PHOTOSYNTHETIC PERFORMANCE OF MAIZE PLANTS UNDER SALINITY AS AFFECTED BY LEAF H_2O_2 PRIMING[1]

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ABSTRACT: Priming with hydrogen peroxide (H_2O_2) has emerged as an important strategy to activate multiple acclamatory responses that reinforce resistance to various environmental stresses, including salt stress. This study aimed to investigate the role of H_2O_2 priming in photosynthetic efficiency of maize plants under salinity. Ten-day old *Zea mays* plants (BR 5011, a salt-sensitive genotype) were primed with H_2O_2 at 0 (negative control) and 10 mM (priming), and then subjected to salt stress with NaCl at 0 (control) and 80 mM (salt treatment). Experiments were carried in a randomized completely design, in a 2 x 2 factorial scheme, consisting of two growth conditions (absence or presence of NaCl-stress) and two priming treatments (not-primed or primed with H_2O_2), with five repetitions. The results suggest that H_2O_2-primed alleviates salt damage effects on photosynthetic efficiency by improving in CO_2 assimilation, effective photosystem II efficiency and electron transport rate, as well as the photosynthetic pigments accumulation.

KEYWORDS: Acclimation, salt stress, hydrogen peroxide, *Zea mays*

DESEMPENHO FOTOSSINTÉTICO DE PLANTAS DE MILHO PRÉ-TRATADAS COM H_2O_2 E SUBMETIDAS À SALINIDADE

RESUMO: O pré-tratamento com H_2O_2 tem se mostrado uma importante estratégia para ativar múltiplas respostas de aclimatação, que atuam na tolerância à vários estresses ambientais, incluindo o estresse salino. O presente estudo teve como objetivo averiguar o papel do pré-tratamento com H_2O_2 na eficiência fotossintética de plantas de milho sob salinidade. Plantas de milho (BR 5011, genótipo sensível ao sal) foram pré-tratadas com H_2O_2 a 0 (controle negativo) e 10 mM (pré-tratamento) e, em seguida, submetidas ao estresse salino com NaCl a 0 (controle)

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e 80 mM (tratamento salino). Os experimentos foram realizados em um delineamento inteiramente casualizado, com arranjo fatorial 2 x 2, consistindo de duas condições de crescimento (ausência ou presença de estresse salino) e duas condições de pré-tratamento com H_2O_2 (ausência ou presença do pré-tratamento com H_2O_2), com cinco repetições. Os resultados obtidos sugerem que o pré-tratamento com H_2O_2 reduz os efeitos negativos da salinidade na eficiência fotossintética através de aumentos na assimilação de CO_2, na eficiência efetiva do PSII e na taxa de transporte de elétrons, bem como no acúmulo de pigmentos fotossintéticos.

PALAVRAS-CHAVE: Aclimatação, estresse salino, peróxido de hidrogênio, *Zea mays*

INTRODUCTION

Salinity is a major abiotic stress restricting crop yield by impairing physiological, biochemical and molecular processes, especially photosynthesis (Gorai & Neffati 2007; Saleem *et al*. 2011; Shavrukov, 2013), which is the most important physiological process related to crop productivity (Najafpour & Pashaei, 2012). The salt harmful effects on photosynthesis, in parallel with the reductions in leaf growth (Munns *et al*. 2006; Nebauer, 2013), can be caused directly by changes in the photosynthetic apparatus, reducing the content of photosynthetic pigments, electron transport in chloroplasts and, consequently, causing decreases in the efficiency of photosystem II (Chaves *et al*., 2011; Yan *et al*., 2012; Huang *et al*., 2014).

To survive under saline conditions, plants depend on their ability to acclimate to such adverse conditions. Acclimatization is a process that involve temporary regulations in plant metabolism (physiological, biochemical and morphological), allowing plant to acquire greater capacity to develop under stress, in comparison to those that are not acclimated. Recently, an important tool to induce the acclimatization to numerous abiotic stresses is the priming with organic, inorganic or growth regulating compounds, which may be applied in the growth medium of roots or by spraying in leaves (Ashraf *et al*., 2008). Some examples of acclimatization inducers are signaling molecules like hydrogen peroxide (H_2O_2) and nitric oxide (NO) (Khan *et al*., 2012; Filippou *et al*., 2013). Some studies have shown that pretreatment with appropriate H_2O_2 concentrations can confer tolerance to environmental stresses by modulating various physiological processes, such as photosynthesis, as well as multiple stress-responsive pathways, including the detoxification pathways of reactive oxygen species (ROS) (Azevedo- Neto *et al*., 2005; Chao *et al*., 2009; Liu *et al*., 2010; Wang *et al*., 2010; Gondim *et al*., 2013).

Given the economic importance of maize and its relative sensitivity to salinity, the studies regarding of H_2O_2 priming in inducing salt stress acclimation become strikingly relevant. Therefore, this study aimed to investigate the role of H_2O_2 priming on modulation of photosynthetic performance of maize plants subjected to NaCl-stress.

MATERIAL AND METHODS

Maize seeds (*Zea mays* L.), genotype 5011, were surface-sterilized in 2 % sodium hypochlorite for 5 min and then sown in vermiculite moistened with distilled water. Seven days after sowning, seedlings were transferred to a hydroponic system containing half-strength Hoagland's nutrient solution during five days, for an acclimation period. After, the pre-treatment with H_2O_2 began, which was verified by foliar spraying with distilled water (control) or 10 mM H_2O_2 solution (both added with 0.025% Tween 20 in order to break the water tension on leaf surface and facilitate H_2O_2 penetration). After 48 h of start pretreatment the addition of NaCl was started, which was done in dose of 40 mM until reaching the concentration of 80 mM to avoid osmotic shock. In each group of pretreated plants, half of them remained in the absence of NaCl, thus constituting the control treatment (NaCl at 0 mM). All nutrient solutions were renewed every 5 days and the plants were harvested twelve days after the last salt dosage. The experiment was carried out under greenhouse conditions with midday photosynthetic photon flux density (PPFD) at approximately 1.300 μmol m^{-2} s^{-1}, main temperature of 32.3 ± 6 ° C, and main relative humidity of 44.8 ± 10.9 %.

Rates of CO_2 assimilation (*A*), transpiration (*E*), stomatal conductance (g_s) and Rubisco carboxylation efficiency (*A*/Ci) were measured employing a portable photosynthetic system (LI-6400 XT, Li-Cor, Lincoln, NE, USA) coupled with a leaf chamber fluorometer (6400-40, Li-Cor, USA). The parameters of maximum quantum yield of PSII in dark-adapted leaves [Fv/Fm = (Fm - Fo)/Fm], effective quantum yield of PSII [ΦPSII = (Fm' - Fs)/Fm'], electron transport rate [ETR = (ΦPSII x PPFD x 0.5 x 0.84)] and photochemical [qP = (Fm' - Fs)/(Fm' - Fo')] and non-photochemical [NPQ = (Fm - Fm')/Fm'] quenching were also determined. To calculate the ETR, 0.5 was used as fraction of the excitation energy distributed to PSII, whereas 0.84 was used as fraction of incoming light absorbed by the leaves. All of the measurements were done in the third fully expanded leaves between 10:00 and 11:00 a.m. Twelve days after salt treatment, five plants from control and salt stressed genotypes were individually harvested and separated in shoots (leaves + stems) and roots. The leaf area (LA) was measured using an area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Subsequently, fresh plant material was

dried by lyophilization and the dry mass of shoots (DMS), roots (DMR) and total (DMT) was determined.

Photosynthetic pigments were extracted from fresh leaves after incubating in $CaCO_3$ saturated in dimethyl sulfoxide (DMSO), during 48 h at room temperature. Contents of chlorophyll *a*, *b* and *total*, and carotenoids were spectrophotometrically measured trough reading at A480, A649 and A665. The concentrations were calculated using equations based on the specific absorption coefficients as reported by Arnon (1949).

Experimental design was completely randomized following a 2 × 2 factorial scheme, composed of two growth conditions [nutrient solution with (salt stress) and without NaCl (control)] and two priming treatments (not-primed or primed with H_2O_2). All analyses were performed using five repetitions per treatment, it being an individual plant considered one repetition. The data were submitted to two-way analysis of variance (ANOVA) and the main values were compared trough Tukey's test ($P \leq 0.05$) using Sisvar® 5.3 program.

RESULTS AND DISCUSSION

Under control conditions, leaf area (LA) and root fresh mass (RFM) did not change by H_2O_2 priming, whereas the shoot fresh mass (SFM) and total fresh mass (TFM) were reduced by 64 and 53%, respectively, as compared to control (Fig. 1). In general, salt stress significantly reduced the growth of maize plants, irrespective of priming treatments; however, the H_2O_2 priming was effective in minimizing the negative effect of saline stress (Fig 1). The leaf area was decreased by 41% in non-primed plants, while it was reduced by only 29% in H_2O_2-primed plants (Fig 1a). Similarly, fresh mass of maize plants was reduced by salinity, except for roots (Fig 1c); nevertheless, under salinity, H_2O_2-primed plants displayed values of SFM and TFM 25 and 19%, respectively, higher than those of non-primed plants (Fig 1b e d).

Evidence that H_2O_2 priming may act as important inducer of salinity tolerance is increasingly concrete. Gondim *et al*. (2013), in studies with exogenous application of H_2O_2 in maize (*Zea mays* L.) plants under salinity, observed that photosynthesis rates, transpiration, stomatal conductance and internal CO_2 concentration were reduced in plants under salinity, but the salt harmful effects were less aggressive in plants from H_2O_2-primin treatments. Furthermore, in the same study, plants sprayed with H_2O_2 had the highest relative water and chlorophyll content, and low foliar H_2O_2 accumulation, which correlated positively with the improvement in gas exchanges compared to control plants. Recently, Ashfaque *et al*. (2014) conducted an experiment to study the role of H_2O_2 in mitigating the effects of salt stress on

wheat plants (*Triticum aestivum* L.). Therein, the authors observed that the primed with H_2O_2 positively influenced on the growth under saline conditions, reducing the accumulation of toxic Na^+ and Cl- ions and increasing the assimilation of nitrogen.

Simultaneous measurements of chlorophyll *a* fluorescence and gas exchange allow a better understanding of salt effects on the photosynthetic apparatus and have been very useful in the selection of plants more tolerant to adverse environmental conditions (Baker *et al*., 2008; Razavi *et al*., 2008; Lima Neto *et al*., 2014). In our study, in absence of NaCl, maize plants showed similar behavior even when submitted to pretreatment with H_2O_2. Yet, salinity significantly reduced all of the gas exchange parameters in plants from studied salt treatments. However, under salt stress, H_2O_2-primed plants displayed values of CO_2 assimilation (*A*), transpiration (*E*) and carboxylation efficiency of Rubsico (*A*/Ci) found to be 20, 25 and 20%, respectively, higher than the ones of NaCl-stressed plants only (Fig. 2a, b e d).

Chlorophyll a fluorescence parameters may estimate the efficiency of photosystem II of photosynthetic machinery. Herein, under salt conditions, although maximum photochemical efficiency of photosystem II (Fv/Fm) was significantly increased by H_2O_2 priming, the effective photochemical efficiency of photosystem II (ϕPSII) did not suffer any significant alteration. In NaCl absence, there was no effect of H_2O_2 priming on the non-photochemical quenching (NPQ), but it improved the photochemical quenching (qP) of energy dissipation. On the other hand, when exposed to salinity and primed with H_2O_2, maize plants showed a 60 and 12% increase in NPQ and electron transport rate (ETR), respectively, as compared to non-primed stressed plants (Table 1). Our data suggest the H_2O_2 priming enhances photochemical efficiency of maize plants under salt stress, which result in an improved photosynthetic performance. Similar results were observed by Uchida *et al*. (2002) in rice (*Oryza sativa*), their reports showed that seedlings treated with low concentrations of H_2O_2 resulted in greener leaves and a higher photosynthetic activity than that of the control plants under conditions of salt. Similarly, Wahidetal *et al*. (2007) reported that exogenous H_2O_2 improved salinity tolerance in *Triticuma estivum*, for also exhibited better photosynthetic capacity.

Several studies have shown that reductions in photosynthetic pigments by salinity impair severely the photosynthetic efficiency of numerous plant species (Sudhir & Murthy, 2004; Miranda *et al*., 2013). In this study, maize plants submitted to salinity had their chlorophyll *b* and *total* contents reduced, however, when plants were submitted to H_2O_2-primed, these variables increased 64 and 31% (Fig. 3b and c). Interestingly, H_2O_2-primed plants displayed reduced Chl *a* contents, but no significant alterations were observed in carotenoids concentrations (Fig. 3a e c).

CONCLUSION

The H_2O_2 priming improves photosynthetic performance of maize plants under salinity by increasing photochemical efficiency of PSII and photosynthetic pigments. Our findings suggest that the priming with H_2O_2 alleviates the negative effects of salinity and may be used as a potent tool to mitigate the losses of productivity of maize plants cultivated in saline areas.

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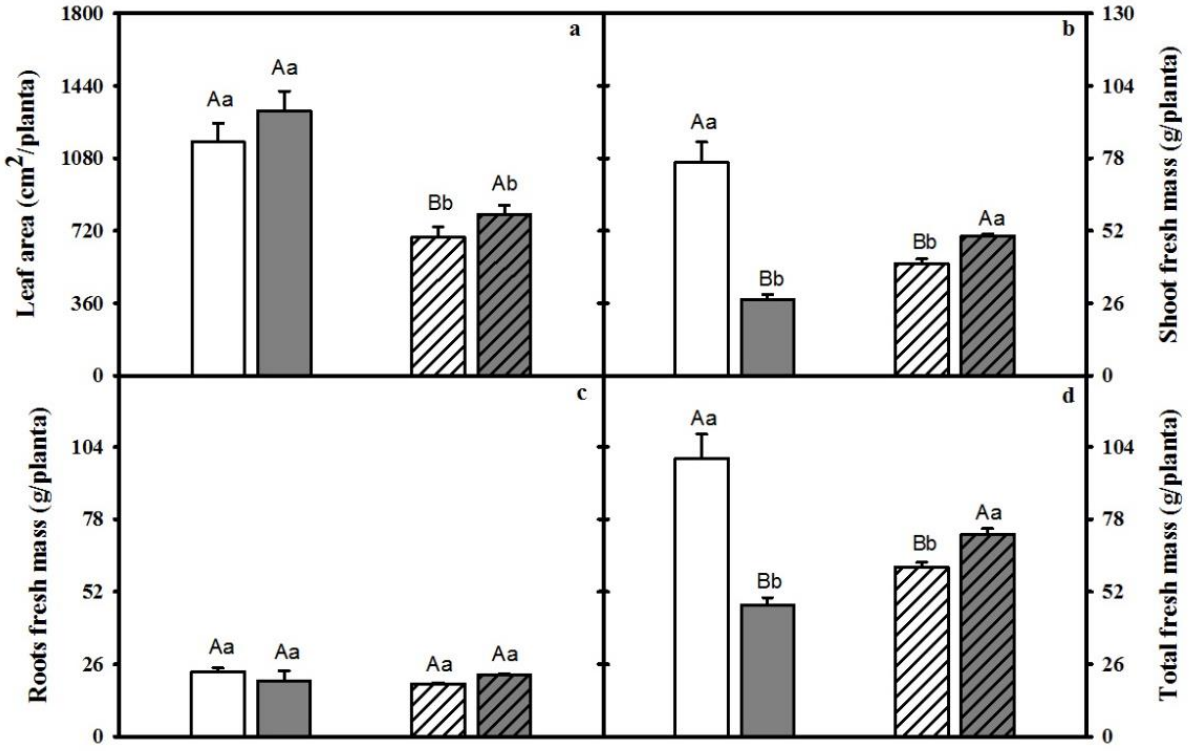

Figure 1. Leaf area (**a**), Shoot fresh mass (**b**), roots (**c**) and total(**d**) of maize plants, genotype 5011, grown in control conditions (*open square*), H_2O_2-primed (*filled square*), presence of 80 mM NaCl-stress (*open and hatched square*) and H_2O_2-primed under salinity (*filled and hatched square*). Measurements were done twelve days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same H_2O_2 pre-treatment, or by equal capital letters in the same salinity level, are not statistically different according to Tukey's test ($p \geq 0.05$).

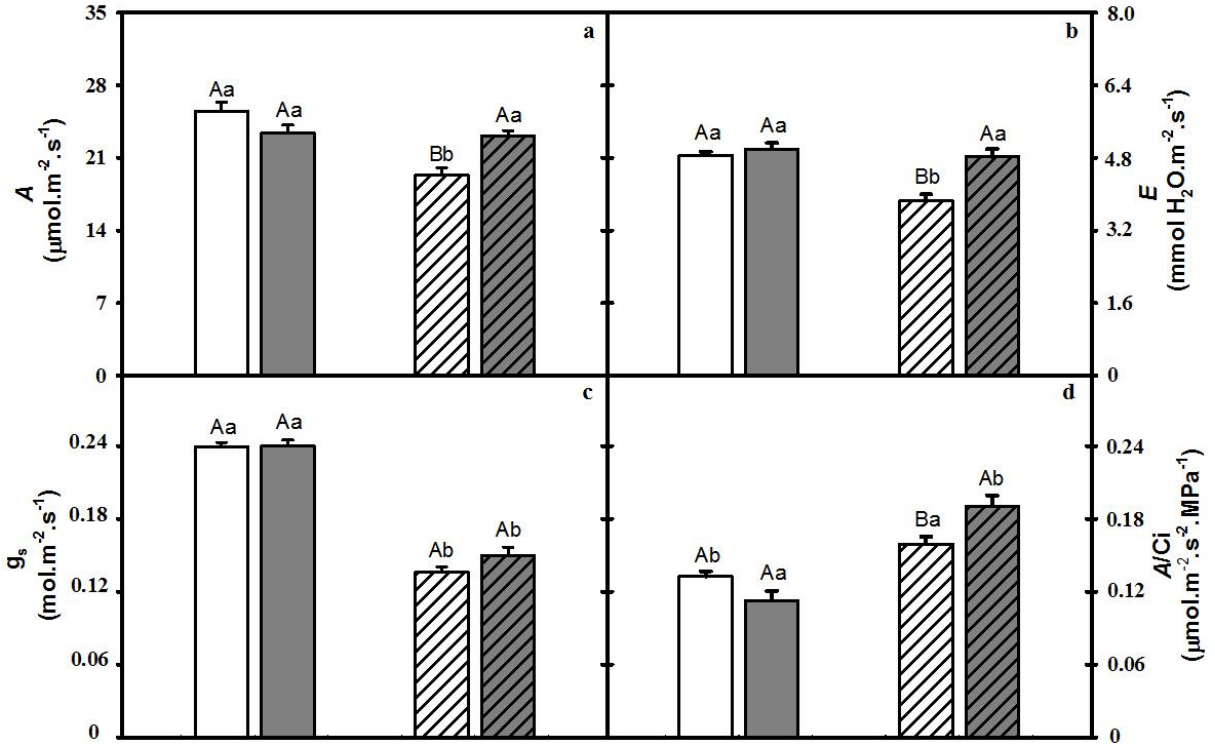

Figure 2. Rates of CO_2 assimilation (*A*, **a**), transpiration (*E*, **b**), stomatal conductance (g_s, **c**) and Rubisco carboxylation efficiency (*A*/Ci, **d**) of maize plants, genotype 5011, grown in control conditions (*open square*), H_2O_2-primed (*filled square*), presence of 80 mM NaCl-stress (*open and hatched square*) and H_2O_2-primed under salinity (*filled and hatched square*). Measurements were done twelve days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same H_2O_2 pre-treatment, or by equal capital letters in the same salinity level, are not statistically different according to Tukey's test ($p \geq 0.05$).

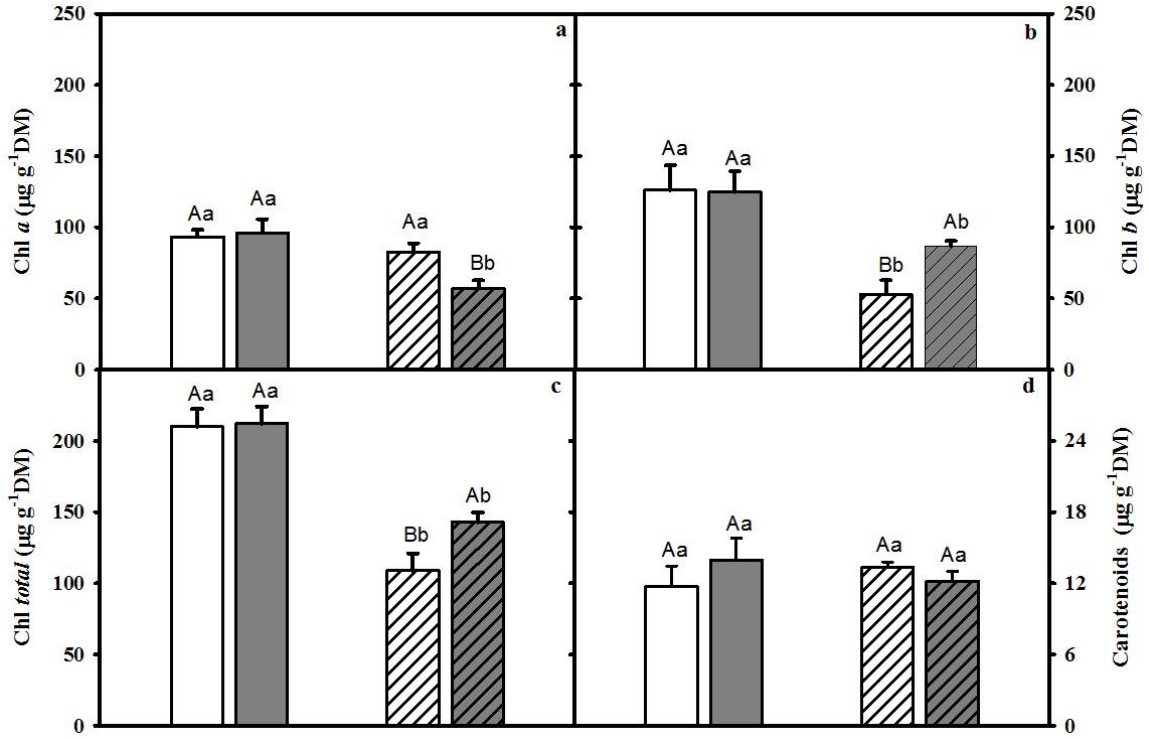

Figure 3. Contents of chlorophyll *a* (Chl *a*, **a**), *b* (Chl *b*, **b**) and *total* (Chl total, **c**) and carotenoids (**d**) of maize plants, genotype 5011, grown in control conditions (*open square*), H_2O_2-primed (*filled square*), presence of 80 mM NaCl-stress (*open and hatched square*) and H_2O_2-primed under salinity (*filled and hatched square*). Measurements were done twelve days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same H_2O_2 pre-treatment, or by equal capital letters in the same salinity level, are not statistically different according to Tukey's test ($p \geq 0.05$).

Table 1. Maximum quantum yield of PSII (Fv/Fm), effective quantum yield of PSII (ΦPSII), electron transport rate (ETR), photochemical (qP) and non-photochemical (NPQ) quenching of maize plants, genotype 5011, grown in control conditions (*open square*), H_2O_2-primed (*filled square*), presence of 80 mM NaCl-stress (*open and hatched square*) and H_2O_2-primed under salinity (*filled and hatched square*). Measurements were done twelve days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same H_2O_2 pre-treatment, or by equal capital letters in the same salinity level, are not statistically different according to Tukey's test ($p \geq 0.05$).

Salt Treatment	Photochemical Parameters				
	Φ PSII	**Fv/Fm**	**NPQ**	**qP**	**ETR**
Control	0,34±0,01Ba	0,61±0,01 Aa	0,55±0,08 Ab	0,56±0,03 Bb	192,74±9,07 Aa
H_2O_2	0,39±0,01Aa	0,58±0,01 Aa	0,70±0,02 Ab	0,67±0,01 Aa	204,44±3,31 Aa
80 mM NaCl	0,36±0,00Aa	0,50±0,01 Bb	1,18±0,03 Ba	0,71±0,02 Aa	182,63±2,52 Ba
H_2O_2 + NaCl	0,36±0,02Aa	0,54±0,01 Ab	1,90±0,08 Aa	0,67±0,03 Aa	200,70±3,08 Aa