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Study on the effect of brassinolide and salicylic acid on vegetative and physiological traits of *Aloe maculata* All. in different substrates in a pot experiment

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ABSTRACT

The effect of different concentrations of salicylic acid (SA) and epi-brassinolide and different substrates was examined on increasing the yield of *Aloe maculata* All. and improving its vegetative quality in a factorial study based on a Randomized Complete Block Design with three factors, three replicates and 27 treatments. The first factor (A) was substrate at three levels (a_1: garden soil; a_2: garden soil + 10% azocompost; a_3: garden soil + 20% vermicompost), the second factor (B) was epi-brassinolide at three levels (b_1: no brassinolide; b_2: 1 mg l^{-1} brassinolide; b_3: 2 mg l^{-1} brassinolide), and the third factor (C) was SA at three levels (c_1: no SA; c_2: 0.7 mM SA; c_3: 1.5 mM SA). It was found that growth regulators epi-brassinolide and SA as well as substrate containing garden soil and vermicompost had positive effect of yield-related parameters including leaf and root fresh and dry weight and plant biomass. Also, vermicompost-containing substrate affected superoxide dismutase antioxidant enzyme positively. According to the results, it can be concluded that although azocompost-containing substrate was not recognized as to be appropriate for higher yield, it had significant impact on the amount of chlorophyll *a* and *b* and total chlorophyll as well as anthocyanin. So, the substrate should be selected on the basis of the cultivation target.

1. Introduction

Aloe from the family of Aloaceae is a fleshy, monoploid and perennial plant involving over 400 species. Aloes have been known as medicinal herbs for centuries used for healing people and animals. *Aloe saponaria* Mill. and *Aloe maculata* All. are important species of the genus of Aloe. The name of the plant is taken from the Latin word *sapo* meaning soap because its sap froths in water and it is traditionally used in soap making. The leaves of *Aloe saponaria* Mill. are used in the tanning of clothes made of animal skin in South Africa (Reynolds, 1950). Most aloes are in the danger of extinction. Their excessive collection for the cultivation, their deterioration for extraction and the destruction of their natural habitats are among factors contributing to the loss of their population (Reynolds, 1966). Given the importance of the aloes and the development of their cultivation, one important issue for growers is its propagation and the production of uniform seedlings. Since the growing and propagation of this plant is carried out simultaneously, attempts have been made to find a substrate appropriate for both yield and the production of suckers. The application of organic substrates has been popularized recently (Norouzi et al., 2011). Vermicompost and Azocompost are examples of organic substrates that have been interested. Vermicompost is generated by the conversion of organic matter to humus by, for example, earthworms. Some practitioners believe that vermicompost has, in general, higher nutrient content than traditional compost. In fact, vermicompost can improve soil physical, chemical and biological fertility. Although vermicompost is shown to increase plant growth considerably, its application at high dosages may interrupt the plant growth due to its high dissolved salt content. So, vermicompost should be applied at moderate concentrations to maximize plant yield (Lim et al., 2014). The use of organic fertilizers like Azocompost as nitrogen source improves nutrients, prevents soil nutrients leaching by water, and helps plant nutrition (Mostafavi-rad et al., 2010). Azocompost is a mixture of organic matter including Azolla and rice straw produced by microorganisms in appropriately ventilated hot and humid envi-

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ronment that makes nutrients available to plants (Farahdahr et al., 2011).

Brassinosteroids are inseparable parts of plant hormones influencing the total hormonal balance and the concentration of other plant hormones. Research shows that they are necessary for appropriate production and the resistance to adverse environmental factors. The lack of brassinosteroids in mutants in which the synthesis of brassinosteroids was interrupted resulted in significant deviation as compared to normal plants, and it was restored by the application of exogenous brassinosteroids (Altmann, 1999; Ephritikhine et al., 1999).

An important feature of brassinosteroids is their influence on increasing the crop yield and quality (Prusakova et al., 1999). Brassinosteroids are used in agriculture and horticulture not only for their ability in improving crop yield, but also for their inducing effect on physiological processes. So, it might be possible to grow crops in adverse (stressful) conditions like high salinity, drought and/or nutrient deficiencies (Prusakova et al., 1999). Reports mostly show the impact of brassinosteroids on fungal pathogens so that their protective effect has been even stronger that plants treated with standard fungicides (Khripach et al., 2000). A newly found aspect of brassinosteroids is their effect on regulating the uptake of ions into plant cells. They can be used to reduce the accumulation of heavy metals and radioactive elements in plants that are grown in polluted regions (Khripach et al., 1996). The amounts of O_2^- and H_2O_2 and lipid peroxidation are significantly increased in the roots under oxygen deficiency, and the exogenous application of 24-epi-brassinolide inhibits this phenomenon. The treatment with 24-epi-brassinolide increases the activities of superoxide dismutase (SOD) and peroxidase (POD) under oxygen deficiency stress. Oxygen deficiency results in higher activities of lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH), whilst the treatment with 24-epi-brassinolide significantly increases ADH activity but reduces LDH activity (Kang et al., 2006).

Salicylic acid is a secondary metabolite produced by a wide range of prokaryotic and eukaryotic organisms including plants. It chemically belongs to a group of phenolic compounds that have an aromatic ring containing hydroxyl group. The exogenous application of SA or its derivatives affects diverse plant processes such as thermogenesis (Raskin et al., 1987), seed germination (Rajou et al., 2006), seedling establishment (Alonso-Ramírez et al., 2009), cell growth (Vanacker et al., 2001), respiration (Norman et al., 2004), stomatal responses (Manthe et al., 1992; Lee, 1998), senescence (Rao et al., 2002), heat tolerance (Clarke et al., 2004), and nod formation (Stacey et al., 2006). Also, studies on genetic mutation in Arabidopsis show that SA is involved in cell growth regulation (Rate et al., 1999), hair formation (Traw and Bergelson, 2003), and leaf senescence (Morris et al., 2000). However, it may affect other processes indirectly because SA heavily interacts with other plant hormones (Robert-Seilaniantz et al., 2007; Pieterse et al., 2009).

Aloe maculata All. is an important species of ornamental aloes. Nonetheless, very few studies have been conducted on factors affecting its growth, propagation and physiological indices. The objective of the present study was to investigate the impact of different hormone levels and organic substrates on some physiological indices and vegetative factors of this species.

2. Material and methods

2.1. Experimental design

The present study was carried out in a greenhouse located in Guilan Greenhouses Center, 17 km away from Rasht City. *Aloe maculata* All. is native to Southern Africa and it recently is imported and cultivated in the Guilan, northern province of Iran. We represented trial specimens to different botanists and they were identified with the help of available Floras and consulting with different herbal literature (Graf, 1979, http://davesgarden.com). It was a factorial experiment on the basis of a completely randomized design involving three factors (Figs. 1 and 2): (A) substrate at three levels (a_1: garden soil; a_2: garden soil + 10% azocompost; a_3: garden soil + 20% vermicompost), (B) brassinolide at three levels (b_1: no brassinolide; b_2: 1 mg l^{-1} brassinolide; b_3: 2 mg l^{-1} brassinolide), and (C) salicylic acid (SA) at three levels (c_1: no SA; c_2: 0.7 mM SA; c_3: 1.5 mM SA) at three replications. Each experimental unit was composed of one pot involving one plant. In total, there were 81 pots (two gallon pot with about 500 g substrates) in the experiment. Almost uniform suckers were cut from maternal plants that had grown in the same conditions. Then, they were immediately transferred to the greenhouse taken care of all precautions. After weighing, they were planted in substrates on September 23. The temperature and humidity of the greenhouse were kept at 18–20 °C and 80–85% in autumn and winter and 22–25 °C and 90–95% in the spring, respectively. Hormones were applied as spraying on the shoots, one month after the planting of suckers, i.e. on October 23. Brassinolide was procured from Sigma Co., and SA was procured from Merk Co. (Germany). The samples were examined once a month for data collection. Also, the required data were read every day. Some chemical properties of trial substrates are provided in Table 1.

Fig. 1. The trial plants at the beginning of experiment in the greenhouse.

2.2. Traits evaluation

2.2.1. Biomass and dry weight

Plant samples were removed from the soil and were transferred to the laboratory after washing. To measure total biomass, fresh weight of whole plant was measured by a digital scale (0.01 g precision). Biomass was measured by weighing fresh weight of all parts of the plant (root and shoot). After separating the leaves and roots, fresh weight of shoot and root were measured. Since the leaves of Aloe are succulent and they are dried too late. Therefore, 100 g of leaves of each treatment were dried in an oven at 105 °C for 24 h for dry weight. All roots were dried at 105 °C for 24 h. Sum of the dry weight of root and shoot was reported as total dry weight.

2.2.2. Suckers

The suckers of the experimental plots were counted every month. Then, sucker production rate was measured using total suckers and the following equation:

$$G = \frac{n_1 t_1 + \ldots}{N} \quad (1)$$

where,

N = total number of produced suckers,