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Variation in physio-chemical attributes and WUE during growth and development of Pak choi (*Brassica rapa* L. subsp. *chinensis* L.) under different drip fertigation and mulching treatments

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ABSTRACT

In the modern era, nutraceutical properties of horticultural crops are indispensable to determine their adaptability to different agro-ecological regions. The present study exploits the potential of mulches (P: plastic mulch; S: straw mulch; N: No mulch) in relation to drip irrigation (I_1: 100%, I_2: 80%, I_3: 60% of crop evapotranspiration (Etc)), and fertigation (F_1: 100%, F_2: 80%, F_3: 60% of recommended dose of fertilizer (RDF): 125 kg N, 62.5 Kg P_2O_5, 62.5 kg K_2O per ha) on Pak choi at three maturity stages in the North West region of India. Plant fresh weight was the highest at 55 days after transplanting (DAT); however, maximum soil plant analysis development (SPAD) values were registered at 45 DAT. Antioxidant activity, FRAP, DPPH, phenols, flavanols, total sugars, ascorbic acid, free amino acids, and irrigation water use efficiency were observed significantly higher in plants grown on plastic mulch at different levels of irrigation and fertigation. It is envisioned that Pak choi plants had the highest nutritional value at 45 DAT

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from plots mulched with silver-black, irrigated at 80% Etc, and fertigated at 100% RDF. The nutrient enriched plants are used for green salad and as ingredients for the preparation of many recipes in the semi-arid and sub-tropic areas of India.

KEYWORDS

fertigation, nutrients, irrigation, mulch, Pak choi

1. INTRODUCTION

Pak choi (*Brassica rapa* L. subsp. *chinensis* L.), belonging to the *Brassicaceae* family, originates from China and its cultivation is documented and dated to the 5th century AD (Pachore et al., 2019). It is an imminent vegetable crop and is widely cultivated for its tender stalks and flavourful leaves in Asian continental areas. In recent years, its cultivation in terms of area and production has been expanding in European and American countries (Stephen and Gobilik, 2022). It is popular among Indonesians due to its nutritive value and health benefits. It is an excellent source of antioxidants, fibre, vitamins (A, B, C, E, and K), and minerals (Mg, Ca, P, and Fe) (Huang et al., 2021). The plant can be used as roughage for preparing salads, soups, stir-fries, or pickles. It complements well with multiple ingredients and dressings so that both oriental and more western-inspired dishes can be prepared. It is a self-fertile plant and suitable for light, heavy, medium alkaline, and non-saline soils. It is a short-season annual vegetable crop, requires mild temperature, and can be grown under wide semi-arid, dry summer, sub-tropical rainfall climatic zone extending from China to Central Europe from spring to winter (Xie et al., 2020). Pak choi leaves are dark green with a thick white or green stalk in the form of loose head and is consumed during various growth and developmental stages. However, Singh et al. (2019a) recommended that rosette should be harvested after 50–60 days from date of sowing or 30–40 days after transplanting (DAT).

Bio-synthesis and assimilation of nutrients and dry matter in a plant is related to climatic factors, soil physicochemical properties, variety under cultivation, and cultural management practices. Although Indian agriculture depends upon canal water and seasonal rainfall for irrigation, irrigation efficiency of ≤40 per cent has been reported by Pandiyan et al. (2018). Resource conservation technology like drip irrigation, fertigation, and mulching sustainably improves water and nutrient use efficiency and productivity of field crops. Drip irrigation is an effective system that distributes nutrient uniformly in the root zone and enhances nutrient use efficiency (Singh et al., 2020). Mulching forms physical barrier that prevents water loss through evaporation and enhances soil temperature and moisture resulting in advancement of harvesting period (Singh et al., 2019b). It also checks weed population, improves soil properties and crop productivity. Drip fertigation coupled with mulching is gaining popularity for improving water use efficiency, productivity, and profitability of vegetable crops (Haoru et al., 2021). Mulching and micro-irrigation substantially improved leaf photosynthesis activity and nutrients uptake that consequently enhances vegetative growth and calorific value of Pak choi plants (Xie et al., 2020).

Moreover, there is a need for standardising precision farming technologies for farmers in the era of diminishing resources to improve the productivity and reduce water foot print per unit of crop produce. Therefore, effectiveness of any new technology innovation needs to be evaluated and should be modified under specific agroclimatic conditions. Hence, the present investigation was planned to elucidate the influence of different irrigation, fertigation, and mulching practices on qualitative characteristics of Pak choi plants grown under humid subtropical condition of Punjab during winter season.

2. MATERIALS AND METHODS

2.1. Plant material and experimental design

The seeds of indigenous 'Pak choi' variety 'Pusa PakChoi-1' (developed by the Center for Protected Cultivation Technology, ICAR-IARI, New Delhi) were procured, and nursery was sown in 96-cells expanded polystyrene trays on the substrate of coco peat and vermicompost (4:1) during the last week of October, 2021. Fifteen days after sowing, seedlings with four fully expanded leaves were transplanted in the Research Farm, Department of Soil and Water Engineering, Punjab Agricultural University, Ludhiana, India in November, 2021.

The experiment was laid out on Factorial Completely Randomized Design with three levels of irrigation, fertigation, and mulching treatments for three harvesting stages *viz.* 35 DAT, 45 DAT, and 55 DAT (Fig. 1). Drip irrigation at alternate days (except rainy days) was given and irrigation quantity was calculated on crop evapotranspiration (Etc) for three levels *viz.*100%, 80% and 60% of Etc (I_1, I_2, and I_3, respectively). The water-soluble fertilisers were applied after

Fig. 1. Field layout of Pak choi seedlings at three horticultural maturity stages

one week from DAT of seedlings through a venturi system at three levels of 100%, 80%, and 60% of the recommended dose of fertiliser (RDF) (F_1, F_2, and F_3, respectively), and subsequently were applied twice a week in 12 equal splits. The recommended doses of fertilisers were 125 kg of N, 62.5 kg of P_2O_5, and 62.5 kg of K_2O per hectare (Package of practices of crops of Punjab, Kharif, 2020). Additionally, three mulching treatments like plastic mulch (P) (silver-black mulch of 30-μm thickness from Blue Stallion Equipments (P) Ltd., Punjab), straw mulch (S), and the control (N) (no mulch) was used for experimentation. In all treatments, effective bed width of 1.2 m and length of 20 m were prepared and planted at 45 × 45 cm accommodating 14,500 plants/acre. The crop was sown in paired rows, spaced at 40 cm in the centre of beds by dibbling. Analysis of various biochemical parameters along with plant fresh weight, soil plant analysis development (SPAD) values, and irrigation water-use efficiency (IWUE) of Pak choi plants was done in triplicates from tagged plants for each treatment.

2.2. Analysis of various parameters

Plant fresh weight analysis was done using top-loading weighing balance with an accuracy of 0.01 g. SPAD-502 plus chlorophyll meter was used to determine the relative chlorophyll content. At 55 DAT, IWUE was calculated by dividing yield by the amount of irrigation water applied in mm.

The extraction and estimation of free radical scavenging activities, phenols, and flavanols were carried out by the methods described in Kaur et al. (2018). Leaf sample (0.5 g) was homogenised with 5 mL of methanol, followed by filtration through Whatman No.1 filter paper. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was estimated by addition of 3 mL of DPPH to 500 μL extract, measuring the colour change at 515 nm, and expressed as percentage inhibition as compared to control. Antioxidant potential in terms of ferric reducing antioxidant power (FRAP) was calculated from $FeSO_4.7H_2O$ standards (5–30 μg) that were run simultaneously. Total phenols and flavanols were estimated and their contents were calculated from standard curves of gallic acid (10–50 μg) and rutin (40–200 μg), respectively, which were run simultaneously along with the samples.

Total soluble sugars were extracted from leaf tissue by 80% methanol and estimated by phenol-sulphuric acid method (Dubois et al., 1956). Quantitative estimation of free amino acids was done according to Lee and Takahashi (1966) using L-glycine (5–25 mg) as a reference standard amino acid. Ascorbic acid was extracted by crushing 0.2 g of leaf tissue in 1.5 mL of 5% ice-cold metaphosphoric acid and centrifuged at 10,000 × *g* for 10 min. Reduction of Fe^{3+} in the presence of ascorbic acid produces Fe^{2+} that reacts with 2,2′-bipyridyl to form pink-coloured 2,2′-bipyridyl ferrous complex that was read by a spectrophotometer at 520 nm (Law et al., 1983). The determination of glucosinolates was based on the formation of coloured complex between hydrolytic products of glucosinolates and sodium tetrachloro palladate (II), which was read at 405 nm (Kumar et al., 2004).

2.3. Statistical analysis

Data for various parameters were statistically analysed by one-way analysis of variance (ANOVA) using multiple comparisons (Tukey's post hoc test) by SPSS V16.0 software ($P \leq 0.05$). Different letters presented in tables (superscript) depict significant differences among different treatments of drip irrigation, fertigation, and mulching at three growth stages of Pak choi.

3. RESULTS AND DISCUSSION

Plant fresh weight increased with the progression of plant growth, i.e., being highest at 55 DAT ($P \leq 0.05$, Table 1). Fresh weight was maximum under plastic (P) mulch in comparison to straw (S) mulch and no mulch. The stimulated plant fresh weight might be due to alteration in soil temperature and compaction, reduction in evaporation, and weed competition caused by the plastic mulch (Xie et al., 2020). Among different drip irrigation and fertigation treatments, higher plant fresh weights were registered at I_2 (80% Etc) and F_1 (100% RDF) at three harvesting stages. At 55 DAT, about 13.65 percent enhancement in plant weight was registered at F_1 compared to F_3 level. The interaction studies ($I \times F \times M$) revealed that the highest plant fresh weight was observed under I_2F_1P with the value of 1,178.8 g followed by I_1F_2P (1,049.4 g) at 55 DAT ($P \leq 0.05$). Our results were in accordance with findings of Pachore et al. (2019), who found that 100% irrigation with plastic mulch was most significant for bok choy.

The SPAD values showed low-high-low trends at 35 DAT, 45 DAT, and 55 DAT, respectively ($P \leq 0.05$, Table 1). According to Gräf et al. (2020), higher temperature and radiation were beneficial for enhancing leaf SPAD values. The possible reasons behind this pattern might be the decrease in day length with progression of Pak choi plant growth when sown during the winter season in the Northern sub-tropical region of India. Higher SPAD values indicate more leaf N content that further depicts better performance at specific harvest stage (Xiong et al., 2015). Among different drip irrigation, fertigation, and mulching practices, maximum SPAD values were observed under 100% Etc, 100% RDF, and plants raised on plastic (P) mulch at 45 DAT and 55 DAT; however, at 35 DAT, the values were observed higher in soil covered with straw mulch, irrigated at I_1 (100% Etc), and fertigated at F_2 (80% RDF). Interaction between $I \times F \times M$ showed that maximum SPAD value of 64.0 was registered in plants subjected to treatment I_1F_3S followed by I_3F_1N (63.4) at 45 DAT ($P \leq 0.05$).

Table 1. Effects of different irrigation and fertigation levels along with different mulching practices on plant fresh weight, SPAD values and IWUE at different growth stages of Pak choi. Different letters depict significant differences among the treatments at $P \leq 0.05$

Treatments	Plant fresh weight (g)			SPAD values			IWUE (kg m^{-3})
	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT	55 DAT
Irrigation level							
I_1 (100% Etc)	198.55^a	380.01^b	1,054.05^a	50.23^a	57.55^a	51.41^a	4.11^c
I_2 (80% Etc)	180.37^b	425.64^a	1,017.58^b	48.99^{ab}	55.45^a	51.32^a	4.36^b
I_3 (60% Etc)	165.05^c	398.39^b	985.14^c	46.92^a	54.51^a	46.18^b	5.76^a
Fertigation level							
F_1 (100% RDF)	185.32^a	397.71^b	1,121.17^a	47.71^a	57.05^a	50.05^a	5.41^a
F_2 (80% RDF)	179.67^b	437.37^a	996.8^b	49.34^a	54.54^a	49.53^a	4.76^b
F_3 (60% RDF)	179^b	368.95^c	938.8^c	49.09^a	55.92^a	49.34^a	4.06^c
Mulching							
P (Plastic)	212.89^a	428.13^a	1,168.58^a	47.59^a	57.25^a	50.09^a	5.87^a
S (Straw)	191.31^b	393.7^b	962.62^b	49.19^a	54.62^a	49.68^a	4.79^b
N (No mulch)	139.78^c	382.21^b	925.56^c	49.35^a	55.65^a	49.15^a	3.57^c

Etc: crop evapotranspiration; RDF: recommended dose of fertilizer; SPAD: soil plant analysis development; IWUE: irrigation water-use efficiency.

Crop evapotranspiration depends on the climatic conditions, soil evaporation, plant species, and harvesting stages (da Silva et al., 2020). Significant variations in IWUE were recorded when plants were grown under different irrigation, fertigation, and mulching conditions during different harvest stages ($P \leq 0.05$, Table 1). The highest IWUE was found in treatments applied alone *viz.* I_3 (60% Etc), F_1 (100% RDF), and plants mulched with silver black. The interaction studies (I × F × M) also revealed that the highest value of IWUE (8.13 kg m^{-3}) was observed under I_3F_1P and the lowest (2.91 kg m^{-3}) in I_1F_3N at 55 DAT ($P \leq 0.05$). Similar results were observed by Xie et al. (2020), who used plastic mulch to improve Pak choi growth and WUE under different irrigation schedule. The higher IWUE under plastic mulch drip-irrigated plots may be attributed to reduced evaporation losses during earlier development stages and relatively weed-free environment near the crop-root zone, resulting in reduced competition for water and nutrients content (Wu et al., 2023).

The DPPH free radical scavenging activity and FRAP activity are significant indicators for measuring the antioxidant capacity of plants (Zheng et al., 2018). Antioxidant activities of Pak choi plants under different irrigation, fertigation, and mulching treatments as alone were significantly improved during three harvest stages except under fertigation (at 35 DAT) and mulching treatments during 45 DAT and 55 DAT for DPPH activity (Table 2). Interaction studies between I × F × M also showed that antioxidant activities were enhanced during different harvested stages; however, the results were statistically non-significant. Total phenol and flavanol contents are active antioxidant compounds with various health benefits to humans and act as defensive phytochemicals in plants that decreases the damage produced by biotic and abiotic stresses (Huang et al., 2021). The assimilation of antioxidants in plants depends upon the intensity, quality, and exposure duration of light (Bian et al., 2015). Total phenol and flavanol content improved with progression of plant growth and development. Total phenol content estimated at different treatments and harvesting stages ranged from 593.3 μg g^{-1}–1,337.92 μg g^{-1}, being maximum at I_1 (100% Etc), F_1 (100% RDF), and soil covered with plastic (P) mulch. However, total flavanol activity was maximal at I_2 (80% Etc) at 35 DAT and 45 DAT; F_1 (80% RDF) at 45 DAT, plants grown under plastic mulch (P) at 35 DAT, and straw (S) mulch at 55 DAT. The combined effect of different treatments (I × F × M) showed the highest total phenol content with a value of 1,513.7 μg g^{-1} and total flavanol (22,518.2 μg g^{-1}) in I_2F_2N at 45 DAT ($P \leq 0.05$).

Sugars synthesised through photosynthesis act as precursor for the synthesis of L-ascorbic acid, whose concentration depends upon the light intensity and temperature during the growing period of vegetables and fruits (Riga et al., 2019). At 45 DAT, significant variability among total soluble sugars, free amino acids, ascorbic acid, and glucosinolates contents were registered and values ranged from 49.59 to 63.27 mg g^{-1}, 3.30–3.54 mg g^{-1}, 955.09–1,224.73 μg g^{-1}, and 15.01–19.67 mg g^{-1}, respectively, under different irrigation, fertigation, and mulching conditions ($P \leq 0.05$, Table 3). These are important parameters for the evaluation of sensory, quality, and nutritive properties of vegetable crops (Arasaretnam et al., 2018). Interaction studies (I × F × M) showed that total soluble sugars (86.5 mg g^{-1}), free amino acids (4.3 mg g^{-1}), and ascorbic acid (1,955.4 μg g^{-1}) contents were maximal for I_2F_1P, I_2F_2N, and I_1F_2N treatments, respectively, whereas the lowest (9.7 mg g^{-1}) under I_3F_3N at 45 DAT ($P \leq 0.05$). Additionally, lower values of total soluble sugars were related to the degradation of chlorophyll and positively correlated with SPAD values. Glucosinolates are sulphur containing compounds found in *Brassica* species that seem to lower the risk of cancer. However, many of these glucosinolates and their hydrolysis products are bitter, and are therefore unpalatable for many

Table 2. Effects of different irrigation and fertigation levels along with different mulching practices on DPPH free radical scavenging activity, FRAP activity, total phenols and total flavanols contents at different growth stages of Pak choi. Different letters depict significant differences among the treatments at $P \leq 0.05$

	DPPH free radical scavenging activity (%)			FRAP activity (mg Fe^{2+}/g)			Total phenols (μg GAE/g)			Total flavanols (μg RE/g)		
Treatments	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT
Irrigation level												
I_1 (100% Etc)	83.55^{b}	85.94^{a}	84.66^{a}	2.67^{a}	1.66^{b}	3.88^{b}	723.38^{a}	1,028.11^{a}	1,337.92^{a}	953.44^{b}	12,455.55^{b}	15,474.87^{a}
I_2 (80% Etc)	86.73^{a}	81.93^{b}	86.16^{a}	2.22^{c}	2.85^{a}	2.81^{c}	622.91^{b}	1,048.82^{a}	1,205.75^{b}	1,002.09^{a}	13,686.57^{a}	14,905.12^{b}
I_3 (60% Etc)	86.19^{a}	84.63^{a}	82.41^{b}	2.35^{b}	2.67^{a}	4.14^{a}	600^{b}	1,038.65^{a}	1,127.45^{c}	805.49^{c}	13,815.15^{a}	13,759.26^{c}
Fertigation level												
F_1 (100% RDF)	86.27^{a}	84.30^{a}	84.09^{b}	2.44^{a}	2.75^{a}	3.75^{a}	679.52^{a}	978.37^{b}	1,287.11^{a}	977.43^{a}	13,432.32^{b}	15,086.12^{a}
F_2 (80% RDF)	84.40^{a}	86.34^{a}	86.54^{a}	2.31^{b}	2.08^{b}	3.23^{b}	673.66^{a}	1,115.43^{a}	1,227.46^{b}	909.30^{b}	14,290.91^{a}	14,459.86^{b}
F_3 (60% RDF)	85.76^{a}	81.87^{b}	82.60^{b}	2.50^{a}	2.35^{b}	3.86^{a}	593.30^{b}	1,027.78^{b}	1,156.55^{c}	874.29^{b}	12,234.34^{c}	14,593.26^{b}
Mulching												
P (Plastic)	84.33^{b}	84.04^{a}	84.03^{a}	2.32^{b}	1.75^{b}	4.00^{a}	654.96^{a}	1,080.52^{a}	1,297.09^{a}	1,186.25^{a}	12,962.63^{b}	14,738.11^{b}
S (Straw)	85.73^{ab}	84.86^{a}	85.42^{a}	2.46^{a}	2.85^{a}	3.44^{b}	640.93^{a}	1,015.64^{b}	1,251.24^{b}	792.94^{b}	13,112.12^{b}	15,649.56^{a}
N (No mulch)	86.40^{a}	83.61^{a}	83.78^{a}	2.46^{a}	2.58^{a}	3.38^{b}	650.58^{a}	1,019.41^{b}	1,122.78^{c}	781.83^{b}	13,882.82^{a}	13,751.58^{c}

Etc: crop evapotranspiration; RDF: recommended dose of fertiliser; DAT: days after transplanting.

Table 3. Effects of different irrigation and fertigation levels along with different mulching practices on total soluble sugars, free amino acids, ascorbic acid, and glucosinolates contents at different growth stages of Pak choi. Different letters depict significant differences among the treatments at $P \leq 0.05$

Treatments	Total soluble sugars (mg g^{-1})			Free amino acids (mg g^{-1})			Ascorbic acid (μg g^{-1})			Glucosinolates (mg g^{-1})		
	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT	35 DAT	45 DAT	55 DAT
Irrigation level												
I_1 (100% Etc)	48.81^{a}	52.23^{b}	34.40^{a}	1.60^{c}	3.39^{a}	0.81^{a}	368.50^{b}	$1{,}189.76^{a}$	384.63^{a}	43.78^{b}	18.34^{a}	32.06^{a}
I_2 (80% Etc)	46.86^{a}	54^{b}	27.22^{b}	1.80^{a}	3.46^{a}	0.65^{b}	421.99^{a}	$1{,}191.62^{a}$	297.20^{b}	46.01^{a}	15.57^{b}	22.98^{b}
I_3 (60% Etc)	39.94^{b}	63.27^{a}	28.54^{b}	1.68^{b}	3.34^{a}	0.62^{b}	429.64^{a}	955.09^{b}	317.82^{b}	43.91^{b}	17.68^{a}	20.77^{c}
Fertigation level												
F_1 (100% RDF)	48.37^{a}	59.52^{a}	30.28^{a}	2.03^{a}	3.54^{a}	0.72^{a}	421.99^{a}	$1{,}183.06^{a}$	335.40^{a}	44.86^{a}	17.02^{b}	31.69^{a}
F_2 (80% RDF)	45.50^{b}	51.32^{b}	30.58^{a}	1.52^{b}	3.34^{b}	0.65^{a}	409.06^{ab}	$1{,}102.76^{b}$	288.15^{b}	43.96^{a}	19.56^{a}	20.50^{c}
F_3 (60% RDF)	41.75^{c}	58.66^{a}	29.22^{a}	1.53^{b}	3.3^{b}	0.72^{a}	389.07^{a}	$1{,}050.65^{c}$	340.10^{a}	44.88^{a}	15.01^{c}	23.62^{b}
Mulching												
P (Plastic)	47.28^{a}	61.42^{a}	34.54^{a}	1.69^{b}	3.37^{a}	0.67^{a}	402.09^{b}	$1{,}224.73^{a}$	298.91^{b}	43.83^{a}	19.67^{a}	26.80^{a}
S (Straw)	44.63^{b}	58.48^{b}	28.20^{b}	1.59^{c}	3.45^{a}	0.73^{a}	378.66^{c}	989.06^{c}	322.08^{ab}	45.41^{a}	16.16^{b}	26.15^{a}
N (No mulch)	43.70^{b}	49.59^{c}	27.42^{b}	1.79^{a}	3.36^{a}	0.69^{a}	439.38^{a}	$1{,}122.67^{b}$	342.65^{a}	44.45^{a}	15.76^{b}	22.87^{b}

Etc: crop evapotranspiration; RDF: recommended dose of fertiliser; DAT: days after transplanting.

consumers (Wu et al., 2021). In the present study, total soluble sugars, free amino acids, and ascorbic acid contents of Pak choi plants depicted disparity in glucosinolates content and showed a reverse trend at three different growth stages. Our results are in accordance with findings of Zhu et al. (2013), who observed that Pak choi plants harvested at 20–25 DAT had high levels of beneficial glucosinolates.

4. CONCLUSIONS

It can be concluded that the maximum potential of Pak choi plants was obtained under I_2F_1P (80% Etc + 100% RDF + plastic mulch) conditions. According to SPAD values, the best performance and appropriate harvesting stage of crop was at 45 DAT. The biochemical parameters *viz.* total soluble sugars, ascorbic acid, free amino acids, and glucosinolates contents were found to be favourable in Pak choi plants grown under cold conditions of Punjab. Thus, Pak choi can be considered an alternative crop for winter in Punjab due to its dietary value, easy cultivation, and relatively short growing period. In addition, the evaluation of data on quality parameters also showed that Pak choi could be harvested at 45 DAT when cultivated under humid subtropical regions of Punjab during winter season.

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