Growth variation and proline accumulation of *Echinacea purpurea* cultivated to CaCl$_2$ salinity

J P Choirunnisa$^1$, Y Widiyastuti$^2$, B Pujiasmanto$^3$, A T Sakya$^3$ and A Yunus$^3*$

$^1$ Post Graduate Departement of Agronomy, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
$^2$ Medicinal Plants and Traditional Medicines Research and Development Center (B2P2TOOT) Tawangmangu, Indonesia
$^3$ Departement of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia

Corresponding author: yunus.uns7@yahoo.com

Abstract. Purple coneflower (*Echinacea purpurea* (L.) Moench) is classified as medicinal plant comes from North America and not much developed in Indonesia. *E. purpurea* can be developed by utilizing suboptimal land such as saline land. This study aims to determine the effect of CaCl$_2$ concentration on growth and proline accumulation of three accessions *E. purpurea*. This study used a Completely Randomized Design (CRD) with 2 factors and five replications. The first factor is three accessions *E. purpurea* (accession 1; 2; and 3). The two factors are four concentrations CaCl$_2$ (0; 2500; 5000; and 10000 ppm). The observations are plant height, number of leaves, number of branches and proline accumulation. Data were analyzed using SPSS to test difference. The results showed that highest concentration of CaCl$_2$ can inhibit growth and increase proline accumulation. The highest proline accumulation in accession 2 was 22.8002 µmol g$^{-1}$, accession 2 as an indicator of accession tolerant to salinity.

1. Introduction

The use of traditional medicine in Indonesia with medicinal plant as raw materials has developed in accordance with global issues. One of the medicinal plants that can maintain and increase endurance is purple coneflower (*Echinacea purpurea* (L.) Moench) from North America [1]. *E. purpurea* has benefits for humans as antioxidant, anti-inflammatory, immunoregulatory and has no hypersensitivity in clinical trials [2]. Since *E. purpurea* is an introduced plant, the development of *E. purpurea* as a medicinal raw material in Indonesia needs to conducted. The development of *E. purpurea* for the production of medicinal plants can utilize suboptimal land such as saline land.

Salinity is defined as the level of excess salt concentration in the soil which made plants tensed or stressed. Salt stress is one of the inhibiting factors for plant growth. The results of previous research by Lolaei [3] showed that treatment of 60 mM NaCl decreased the number of leaves of tomato plants from 29.5 (control) to 19.4 (NaCl 60 mM). This is in accordance with the result of research Petretto et al. [4] in Rocket leaves (*Eruca sativa*); where without salinity treatment resulted in an average plant height of 17.6 cm, while plant height decreased 5.7 cm under salinity stress of 130 mM. The stunted growth is caused by osmotic stress, makes it difficult for plants to absorb water and inhibits cell division [5]. Plants under salinity stress will undergo adaptation through physiological mechanisms; namely by
osmoregulation in the form of osmotic potential regulation [6]. Osmoregulation implicated osmotic regulation by synthesizing amino acids and organic acids to produce proline [7]. Indicators in the selection of tolerant plant responses to stress can be categorized based on the accumulation of proline [8].

Information on the effect of salinity with the addition of CaCl₂ levels in *E. purpurea* especially with 3 different flower accessions in tropical countries such as Indonesia is very limited. This research aims to examine the dynamics of growth and accumulation of proline at different levels of CaCl₂, and to obtain *E. purpurea* plants tolerant to salinity.

2. Materials and methods

The research was conducted from December 2020 to June 2021 at the Jumantono Screen House within altitudinal ranges 293 meters above sea level (m asl), Sebelas Maret University, Karanganyar, Central Java, Indonesia. The materials used were 3 accessions of *E. purpurea* from Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT) Tawangmangu and CaCl₂.

The study used a factorial Completely Randomized Design (CRD) consisting of two factors with five replications. The first factor was accessions of *E. purpurea* with difference in morphology and flower color consists of 3 levels, namely accessions 1 (E1), accessions 2 (E2) dan accessions 3 (E3). The second factor was concentrations of CaCl₂ consists of 4 levels, namely 0 ppm or without CaCl₂ (C1), 2,500 ppm (C2), 5,000 ppm (C3) dan 10,000 ppm (C4).

*E. purpurea* seeds were sown until the age of 5 weeks then moved to the planting medium in the form of soil, manure and rice husk with ratio 2:1:1. The watering volume was determined based on the field capacity from method of Hakim et al. [9]. The treatment of CaCl₂ solution was applied to the vegetative phase of the plant as much 4 times starting at 4, 5, 6 and 7 weeks after planting with watering interval 1 week once with watering volume of 500 ml/flower pot. Plant maintenance included watering, weeding and pest control done by manual. Harvesting was done on the age plant of 16 weeks after planting (80% flowering).

Observations were conducted on growth and accumulation of proline. Growth observations included plant height, number of leaves and number of branches measured at the age of 1 to 16 weeks after planting. Observation of proline accumulation was done on the plant aged 8 weeks after planting with [10] method on the leaf segment number 2-3 from the shoot. The research data were analyzed on SPSS (Statistical Product and Service Solution) with analysis of variance and simple correlation analysis followed by DMRT (Duncan Multiple Range Test) method level of 5%.

3. Results and discussion

Salinity induces stunted growth on *E. purpurea*. According to Purwaningrahayu and Taufiq [11], the cause of stunted plants is salinity stress which has negative impact on plant growth. The results of the study were that CaCl₂ concentration decreased growth in three accessions of *E. purpurea* aged 16 weeks after planting. The decrease in growth occurred in plant height, number of leaves and number of branches (Table 1).

3.1. Plant height

Based on analysis of variance, the effect of CaCl₂ concentration on plant height of *E. purpurea* indicated significant results, while the height of plants of all accessions were not significantly different. The higher concentration of CaCl₂ resulted in the plant height decreasing with 34.62%, this was caused by the dissolved CaCl₂ salt in the soil causes the soil to become saline so that plants were stressed and affect the characteristics. The result is in accordance with research conducted by Dachlan et al. [12], the high NaCl concentration of 5 g L⁻¹ caused plant height of corn to decrease by 12.31%. The same thing happened to green bean (*Vigna sinensis*) with the treatment of seawater that has electrical conductivity (EC) 15.8 dS/m (7,900 ppm) had a significant effect to decrease the average plant height [13]. According to Romadloni and Wijcaksono [14], stunted plant growth is caused by osmotic stress which makes it difficult for plants to absorb water, so that causing inhibition of plant cell division and enlargement.
Plant height is inhibited due to salt stress also caused by decreased hormone concentrations; according to Larcher [15] the concentration of cytokinin and auxin hormones decreases but the ethylene hormone increases under salt stress conditions in plants. The increased concentration of the hormone ethylene induces stunted growth, because ethylene is an inhibitory hormone, which is a hormone that works to inhibit cell elongation [16].

Table 1. Effect of CaCl$_2$ concentration on growth in three accessions of *E. purpurea* age 16 weeks after planting.

| Accession | Plant height (cm) | Growth observations | Number of leaves | Number of branches |
|-----------|------------------|---------------------|------------------|--------------------|
|           |                  |                     |                  |                    |
| 1         | 29.53$^a$        |                     | 23.90$^a$        | 2.75$^a$           |
| 2         | 29.57$^a$        |                     | 25.40$^a$        | 3.15$^a$           |
| 3         | 29.55$^a$        |                     | 25.00$^a$        | 2.95$^a$           |

| Concentration of CaCl$_2$ | Plant height (cm) | Growth observations | Number of leaves | Number of branches |
|---------------------------|------------------|---------------------|------------------|--------------------|
| 0 ppm                     | 36.79$^a$        |                     | 35.80$^a$        | 4.20$^a$           |
| 2,500 ppm                 | 30.07$^b$        |                     | 26.40$^b$        | 3.40$^b$           |
| 5,000 ppm                 | 27.29$^c$        |                     | 20.20$^c$        | 2.60$^c$           |
| 10,000 ppm                | 24.05$^d$        |                     | 16.67$^d$        | 1.60$^d$           |
| CV (%)                    | 18.62            |                     | 33.41            | 38.68              |

Numbers followed by different lowercase letters in each column showed a significant difference in DMRT at $\alpha = 5\%$.

3.2. Number of leaves

The results from analysis of variance on the effect of CaCl$_2$ concentration to number of leaves *E. purpurea* indicated significant results. The higher concentration of CaCl$_2$ resulted in the number of leaves decreasing 53.43%. This is in accordance with the research by Andriani [17] that stated, number of leaves on control mustard pakcoy plant was 42% more than number of leaves mustard plant pakcoy in the 300 mM NaCl treatment. The rate of leaf growth is disturbed at high salt concentrations due to disruption of the water and nutrient transport system. The inhibited absorption of water and nutrients causes a deficit of water and nutrients in plants, while water is the raw material needed for metabolic activities. One form of plant response to reduce evaporation due to water deficit that is dropping the leaves [18]. Leaf fall causes the number of leaves to decrease. This is revealed in research by Junandi et al. [19], salinity of 7,500 ppm resulted in the lowest number of leaves in cowpea.

3.3. Number of branches

The concentration of CaCl$_2$ had a significant effect on the number of branches on *E. purpurea*. The higher concentration of CaCl$_2$ resulted in the number of branches decreasing by 61.90%. The result is in accordance with research by Sarijan and Ekowati [20], salt stress reduced the number of branches in tomato plant by 17.06%. The number of branches decreased with increasing CaCl$_2$ concentration due to inhibition of hormones translocation that have a role an important in growth. According to Hamayun et al. [21], salt stress can increase abscisic acid hormone (ABA), but hormone concentrations of auxin, cytokinin and gibberellins decreased. Decreased cytokinin hormones will inhibit cell division so that branch formation is inhibited. Research result by Wijayanti et al. [22] stated that the number of branches in tomato plant decreased on the 7 g/l NaCl treatment. Inhibited branch formation is also caused by limited water available due to high osmotic pressure in soil solution, so that the absorption of water by roots will be reduced which causes limited water availability, while water have a role in cell division and enlargement to support branch forming [23].

3.4. Proline accumulation

Plants responded to salinity stress by producing proline compounds to protect plants with maintaining cell turgor in stressed conditions [24]. Proline is one of the amino acids synthesized from organic compounds as a form of plant physiological mechanisms when experiencing salinity stress [25].
results of the study showed that the effect of CaCl$_2$ concentration increased proline accumulation in all three accessions of *E. purpurea* (Table 2); and the growth of *E. purpurea* was negatively correlated with proline accumulation (Table 3).

The results showed that the accumulation of proline of all accessions *E. purpurea* was not significantly different; while the concentration of CaCl$_2$ resulted in a significantly different accumulation of proline. Along with the higher concentration of CaCl$_2$, then the proline content produced in *E. purpurea* increased by 83.56%. The same thing happened to tomato (*Solanum lycopersicum* L.) plants with with 200 mM NaCl treatment had a significant effect on increasing proline content by 84% compared without NaCl treatment [26]. Proline content increases in stressed conditions caused by the ability of plants to regulate osmotic pressure with increasing non-toxic soluble compounds such as proline as a physiological response to salinity stress conditions. Research result by Hakem et al. [27] stated that proline levels in Pusa-37 soybeans increased 5-fold at 150 mM NaCl concentration compared to treatment without NaCl. Plants tolerant to salinity stress will produce proline as a mechanism to maintain turgor in preserving the water potential of the cell to be constant against external water potential, so that plasmolysis does not occur [28]. The highest proline accumulation was shown in accession 2, which means accession 2 was more tolerant of salinity stress.

### Table 2. Effect of CaCl$_2$ concentration on proline accumulation in three accessions of *E. purpurea*

| Accessions | Proline accumulation (µ mol/g$^{-1}$) |
|------------|-------------------------------------|
| 1 (E1)     | 19.0498$^a$                          |
| 2 (E2)     | 22.8002$^a$                          |
| 3 (E3)     | 20.3005$^a$                          |

### Table 3. Correlation analysis of proline accumulation and growth in *E. purpurea*.

| Observation | Plant height | Number of leaves | Number of branches |
|-------------|--------------|-----------------|-------------------|
| Proline accumulation | -0.896** | -0.921** | -0.911** |

The sign ** indicates very significant difference in simple correlation analysis level of 5%

Proline accumulation was negatively correlated with plant growth of *E. purpurea* (Table 3). It was suspected that ABA (abscisic acid) hormone increased in salt stressed conditions. ABA hormone can induce genes to produce enzymes functions in synthesis of proline through the glutamate line [29]. ABA is a hormone which role is inhibiting plant growth [30], so that the increase of ABA cause stunted growth.

### 4. Conclusion

CaCl$_2$ concentration of 10,000 ppm in 3 accessions of *E. purpurea* inhibited growth through reducing plant height by 34.62%, leaf number by 53.43% and number of branches by 61.90%, otherwise increasing concentration of CaCl$_2$ can increase proline accumulation in *E. purpurea* leaves by 83.56%. Accession 2 of *E. purpurea* had a high proline content which indicated characteristics which were more tolerant of salinity stress compared to accession 1 and accession 3.

### Acknowledgment

The authors thank the Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT), Tawangmangu, Indonesia for supported *E. purpurea* seeds and access for analysis in the laboratory and to the KEMRISTEKDIKTI for financial support.
References
[1] Sudrajad H and Saryanto 2011 Prosiding Nasional “Peranana dan kontribusi herbal dalam terapi penyakit degeneratif vol 1 ed D A Kunti (Semarang: Universitas Wahid Hasyim) pp 102–7
[2] Sidhiq D F, Widiyastuti Y, Subositi D, Pujiasmanto B and Yunus A 2020 Biodiversitas 21 1265–71
[3] Lolaei A 2012 J. of Ornamental and Horticultural Plants 2 155–60
[4] Petretto G L, Urgeghe P P, Massa D and Melito S 2019 Plant. Physiology and Biochemistry 141 30–9
[5] Romadloni A and Wicaksono K P 2018 J. Produksi Tanaman 6 1663–70
[6] Rahmawati H, Sulistyaningsih E and Putra E T S 2012 Vegetalika 1 1–11
[7] Mardhiana F, Soeparjono S and Handoyo T 2018 Agriprima 2 1–8
[8] Ra gmawati H, Sulistyaningsih E and Putra E T S 2012 Vegetalika 1 1–11
[9] Hukim N, Nyakpa M, Lubis A, Pulung A, Saul R, Diha M, Hong G and Bailey H 1984 Practical Materials for Basic Soil Science (Lexington: Soil Science Cooperation Agency PTN/USAID, University of Kentucky)
[10] Bates L S, Waldren R P and Teare I D 1973 Plant Soil 39 205–7
[11] Purwaningrahayu R D and Taufiq A 2017 J. Biologi Indonesia 13 175–88
[12] Dachlan A, Kasim N and Sari A K 2013 Biogenesis 1 9–17
[13] Taufiq A and Purwaningrahayu R D 2013 Penelitian Pertanian Tanaman Pangan 32 159–70
[14] Rahmawati H, Sulistyaningsih E and Putra E T S 2012 Vegetalika 1 1–11
[15] Larcher W 1995 Physiological Plant Ecology Ecophysiology and Stress Physiology of Functional Group 3rd ed. (Berlin: Springer-Verlag)