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# Omethoate treatment mitigates high salt stress inhibited maize seed germination

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ABSTRACT

Omethoate (OM) is a highly toxic organophosphate insecticide, which is resistant to biodegradation in the environment and is widely used for pest control in agriculture. The effect of OM on maize seed germination was evaluated under salt stress. Salt (800 mM) greatly reduced germination of maize seed and this could be reversed by OM. Additionally, $H_2O_2$ treatment further improved the effect of OM on seed germination. Higher $H_2O_2$ content was measured in OM treated seed compared to those with salt stress alone. Dimethylthiourea (DTMU), a specific scavenger of reactive oxygen species (ROS), inhibited the effect of OM on seed germination, as did IMZ (imidazole), an inhibitor of NADPH oxidase. Abscisic acid (ABA) inhibited the effect of OM on seed germination, whereas fluridone, a specific inhibitor of ABA biosynthesis, enhanced the effect of OM. Taken together, these findings suggest a role of ROS and ABA in the promotion of maize seed germination by OM under salt stress.

## 1. Introduction

Natural soil forming processes in dry and warm regions always produce saline soils with low agricultural potential. > 800 million hectares of salt-affected land are recorded throughout the world [1]. Salinity is the major environmental factor limiting plant growth and crop yield. In addition, it is also known to decrease seed germination, reduce seedling growth and also affect other metabolic processes [2,3].

Omethoate or OM, an organophosphate pesticide, is widely used to control pests and to increase harvest productivity in many developing countries due to its low price and high efficiency [4]. In China, high dosage of OM (2–8 mM) is commonly used by farmers. Thus, high residual of this pesticide can be expected due to it's inevitable spray onto the soil. In addition, OM has long persistence in the soil. Because of such abundant usage and the potential for environmental transport, OM contamination can be detected throughout the environment [5].

Environmental stress (including salinity and chemical pollution) can cause oxidative damage in plants and lead to ROS production. In general, ROS are mainly produced in the chloroplasts, mitochondria, peroxisome and by certain flavin-containing enzymes such as NADPH oxidase [6]. However, the level of ROS can be tightly controlled by enzymatic and non-enzymatic antioxidants in plants [7].

ROS play a dual role in seed germination: to activate cellular signaling pathways or lead to oxidative damage under stress conditions [3,8]. In general, low levels of ROS can favor seed germination or act as a positive signal for seed dormancy release [8]. For example, inhibition of catalase (a specific scavenger of $H_2O_2$) activity can promote the dormancy release of lettuce and pigweed seeds [9]. However, ROS overproduction can arrest seed germination [3,8] and accelerate seed ageing [10].

Seed germination can be profoundly affected by abiotic stress such as salt, heavy metal and chemical pollutants. Numerous studies report the effect of single stress on seed germination [3,11,12]. However, seed germination, as well as plant growth, would always encounter multiple stresses in the field [13]. For example, salt stress and chemical pesticide toxicity can be found simultaneously in the farmland.

Plants have various detoxification systems to cope with the phytotoxicity of the wide variety of natural and synthetic chemicals — xenobiotics — present in the environment [14,15]. The toxicity and mechanisms of xenobiotics (including OM) on plant and animal have been demonstrated previously [16,17]. Reports show that OM can affect soil rhizobia when seed pre-treated with this pesticide [18]. However, there is little information about OM effects on seed germination under favorable or adverse conditions.

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The aim of this study was to investigate seed germination under combination stresses of chemical and salt and the possible mechanisms. This work will help to better understand the positive roles of OM on seed germination under salt stress conditions.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All chemical reagents used in this work were of analytical grade. ABA and IMZ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were purchased from a biochemical reagent Co., Ltd. in Harbin city of China.

### 2.2. Seed treatment

The maize seeds *jingxiangtiannuo* (*Zea mays* L.) were obtained from a seed distributor in Daqing city of China and used for these studies. These seeds were sown in plastic boxes and placed in a seed germinator (LRH-250-A, made in Guangdong Medical Instruments Factory, China) at 25 ± 1°C for the entire germination period. Germination trials were conducted in plastic boxes equipped with 2-cm deep sand (diameter about 0.5 mm) moistened with distilled water. The seeds were placed on the sand moistened with 80 or 800 mM NaCl for salt stress. Before the stress treatment, the seeds were moistened with distilled water for 120 mins. After treatment, the seeds were transferred to plastic boxes.

This experiment consisted of six treatment groups. For group 1, three concentrations of NaCl (0, 80 and 800 mM) were applied to these maize seeds. For group 2, three concentrations of OM (0, 2 and 10 mM) were applied to these maize seeds. For group 3, two concentrations of OM (2 and 10 mM) was applied to these seeds under high salt stress (800 mM). For group 4, $H_2O_2$ (10 mM) was applied to the OM (10 mM)-treated seeds under high salt stress (800 mM). For group 5, DMTU (20 mM) or IMZ (5 mM) were applied to the OM (10 mM)-treated seeds under high salt stress (800 mM). For group 6, ABA (0.5 mM) or fluridone (1 mM) were applied to the OM (10 mM)-treated seeds under salt stress (800 mM).

Approximately 1 mL solution was used during each spraying treatment in this experiment (sprayed 3 times after sowing at 0, 6 and 12 h). All assays were replicated at least five times; each replicate was carried out on 50 seeds.

### 2.3. Seed germination assay

When the radicle emergence of seeds exceeded 1 mm, they were considered to have germinated. The number of germinated seeds was counted three times per day, for the time necessary to achieve the final percentage of germinated seeds. Germination rates (GR) were calculated as the percentage of germinated seeds after sowing for different hours.

### 2.4. $H_2O_2$ extraction and assay

The $H_2O_2$ extraction and assay were performed with the methods of Gay et al. [19] with some modification. Here, the treated seeds (water control, salt and OM treatment) were collected after sowing for 2, 10 and 18 h for the $H_2O_2$ assay. All germinating seeds were collected and immersed in acetone to terminate germination. The collected seeds were weighed and immediately frozen in liquid $N_2$. Samples (~1 g FW) were ground to a powder in liquid $N_2$ using a mortar and pestle. The ground seed was homogenized with 10 mL of 5% TCA for $H_2O_2$ extraction. After centrifugation at 10,000*g* for 30 min (25 °C), the supernatants were collected. 1 mL of assay reagent (25 mM $FeSO_4$ and 25 mM $(NH_4)_2SO_4$, dissolved in 2.5 M $H_2SO_4$) was added to 100 mL of 125 μM xylenol orange and 100 mM sorbitol. The supernatant (100 μL) was added to 1 mL of xylenol orange reagent. After 30 min of incubation, absorbance by the $Fe^{3+}$–xylenol orange complex was recorded at 560 nm.

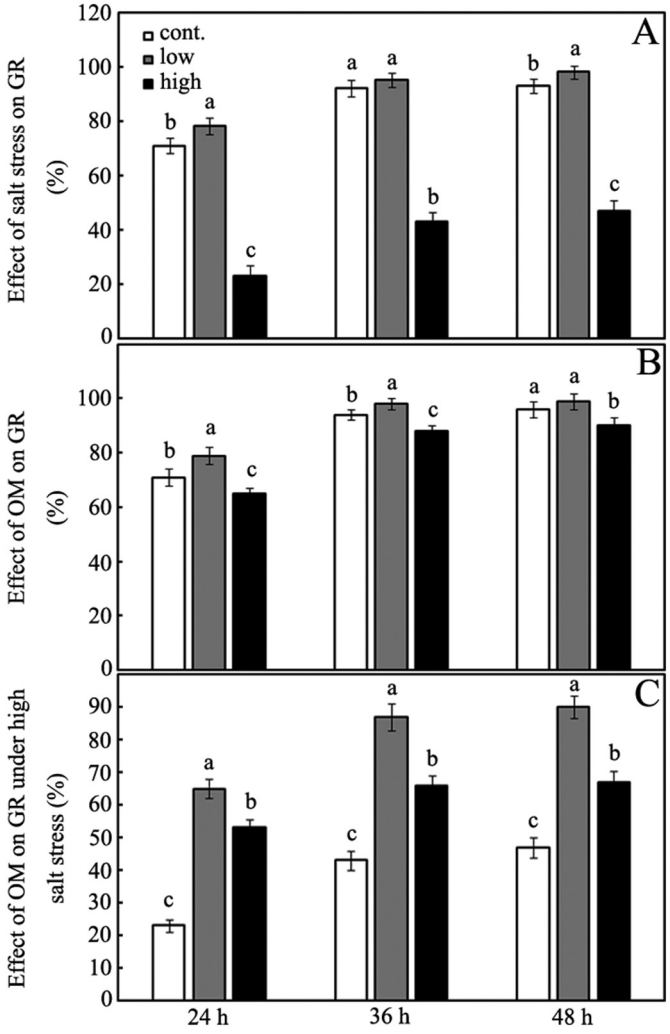


**Fig. 1.** Effect of salt and OM stress on GR.
Effect of low and high salt stress (A), OM stress (B) and OM combined with high salt stress (C) on GR of maize seeds was measured during the first 48 h after sowing. Bars represent standard deviations of the means ($n = 5$). Different letters indicate significant differences among treatments at $P < 0.05$ in GR at same timepoint. GR, germination rate; OM, omethoate.

### 2.5. Statistical analysis

Statistical analyses were performed using SPSS 13.0 software. Differences among groups of means were examined using one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$).

## 3. Results

### 3.1. Effect of salt, OM and OM + salt on GR

The effect of salt (80 and 800 mM NaCl), OM and salt + OM stresses on the GR of maize seed was determined during the first 48 h after imbibition (Fig. 1). Compared with the water control, high salt stress (800 mM NaCl) significantly reduced GR (Fig. 1A). Approximately, 71%, 92% and 93% GR were recorded in the water control group after imbibition for 24, 36 and 48 h, respectively. However, approximately 23%, 43% and 47% GR was observed in the high salt treated seeds but 78%, 95% and 98% GR in the low salt treated seeds after imbibition for