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EXPLORING TOLERABLE AND SENSITIVE CANOLA VARIETIES FOR CULTIVATION WITH PB CONTAMINATED MUNICIPAL WASTEWATER BY USING IRRIGATION DILUTION TECHNIQUE

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Abstract

Agriculture and food security are seriously threatened by water shortage, a worldwide problem that is quickly becoming more of a concern in many countries. By reducing water shortages and enhancing soil fertility by recycling nutrients and organic matter found in the wastewater, using municipal wastewater as an irrigation source offers the potential to address this issue. However, the high contamination of heavy metals in such wastewater is also a major concern for its usage. Despite ongoing efforts to treat municipal wastewater for agricultural use through physical, chemical, and biological methods, the use of dilution techniques remains a crucial area of study. It can minimize the toxic concentration of heavy metals in municipal wastewater via the dilution effect. In this regard, a laboratory screening experiment was conducted to determine the effectiveness of municipal wastewater irrigation (WWI) on the growth of 15 varieties of canola plants. The diluted WWI was applied at a rate of control (no wastewater and 1000 ml of tap water), 20%, 40%, 60%, 80%, and 100%. The findings showed that Punjab Canola and Dunkeld seedlings had the best growth outcomes at all irrigation levels. On average, 60% of WWI emerged as the optimal dilution for enhancing canola seedling's growth and minimizing stress caused by contaminations in WWI. CON-III and AC Excel were found to be more sensitive to stress, while Punjab Canola and Dunkeld showed resistance to 100% WWI. Further investigation under pot and field conditions is required to determine the best varieties of canola and to declare 60% WWI as the appropriate dilution for using municipal wastewater as irrigation.

Key words: Irrigation; Growth attributes; Antioxidants; Dilution technique; Canola.

Introduction

The shortage of fresh water for irrigation in arid and semi-arid zones is a major concern for agricultural production and food security (Danish & Zafar-ul-hye, 2020, Danish *et al*., 2020). These areas receive low amounts of rainfall and have limited water resources, making it difficult for farmers to grow crops and maintain their livelihoods (Priyan, 2021). Due to population increase, urbanization, and industrialization, there is a rising need for water for irrigation, further depleting already scarce water supplies (Priyan, 2021). The limited water availability in arid and semi-arid zones has resulted in the over-extraction of groundwater, leading to its depletion (Mizyed, 2013). In some cases, the water table has dropped to such low levels that it is no longer economically viable to pump water for irrigation (Mizyed, 2013). Additionally, the poor quality of water in these areas, due to high levels of salinity and other contaminants, limits its use for irrigation (Priyan, 2021).

To address the shortage of fresh water for irrigation, several strategies have been proposed. One approach is the dilution of sewerage water with good quality water for its usage as irrigation (Minhas *et al*., 2022). In addition to reducing the quantity of garbage produced by cities, using municipal waste as irrigation water for agricultural production has the potential to ease water shortages in arid and semi-arid countries (Bakari *et al*., 2022). Municipal waste, also known as wastewater, is generated from households, institutions, and industries and contains a variety of pollutants and organic matter. However, if treated properly, it can be used as an alternative source of water for irrigation (Bakari *et al*., 2022).

The potential benefits of using treated wastewater for irrigation include increased water availability, reduced pressure on freshwater resources, and improved soil fertility (Lucia *et al*., 2022). The organic matter and nutrients present in wastewater can provide essential nutrients to crops, improving their growth and yields (Asirifi *et al*., 2023). Additionally, using wastewater for irrigation might lessen the quantity of waste that cities produce, aiding in the solution of the waste management problem. Municipal garbage, however, has harmful effects on the environment and agriculture when used as irrigation water. Untreated wastewater that contains pollutants and germs can be hazardous to the environment and to people's health (Ait-Mouheb *et al*., 2022). The use of untreated wastewater for irrigation can lead to the contamination of crops with harmful substances, reducing their quality and safety for human consumption (Ait-Mouheb *et al*., 2022).

To minimize the negative impacts of using municipal waste for irrigation, it is important to properly treat the wastewater before use (Bakari *et al*., 2022). This may be done by using wastewater treatment techniques that remove pathogens and contaminants through physical, chemical, and biological means. One way of treating municipal wastewater that can be utilized to partially enable irrigation is dilution (Bakari *et al*., 2022). Dilution involves mixing the wastewater with a large volume of clean water, such as freshwater or rainwater, to reduce the concentration of pollutants and pathogens. This process can help to minimize the risks associated with using wastewater for irrigation, such as the contamination of crops with harmful substances (Bakari *et al*., 2022).

Furthermore, research on canola is crucial due to its significance as an agricultural crop and its economic importance (El Gafary *et al*., 2022). It is a major oilseed crop and plays a significant role in the agriculture industry, therefore research can help to improve its yield, quality, and resistance to environmental stressors like drought, pests, and diseases (Viana *et al*., 2022). Additionally, using wastewater for irrigation might lessen the quantity of waste that cities produce, aiding in the solution of the waste management problem. Municipal garbage, however, has harmful effects on the environment and agriculture when used as irrigation water. Untreated wastewater that contains pollutants and germs can be hazardous to the environment and to people's health (Barthet, 2015). That's why the current study was conducted to address the issue of low canola production due to the limited availability of good quality irrigation water. This experiment is covering the knowledge gap regarding the use of municipal wastewater as irrigation after dilutions with good quality water.

Comparing irrigation with diluted municipal wastewater to irrigation with tap water is thought to potentially improve the growth and development of canola crops. This theory is based on the idea that the dilution process may help to lessen the amount of pathogens and contaminants that are present in the wastewater, potentially reducing any adverse effects on crop health. Additionally, the presence of helpful plant nutrients like nitrogen and phosphorus in wastewater may help canola crops develop and produce more. This theory has to be investigated further to establish the ideal concentration and make-up of diluted municipal wastewater for canola irrigation.

Material and Methods

To select canola-resistant, moderate, and sensitive types for growing under various diluted levels of municipal wastewater irrigation, a laboratory hydroponic experiment was done.

Canola varieties: The Ayub Agriculture Research Institute provided 15 different canola types for the trial. There were several different kinds, including Dunkeld, Super Raya, Rainbow, Punjab Canola, Oscar, Legend, AARI Canola, AC Excel, CON-II, CON-III, Cyclone, Faisal Canola, Super Canola, Sandal Canola, and Shiralee. A manual screening procedure was performed to weed out any damaged seedlings before the experiment began.

Municipal wastewater: The collection of municipal wastewater was carried out from a local sewerage line (30°50'44.2"N 70°57'38.1"E). To ascertain the samples' pre-experimental characteristics, which are shown in (Table 1), the samples were evaluated in a lab. By combining the wastewater with deionized water, successive dilutions were created based on the properties of the effluent. Following were the wastewater concentrations used in the experiment: Control (no wastewater and 1000 ml of tap water), 20% (200 ml of wastewater and 800 ml of tap water), 40% (400 ml of wastewater and 600 ml of tap water), 60% (600 ml of wastewater and 400 ml of tap water), 80% (800 ml of wastewater and 200 ml of tap water), and 100% (1000 ml of wastewater and no tap water).

Treatment plan: The experiment involved six levels of municipal wastewater irrigation (WWI), namely Control, 20, 40, 60, 80, and 100%, in combination with 15 canola varieties, namely Dunkeld, Super Raya, Rainbow, Punjab Canola, Oscar, Legend, AARI Canola, AC Excel, CON-II, CON-III, Cyclone, Faisal Canola, Super Canola, Sandal Canola, and Shiralee. A total of 90 treatment combinations were evaluated in three replications to ensure robust results.

Table 1. Pre-experimental characteristics of wastewater irrigation.

Attribute	Unit	Value	References
pH	-	7.65	(Estefan *et al*., 2013)
EC	dS/m	3.46	
Nitrogen	mg/L	21	
Phosphorus		6.9	
Potassium		288	
Sodium		201	
Calcium		51	
Chloride		190	
Carbonates	meq./L	2.31	
Bicarbonates		5.67	
Magnesium		4.54	
Cadmium	mg/L	0.002	
Lead		0.225	
Arsenic		0.04	
Chromium		0.021	

Sowing and incubation conditions: Ten sterilized seeds were seeded in sterile petri plates for each of the three repetitions. The seeds were soaked in a sodium hypochlorite solution (5%). The soaking duration varied accordingly, from a few minutes to several hours. Subsequently, the sterilized seeds were thoroughly rinsed with ethanol (95%) and sterile distilled water multiple times to remove any residual sodium hypochlorite (Ahmad *et al*., 2014). For the best germination, the seeds were sandwiched between sterile filter sheets. Throughout the experiment, the petri dishes were incubated at a temperature of 25°C±3°C. The humidity in the incubator was kept at 70% throughout the experiment to provide ideal conditions for seed germination and development.

Data collection and harvesting: The number of seeds that germinated on the third and seventh day after planting was counted to determine the germination percentage. After 15 days of seeding, the first harvest was carried out, and three healthy seedlings were taken from each petri dish. A standard scale was used to measure the root and shoot lengths, and an analytical-grade balance was used to weigh the fresh root and shoot. After being oven-dried for 48 hours at 65°C to ascertain their dry weights, the shoot and root samples were then reweighed on an analytical-grade scale.

Transplantation: The remaining seedlings from the petri dishes were transplanted into plastic cups with a diameter of 3 inches and a depth of 5 inches. A rolled filter paper was used to support the shoots of the seedlings. The experiment continued for a total of 30 days after sowing.

Harvesting for antioxidants: After 30 days of seeding, three healthy seedlings were harvested from each petri dish for the second harvest. To measure the amount of antioxidants in fresh leaf samples, a spectrophotometer was used.

POD assay: For the peroxidase (POD) assay in fresh plant leaves, we collected and rinsed the leaves with distilled water to remove any dirt or debris, then blotted them dry with filter paper. The leaves were ground in a mortar and pestle with 50 mM phosphate buffer (pH 7.0) to obtain a homogenate. The POD enzyme was extracted from the homogenate by centrifugation at 10,000 x g for 10 minutes at 4°C to obtain the supernatant. The POD activity was assayed by mixing 0.1 ml of the POD supernatant with 1.9 ml of the assay buffer (50 mM phosphate buffer, pH 7.0) and 0.1 ml of guaiacol as the substrate. The reaction mixture was incubated at room temperature for 5 minutes, stopped by adding 1.0 ml of 1 M H_2SO_4, and the optical density was immediately measured at 470 nm using a spectrophotometer. The POD activity was calculated using a standard curve (Pütter, 1974).

CAT assay: For the catalase assay, fresh plant leaves were collected, rinsed with distilled water, and blotted dry with filter paper. The leaves were then ground in a mortar and pestle with a buffer solution (e.g., 50 mM phosphate buffer, pH 7.0) to obtain a homogenate. The CAT enzyme was extracted from the homogenate by centrifugation at 10,000 x g for 10 minutes at 4°C to obtain the supernatant. The CAT activity was assayed by mixing 0.1 ml of the CAT supernatant with 1.9 ml of the assay buffer (e.g., 50 mM phosphate buffer, pH 7.0) and 0.1 ml of hydrogen peroxide (H_2O_2) as the substrate. The decrease in H_2O_2 concentration was monitored spectrophotometrically by measuring the decrease in absorbance at 240 nm over time. The CAT activity was calculated using the change in absorbance per unit protein (Teranishi *et al*., 1974).

SOD assay: For the superoxide dismutase (SOD) assay, fresh plant leaves were collected, rinsed with distilled water, and blotted dry with filter paper. The leaves were then ground in a mortar and pestle with a buffer solution (e.g., 50 mM phosphate buffer, pH 7.0) to obtain a homogenate. The SOD enzyme was extracted from the homogenate by centrifugation at 10,000 x g for 10 minutes at 4°C to obtain the supernatant. The SOD activity was assayed using a spectrophotometric method based on the inhibition of the photochemical reaction of nitro blue tetrazolium (NBT) with superoxide radicals. The reaction mixture consisted of 0.1 ml of the SOD supernatant, 1.9 ml of the assay buffer (e.g., 50 mM phosphate buffer, pH 7.0), and 0.1 ml of a solution containing NBT and riboflavin. The increase in absorbance at 560 nm was measured over time, and the SOD activity was calculated using a standard curve or the change in absorbance per unit protein (Giannopolitis & Ries, 1977).

MDA assay: For the malondialdehyde (MDA) assay, fresh plant leaves were collected, rinsed with distilled water, and blotted dry with filter paper. The leaves were then ground in a mortar and pestle with a buffer solution (e.g., 50 mM phosphate buffer, pH 7.0) to obtain a homogenate. The homogenate was used to extract the lipid peroxidation products, including MDA, using a solvent extraction procedure (e.g., using a mixture of butanol and acetic acid). The MDA concentration was then measured using a colorimetric assay based on the reaction of MDA with thiobarbituric acid (TBA) to form a pink-colored complex. The reaction mixture consisted of 0.1 ml of the extracted sample, 1.9 ml of a reaction buffer (e.g., 0.67% (w/v) TBA in 20% (v/v) acetic acid), and the mixture was heated at 95-100°C for 20 minutes. The absorbance of the reaction mixture was measured at 532 nm, and the MDA concentration was calculated using a standard curve or a calibration equation (Király & Czövek, 2002).

Statistical analyses

A standard statistical procedure was followed for the statistical analysis of the data (Steel *et al*., 1997). For the comparison of treatments, a two-way analysis of variance (ANOVA) was carried out along with Fisher's least significant difference test (LSD) at a significance level of p 0.05. The study variables, irrigation levels, and canola cultivars were correlated positively and negatively using Pearson correlation. Utilizing the program OriginPro2021, data analysis, and graph development were carried out (OriginLab Corporation, 2021).

Results

Effects of different levels of wastewater irrigation (WWI) were significant in the 3rd day germination of different canola varieties. Results showed that Dunkeld and Punjab Canola were significantly best for showing the highest 3rd day germination compared to Super Raya, Rainbow, Super Canola, Sandal Canola and Shiralaee where tap water was applied as a source of irrigation. Under 20, 40 and 60WWI, Punjab Canola, Dunkeld and Cyclon performance was significantly better for 3rd day germination compared to Super Raya, Rainbow, Super Canola, Sandal Canola and Shiralaee. It was noted that Punjab Canola differed significantly better than Dunkeld at 80 and 100WWI for 3rd day germination. However, Dunkeld showed significantly better results for 3rd day germination over Super Raya, Rainbow, Super Canola, Sandal Canola and Shiralaee (Fig. 1A). For 7th day germination, Dunkeld and Punjab Canola showed significantly better results compared to all other canola varieties under tap water irrigation, 20, 40 and 60 WWI. On the other hand, Punjab Canola performed significantly better than Dunkeld for 7th day germination yet, both were significantly better than Super Raya, Rainbow, Super Canola, Sandal Canola and Shiralaee. Super Raya, AARI Canola and Shiralaee showed minimum 7th day germination among all the varieties under all levels of irrigation (Fig. 1B).

Results showed that Punjab Canola differed significantly better than all the varieties under tap water, 20, 60, 80 and 100 WWI for shoot length. No significant change was noted in the shoot length of Dunkeld and Punjab Canola at 40WWI. AARI Canola and CON-III were the varieties that showed the lowest shoot length under all levels of irrigation. On average 60WWI irrigation was the best level which showed the maximum shoot length of all the varieties compared to tap water, 20, 40, 80 and 100WWI. It was also observed that 100WWI showed relatively lower, shoot lengths of canola varieties compared to tap water (Fig. 2A). For root length, Punjab Canola performance was significantly better compared to all other varieties at tap water, 20, 80 and 100 WWI. No significant alteration in root length was observed in Dunkeld and Punjab Canola at 40 and 60 WWI. However, both remained significantly better for the enhancement in the root length compared to AARI Canola and CON-III. On average 60WWI irrigation was the best level which showed the maximum root length of all the varieties compared to tap water, 20, 40, 80 and 100WWI. It was also observed that 100WWI showed relatively lower, root lengths of canola varieties compared to tap water (Fig. 2B).

It was noted the Punjab Canola showed significantly higher shoot fresh weight under tap water, 20, 80 and 100WWI compared to all other canola cultivars. Dunkeld and Punjab Canola remained statistically similar to each other for shoot fresh weight under 40WWI and 60WWI. However, CON-III and AARI Canola performance was poor for the improvement in shoot fresh weight under all levels of WWI and tap water (Fig. 3A). For shoot dry weight similar trend was also noted where Punjab Canola differed significantly better at tap water, 20, 60, 80 and 100WWI compared to all other canola cultivars. However, only at 40WWI Dunkeld and Punjab Canola did not show any significant variation in shoot dry weight. Like shoot fresh weight, CON-III and AARI Canola showed minimum shoot dry weight among all the canola cultivars under tap water and WWI (Fig. 3B).

For improvement in root fresh weight, Punjab Canola differed significantly better compared to all other varieties under tap water and at all levels of WWI. At 40 and 60 WWI no significant variations were observed in root fresh weight of Dunkeld and Punjab Canola. However, AARI Canola and CON-III showed minimum root fresh weight than all other canola varieties under tap water and variable levels of WWI. Results clearly showed that 60WWI provided the maximum root fresh weight compared to other levels of WWI and tap water (Fig. 4A). In case of improvement in root dry weight, Punjab Canola performed as significantly better compared to all other canola varieties under tap water and variable levels of WWI. CON-III and AARI Canola did not differ significantly at 20, 40, 60, 80 and 100 WWI with each other for root dry weight. It was also observed that CON-III and AARI Canola showed the lowest values of root dry weight under tap water and different levels of WWI (Fig. 4B).

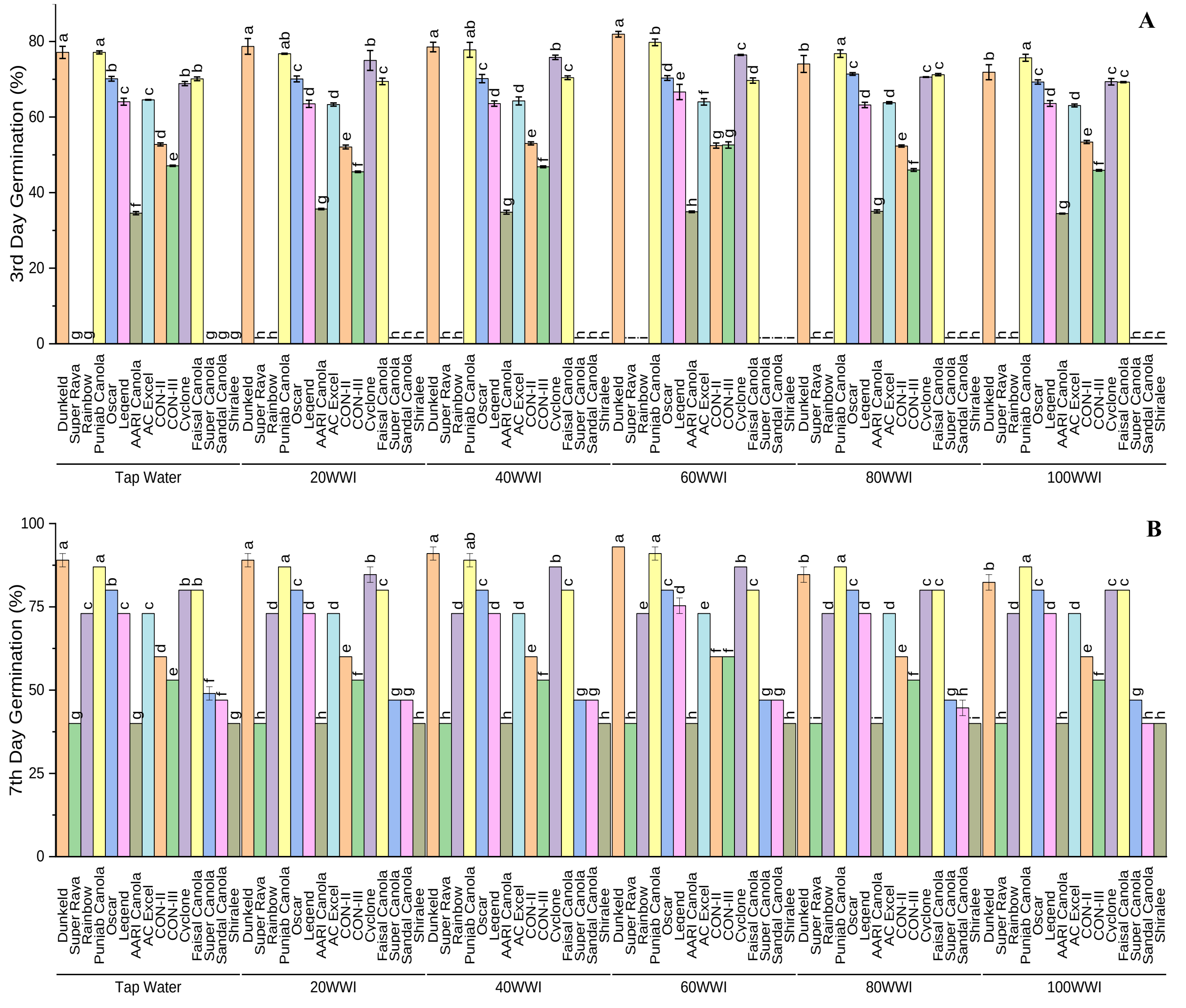

Fig. 1. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on 3rd day and 7th day germination of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \leq 0.05$: Fisher LSD.

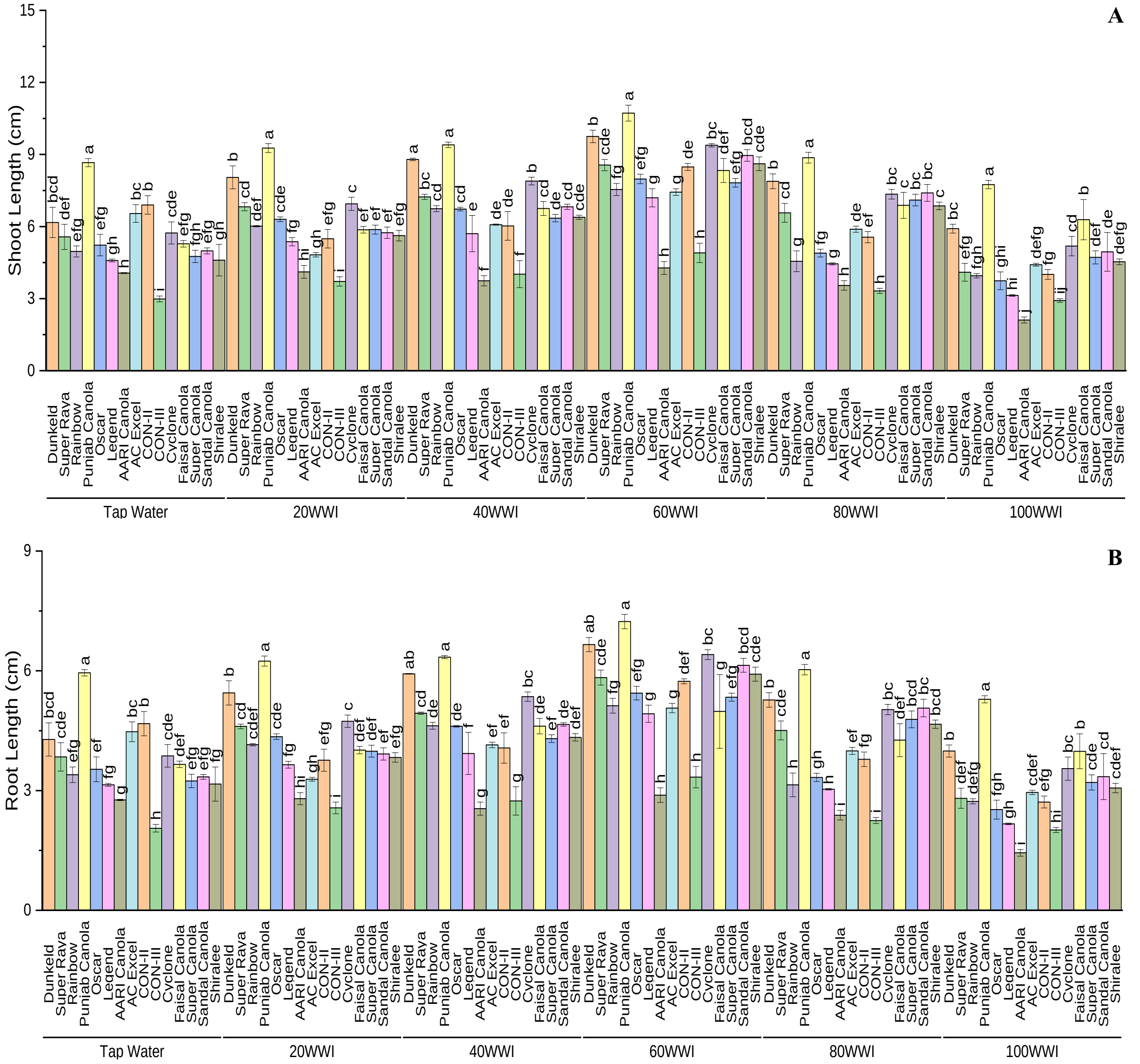

Fig. 2. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on shoot and root lengths of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \leq 0.05$: Fisher LSD.

A significant variation in seedling's fresh and dry biomass was observed in Punjab Canola under tap water and 20 WWI compared to all other canola varieties. Dunkeld and Punjab Canola performance was statistically similar to each other, but both differ significantly for enhancement in seedling's fresh and dry weight compared to all other canola varieties. It was also recorded that most canola cultivars showed their maximum potential for seedlings fresh (Fig. 5A) and dry weight (Fig. 5B) at 60WWI compared to all other levels of irrigation. However, on average AARI Canola and CON-III performance was lowest compared to other canola varieties for seedlings fresh and dry weight.

In Peroxidase (POD) of the shoot, Rainbow, Shiralaee and Super Canola showed the highest value compared to all other canola cultivars in tap water irrigation, 20, 40, 60, 80 and 100WWI. On the other hand, Dunkeld, Punjab Canola and Oscar showed significantly lower POD shoot compared to Rainbow, Shiralaee and Super Canola in tap water irrigation, 20, 40, 60, 80 and 100WWI (Fig. 6A; S1). A similar kind of trend was also noted in shoot catalase (CAT) where Rainbow, Shiralaee and Super Canola showed significantly higher values in tap water irrigation, 20, 40, 60, 80 and 100WWI. However, Dunkeld and Punjab Canola showed significantly lower shoot CAT compared to Rainbow, Shiralaee and Super Canola in tap water irrigation, 20, 40, 60, 80 and 100WWI (Fig. 6B; S2). The superoxide dismutase (SOD) and malondialdehyde (MDA) were significantly highest in Super Canola, Sandal Canola and Shiralaee compared to all other canola varieties in tap water irrigation, 20, 40, 60, 80 and 100WWI. Dunkeld and Punjab Canola showed significantly lower SOD (Fig. 7A; S3) and MDA (Fig. 7B; S4) than Super Canola, Sandal Canola and Shiralaee under tap water irrigation, 20, 40, 60, 80 and 100WWI.

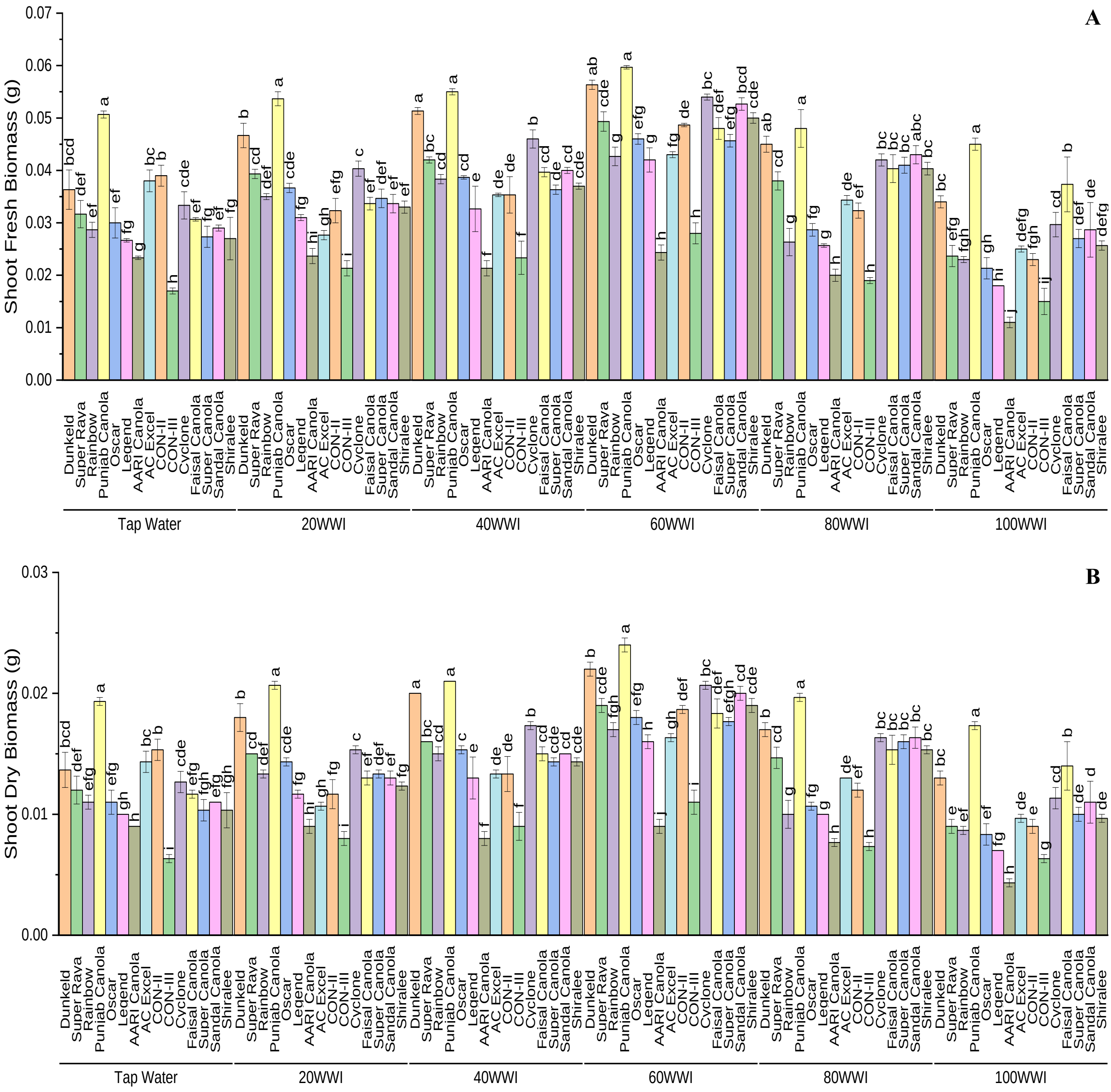

Fig. 3. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on shoot fresh and dry biomass of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \leq 0.05$: Fisher LSD.

Pearson correlation showed that the impact of irrigation was significantly native to CAT and SOD of the shoot in different canola varieties. However, a significant negative correlation was also established by different canola varieties with 3rd and 7th days germination, Shoot length, root length, seedling height, fresh and dry weight of shoot, fresh weight of root and seedlings dry biomass. POD, SOD, CAT and MDA were positively correlated with the variations in varieties. It justified that some varieties were sensitive, and some were resistant against different levels of WWI irrigation which were applied as treatment. MDA and POD showed a significant negative correlation with shoot and root length, shoot and root fresh and dry weight, seedlings height, fresh and dry weight. However, both MDA and POD showed significant positive correlation with SOD and CAT (Fig. 8).

The cluster plot convex hull analysis was conducted to explore the distribution and separation of irrigation conditions based on the first two principal components, PC1 and PC2. These components accounted for 62.31% and 28.14% of the total variance, respectively. Noteworthy examples include the "Dunkeld" variety, which exhibits scores of approximately 2.82 and -3.15 on PC1 and PC2, respectively. Contrarily, the "Super Raya" variety is characterized by a score of 0.06 on PC1 and 1.64 on PC2, while "Rainbow" showcases scores of -0.15 on PC1 and 2.01 on PC2. Additionally, "Punjab Canola" demonstrates scores proximate to 4.40 on PC1 and -2.33 on PC2, whereas "Oscar" occupies a position of approximately 0.28 on PC1 and -2.18 on PC2. This dataset's intricate portrayal extends to "Legend," which manifests values of about -2.02 on PC1 and -1.88 on PC2, and "AC Excel," attaining scores of around 2.68 on PC1 and 0.92 on PC2. Furthermore, nuanced insights emerge from the scores of "CON-II" (approximately 2.35 on PC1 and -0.02 on PC2) and "CON-III" (circa -4.61 on PC1 and -1.28 on PC2), delineating distinct positioning patterns (Fig. 9A). For the "Tap Water" irrigation treatment, data points were grouped within a specific region of the plot characterized by scores around -3.15 on PC2 and varying scores on PC1. This indicated a common clustering of samples subjected to this irrigation condition. Similarly, the "20WWI," "40WWI," "60WWI," and "80WWI" irrigation treatments exhibited distinct clusters, each occupying a specific region on the plot based on their corresponding scores on both PC1 and PC2. Notably, the "100WWI" irrigation treatment samples formed their cluster, demonstrating a clear separation from other treatments in the plot (Fig. 9B).

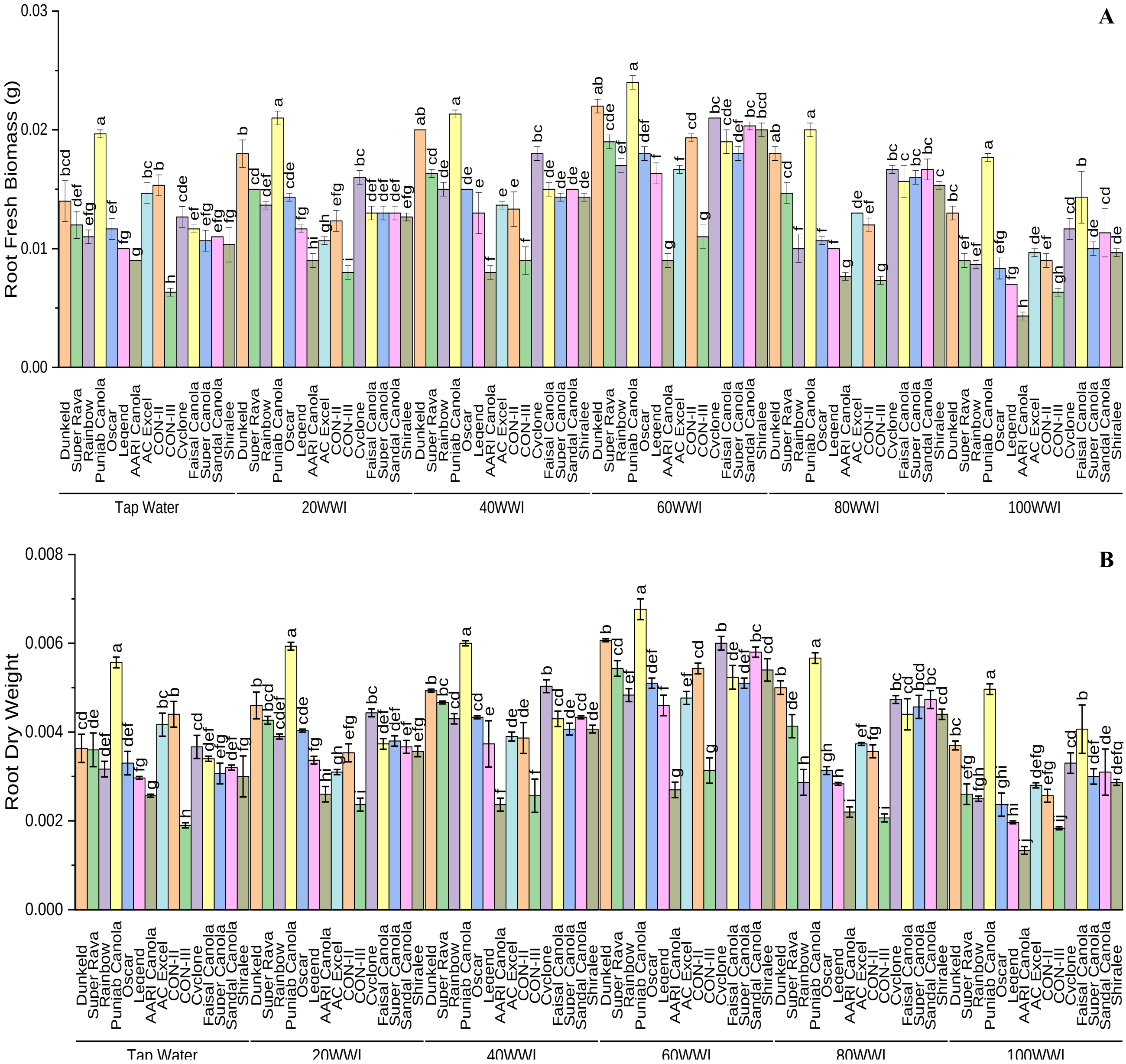

Fig. 4. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on root fresh and dry biomass of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \leq 0.05$: Fisher LSD.

Discussion

Results of the current study indicated that, notably in Dunkeld and Punjab Canola, 60% of WWI induced a considerable improvement in canola growth parameters. The enrichment of N, P, and K in the irrigation system's municipal effluent might be to blame for the improvement in growth characteristics (WWI) (Martín-Hernández *et al.*, 2009, Xue *et al.*, 2015). Second, diluting WWI also lessens the damaging effects of heavy metals, which ultimately have a significant impact on the lowering of canola's growth characteristics and antioxidant content. In hydroponic tests, it was shown that higher amounts of potassium and nitrogen in municipal wastewater had a favorable impact on canola growth (Martín-Hernández *et al.*, 2009, Xue *et al.*, 2015).

Nitrogen (N) is essential for the growth and development of plants. It is a crucial part of amino acids, which serve as the foundation for proteins (Hawkesford *et al*., 2012, Dawar *et al*., 2023). The essential component of chlorophyll, which is required for photosynthesis, is N. Nitrogen is also involved in the production of nucleic acids, which are necessary for cell division and DNA replication (Hawkesford *et al*., 2012). All things considered, plants need enough nitrogen to grow, develop, and reproduce. By promoting root development, improving photosynthesis, and boosting the production of crucial proteins and enzymes, phosphorus (P) intake can stimulate plant growth. Phosphorus is also essential for the transport and storage of energy inside the plant, which is necessary for metabolic activities (Hawkesford *et al*., 2012, Danish *et al*., 2015, Bibi *et al*., 2020, Rafiullah *et al*., 2020).

Potassium, as an essential nutrient for plant growth, holds great significance in the regulation of various plant functions such as water balance, stomatal opening and closing, and photosynthesis (Johnson *et al*., 2022). The activation of approximately 60 enzymes in the plant system is dependent on potassium, which plays a crucial role as a regulator. Potassium plays a key role in protecting plants against abiotic stress conditions in the environment (Johnson *et al*., 2022). Similarly, nitrogen, which is an essential part of chlorophyll, is essential to photosynthesis and the production of energy in plants. The enrichment of these two elements in municipal wastewater may be considered as having a good impact on the growth of the crop since they are essential for the general growth and development of canola (An *et al*., 2022, Xie *et al*., 2022).

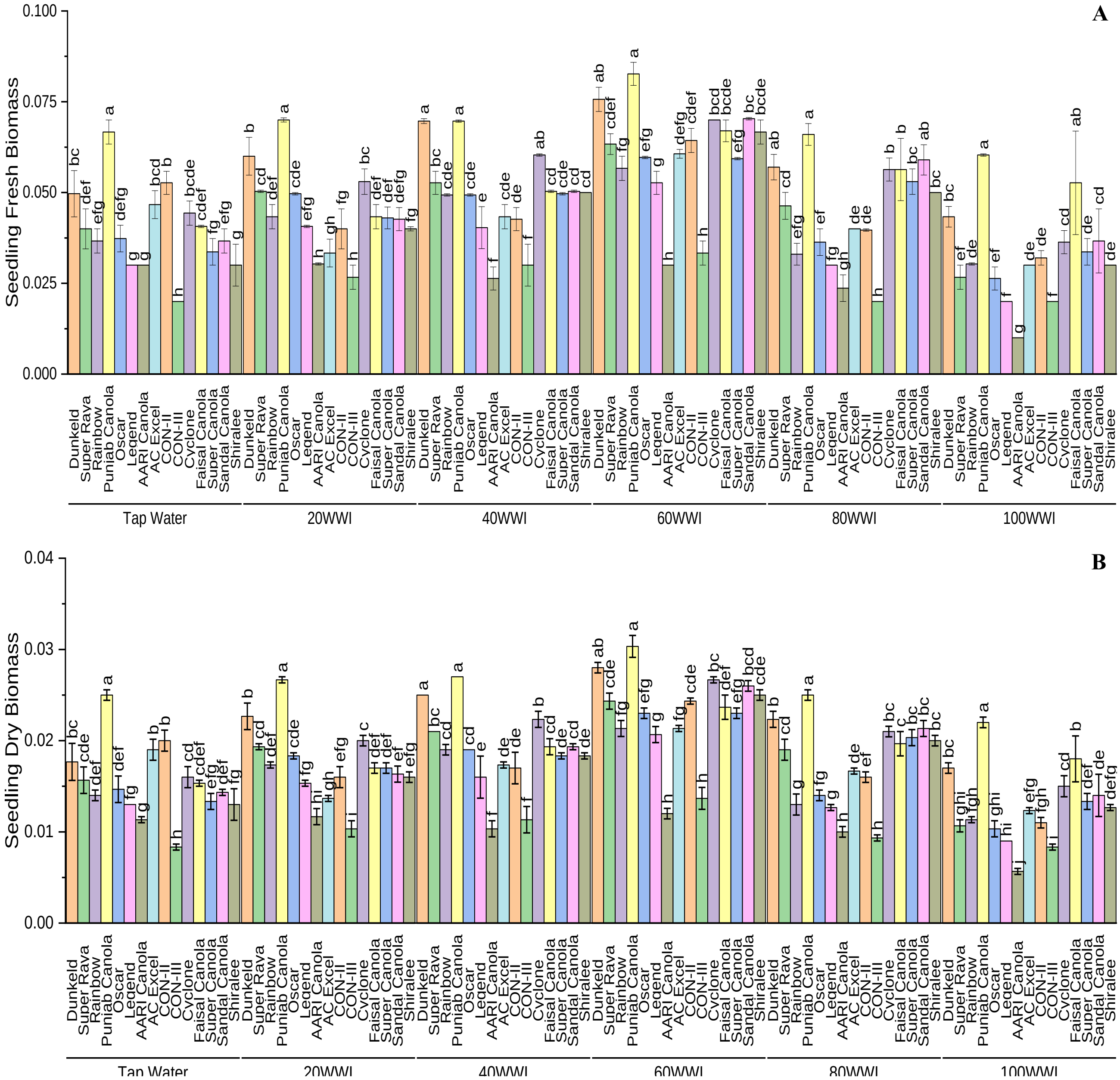

Fig. 5. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on root fresh and dry biomass of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \le 0.05$: Fisher LSD.

The presence of magnesium and calcium in municipal wastewater can also have a positive impact on the growth of canola seedlings. Magnesium is an integral part of chlorophyll and is thus crucial for photosynthesis, as well as the activation of various enzymes and the regulation of plant metabolism (Hawkesford *et al*., 2012). Calcium, on the other hand, serves as a signaling molecule, regulating cell division and elongation, root and shoot development, and cell wall formation. By strengthening the plant structure, calcium helps to enhance the seedlings' ability to resist environmental stressors, both biotic and abiotic (Hawkesford *et al*., 2012).

The measurement of antioxidant activity in plants is a well-established method for assessing stress levels. Antioxidants are critical components in the defense mechanism of plants, helping to protect against the harmful effects of reactive oxygen species (ROS) (Mansoor *et al*., 2022). The ROS are produced during cellular metabolism and can cause oxidative stress in plants, leading to cellular damage and reduced growth (Hashmi *et al*., 2019, Abbas *et al*., 2020, Ramzan *et al*., 2020, Shah *et al*., 2020, Haider *et al*., 2021). Therefore, high levels of antioxidant activity are a sign of a plant's capacity to handle oxidative stress well, whereas low levels might be a sign of oxidative stress. It's also crucial to remember that heavy metals, which are frequently found in urban wastewater, might make plants more susceptible to oxidative stress and decrease their antioxidant activity (Xiang *et al*., 2022). As such, it is important to monitor both the levels of heavy metals and antioxidant activity in plants grown in municipal wastewater to ensure that the plant is not being subjected to harmful levels of oxidative stress (Younis *et al*., 2015, Zafar-Ul-hye *et al*., 2020, Zafar-ul-hye *et al*., 2020, Khan *et al*., 2021, Malik *et al*., 2021, Xiang *et al*., 2022)

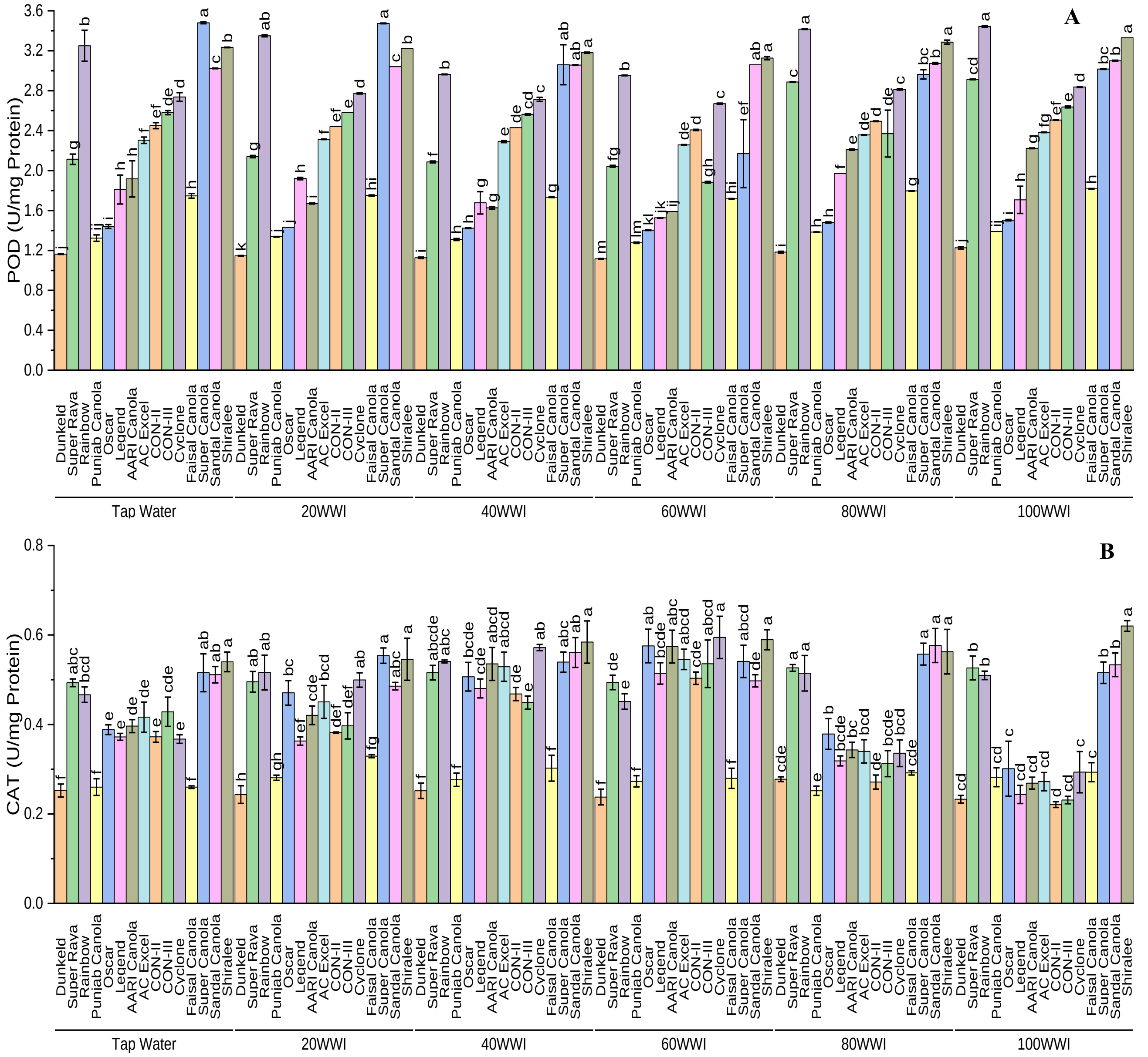

Fig. 6. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on POD and CAT of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at *p*≤0.05: Fisher LSD.

Low antioxidant production in resistant canola cultivars under municipal wastewater treatment can be an indication of stress tolerance in these varieties (Demirci-Çekiç *et al.*, 2022). In these cultivars, the plant may have developed alternative mechanisms to deal with the oxidative stress caused by the presence of heavy metals and other pollutants in municipal wastewater (Demirci-Çekiç *et al.*, 2022). As a result, the body produces less antioxidants, which is a typical reaction to oxidative stress being present at lower levels. It's crucial to remember, though, that inadequate antioxidant synthesis can also be caused by other things, such as dietary deficits or modifications in the growth environment (Demirci-Çekiç *et al.*, 2022).

Increased levels of nitrogen (N), phosphorus (P), and potassium (K) can promote better growth and higher biomass production in plants. However, higher levels of these nutrients can also suppress the production of antioxidants, as plants allocate more resources towards growth (Hawkesford *et al.*, 2012). Plants may boost the synthesis of several metabolic components, resulting in greater shoot and root development as well as increased fresh and dry weight, by providing sufficient levels of N, P, and K. Nitrogen serves as the building blocks for the synthesis of proteins and other structural elements, which promotes an increase in the development of shoots and leaves. Phosphorus contributes to the synthesis of energy, which powers root growth and plant growth. Potassium aids in maintaining the proper balance of water, which increases photosynthesis and promotes overall plant development (Hawkesford *et al.*, 2012).

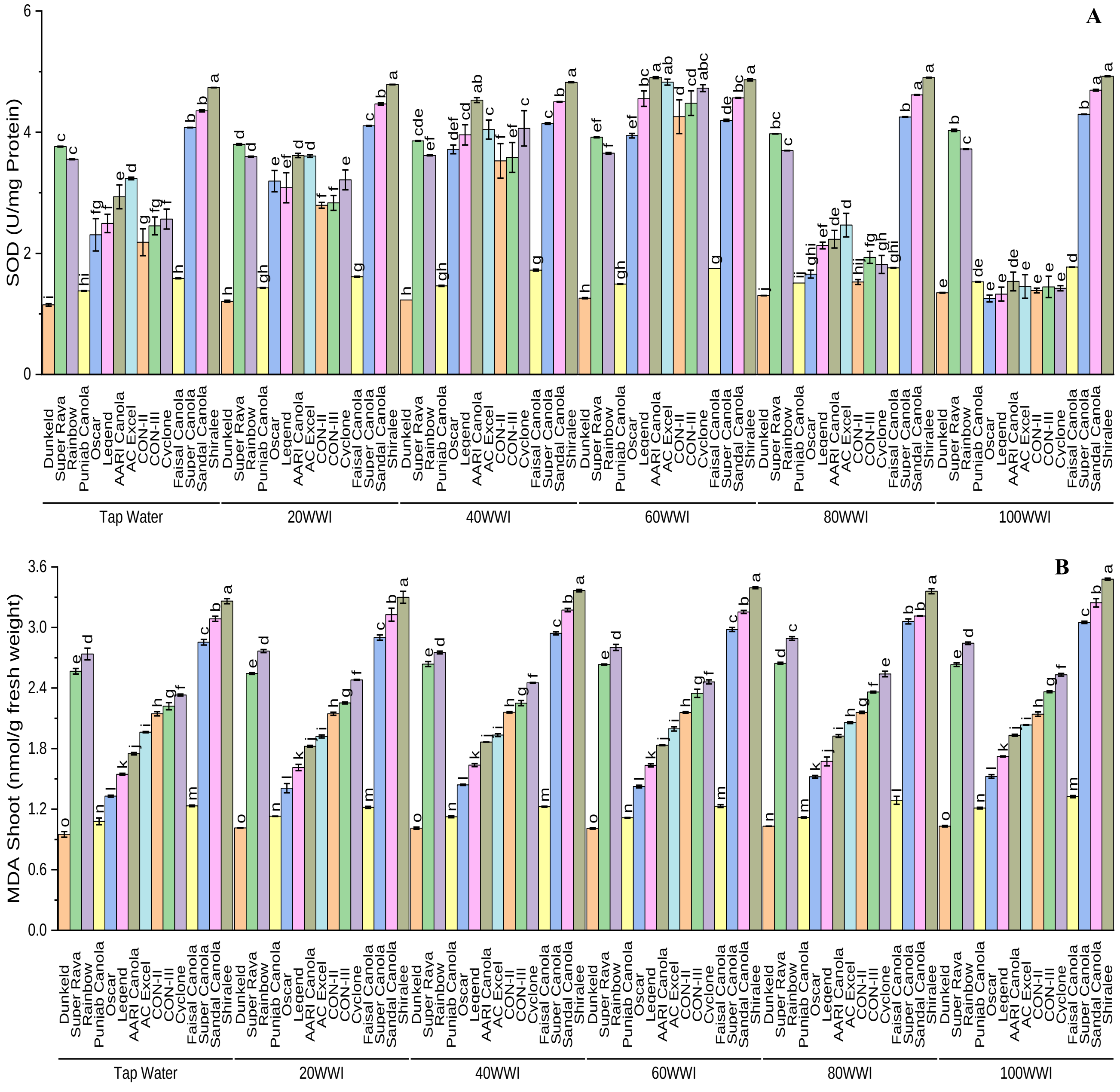

Fig. 7. Effect of variable levels of wastewater irrigation (WWI) i.e., tap water (0%WWI), 20, 40, 60, 80 and 100% WWI, on SOD and MDA of different canola varieties. Bars are means of 3 replicates ± SE. Different letters on bars are showing significant changes at $p \leq 0.05$: Fisher LSD.

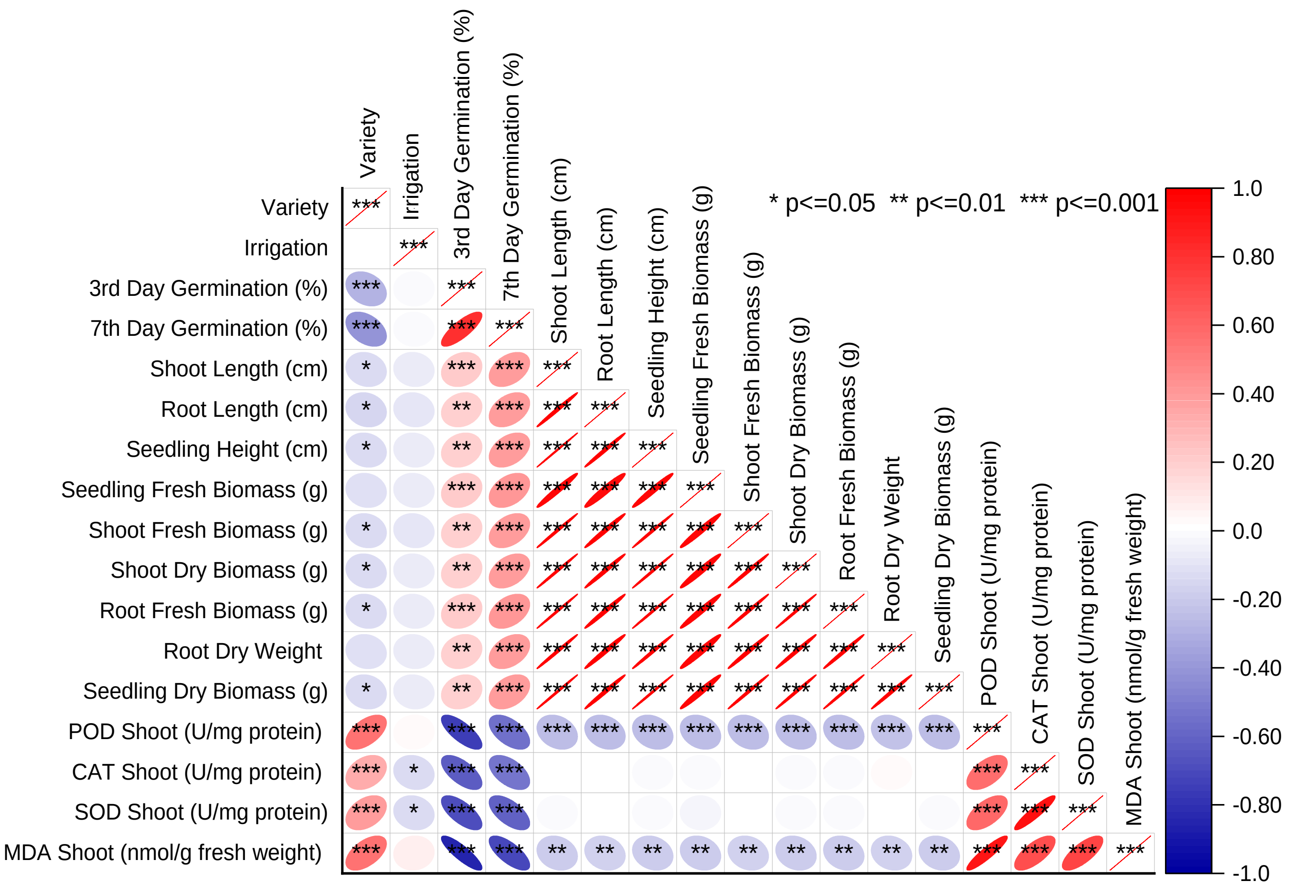

Fig. 8. Pearson correlation for studied attributes of different canola varieties under variable levels of diluted municipal waste water irrigation.

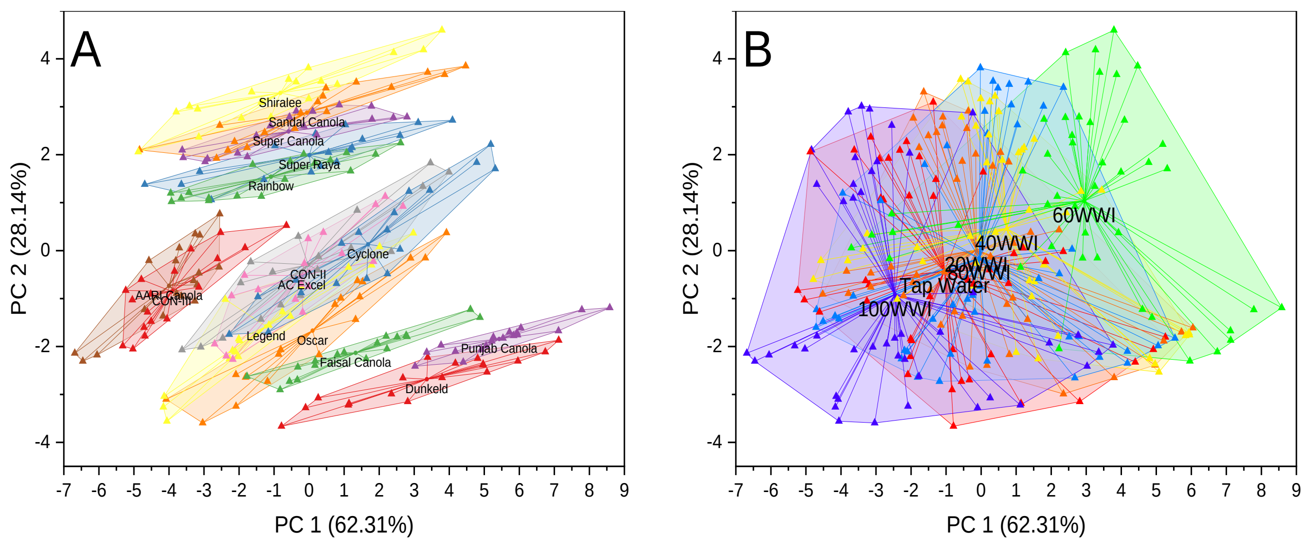

Fig. 9. Convex hull cluster plot showing score values for canola varieties (A) and irrigation dilutions (B).

Conclusion

In conclusion, diluted WWI had a positive effect on the growth of 15 varieties of canola plants, with Punjab Canola and Dunkeld exhibiting the best growth results under all levels of irrigation. Overall, a dilution of 60% WWI was shown to be the best option for promoting canola seedling development and reducing stress brought on by Pb contaminations in WWI. The canola plants under 60% WWI also had low levels of POD, SOD, and MDA, which implies a lower degree of oxidative stress brought on by Pb contaminations in WWI, in addition to the beneficial growth outcomes. Therefore, it can be inferred that using wastewater as irrigation not only encourages development but also lessens the negative consequences of heavy metal poisoning. However, further investigation under pot and field conditions is necessary to declare Punjab Canola and Dunkeld as the best varieties of canola and to confirm the 60% dilution as a better level for using municipal wastewater as irrigation.

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