ENZYMATIC ACTIVITY ON QUINOA LEAVES (*CHENOPODIUM QUINOA,* WILLD.), UNDER ABIOTIC STRESS

ACTIVITATEA ENZIMATICĂ DIN FRUNZELE DE QUINOA (*CHENOPODIUM QUINOA*, WILLD.), SUB INFLUENŢA STRESULUI ABIOTIC

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Abstract.
Quinoa (Chenopodium quinoa sp.), is a species known throughout the world, native to Latin America, and is increasingly appreciated in many parts of the world. The aim of the research was to evaluate fertigation and irrigation on the enzyme activity in the leaves of two cultivars of quinoa (Vikinga and Puno), for its introduction as vegetable crop. The experiment was carried out in the IULS Iasi greenhouse, in vegetative pots, of 42 variants, the experiment was organized in randomized blocks. The results obtained, under the influence of the factors: cultivar, fertilization and irrigation, show that the most intense enzymatic activity was evidenced in Vikinga cultivar - chemically fertilized variant, with irrigation 50% of the substrate field capacity. The results are positively correlated with the increase in the amount of chemical fertilizer administered. The most evident decreases in CAT activity were evidenced in the experimental variants to which Micoseed MB® was applied, in both cultivars.
Key words: quinoa, fertilization, irrigation, enzyme activity.

Rezumat.
Quinoa (Chenopodium quinoa sp.), este o specie cunoscută pe întreg mapamondul, originară din America Latină, fiind tot mai apreciată în mai toate zonele lumii. Studiile și cercetările proprii ne-au arătat că anumite populații pot fi utilizate ca legume pentru frunze. Scopul cercetării a avut in vedere evaluarea ferilizării și irigării asupra activității enzimatice în frunzele a două cultivare de quinoa (Vikinga și Puno), în vederea introducerii acesteia în cultură, ca plantă legumicolă. Experiența a fost organizată în sera USV Iasi, în vase de vegetație, în 42 de variante, experiența fiind organizată în blocuri randomizate. Rezultatele obținute, sub influența factorilor: cultivar, fertilizare și irigare arată că activitatea enzimatică cea mai intensă s-a evidențiat la cultivarul Vikinga- varianta fertilizată chimic, cu irigare 50 % din capacitatea de câmp a substratului. Rezulattele sunt pozitiv corelate cu creșterea cantitații de îngrăsămant chimic administrat. Cele mai evidente scăderi ale activității CAT au fost

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evidențiate în variantele experimentale la care s-a aplicat Micoseed MB®, la ambele cultivare.

Cuvinte cheie: quinoa, fertilizare, irigare, activitate enzimatică.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is considered a pseudo-cereal, native from Latin America. The species still has a strong traditional imprint, even if new modern practices appear, due to studies conducted at the University of Colorado (USA) and in Europe [Pedersen *at al.*, 2015; Mujica, 2001].

The cultivation of the species was largely abandoned with the arrival of the Spanish conquerors, who replaced the quinoa plant with cereals brought from Europe (wheat and barley), much more productive at that time. The quinoa plant is currently grown throughout the Andean region, in the USA, in Europe, Asia and Africa [Bazile *et al.*, 2016; Mazoyer *et al.*, 2006]. Quinoa is a plant grown mainly for its edible seeds, with a high degree of digestibility [Asao et al., 2010]. Also, the leaves can be eaten as a substitute for spinach, in various dishes, well known in the area of origin [Stoleru *et al.*, 2022 B; Vitanescu, 2020].

The nutritional value of quinoa leaves is special, quinoa is a very interesting food, being a precious source of protein, vitamins and minerals [FAO, 1992]. According to the Food and Agriculture Organization of the United Nations (FAO), quinoa can assure the global food security due to its high nutritional qualities as well as tolerance to various abiotic stresses including salinity [FAO, 2013].

Due to the fact that it can be grown in the fields, as well as in tunnels and greenhouses, quinoa can ensure also a sustainable production throughout the year [Stoleru *et al.*, 2022 A; Pedereson *et al.*, 2020].

In this respect, the efforts of our researcher is mainly focused on the following research direction, namely: evaluation of enzymatic activity on quinoa leaves against abiotic stressors - drought and fertilization.

One of the most important environmental factors affecting the success of plant growth and productivity is the amount of water available during the growing season of plants [Shahnaz *et al.*, 2022]. Reduced water availability in plants induces drought stress, which causes numerous morphological and physiological changes [Seleiman *et al.*, 2021]. This natural phenomenon alters chlorophyll content and components, inhibits plant photosynthesis and harms the development of photosynthetic apparatus [Nayyar and Gupta, 2006].

Water stress affects all metabolic processes by impairing enzyme activity [Esfandiari *et al.*, 2008].

Application of biofertilizers under water stress conditions increases production, chlorophyll content and antioxidant enzyme activity. Plants are believed to apply defensive mechanisms such as synthesis of antioxidant enzymes and phenolic compounds to mitigate the effects of water stress [Shahnaz *et al.*, 2022].

In order to study the influence of the experimental factors on the growth and development of Vikinga and Puno varieties under controlled conditions, biochemical determinations of leaf enzyme activity were performed. Evidence of the cumulative influence of the two experimental factors on the growth of the two quinoa varieties was obtained by determining the enzymatic activity of ascorbate peroxidase and catalase during the growing period when the plants reached the 8 true leaf stage.

The results obtained on APX values in the two varieties were compared with the control. The increase of ascorbate peroxidase activity can be considered as an indicator to assess the stress level caused by growing conditions or by the unfolding of physiological processes specific to the vegetation phenophase.

MATERIAL AND METHOD

The research was carried out in a greenhouse of Iasi University of Life Sciences (IULS), Romania, during March to April 2021-2024. vegetation pots (2700 cm^3), chemically and organically fertilized substrate, resulted 42 variants, of 5 replicates, 8 plants for each repetition.

The biological material - seeds of two cultivars Puno and Vikinga. The plants were grown (16-18ºC/20-22ºC) humidity (70-75%/60-65%) and natural light (13/11hours).

The fertilizers used were represented by biologic fertilizer, Micoseed MB® and chemical fertilizer, KSC® II, in different quantities.

To test the influence of type of fertilizations on plant growth were used the following amounts:

- Biological fertilization: 500g/m^3 (F1); 1000 g/m^3 (F2); 1500 g/m^3 (F3)- Micoseed MB®;
- Chemical fertilization: 1000 g /m^3 (F4); 2000 g/m^3 (F5); 3000 g/m^3 (F6) KSC®.

For the watering of the substrate, water was used in different percentages quantities 50%/75%/100 % of substrate capacity.

The experience was organised in vegetation pots (2700 cm^3 capacity). Corresponding to the proposed factors resulted 42 variants, of 5 replicates, 8 plants for each repetition.

The determination of catalase (CAT) and ascorbate peroxidase APX was carried out by the spectophotometric method.

For the determination of catalase (CAT) and ascorbate peroxidase (APOX) activity, 0.5 g of plant material was soaked on ice in K-phosphate buffer pH 7.0. To obtain the supernatant from which the enzyme activity was analyzed, the extract obtained from the soaking was centrifuged at 12 000 rpm for 20 min at 40C.

Total CAT activity was tested by measuring the initial rate of H_2O_2 disappearance according to the method of Aebi [1984].

APX activity was determined according to the method of Chen and Asada (1989) by monitoring the decrease in absorbance at 290 nm.

For statistical analyses the data are expressed as the means ± standard deviation (SD). The analysis of variance (ANOVA) was used to see the influence of cultivar, fertilization and irrigation on the number of leaves, chlorophyll pigments, leaf surface and green leaf biomass of quinoa. To determine the significant differences between treatments were established by using Tukey's post hoc test with a degree of confidence of 95% ($p \leq 0.05$), using a SPSS ver. 21.

RESULTS AND DISCUSSIONS

Enzyme activity in quinoa leaves play a vital role in numerous biochemical reactions necessary for their growth, development, defense against pathogens and survival.

The limitations of this study and the current research note that the most intense enzymatic activity was evidenced in chemically fertilized variants.

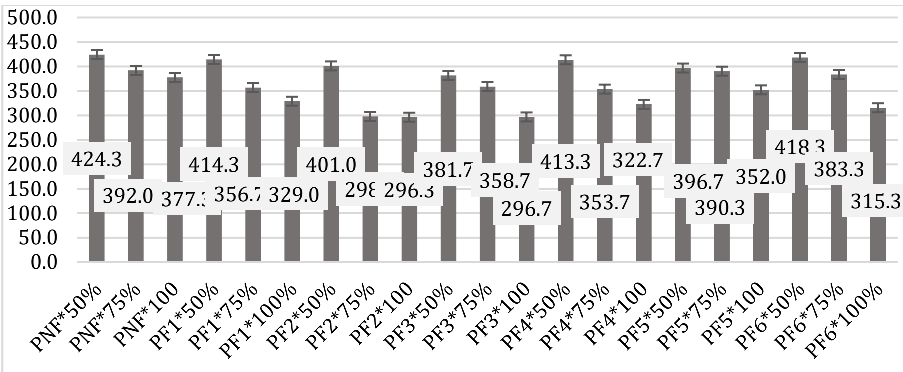

Fig. 1. The influence of the fertilization regime on the enzymatic activity in quinoa leaves (Puno), APX(U/min/g) S.P.

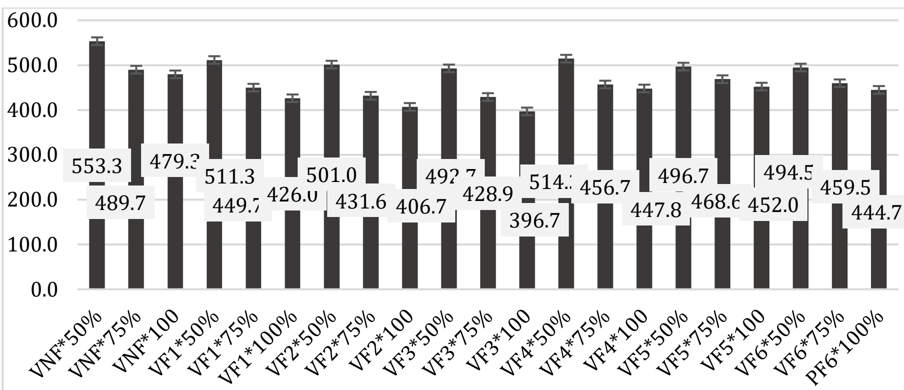

Fig. 2. The influence of the fertilization regime on the enzymatic activity in quinoa leaves (Vikinga), APX(U/min/g) S.P.

The increase in ascorbate peroxidase activity can be considered an indicator of the level of stress caused by the culture conditions or the development of physiological processes specific to the vegetation phenophase. By comparing the results, the greatest increases in APX activity were highlighted in the amount of 553.3 U/g/min S.P., in the Vikinga variety, the non-fertilized-NF variant, with an irrigation regime of 50% (Figure 2) and values the lowest of APX in the Puno variety, amounting to 296.3 U/g/min S.P. in the F2 variant, biologically fertilized, with 100% irrigation regime (Figure 1). It can be seen that under drought conditions APX values increase, while high amounts of water cause enzyme activity to decrease.

The results obtained regarding the APX (ascorbate peroxidase) values in the two varieties, in which biochemical determinations were made regarding the enzymatic activity in the leaves, were compared with the non-fertilized variety – NF.

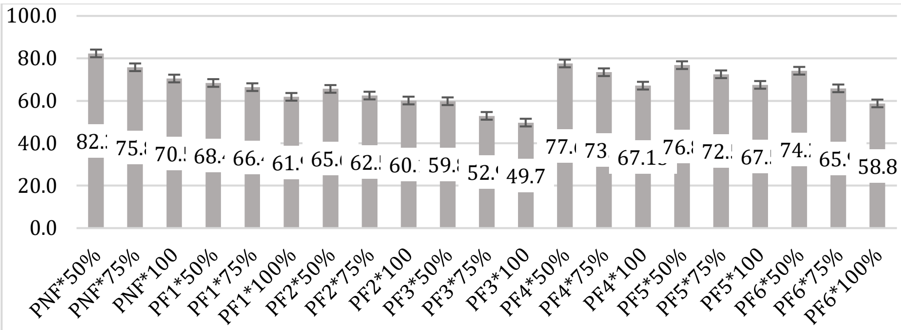

Fig. 3. The influence of the fertilization regime on the enzymatic activity in quinoa leaves (Puno) CAT(U/min/g) S.P

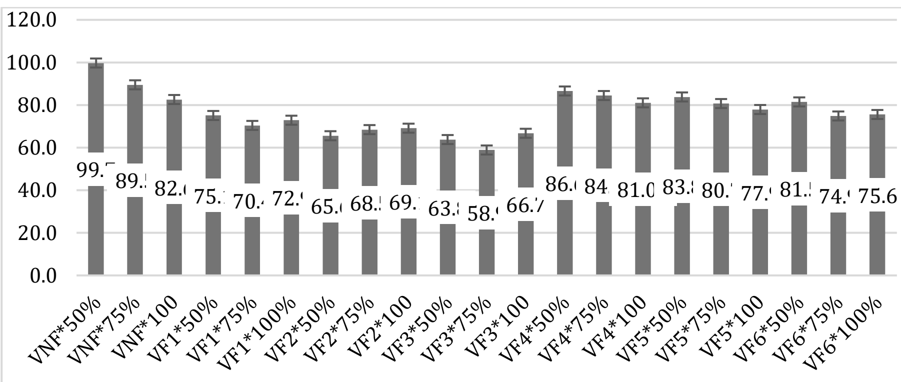

Fig. 4. The influence of the fertilization regime on the enzymatic activity in quinoa leaves (Vikinga), CAT(U/min/g) S.P.

Regarding the results of catalase activity (CAT), by comparing the results from the experimental variants with those from the control, the same trend was highlighted as in the case of APX. The lowest values of CAT activity were obtained in the biologically fertilized variant - F3, with 100% irrigation regime, for the cultivar Puno, the value being 49.7 U/g/min S.P. (Figure 3), and the highest values of CAT activity were noted in the F4 variant, with a 50% irrigation regime, in the case of the Vikinga cultivar, the value being 86.6 U/g/min S.P. (Figure 4).

As an observation, according to the graphs, we can say that the most obvious decreases in CAT activity were highlighted in the experimental variants to which Micoseed MB® was applied, in both cultivations.

Decreases in CAT activity compared to the non-fertilized variant were highlighted in all the experimental variants to which fertilizer was applied, with the irrigation regime above 50%.

In the case of the Puno cultivar, comparing the results obtained from the control variant, NF, with those from the variants fertilized with Micoseed MB® and KSC I®, an increase in the total content of assimilatory pigments is observed, along with the increase in the concentration of fertilizer in the substrate. In the Puno cultivar, the variants fertilized with Micoseed MB® presented a total content in chlorophyll pigments that varied between 2.29mg/g s.p. in the NF variant, irrigation regime of 50% and 4.01 mg/g s.p. in the version with the highest concentration of biofertilizer PF3, 100% irrigation regime (biofertilizer was administered in a concentration of 1500 g/m3 Micoseed MB® in the substrate). Also, the values register a slight increase in the chemically fertilized variants, KSC I®, directly proportional to the increase in the amounts of administered water (irrigation regime of 75% and 100%).

CONCLUSIONS

The most obvious decreases in CAT activity were highlighted in the biological experimental version - Micoseed MB®, in both cultivations.

By comparing the results, the greatest increases in APX activity were highlighted in the Vikinga variety, the non-fertilized-NF variant, with an irrigation regime of 50%. It can be seen that in drought conditions the APX values increase, while large amounts of water cause the enzymatic activity to decrease.

The result regarding all the factors shows that the specie is suitable for cultivation in protected areas.

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