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Urban wastewater effects on chlorophyll fluorescence parameters, water productivity, and soybean yield under drought stress

Saeid SHIUKHY-SOQANLOO (✉), Bahareh SHAMGANI MASHHADI

Department of Water Engineering, Faculty of Agricultural Engineering, Sari Agricultural Sciences and Natural Resources University, Mazandaran, Iran

✉ Corresponding author: s.shiukhy@sanru.ac.ir

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ABSTRACT

Water resource depletion and recurrent drought events pose significant challenges to sustainable agricultural production, particularly in regions such as northern Iran. The study was carried out as a split-plot experiment arranged in a randomised complete block design with three replications over the 2022 and 2023 growing seasons in the Caspian Sea region, Sari, Iran. The experimental treatments consisted of three drought stress levels (non-stress (FC100), moderate (FC75), and severe (FC50))-as the main plot, and two irrigation source-urban wastewater (UWW) and well water (WW)-as the subplot. The results indicated that the highest plant height (119.5 cm), number of pods per plant (64), 100-seed weight (19.1 g), and grain yield (1109 kg/ha) were obtained under irrigation with UWW combined with FC100 conditions. In contrast, the lowest values for these traits, including plant height (93.8 cm), number of pods per plant (34.4), 100-seed weight (16.8 g), and grain yield (311 kg/ha), were recorded under WW irrigation and severe drought stress (FC50). Moreover, the highest water productivity (0.65 kg/m^3) was achieved under UWW and FC100 conditions, while the lowest value (0.25 kg/m^3) was observed under WW irrigation combined with FC50. Based on the results, the highest value of F_v/F_m (0.79), qP (0.99), and qN (0.06) was observed under FC100 conditions, whereas the lowest values for these parameters, 0.33, 0.83, and 0.03, respectively, were observed under severe stress (FC50). It can be concluded that the use of urban wastewater for irrigation represents a practical and feasible strategy to mitigate the adverse effects of drought stress during the soybean growing season.

Keywords: Caspian Sea, field capacity, pod number, non-conventional waters, water scarcity

INTRODUCTION

Soybean (*Glycine max* L.) is a key global oilseed crop, serving as a primary source of vegetable oil and protein. It contains approximately 18–22% oil and 35–40% protein, making it a vital component of human and animal nutrition. Globally, soybean holds the largest cultivated area among oil crops.

In Iran, soybean cultivation covers around 110,000 hectares annually, producing approximately 260,000 tons. The global average soybean yield is 2,190 kg/ha, with Iran slightly exceeding this at 2,360 kg/ha. Specifically, in Mazandaran province, soybean is cultivated on 87.8 hectares under irrigation and 121.7 hectares under rainfed conditions, yielding 122.3 and 157.2 tons per hectare, respectively (FAOSTAT, 2022).

The growing global population, expansion of agricultural and industrial activities, and recurring droughts have intensified pressure on freshwater resources. This increasing scarcity has led to the consideration of urban wastewater as an alternative irrigation source (Khawla et al., 2019). Urban wastewater can be utilised for irrigation, aquifer recharge, and nutrient recycling, contributing to sustainable agricultural development goals (Fridman et al., 2023). The reuse of wastewater in agriculture is crucial in areas with limited freshwater availability and declining groundwater levels, as it helps recycle nutrients back into biogeochemical cycles (Santos et al., 2023). Research indicates that irrigation with treated wastewater can enhance plant growth parameters such as height, biomass, chlorophyll content, and water productivity without significant reductions in crop yield (Rodríguez-Espinosab et al., 2023; Singh et al., 2023; Du et al., 2022). However, it did not affect the amount of flavonoids, phenolic compounds, and antioxidant activity (Ali et al., 2022).

The utilization of treated or untreated wastewater for agricultural irrigation has emerged as a promising strategy to alleviate water scarcity and enhance crop productivity and physiological performance. Nevertheless, the potential environmental and health risks associated with wastewater application, particularly the accumulation of toxic elements such as heavy metals in soil-plant systems, have raised significant concerns regarding its long-term sustainability and safety. Several studies have highlighted these dual aspects. For instance, Mahfooz et al. (2020) reported a statistically significant positive correlation between the use of wastewater for irrigation and the accumulation of heavy metals within plant tissues and agricultural soils, underscoring potential risks to food security and ecosystem health. However, their findings also demonstrated that, under controlled conditions, wastewater irrigation could lead to substantial improvements in both the quantitative and qualitative attributes of crop yield. Similarly, Raychaudhuri et al. (2014) emphasised that the substitution of conventional irrigation sources with wastewater should be preceded by rigorous physicochemical characterisation of both water and soil matrices to ensure that the practice does not pose adverse ecological or human health impacts over the long term.

Soybean is highly sensitive to water availability, with drought stress recognized as one of the most critical factors limiting its growth, yield, and quality (Morales Santos et al., 2022). The vulnerability of soybean to drought extends across all phenological stages, with the flowering and podding phases being particularly susceptible (Song et al., 2022). The detrimental effects of drought on plants are largely attributed to the disruption of photosynthesis, the fundamental physiological process governing plant growth and the stability of natural ecosystems. Leaf tissues, serving as the primary photosynthetic organs, play an essential role in capturing light energy and converting it into chemical energy through photosynthesis (Roth-Nebelsick and Krause, 2023; Yang et al., 2023). Drought stress adversely affects both the structural integrity and functional components of the photosynthetic apparatus. Biochemical limitations induced by water deficit lead to reduced photosynthetic efficiency, impaired pigment biosynthesis, and ultimately, disruption of the photosynthetic system (Zahra et al., 2023). In soybean, severe drought stress significantly diminishes key physiological parameters such as net photosynthetic rate, stomatal conductance, and the actual photochemical quantum yield of Photosystem II (PSII) (Wang et al., 2018).

Chlorophyll fluorescence has been widely recognised as a rapid, non-invasive, and reliable tool for evaluating photosynthetic performance under various environmental stresses. The analysis of chlorophyll fluorescence parameters provides critical insights into plant responses to drought, extreme temperatures, radiation intensity, water deficit, and salinity stress (Banks, 2017; Xia et al., 2018; Li et al., 2021; Azhar et al., 2020; Tsia et al., 2019). Parameters such as the maximum quantum efficiency of PSII photochemistry (F_v /F_m), initial fluorescence (F_0), maximum fluorescence yield (F_m), non-photochemical quenching (NPQ), and PSII operating efficiency have been established as reliable indicators of drought-induced alterations in photosynthetic performance (Ni et al., 2019). Previous studies have demonstrated the profound impact of drought stress on soybean physiology and growth. For instance, Lumactud et al. (2020) reported significant reductions in the number of nodes, root and shoot biomass, total shoot nitrogen content, and stem carbon-to-nitrogen ratio under drought stress, whereas root-to-shoot ratio and shoot nitrogen concentration increased compared to well-irrigated controls.

Similarly, Song et al. (2022) examined the influence of drought stress on the activity of key enzymes involved in carbon metabolism and the photosynthetic characteristics of soybean. Their findings revealed that drought stress altered several photosynthetic parameters, with Phi2, PhiNO, and F_m increasing under severe drought conditions, while PhiNPQ and F_v/F_m exhibited a marked decline.

In the present study area, reduced precipitation and its irregular distribution throughout the soybean growing season, particularly during the critical mid to late growth stages, have exacerbated the risk of drought stress, posing a serious challenge to sustainable production. Consequently, there is an urgent need to evaluate the effects of drought stress on soybean yield and its physiological determinants. In parallel, the application of urban wastewater has been reported to improve soil physical properties and fertility by supplying essential nutrients, which may partially mitigate the adverse effects of water deficit. Therefore, the present study was conducted to investigate the effects of urban wastewater on chlorophyll fluorescence parameters, water productivity, and soybean yield under drought stress conditions.

MATERIAL AND METHODS

This research was carried out in the Caspian Sea region, Sari (research farm of Sari University of Agricultural Sciences and Natural Resources, SANRU), Mazandaran, Iran. The studied area has latitude and longitude (36° 33′ N and 53° 00′ E, respectively), altitude (14 m, sea level), average temperature (17.9 °C), and average total rainfall (550 mm). The region is characterised by a temperate-humid climate, as classified by the Extended De Martonne climate classification system (Shamgani-Mashhadi et al., 2024). More details of the weather conditions of the study area during the growing season are presented in Table 1.

The study was carried out as a split-plot experiment arranged in a randomised complete block design with three replications over the 2022 and 2023 growing seasons in the Caspian Sea region, Sari, Iran. The Experimental treatments consisted of three drought stress levels (non-stress (FC100), moderate (FC75), and severe (FC50))-as the main plot, and two irrigation source-urban wastewater (UWW) and well water (WW)-as the subplot. The information related to the characteristics of urban wastewater and well water, as well as the physicochemical characteristics of the soil, was reported in Table 2 (Water Quality Laboratory of Mazandaran Regional Water Company).

Before sowing, the required fertilisers were added to the soil based on the field soil decomposition test. Depending on the needs of the plant during the growth period, fertilisation was done with NPK 20-20-20 fertiliser (nitrogen as nitrate: 6.5%, ammonium, 0.4%; urea, 4.10%; phosphate as P_2O_5, 0.20%; and potassium as K_2O, 0.20%) (EDTA). Soybean seeds of the Williams variety were planted manually on 13 June in plots with dimensions 1.2 m × 3 m in 4 rows with a distance of 10 cm within the rows and 30 cm between the rows. In addition, the distance between the blocks from each other was considered to be 2 m. Irrigation was done immediately after the planting operation. Soil water content (SWC) was monitored during the growing season using a tensiometer, and plants were re-watered when the SWC dropped to 70 % of soil field capacity. After the 6-8 leaf stage of soybean, irrigation was performed at 100% of field capacity in the control treatment, and at 75 and 50% of field capacity in the drought stress treatments, respectively. To prevent the effect of rainfall on the drought stress treatments, a rain shelter was used to control the amount of water used for irrigation.

Measurement of yield and yield components

To measure plant height, samples were taken from the soil surface, and the plant height was measured from the lowest point to the tip of the main stem of the plant (cm). The number of pods per plant was also counted. The 100-seed weight and the total yield were also measured using a digital scale with an accuracy of 0.01 g.

Table 1. The weather conditions of the experimental site during the growth season 2022 and 2023 (Iran Meteorological Organisation, IRIMO)

Growth season (month)	Evaporation (mm)		Precipitation (mm)		Minimum temperature (°C)		Maximum temperature (°C)		Average temperature (°C)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
June	159.9	165.3	22.1	17.3	27.4	28.1	33.6	34.7	23.4	24.3
July	178.2	165.8	14.8	13.4	32.7	30.6	37.8	36.6	28.7	29.1
August	205.8	215.7	2.9	0.7	28.6	29.1	35.1	35.8	22.8	23.4
September	135.8	129.4	36.1	44.1	23.9	24.7	30.1	31.4	19.3	20.8
October	67	65	145	136.5	19.5	20.8	26.1	27.6	14.4	15.7
Average	149.3	144.4	44.1	43.3	26.4	26.6	32.5	33.2	21.7	22.6

Table 2. Physico-chemical characteristics of soil, source of irrigation, and maximum permissible limits for wastewater application in agriculture

Characteristics	pH	NTU	TDS	TSS	EC	TP	COD	N	Ca	Na	Mg
	-	-	-	-	(ds/m)	(mg/l)					
UWW	8.1	2.9	663	27	1.06	4.9	17	0.5	100	58	18.1
WW	7.2	9.9	629.3	16	0.78	2.2	11.1	0.5	60	69	16.1
IRNDOE	6-8.5	50	-	100	-	6	200	-	-	-	100
WHO, 2006	6-8.5	-	450	-	0.7	4	-	-	-	-	-
FAO, 2017	6-8.5	-	450	-	0.7	4	-	-	-	-	-
Soil	Depth	Texture	pH	EC (ds/m)		OM (%)	OC (%)	P (ppm)		K (ppm)	N (%)
	(0-30cm)	Sand-loam	7.2	0.55		1.72	0.52	4		146	0.1

NTU: Nephelometric Turbidity unit; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; EC: Electrical Conductivity; TP: Total Phosphorus; COD: Chemical oxygen demand; N: Nitrogen; Ca: Calcium; Na: Sodium; and Mg: Magnesium

Measurement of water productivity

Water productivity was calculated from equation (1).

$$WP = GY / Wap \qquad (1)$$

where, *WP*: water productivity (kg/m^3), *GY*: grain yield (kg/ha), and *Wap*: water consumption (m^3/ha) (Farre and Faci, 2006).

Measurement of chlorophyll fluorescence parameter

A fluorometer (PAM 2500, Walz, Germany) was used to measure the fluorescence parameters. The length of the dark period in this measurement was considered 20 minutes. For this purpose, 5 plants were selected from each plot, and three young and fully opened leaves were selected from each plant. Each of the fluorescence parameters was calculated using the following equations (Wang et al., 2018).

$$F_v/F_m = (F_m - F_o) / F_m \qquad (2)$$

$$qP = 1 - (F_s - F_o') / (F_m' - F_o') \qquad (3)$$

$$qN = 1 - (F_m' - F_o'') / (F_m - F_o) \qquad (4)$$

$$NPQ = F_m / F_m' - 1 \qquad (5)$$

where, F_m (chlorophyll fluorescence maximum), *Fo* (chlorophyll fluorescence minimum) in dark-adapted leaves, F_m' (chlorophyll fluorescence maximum), *Fo′* (chlorophyll fluorescence minimum) in light-adapted leaves, F_v (difference between F_m and F_o as variable fluorescence), F_v/F_m (maximum PS II), F_s (steady-state fluorescence), *qP* (Photochemical quenching coefficient), *qN* and *NPQ* (non-photochemical quenching coefficients). The data were analysed using ANOVA of SAS software (SAS 9.2), and the Duncan post hoc test was employed to compare treatment means.

RESULTS AND DISCUSSION

Yield and yield components

The results of the analysis of variance examining the combined effects of irrigation source and drought stress on soybean yield and its components, as well as water productivity, are presented in Table 3. The findings indicate that both irrigation source and drought stress had a significant impact ($P \leq 0.01$) on plant height, number of pods per plant, 100-seed weight, yield, and water productivity.

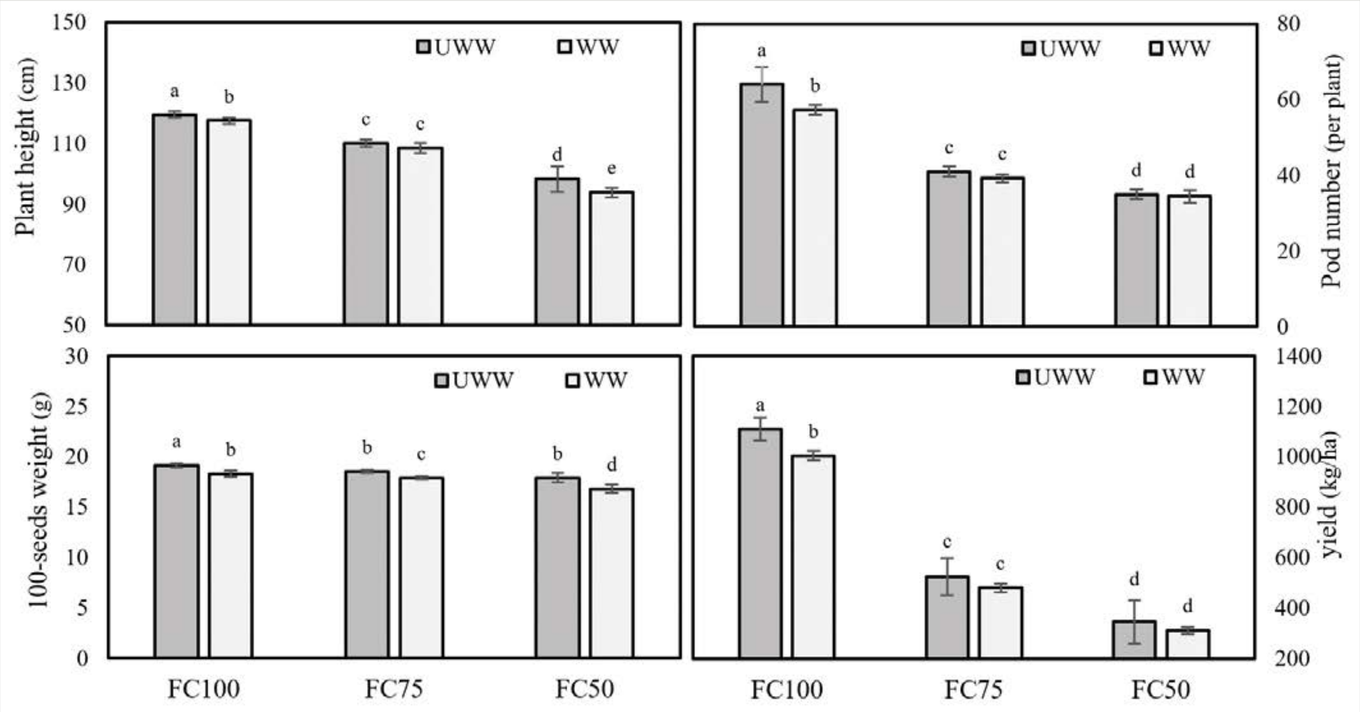
Based on the results, irrigation with urban wastewater led to the highest average values for plant height, number of pods per plant, 100-seed weight, and yield, measuring 119.5 cm, 46.6 pods, 18.5 g, and 705.2 kg/ha, respectively (Figure 1). As illustrated in Figure 1, urban wastewater irrigation significantly outperformed well water irrigation in all measured traits. The results also indicated that under non-stress conditions (FC100), the highest averages for plant height, number of pods per plant, 100-seed weight, and yield were observed, measuring 118.5 cm, 60.7 pods, 18.7 g, and 1056.7 kg/ha, respectively. In contrast, under moderate (FC75) and severe (FC50) drought stress conditions, these traits declined significantly as the intensity of drought stress increased. For instance, seed yield under non-stress conditions (FC100) was 1056.7 kg/ha, which decreased by 58% and 62% under moderate and severe stress conditions, respectively. Furthermore, the highest average plant height (119.5 cm) was recorded under irrigation with urban wastewater combined with non-stress conditions, while the lowest (93.8 cm) was observed under well water irrigation and severe drought stress (Figure 1). The highest average number of pods per plant and seed yield showed similar trends, with both peaking under irrigation with urban wastewater and non-stressed conditions—64 pods per plant and 1109 kg/ha seed yield, respectively. Furthermore, the highest average 100-seed weight of 19.1 g was also recorded under these same conditions. In contrast, the lowest average 100-seed weight of 16.8 g occurred under irrigation with well water combined with severe drought stress (Figure 1).

Plant responses to stress conditions are complex and influenced by various factors, including species and genotype, plant age and size, growth rate, as well as the intensity and duration of the stress (Ma et al., 2020). Drought stress significantly reduces soybean growth and yield by inhibiting photosynthetic product formation, lowering leaf photosynthetic capacity, accelerating leaf senescence, and increasing oxidative stress (Farooq et al., 2017).

Table 3. Results of analysis of variance for the effects of irrigation source and drought stress on soybean yield and yield components during the growth season

Source of variation (S.O.V)	df	Mean square				
		Plant height (cm)	Pod number (per plant)	100-Seed weight (g)	yield (kg/ha)	Water productivity (kg/m^3)
Year	1	6.25^{ns}	0.5877^{ns}	0.0002^{ns}	5181.60^{ns}	0.0040^{ns}
Year × Block	4	13.55^{ns}	8.6652^{ns}	0.0913^{ns}	2118.874^{ns}	0.0013^{ns}
Source of Irrigation (SI)	1	66.695**	79.804**	5.840**	207586.54**	0.1248**
Drought stress (DS)	2	1524.195**	2253.3369**	5.590**	1648522.302**	0.3108**
Year × SI	1	0.02778^{ns}	0.36^{ns}	0.0136^{ns}	6831.023^{ns}	0.0053^{ns}
Year × DS	2	0.0834^{ns}	0.5036^{ns}	0.0144^{ns}	2453.035^{ns}	0.0018^{ns}
SI × DS	2	57.1945*	32.50*	4.2887*	25073.424*	0.0078*
Year × SI × DS	2	0.1945^{ns}	0.0325^{ns}	0.0044^{ns}	1905.386^{ns}	0.0014^{ns}
Error	20	3.423	5.658	0.1279	2348.67	0.0015
Ttotal	35	-	-	-	-	-
C.V	-	1.7	5.2	1.9	7.7	9

**, * and ns: They indicate significance at 1%, 5% and non-significance levels, respectively (Duncan, *P* ≤ 0.05)



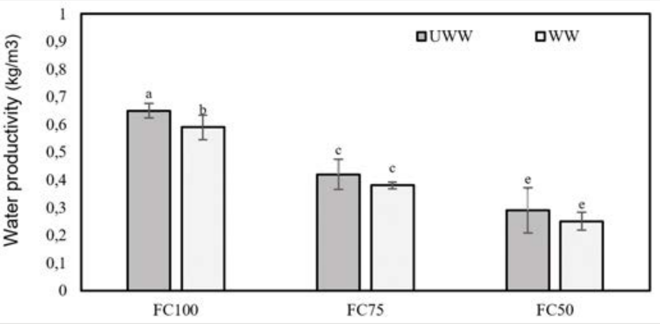
Note: Columns sharing the same letters indicate no significant difference between treatments. The lines on each column represent the error bars. Abbreviations: UWW = Urban Wastewater; WW = Well Water.

Figure 1. Interaction effect of irrigation source and drought stress on soybean yield and yield components

Drought stress reduces the biomass of soybean stems, roots, and seeds, and decreases the allocation of biomass to seeds compared to non-stress conditions, ultimately leading to lower seed weight. Additionally, drought stress lowers the dry matter distribution ratio in the seeds and pod walls but increases it in the roots and stems (Du et al., 2020). Soybean is considered one of the most important oilseed crops worldwide. However, its growth and productivity are highly influenced by environmental stresses, particularly drought stress during critical growth stages such as flowering, pod formation, and pod filling (Poudel et al., 2023). Previous studies have shown that drought stress at these stages results in significant reductions in yield quantity and quality (Gebre et al., 2022). Under optimal irrigation conditions, increasing the number of seeds per plant directly contributes to higher seed yield. Conversely, drought stress during the reproductive phase significantly reduces both seed number and seed weight (Galić Subašić et al., 2022). Drought stress not only limits evapotranspiration and biomass accumulation but also induces long-term physiological and developmental consequences, which negatively affect subsequent growth stages (Cui et al., 2021). Aziez and Prasetyo (2022) reported that drought stress at various growth stages significantly reduces leaf area index, net assimilation rate, and seed weight per plant. In addition, the plant response to drought involves complex physiological and molecular mechanisms, including the differential expression of drought-resistance-related genes. Severe drought conditions can reduce soybean yield by up to 50% (Heatherly, 2022). Given the growing importance of sustainable water management in agriculture, alternative water sources such as treated urban wastewater have been considered to mitigate the negative effects of water scarcity. Wastewater contains considerable amounts of nutrients, which can improve soil properties, increase nutrient availability, and consequently enhance plant growth and yield (Gatta et al., 2015). The application of wastewater has been shown to positively influence plant organ development, photosynthetic efficiency, and overall crop productivity compared to well water irrigation (Mahfooz et al., 2020). Furthermore, Swain et al. (2021) observed significant improvements in morphological traits and nutrient uptake in spinach under wastewater irrigation. Yield components such as the number of pods per plant, flower retention, and pod retention play a critical role in determining final seed yield. Tavares et al. (2011) emphasised that reductions in pod number and increases in flower and pod abortion are major factors leading to decreased seed yield. Recent research indicates that nutrient-rich wastewater can partially compensate for drought-induced damage by improving soil conditions and plant nutrition, which helps maintain yield under water-limited conditions (Ray et al., 2018; Tripathi et al., 2016). In this context, irrigation with treated wastewater appears to be an effective strategy to enhance plant resilience to drought stress and sustain crop yield under adverse environmental conditions. However, further research is needed to optimise wastewater application methods and assess potential environmental risks associated with its use.

Water productivity

Based on the results, water productivity under irrigation with urban wastewater in non-stress conditions was measured at 0.65 kg/m^3, while the lowest values were observed under severe drought stress, reaching 0.29 kg/m^3 and 0.25 kg/m^3 for irrigation with urban wastewater and well water, respectively. Water productivity is defined as the ratio of crop yield to the volume of water consumed by the crop during the growth season. Since crops irrigated with urban wastewater produced higher yields compared to those irrigated with well water, water productivity was consequently enhanced (Dubey et al., 2024). The decline in carboxylation activity during periods of drought stress and factors affecting CO_2 release into the chloroplast are primarily due to reduced water uptake, which leads to a decrease in water productivity as drought intensity and duration increase (Tabatabaei et al., 2020). Irrigation with wastewater supplies plants with higher nutrient levels, particularly nitrogen, compared to well water, promoting enhanced growth of plant organs. This nutrient availability improves the efficiency of the photosynthetic apparatus, ultimately increasing crop yield (Mishra et al., 2023). The findings of this study demonstrated that, although the total water applied under non-stress conditions was similar, irrigation with wastewater resulted in increased soybean yield and thus higher water productivity compared to well water irrigation. This improvement can be attributed to the better cultivation conditions and nutrient availability provided by wastewater irrigation. Although no statistically significant differences in water productivity were detected under moderate and severe drought stress between irrigation treatments, the average water productivity remained higher for plants irrigated with wastewater than those receiving well water. Hao et al. (2022) reported that combining organic wastewater with chemical fertilizers reduces the need for chemical fertilizers and enhances their utilization efficiency, achieving high cotton yields and improved water productivity under mulch drip irrigation in arid regions. Similarly, Giri et al. (2022) found that using treated and untreated wastewater in irrigation schedules increased soybean yield, yield components, and water productivity relative to well water irrigation. Moreover, studies conducted in diverse geographical regions such as Austria (Santos et al., 2023), Antalya, Turkey (Aydinsakir et al., 2021), the Volga region in Russia (Tolokonnikov et al., 2023), and South-eastern Australia (Zeleke and Nendel, 2024) emphasize the critical role of water productivity in soybean cultivation under varying irrigation regimes.



Note: Columns sharing the same letters indicate no significant difference between treatments. The lines on each column represent the error bars. Abbreviations: UWW = urban wastewater; WW = well water.

Figure 2. Interaction effect of irrigation source and drought stress on water productivity in soybean

Chlorophyll florescence parameter

The analysis of variance for the combined effect of irrigation source and drought stress on fluorescence parameters is presented in Table 4. The results revealed that drought stress had a highly significant effect on fluorescence parameters ($P \leq 0.01$), indicating the sensitivity of the photosynthetic apparatus to water limitation. In contrast, neither the main effect of irrigation source nor the interaction between irrigation source and drought stress showed a statistically significant influence on fluorescence parameters. had no significant difference. The findings suggest that while drought stress independently alters fluorescence characteristics, the source of irrigation (UWW or WW) does not significantly modify these parameters under the conditions tested.

The results indicated that chlorophyll fluorescence parameters in soybean exhibited a consistent decreasing trend across different levels of drought stress. A clear reduction was observed from non-stress conditions to severe drought stress. The only exception to this trend was observed in the NPQ (non-photochemical quenching) parameter, where no significant difference was detected between non-stress and moderate drought stress conditions; however, both conditions recorded higher NPQ values compared to severe drought stress. According to the findings, the highest average values for F_v/F_m ratio, qP, and qN were recorded under non-stress conditions (FC100), reaching 0.79, 0.99, and 0.06, respectively. Conversely, the lowest average values for these parameters were observed under severe drought stress conditions (FC50), with values of 0.33, 0.83, and 0.03, respectively. Similarly, the highest NPQ value of 0.05 was observed under both non-stress and moderate stress conditions, while the lowest NPQ value of 0.02 was recorded under severe drought stress (Figure 3). As shown in Figure 3, a reduction of 25% (moderate stress) and 50% (severe stress) in soil moisture based on field capacity resulted in a corresponding decrease in F_v/F_m ratio by 22% and 58%, respectively, compared to non-stress conditions. Furthermore, qP and qN decreased by 10% and 16%, and by 17% and 50%, respectively, under moderate and severe drought stress. While a 25% reduction in soil moisture had minimal effect on NPQ, a 50% reduction led to a significant 60% decrease in NPQ compared to the non-stress condition.

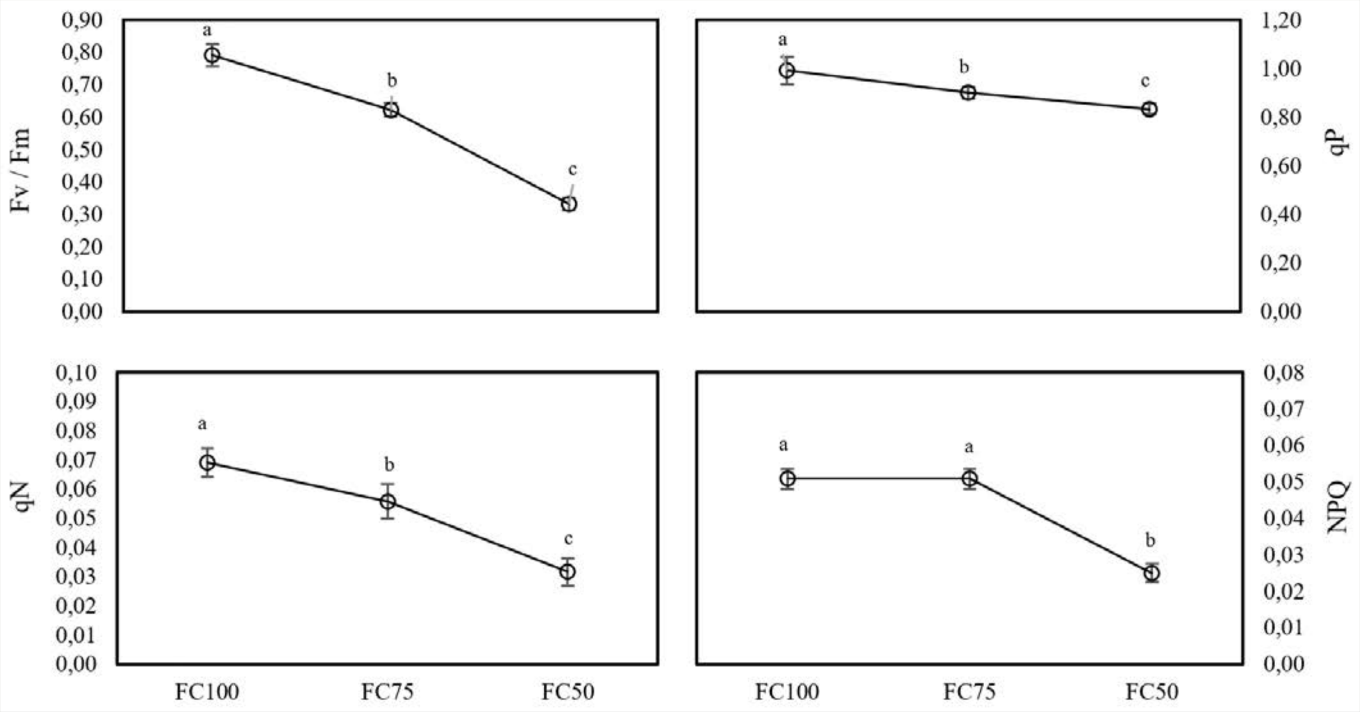
Drought stress is widely recognized as a major factor disrupting plant physiological processes. A reduction in photosynthetic pigment activity, which is closely associated with plant genotype and species, serves as a reliable indicator of plant exposure to environmental stresses, particularly water stress (Teles et al., 2023). Among the factors contributing to the maintenance and stability of photosynthetic capacity, chlorophyll content plays a key role and is significantly reduced under drought stress conditions (Jianguo et al., 2018). The F_v/F_m ratio, representing the maximum quantum efficiency of photosystem II (PSII), typically ranges from 0.78 to 0.84 across most plant species under optimal conditions. A reduction in this ratio reflects damage or inhibition in the reaction centers of PSII, often as a result of photoinhibition induced by environmental stress (Strasser and Stirbet, 2001). In this study, drought stress significantly decreased the F_v/F_m ratio, highlighting the adverse effects on the photosynthetic apparatus. Wang et al. (2022) similarly reported that chlorophyll fluorescence parameters in various soybean cultivars declined progressively with the duration of drought stress and were consistently lower under stress compared to well-watered conditions.

Song et al. (2022) also observed that increasing drought severity significantly reduced fluorescence parameters, including F_m, F_0, F_s, NPQ, and F_v/F_m, relative to non-stress conditions. The present results further revealed that irrigation with urban wastewater did not lead to an improvement in chlorophyll fluorescence parameters compared to well water irrigation. This may be attributed to the severe reduction in photosynthetic pigment activity under drought stress, which likely overshadowed the potential benefits of wastewater irrigation, such as improved soil fertility and partial nutrient supplementation. It is important to note that light intensity and water availability have a more direct and substantial influence on the performance of photosynthetic pigments than nutrient availability or improved soil conditions. These findings are consistent with those of Kaczmarska and Michalek (2013) and Na

Table 4. Results of analysis of variance for the effects of irrigation source and drought stress on chlorophyll fluorescence parameters of soybean during the growth season

Source of variation (S.O.V)	df	Mean square			
		Fv/F_m	qP	qN	NPQ
Year	1	0.00054^{ns}	0.0025^{ns}	0.00001^{ns}	0.00001^{ns}
Year × Block	4	0.00082^{ns}	0.0010^{ns}	0.00008^{ns}	0.00002^{ns}
Source of Irrigation (SI)	1	0.0013^{ns}	0.0009^{ns}	0.00017^{ns}	0.00001^{ns}
Drought stress (DS)	2	0.6475**	0.0750**	0.0043**	0.0026**
Year × SI	1	0.0001^{ns}	0.0004v	0.00001^{ns}	0.00004^{ns}
Year × DS	2	0.00003^{ns}	0.0004^{ns}	0.00005^{ns}	0.00003^{ns}
SI × DS	2	0.0014^{ns}	0.0002^{ns}	0.00005^{ns}	0.0000002^{ns}
Year × SI × DS	2	0.00007^{ns}	0.0009^{ns}	0.000002^{ns}	0.000002^{ns}
Error	20	0.0005	0.0017	0.00008	0.00006
Ttotal	35	-	-	-	-
C.V	-	3.8	4.6	18.11	18.51

**, * and ns: They indicate significance at 1%, 5% and non-significance levels, respectively (Duncan, $P \leq 0.05$)



Note: Columns with the same letters are not significantly different at the 5% probability level. Error bars represent the standards error of the mean.

Figure 3. Effect of drought stress on chlorophyll fluorescence parameters of soybean during the growth season

et al. (2014), who also reported that the beneficial effects of nutrient enrichment under wastewater irrigation could not compensate for the physiological damage caused by water stress in plants.

CONCLUSIONS

The occurrence of drought stress, irregular rainfall distribution, and limited water resources during the soybean growing season highlights the importance of understanding soybean responses to stress conditions and evaluating the effectiveness of alternative irrigation sources, such as urban wastewater. The results of this study demonstrated that moderate and severe drought stress significantly reduced morphological traits, yield, yield components, and chlorophyll fluorescence parameters in soybean plants. However, irrigation with urban wastewater, due to its nutrient content and positive effects on soil fertility, led to improved plant growth and yield, effectively mitigating some of the negative impacts of drought stress compared to well water irrigation. Although wastewater irrigation did not fully eliminate the adverse effects of water deficit, it proved to be more effective than well water in enhancing growth characteristics and yield under both moderate and severe drought stress conditions. These findings suggest that urban wastewater can serve as a potential alternative to conventional water sources to reduce drought-induced yield losses in soybean cultivation. However, its use requires careful management, including regular monitoring of water and soil quality, to prevent long-term negative impacts such as heavy metal accumulation and environmental degradation.

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