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Screening of Locally Adapted Chilli (*Capsicum annuum* L.) Genotypes for Drought Tolerance at Seedling Stage

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ABSTRACT

This study was conducted to explore the mechanisms of oxidative stress tolerance in Chilli (*Capsicum annuum L.*) under drought conditions by evaluating various morphological, physiological, and biochemical. A total of 8 genotypes were assessed for their genetic potential to tolerate drought stress at 45 days of age for 6 days. Later, 3 genotypes exhibited tolerance to drought stress; those 3 genotypes were selected to impose the drought for 12 days and investigate oxidative stress tolerance mechanisms. Drought stress resulted in a decrease in root & shoot length and dry weight parameters compared to control plants. Tolerant genotypes showed a smaller decrease in electrolyte leakage, photosynthetic pigment, relative water content, and specific leaf area characters than the control plants. Although drought plants exhibited a higher level of proline amino acid than the control plants. Eventually, Nepolean-5575 showed high tolerance, Lucky-2 exhibited moderate tolerance, and Tamanna exhibited less tolerance.

Introduction

Drought is becoming an increasing concern globally due to higher evapotranspiration and evaporation losses from plants and exposed soils, respectively. Water is a critical factor affecting plant productivity, and when water availability in the soil is insufficient and atmospheric conditions lead to continuous water loss, drought stress occurs. Drought is one of the leading causes of yield reductions, accounting for more than a 50% decrease in the average yield of many crops worldwide (Wang *et al.,* 2003; Bayoumi *et al.,* 2008). Chilli (*Capsicum annuum L.*), a member of the Solanaceae family, is highly sensitive to drought stress, likely due to its shallow root system, which makes it especially vulnerable to significant reductions in yield under water stress. Chilli is an important spice and one of India's key cash crops. Several studies have reported that drought stress drastically reduces Chilli yield. The impact of drought becomes particularly severe when there is extremely low soil water availability, leading to either very low or no yield. Drought can affect plants at any stage of their growth, but the most critical stages are germination and seedling development (Pessarakli *et al.,* 1999). Drought stress causes changes in the morphology, physiology, biochemistry, and stomatal function of plants due to a reduction in tissue water content and water potential (Misra *et al.,* 2011). In India, drought has been a significant factor in reducing crop growth and yield in recent decades. Several studies have highlighted the usefulness of seedling growth parameters—such as root and shoot length and dry weights as traits for selecting drought-tolerant genotypes in crops like wheat (Awan *et al.,* 2011; Baloch *et al.,* 2012; Ahsan, 2018), soybean (Kim *et al.,* 2015), safflower (Farooq *et al.,* 2020), and tomato (Ghebremariam *et al.,* 2013). However, limited research is available on the effects of drought stress in Chilli. The present study was undertaken to examine the morphological, physiological, stomatal, and biochemical responses of both drought-tolerant and drought-susceptible Chilli genotypes at the seedling stage. This research aims to aid in the selection of tolerant genotypes and improve understanding of oxidative stress under drought conditions.

Materials and Methods

Plant materials and field preparations

To assess drought tolerance during the 45 days of age, 8 Chilli genotypes were screened initially by applying drought stress for 6 days. Among these 8, we found 3 genotypes (Nepolean-5575, Lucky-2, and Tamanna) performed well in response to drought stress.

Again, these three varieties were taken, and a drought stress experiment was conducted to impose 12 days without irrigation. The germinated seeds were initially watered for 45 days, after which watering was stopped for the next 12 days. During this period, the number of dead and surviving seedlings was recorded daily. A portable soil moisture meter (PMS-714, LUTRON, Taiwan) was used to measure soil moisture content.

Determination of Growth Parameters

Root and shoot lengths were measured from three randomly selected fresh seedlings per treatment and recorded in centimeters (cm). Similarly, three fresh seedlings from each treatment were dried at 80°C for 48 hours and then weighed to determine their dry weight (DW), which was expressed in grams (g). The root-to-shoot weight ratio was calculated based on the dry weights. The average weight of three leaf petioles was recorded using a precision balance and expressed in grams (g).

Measurement of Leaf Relative Water Content (RWC)

The relative water content (RWC) of the leaves from plants in different treatments and genotypes was determined using the method outlined by Savita (2016). Leaf samples of similar sizes were weighed immediately to obtain fresh weight. The samples were then fully hydrated by floating them on deionized water in a closed petri dish for 4 hours under normal room light and temperature.

After 4 hours, the samples were removed from the water, and any surface moisture was quickly blotted off using filter paper before weighing them to obtain turgid weight. The samples were then dried in an oven at 70°C for 48 hours, and after cooling in a desiccator, they were weighed again to determine the dry weight. The RWC was calculated using the following formula and expressed as a percentage.

$$RWC = \frac{\text{Fresh Leaf Weight} - \text{Dry Leaf Weight}}{\text{Turgid Leaf Weight} - \text{Dry Leaf Weight}}$$

SPAD (Soil Plant Analysis Data) Assay

SPAD Chlorophyll Meter Reading (SCMR) was recorded at 45 days after sowing (DAS) by a Minolta handheld portable SCMR meter (SPAD-502, Konica Minolta, Japan) using the third leaf for a sample at 9.00-10.00 A.M. across all control and drought stress Chilli plant genotypes.

Measurement of Electrolyte Leakage (EL)

Cell damage was assayed by measuring electrolyte leakage. Twenty-five leaf discs of 0.3 cm² were excised from the third/fourth leaf using a cork borer, rinsed briefly with water, and floated on 5 ml of double distilled water for 6 hours at room temperature.

The conductivity of the water was measured using a Crison conductivity meter. This represented the electrolyte leakage from the leaf discs (reading 1). Then, the samples were boiled for 20 min at 90°C. After the liquid cooled down, the conductivity of the water was measured again. This represented the total ions present in the leaf discs (reading 2).

$$\text{Electrolyte leakage} = \frac{Reading\ 1}{Reading\ 2} X100$$

Specific Leaf Area (SLA)

Specific leaf area of control and drought-treated plant leaves was measured using a leaf area meter (LI-3000C LI-COR Biosciences) according to Sebahattin and Necdet (2007). SLA readings were taken from the fully expanded third leaf of every plant. To determine the leaf dry weight, leaf samples were oven dried at 80°C for 48 h. SLA was calculated using the following formula.

$$\text{SLA} = \frac{\text{Leaf area (cm2)}}{\text{Leaf dry weight (g)}}$$

Measurement of Proline

The proline content was determined calorimetrically following the method described by Bates *et al.,* (1973), which involves proline's reaction with ninhydrin. Fresh leaf tissue (0.5 g) was ground in 10 ml of 3% sulfosalicylic acid on ice. The resulting homogenate was centrifuged at 11,500 rpm for 15 minutes. A 2 ml aliquot of the filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid.

The mixture was incubated at 100°C for 1 hour, then cooled, and 4 ml of toluene was added. The absorbance of the toluene solution containing the chromophore was measured at 520 nm using toluene as a blank. Proline content was quantified by comparison with a standard curve.

Experimental Design

The experiment was laid out in a two-factor completely randomized design. Each experiment was repeated twice independently with three replications.

Statistical Analysis

Data of all parameters were reported as the mean ± SE. The data were statistically analyzed by analysis of variance (ANOVA) using Statistix 10 software. Least significant difference (LSD) at a 5% level of probability was used to compare the mean values. Comparisons with $P \leq 0.05$ were considered significantly different.

Results and Discussion

Shoot Length (cm)

Total plant height was measured in control and drought-treated plants (Fig. 1A). Shoot length (SL) showed significant variation in genotypes, treatments, and interactions. It was clear that drought stress decreased more SL in the Tamanna genotype than in the Nepolean-5575 and Lucky-2 (Fig. 1B).

Root Length (cm)

The significant root length differences were observed between genotypes and treatments. The reduction in root length was notably greater in the Tamanna genotypes compared to the Nepolean-5575 and Lucky-2. Root density was also higher in the Nepolean-5575 and Lucky-2 tolerant genotypes compared to the Tamanna (Fig. 1C).

Shoot and Root Fresh Weight

Drought stress significantly impacted the shoot and root fresh weights because of the unavailability of water in the plant cells. Nepolean-5575, Lucky-2, and Tamanna exhibited less root and shoot fresh weights than the irrigated plants (Fig. 1D & 2A).

Shoot and Root Dry Weight

Drought stress significantly impacted the shoot and root dry weights because of the unavailability of water in the plant cells. After drying the plants, a less amount of water can also get dry. Nepolean-5575, Lucky-2, and Tamanna exhibited less root and shoot dry weights than the irrigated plants (Fig. 2B & C).

Relative Water Content in Leaf

The relative water content (RWC) of Chilli leaves decreased under drought conditions. Significant variations were observed among the genotypes. After 12 days of stress, Nepolean-5575 exhibited more tolerance than the remaining Lucky-2 and Tamanna genotypes (Fig. 3A).

Chlorophyll Content

Although drought caused a loss of chlorophyll content in unirrigated plants. The more chlorophyll degradation was observed in Tamanna genotypes than in the Nepolean-5575 and Lucky-2 genotypes (Fig. 3B).

Electrolyte Leakage

Generally, when the plants are affected by any stress, the cell membrane becomes weak so that leakage of electrolytes will be more from the cell in genotypes. Control plants cell membranes will be less because those plants are irrigated with sufficient water than the drought-stressed plants. Nepolean-5575 exhibited less electrolyte leakage than the other two Lucky-2 and Tamanna genotypes (Fig. 3C).

Specific Leaf Area

Leaf area influences the photosynthesis rates in plant leaves; more leaf area plants perform more photosynthetic rates. Irrigated plants showed more leaf area than the drought-affected plants. Nepolean-5575 exhibited more leaf area than the Lucky-2 and Tamanna genotypes under drought stress (Fig. 3D).

Proline (Pro) Content

The data on the impact of drought on proline (Pro) accumulation is shown in Fig. 3E, Significant variations were observed in Pro content across three genotypes. Stress effects revealed that both tolerant and susceptible genotypes accumulated more Pro under drought stress compared to the control. However, the accumulation was notably higher in the Nepolean-5575 genotype (Fig. 3E).

Estimation of Survival Performance

Drought-tolerant genotypes typically exhibit lower death percentages and higher survival percentages at the 45-day stage. After imposing the stress, susceptible plants showed higher death percentages. Nepolean-5575 showed better performance in all parameters than the Lucky-2 and Tamanna genotypes under drought stress.

Evaluation of Morphological Parameters

A well-developed root system under drought stress is a key criterion for selecting drought-tolerant genotypes, as such a root system enables better moisture uptake from deeper soil layers, making it a critical trait for genotype selection at the 45-day age stage (Morgan *et al.,* 1986; Dhanda, 1995; Baloch *et al.,* 2012). Therefore, genotypes with greater root length and volume tend to exhibit greater resistance to drought stress (Farooq *et al.,* 2020; Leishman *et al.,* 1994; Cardoso *et al.,* 2014; Molla *et al.,* 2021). In this study, drought-treated plants showed a reduction in both shoot and root length and dry weights compared to the controls (Fig. 1B, C, D & 2A, B & C). Similar trends were observed for shoot length and dry weight. The reduction in root-shoot length may be attributed to impaired cell division and elongation (Raziuddin *et al.,* 2010; Khakwani *et al.,* 2011; Ricardez-Miranda *et al.,* 2021), leading to a decrease in both root-shoot length and overall dry weight (Ahsan *et al.,* 2018). These findings are consistent with studies by Farooq *et al.,* (2020) and Mahmoud *et al.,* (2014), which demonstrated that drought stress reduces root-shoot growth in safflower and wheat, respectively. Pace *et al.,* (1999) suggested that drought-induced increases in taproot length, at the expense of root thickening, are a common response in cotton, allowing the plant to access water from deeper soil layers, which helps it survive drought. Jayanna (2014) reported reduced shoot and root dry weight under nickel-stressed groundnut plants.

Ahmad *et al.,* (2007) also reported reduced dry weight in wheat genotypes under water stress, supporting our results. This reduction is likely due to suppressed cell expansion and growth as a result of low turgor pressure (Rouhi *et al.,* 2007; Jaleel *et al.,* 2008). These results suggest that genotypes with a higher proportion of roots are better able to compete for soil nutrients, while those with a greater shoot proportion can capture more light energy. Several studies have reported that seedling growth parameters such as root and shoot length and dry weight are valuable traits for selecting drought-tolerant genotypes in crops such as tomato (Rana *et al.,* 1989; Ghebremariam, 2013), wheat (Ahsan, 2018; Mahmoud *et al.,* 2014), safflower (Farooq *et al.,* 2020), and cotton (Pace *et al.,* 1999).

Evaluation of Physiological and Biochemical Parameters

Relative Water Content

Relative water content (RWC) is closely related to cell volume and can effectively reflect the balance between water absorbed by the plant and the water lost through transpiration (Hassanzadeh *et al.,* 2009; Lugojan *et al.,* 2011; Okunlola *et al.,* 2017). As such, it is considered an important indicator for selecting drought-tolerant plants (Kumutha *et al.,* 2008; Sairam *et al.,* 2009; Prasanna *et al.,* 2014; Soltys-Kalina *et al.,* 2016). In this study, a decrease in RWC was observed in the Lucky-2 and Tamanna genotypes compared to the Nepolean-5575 under drought conditions (Fig. 3A). For example, while screening water stress tolerance in long-storage tomato genotypes, it was found that leaf RWC significantly decreased as drought stress intensified (Patane *et al.,* 2016). In another study on water stress tolerance in wheat, Arjenaki *et al.,* (2012) showed that wheat cultivars with higher RWC are more tolerant to drought stress. Therefore, higher RWC in drought-tolerant plants is a result of better water regulation during transpiration and photosynthesis processes (Jones *et al.,* 1998; Lobato *et al.,* 2009; Kanala *et al.,* 2023).

Chlorophyll and Leaf Area

Photosynthetic pigments are key factors driving a plant's photosynthetic capacity because of their essential roles in light absorption (chlorophyll) and light energy dissipation (carotenoids) (Kamanga *et al.,* 2018). A reduction in the levels of these pigments due to abiotic stresses, such as drought, is a major cause of photosynthesis inactivation. Consequently, a decrease in photosynthetic capacity is expected in plants subjected to water stress. Several studies have indicated that the inhibition of photosynthesis results from the degradation of photosynthetic pigments or chlorophyll, either due to pigment oxidation or by blocking their synthesis (Montagu and Woo, 1999; Anjum *et al.,* 2011; Saraswathi and Paliwal, 2011; Rohman *et al.,* 2016; Widuri *et al.,* 2020; Madhavi *et al.,* 2024). This process is likely triggered by reactive oxygen species (ROS), particularly H_2O_2, which accumulates under water stress and promotes the oxidation/inactivation of pre-existing pigments in chloroplasts (Almási *et al.,* 2000). In the present study, both leaf area and photosynthetic pigment levels decreased due to reduced water availability in Chilli genotypes. Among the genotypes, Nepolean-5575 exhibited higher chlorophyll content than the other two genotypes, Lucky-2 and Tamanna (Fig. 3B).

Electrolyte Leakage

Electrolyte leakage is a common feature of plant responses to various stress factors, including salinity, pathogen invasion, drought, heavy metals, extreme heat, and cold. Growing evidence suggests that electrolyte leakage is primarily associated with the efflux of K+ from plant cells, a process regulated by the plasma membrane's cation conductance. Electrolyte leakage decides the cell membrane stability to plant survival by overcoming the stress (Hareem *et al.,* 2024). Recent research indicates that these conductances involve components with distinct activation kinetics and cation selectivity, likely encoded by the GORK, SKOR, and annexin genes (Rajeswari *et al.,* 2020). In the present study, Nepolean-5575 showed less electrolyte leakage, which indicates the Nepolean-5575 genotype is more drought tolerant than the remaining two genotypes under drought stress (Fig. 3C).

Proline Content

Proline, a vital amino acid, plays a key role in the biosynthesis of primary metabolism during growth and development (Hare *et al.,* 1999; Funck *et al.,* 2012; Jayanna *et al.,* 2014; Banavath *et al.,* 2018). It also accumulates in response to various stress conditions in plants. Well-established as an osmotic regulator, proline helps reduce osmotic damage (Slama *et al.,* 2008; Reddy *et al.,* 2015; Jayanna *et al.,* 2018; Fiasconaro *et al.,* 2019).

Additionally, it is believed that proline accumulation in leaves may serve as a protective function beyond osmoregulation during water stress (Jungklang *et al.,* 2017). Dien *et al.,* (2019) proposed that osmotic adjustment is a biochemical mechanism that assists plants in adapting to water deficits, with one of the main strategies being the accumulation of compatible solutes like proline. The results from this study indicated that proline accumulated significantly in the leaves of drought-tolerant chilly seedlings under stress, compared to the control plants (Fig. 3E). For example, Dien *et al.,* (2019) found significantly higher proline levels in the drought-tolerant rice cultivars DA8 and Thierno Bande.

Table.1 Different Chilli genotypes and their germination percentage rates

S.No	Genotypes	Germination percentage (%)
1	INDAM-67	85
2	RUPALI	86
3	USM PAVITHRA	85
4	NEPOLIAN-5575	92
5	BULLET RAJA	86
6	LUCKY-2	90
7	SWARNA	87
8	TAMANNA	90

Figure.1 Morphological and Shoot and Root weights of control and Drought stressed plants: **A.** Total Plant Height; **B.** Shoot Length; **C.** Root Length; **D.** Shoot Fresh Weight

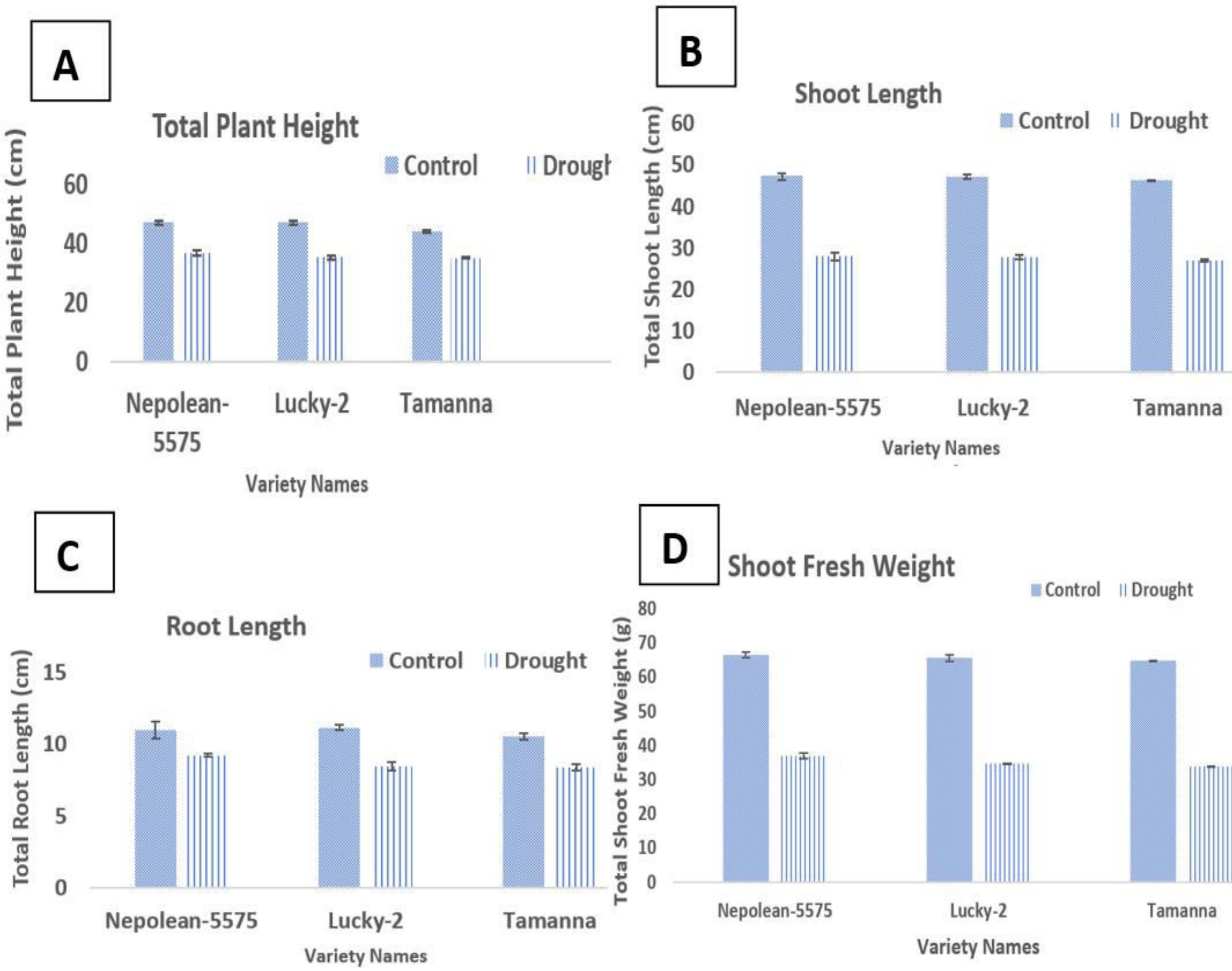


Figure.2 Shoot and Root weights of control and Drought stressed plants:
A. Root Fresh Weight. **B.** Shoot Dry Weight; **C**. Root Dry Weight.

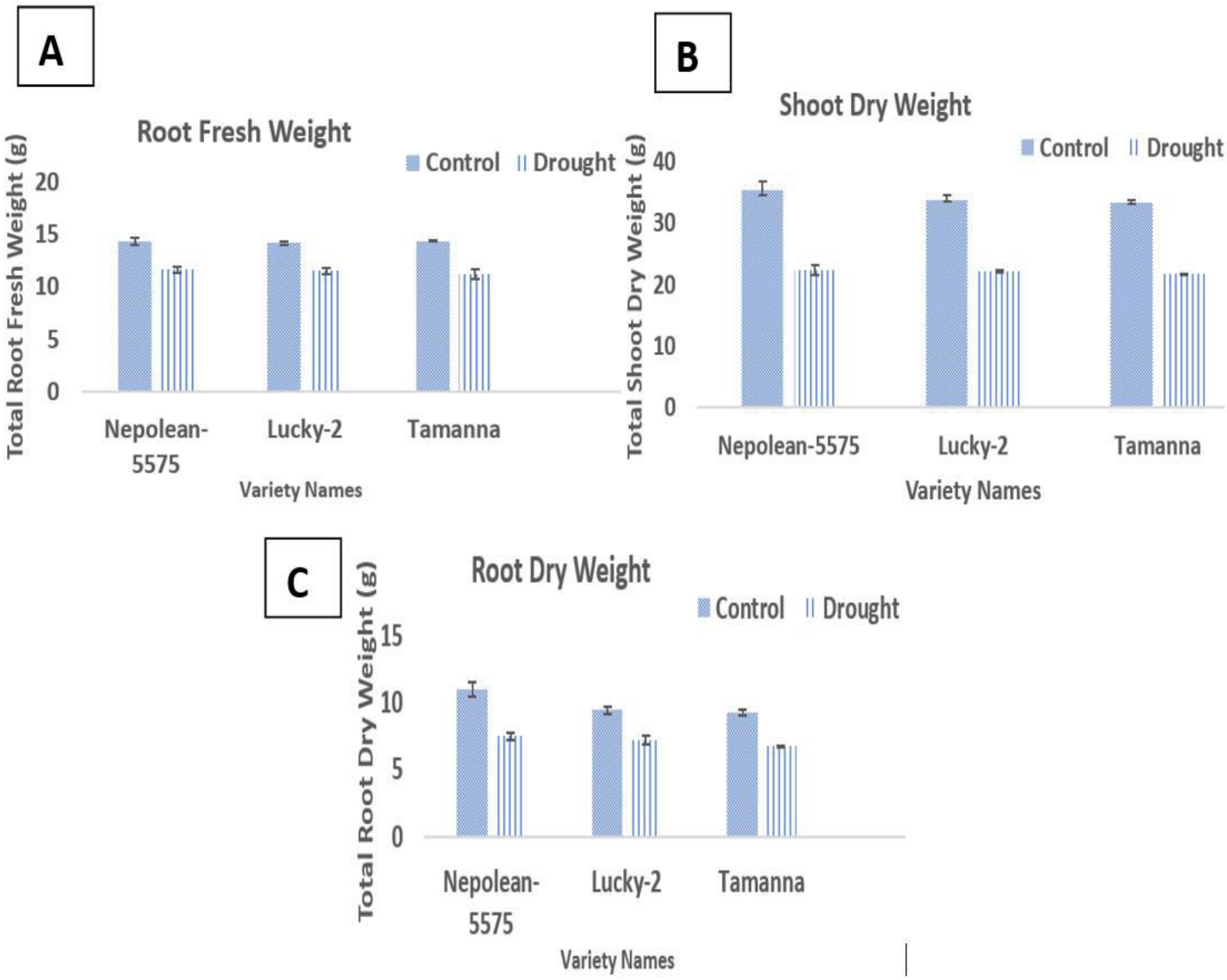
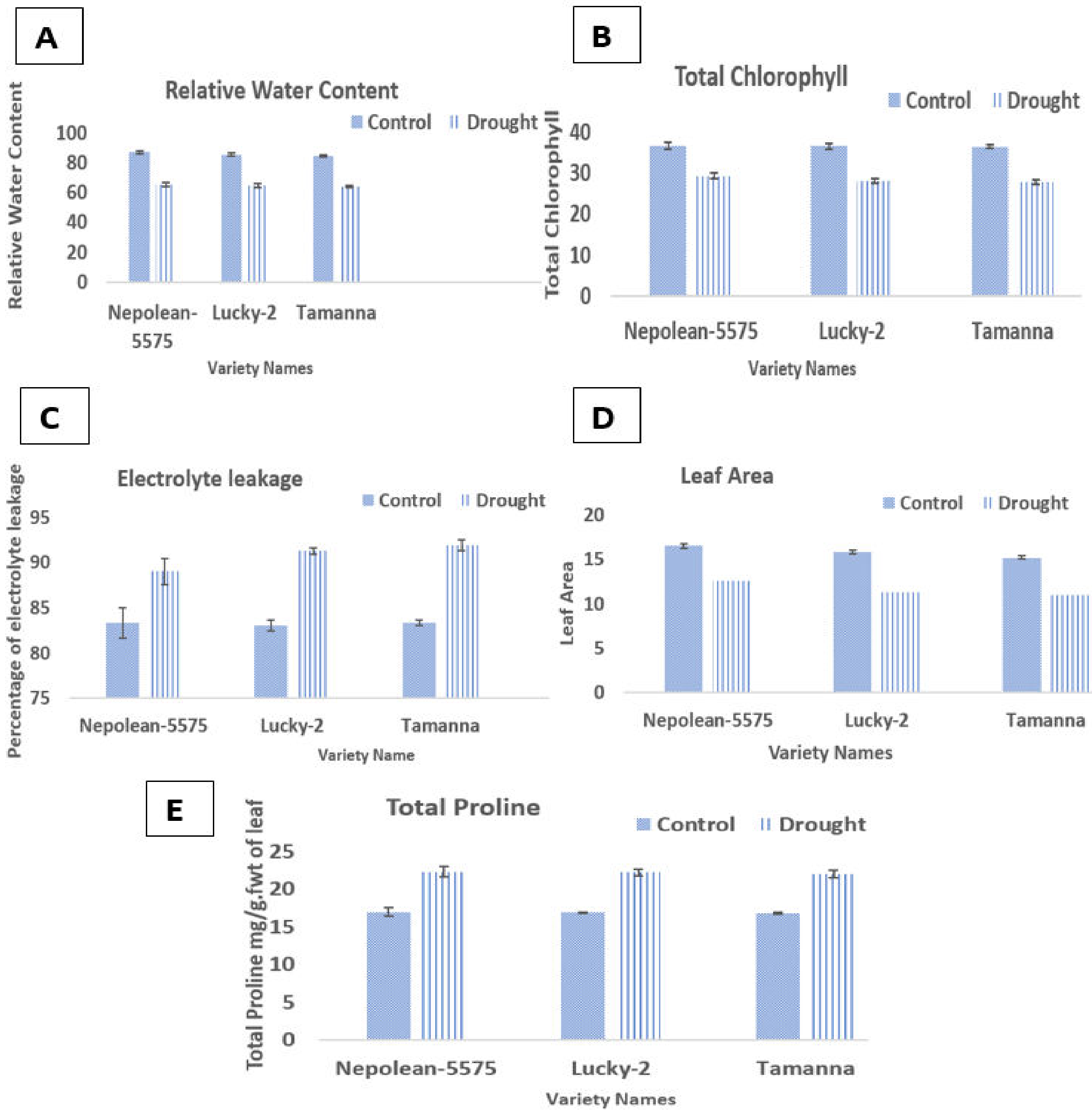


Figure.3 Physiological and Biochemical analysis of control and drought stress plants: **A.** Relative water content; **B.** Total chlorophyll content; **C.** Electrolyte leakage; **D.** Leaf Area; **E.** Total Proline



Likewise, Ranganayakulu *et al.,* (2015) reported that proline accumulation increased in both drought-treated tolerant and susceptible cultivars, with a greater increase observed in the tolerant cultivar K134 compared to the susceptible cultivar JL-24.

In this study, we examined and compared the role of various morphological, physiological, biochemical, and anatomical parameters in the 8 genotypes of 45-day-old seedlings for 6 days with water stress. Among 8 genotypes, 3 genotypes (Nepolean-5575, Lucky-2, and Tamanna) showed adaptation to drought tolerance. Hence, those 3 genotypes were forwarded to conduct the drought-tolerant experiment at 45-day-old seedlings for 12 days. Our results show that the Nepolean-5575 showed high tolerance, and Lucky-2 showed moderate tolerance compared to the Tamanna genotype under water stress conditions. However, irrigated plants exhibited significant symptoms in all parameters because of the daily watering. Additionally, the expanded leaves of the tolerant genotypes contained average levels of photosynthetic pigments in non-irrigated plants compared to the irrigated plants.

The increased accumulation of stress metabolites such as proline (Pro) in the tolerant genotypes could protect them from osmotic damage. As a result, the tolerant genotypes displayed higher antioxidant activity than the susceptible ones. Furthermore, the tolerant genotypes were able to close their stomata more rapidly, which likely contributes to their increased drought tolerance. This study highlights distinct differences between tolerant and susceptible genotypes across morphological, physiological, biochemical, anatomical, and overall antioxidant parameters.

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Author Contributions

Bonam Blessy, Bhukya Shivani, Yerra Laxshminarayana, P. V. Sai Sree Harsha, Banavath Jayanna Naik and Dowlathabad Muralidhara Rao: Conceived the original idea and designed the model and wrote the manuscript.; Shaik Anjuma, Manjunath S. Patil and Chadive Dhanunjaya Kumar: Designed the model and the computational framework and analysed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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