Effects of tobacco (*Nicotiana tabacum*) applied with gibberellic acid (GA3) in response to different durations of drought and flooding

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ABSTRACT

Background and Objective: Tobacco (*Nicotiana tabacum*) is a major industrial crop used in cigars and cigarettes. Drought and flooding can significantly impact its growth. Gibberellic acid (GA3) explored its potential to enhance the resilience. Therefore, this study investigates the effects of GA3 on tobacco growth under varying drought and flooding conditions.

Methodology: The study utilized 6 × 2 factorials in a randomized complete block design (RCBD) with three replications. Factor A included stress duration treatments: control (Con), drought for three days (D), prolonged drought for five days (PD), flood for three days (F), prolonged flood for five days (PF), flood followed by prolonged drought (F + PD), and prolonged flood followed by prolonged drought (PF + PD). Factor B involved gibberellic acid 3 (GA3) application, with B1 representing GA3 application (WG) and B2 without GA3 (WOG). Data was analyzed with two-way ANOVA. Treatment means differences assessed via Tukey's HSD test.

Main Results: Gibberellic acid 3 (WG) significantly influenced ($P < 0.05$) plant height (PH), number of leaves (NL), leaf length (LL), root length (RL), and biomass yield (BY), averaging 35.71 ± 2.34 cm, 5.00 ± 0.53, 31.14 ± 1.87 cm, 25.14 ± 1.42 cm, and 195.43 ± 7.65 g, respectively. Con + WG notably had significantly higher PH (63.00 ± 3.12 cm), NL (7.00 ± 0.53), LL (50.00 ± 2.34 cm), RL (36 ± 1.87 cm), and BY (255.65 ± 9.72 g). Different durations of drought and flooding stress conditions high significantly impaired tobacco growth, resulting in decreased PH from 60.00 ± 17.07 to 17.50 ± 7.79 cm and LL from 43.00 ± 8.60 to 17.50 ± 0.71 cm.

Conclusions: GA3 application enhances tobacco growth, boosting plant height, leaf count, length, and biomass yield, counteracting water stress's adverse effects. Water stress diminishes tobacco metrics like plant height, leaf area, and fresh weight.

Keywords: Drought, flood, gibberellic acid, tobacco, water stress

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INTRODUCTION

Tobacco (*Nicotiana tabacum*) is a widely cultivated crop, covering approximately 30,352 hectares across 23 provinces in 2020, with the Ilocos Region being the leading producer, accounting for 69.2% of total production, followed by Cagayan Valley at 23.0% (PSA, 2023). The dominant variety is Virginia tobacco, contributing 53.3% to overall tobacco production, typically planted from October to December. Water stress significantly affects tobacco, which has considerable economic importance due to its use in various products such as cigarettes, cigars, and smokeless tobacco. Water stress, caused by drought and flooding, poses a major constraint on tobacco growth and yield. Gibberellic acid 3 (GA3), a plant growth regulator, has shown potential in enhancing plant development, but its effects under different durations of water stress in tobacco remain poorly understood (Biglouei *et al*., 2010).

Research into the interaction between GA3 and water stress in tobacco is essential for developing sustainable strategies to boost tobacco productivity under water-limited conditions. Studies on GA3's impact on plant growth and development under water stress conditions have yielded promising results. For instance, Abd El-Samad (2013) reported that the application of GA3 under saltwater stress conditions led to improved growth, photosynthesis, and yield in wheat. Similarly, Zhang *et al.* (2000) observed increased growth, yield, and water use efficiency in chickpeas with GA3 treatment. However, the specific effects of GA3 on tobacco subjected to varying durations of drought and flooding have not been adequately documented. While some individual studies have examined the impact of GA3 and water stress on tobacco growth—such as Yang *et al.* (2019), who found that GA3 application increased leaf area, fresh and dry weight, and photosynthetic rate, even though water stress decreased tobacco growth—the combined effects of GA3 and water stress on tobacco still require further investigation.

Therefore, the objective of this study is to investigate the specific effects of GA3 application on tobacco plants under varying durations of drought and flooding. By doing so, we aim to enhance our understanding of how GA3 can bolster tobacco plants' tolerance to water stress, ultimately improving their growth and yield. This research is essential in establishing sustainable strategies to enhance tobacco productivity in the face of challenging environmental conditions.

MATERIALS AND METHODS

The experiment was conducted in the Nursery Greenhouse of the College of Agricultural Sciences and Technology, Isabela State University, Cabagan Campus, Cabagan, Isabela, Philippines. The geographical coordinates of the experimental area are 17.4144° North latitude and 121.7670° East longitude. The study was carried out in May 2023. During the three-month experimental duration, pot trials were conducted using pails measuring 11 × 8.5 inches. Each pail was filled with 15 kg of air-dried soil supplemented with specific quantities of essential nutrients. These nutrients included 2 grams of nitrogen (N), 1.5 grams of phosphorus (P), 0.50 grams of potassium (K), and 1.0 grams of sulfur (S), administered as urea and complete fertilizer (14-14-14). The experiment was structured as a 6 × 2 randomized complete block design (RCBD) with a two-factorial approach. Factor A was designed to evaluate the effects of different durations of drought and flood stress, consisting of the following treatments: A1- Control (Con), A2- Drought for three days (D), A3- Prolonged drought for five days (PD), A4- Flood for three days (F), A5- Prolonged flood for five days (PF), A6- Flood + Prolonged drought (F + PD), and A7- Prolonged flood + Prolonged drought (PF + PD). Furthermore, the control group denoted as A1 in the experiment, served as the baseline reference point against which the other treatment groups were compared. A1- Control refers to the plants that were not subjected to any specific duration of drought or flood stress and did not receive any application of GA3. The purpose of the control group was to provide a standard for comparison, enabling the assess the effects of the applied treatments accurately. On the other hand, Factor B was implemented to investigate the impact of the presence or absence of GA3, comprising B1- With GA3 (WG) and B2- Without GA3 (WOG). To ensure the reliability and robustness of the findings, each treatment group, which encompassed the various combinations of drought and flood durations and the presence or absence of GA3, was represented by two sample plants. Moreover, the entire experiment was replicated three times, thereby allowing for assessing any potential variations in the experimental setup and strengthening the validity of the results.

The data collection included the recording of various growth parameters such as plant height (PH), which was measured from the base to the tip of leaves after subjecting plants to water stress, number of leaves (NL), which was counted after applying water stress, length of leaves (LL), this was measured from the base to the tip of leaves post-water stress, biomass yield (BY), this was determined by weighing the fresh weight, including stalks and leaves and root length (RL), this was measured from the base to the tip of the longest root. A two-factorial analysis of variance (ANOVA) was employed to analyze the effects of GA3 application and different stress durations on tobacco growth parameters. The significance of the differences among treatment means was determined using Tukey's Honestly Significant Difference (HSD) test. The results were presented as mean ± standard deviation (SD). The significance level was set at $P < 0.05$ to determine the statistical significance of the findings.

Furthermore, all the necessary materials were gathered, which included a pail with dimensions of 11 × 8.5 inches, tobacco seeds, water, seedling tray, knife or bolo, hand shovel, transparent roll bag, and a moisture meter. Once all the materials were ready, the tobacco seeds were planted in a plastic pot filled with soil, and water was added to ensure moisture. The seedling tray was then placed in a warm and sunny area, with the transparent roll bag covering the pot to create a greenhouse effect. The moisture meter was used to monitor the soil's moisture levels to ensure it remains moist but not waterlogged. After 2–3 weeks, the tobacco seedlings were transferred to the pail to continue growing until they reached maturity. The pail comprises a soil media mix created by combining one part vermicompost, one part garden soil, and one part sand or rice hull. Vermicompost, rich in organic matter, provided essential nutrients and improved soil structure and water retention. Garden soil contributed a balanced mixture of sand, silt, clay, and beneficial microorganisms. Sand or rice hulls were added to enhance soil drainage and aeration. Mixing these components thoroughly resulted in a nutrient-rich and well-balanced soil media for plant cultivation. During water management, it provided when the soil moisture content fell below 5–7% until the plants developed 3–5 true leaves. This ensured healthy root development and growth while preventing overwatering. Afterward, controlled water stress was applied to enhance root development and drought tolerance, optimizing plant growth and conserving water. GA3 application required diluting five ppm of GA3 in 8.5 mL of water. This diluted solution was applied to the plants when they had 3–5 true leaves or just before subjecting them to water stress, such as drought or flooding. In pest management, manual removal was employed to eliminate insects and diseased plants physically, minimizing the spread of pests and diseases and ensuring healthy tobacco plant growth. When the tobacco plants reach 16 days after transplanting or are composed of 3–5 leaves, the experiment starts. Drought treatment involved reducing irrigation to maintain soil moisture below 6%. Flood treatment submerged plants in water up to 3–5 cm above the soil surface within a plastic pot placed inside a larger pail to prevent water escape.

RESULTS AND DISCUSSION

Plant Height

The data presented in Table 1 shows the response of tobacco PH to different durations of drought and flooding. Control (Con) had the tallest PH at 60.00 ± 17.07 cm. This was followed by PD, D, PF, F, F + PD, and PF + PD with means of 44.50 ± 10.36, 35.50 ± 9.79, 25.00 ± 6.36, 24.50 ± 10.61, 18.50 ± 7.79, and 17.50 ± 7.79 cm, respectively. The analysis of variance further revealed that the application of GA3 had a significant effect on PH after exposure to drought and flooding when compared to the control. This indicates that using GA3 can enhance plant growth and development even in the presence of environmental stressors. The study of Wu *et al.* (2022) found that applying GA3 to plants enhanced their resistance to both drought and flooding, as well as improved their ability to recover from these stresses. The study showed that GA3-treated plants had higher leaf water potential and leaf relative water content, indicating better water use efficiency and that the plants were less susceptible to wilting under drought conditions. Additionally, the GA3-treated plants had higher photosynthetic rates and chlorophyll content, indicating better recovery after flooding.

Table 1 Response of tobacco plant after being subjected to different durations of drought and flooding

Factor A	PH (cm)	NL	LL (cm)	RL (cm)	BY (g)
Con	60.00 ± 17.07[a]	6.50 ± 1.50[a]	43.00 ± 8.60[a]	33.00 ± 10.22[a]	254.95 ± 46.24[a]
D	35.50 ± 9.79[b]	6.00 ± 0.00[a]	33.00 ± 0.00[b]	27.50 ± 0.00[a]	232.80 ± 0.00[a]
PD	44.50 ± 10.36[b]	6.00 ± 0.00[a]	32.00 ± 0.00[b]	25.00 ± 0.00[a]	225.29 ± 0.00[a]
F	24.50 ± 10.61[c]	3.00 ± 0.00[b]	22.50 ± 0.00[c]	12.00 ± 0.00[b]	196.12 ± 0.00[a]
PF	25.00 ± 6.36[c]	2.50 ± 0.71[b]	17.50 ± 12.02[c]	17.00 ± 3.61[b]	144.40 ± 30.65[b]
F + PD	18.50 ± 7.79[c]	3.00 ± 0.00[b]	21.00 ± 0.00[c]	13.50 ± 0.71[b]	135.48 ± 4.95[b]
PF + PD	17.50 ± 7.79[c]	2.50 ± 0.71[b]	17.50 ± 0.71[c]	18.00 ± 5.66[b]	120.88 ± 15.33[b]

Note: Con = control, D = drought for three days, PD = prolonged drought for five days, F = flood for three days, PF = prolonged flood for five days, PH = plant height, NL = number of leaves, LL = length of leaves, RL = root length, and BY = biomass yield. [a,b] Means within the same column with different subscript differ ($P < 0.05$)

The effects of GA3 on tobacco PH under varying durations of drought and flooding are depicted in Table 2. The tallest PH, at 35.71 ± 2.34 cm, was recorded for the WG treatment, while the lowest, at a mean of 28.71 ± 2.12 cm, was observed for WOG. There was a significant difference between the PH of B1 and B2, suggesting that the application of GA3 can aid in the survival of tobacco plants subjected to drought and flooding conditions of varying durations. This indicates that GA3 has a positive impact on the growth and development of tobacco plants under stressful environmental conditions.

Table 2 Response of tobacco plant with and without GA3 after subjected to different duration drought and flooding

Factor B	PH (cm)	NL	LL (cm)	RL (cm)	BY (g)
WG	35.71 ± 2.34[a]	5.00 ± 0.53[a]	31.14 ± 1.87[a]	25.14 ± 1.42[a]	195.43 ± 7.65[a]
WOG	28.71 ± 2.12[b]	3.43 ± 0.61[b]	22.14 ± 1.29[b]	16.57 ± 0.92[b]	178.83 ± 6.94[b]

Note: WG = with gibberellic acid 3, WOG = without gibberellic acid 3, PH = plant height, NL = number of leaves, LL = length of leaves, RL = root length, and BY = biomass yield. [a,b] Means within the same column with different subscript differ ($P < 0.05$)

In Table 3, the PH of tobacco under varying durations of drought and flooding in the presence of GA3 is presented. Con + WG had the highest mean of 63.00 ± 3.12 cm, followed by Con + WOG with a mean of 57.00 ± 2.68 cm, while the lowest was the PF + PD + WOG with a result of 12.00 ± 1.29 cm. However, the ANOVA result showed that Con + WG significantly differed from Con + WOG, PD + WG, and PD + WOG. This suggests that prolonged drought and flooding have an adverse effect on tobacco plant height, resulting in either stunted growth or reduced height. Overall, the results indicate that applying GA3 can help mitigate the negative effects of drought and flooding on tobacco plant height, especially under moderate stress conditions.

Table 3 Interaction of presence in GA3 and different durations of drought and flooding in tobacco plants

Treatment	PH (cm)	NL	LL (cm)	RL (cm)	BY (g)
Con + WG	63.00 ± 3.12[a]	7.00 ± 0.53[a]	50.00 ± 2.34[a]	36 ± 1.87[a]	255.65 ± 9.72[a]
Con + WOG	57.00 ± 2.68[b]	6.00 ± 0.53[a]	36.00 ± 1.87[b]	30 ± 1.29[a]	254.25 ± 8.65[a]
D + WG	42.00 ± 2.12[b]	7.00 ± 0.53[a]	36.00 ± 1.87[b]	35 ± 1.42[a]	235.15 ± 7.65[b]
D + WOG	29.00 ± 1.87[c]	5.00 ± 0.61[b]	30.00 ± 1.29[b]	20 ± 0.92[b]	230.45 ± 6.94[b]
PD + WG	46.00 ± 2.12[b]	7.00 ± 0.53[a]	36.00 ± 1.87[b]	30 ± 1.29[a]	230.25 ± 7.12[b]
PD + WOG	43.00 ± 1.87[b]	5.00 ± 0.61[b]	28.00 ± 1.29[b]	20 ± 0.92[b]	220.33 ± 6.78[b]
F + WG	26.00 ± 1.42[c]	4.00 ± 0.61[b]	27.00 ± 1.29[b]	14 ± 0.92[c]	205.45 ± 6.32[b]
F + WOG	23.00 ± 1.29[c]	2.00 ± 0.53[c]	18.00 ± 1.29[c]	10 ± 0.92[c]	186.78 ± 5.75[c]
PF + WG	30.00 ± 1.42[c]	3.00 ± 0.53[c]	19.00 ± 1.29[c]	25 ± 1.29[b]	155.55 ± 5.12[c]
PF + WOG	20.00 ± 1.29[c]	2.00 ± 0.53[c]	16.00 ± 1.29[c]	9 ± 0.92[c]	133.25 ± 4.65[c]
F + PD + WG	20.00 ± 1.29[c]	4.00 ± 0.61[b]	30.00 ± 1.29[b]	15 ± 0.92[c]	150.45 ± 5.32[c]
F + PD + WOG	17.00 ± 1.29[c]	2.00 ± 0.53[c]	12.00 ± 1.29[c]	12 ± 1.29[c]	120.50 ± 4.65[d]
PF + PD + WG	23.00 ± 1.29[c]	3.00 ± 0.53[c]	20.00 ± 1.29[c]	21 ± 1.29[b]	135.50 ± 5.12[c]
PF + PD + WOG	12.00 ± 1.29[c]	2.00 ± 0.53[c]	15.00 ± 1.29[c]	15 ± 1.29[c]	106.25 ± 4.65[d]

Note: Con = control, D = drought for three days, PD = prolonged drought for five days, F = flood for three days, PF = prolonged flood for five days, WG = with gibberellic acid 3, WOG = without gibberellic acid 3, PH = plant height, NL = number of leaves, LL = length of leaves, RL = root length, and BY = biomass yield. [a,b] Means within the same column with different subscript differ ($P < 0.05$)

Number of Leaves

The data shown in Table 1 revealed that there was variation in the NL between the treatments, with a range of 2.50 ± 0.71 to 6.50 ± 1.50. The lowest NL was observed in treatments F, PF, F + PD, and PF + PD, with only three leaves in F and F + PD and 2.5 leaves in PF and PF + PD. On the other hand, the highest NL was observed in the control treatment, with 6.50 ± 1.50. The other treatments, D and PD, had 6.00 ± 0.00. The results suggest that the duration of drought and flooding had a significant impact on the number of leaves in tobacco plants. Prolonged drought and flooding stress can lead to a reduction in the number of leaves in plants, which can affect their growth and yield.

ANOVA showed that the control treatment did not have a significant difference compared to D and PD, but it differed significantly from F to PF + PD (Table 2). This implies that as the duration of stress becomes longer, the performance of tobacco decreases. Similar observations have been reported in previous studies, which demonstrated a decline in the number of leaves in various crops subjected to drought and flooding stress (Striker, 2012; Akhtar and Nazir, 2013; Mangani *et al.,* 2018). The reduction in the NL in tobacco plants under drought and flooding stress can be attributed to several physiological and molecular mechanisms, such as decreased leaf expansion, leaf abscission, and altered hormonal balance (Striker, 2012; Mangani *et al.,* 2018).

Table 2 displays the effects of the presence of GA3 in tobacco plants in terms of the NL after being subjected to different durations of drought and flooding. WG had a mean NL of 5.00 ± 0.53, while WOG had a mean NL of 3.43 ± 0.61. The significant difference between the two means indicates that the presence of GA3 in tobacco plants can have a positive effect on their performance under drought and flooding stress. According to ANOVA, WG showed significant differences compared to WOG. These findings align with previous studies that have reported the positive impact of GA3 application on plant growth and development, including an increase in the NL, by promoting cell elongation and division (Miceli *et al.,* 2019). Additionally, GA3 has been shown to potentially alleviate the negative effects of abiotic stress, such as drought and flooding stress, on plant growth. For instance, GA3 can help improve the water use efficiency of plants by regulating stomatal conductance and reducing transpiration rates, ultimately conserving water during drought stress (Li *et al*., 2012).

The interaction between the presence of GA3 and different durations of drought and flooding stress on NL in tobacco plants was investigated in this study (Table 3). The results revealed that the presence of GA3 had a significant effect on NL in tobacco plants under drought and flooding stress. As seen in Treatment: Con + WG (7.00 ± 0.53) to F + PD + WG (4.00 ± 0.61), the NL in tobacco plants treated with GA3 was higher than those without GA3. Moreover, the duration of drought and flooding stress also had a significant effect on the number of leaves in tobacco plants because ANOVA revealed that Con did not vary significantly with D + WG (7.00 ± 0.53) and PD + WG (7.00 ± 0.53), Con + WOG (6.00 ± 0.53) but vary significantly differences with D + WOG (5.00 ± 0.61), PD + WOG (5.00 ± 0.61), F + WG (4.00 ± 0.61), F + PD + WG (4.00 ± 0.61), PF + PD + WG (3.00 ± 0.53), PF + WG (3.00 ± 0.53), PF + WOG (2.00 ± 0.53), F + WOG (2.00 ± 0.53), F + PD + WOG (2.00 ± 0.53), and PF + PD + WOG (2.00 ± 0.53). As the duration of stress increased, the NL decreased. However, the effect of drought stress was more significant compared to flooding stress. This finding is consistent with previous studies that have reported a reduction in the NL under drought and flooding stress in various crops (Striker, 2012; Akhtar and Nazir, 2013; Mangani *et al*., 2018). The interaction between GA3 and drought/flooding stress was also observed in this study. The application of GA3 significantly increased the NL in tobacco plants under both drought and flooding stress. This result aligns with previous studies that have demonstrated the positive effects of GA3 on plant growth and development, including the NL (Shomeili *et al.,* 2011; Miceli *et al.,* 2019). GA3 application has been reported to promote cell elongation and division, stimulating plant growth and development, particularly under abiotic stress conditions (Mafakheri *et al.,* 2011; Li *et al.,* 2016).

Length of Leaves

As seen in Table 1, the LL in tobacco plants decreased as the duration of stress increased. Con, representing the control group without stress, had the highest LL at 43.00 ± 8.60 cm. On the other hand, PF + PD and PF, which were subjected to the longest duration of stress, had the lowest LL at 17.50 ± 0.71 and 17.50 ± 12.02 cm, respectively. In addition, the duration of drought and flooding stress also had a significant effect on the LL in tobacco plants because, according to the result of ANOVA, it revealed that Con varied significantly from D (33.00 ± 0.00 cm) to PD (32.00 ± 0.00 cm) and highly significant to F (22.50 ± 0.00 cm), PF (17.50 ± 12.02 cm), F + PD (21.00 ± 0.00 cm), and PF + PD (17.50 ± 0.71 cm). As the duration of stress increased, the LL decreased. This agrees with previous studies that have reported reduced LL under drought and flooding stress in various crops. The decrease in LL in tobacco plants under drought and flooding stress is a common response observed in various crops, as reported in previous studies (Striker, 2012; Akhtar and Nazir, 2013; Mangani *et al.,* 2018). It is worth noting that the reduction in the LL in tobacco plants under stress was not uniform across all groups. For instance, F and PF had a larger reduction in the LL than D and PD. This variation could be attributed to the differences in the severity of stress and the timing of stress application. Plants respond differently to stress depending on the intensity, duration, and timing of stress application. In this case, it is possible that F and PF were subjected to a more severe stress condition or were exposed to stress at a more critical developmental stage compared to D and PD.

The average LL in tobacco plants in WG was 31.14 ± 1.87 cm, while the average LL in tobacco plants in WOG was 22.14 ± 1.29 cm, as shown in Table 2. ANOVA revealed that WG varies significantly more than WOG. The LL in tobacco plants was affected by the presence of GA3 after exposure to varying durations of drought and flooding stress. It illustrates that the average LL of tobacco plants treated with GA3 is greater than those that were not treated with GA3.

The interaction between the presence of GA3 and different durations of drought and flooding has been shown to significantly affect the growth of tobacco plants, including the LL they produce. The data presented in Table 3 shows the LL produced by tobacco plants subjected to varying durations of drought and flooding stress in the presence and absence of GA3. Based on the data, it is apparent that the LL produced by tobacco plants was affected by the presence of GA3 and the duration of drought and flooding stress. Con + WG produced leaves of 50.00 ± 2.34 cm, which is higher compared to those in Con + WOG (36.00 ± 1.87 cm), D + WG (36.00 ± 1.87 cm), PD + WG (36.00 ± 1.87 cm), D + WOG (30.00 ± 1.29 cm), PD + WOG (28.00 ± 1.29 cm), and F + WG (27.00 ± 1.29 cm), respectively. However, ANOVA revealed that Con + WOG, D + WG, PD + WG, D + WOG, PD + WOG and F + WG had no statistical difference between each other but were significantly different from Con + WG and highly significant from F + PD + WG (30.00 ± 1.29 cm), PF + PD + WG (20.00 ± 1.29 cm), PF + WG (19.00 ± 1.29 cm), F + WOG (18.00 ± 1.29 cm), PF + WOG (16.00 ± 1.29 cm), PF + PD + WOG (15.00 ± 1.29 cm), and F + PD + WOG (12.00 ± 1.29 cm) respectively had no statistical differences but significantly difference to other treatments from Con + WG to F + WG. A study conducted by Peng *et al*. (2014) investigated the effects of GA3 on the growth of maize seedlings under different soil moisture conditions. The results showed that GA3 treatment significantly increased the growth of maize seedlings under both well-watered and drought conditions. The study concluded that GA3 could be used as a potential plant growth regulator to improve crop yields, especially under drought-stress conditions. Another study by Farooq *et al*. (2009) examined the effects of GA3 on wheat growth under drought stress. The results showed that GA3 treatment significantly increased plant growth, including the number of leaves produced, under drought-stress conditions. The study concluded that GA3 treatment could be a potential strategy for mitigating the negative effects of drought stress on wheat growth and yield.

Root Length

The effects of drought and flooding stress on plant growth have been extensively studied in recent decades. Among the different plant growth parameters that are affected by these stresses, RL has been shown to be an important indicator of plant tolerance and adaptation to water stress conditions. Table 1 under RL shows the root length of tobacco plants subjected to different durations of drought and flooding stress. The RL of tobacco plants decreased with increasing durations of drought and flooding stress. Con obtained the highest RL with a mean of 33.00 ± 10.22 cm, while the lowest RL was found in F with a mean of 12.00 ± 0.00 cm. However, Con did not vary significantly with D (27.50 ± 0.00 cm) and PD (25.00 ± 0.00 cm), which means that tobacco is resistant to drought while these factors are significantly differences F, PF (17.00 ± 3.61 cm), F + PD (13.50 ± 0.71 cm) and PF + PD (18.00 ± 5.66 cm) means when there is a subjection of flooding regardless of drought, it affects its root development. These findings are consistent with previous studies that have reported a reduction in RL in various plant species, including tobacco, as a response to water stress conditions (Farnia and Omidi*,* 2015; Ali *et al.,* 2023). The reduction in RL has been attributed to the inhibition of cell division and elongation, as well as the alteration of hormonal balance, particularly the increase in abscisic acid (ABA) levels (Tardieu, 2012). To mitigate the negative effects of water stress on plant growth, various strategies have been proposed, including the use of plant growth regulators such as GA3. A study conducted by Farooq *et al*. (2009) investigated the effects of GA3 on wheat growth under drought-stress conditions. The results showed that GA3 treatment significantly increased RL, as well as other plant growth parameters, under drought-stress conditions.

The average RL of tobacco plants in WG was 25.14 ± 1.42 cm, which was higher than the average RL of tobacco plants in WOG at 16.57 ± 0.92 cm (Table 2). However, ANOVA revealed that

WG is significantly different from WOG. This finding suggests that applying GA3 has a positive effect on root development in tobacco plants subjected to drought and flooding stress conditions. This result is consistent with previous studies investigating the effect of GA3 on plant growth under water stress conditions. For example, a study conducted by Li *et al*. (2016) found that the application of GA3 increased the RL and biomass of maize plants under drought-stress conditions.

The RL of tobacco plants varied depending on the duration of stress and the presence of GA3. Con + WG obtained the highest RL, with a mean of 36 ± 1.87 cm, while the lowest RL was observed in PF + WOG, with a mean of 9 ± 0.92 cm. However, ANOVA revealed that Con + WG is not varied significant difference with Con + WOG (30 ± 1.29 cm), D + WG (35 ± 1.42 cm), and PD + WG (30 ± 1.29 cm) but varied significantly differences with PF + WG (25 ± 1.29 cm), PF + PD + WG (21 ± 1.29 cm), D + WOG (20 ± 0.92 cm), PD + WOG (20 ± 0.92 cm), F + PD + WG (15 ± 0.92 cm), F + WG (14 ± 0.92 cm), F + PD + WOG (12 ± 1.29 cm), F + WOG (10 ± 0.92 cm), PF + WOG (9 ± 0.92 cm), respectively. The results of this study are consistent with previous research that has shown the positive effects of GA3 on plant growth and development under stress conditions. GA3 has been shown to enhance root growth in various plant species, including tobacco, by promoting cell division and elongation (Shi *et al*., 2010; Jiang *et al*., 2013). In addition, GA3 has been reported to improve plant tolerance to water stress conditions by regulating the balance of plant hormones, such as abscisic acid and cytokinins (Farooq *et al*., 2009; Li *et al*., 2016). The findings also suggest that the duration of stress significantly affects the RL of tobacco plants, as seen in the lower means observed in D + WOG, PD + WOG, F + WOG, and PF + WOG. This result is consistent with previous studies that have reported a reduction in RL as a response to water stress conditions in various plant species, including tobacco plants (Shah *et al*., 2023).

Biomass Yield

The BY of tobacco plants subjected to different durations of drought and flooding stress. Con had the highest BY with a mean of 254.95 ± 46.24 g, while PF + PD had the lowest BY with a mean of 120.88 ± 15.33 g. The results indicate that tobacco plants are sensitive to both drought and flooding stress, which are major factors affecting BY. ANOVA revealed that Con did not vary significantly to D, PD, and F with a mean BY of 232.80 ± 0.00, 225.29 ± 0.00, and 196.12 ± 0.00 g, but varied significant difference with PF, F + PD, and PF + PD with a total mean BY of 144.40 ± 30.65, 135.48 ± 4.95, and 120.88 ± 15.33 g, respectively. Several studies have investigated the effects of drought and flooding stress on BY in tobacco plants. For instance, Xu *et al.* (2022) conducted a study that reported a notable decrease in biomass yield in tobacco plants under drought stress. The study also revealed that water deficit stress caused a decrease in the number of leaves, stem length, and root volume in tobacco plants, which are important factors that influence biomass yield. Similarly, flooding stress has also been reported to have a negative impact on biomass yield in tobacco plants. A study by Wu *et al*. (2022) found that flooding stress resulted in a notable decrease in biomass yield, alongside reductions in chlorophyll content and photosynthesis rate in tobacco plants.

The average BY of tobacco plants in WG was 195.43 ± 7.65 g, while the average BY of plants in WOG was 178.83 ± 6.94 g (Table 2). This indicates that the presence of GA3 in tobacco plants can enhance their BY even under stressful conditions. However, further analysis revealed that the difference between the means of WG and WOG was statistically significant, suggesting that the effect of GA3 on BY may be influenced by other factors, such as the severity and duration of stress. Previous studies have reported the positive effect of GA3 on BY in various plant species under different stress conditions. For instance, a study conducted by Farooq *et al*. (2009) investigated the effect of GA3 on wheat growth under drought-stress conditions. The results showed that GA3 treatment significantly increased biomass yield, as well as other plant growth parameters such as plant height, leaf area, and root length. Similarly, a study conducted by Vanaja *et al.* (2015) reported that GA3 treatment increased the biomass yield of pigeon pea plants under drought-stress conditions. The mechanism behind the positive effect of GA3 on plant biomass yield under stress conditions is not fully understood. However, it has been suggested that GA3 can enhance photosynthesis and carbon assimilation in plants, which in turn can increase biomass yield (Morales, 2000). GA3 can also stimulate cell division and elongation, leading to increased biomass accumulation in plants (Farooq *et al*., 2009).

It was observed that the BY of tobacco plants decreased with increasing durations of both drought and flooding. However, the presence of GA3 had a positive effect on BY, particularly under stressful environmental conditions. The treatments with the highest BY were Con + WG (255.65 ± 9.72 g) and Con + WOG (254.25 ± 8.65 g), which had the shortest durations of drought and flooding and the presence of GA3. Conversely, the treatments with the lowest BY were PF + PD + WG (135.50 ± 5.12 g) and PF + PD + WOG (106.25 ± 4.65 g), which had the longest durations of drought and flooding and the absence of GA3, However, Con + WG was not vary significantly difference with Con + WOG but highly significantly to PF + WG (155.55 ± 5.12 g) to PF + PD + WG (135.50 ± 5.12 g) and vary significantly with D + WG (235.15 ± 7.65 g), D + WOG (230.45 ± 6.94 g), PD + WG (230.25 ± 7.12 g), PD + WOG (220.33 ± 6.78 g), and F + WG (205.45 ± 6.32 g). The results of the experiment suggest that GA3 has a positive effect on tobacco plant biomass yield under drought and flooding stress. This finding is consistent with previous studies that have reported the beneficial effects of GA3 on plant growth and yield under stressful environmental conditions (Shah *et al*., 2023). It is worth noting that the effects of GA3 on plant growth and yield can vary depending on the plant species, developmental stage, and environmental conditions. Therefore, further studies are needed to validate the findings of this experiment and to explore the underlying mechanisms of the GA3-mediated response to drought and flooding stress in tobacco plants.

CONCLUSIONS

Based on our research investigating the effects of applying GA3 to tobacco (*Nicotiana tabacum*) under varying water stress conditions, several key conclusions and recommendations emerge. Firstly, GA3 application yields positive growth effects, enhancing parameters like plant height, leaf count, leaf length, and biomass yield. Conversely, water stress adversely impacts tobacco growth, diminishing metrics like plant height, leaf area, and fresh weight. Notably, GA3 application mitigates these negative water stress effects, particularly at lower concentrations. However, determining the optimal GA3 concentration and water stress level depends on specific conditions such as tobacco variety, soil type, and climate. In light of these findings, recommendations include using GA3 as a tool to enhance tobacco growth under water stress, but with a focus on tailored applications based on local conditions. Growers in water-stressed regions should consider GA3 application to counteract growth limitations. Furthermore, future research should delve into the mechanisms by which GA3 aids tobacco in coping with water stress and explore its potential application in other water-stressed crops.

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