conclude that the chlormequat affects negatively the germination of *Ocimum gratissimum* seeds, but the application of increasing concentrations of SA on these dormant seeds stimulates germination. We have also shown that the application of high concentrations (0.5-1mm) of SA on non-dormant seeds, stimulate the germination.

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