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Physiological tolerance of shallot varieties to airborne salinity in coastal sandy soils

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Abstract: Shallot as a horticultural crop has various benefits and important uses as a provider of nutritional needs. Its uniqueness in aroma and flavor makes it commonly used as a seasoning so that it has a good economic value as an increase in farmers' income. Sandy land on the coast has the potential for shallot cultivation. The presence of wind that airborne salinity on coastal land requires the selection of tolerant varieties and knowledge of the level of airborne salinity concentration that shallot plants can tolerate. Experiments have been conducted from July to December 2023 in the screenhouse and horticultural agronomy lab, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto (7°24'27.7"S, 109°15'19.1"E). Treatments consisted of the use of shallot varieties Bali Karet (B_1) and Bima Brebes (B_2), with the application of several concentrations of airborne salinity consisting of 0, 6, 12, and 18 mS cm^{-1}. The Bali Karet variety excels in plant height and root dry weight morphologically. Physiologically, Bima Brebes has higher levels of chlorophyll a and stomatal density, while Bali Karet is superior in chlorophyll b. Harvest results show Bima Brebes produces more tubers, while Bali Karet produces higher fresh tuber weight per clump. Morphological parameters (plant height, root dry weight), physiology (chlorophyll a, chlorophyll b, stomatal aperture, stomatal density), and yield showed the highest value at the lowest air salinity concentration (0 mS cm^{-1}). Both varieties increased proline as a tolerance mechanism to 18 mS cm^{-1} air salinity. The best interaction occurred between Bali Karet and 0 mS cm^{-1} salinity on stomatal opening, and between Bima Brebes and 0 mS cm^{-1} salinity on stomatal density. Both varieties were classified as having moderate tolerance to 18 mS cm^{-1} salinity, but total chlorophyll was very sensitive to this salinity concentration.

1. Introduction

Horticultural crops play an important role in providing food nutrition as well as increasing farmers income. Horticultural development continues as technology advances. Horticultural products are an important source of valuable nutritional and nutraceutical compounds as nutrients needed by humans (Durazzo and Lucarini, 2022). Horticultural crops include fruit plants, medicinal plants, vegetable plants, plantation plants, spices, and ornamental plants, playing an important role in the economic development and prosperity of a country (Kour *et al*., 2022). The export of horticultural crop commodities provides a great opportunity globally in increasing the country's income and the welfare of farmers. One of the potential commodities in horticultural production activities is shallots.

Shallot (*Allium ascalonicum* L.) as a commodity type of horticulture with high economic potential for farmers' income. Shallot cultivation plays an important role in the national economy and globally. There is a high market demand for shallots domestically and internationally, increased production and technological development are required, contributing to food security. Shallot production reached 1.985 million tons in 2023, marking a 0.14% increase (2.87 thousand tons) compared to 2022. Household consumption of shallots in 2023 decreased by 4.07% (33.83 thousand tons), totaling 797.32 thousand tons compared to the previous year. The import value of shallot in 2023 reached US $1.82 million, increased of 21.94% (US $327.46 thousand) from 2022. The consumption needs of shallots by households in Indonesia have fluctuated in the last five years, respectively in 2019 by 750.63 thousand tons; 2020 by 729.82 thousand tons; 2021 by 790.63; 2022 by 831.14 thousand tons; and 2023 by 797.32 thousand tons (Badan Pusat Statistik, 2024).

Based on the high interest and potential, a strategy is needed to increase shallot productivity. As an archipelago, Indonesia has many islands spread across its territory. Indonesia as an archipelago consists of 17,504 islands, has a coastal area of 95,118 kilometers (Syamsuddin *et al*., 2019). The amount of sandy beach land is a potential in increasing agricultural land for farmers. Sand land is one of the potentials to overcome the problem of agricultural land conversion, as well as in horticultural development (Fikri, 2021). Seeing the increasingly limited cultivation land provides a highlight of the potential of coastal land as a feasible marginal land utilization effort. Extensification activities on coastal land can significantly increase the total shallot planting area, thereby increasing total production in an area. One of the efforts to meet shallot production needs is done with off season cultivation (Susanawati and Fauzan, 2019). Off season shallot cultivation can be done on coastal sand land (Fauzan, 2020). Different soil and climatic conditions make coastal land a challenge in conducting shallot cultivation activities. However, coastal sand land is easy to cultivate because of its loose texture so that it can save time and cost of land treatment and land is relatively safe from disease (Iriani, 2013).

Another major problem that needs to be considered in cultivation on coastal land is the presence of airborne salinity. A simple sensor exposure method with a wet sponge in coastal areas showed air salinity of 19.69 mS at 6 hours and 151.19 mS at 24 hours (Saparso *et al*., 2023). This shows that the air salinity in coastal areas is very high as indicated by the salt particles captured on the wet sponge. Evaporation that occurs in the sea around the coast causes salt particles to be carried into the atmosphere. Winds in coastal areas carry water vapor that has a certain level of salinity originating from the sea area. When carried inland on agricultural land, water vapor with a certain level of salinity can affect plants. Deposition of salt particles on the surface of leaves and other organs, allowing uptake by plants. Growth reduction due to high salinity results from a combination of osmotic stress causing water deficit and the impact of excess Na^+ and Cl^- ions on crucial biochemical processes (Munns and Tester, 2008). NaCl in high concentrations is toxic when accumulated in plant tissues. High concentrations of Na^+ disrupt the uptake of K^+ and Ca^{2+} nutrients, while high concentrations of Cl^- decrease photosynthetic capacity due to chlorophyll degradation (Tavakkoli *et al*., 2010). Salinity stress in plants influences numerous cellular mechanisms, such as disturbing cellular homeostasis, hindering photosynthesis, affecting mRNA processing, transcription, and protein synthesis, as well as disrupting energy metabolism, amino acid biosynthesis, and lipid metabolism (Hameed *et al*., 2021). Salinity stress can cause a reduction in photosynthesis efficiency, chlorophyll, total protein, biomass, stomatal closure and increasing the oxidative stress (Gupta and Huang, 2014).

Salinity stress in plants increases the production of reactive oxygen species (ROS) through oxidative stress mechanisms. ROS are normal products of cell metabolism, but environmental stress increases their production excessively, damaging biomolecules and organelles. The role of ROS as signals or stressors is determined by the balance between their formation and elimination by the antioxidant system, and disruption of this balance leads to oxidative stress (Hasanuzzaman *et al*., 2021). Due to the presence of high salinity there is a water deficit and an increase in free radicals that damage cell structures, plants respond by synthesizing osmolytes such as proline and sugar. Proline has antioxidant activity, activates the detoxification system, contributes to cellular homeostasis by protecting redox balance, and serves as a protein precursor and energy source in the recovery process from stress (Mansour and Ali, 2017). Proline is able to minimize damage from ROS thereby reducing lipid peroxidation, which results in protection of the photosynthetic apparatus in various plant species (Ashraf and Foolad, 2007; Wani *et al.*, 2012).

Each crop variety has a different genetic makeup that determines its adaptability to environmental stress, such as salinity. Research shows that Allium species, including shallots, are plants that are quite sensitive to salinity stress (Kadayifci *et al*., 2005; Kiremit and Arslan, 2016). To investigate the effect of salinity on shallot, two different varieties were used: Bima Brebes and Bali Karet. Genetic differences in shallots of Bima Brebes and Bali Karet varieties cause differences in morphology, physiology, and yield in plants. Alavan *et al*. (2015), stated that different varieties affect the diversity of plant appearance, due to differences in plant traits (genetic) or environmental influences. The results of research by Karo and Manik (2020), showed that differences in shallot varieties had a significant effect on the number of flowers with the highest value being the Pancasona variety 2.93 stalks and the lowest Birma 0.07 stalks. According to Azmi *et al*. (2011), that several varieties planted on the same land have different bulb sizes for each variety.

To improve productivity on land with exposure to airborne salinity, it is necessary to select varieties that can adapt to salinity exposure. This selection of plant varieties is based on morphological, physiological and molecular markers (Soltabayeva *et al*., 2021). Currently, there is still no information and research on the impact of airborne salinity on shallots grown on the coast. Therefore, this study aims to determine the impact of airborne salinity on the morphology, physiology, and yield of shallot plants in two different varieties on coastal land.

2. Materials and Methods

Experimental design

Experiments have been conducted from July to December 2023 in the screenhouse and horticultural agronomy lab, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto (7°24’27.7”S, 109°15’19.1”E). Screenhouse microclimate with daytime peaks of 33.17°C (36,7% RH) under solar radiation and nighttime lows of 27.03°C (55,74% RH) due to radiative cooling.

Experiment with factorial research with a two-factor completely randomized design (CRD) instrument. The first factor shallot varieties consisted of Karet Bali (B_1) and Bima Brebes (B_2), the second factor airborne salinity at a concentration of 0 mS cm^{-1} (A_0), 6 mS cm^{-1} (A_1), 12 mS cm^{-1} (A_2), and 18 mS cm^{-1} (A_3). There were 8 treatment combinations with 3 replications, there are 24 units, with 5 polybags each, making a total of 120 polybags.

Plant material

The shallot variety Bima Brebes originates from Brebes. The plant starts flowering in 50 days and can be harvested in 60 days. It reaches 34.5 cm in height and produces 7-12 bulbs per clump. The leaves are green, cylindrical, and 14-50 in number. Dry tuber production reaches 9.9 tons per hectare. This variety is quite resistant to tuber rot but susceptible to leaf tip rot. The tubers are oval and pink in color, suitable for lowlands (Annex to the Regulation of the Indonesian Minister of Agriculture Number: 594/Kpts/TP.240/8/1984 Dated: August 11, 1984).

The Bali Karet (Batu Ijo) variety of shallots originates from Batu, Malang. Plants start flowering in 45-50 days and are harvested in 55-60 days in the lowlands or 65-70 days in the highlands. It is between 45-60 cm tall and produces 2-6 bulbs per clump. The leaves are dark green, cylindrical, and number 45-50. The dry tuber production reaches 18.5 tons per hectare. The tubers are round and pink in color, and this variety is well adapted to areas with an altitude of 50-1000 meters above sea level (Annex to the Regulation of the Indonesian Minister of Agriculture Number: 366/Kpts/LB.240/6/2004 Dated: June 2, 2004).

Agronomic variables

Plant height (cm) was determined from the soil surface to the uppermost shoot. The roots were dried in an air-circulated oven at a constant temperature of 70°C until constant weight (72 hours). Root dry weight was then weighed using an analytical balance with an accuracy of 0.01 g and expressed in grams (g) per plant. Counting the number of tubers per clump was done at harvest time. Uniform and healthy sample plants were uprooted along with the tubers. After being cleared of soil, the clumps of tubers were manually separated from the remains of dried roots and leaves. Each bulb in a clump was counted manually, and the results were expressed as the number of bulbs per clump (bulbs per clump). Fresh bulb weight per clump was measured at harvest. Each whole clump was directly weighed using an analytical balance (accuracy 0.01 g). Measurement results were expressed in grams per clump (g).

Assessment of leaf greenness

Data on the greenness value of shallot leaves were observed in the late vegetative and late generative phases 34 and 47 days after planting, respectively. Leaf greenness value was determined with the SP3 leaf chlorophyll meter on the SPAD-502 plus device. Data on chlorophyll content in the leaves were taken randomly in the sample unit. The leaf greenness each leaf sample observed was then taken as the average value. The results of the average value of SPAD-502 plus as sample data are processed. Data collection in sunny weather to increase the accuracy of data collection.

Assessment of chlorophyll content

Chlorophyll concentration was determined using the modified International Rice Research Institute (IRRI) method (Alsuhendra, 2004). A total of 0.01 g of shallot leaves were weighed on a balance sheet, pulverized in a mortar with the addition of 10 ml of 80% acetone. Leaves that have been pulverized, filtered with filter paper. The shallot leaf extract was analyzed for chlorophyll content on a spectrophotometer, 663 and 645 nm wavelengths.

$$\text{Chl Content (mg L}^{-1}) = (20.2 \times A645) + (8.02 \times A663) \quad (1)$$

A663 = Absorbance at 663 nm wavelength
A645 = Absorbance at 645 nm wavelength

Assessment of stomatal characteristics

Stomatal opening was quantified by identifying epidermal impressions which were obtained from the abaxial leaf surface using clear nail polish. After application, transparent adhesive tape was pressed onto the coated section and carefully peeled to transfer the imprint. The tape-mounted impression was then affixed to a glass slide for stomatal aperture observation at 400× magnification. Imprints were examined under a compound light microscope equipped with a calibrated ocular micrometer. Stomatal opening width (µm) was measured as the maximum pore distance between guard cells.

Stomatal density was quantified by counting stomata within a defined microscopic field of view (area = 0.1589 mm^2 at 400× magnification). The density was calculated using the formula:

$$\text{Density} = \text{Number of stomata/Field of view area}$$

Proline content determination

Proline (µmol g^{-1} fresh weight) was determined based on the technique (Bates *et al.*, 1973), in 0.5 g fresh leaves that have been mashed given 10 mL of 3% 5-sulfosalicylic acid, then filtered. The filtrate was then given 2 mL ninhydrin (2,2-dihydroxyindane-1,3-dione) and 2 mL glacial acetic acid, put in a tube, for one hour heated at 100°C (212.0°F) with the addition of 4 mL toluene. The extract solution turned dark red indicating proline content, measured by Milton Roy 2D Spectrophotometer, wavelength 520 nm. The value on the spectrophotometer was calculated by the formula:

$$\text{Proline content } (\mu\text{mol g}^{-1} \text{ fresh weight}) = (64.3649 \times \text{absorbance}) + (-5.2987 \times 0.347) \quad (2)$$

64.3649 = The slope value of the standard curve, which indicates the increase in proline content (µmol g^{-1}) per unit increase in absorbance.
Absorbance = Spectrophotometric measurement value that is directly proportional to the concentration of proline in the sample.

Assessment of stress tolerance index (STI)

The stress tolerance index (STI) quantifies shallot yield under salinity stress relative to yield under normal conditions. This index was calculated using the formula established by Hooshmandi (2019):

$$STI = (Hp \times Hs) / (\bar{H}p)^2 \quad (3)$$

where STI is stress tolerance index, Hp= Yield of a genotype under non-stressed conditions, Hs the yield of a genotype under stressed conditions, and Ȳp is Mean yield of all genotypes under non-stressed conditions.

Data analysis

Analysis of variance (ANOVA), was used in data analysis. Duncan's Multiple Range Test (DMRT) was then used on data significantly different at 5% standard error. Statistical data were processed using SPSS 26 supported by Microsoft Excel.

3. Results

The results show that salinity in several levels affects the morphological variables of shallots (Table 1, Fig. 1) . Plant height and root dry weight of shallots of Bali Karet varieties are 58.53 cm and 0.11 g plant^{-1} higher than Bima Brebes by 20.16% and 120%. While the leaf greenness of both varieties is not significantly different. Bali Karet variety is higher than Bima Brebes in all morphological parameters, indicating it is more tolerant to salinity stress. Airborne salinity treatment significantly reduces plant height, leaf greenness, and root dry weight variables with the highest values of 57.69 cm; 48.16;

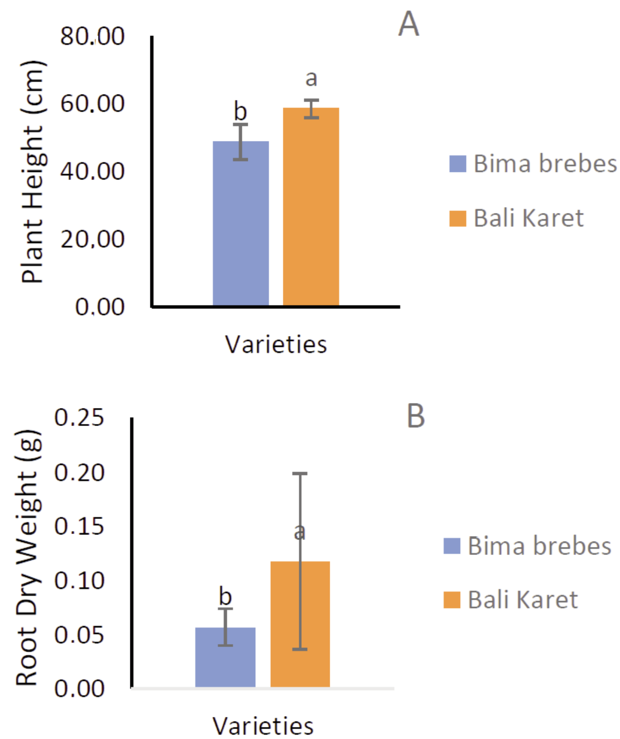

Fig. 1 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on plant height (A) and root dry weight (B). Data are expressed as the mean of determination ± SD in 3 replicates.

Table 1 - Varietal effect and air salinity on shallot morphology

Treatment	Plant height (cm)	Leaf greenness	Root dry weight (g plant^{-1})
Varieties (B)			
Bali Karet (B_1)	58.53±2.55 a	43.67±6.24 a	0.11±0.06 a
Bima Brebes (B_2)	48.71±4.68 b	44.18±3.36 a	0.05±0.01 b
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	57.69±5.18 a	48.16±2.43 a	0.12±0.08 a
6 mS cm^{-1} (A_1)	53.98±6.01 ab	45.00±4.17 ab	0.08±0.03 ab
12 mS cm^{-1} (A_2)	52.81±6.06 b	42.93±4.26 bc	0.08±0.04 ab
18 mS cm^{-1} (A_3)	49.99±6.49 b	39.57±4.71 c	0.06±0.03 b
Varieties (B) x Airborne salinity (A)			
B_1A_0	61.01±2.44 a	49.00±2.86 a	0.18±0.08 a
B_1A_1	59.30±1.60 ab	45.45±5.23 abc	0.10±0.03 b
B_1A_2	58.03±2.15 ab	42.53±5.96 bcd	0.10±0.04 b
B_1A_3	55.80±1.05 b	37.68±6.34 d	0.09±0.03 b
B_2A_0	54.37±5.30 b	47.34±2.35 ab	0.06±0.02 b
B_2A_1	48.68±1.76 c	44.56±3.93 abc	0.06±0.01 b
B_2A_2	47.60±2.34 c	43.34±3.06 bcd	0.06±0.02 b
B_2A_3	44.19±1.75 c	41.47±2.16 cd	0.05±0.00 b

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different (p<0.05).

and 0.12 g plant-1 at 0 mS cm-1 (A_0), respectively, with differences reaching 15.40%; 21.71%; and 100% at 18 mS cm-1 (A_3). The analysis of two shallot varieties at several levels of airborne salinity shows that Bali Karet and Bima Brebes varieties are slightly tolerant to airborne salinity and both have the same decreasing trend in morphology (plant height, leaf greenness, and root dry weight) as airborne salinity increases (Fig. 2); however, both varieties have different mechanisms to salinity stress.

In Table 2 and figure 3 can be observed that the shallot variety Bima Brebes has a value of 9.33 mg L^{-1} 13.69% greater than the value of chlorophyll a Bali Karet. In contrast, the Bali Karet variety has values of 6.85 mg L^{-1} and 16.19 mg L^{-1} respectively 86.14% and 11.58% greater than the chlorophyll b and total values of Bima Brebes. Physiological characteristics were significantly affected by the level of airborne salinity in chlorophyll a, b, and total variables (Fig. 4) with the highest values of 12.28 mg L^{-1}; 8.54 mg L^{-1}; and 20.83 mg L^{-1} at 0 mS cm^{-1} (A_0), these values were 57.44%; 288.18%; and 108.09% higher than the 18 mS cm^{-1} treatment (A_3). Considering the results of the two varieties under escalating airborne salinity, Bali Karet and Bima Brebes deploy contrasting chlorophyll strategies. Bali Karet boosts chlorophyll b to maximize light harvesting for growth, while Bima Brebes prioritizes chlorophyll a to protect

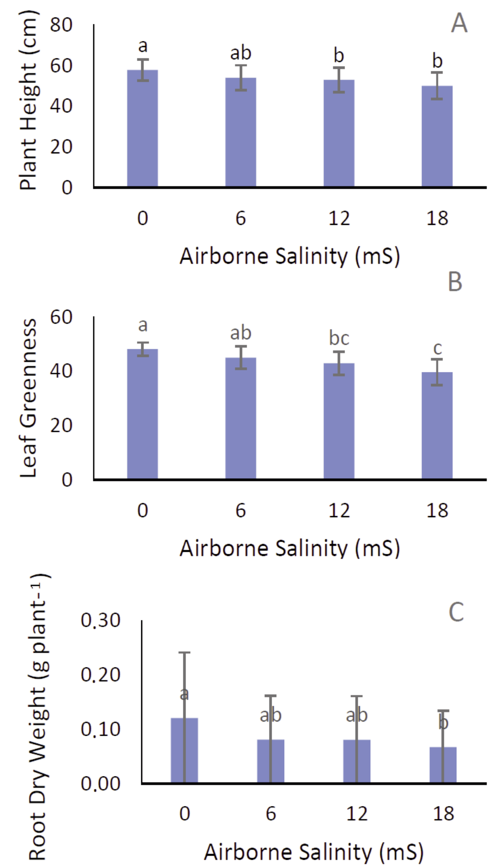

Fig. 2 - Effects of airborne salinity (0, 6, 12, and 18 mS) on plant height (A), leaf greenness (B), and root dry weight (C). Data are expressed as the mean of determination ± SD in 3 replicates.

Table 2 - Varietal effect and air salinity on shallot physiology (chlorophyll)

Treatments	Chlorophyll a (mg L-1)	Chlorophyll b (mg L^{-1})	Total chlorophyll (mg L^{-1})
Varieties (B)			
Bali Karet (B_1)	9.33±2.35 b	6.85±4.52 a	16.19±5.13 a
Bima Brebes (B_2)	10.81±2.61 a	3.68±2.58 b	14.51±4.40 a
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	12.28±2.33 a	8.54±4.22 a	20.83±2.70 a
6 mS cm^{-1} (A_1)	10.88±1.66 ab	5.54±3.76 ab	16.43±2.56 b
12 mS cm^{-1} (A_2)	9.32±1.41 bc	4.78±3.63 ab	14.12±3.19 bc
18 mS cm^{-1} (A_3)	7.80±2.47 c	2.20±1.40 b	10.01±2.54 c
Varieties (B) x Airborne salinity (A)			
B_1A_0	10.88±2.49 bc	11.13±2.50 a	22.01±1.20 a
B_1A_1	10.25±1.88 bcd	7.04±5.30 b	17.29±3.49 bc
B_1A_2	8.71±1.65 bcd	6.17±5.19 b	14.89±4.63 cd
B_1A_3	7.50±2.71 d	3.07±1.19 bc	10.58±2.74 de
B_2A_0	13.68±1.22 a	5.97±4.27 b	19.65±3.56 ab
B_2A_1	11.52±1.47 ab	4.05±0.69 bc	15.57±1.44 bc
B_2A_2	9.94±1.07 bcd	3.40±0.54 bc	13.35±1.47 cde
B_2A_3	8.11±2.75 cd	1.34±1.11 c	9.45±2.76 e

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different (p<0.05).

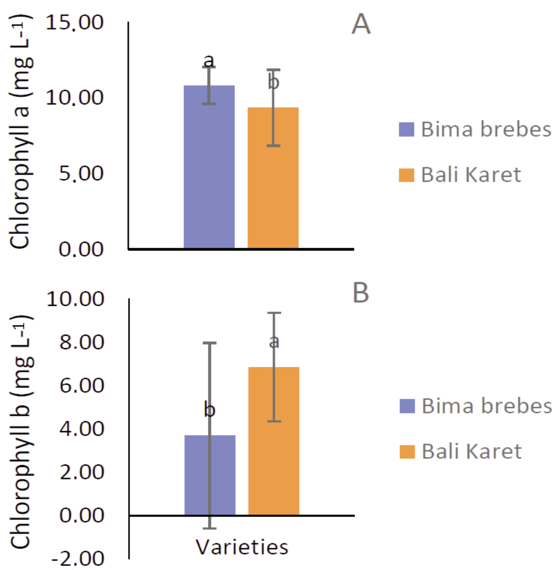

Fig. 3 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on chlorophyll a (A) and chlorophyll b (B). Data are expressed as the mean of determination ± SD in 3 replicates.

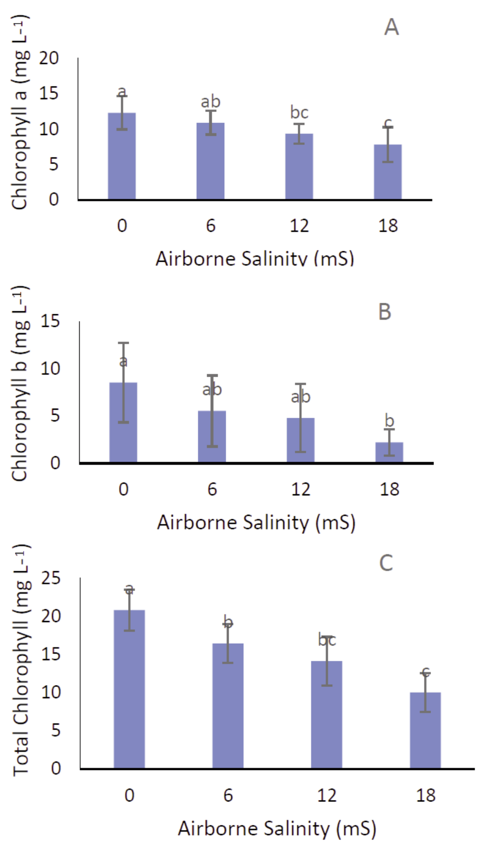

Fig. 4 - Effects of airborne salinity (0, 6, 12, and 18 mS) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C). Data are expressed as the mean of determination ± SD in 3 replicates.

photosynthetic reaction centers. This reflects a fundamental trade-off between photon capture (Bali Karet) and photochemical resilience (Bima Brebes), a divergence critical for variety-specific airborne salinity adaptation.

Table 3 shows that the use of different varieties on stomatal physiology impacts only density of stomata, with the variety of Bima Brebes having a stomatal density of 55.55 stomatal mm^{-2} greater 17.79% than the variety of Bali Karet. No significant differences are found on stomatal opening and proline content. Nevertheless, it can be noticed that Bali Karet variety has a value of 3.58 µm 4.68% greater than Bima Brebes on stomatal opening, and that Bali Karet variety has a value of 1.15 µmol g^{-1} fresh weight 19.01% lower than Bima Brebes on proline content. Several treatments at the airborne salinity level had an effect on decreasing stomatal opening and density and increasing proline. The highest value of stomal opening and stomatal density are 4.66 µm and 58.70 stomatal.mm^{-2} at 0 mS cm^{-1}, 100% and 36.61% surpassed the 18 mS cm^{-1} treatment. In opposite, the highest value on proline 2.24 µmol g^{-1} fresh weight fresh leaves at 18 mS cm^{-1} on proline up to 397.78% greater than the control (0 mS cm^{-1}). In the physiological characteristics, the interaction between the use of different varieties and the level of airborne salinity influenced considerably stomatal mechanism of stomatal opening and stomatal density with the highest values of 5.33 µm and 54.50 stomatal mm^{-2} (B_1A_0), respectively 128.76% and 52.96% surpassed B_1A_3 and B_2A_3 on stomatal opening and B_1A_3 on stomatal density (Fig. 5). Although there was no interaction on proline between the use of two shallot varieties and airborne salinity at several levels, it can be observed that the Bima Brebes variety accumulated higher proline than Bali Karet with the same increasing trend. This shows the type of adaptation of Bima Brebes on cellular adaptation, compared to Bali Karet which focuses on growth optimization (Fig. 6).

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

Table 4 shows that there is an influence of both varieties on yield characteristics, variable number of bulbs per clump Bima Brebes 6.66 pieces greater 55.61% than Bali Karet. In fresh bulb weight per clump on the contrary, Bali Karet has a value of 45.38

Table 3 - Varietal effect and air salinity on shallot physiology (stomatal and proline)

Treatment	Stomatal opening (µm)	Stomatal density (Stomatal mm^{-2})	Proline (µmol g^{-1} fresh weight)
Varieties (B)			
Bali Karet (B_1)	3.58±1.31 a	47.16±8.69 b	1.15±0.78 a
Bima Brebes (B_2)	3.42±0.90 a	55.55±6.48 a	1.42±0.92 a
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	4.66± 0.82a	58.70±6.50 a	0.45±0.38 c
6 mS cm^{-1} (A_1)	4.16±0.41 a	52.41±8.59 ab	0.95±0.32 bc
12 mS cm^{-1} (A_2)	2.83±0.41 b	51.36±2.57 b	1.47±0.44 ab
18 mS cm^{-1} (A_3)	2.33±0.52 b	42.97±8.36 c	2.24±0.87 a
Varieties (B) x Airborne salinity (A)			
B_1A_0	5.33±0.58 a	54.50±3.63 abc	0.21±0.05 d
B_1A_1	4.00±0.00 abc	46.12±7.26 c	0.91±0.33 bcd
B_1A_2	2.66±0.58 cd	52.41±3.63 bc	1.53±0.30 abc
B_1A_3	2.33±0.58 d	35.63±3.63 d	1.94±0.76 ab
B_2A_0	4.00±0.00 abc	62.89±6.29 a	0.71±0.42 cd
B_2A_1	4.33±0.58 ab	58.70±3.63 ab	1.00±0.37 bcd
B_2A_2	3.00±0.00 bcd	50.31±0.00 bc	1.42±0.61 bc
B_2A_3	2.33±0.58 d	50.31±0.00 bc	2.55±1.03 a

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

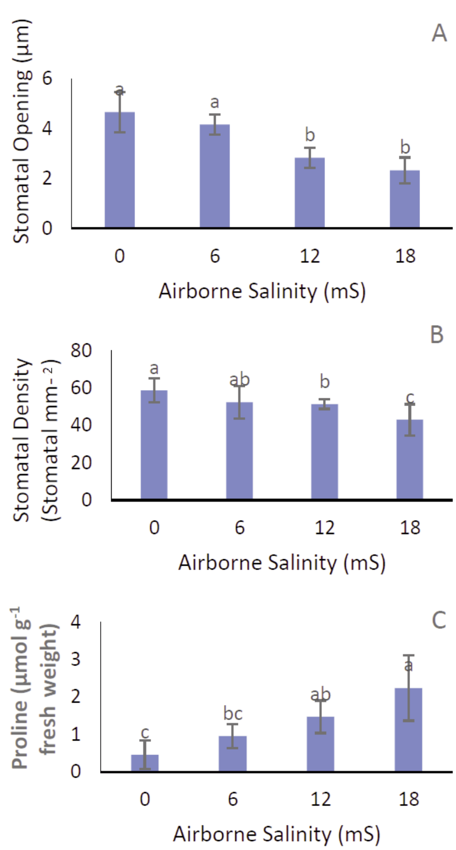

Fig. 5 - Effects of airborne salinity (0, 6, 12, and 18 mS) on stomatal opening (A), stomatal density (B), and proline (C). Data are expressed as the mean of determination ± SD in 3 replicates.

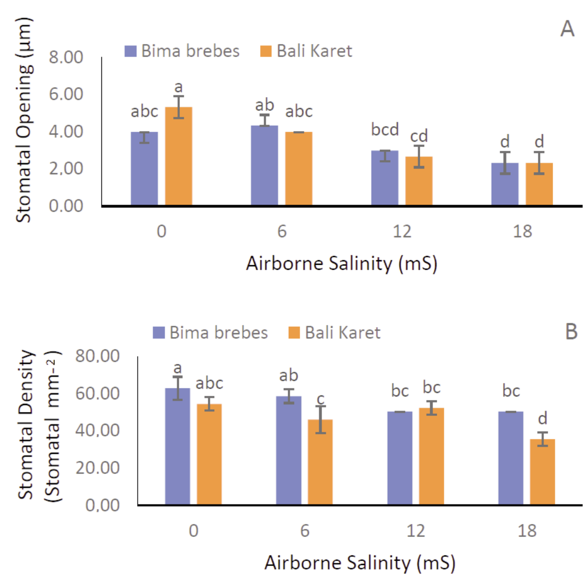

Fig. 6 - Interaction of different varieties (B1= Bali Karet, B2= Bima Brebes) with different levels of airborne salinity (0, 6, 12, and 18 mS) on stomatal opening (A) and stomatal density (B). Data are expressed as the mean of determination ± SD in 3 replicates.

g, 95.09% greater than Bima Brebes. The yield parameter in the airborne salinity treatment in the research conducted had no effect. The interaction of two shallot varieties at several levels of airborne salinity was not significant on yield. Bali Karet variety

Table 4 - Varietal effect and air salinity on shallot yield

Treatment	Number of bulbs per clump	Fresh bulb weight per clump (g)
Varieties (B)		
Bali Karet (B_1)	4.28±0.77 b	45.38±11.15 a
Bima Brebes (B_2)	6.66±1.23 a	23.26±10.46 b
Airborne salinity (A)		
0 mS cm^{-1} (A_0)	4.80±0.49 a	41.17±22.01 a
6 mS cm^{-1} (A_1)	5.90±1.52 a	32.78±14.57 a
12 mS cm^{-1} (A_2)	5.47±2.15 a	33.08±15.50 a
18 mS cm^{-1} (A_3)	5.73±1.85 a	30.27±9.15 a
Varieties (B) x Airborne salinity (A)		
B_1A_0	4.40±0.35 b	54.68±12.97 a
B_1A_1	4.67±0.42 b	43.85±12.53 abc
B_1A_2	3.73±1.21 b	45.49±10.19 ab
B_1A_3	4.33±0.92 b	37.51±5.36 abcd
B_2A_0	5.20±0.00 b	27.65±22.26 bcd
B_2A_1	7.13±1.01 a	21.71±2.34 cd
B_2A_2	7.20±1.06 a	20.66±5.88 d
B_2A_3	7.13±1.36 a	23.03±4.81 cd

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different (p<0.05).

has an escape response shown in fresh bulb weight per clump which is higher than Bima Brebes, although Bima Brebes is higher in the number of bulbs per clump due to the defense response from airborne salinity stress (Fig. 7).

Table 5 show that shallot varieties Bali Karet and Bima Brebes were medium tolerant variety (mt) on 6, 12, and 18 mS cm^{-1} airborne salinity. This shows the ability of both varieties to tolerate salinity stress, but have different response mechanisms. The responses of the two varieties to physiology, morphology, and yield are shown in Tables 1-4.

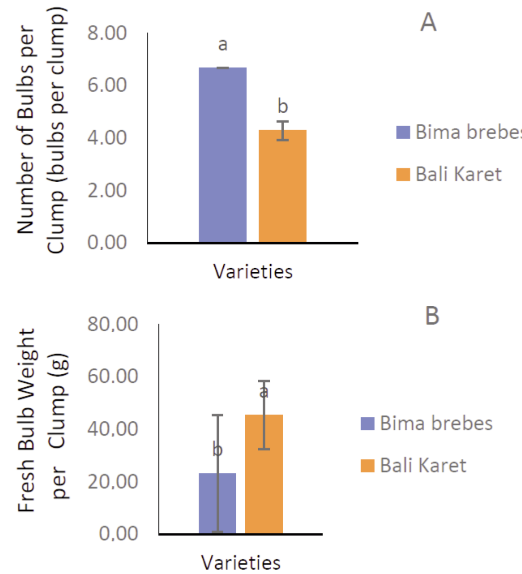

Fig. 7 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on number of bulbs per clump (A) and fresh bulb weight per clump (B). Data are expressed as the mean of determination ± SD in 3 replicates.

4. Discussion and Conclusions

Salinity on certain levels can affect morphology, physiology and yield in plants. According to Shokat and Großkinsky (2019), salinity stress is one of the major problems in agriculture studied globally. Dry weight loss is one of the signs that plant growth is affected by salinity (Suharjo *et al.*, 2021). The results in Table 1 show that the higher the airborne salinity, the lower the morphological variables in shallots. Salinity determines the ability of plants to grow because it can damage cells. High salinity levels affect water uptake by plants due to salt around the plant roots, which causes oxidative stress (Anwar *et al.*, 2024), prolongs shoot emergence, slows leaf growth, reduces plant height, changes the form of tubers, and reduces their overall mass and size (Alam *et al.*, 2023).

Table 5 - Varieties effect and airborne salinity on stress tolerance index

Variables	Varieties	Airborne salinity (mS cm^{-1})		
		6	12	18
Stress tolerance index	Bali Karet	0.802 (mt)	0.832 (mt)	0.686 (mt)
	Bima Brebes	0.785 (mt)	0.747 (mt)	0.833 (mt)

Stress Tolerance Index <0.5 sensitive variety (tt), 0.5-1.0 medium tolerant variety (mt). STI >1 tolerant variety (t) (Saparso *et al.*, 2024).

This research found that higher airborne salinity reduced morphological characteristics such as plant height, leaf greenness, and root dry weight. Those effects may be explained by the fact that saline environments generally have the same or even higher osmotic pressure than in plant cells, which can inhibit water from entering plant cells. Water flows from areas of low osmotic pressure to areas of higher osmotic pressure, causing plants in saline conditions to experience water stress. In *Allium cepa*, stress inhibits cell division so that the number of new cells is reduced and the meristem shrinks in size (Kielkowska, 2017). Osmotic pressure also affects the speed at which cells absorb nutrients (Zainuddin *et al*., 2017). Compared to the control, root fresh weight in pomegranate cultivars decreased by 46.3%, 57.4%, and 66% under 6, 9, and 12 dS m^{-1} salinity treatments, respectively, while root dry weight decreased by 45.4%, 52.5%, and 59% at the same salinity levels (Jadidi *et al*., 2020). Salinity stress significantly reduced pepper plant height (Badem and Söylemez, 2022), leaf greenness values were lower under higher NaCl stress (Rustikawati *et al*., 2023). According to research by Kul *et al*. (2021), water salinity caused 22.0% decrease in root fresh weight and 36.0% decrease in root dry weight of tomato compared to non-saline control and unamended control. Plants have evolved biochemical and molecular mechanisms, which work in sync as an integrated physiological response to soil salinity (Ruiz-Lozano *et al*., 2012). High salinity reduces crop production, subsequent growth, and cause physiological defects threatening global food security and prosperity (Balasubramaniam *et al*., 2023).

Furthermore, high salinity environments can damage plant membranes and chlorophyll in *Zea mays* and *Cyperus rotundus*, causing disturbances in nutrient absorption due to disturbed ion balance in plant roots (Pranasari *et al*., 2012). The accumulation of Na^+ and Cl^- in tissues disrupts enzyme function, photosynthesis, and cell division, especially in young leaves (Munns and Tester, 2008). Other responses in plants include selective buildup or exclusion of salt ions as maintenance on the photosynthesis process to reach adequate values for plant growth, changes in membrane structure, and phytohormone synthesis (Türkan and Demiral, 2009). The present study shows that as increasing airborne salinity from 0 to 18 mS cm^{-1} decreased total chlorophyll by 20.83, 16.43, 14.12, 10.01 mg L^{-1} recpectively. The highest value of stomal opening and stomatal density are 4.66 µm and 58.70 stomatal.mm^{-2} at 0 mS cm^{-1}, 100% and 36.61% surpassed the 18 mS cm^{-1} treatment. Accordingly, the results of research by Fakhri and Ekawati (2020), explained that different salinities had a significant effect on the chlorophyll a content in *Dunaliella* sp., an increase in salinity from 15 to 35 ppt caused a 32.65% decrease in chlorophyll a content with the highest concentration (11.27 mg L^{-1}) produced at 15 ppt salinity. Salinity inhibits the osmotic uptake of water, which negatively affects the carbon assimilation process, salinity decreases photosynthetic rate, transpiration, stomatal conductance and chlorophyll levels in plants, affecting the ability of plants to photosynthesize optimally (Ashraf and Ali, 2008). Salinity stress lowers the osmotic potential of the soil solution reducing the availability of water for plants and increasing the concentration of ions that are toxic to plants (Anugrah *et al*., 2022). Plants have mechanisms to deal with stress. Exposed to salinity stress on plants, stomatal will be closed to protect against water loss, leading to increased leaf temperature, salinity-induced stress resulting in stomatal regulation, with strategies to cope with ionic and osmotic pressures induced by NaCl (Orzechowska *et al*., 2021).

Accumulations of cytotoxic-dependent toxic ions such as Na^+ and Cl^- and formation of reactive oxygen species (ROS), can occur due to salinity stress disrupting plant development and growth through water stress (Isayenkov, 2012). Under conditions of oxidative stress, changes in cell metabolic processes occur, causing the production of ROS to increase excessively, damaging proteins, fats, nucleic acids, and can cause plant cell death (Ahmad *et al*., 2019). Plants activate antioxidants (SOD, CAT) and accumulate compatible solutes (proline, glycine betaine) for mitigation (Hasegawa *et al*., 2000). According to the research Saparso *et al*. (2023), higher proline content makes plants more tolerant of air salinity stress, proline content in the plant increases the higher the level of air salinity applied, where the highest proline content of corn plants treatment of 18 mS air salinity, which is 3.58 µmol g^{-1} and the lowest proline content in the treatment of 0 mS air salinity, which is 1.75 µmol g^{-1}. This is consistent with the results of this study, that increased exposure to airborne salinity increases proline levels. The proline functions as an osmolyte helping to maintain osmotic balance in plant cells, at high salinity water tends to escape from cells due to differences in ion concentration, the presence, accumulation of proline so that plant cells can draw water in, prevent dehydration and maintain cell turgor. According to Khanna-Chopra *et al*. (2019), plants produce proline and accumulate in the cytosol, in response to stresses such as salinity, to modify the osmotic properties of the cytoplasm thereby increasing tolerance in plants. However, it is also known that proline can increase the resistance and growth ability of plants under stressful conditions, such as high salinity. Increase in proline under salinity stress as extra Nitrogen (N) and energy storage achieved through salinity-induced growth reduction for plant survival and growth under stress conditions (Kubala *et al*., 2015).

Table 3 and 5 indicate that the increase in proline due to exposure to 6 mS cm^{-1} to 18 mS cm^{-1} represents the ability of both varieties to maintain cell osmoregulators so as not to cause physiological and metabolic plant stress. According to Ayub *et al*. (2015), high proline in plants tolerant of environmental stress plays a role in regulating plant cell osmoregulators. The defense mechanism from cell damage due to ROS as free radicals, plants respond through the antioxidant defense system (Denaxa *et al*., 2020). Proline plays a very important role in reducing the negative effects of plant salinity stress by neutralizing free radicals formed due to increased ROS. Plants have enzymatic and non-enzymatic antioxidant defense systems, which play an important role in detoxifying ROS generated under stress conditions, it is known that proline acts as an enzyme protector and ROS antioxidant. (Khatun *et al*., 2020). According to Silva-Ortega *et al*. (2008), proline accumulates dominantly in leaves to maintain chlorophyll levels and cell turgor pressure, which is crucial for preserving photosynthetic productivity when facing salinity stress. Accumulation of proline in stressed plants occurs both through induction of proline bio-synthesizing gene expression (P5CR and P5CS) and by inhibition of genes associated with the degradation pathway.

Under osmotic stress conditions, proline synthesis is mediated by the enzymes encoded by the P5CS and P5CR genes in most plants (Furlan *et al.,* 2020).

Salinity has three effects on crop growth and yield in the form of ion unbalance, ionic and osmotic stress (Anshori *et al*., 2018). However, experiment results reported in this study showed that airborne salinity had no effect on shallot production. There was a 19.38% decrease in the number of bulbs per clump in the comparison of control and 18 mS cm^{-1} treatment, in addition to the fresh bulb weight per clump control was 26.48% greater than 18 mS cm^{-1} treatment, but both parameters were not significantly different. This is in line with research Saparso *et al.* (2024), in cauliflower and cabbage, unlike the physiological response, plants in the air salinity level treatment had no impact on yield. Salinity conditions affect plant nutrient uptake due to the presence of excess Na^+ and Cl^- ions that prevent the uptake of NO_3^-, Ca_2^+, and K^+ ions respectively (Kharisun *et al*., 2022), the decrease may be due to the fact that these elements are very important in the initiation of bulbs (Mardhiana *et al*., 2018). In research, some crops showed a decrease in yield due to salinity. Tomato yield decreased by 7.2% at 5 mS cm^{-1} salinity and increased at higher salinities (Zhang *et al*., 2016). Research on Onion Granex 33 variety against 6 NaCl concentrations showed that increasing NaCl concentration resulted in a decrease in the fresh weight of mature plant bulbs, even plants could not survive at 125 mM NaCl concentration (Ratnarajah and Gnanachelvam, 2021). According to research by Syamsiyah *et al*. (2020), high salinity levels did not significantly affect yield components such as growth, yield, number of tubers, fresh and dry tuber weight of local shallots of Brebes and Purbalingga which varieties are tolerant of salinity up to salinity levels of 3 mS cm^{-1}. Meanwhile, this study shows that exposure to airborne salinity at the highest level up to 18 mS cm^{-1} (A_3) does not significantly affect the yield of shallots Bima Brebes and Bali Karet on the variable number of bulbs per clump and fresh bulb weight per clump. In this study, it can be said that airborne salinity stress does not affect the yield of shallot varieties of Bima Brebes and Bali Karet because both varieties are tolerant and able to adapt to certain levels of salinity. This adaptability allows them to maintain physiological and morphological stability, resulting in consistent yields despite saline conditions. Bali Karet variety has an increased response on growth variables, while Bima Brebes has a response on physiological variables. The results of research by Hadianti and Damanhuri (2019), that the six varieties of shallots: Bima Brebes, Bauji, Super Philip, Tajuk, Katumi, and Trisula are tolerant of high salinity concentrations, at ppm 12,000 experiencing severe stress. Sidabariba and Sudjatmiko (2023), stated that with its advantages, the Bali Karet (Batu Ijo) variety can adapt well to its growing environment.

A schematic representation of the different mechanisms performed by the two shallot varieties at increasing salinity levels is shown in figure 8.

This study shows that both varieties are medium tolerant (mt) or tolerant (t) to 18 mS cm^{-1} airborne salinity for most of the parameters, except that for total chlorophyll. More in detail, the following differences in the performances of the two varieties can be highlighted, where Bali Karet and Bima Brebes are tolerant to airborne salinity, but both have different response mechanisms to airborne salinity stress. The Bali Karet variety increases the ability to optimize growth, indicating escape type adaptation with a focus on short term productivity (higher plant height and root dry weight) and bulb weight; while the Bima Brebes variety reflects cellular defense based tolerance type adaptation (higher chlorophyll a and stomatal density) and number of bulbs. Of course different level of salinity determined different responses. Interestingly, both varieties of Bali Karet and Bima Brebes show a tolerance mechanism through increased osmoregulators with an increase in proline at the highest airborne salinity concentration (18 mS cm^{-1}).

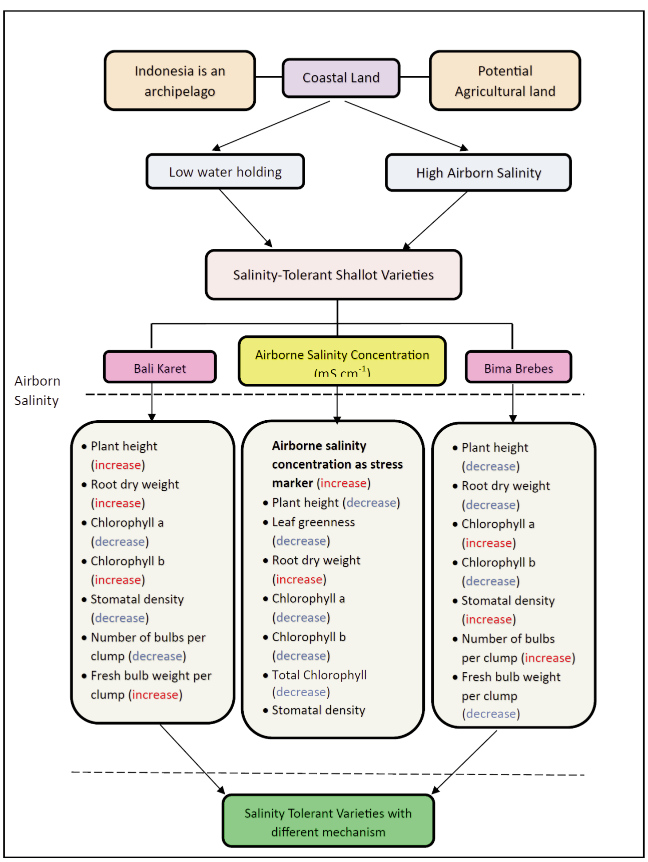

Fig. 8 - Effect of airborne salinity on morphology, physiology, and yield parameters in two shallot varieties.

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