

## Cultivation and cold stress protection in *Crassula* with zeolites

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### ABSTRACT

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*With the aim of improving certain growth and quality characteristics of *Crassula Ovata* plants, an experiment was conducted at CREA-OF in Pescia (PT), where conventional cultivation substrates were compared with substrates added with chabazitic-zeolites. The result of the use of zeolites added to the growing medium was a significant increase in plant height, number of leaves, total plant weight, number of new shoots and stem diameter. It is clear that chabazite can increase plant growth, probably due to an increased supply of water and nutrients. In addition, zeolite-treated plants showed greater resistance to cold, which was also demonstrated by the use of the spectroradiometer, which showed significant differences in parameters  $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E^*$ . These data therefore underline how the use of zeolites and in particular chabazite, in addition to improving certain aspects of plant quality and production can be crucial for the prevention and protection of certain abiotic stresses, especially from cold.*

**Key-words:** *chabazite, plant protection, plant stress, succulent plants, cold stress, abiotic stress, inorganic amendment, alternative substrate*

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## I. INTRODUCTION

### Cold stress on plants

Cold stress is one of the main abiotic stresses that can cause a reduction in plant growth and quality depending on the duration of exposition. Normally, exposition to low temperatures shifts the thermodynamic balance so that the non-polar lateral chain of proteins comes into contact with the aqueous medium of the cell. This affects protein stability and metabolic mechanisms in plants, particularly in the young plant stage [16].

The main symptoms in response to cooling stress are dehydration, osmotic imbalance, discoloration, tissue degradation, accelerated senescence, ethylene production, reduced longevity and decay due to loss of plant metabolites [15]. Cold also causes the accumulation of reactive oxygen intermediates, swelling and degeneration of mitochondria [5] and the qualitative and quantitative fluctuation of the position of membrane lipids [6]. The formation of ROS causes cellular lesions and apoptosis which eventually leads to plant death due to impairment of the system II reaction center and membrane lipids [17]. Cold-sensitive plants have a higher transition temperature than cold-resistant plants due to the higher percentage of saturated fatty acids. However, the real reason for the damage caused by frost to plants is the formation of ice rather than low temperatures. This ice formation in plants initially begins in the apo-plastic spaces due to the relatively lower solute concentration.

### Zeolites and zeolites

Natural Zeolites are a mineral family composed by 54 different species chemically defined as “hydrated allumino-silicates of alkaline and alkaline earth elements” and structurally belonging to the tectosilicates. Due to their crystal chemistry, zeolites show physical-chemical peculiarities such as high and selective cation exchange capacity (CEC), reversible dehydration, selective molecular absorption, and catalytic behaviour. Therefore, rocks containing more than 50% of zeolites (zeolites) are widely and profitably utilized in the purification of municipal, zootechnical and industrial wastewaters, as additive in animal nutrition, agriculture and floriculture. Because of both the presence of the zeolites and texture of the rocks, zeolites exhibit high (130-200 meq/100g) and selective (mainly for  $\text{NH}_4$  and  $\text{K}^+$ ) cation exchange capacity, reversible dehydration, permeability, and high water retention, which are all useful in agricultural, horticultural and floricultural applications. The zeolites were used in this experiment because they exhibit several interesting features for use in agriculture, horticulture and in particular in tomato [7], celery [1], courgette and melon [8], and vegetables and fruit [9] and ornamentals [11][12][13].

To improve the quality and resistance of cold stress in succulent plants, the addition of zeolites on plants of *Crassula Ovata* was evaluated in the experiment.

## II. MATERIALS AND METHODS

### Greenhouse experiment and growing conditions

The experiments began in early December 2018 (mean temperature 7.5°C), were carried out in experimental greenhouses of the CREA-OF of Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on plants of *Crassula Ovata*. The plants were placed in pots ø 12 cm; 36 plants for thesis divided into replicas of 12 plants each. The chabazitic-zeolites supplied by BalCo of Sassuolo (MO) (company specialized in the sale of minerals and rocks for ceramics and agriculture) had the following characteristics: 1) qualitative-quantitative mineralogical analysis (% by weight with standard deviations in brackets) carried out by X-ray powder diffractogram according to the RIETVELD-RIR methodology [4]: chabazite 66.2 (1.0); phillipsite 2.4 (0.5); mica 5.6 (0.6); K-feldspat 10.3 (0.8); pyroxen 2.2 (0.5); volcanic glass 13.3 (1.5); 2) Total zeolitic content (%): 68.6 (1.3), of which 66.2 due to chabazite and 2.4 from phillipsite. Cation exchange capacity (in meq/g with standard deviation in brackets) determined using the methodology described in Gualtieri et al. (1999) [3]: 2.15 (0.15) of which 1.42 due to Ca, 0.04 to Mg, 0.05 to Na and 0.64 to K. The 2 experimental theses in cultivation were:

- control (CTRL): soil for acidophilic 40%, volcanic lapillus 30%, quartz sand 30%, (root wetting every 20 days), trivalent fertilizer 7-14-21;
- treated (T1): soil for acidophilic 40%, chabazitic-zeolites 20%, quartz sand 40%, (root wetting every 20 days), trivalent fertilizer 7-14-21.

For each cultivated species the following were evaluated: plant height, stem diameter, number of leaves per plant, total fresh weight, number of new shoots. The colour of leaves was evaluated using a portable sphere spectrophotometer (Xrite, SP64, Grandville, Michigan, Usa). The colorimetric data were formatted according to CIE L\*a\*b\* coordinates, with L\* defined as the brightness, ranging from 0 (black) to 100 (white), and a\*b\* as the chromatic coordinates, with a\* ranging from - 60 (green) to + 60 (red), and b\* ranging from - 60 (blue) to + 60 (yellow). Colorimetric differences ( $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ ). [2]. The colorimetric analysis was carried out on 10 leaves per plant, for 20 plants per experimental thesis.

### Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ( $P \leq 0.05, 0.01$  and  $0.001$ ) differences among treatments. Mean values were then separated by LSD multiple-range test ( $P = 0.05$ ). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

## III. RESULTS

In the experiment, the use of chabazitic-zeolites resulted in a significant increase in plant height, number of leaves, total plant weight, number of new shoots and stem diameter.

A significant value of 12.65 cm was determined for the height of the plants compared to 7.92 cm for the control (fig.1a, fig.3). For the number of leaves the experiment provided a significant value of 27.60 in the substrate with added chabazite against 16 of the control (fig.1b). The total plant weight ranges from 126.30 g of the thesis treated to 55.48 g of the control thesis (fig.1c). The test also showed a significant increase in the number of shoots ranging from 14.60 of the treated with chabazite to 7.40 of the control (fig.1d, fig.2) and stem diameter 6.64 cm in chabazite compared to 5.72 of the conventional substrate (fig.1e).

It is clear that chabazite can increase plant growth, probably due to an increased supply of water and nutrients. This aspect can also be seen from a greater radical development of the theses treated with zeolites (fig.4). It is also evident an increase in the speed of growth and development of new shoots even in plants subjected to cold stress.

The spectrophotometer represents a new methodology for assessing the colour of flowers and leaves. In this experiment it was possible to verify if there were colour differences in the leaves due to cold stress according to the different type of growing substrate. The parameters L\*, a\* and b\* and the index  $\Delta E^*$  express respectively: the brightness, the trend from green to red, the trend from yellow to blue and finally  $\Delta E^* = (\Delta E^*_{ab} - [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2})$ .

The treatment with chabazite in the substrate has already shown to the naked eye a reduction in cold stress compared to the control substrate. The spectrophotometer data, in fact, show that there is a significant reduction in luminosity (L\*), a trend towards red (a\*), and towards blue (b\*) in the control thesis. This aspect is also confirmed by the  $\Delta E$  with a value of 12.4, which therefore shows significant differences in treatments perceptible to the eye ( $\Delta E \geq 3$ ) (tab.1) (fig.5).

#### IV. DISCUSSION

The use of zeolites, in particular chabazite, can lead to a significant improvement in plant growth conditions, in particular related to the saving of water, fertilizers and energy for crops.

Zeolites, in fact, once introduced in the cultivation substrata or in open field, can increase the quality of the plants, retaining water and fertilizers and making them available when needed [14].

In this experiment it is evident that the addition of chabazitic-zeolites to the growth substrates of *Crassula Ovata* plants resulted in a significant increase in plant height, number of leaves, stem diameter, total plant weight and number of new shoots. In addition, zeolite-treated plants showed greater resistance to cold, which was also demonstrated by the use of the spectrophotometer, which showed significant differences in parameters  $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E^*$ .

The ability of zeolites chabazite to determine greater resistance to cold is probably related to the fact that zeolites contain in their cavities structural molecules of water always in perfect balance with the conditions of temperature and humidity of the environment in contact.

The structural water content (up to 10% in the case of chabazite) then suffers a gradual loss (dehydration = endothermic process) as a result of an increase in temperature and/or a decrease in the degree of surrounding humidity and, subsequently, a gradual recovery of water (rehydration = exothermic process) as a result of a lowering of the temperature and/or an increase in the degree of surrounding humidity. This process, which can be infinitely reversed, is the basis of the peculiar characteristics of zeolites to be maintained in an environment where temperature and humidity conditions are almost constant, thus eliminating both positive and negative peaks.

These data therefore underline how the use of zeolites and in particular chabazite, in addition to improving certain aspects of plant quality and production can be crucial for the prevention and protection of certain abiotic stresses, especially from cold. A decisive factor in zeolites is the purity of the mineral used. The determination of the chemical-physical characteristics is in fact of particular importance in order not to run into problems during the cycle of cultivation in pots or in the open field. A new decree of March 3, 2015, proposed by Prof. Passaglia [10], indicates the non-marketability of zeolites that do not contain in their structure a content greater than 50% of that particular mineral. This assessment can only be made with the RIETVELD-RIR [4]. All this to limit the marketing of products that are not quality and to ensure that those who use this type of mineral can not then encounter problems of phytotoxicity on plants in cultivation.

#### V. CONCLUSION

The test has shown how the addition of zeolites in the growing substrate can bring several benefits to the plants: significant increase in growth and different quantitative and qualitative aspects of the plants of *Crassula*, better use of water and fertilizers, greater prevention against some abiotic stresses, especially from cold. Thanks to zeolites, plants have a better chance of overcoming this type of stress, as the surfaces that are in contact with chabazite are less affected by sudden changes in temperature.

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**TABLE**

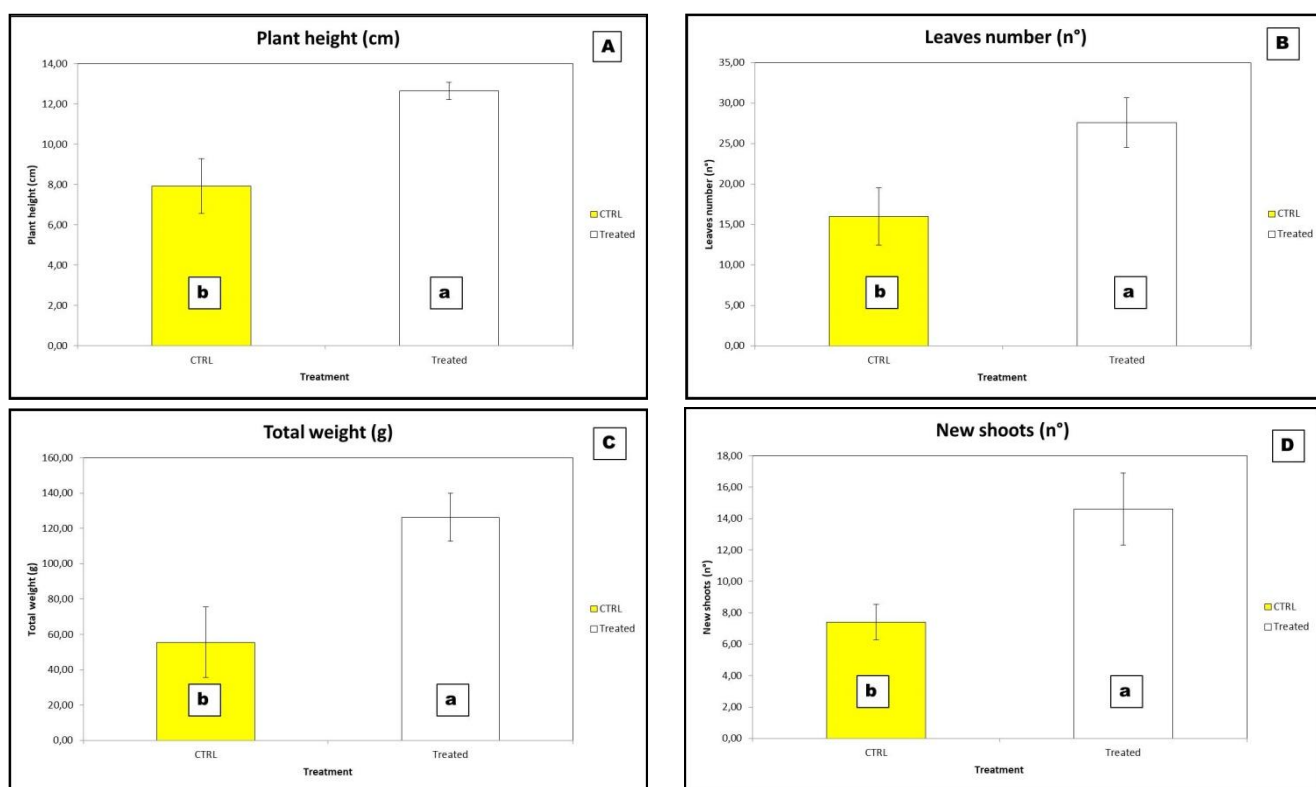
**Tab.1** Average values of L\*, a\*, b\* and ΔE\* (Treatment vs. Control) for the evaluation of cold stress in *Crassula Ovata*

| Treatment | L*      | a*   | b*      | ΔE*  |
|-----------|---------|------|---------|------|
| CTRL      | 34,26 b | 42 b | 17,80 b | -    |
| T1        | 42,48 a | 35 a | 23,88 a | 12,4 |

Each value reported in the graph is the mean of three replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

**FIGURE**

**Fig.1** - Effect of zeolites treatment on the agronomic parameters of *Crassula Ovata*



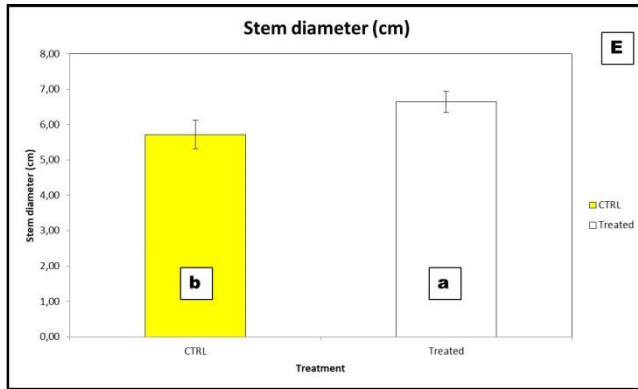


Fig.2 - View of *Crassula Ovata* in greenhouse cultivation

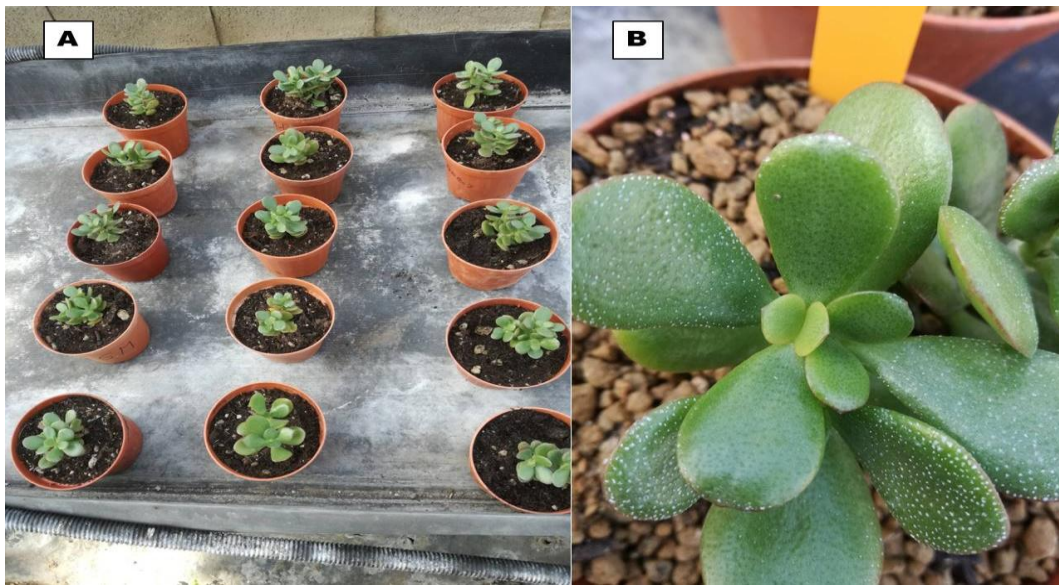
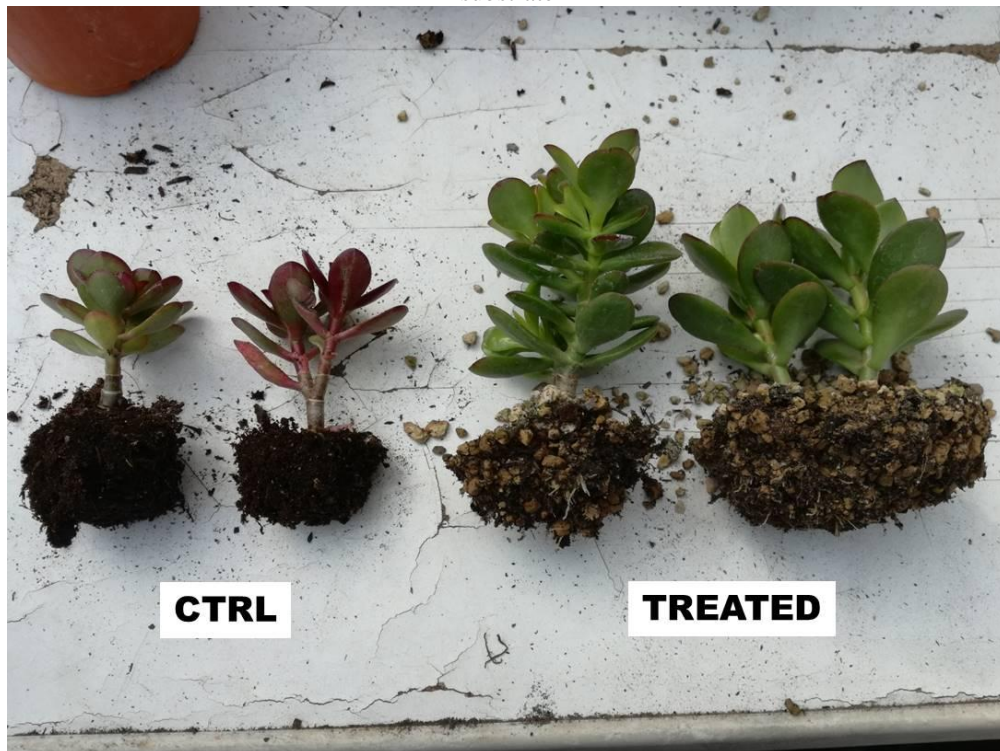


Fig.3 - Comparison of *Crassula Ovata* plants grown in a substrate with zeolitites added and a control substrate



**Fig. 4** - Comparison of root growth of *Crassula Ovata* plants in substrate with chabazitic-zeolites and control substrate



**Fig.5** - Effect of chabazitic-zeolites in the control of cold stress. Plant grown in a substrate with added zeolite which have not undergone discolouration due to cold (left) and plant which have instead suffered from cold (right).

