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# Identifying Drought-Tolerant Impatiens Genotypes by Using Water Stress Treatment

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## Abstract

The drought-tolerant Impatiens genotypes are known for their resistance to limited or stressed water. The study aimed to identify drought-tolerance of Impatiens clones. The experiment used a split-plot design replicated three times, with the water stress treatment as the main plot and Impatiens clones as subplots. The main plot consists of 100% and 60% of field capacity. The subplots consist of five Impatiens clones, 17.12; 12; 33.3; 40 B and Impatiens cv of Impala Agrihorti as a control. The results showed that 60% field capacity decreased morphological and physiological traits. Still, the drought-tolerant clones were less affected by the stress and produced more flowers than the others. The most drought-tolerant Impatiens was clone 12. The mechanism of drought tolerance Impatiens was by stomatal closure when the humidity in the growing medium was decreasing. The stomata closure did not significantly reduce the fresh and dry weight in drought-tolerant plants, but it affected the delay in flower initiation. The plant accumulated assimilate for plant height and diameter growth but is not sufficient for generative initiation. They assimilate in the vegetative phase and can be used as sources for flower formation, which show no significant decrease in the number of flowers. The study implies that the drought-tolerant Impatiens clones can be used as genotype sources for drought-tolerant or can be released as new varieties of Impatiens for landscape plants with the superiority in having drought tolerant.

**Keywords:** abiotic stress; morpho-physiological characters; stress tolerance index

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## INTRODUCTION

The water use efficiency in the agricultural sector needs to be done to ensure food security in the following years (Zuccarini et al., 2020). Nowadays, water use efficiency has become an essential aspect of agriculture since the world is facing climate change. Climate change is an anomaly of atmospheric conditions and sudden unexpected climatic events, such as hurricanes, floods, severe and/or durational droughts and extreme temperatures (Gulser and Cig, 2021). Climate change has a negative effect on plants (Pareek et al., 2020). This condition influences physiological and metabolic activities of plants, the degradation of plant growth and productivity and pest attacks (Malhi et al., 2021). Plants, particularly ornamentals plants, can be utilized for natural landscapes (controlling erosion, reducing energy for climate and air consumption, and controlling erosion) as well as for recreational areas, interiors and commercial sites. Many ornamental species have suitable genotypes that can cope with drought stress and can be used for landscape (Savé, 2009). Different genotypes may respond to water stress differently, especially

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under limited irrigation conditions (Giordano et al., 2021). Identification of drought-tolerance ornamentals supports a sustainable agricultural system.

Impatiens are native to Eurasia, North and Central America, New Guinea, tropical Africa and Madagascar, most concentrated in the tropics and subtropics areas (Yu et al., 2015; Hassemer and Pereira dos Santos, 2017). Naturally, Impatiens are also forest plants (Čuda et al., 2016; 2017) and they can remediate soil contamination such as lead (Pb), polychlorinated biphenyls (PCBs) (Liu et al., 2020) and tin (Sn) (Liu et al., 2021). Meanwhile, New Guinea Impatiens are perennial evergreen herbs with a long flowering period, rich flower colors and high ornamental value. Therefore, the flowers become important put in pots and flower beds (Zhang et al., 2021). Impatiens also have the potential for landscaping ornamentals as flowers in pots and flower beds.

As an ornamental plant in landscaping, the beautiful Impatiens are seen from decorative traits and long flowering period (Nurul et al., 2010). According to Hassemer and Pereira dos Santos (2017), Impatiens are highly valued for their ornamental properties, principally the largeness, showy flowers, and relatively easy culture. Naturally, Impatiens are commonly cultivated rainy season (Dikshit and Girjesh, 2007). In the long term, the beauty of Impatiens needs to be maintained. However, Impatiens often drop their leaves and flowers or cannot produce flowers when drought stress occurs (Kaczperski and Carlson, 1989).

According to Antonic et al. (2020), abiotic stress such as drought has detrimental effects on Impatiens growth and development. This is the main problem in commercial production and market placement of these plants. Drought is a factor that decreases plant growth, development (Đurić et al., 2020), and flowering (Descamps et al., 2021) in Impatiens. Heat stress can also be caused by drought stress. Increasing temperature or prolonged exposure to heat stress could significantly affect insect survival, growth and development (Gou et al., 2020). Meanwhile, *Impatiens hawkeri* was crossed with *I. platypetala* to produce New Guinea Impatiens, whereas it was a heat and drought-resistant cultivar (Stephens, 1998). Several clones have been made from crosses of *I. hawkeri* and *I. platypetala*. To date, Indonesia has produced the crosses of *I. hawkeri* and *I. platypetala,* such as Impala Agrihorti variety. The drought tolerance of Impala has been tested the paraquat method (Soehendi et al., 2022). However, some tolerant genotypes of Impatiens are still very much needed. Therefore, several clones need to be tested for their superiority to drought stress.

A reported study has identified the drought tolerance of Impatiens cultivars. The exogenous salicylic acid (SA) counteracted the effects of polyethene glycol (PEG) used to select Impatiens drought tolerance through *in vitro* culture (Antonić et al., 2016). In addition, the selection of drought stress-tolerant Impatiens and the other ornamentals could be made using the method of managing watering frequency (Heschel and Riginos, 2005; Riaz et al., 2013; Cirillo et al., 2014; Tribulato et al*.*, 2019). This method is considered the easiest and can be directly applied to the field. However, no study identified the drought tolerance of Impatiens clones or varieties in Indonesia using water stress treatment. Therefore, the study aimed to determine the drought tolerance of Impatiens clones using water stress treatment. The hypotheses are there is new genotype that is more drought-tolerant than Impala Agrihorti. Therefore, to obtain the drought-tolerant Impatiens for landscape plant in a drought area.

## MATERIALS AND METHOD

### Plant materials

The study was conducted in the screenhouse at the experimental station of the Indonesian Ornamental Crops Research Institute, Cianjur, West Java, Indonesia, at an altitude of 1,100 m above sea levels from May to December 2021. The plant materials were five genotypes of Impatiens, including clones 17.12; 12; 33.3; 40 B and Impala Agrihorti as a control. Four weeks of root cuttings of Impatiens with 4 to 5 leaves were used in the experiment. These cuttings were transplanted into pots at the four-leaf stage (17 cm diameter by 20 cm height) filled with 2 kg of soil, rice husk and bamboo leaf (1:1:1, by volume) substrate. After transferred to the pots, the plants were irrigated at level field capacity for two weeks to prevent drought and establish the plant well. The plants were fertilized with completely solid fertilizers as much as 0.5 g $l^{-1}$ (N:P:K = 20:20:20) every two weeks before treatments. The plants were fertilized at 2 up to 12 weeks after planting (WAP).

### Soil properties and field capacity measurement method

The field capacity to determine the watering volume was done by pouring the planting media in polybags with water until it dripped (saturated). Then, the media were allowed to stand for three days until no water dripping. Next, the planting media were measured for the fresh weight and dry weight. The new fresh weight was measured after no water dripped from the polybag, while the new dry weight was estimated after the growing media were dried at 100 °C for 24 hours until a constant weight was obtained. The field capacity of 100% was determined using a formula by Hendriyani and Setiari (2009) (Equation 1).

$$FC = \frac{FW\text{-}DW}{DW} \times 100\% \qquad (1)$$

Where: FC = field capacity, FW = fresh weight, and DW = dry weight. Furthermore, the plant was watered every three days until 12 weeks.

**Experimental design**

The study laid a split-plot design with three replications. There were 10 plants in every unit of treatment. Field capacity level and genotypes were the main plot and subplot factors, respectively. Field capacity levels included 100% and 60%. This study used five genotype clones, number 17.12; 12; 33.3; 40 B and Impala Agrihorti as a control.

**Observation variables**

*Environmental*

The temperature, relative humidity and light intensity during treatment were measured every day at 7 a.m., 12 p.m., and 5 p.m., and averaged every two weeks. The temperature and relative humidity were measured by digital thermometer. The light intensity was evaluated by luxmeter on the top of plant canopy. Meanwhile, soil moisture and soil temperature were assessed every three days immediately after treatment, then averaged every 2 weeks up to 12 WAP. The soil moisture and soil temperature were estimated by soil moisture tester inserted into medium.

*Morphology variables*

The morphological variables include plant height, stem diameter, flower initiation, number of flowers and flower diameter, which were measured as proposed by Safari et al. (2022). The plant height was measured from the base of the stem to the top of the plant. The stem diameter was measured at 5 cm from the base of the stem. The flower initiation was noted when the first-time flower bud size reached 2 mm. The number of flowers was calculated when the flower fully opened. The flower diameter was measured by the length from the left up to the right. These variables were evaluated at 12 WAP.

*Physiology variables*

The physiological variables include plant dry weight, chlorophyll content on SPAD, stomata characters, and proline. These characters were measured at 12 WAP. The fresh weight of all plants was estimated and subsequently dried at 70 °C for 48 hours, after which the dried samples were measured (Gaur et al., 2000). The chlorophyll was measured using the Minolta SPAD meter to evaluate 10 random leaves per plant and the mean value was recorded (Smith et al., 2004). Following Savvides et al. (2012), the stomata measurement variables, which include the width of stomata pore aperture, length of stomata and the number of stomata, were estimated. The proline was measured following the Bates methods (Bates, 1973). The 0.5 g leaves were extracted in 10 ml of 3% sulfuric acid and then the extract was filtered.

Meanwhile, a 1.25 g of ninhydrin was dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 M of $H_3PO_4$ until dissolved. Furthermore, it was cooled and stored at a temperature of 40 °C. Then, 2 ml of filtrate was reacted with 2 ml of ninhydrin acid solution and 2 ml of glacial acetic acid in the test tube for 1 hour at 100 °C. The reaction process ended in an “ice bath”. The mixture was then extracted with 4 ml of toluene and firmly soaked using a “test-tube stirrer” for 15 to 20 seconds. This extract was then assessed at 520 nm with a spectrophotometer. The proline content in µm $g^{-1}$ unit was determined using the formula = (64.3649 x A520 nm – 5.298) x 0.347.

*Drought tolerance index*

All variables were observed under normal and stress conditions to calculate the level of drought tolerance based on the stress tolerance index (STI) by Fernandez (1991) (Equation 2) and Yield Stability Index (YSI) by Bouslama and Schapaugh (1984) (Equation 3).

$$STI = \frac{(Ys \times Yp)}{mYp2} \qquad (2)$$

$$YSI = \frac{Ys}{Yp} \qquad (3)$$

Where: Ys = character value of a cultivar under stress (60% field capacity); Yp = character values of a cultivar in non-stress conditions (100%

field capacity); mYs = average of a character under stress conditions; mYp = the average of a character in normal (non-stress) conditions.

**Data analysis**

One-way ANOVA assessed statistical differences between treatments while the mean differences were compared using the Duncan Multiple Range test (DMRT), with a statistical significance of $p \leq 0.05$. The analysis statistic used SAS program.

## RESULTS AND DISCUSSION

**Environmental condition**

The ideal conditions for Impatiens growth in seedlings stadia are a temperature of 24.3 to 25 °C for 10 to 14-day-old cuttings and 23.0 to 24.0 °C for 16- to 20-day-old cuttings (Dreesen and Langhans, 1992). The optimum cultivation of net photosynthesis was at 24 to 32 °C and the light saturation was reached at light intensities above 700 mol photons $m^{-2}$ $s^{-1}$ (Schmitz and Dericks, 2010). The temperature during the study ranged from 21.50 to 26.50 °C, while the relative humidity was 45.96% to 64% (Table 1).

Soil moisture is one indicator that can determine water availability in the soil. There was no interaction between genotypes and watering treatment for soil moisture. The watering treatment started at 2 WAP. Furthermore, the observation of soil moisture started at 4 WAP. The range of watering treatment in 100% and 60%. The field capacity ranged from 82.02% to 95.02% and 74.18% to 87.87%, respectively. The genotype factor was not influenced by soil moisture at 4 to 12 WAP (Table 2), and also soil temperature at 4 to 12 WAP (Table 3).

**Plant morphology variables**

The interaction did not occur between watering treatment and genotype on the plant morphology variables, including stem diameter, plant height, flower bud initiation, flower diameter and the number of flowers. Impala Agrihorti was used as a control with relatively high plant, larger stem diameter, faster flower initiation time, a large number of flowers and large flower diameter compared to other tested genotypes in watering treatment in 100% field capacity. The treatment of watering plants with 60% field capacity did not significantly reduce the stem diameter and number of flowers in Impala and even the initiation of flowering was faster. In the other genotypes tested, clone 12 had characters that are close to Impala. There was no reduction in stem diameter, plant height, flower number and flower diameter compared to other genotypes (Table 4). According to Schmitz and Dericks (2010), Impatiens would reach maximal height under optimal soil conditions. In the genotype of drought resistance, there was a reduction in flowering indexes under drought stress observed in Impatiens as bedding plants (Chylinsi et al., 2007). Also, there was a decrease of plant weight associated with the loss of water from leaves during drought stress (Heidari et al., 2019).

Table 1. Environmental conditions during water stress treatment in Impatiens

| Variables | WAP | | | | | | Average |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 | |
| Air temperature (°C) | 21.50 | 26.50 | 24.10 | 25.30 | 26.04 | 25.67 | 24.79 |
| Relative humidity (%) | 64.00 | 58.05 | 62.50 | 52.05 | 45.96 | 55.25 | 56.37 |

Table 2. Soil moisture during water stress treatment in Impatiens

| Treatment | WAP | | | | |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 |
| Watering | | | | | |
| Field capacity of 100% | 82.02a | 82.20a | 95.02a | 84.98a | 86.15a |
| Field capacity of 60% | 74.18a | 73.35a | 87.87a | 77.95a | 77.06a |
| Genotype | | | | | |
| Clone 17.12 | 77.50a | 78.17a | 94.58a | 82.92a | 83.33a |
| Clone 12 | 80.00a | 76.75a | 91.67a | 83.75a | 76.92a |
| Clone 33.33 | 81.08a | 77.83a | 92.08a | 78.92a | 77.08a |
| Clone 40 B | 77.33a | 75.41a | 94.75a | 89.08a | 89.75a |
| Impala Agrihorti (control) | 77.83a | 77.42a | 93.33a | 77.08a | 82.08a |
| CV (%) | 9.15 | 7.59 | 5.48 | 13.26 | 11.76 |

Notes: CV = coefficient of variation. Means in the same column followed by the same letter are not significantly different based on DMRT, p-value = 0.05

Table 3. Soil temperature content during water stress treatment in Impatiens

| Treatment | WAP | | | | |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 |
| Watering | | | | | |
| Field capacity of 100% | 21.70a | 21.31a | 18.79a | 19.35a | 19.70a |
| Field capacity of 60% | 21.85a | 21.56a | 19.83a | 19.06a | 19.68a |
| Genotypes | | | | | |
| Clone 17.12 | 21.58a | 21.58a | 20.33a | 19.50a | 19.83a |
| Clone 12 | 21.75a | 21.33a | 19.58a | 19.08ab | 19.50a |
| Clone 33.33 | 21.67a | 21.42a | 17.92a | 18.83b | 19.75a |
| Clone 40 B | 21.83a | 21.42a | 17.83a | 18.92b | 19.50a |
| Impala Agrihorti (control) | 21.83a | 21.58a | 19.83a | 19.42ab | 10.50a |
| CV (%) | 1.31 | 1.55 | 10.36 | 2.78 | 2.38 |

Notes: CV = coefficient of variation. Means in the same column followed by the same letter are not significantly different based on DMRT, p-value = 0.05

Table 4. Plant morphology variables

| Variables | | Normal (FC 100%) | Stress (FC 60%) | Delta (%) | Average |
|---|---|---|---|---|---|
| Stem diameter | Clone 17.12 | 5.11 | 4.62 | -10.98 | 4.86d |
| | Clone 12 | 8.07 | 7.47 | -7.43 | 7.76ab |
| | Clone 33.33 | 7.70 | 6.16 | -20.00 | 6.92bc |
| | Clone 40 B | 6.14 | 5.85 | -4.72 | 6.00c |
| | Impala Agrihorti (control) | 8.77 | 8.58 | -2.17 | 8.66a |
| | CV = 12.33% | 7.15a | 6.53a | | (-) |
| Plant height | Clone 17.12 | 24.89 | 23.98 | -3.66 | 24.43c |
| | Clone 12 | 40.93 | 39.87 | -2.59 | 40.40a |
| | Clone 33.33 | 35.18 | 32.43 | -7.82 | 34.13b |
| | Clone 40 B | 25.76 | 27.30 | 5.64 | 26.53b |
| | Impala Agrihorti (control) | 41.87 | 38.59 | -7.83 | 40.23a |
| | CV = 12.42% | 33.85a | 32.44a | | (-) |
| Flower bud initiation | Clone 17.12 | 53.89 | 53.67 | -0.41 | 53.78bc |
| | Clone 12 | 43.56 | 50.00 | 12.88 | 46.78c |
| | Clone 33.33 | 71.78 | 73.11 | 1.82 | 72.44a |
| | Clone 40 B | 62.55 | 64.89 | 3.61 | 63.72ab |
| | Impala Agrihorti (control) | 44.11 | 39.56 | -10.32 | 41.83c |
| | CV = 16.82% | 55.17a | 56.24a | | (-) |
| Flower diameter | Clone 17.12 | 4.94 | 4.60 | -6.88 | 4.78b |
| | Clone 12 | 4.81 | 4.64 | -3.53 | 4.72b |
| | Clone 33.33 | 5.36 | 4.74 | -11.57 | 4.92b |
| | Clone 40 B | 5.38 | 5.49 | 2.00 | 5.44a |
| | Impala Agrihorti (control) | 5.72 | 4.51 | -21.15 | 5.67a |
| | CV = 4.48 % | 5.24a | 4.96b | | (-) |
| Number of flowers | Clone 17.12 | 29.11 | 13.32 | -54.24 | 21.17a |
| | Clone 12 | 28.22 | 24.33 | -13.78 | 26.28a |
| | Clone 33.33 | 7.43 | 5.00 | -32.71 | 6.22b |
| | Clone 40 B | 9.00 | 10.77 | 16.43 | 9.88b |
| | Impala Agrihorti (control) | 25.57 | 24.43 | 4.45 | 25.00a |
| | CV = 31.39% | 19.87a | 15.56a | | (-) |

Notes: CV = coefficient of variation; FC = field capacity. Means in the same column followed by the same letter are not significantly different based on DMRT, p-value = 0.05. Character “-” shows no interaction, and character “+” indicates an interaction between the watering treatment and genotypes

**Variables of stomata**

There was no interaction between water stress treatment and the genotype (Table 5). All genotypes had the mechanism of stomata closing when drought stress occurred. It was reported that the stomata of the leaves closed with increasing Vapour Pressure Deficit (VPD), where VPD above 4.7 kPa caused the stomata to be completely closed (Schmitz and Dericks, 2010). Besides, all genotypes treated with drought stress treatment showed a decrease in stomata length. This was because the formation of stomata positively related to the growth of leaf cells (Spiegelhalder and Raissig, 2021), while the leaves of plants stressed usually demonstrated a narrowing of leaf width (Toscano et al., 2016). However, the length of stomata increased in both genotypes clone 12 and Impala Agrihorti, because of increasing in leaf cells thickness.

**Physiology variables**

There was no interaction between watering stress treatment and the genotype tested on chlorophyll content and plant dry weight. However, there was an interaction between watering stress treatment and the genotype on proline content. The lowest change was found in proline content in Impala Agrihorti (control) when treated after stress, which was 9.33%, followed by clone 12, clone 17.12, clone 33.33 and clone 40.3, reaching 22.30, 40.94, 46.73 and 66.66%, respectively (Table 6). Leaves under intense light conditions had more densely packed palisade parenchyma and more chloroplasts compared to the leaves developed in low light conditions (Langkamp et al., 2015). The water potential of plant cells decreased when there was a lack of water (Toscano et al., 2014). The cells could maintain cell turgor through biosynthesis and accumulation of compatible solutions, such as proline. Proline accumulation is often associated with drought tolerance. Proline functions are osmotic control, osmotic protection, and antioxidant and reactive oxygen system scavenger. Proline is also involved in membrane and protein stability, buffers redox potential under stressed conditions, and acts as a sink for carbon and nitrogen used to release stressed conditions (Antonic et al., 2020).

Table 5. Variables of stomata

| Variables/clone | Normal (FC 100%) | Stress (FC 60%) | Delta (%) | Average |
|---|---|---|---|---|
| Width of stomata | | | | |
| Clone 17.12 | 26.00 | 13.00 | -50.00 | 21.33a |
| Clone 12 | 24.00 | 12.33 | -48.62 | 20.56a |
| Clone 33.33 | 22.33 | 12.67 | -43.26 | 21.78a |
| Clone 40 B | 29.67 | 18.33 | -38.22 | 24.78a |
| Impala Agrihorti (control) | 32.33 | 10.33 | -68.04 | 26.11a |
| CV = 28.75% | 26.86b | 11.40a | | (-) |
| Length of stomata | | | | |
| Clone 17.12 | 130.67 | 101.67 | -22.19 | 116.17ab |
| Clone 12 | 151.33 | 100.00 | -33.92 | 125.67a |
| Clone 33.33 | 132.33 | 92.67 | -29.97 | 112.50ab |
| Clone 40 B | 128.67 | 122.33 | -4.92 | 125.50a |
| Impala Agrihorti (control) | 127.67 | 82.00 | -35.77 | 104.83ab |
| CV = 14.84% | 134.13a | 99.73b | | (-) |
| Number of stomata | | | | |
| Clone 17.12 | 9,772 | 8,886 | -9.06 | 9,329ab |
| Clone 12 | 9,410 | 11,640 | 19.15 | 10,525ab |
| Clone 33.33 | 11,870 | 9,536 | -19.66 | 10,703ab |
| Clone 40 B | 8,527 | 7,334 | -13.99 | 7,931b |
| Impala Agrihorti (control) | 8,605 | 9,248 | 6.95 | 8,925ab |
| CV = 30.60% | 9,329a | 9,636a | | (-) |

Notes: CV = coefficient of variation; FC = field capacity. Means in the same column followed by the same letter are not significantly different based on DMRT, p-value = 0.05. Character "-" shows no interaction, and character "+" indicates an interaction between the watering treatment and genotypes

According to Đurić et al. (2020), proline had no significant role as an osmoprotectant under drought stress conditions in *I. walleriana*. Drought stress controls biosynthesis, negatively affecting the activities involved in amino acid biosynthesis. The absence of a positive relationship between proline content and drought stress in *I. walleriana* may indicate a significant role for other types of osmotic solutions involved in regulating the drought mechanism in Impatiens. The plants' initial and most essential responses to water shortage are the reduction in growth ability and biomass (Cirillo et al., 2014). The increasing water stress caused a reduction in the assimilation process (Toscano et al., 2016). Shoot dry weight as plants' biomass under different irrigation levels changed significantly. The highest shoot fresh and dry weight were found in *Echinacea purpurea* with 100% fields capacity treatment (Heidari et al., 2019).

**Cultivar identification based on drought tolerance index**

According to Sakya et al. (2020), tolerance is based on the decreasing yield under stress conditions. Flowers are the main indicators of the quality of Impatiens. The higher number of flowers in water stress conditions indicates a drought-tolerant plant (Descamps et al., 2021). The drought tolerant genotype as control was Impala Agrihorti. In comparison, the selected clone that was the most drought-tolerant was Clone 12 based on tolerance indexes, namely STI and YSI (Table 7).

**Correlation test of tolerance drought stress genotype**

The correlation test between variables could describe the mechanism of tolerance drought stress genotype, especially in clone 12. When there was drought stress or water limitation, the humidity of the plant medium decreased. The relative humidity of the plant medium had a strong positive correlation with the flower bud initiation (0.92), stomata length (0.89) and proline content (0.84) and negative correlation with stem diameter (-0.74), flower diameter (-0.61), and the number of flowers (-0,53). The number of flowers is an indicator of the quality of Impatiens. The number of flowers was strongly and positively correlated with dry weight (0.64), stem diameter (0.59), and flower diameter (0.57), as well as strongly and negative correlated with flower bud initiation (-0.58) (Table 8).

Table 6. Variables of physiology

| Variables | Normal watering (FC 100%) | Stress watering (FC 60%) | Delta (%) | Average |
|---|---|---|---|---|
| SPAD 12 | | | | |
| Clone 17.12 | 36.35 | 33.65 | -7.42 | 35.00ab |
| Clone 12 | 37.13 | 34.88 | -6.05 | 36.08ab |
| Clone 33.33 | 34.43 | 34.02 | -1.19 | 34.25ab |
| Clone 40 B | 34.30 | 38.73 | 4.43 | 36.52a |
| Impala Agrihorti (control) | 36.18 | 36.18 | 0.00 | 37.88a |
| CV = 9.65% | 35.68a | 36.17a | | (-) |
| Proline | | | | |
| Clone 17.12 | 1.99e | 3.37d | 40.94 | 2.68 |
| Clone 12 | 5.05b | 6.50a | 22.30 | 5.77 |
| Clone 33.33 | 1.79ef | 3.36f | 46.73 | 2.57 |
| Clone 40 B | 1.29g | 3.87c | 66.66 | 2.58 |
| Impala Agrihorti (control) | 1.36g | 1.50fg | 9.33 | 1.43 |
| CV = 7.26% | 2.29 | 43.72 | | (+) |

Notes: CV = coefficient of variation; FC = field capacity. Means in the same column followed by the same letter are not significantly different based on DMRT, p-value = 0.05. Character "-" shows no interaction, and character "+" indicates an interaction between the watering treatment and genotypes

Table 7. Drought tolerance index

| | Cultivar | Conditions | | Drought indices | | | |
|---|---|---|---|---|---|---|---|
| | | Normal (FC 100%) | Stress (FC 60%) | STI | T/S | YSI | T/S |
| Fresh weight (g) | Clone 17.12 | 159.6 | 79.5 | 0.49 | S | 0.50 | S |
| | Clone 12 | 171.8 | 169.6 | 0.97 | T | 0.99 | T |
| | Clone 33.33 | 248.3 | 245.2 | 0.97 | T | 0.99 | T |
| | Clone 40 B | 176.0 | 109.3 | 0.68 | S | 0.62 | S |
| | Impala Agrihorti | 196.2 | 151.2 | 0.77 | S | 0.77 | T |
| | average | | | 0.78 | | 0.77 | |
| Dry weight (g) | Clone 17.12 | 19.2 | 7.5 | 0.38 | S | 0.39 | S |
| | Clone 12 | 18.7 | 16.9 | 0.91 | T | 0.90 | T |
| | Clone 33.33 | 30.4 | 22.0 | 0.67 | S | 0.73 | T |
| | Clone 40 B | 13.1 | 8.3 | 0.69 | T | 0.64 | S |
| | Impala Agrihorti | 28.8 | 20.9 | 0.72 | T | 0.73 | T |
| | average | | | 0.67 | | 0.68 | |
| Number of flowers | Clone 17.12 | 29.11 | 13.22 | 0.45 | S | 9.46 | S |
| | Clone 12 | 28.22 | 24.33 | 0.86 | T | 16.24 | T |
| | Clone 33.33 | 7.44 | 5.00 | 0.67 | S | 3.02 | S |
| | Clone 40 B | 9.00 | 10.78 | 1.19 | T | 6.59 | S |
| | Impala Agrihorti | 25.56 | 24.44 | 0.96 | T | 15.22 | T |
| | average | | | 0.82 | | 10.11 | |
| Proline (mg $g^{-1}$) | Clone 17.12 | 2.0 | 3.4 | 1.70 | S | 1.69 | S |
| | Clone 12 | 5.1 | 6.5 | 1.29 | S | 1.29 | S |
| | Clone 33.33 | 1.8 | 3.4 | 1.87 | T | 1.88 | T |
| | Clone 40.B | 1.3 | 3.9 | 2.99 | T | 2.99 | T |
| | Impala Agrihorti | 1.4 | 1.5 | 1.10 | S | 1.13 | S |
| | average | | | 1.79 | | 1.79 | |

Note: STI = stress tolerance index; YSI = yield stability index; FC = field capacity. *S and T compare with the drought index value in each category. For STI and YSI: S = susceptible (value < mean in each character), T = tolerant, value > mean in each character

Flower growth is the main indicator of Impatiens quality. The higher number of flowers in water stress indicates a drought-tolerant plant. Clones 12 was a drought tolerant plant because it showed a slight decrease in flower numbers during drought stress. There was a decrease in the humidity of the growing media when the plant was stressed. The stomata closed when there was a decrease in the humidity of the media. According to Quinet et al. (2015), plants close their stomata to limit water loss through transpiration in response to water stress. The decreasing stomatal conductance usually reduces plant photosynthesis and consequently affects plant biomass production. However, despite stomatal closure, the $CO_2$ assimilation rate was only slightly affected by water stress exclusively under full light. The increasing water use efficiency (WUE) under water stress contributed to maintaining the net photosynthesis rate. The stomata closure did not significantly reduce the fresh and dry weight in drought-tolerant plants, but it affected the delay in flower initiation. The plant accumulated assimilate for plant height and diameter growth but were not sufficient for generative initiation. In line with Toscano et al. (2019), they showed lines with low WUE, increased stomatal conductance and early flowering.

The assimilation of the vegetative phase could be used as a source for flower formation, which showed no significant decrease in the number of flowers. This Impatiens drought-resistance mechanism had no positive correlation between proline content and drought stress. The main role of other types of osmotic solutions is probably in regulating the Impatiens drought mechanism. Drought stress controls biosynthesis negatively affected the activities involved in amino acid biosynthesis. Drought stress affects plant growth in many ways. Reduction in growth ability and biomass are the plants' initial and most important responses to water shortage. Furthermore, decreased plant weight is associated with leaf water loss during drought stress. However, plants that were tolerant of drought stress would adapt to high carbon assimilation in drought stress conditions (Tribulato et al., 2019).

Table 8. Correlation between observed variables of clone 12

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | | | | | | | | | | |
| 2 | 0.55 | 1.00 | | | | | | | | | | | | |
| 3 | -0.38 | 0.30 | 1.00 | | | | | | | | | | | |
| 4 | 0.89 | 0.47 | -0.62 | 1.00 | | | | | | | | | | |
| 5 | 0.06 | 0.42 | 0.51 | -0.27 | 1.00 | | | | | | | | | |
| 6 | -0.08 | 0.37 | 0.23 | -0.17 | 0.38 | 1.00 | | | | | | | | |
| 7 | -0.14 | -0.45 | -0.22 | -0.19 | 0.29 | -0.55 | 1.00 | | | | | | | |
| 8 | -0.42 | -0.88 | -0.10 | -0.49 | -0.12 | -0.59 | 0.71 | 1.00 | | | | | | |
| 9 | 0.92 | 0.67 | -0.22 | 0.77 | 0.16 | 0.29 | -0.41 | -0.63 | 1.00 | | | | | |
| 10 | -0.14 | 0.54 | 0.13 | -0.01 | 0.33 | 0.76 | -0.35 | -0.73 | 0.11 | 1.00 | | | | |
| 11 | -0.74 | -0.38 | -0.11 | -0.48 | 0.00 | 0.14 | 0.36 | 0.19 | -0.72 | 0.48 | 1.00 | | | |
| 12 | -0.61 | -0.33 | -0.25 | -0.30 | -0.08 | 0.01 | 0.41 | 0.16 | -0.65 | 0.45 | 0.97 | 1.00 | | |
| 13 | -0.53 | -0.90 | -0.52 | -0.34 | -0.46 | -0.11 | 0.35 | 0.64 | -0.58 | -0.20 | 0.59 | 0.57 | 1.00 | |
| 14 | 0.84 | 0.30 | -0.53 | 0.91 | -0.36 | -0.52 | -0.03 | -0.20 | 0.62 | -0.38 | -0.62 | -0.43 | -0.33 | 1.00 |

Note: 1 = RH of medium; 2 = temperature of medium; 3 = width of stomata; 4 = length of stomata; 5 = number of stomata; 6 = SPAD; 7 = fresh weight; 8 = dry weight; 9 = flower bud initiation; 10 = height of plant; 11 = stem diameter; 12 = flower diameter; 13 = number of flowers; 14 = proline

## CONCLUSIONS

The results showed that the water stress treatment (60% field capacity) decreased morphological and physiological traits performance. Still, the drought tolerance clones were less affected by the stress and produced more flowers than the others. The best drought-tolerant Impatiens was clone 12. The study implies that the drought-tolerant Impatiens genotypes can be used as sources for drought tolerant genotypes and the landscape plants in a drought area.

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