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Effect of wood vinegar on vegetative growth and nutrient uptake in two citrus rootstocks

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ABSTRACT

Purpose: It is believed that wood vinegar (WV) can improve soil nutrient availability and uptake, thereby improving plant growth and development. In this study we investigate the effect of WV on the availability of macro- and micro elements in the soil and the uptake, translocation and efficiency of these elements in seedlings of sour orange (SO) and Mexican lime (ML) as well as on plant growth. **Research method:** The applied WV (1 and 2%) (v/v) was added to the irrigation water at intervals of 3, 6, 9 and 12 weeks after planting. **Findings:** The results showed that the use of WV at both concentrations reduced the phosphorous (P) and potassium (K) concentration in the leaves of ML, reduced the percentage of calcium (Ca) uptake and efficiency of copper (Cu) in SO and increased the iron (Fe) in ML root (1150 to 1320 mg kg^{-1} DW). Although 1% WV increased soil availability of Ca, sodium (Na), zinc (Zn) and manganese (Mn) and thus decreased root K/Na and Ca/Na, WV 2% improved Mn and K availability but decreased Ca in the soil solution. Application of 1 and 2% WV reduced root dry weight by 16.1 and 12.9% in SO seedling, respectively and in ML seedlings 2% WV reduced total chlorophyll and leaf greenness. **Research limitations:** No limitations were found. **Originality/Value:** The results showed that although the addition of WV to the soil can reduce the pH and thereby increase the availability of some elements such as K and Mn, the increase in EC prevents the effective absorption and translocation of elements and thus plant growth such as root dry weight and greenness.

INTRODUCTION

Annually, 298788 tons of citrus fruits are produced in six southern provinces and three northern coastal provinces of Iran in an area of 5613130 hectares (Ahmadi et al., 2021). In the citrus production industry, various rootstocks are used and they are effective on characteristics (more than 20 horticultural characteristics) such as resistance to pests and soil diseases, early ripening of the crop (Davies & Albrigo, 1999), external and internal fruit quality (Aguilar-Hernández et al., 2020; Castle et al., 2016), depth of the root and tree growth (Albrigo et al., 2019). The citrus rootstocks have significant differences in physiological and morphological characteristics of the root system (Eissenstat & Achor, 1999) and then absorption of nutrients (Romero et al., 2006). Different ability of citrus rootstocks to absorb the elements from the soil and translocation those into shoot has been proven in many other previous studies (Albrigo et al., 2019; Romero et al., 2006). These differences can affect the water and mineral uptake efficiency through changes in root distribution, root growth and carbohydrate distribution (Pedrero et al., 2015). On the other hand, in many citrus production areas in Iran, soils have mineral imbalances due to high pH and alkalinity and in many cases, despite the addition of large amounts of micro and macro elements in the form of chemical fertilizers, trees suffer from disorders caused by deficiency or toxicity of these elements. Although Mexican lime (*Citrus aurantifolia*) are sensitive to chilling and Tristiza virus but is highly used by citrus growers, especially in the southern regions of Iran, due to its high percentage of seed germination and high transplant ability. Sour orange (*Citrus aurantium* L.) is a medium-sized rootstock with a deep root system in which the root density is low and the ability to produce secondary roots is moderate and despite having a positive effect on fruit yield and quality, it is sensitive to soils salinity, soil nematodes and especially Tristiza disease (Davies & Albrigo, 1999). In recent years, the use of some compounds such as humic acid, wood vinegar (WV) and biofertilizers has been increased with the aim of increasing the availability of elements in the soil (Azarmi-Atajan et al., 2023; Fani, 2023; Ziatabar Ahmadi et al., 2024). Wood vinegar (pyroligneous acid) is one of the main liquid by-products that obtained from the condensed vapors generated during the biomass pyrolysis and consisted of many complex organic components and compounds (Hou et al., 2018). Wood vinegar is composed mainly of water (80-90%) and more than 200 organic compounds such as acids, alcohols, phenols, aldehydes and esters (10-20%) but the main component of it is acetic acid (Aguirre et al., 2020). Its composition depends on the type of biomass used, the moisture content of feedstock and pyrolysis process used (Fagernäs et al., 2012; Martín et al., 2017; Mathew & Zakaria, 2015; Wang et al., 2010) and used in various areas such as food additive, anti-inflammatory agent, anti-fungal drug, pest control agent (Grewal et al., 2018) and as weed killing agent (Aguirre et al., 2020). Agriculture is one of the most important application fields of WV and it can be used as plant fortifier (Jothityangkoon et al., 2008; Mu et al., 2004) and soil amendments (Mahmud et al., 2016; Zulkarami et al., 2011). There is also evidence that WV application improve soil microbial conditions (Koç et al., 2019; Steiner et al., 2008), enhancing soil enzyme activity (Lashari et al., 2013), nutrient availability (Jeong et al., 2015), nutrient uptake (Pan et al., 2017), reduce the concentrations of metal ions in the soil and prevent their uptake by plants (Theapparat et al., 2015), immobilize metal contaminants such as nickel, zinc (Zn) and copper (Cu) in compost solid wastes and charcoal (Zhu et al., 2021a), has a beneficial effect on leaching of soluble salts, decrease the soil pH resulting in the improvement of crop productivity in saline soils (Lashari et al., 2013), affect the germination and growth of crop seed (Ling-jie et al., 2014; Pan et al., 2010) and promote the nitrogen utilization efficiency (Jianming, 2003; Tsuzuki et al., 2000). Wood vinegar has been shown to contain different levels of macro-elements such as K, P, Ca and mico-elements such as Fe,

Mn, Cu, boron, Zn and molybdenum and some other elements such as aluminum, cadmium, arsenic, Na, lead and chromium (Zulkarami et al., 2011). Most of the previous syudies focused on the effect of WV on the growth of annual and herbaceous plants and had conflicting results on their growth. On the other hand, the effect of WV on the availability of soil elements, especially in alkaline soils with high percentage of lime and calcium, has not been investigated. It is supposed that WV application to soil may affect availability, uptake and translocation of nutrients from calcareous soils and the citrus rootstocks may vary in their reaction to the soil changes by WV. Therefore, the aim of this study was to investigate the effect of WV on soil availability and plant uptake, translocation and consumption of nutrients as well as vegetative growth of two common citrus rootstock seedlings in Iran.

MATERIALS AND METHODS

Preparation of the soil, plant culture and design of treatments

The pots (30 pots) were filled with 7 kg of surface soil (0-30 cm depth) collected from a field in the College of Agriculture and Natural Resources of Darab. The soil was classified as Coarse-loamy, carbonatic, hyperthermic Typic Haplustepts according to the USDA Soil Taxonomy. A soil sample (prior to use) was airdried, sieved (< 2 mm) and then particle size distribution, calcium carbonate equivalent, organic carbon, soil pH, electrical conductivity (EC) and cation exchange capacity (CEC) as well as the concentration of nitrogen (N), phosphorus (P), potassium (K), iron (Fe), magnesium (Mn), Zn and Cu were determined using standard soil analysis methods (Table 1). One-year-old seedling of a sour orange (SO) or Mexican lime (ML) was planted in each pot (5 replicates). Pots were irrigated to reach field capacity moisture content (approximately 50% of saturation percentage) and soil moisture remained nearly constant with pots weighting. The WV was prepared from a biochar production factory (Fasl5 Company) in Jannatshahr Rigone, Darab city which used fruit tree wood as raw material. The WV (1 and 2%) (v/v) required for each treatment and pot was diluted with water and added to the pots four times at intervals of 3, 6, 9 and 12 weeks after planting. The plants were placed in greenhouse conditions (30°C during the day and 22°C at night) for the first three months of the experiment and in the outdoor shed for the last month.

Determination of plant traits

At the end of the experimental period (120 days after planting), the greenness index of the developed mature leaves (leaves 4 and 5 from the tip of the shoot) was determined by SPAD-502 (Minolta, Japan), leaf surface area was determined for 10 leaves of each seedling using a leaf area meter (Delta-T Devices, England) using WinDIAS3 software and then the chlorophyll concentration was determined in two fully developed leaves. For this purpose, 50 mg of fresh sample was macerated with 1 mL of methanol, vortexed for 20 seconds and centrifuged (16870 rcf) for 4 min at 4°C. The supernatant was separated and 1 mL of methanol was added to the solid again, and the vortexing and centrifugation steps were repeated as in the previous step, and the liquid phase was separated again and its absorbance was measured at two wavelengths of 652 and 665 nm using a microplate spectrophotometer (Synergy 2, BioTek, Winooski, USA) (Warren, 2008). The length of the main branch of each seedling (plant height) was recorded with a ruler. The fresh weight of the roots, stems and leaves were measured and then the samples were placed in an oven at 70°C for 48 h and then their dry weight was measured.

Table 1. Some properties of the wood vinegar used.

Property	pH	EC (dS m^{-1})	Organic carbon (%)	N (%)	P (%)	K (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
	3.54	5.15	10.1	0.05	trace	0.01	40.4	trace	5.5	2.8

Determination of nutrient concentration in soil, roots, stem and leaves

At the end of the experiment, the roots, stems and leaves of the plants were harvested separately, rinsed with deionized water and oven-dried at 70°C for three days. The dried samples were powdered using an electric mill and after ashing at 550°C and acid dissolution, the P concentration in the extracts was determined using the yellow color method (Jackson, 2005), as well as the concentrations of calcium (Ca), magnesium (Mg) and sodium (Na) was determined using a flame photometer (ELE, UK) and Fe, Mn, Zn and Cu were determined using an atomic absorption spectrophotometer (AAS; PG 990, PG Instruments Ltd. UK).

After removing the roots from the pots, the soil was dried and the characteristics of each treatment were determined (Table 2). To calculate the uptake and translocation percentage of each element, the root nutrient content was divided by the soil nutrient content or the content of that element in the shoot by its root content. To calculate the efficiency of the element, the total dry weight of the plant was divided by the total amount of elements accumulated in it (Gourley et al., 1994; Yan et al., 2019).

Statistical analysis

A factorial experiment in a completely randomized design was conducted under greenhouse and shed conditions. Treatments included seedling of two rootstocks (sour orange and Mexican lime) and WV application: without WV (WV_0), 1% WV ($WV_{1\%}$) and 2% WV ($WV_{2\%}$). Each treatment included five replicates and each replicate included one pot containing one seedling. Statistical analysis of the data was performed using SAS software for Windows V9 (SAS Institute Inc., Cary, NC, USA). Differences between means were detected by the least significant differences (LSD) test at a significance level of 5% and graphs created using Microsoft Office Excel 2016 software.

RESULTS

Soil properties

$WV_{1\%}$ treatment had no significant effect on soil pH and EC, while $WV_{2\%}$ treatment significantly decreased and increased them from 7.86 to 7.76 and 0.40 to 0.45 dS m^{-1} respectively (Table 2). The use of WV had no effect on the availability of P, Fe and Cu in the soil (Table 2). Although soil K availability was not affected by using 1% WV, it was significantly increased (2.6%) by 2% WV. Treatment with $WV_{1\%}$ and $WV_{2\%}$ increased and decreased soil soluble Ca concentration, respectively. Although $WV_{1\%}$ treatment increased the concentration of Na and Zn in the soil, $WV_{2\%}$ treatment had no effect on the concentration of these elements. Addition of WV at concentrations of 1 and 2% increased soil Mn availability by 12 and 15.7%, respectively (Table 2).

Table 2. Changes in soil properties after the application of wood vinegar (WV).

Treatment	EC (dS/m)	pH	Macro nutrient concentration (g kg^{-1})				Micro nutrient concentration (mg kg^{-1})			
			P	K	Ca	Na	Fe	Mn	Zn	Cu
WV_0	0.40^{b}	7.86^{a}	4.73^{a}	0.22^{b}	0.20^{b}	0.13^{b}	3.48^{a}	5.69^{c}	1.56^{b}	1.09^{a}
$WV_{1\%}$	0.41^{b}	7.79^{ab}	4.87^{a}	0.23^{ab}	0.23^{a}	0.14^{a}	3.59^{a}	6.47^{b}	1.70^{a}	1.10^{a}
$WV_{2\%}$	0.45^{a}	7.76^{b}	4.96^{a}	0.23^{a}	0.19^{c}	0.12^{b}	3.54^{a}	6.75^{a}	1.57^{b}	1.09^{a}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Table 3. Means comparison of wood vinegar (WV) effects on leaf nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

Treatment	Leaf nutrient concentration									
	P (%)		K (%)		Ca (%)		K/Ca		Cu (mg kg^{-1} DW)	
	SO	ML	SO	ML	SO	ML	SO	ML	SO	ML
WV_0	0.26^{d}	0.41^{a}	1.89^{bc}	2.05^{a}	2.43^{a}	2.31^{b}	0.78^{bc}	0.89^{a}	9.25^{bc}	10.34^{ab}
$WV_{1\%}$	0.27^{d}	0.31^{bc}	1.85^{bc}	1.82^{c}	2.40^{a}	2.41^{a}	0.77^{bc}	0.76^{c}	10.35^{ab}	7.67^{d}
$WV_{2\%}$	0.29^{cd}	0.35^{b}	1.85^{bc}	1.94^{b}	2.29^{b}	2.19^{c}	0.80^{b}	0.88^{a}	11.42^{a}	8.49^{cd}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Plant macro nutrient concentration

The results showed that the use of WV had no effect on the P concentration in SO seedlings (in any organ) and only at a concentration of 2%, reduced the efficiency of this element. In ML seedlings, although the use of both WV levels (1 and 2%) reduced the P concentration in the leaves by 23 and 14%, respectively it had no effect on this element in the roots and stems (Table 3). In general, the P concentration of leaf (Table 3) and root (0.19 vs. 0.25%) and its translocation efficiency were higher in ML seedlings than in SO seedlings (Fig. 4A).

The K concentrations in roots and stems were different in two rootstock seedlings but the use of WV had no effect on it. Although the use of WV did not change the K concentration of SO leaves, the use of 1 and 2% WV in ML seedlings reduced the K concentration of leaves by 11.2 and 56.5%, respectively (Table 6). The results showed that the concentration of K in the root (Fig. 3B), stem (Fig. 2A) and the percentage of K uptake were higher in ML seedlings than in SO, but the translocation efficiency and efficiency of this element in SO was higher than that of ML seedlings (Fig. 4).

Although the leaf Ca concentration of SO did not change with the use of 1% WV, it was significantly reduced by the addition of 2% WV (Table 3). In ML seedlings, the use of 1 and 2% WV increased and decreased leaf Ca concentration, respectively (Table 3). In SO, root Ca concentration decreased by 1% of WV application, while it had no effect on ML root Ca concentration. Regardless of the effect of WV, the Ca concentration in the ML root was significantly lower than that of SO (Table 5). The use of WV did not affect Ca uptake percentage in ML seedlings, but at concentrations of 1 and 2%, it reduced this trait by 26.7 and 11.9% in SO seedlings, respectively (Table 6). Regardless of the WV effect, the percentage of Ca uptake in SO was about 69% higher than that in ML seedlings, while the translocation efficiency and Ca efficiency in ML were higher than that in SO seedlings (Fig. 4A, 4B). The use of 1 and 2% WV in SO reduced the percentage of Ca uptake and the use of 1% WV reduced the concentration of this element in the roots, while it had no effect in ML (Table 6).

In SO seedlings, none of the WV levels and in ML seedlings, $WV_{2\%}$ treatment had no effect on the Na concentration in the root and only 1% WV in ML seedlings increased the concentration of this element in the roots in comparison to control (Table 5). Regardless of the effect of WV, the Na concentration of leaves (Fig. 1B) and stems (Fig. 2C) of SO seedlings was 24.5 and 15.7% higher than that of ML, respectively. In both rootstocks,

application of 2% WV reduced the Na concentration in the stem (Fig. 2C). The uptake percentage of Na varied between two rootstocks and its efficiency also varied between rootstocks and WV levels. The results showed that ML seedlings had higher Na efficiency than SO (0.88 vs. 0.74) and in both plants, 1% WV caused a significant decrease in Na efficiency (Fig. 4B).

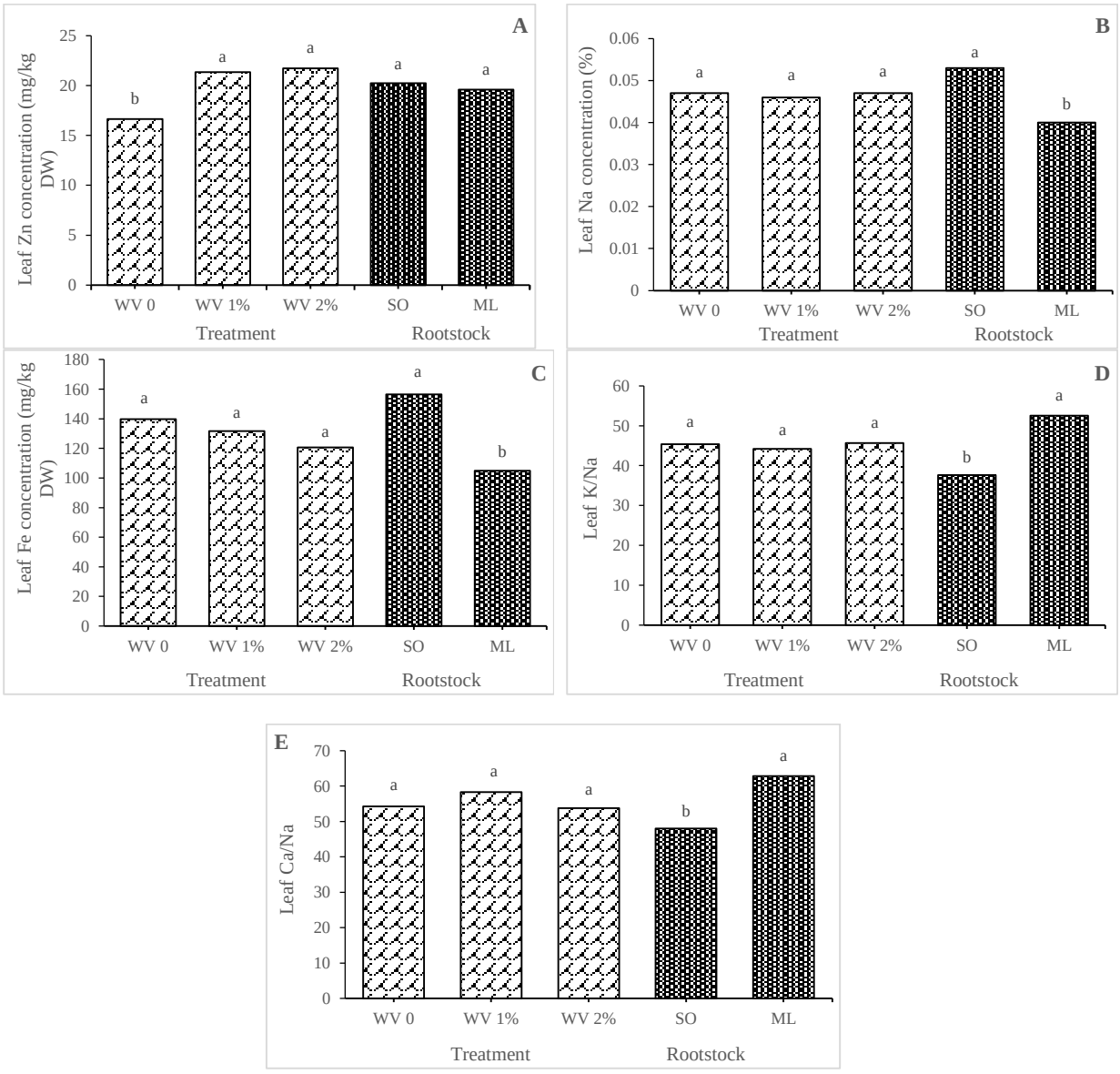

Fig. 1. Effects of wood vinegar (WV) and rootstock on leaf nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: Zn, B: Na, C: Fe, D: K/Na, and E: Ca/Na. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

Table 4. Means comparison of wood vinegar (WV) effects on stem nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

	Stem nutrient concentration					
	Mn (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	5.53^{b}	6.31^{a}	11.03^{b}	15.37^{a}	4.69^{ab}	5.21^{a}
$WV_{1\%}$	5.94^{ab}	5.42^{b}	15.01^{a}	13.38^{ab}	5.49^{a}	3.71^{c}
$WV_{2\%}$	5.73^{ab}	6.28^{a}	13.51^{ab}	13.83^{a}	5.35^{a}	3.89^{bc}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

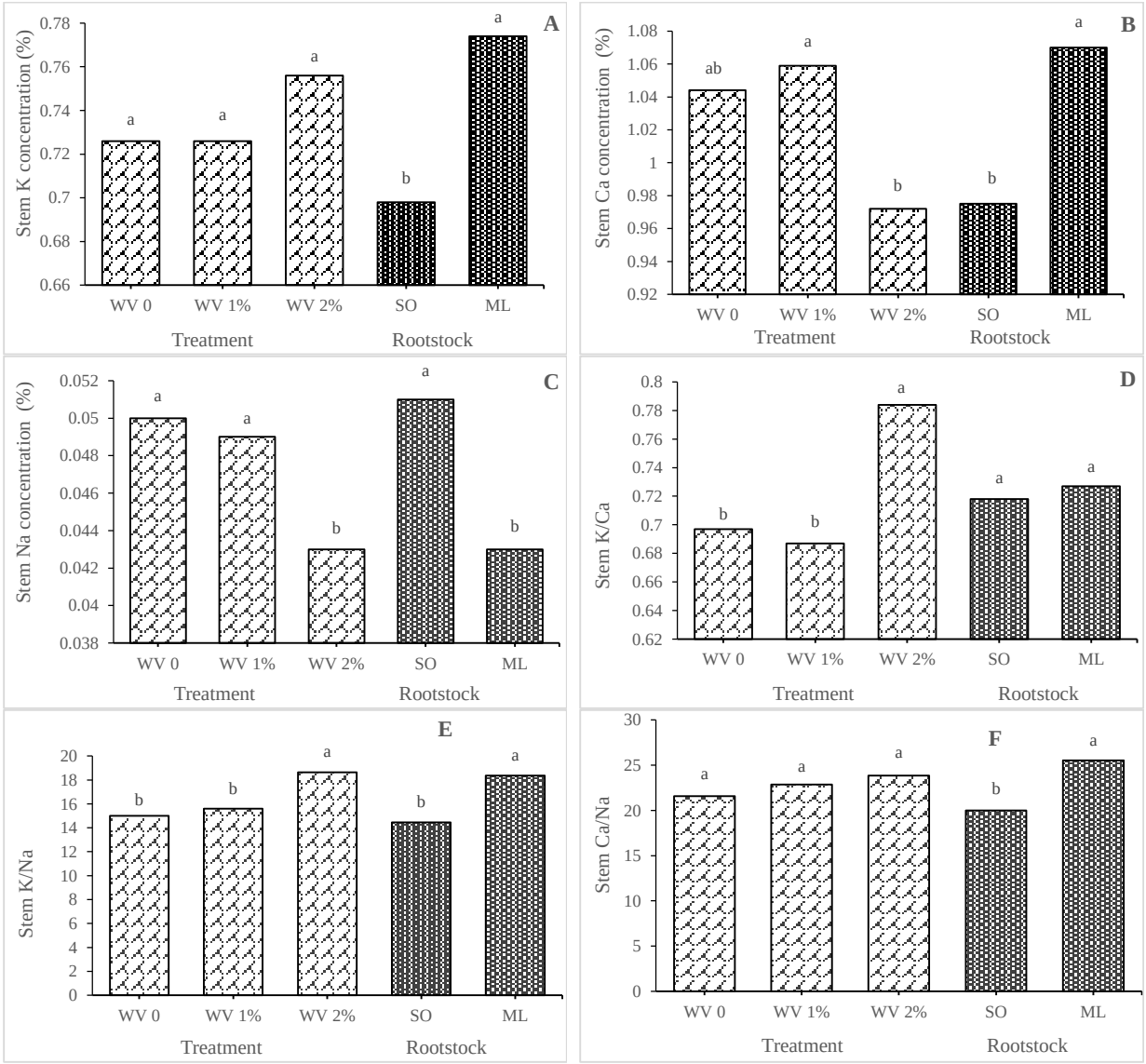

Fig. 2. Effects of wood vinegar (WV) and rootstock on stem nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: K, B: Ca, C: Na, D: K/Ca, E: K/Na, and F: Ca/Na. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

The addition of WV had no effect on the K/Ca in the leaves of SO seedlings, but in ML seedlings, $WV_{1\%}$ treatment resulted in a significant reduction in this ratio due to the reduction in both K and Ca concentrations (Table 3). Although the use of 1% WV had no effect on K/Ca in the stem, the use of 2% WV resulted in a significant increase (11%) of this ratio in

both rootstocks (Fig 2D). Overall, the K/Ca value in the ML root (1.58) was higher than that in SO (0.41).

In roots, stems and leaves of ML seedlings, K/Na was 46.4, 21.4 and 28.3% higher than that of SO seedlings (Fig. 3D, 2E, 1D). Although the use of 1% WV had no effect on the K/Na ratio in the stems of both rootstocks, the $WV_{2\%}$ treatment increased this ratio by 19.5% in both rootstocks and in the roots of both rootstocks only $WV_{1\%}$ treatment decreased this ratio. In the roots of ML seedlings, the ratio of Ca/Na was 51% lower than that in SO seedlings, but this ratio in the stems and leaves of ML seedlings was 21.6% and 23.6% higher respectively in SO seedlings (Fig. 2F, 1E). In the roots of both rootstocks, application of 1% WV significantly reduced the ratio of Ca/Na, but $WV_{2\%}$ treatment had no effect on this ratio (Fig. 3E).

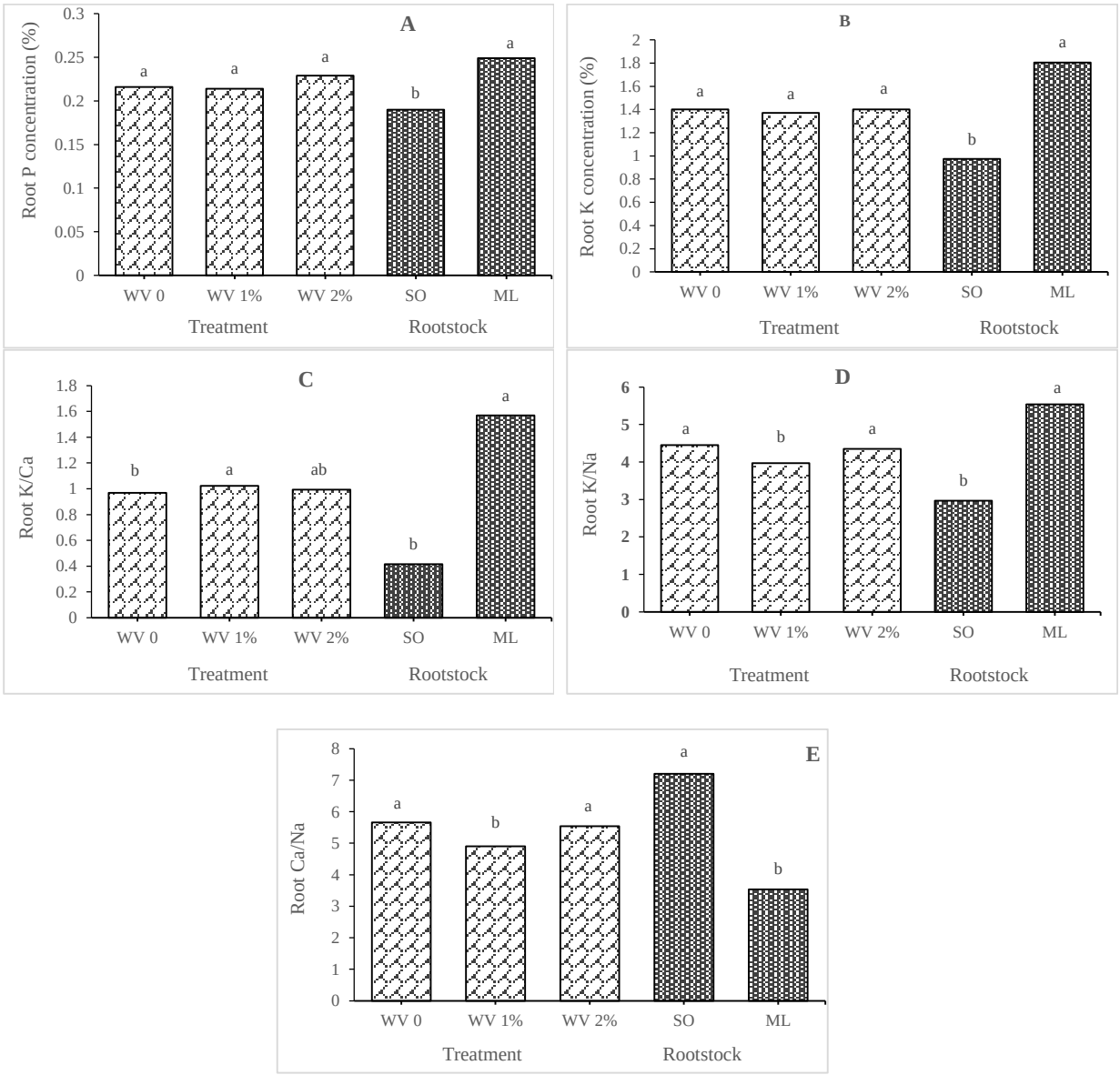

Fig. 3. Effects of wood vinegar (WV) and rootstock on root nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: P, B: K, C: K/Ca, D: K/Na, and E: Ca/Na. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

Table 5. Means comparison of wood vinegar (WV) effects on root nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

	Root nutrient concentration					
	Ca (%)		Na (%)		Fe (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	2.44^{a}	1.17^{c}	0.33^{b}	0.31^{b}	1960.0^{a}	1150.0^{d}
$WV_{1\%}$	2.17^{b}	1.12^{c}	0.33^{b}	0.36^{a}	2050.0^{a}	1300.0^{c}
$WV_{2\%}$	2.45^{a}	1.15^{c}	0.33^{b}	0.32^{b}	1700.0^{b}	1320.0^{c}
	Mn (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	113.2^{b}	80.8^{c}	31.3^{c}	40.2^{b}	18.6^{e}	42.8^{b}
$WV_{1\%}$	134.3^{a}	83.8^{c}	31.8^{c}	51.5^{a}	23.7^{d}	36.4^{c}
$WV_{2\%}$	113.5^{b}	108.3^{b}	37.2^{bc}	58.7^{a}	24.1^{d}	59.8^{a}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Table 6. Means comparison of wood vinegar (WV) effects on uptake percentage, translocation efficiency and nutrient efficiency in sour orange (SO) and Mexican lime (ML) seedlings.

		Ca (%)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Uptake percentage	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	0.55^{a}	0.15^{d}	0.90^{a}	0.36^{c}	0.75^{c}	1.02^{b}
	$WV_{1\%}$	0.41^{c}	0.14^{d}	0.90^{a}	0.36^{c}	0.96^{bc}	0.89^{bc}
	$WV_{2\%}$	0.49^{b}	0.16^{d}	0.61^{b}	0.41^{c}	0.87^{bc}	1.29^{a}
		Zn (mg kg^{-1} DW)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Translocation efficiency	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	51.32^{ab}	53.77^{ab}	36.42^{dc}	53.50^{a}	47.99^{a}	34.77^{b}
	$WV_{1\%}$	56.88^{a}	48.53^{bc}	32.84^{d}	49.76^{a}	44.48^{a}	31.00^{b}
	$WV_{2\%}$	55.27^{a}	47.10^{c}	39.68^{c}	45.38^{b}	48.34^{a}	23.69^{c}
		P (%)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Nutrient efficiency	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	0.46^{a}	0.36^{d}	18.40^{b}	22.55^{a}	92.24^{a}	59.37^{c}
	$WV_{1\%}$	0.46^{a}	0.39^{bc}	15.78^{c}	23.09^{a}	75.67^{b}	72.98^{b}
	$WV_{2\%}$	0.42^{b}	0.38^{cd}	17.98b^{c}	20.14^{b}	72.78^{b}	51.07^{c}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Plant micro nutrient concentration

In SO seedlings, $WV_{2\%}$ treatment significantly reduced the concentration of root Fe, while in ML seedlings; the use of both concentrations of WV increased the concentration of this element (Table 5). Although the efficiency and translocation efficiency of Fe were higher in ML than in SO, but due to the lower Fe uptake in ML than SO (Fig. 4B), finally the concentration of Fe in the leaves of ML seedlings (104.8 mg/kg) was lower than that of SO seedlings (156.5 mg/kg).

In SO seedlings, the concentration of Mn in the stem was not affected by WV, but in ML seedlings, the use of 1% WV reduced the concentration, and 2% WV had no effect on it (Table 4). Root Mn concentration increased by 1% WV in SO seedlings and by 15.7 and 25.5% in ML seedlings using 2% WV, respectively, compared to the control treatment (Table 5). Regardless of the effect of WV, the percentage of Mn uptake was higher in SO seedlings than in ML seedlings, but increasing the translocation efficiency of this element in ML resulted in a higher efficiency of Mn in ML than in SO seedlings (Table 6). Although the use of 1% WV in SO and 2% in ML increased the Mn concentration in the roots (Table 5), it did not increase due to the ineffectiveness of WV on Mn translocation in SO and its reduction in

ML (Table 6), only the Mn efficiency of SO and ML in $WV_{1\%}$ and $WV_{2\%}$ decreased and the Mn concentration in the leaves also did not change.

Addition of WV to the soil increased the Zn concentration in the leaves of both rootstocks (Fig. 1A). Although before the addition of WV to the soil (control treatment), the Zn concentration was higher in the ML stem than in the SO stem (15.4 vs. 11.0), only the use of 1% WV increased the concentration of this element in the SO stem and the addition of WV had no effect on the Zn concentration in the stem of ML (Table 4). The addition of WV to the soil did not change the Zn concentration in the SO root, but in ML, the addition of 1 and 2% WV increased the Zn concentration in the root by 22.11 and 31.54%, respectively (Table 5). The percentage of Zn uptake was not affected by the type of rootstock and the concentration of WV, and on the other hand, the translocation efficiency of this element decreased by $WV_{2\%}$ treatment only in ML. The efficiency of Zn in SO seedlings (54.04%) was higher than that of ML (39.9%) and the use of 1 and 2% WV reduced the efficiency of this element in both rootstocks by 15.51 and 20.97%, respectively (Fig. 4C).

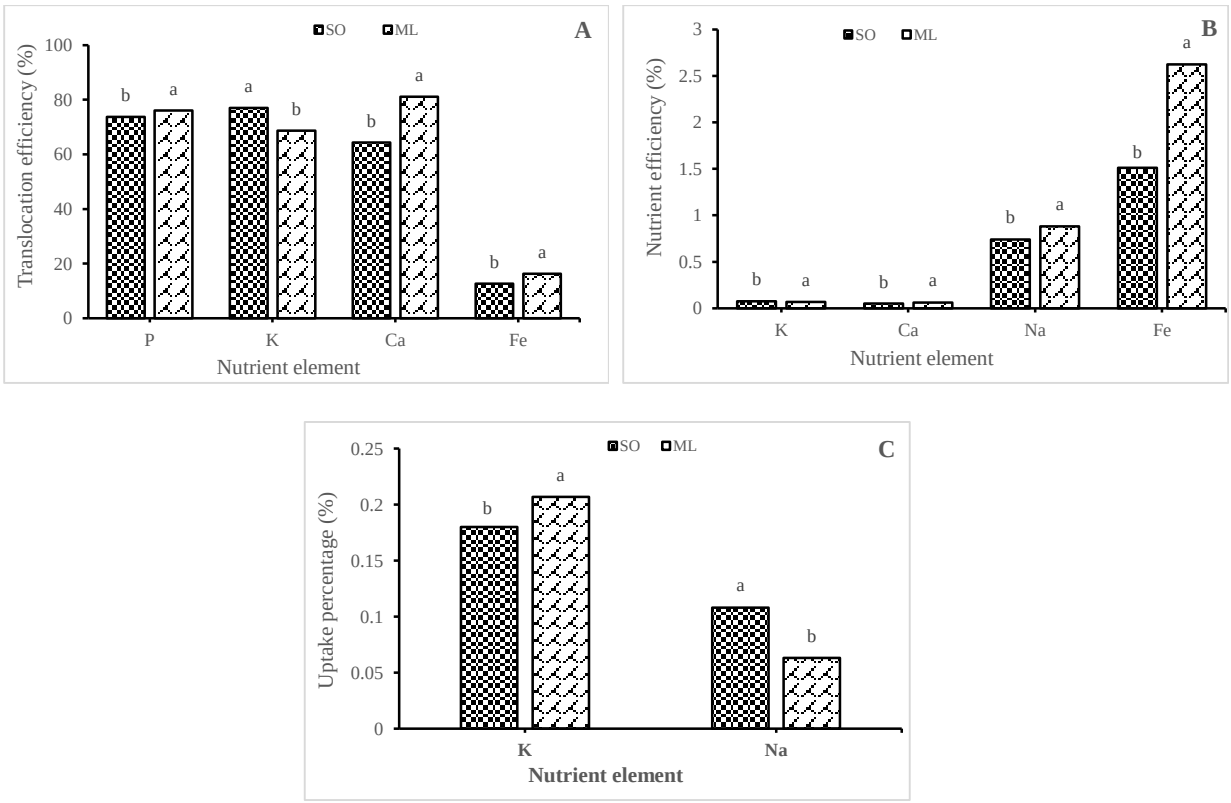

Fig. 4. Comparison of translocation efficiency (A), nutrient efficiency (B), and uptake percentage (C) in sour orange (SO) and Mexican lime (ML) seedlings. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

The results showed that in SO seedlings, the application of 2% WV increased the Cu concentration in the leaves by 0.19%, but in ML seedlings, the addition of 1 and 2% WV reduced the concentration of this element in the leaves by 25.8 and 17.9%, respectively (Table 3). Addition of WV to the soil did not affect the concentration of stem Cu in SO seedlings, but in ML seedlings, both concentrations of WV resulted in a significant decrease in stem Cu concentration (Table 4). The concentration of root Cu in SO seedlings increased due to the application of both WV concentrations, but in ML seedlings, the use of 1% WV decreased the concentration (42.8 vs. 36.4) and 2% WV increased the concentration (42.8 vs. 59.8) of this element in the root (Table 5). Although WV use had no effect on Cu uptake in SO seedlings,

$WV_{2\%}$ treatment in ML increased Cu uptake (Table 6). The translocation efficiency of Cu in SO was not affected by the use of WV, while in ML, the use of 2% WV reduced this feature (Table 6). The efficiency of Cu in SO decreased in both WV concentrations, but in ML, $WV_{1\%}$ increase the efficiency of this element by 18.6% (Table 6).

Changes in physiological and morphological traits

Although in SO seedlings, the leaf chlorophyll concentration and leaf greenness showed no changes by the use of WV, but in ML seedlings, 2% WV reduced leaf greenness and total chlorophyll compared to the control treatment (Table 7). Application of 2% WV reduced the root dry weight by 16.1% and 12.9% compared to the control treatment but did not effect on shoot and total dry weight of SO seedlings (Table 7). Of course, the results showed that the WV application had no effect on plant height; shoot fresh and dry weight, root-to-shoot ratio and chlorophyll a and b concentrations in the seedlings.

Table 7. Means comparison of the effects of wood vinegar (WV) and rootstock interaction on some morphological features in sour orange (SO) and Mexican lime (ML) seedlings.

	SPAD		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)		Total dry weight (g)	
Treatment	SO	ML	SO	ML	SO	ML	SO	ML	SO	ML
WV_0	62.66^{ab}	62.40^{ab}	18.4^{b}	26.4^{a}	3.10^{a}	1.82^{c}	6.92^{a}	5.34^{b}	9.98^{a}	7.17^{c}
$WV_{1\%}$	64.69^{a}	59.95^{b}	22.1^{ab}	26.0^{a}	2.60^{b}	1.90^{c}	6.25^{ab}	5.65^{b}	9.19^{a}	7.65^{bc}
$WV_{2\%}$	64.37^{a}	52.65^{c}	21.0^{ab}	19.6^{b}	2.70^{b}	1.71^{c}	6.26^{ab}	5.19^{b}	8.89^{ab}	6.90^{c}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

DISCUSSION

In this study the use of 1% WV increased the concentration of K, Ca, Na, Mn and Zn and 2% WV increased the concentration of K and Mn in the soil. Increasing the Ca, K and Na in the soil by the use of WV may be due to the dissolution of soil salts such as calcium carbonate (Najafi-Ghiri et al., 2022) but increasing the concentration of Mn and Zn can be due to the effect of WV on soil pH and prevent the stabilization of these elements on soil particles and thus increase their availability (Yamato et al., 2006). It has been shown that the use of WV in saline soils can cause leaching of soluble salts, reduction of pH and thus improve the performance of plants in these soils (Lashari et al., 2013) and in soils contaminated with heavy metals, by helping to stabilize this metals on soil particles, can remediate these soils (Theapparat et al., 2015).

In SO seedlings, the main reason for the reduction of leaf P, K and Ca by WV application, was the reduction in the uptake of these elements by the roots, while in ML seedlings; it is due to the increase in leaf area and the dilution effect. In confirmation of the results, it was reported that the addition of WV to the root environment reduced the absorption of K and P in lettuce (Chen et al., 2016) and K and Mg in cicer (*Cicer arietinum* L.) (Fedeli et al., 2022). Although WV increases the availability of some nutrients by reducing the pH of the root environment, the presence of various organic compounds in its composition can lead to the formation of complexes with some ions and reduce their availability (Pan et al., 2017). Also it has been shown that in basil (*Ocimum basilicum*) and cucumber (*Cucumis sativus*), the use of pine WV, although increased the leaf N and had no effect on the leaf K concentration, but significantly reduced the P, Mg, Fe and Ca (Abdolahipour & Haghighi, 2019). The cell walls and especially middle lamella consist of polygalactrunic acid and their carboxylic groups (R-COO) act as cation exchangers in this site. Plant species differ considerably in their root cation exchange capacity (CEC) and the changes in rhizosphere conditions such as pH effect on root CEC (Marschner, 2011). The change in the content of Ca and K in the root by adding

WV can be due to the change in the CEC of the root tissue caused by changes in the pH of the root environment.

The use of WV did not affect the availability of Fe in the soil and Fe uptake percentage in both rootstocks was reduced by adding 2% of WV. It is believed that Fe has a high affinity with some compounds such as organic acids (Marschner, 2011). On the other hand, WV contains large amounts of organic acids and phenolic compounds, and probably the binding of Fe with these compounds has reduced its availability to plant roots.

Although the application of WV increased the Mn in the roots of both rootstocks, but due to the inhibition of the translocation, the concentration of this element in the leaves did not change. The behavior of both seedlings was very similar to Mn, so that there was no difference between two rootstocks in terms of Mn nutritional traits except the percentage of uptake. In confirmation of these results, it is stated that regardless of the species or cultivar and environmental conditions, the Mn critical concentration in the developed leaves of different plants is the same (10 to 20 mg kg^{-1} dry weight) (Marschner, 2011).

Application of WV increased the leaf Zn concentration in both rootstocks and in both levels but the Zn efficiency in both rootstocks decreased with WV application. Low availability of Zn in calcareous soils (with high pH) is mainly due to the adsorption of Zn on clay or $CaCO_3$, rather than from the formation of sparingly soluble $Zn(OH)_2$ or $ZnCO_3$ (Rengel, 2015; Trehan & Sekhon, 1977). In addition, Zn uptake and translocation to the shoot are inhibited by high concentrations of bicarbonate and HCO_3^- (Marschner, 2011). It seems that WV by decreasing the pH of the root environment and preventing the stabilization of ions on soil particles could increase Zn and Mn availability and increase it in the roots of ML, SO stems and leaf of both rootstocks. The pattern of changes in leaf Cu concentration due to the use of WV in two rootstocks was the opposite, so that it increased in SO and decreased in ML seedlings. The increase in Cu concentration of SO leaves due to the use of WV was due to the increased uptake of this element. However, in ML seedlings, reduction in leaf Cu concentration was mainly due to the decrease in translocation and stem Cu concentration. Contrary to the obtained results in current study, it has been observed that adding WV to animal waste and charcoal can stabilize heavy elements such as nickel, Zn and Cu on their particles and thus reduce the concentration of these elements in soil solution (Zhu et al., 2021a). The results of some studies have shown that the use of WV can increase the vegetative growth of the plant sometimes by 70 to 80% (Jeong et al., 2015). For example, the use of WV increased leaf area, number of fruits and plant dry weight (Abdolahipour, 2019) and the area and volume of roots (Burnette, 2010) in cucumber (*Cucumis sativa*) seedlings. Also, it increased leaf area index, number of pods per plant, plant height and dry weight in rapeseed (*Brassica napus* L.) (Zhu et al., 2021b). The acids in WV increase the concentration of protons (H^+) in leaf tissue and changing the pH of leaf cell sap increases the activity of these cells and plant growth (Zhu et al., 2021b). In the present study, the use of WV with 2% concentration increased leaf area and decreased root dry weight of both rootstocks and did not affect other morphological traits. Due to the presence of some growth inhibitor compounds such as phenol and chrysol in the composition of WV, researchers believe that WV can in some cases reduce plant growth (Jeong et al., 2015; Pan et al., 2017). The use of WV has a positive effect on plant growth if the concentration of all compounds in it is appropriate and balanced (Zhu et al., 2021b).

Changes in the ratio of mineral elements in two studied rootstocks showed that SO seedlings tend to absorb and accumulate Ca in their roots, while ML seedlings accumulate more K in their roots. The reason for this difference can be attributed to the difference in the apoplastic space and the root CEC of two plants (Marschner, 2011), or the difference in their leaf surface and, as a result, their transpiration rates.

The effect of 1% WV on reducing the K/Na and Ca/Na in the roots of both rootstocks can be due to the increase in the availability of Na in the soil and increased its absorption by the roots of both plants (Table 2). Contrary to these results, some researchers emphasize the effect of WV on reducing the adverse effects of salinity on plants (Jayasankaran et al., 2022) and consider this effect to be due to the effect of WV on reducing osmotic stress and stimulating the plant to absorb water, thereby reducing the toxicity of Na and Cl ions (Theerakulpisut et al., 2017).

CONCLUSION

Without considering the effects of WV, the two rootstocks used in this research were different from each other in terms of uptake percentage and especially translocation and efficiency of mineral elements and this should be considered in practice. Addition of WV to the soil decreased the pH but did not significantly change the availability of elements such as P, Fe and Cu, increased the availability of K, Mn and Zn, and increased its EC. The effect of WV on the concentration of mineral elements and their ratio in leaves and roots of plants was different depending on the rootstock type. Although the application of WV had no effect on P, K and K/Ca in the leaves and Zn in the root of SO, it caused a reduction in P, K and increase in the leaf K/Ca, Zn and Fe in the roots of ML seedlings. On the other hand, the application of WV did not have a significant effect on the vegetative growth of the seedlings. It is suggested to investigate the effect of higher concentrations and other WV sources on citrus rootstock seedlings, but application of higher WV concentrations will likely to further reduce soil pH and increase the availability of some elements, thereby increasing soil EC and inhibitor compounds and the formation of complexes may prevent plant growth. The responses of two rootstocks to the application of WV were different and this should be considered in practice.

Conflict of interest

The authors declare that they have no conflict of interest.

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Determining an appropriate integrated nutrition system for saffron (*Crocus sativus* L.) cultivation as affected by maternal corm weight

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ABSTRACT

Purpose: The aim of this study was to determine the response of saffron (*Crocus sativus* L.) to nutrient resources and maternal corm weights. **Research method:** The experiment was conducted as a split-plot based on a randomized complete block design with three replications. The main plots included four integrated nutrition programs (NP0: control (without fertilization); NP1: cow manure, amino acids, humic acid; NP2: slaughterhouse waste, mono-potassium phosphate, humic acid, mix (macro-micro) fertilizer; and NP3: poultry manure, ammonium nitrate, humic acid, amino acid, hormone biofertilizer, NP fertilizer, micronutrients) and the subplots included three maternal corm weights (CW1: 4-8, CW2: 8-12, and CW3: 12-16 g). **Findings:** The difference between the experimental treatments in terms of the effect on the studied traits of saffron was more significant in the second year than in the first year of the experiment. The NP3 treatment resulted in the highest values of the flower number (85.6), fresh and dry flower yield (28.21 and 5.48 g m^{-2}, respectively), and fresh and dry stigma yield (3.35 and 0.67 g m^{-2}, respectively). Also, planting the heaviest corms led to the highest values of the mentioned traits (79.9, 24.57, 4.85, 2.90, and 0.58 g m^{-2}, respectively). The highest value of corm number (312.1), corm diameter (4.27 cm), corm weight (6.86 g), and corm yield (1562.4 g m^{-2}) belonged to the NP3 treatment. The CW3 treatment obtained the highest values of the mentioned traits (322.7, 3.72 cm, 6.87 g, and 1476.5 g m^{-2}, respectively). Furthermore, the highest dry stigma water productivity (1.942 g m^{-3}) and corm water productivity (4.45 kg m^{-3}) were found under the NP3 treatment. Also, the highest value of the mentioned traits (1.681 g m^{-3} and 4.18 kg m^{-3}, respectively) was recorded in the CW3 treatment. **Research limitations:** No limitations were identified. **Originality/Value:** The results of this research clearly show the profound importance of the characteristics of the maternal corms and the integrated nutrition in changes in saffron yield. Overall, we conclude that the NP3CW3 treatment is the best treatment for obtaining the highest values of saffron flower and corm indices as well as the stigma and corm water productivity.

INTRODUCTION

Medicinal plants are the main and essential elements of the indigenous medical systems worldwide (Hosseinzadeh et al., 2015). Currently, the dependence of developing countries on the herbal medicinal sources is much higher than on the modern medicinal sources. According to the estimates, about 80% of the population of these countries depends on the traditional medicine and the use of medicinal sources for medical treatment and healthcare (Tareen et al., 2016). Nowadays, the demand for the use of the medicinal plants for medicinal and medical purposes, especially in traditional medicine in different countries, is increasing rapidly, and this issue has increased the economic importance of the medicinal plants (Agra et al., 2007). The prominent advantage of medicinal plants cultivation in the arid and semi-arid areas is that some environmental stresses can stimulate the plant to produce secondary metabolites, which may improve the crop quality, thereby pharmaceutical and economic superiority of the medicinal plants in these regions rather than other regions (Omidbaigi, 2000).

Saffron (*Crocus sativus* L.) is a botanically annual and agronomically perennial plant species belonging to the Iridaceae family, which is widely cultivated in the arid and semi-arid regions, especially in Iran (Fallahi et al., 2021). Saffron has a variety of uses in food, industry, and medicine, as well as cosmetics and sanitary products (Ben El Caid et al., 2020; Leone et al., 2018; Nazari & Feizi, 2021). The value and importance of saffron are due to its significant biochemical compounds, including sugars, minerals, fats, vitamins, and secondary metabolites, including terpenes, flavonoids, anthocyanins, and carotenoids, among which carotenoids are the most important because of the color and taste properties of saffron (Gismondi et al., 2012; El Grah et al., 2022).

Soil is known as the substrate for plant growth and development, and its fertility and quality are the most essential factors for the sustainable production of crops (Ayoub, 1999). In the past few decades, Iranian farmers have heavily depended on chemical fertilizers to provide the nutrient requirements of crops that, in addition to reducing the production growth rate and the stability of crop production, has caused a decrease in the biodiversity, especially in fauna and flora of the ecosystem, water and soil resources pollution and numerous harmful effects on the health of many kinds of living organisms, especially humans (Esmaeilian et al., 2022b). In such a situation, it is essential to choose a plant nutrition system that, in addition to providing a balanced supply of nutrients, is compatible with the conditions of the agro-ecosystem and causes the least damage to the living and non-living components of the ecosystem. In recent years, integrated nutrient management (INM), as an environment friendly approach characterized by a balanced mixture of the inorganic, organic and biological compounds, has received much attention (Janssen, 1993). The purpose of INM is to achieve the best combination of fertilizers, which, while using sufficient and balanced types of fertilizers, increases the effectiveness of plant nutrition on the improvement of quantitative and qualitative traits of the crops, while maintaining the health and quality of the soil (Selim, 2020). Several researches show a positive and significant response of saffron to INM systems compared to applying fertilizer compounds alone. For example, Sarfraz et al. (2023b) reported that the integrated nutrition systems improved saffron flower and stigma indices by 35-70% and saffron corm indices by 35-96%. Kirmani et al. (2022) conducted a large-scale study and concluded that the highest yields of saffron flowers and corms were obtained in farms where the integrated nutrition system was implemented. The results of another research showed that the integrated application of organic and inorganic nitrogen sources has the potential to improve the quality of saffron stigma (Sarfraz et al., 2023a). Studies have shown significant and positive effects of combined application of cow manure (Turhan et al., 2007), poultry manure (Saeidi Aboueshaghi et al., 2022), vermicompost (Feli et al., 2018), and

biological fertilizers (Ahmadi & Nazari Alam, 2015; Alizadeh et al., 2018) with chemical fertilizers on the growth and yield of saffron.

One of the fertilizer sources that has been considered as an alternative to chemical fertilizers is animal waste that, if properly managed, can be an attractive option in the sustainable agriculture and, as a good source of nutrients, can play an influential role in improving the soil organic matter and fertility, and reduce the costs of the crop nutrition system (Roy et al., 2013). This organic amendment also plays an essential role in improving soil health (Adesina et al., 2020). The Organic fertilizers, unlike the chemical fertilizers, which have high solubility and availability (Barker & Pilbeam 2007), need more time to be mineralized and provide their elements to the plant (Tejada et al., 2014). For this reason, the release of nutrients from these fertilizer sources is done gradually, and thereby, the process of absorbing their elements by plants takes place over a long time. The use of organic and biological fertilizers has positive ecological effects, especially on the physical, chemical, and biological properties of soil, which is closely related to the food quality and safety, environmental health, and human health maintenance (Alfa et al., 2014).

Biofertilizers, containing various microorganisms (e.g., bacteria, fungi, and algae) and growth-promoting compounds (e.g., plant hormones and amino and organic acids) as ecofriendly, low-cost, and effective compounds, play an essential role in the implementation of sustainable agricultural strategies to replace chemical fertilizers and reduce off-farm inputs in low input farming systems (Kawalekar, 2013; Al-Taey et al., 2019; Azarmi-Atajan & Sayyari-Zohan, 2022). Microorganisms in biofertilizers cause the mineralization of organic compounds and the conversion of inorganic compounds from non-absorbable form to absorbable form through various biological processes (Ekta et al., 2017). By improving the physical, chemical, and biological characteristics of the soil, these materials improve soil fertility and, ultimately, increase the growth and quantitative and qualitative yield of crops (Abdel-Raouf, et al., 2012; Al-Taey et al., 2019).

Saffron is a plant whose growth and yield are strongly affected by the environmental and management factors among which, plant density, maternal corm properties, and plant nutrition are critical (Temperini et al., 2009). It has been determined that to produce saffron flowers, a minimum amount of saffron corm weight and size is required (Douglas et al., 2014). It has been reported that planting larger maternal corms due to more reserves, not only improves the vegetative growth of saffron and flower production in the first year, but also, due to better growth and use of environmental resources, leads to more daughter corms production and, ultimately, to more flower and corm yield during the consecutive years (Renau-Morata et al. 2012; Fallahi et al., 2017). Ebrahimi et al. (2021) reported a significant increase in traits such as flowers number, style-stigma dry weight, number and weight of daughter corms as a result of planting large-sized corms. The results of another research showed that planting heaver maternal corms (>12 g) caused earlier flowering and more extended flowering period, which resulted in the significantly higher flower and stigma yields. Also, corm yield was significantly higher in plots with higher maternal corm weight (Alie et al., 2023).

The important challenge facing the agricultural sector is the enhancement of per person crop production in line with the population growth on the one hand, and the limitation of environmental resources, especially water, on the other hand. This issue is more vital in the arid and semi-arid areas, which has utilized the strategies of reducing water consumption or increasing crop production per unit of water consumption in order to improve water productivity making it an inevitable issue. This issue, in the case of saffron plant, which is produced in the important saffron growing areas in the world, including Iran, by basin irrigation, is especially important. Furthermore, the complexity of the saffron plant and its

variable responses to the environmental and management factors are among the main challenges in the main areas of saffron cultivation, especially in Iran. Saffron nutrition and choosing the proper fertilizing system, which, like the eco-friendly agricultural system, guarantee the achievement of the appropriate yields, have always been parts of the basic challenges in the saffron production. On the other hand, the interaction effects of maternal corm characteristics and other effective factors in the growth and yield of saffron are other important questions in the saffron cultivation management. Therefore, the purpose of this research was to compare different types of the integrated nutrition systems in terms of their effect on the quantitative characteristics and water productivity of saffron flowers and corms and to choose the best nutrition system according to different maternal corm weights.

MATERIALS AND METHODS

Site description

The experiment was conducted at the research farm of University of Torbat Heydarieh, Torbat Heydarieh, Iran (59° 133′ E, 35° 20′ N, and 1450 m a.s.l.) during two consecutive growing seasons (2016-17 and 2017-18). The experimental site location is shown in Fig. 1. According to the Köppen climate classification, the area has an arid climate. The average annual temperature of the area is 14.3 °C, while the minimum and maximum temperatures are -4.3 and 33.5 °C in February and August, respectively. The average long-term annual rainfall is 274.8 mm and, mainly, occurs between January and April. The average annual relative humidity is 46%, with a minimum of 33% and a maximum of 64% during August and February, respectively. Monthly rainfall and average maximum and minimum temperature data of the study area during the experiment years are given in Fig. 2. The soil of the experimental site was classified as clay loam with low organic matter. The soil with electrical conductivity (EC)=2.19 dS m^{-1} and pH=8.01 is classified as normal soil (Scherer et al., 1996). Some physicochemical properties of the experimental field soil are presented in Table 1.

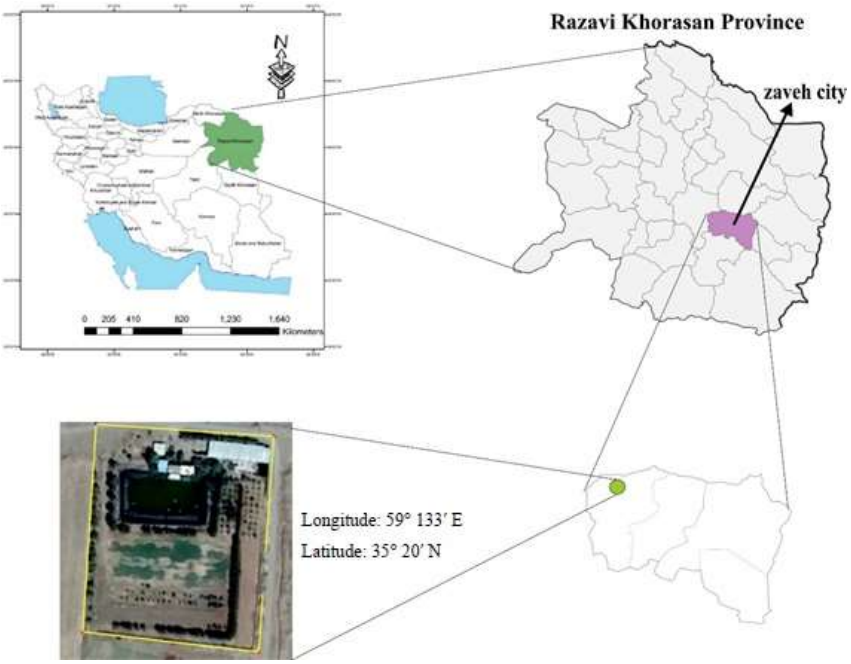

Fig. 1. Location of the studied area, Zaveh, Torbat Heydarieh, Razavi Khorasan province.

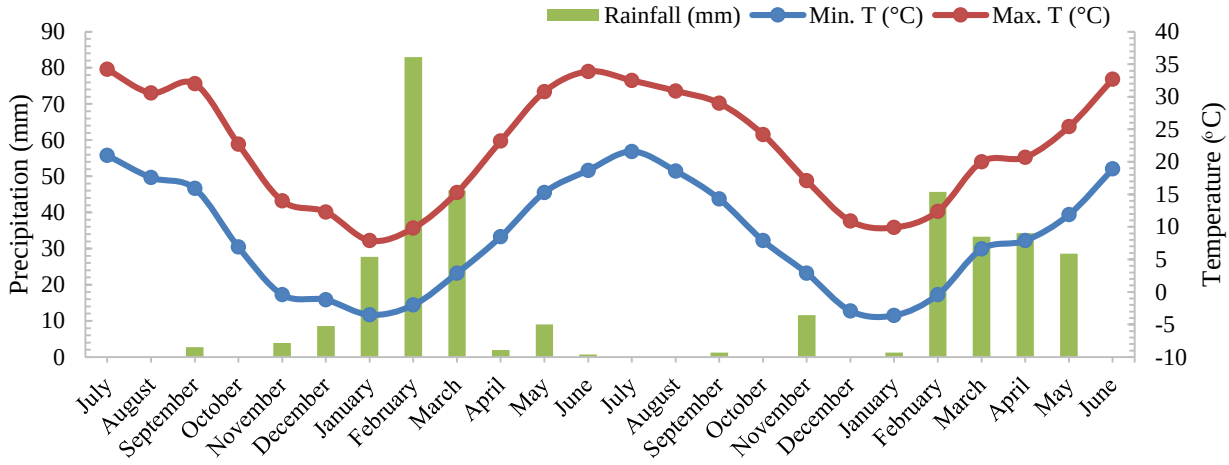

Fig. 2. Regional meteorological records of the experiment site during the two growing seasons (2016-17 and 2017-18).

Table 1. Physical and chemical properties of experimental site soil.

Soil texture	Sand	Silt	Clay	$CaCO_3$	Total N	Organic carbon	Available P	Available K	pH	EC
	%						(mg kg^{-1})		-	dS m^{-1}
Clay loam	13	53	34	17.5	0.049	0.51	28	360	8.01	2.19

Experimental design and treatments

The field experiment was conducted as split plot based on a randomized complete block design with three replications. The main plots included four nutrition treatments described below, and the subplots consisted of three levels of maternal corm weight groups (4-8, 8-12, and 12-16 g).

One of the main objectives of this research was to investigate the effects of the integrated nutrition systems on the studied characteristics of saffron and to choose and recommend the best fertilizer combination for the saffron farming system. So, for the selection of experimental treatments, the literature review and the opinions of experts in the agricultural sector were used. The four levels of the nutrition programs were:

Treatment 1) Control (no fertilization).

Treatment 2) before planting (July 2016), 3400 kg ha^{-1} cow manure was incorporated into the soil. After planting, 5 L ha^{-1} humic acid was applied during the first irrigation (September 2016). Then, in November 2016, the amino acid (0.3 kg ha^{-1}) and humic acid (5 L ha^{-1}) fertilizers were applied with irrigation and repeated at the same rate in January, March, September, November 2017, and January 2018.

Treatment 3) before planting (July 2016), 6600 kg ha^{-1} slaughterhouse waste was mixed with the soil. 50 kg ha^{-1} mono-potassium phosphate, 2 kg ha^{-1} Fe chelated fertilizer and 4 kg ha^{-1} humic acid powder fertilizer was applied during the first irrigation (November 2016). In January 2017, 15 kg ha^{-1} mix (macro-micro) fertilizer (N, P, K, Fe, Zn, Mg, and Cu chelates) was applied during the irrigation operation. In March 2017, 5 L ha^{-1} humic acid fertilizer was applied with irrigation. 25 kg ha^{-1} 15-5-30 NPK fertilizer and 4 kg ha^{-1} humic acid powder fertilizer were applied as incorporated within the irrigation water. In November 2016 and January 2017, the fertilization program of the previous year was repeated. In March 2017, 5 L ha^{-1} humic acid fertilizer was incorporated into the irrigation water.

Treatment 4) before planting (July 2016), 3400 kg ha^{-1} poultry manure was incorporated into the soil. After planting (September 2016) 400 kg ha^{-1} ammonium nitrate and 300 kg ha^{-1}

humic acid granular fertilizer were added to the soil before the first irrigation. In November 2016, 100 kg ha^{-1} urea, 25 kg ha^{-1} 20-20-20 NPK, 1 kg ha^{-1} amino acid, and 200 kg ha^{-1} sulfur granular fertilizers were applied as incorporated within the irrigation water. In January 2017, 1 liter per 1000-liter water of hormone biofertilizer (containing 250 ppm of auxin, gibberellin, and cytokinin), 40-30 NP fertilizer, and micronutrients fertilizer (containing 0.3, 0.4, 0.4, 0.25, 0.8, and 0.65% of B, Cu, Fe, Mg, Mn, and Zn, respectively) were foliar sprayed two times. Also, 3 kg ha^{-1} humic acid powder fertilizer was applied and incorporated into the irrigation water. This nutrition program was repeated in September and November 2017 and January and March 2018.

The chemical properties of the organic fertilizers applied in the experiment are presented in Table 2. Urea (150 kg ha^{-1}), super phosphate triple (120 kg ha^{-1}), and potassium sulphate (100 kg ha^{-1}) were used as fertilizer source of N, P, and K fertilizer, respectively.

Experiment layout and agronomic practices

After plowing, disk, and leveling, the experimental plots were constructed manually with a 4.5 m^2 area (2.25 m × 2.0 m), 1.0 m distance between the adjacent plots, and 1.5 m between the blocks. According to the nutrition program treatments, the required fertilizers were incorporated into the soil of each plot. The saffron corms used in this experiment were provided from Zaveh, Razavi Khorasan Province ecotype. Based on the saffron corm weight treatments, the maternal corms were separated according to each weight group. Saffron corms were planted as the basin method in July 2016 at 100 corms m^{-2} density at 15 cm soil depth. Therefore, no new planting was done in the second year. Irrigation of saffron was carried out as the basin irrigation. Table 3 shows the irrigation date and volume during the saffron growth period. Five days after the first irrigation, breaking the soil crusting was done manually. Weeding was done manually in two stages in December and January of each experiment year.

Measurements and data collection

Saffron flowers were accounted and picked up daily. The duration of the saffron flowering stage in the first and the second years was 5 to 30 November 2016 and 7 November to 2 December 2017, respectively. The flowers were transferred to the laboratory and weighed using a precision digital scale (±0.0001 g), and then the fresh flower yield was calculated. Then, saffron stigmas were separated from the flowers, weighed, and recorded as fresh stigma yield. After drying the flowers and stigmas in the shade at room temperature, dry flower yield and dry stigma yield were measured.

Table 2. Chemical analysis of organic fertilizers used in the experiment.

Parameter / Fertilizer	O.C	N	P	K	Zn	Cu	Fe	Mn	EC	pH
	%				mg kg^{-1}				dS m^{-1}	-
Cow manure	33.1	2.2	0.8	0.9	56.3	35.0	743	70.4	5.7	6.8
Poultry manure	43.7	4.5	1.4	1.9	281.1	26.8	1174	47.6	8.5	6.4
Vermicompost	23.9	2.7	1.5	1.3	41.5	8.1	143	28.5	6.3	7.8
slaughterhouse waste	61.4	6.3	2.0	0.71	76.2	22.7	1038	19.9	4.9	6.1

Table 3. Irrigation dates and volumes applied during the saffron growth period.

Irrigation date					
First year	2016-10-17	2016-11-23	2017-01-04	2017-02-08	2017-04-01
Second year	2017-10-19	2017-11-20	2018-01-09	2018-02-12	2018-03-28
Irrigation volume (m^3 ha^{-1})					
First year	750	600	530	560	550
Second year	630	620	570	580	580

At the end of each growing season (26 and 27 July 2017 and 2018, respectively), daughter corms were harvested from the 0.5 m^2 area of each plot, and the related traits such as replacement corm number, diameter, weight, and corm yield were determined. The corm weight and diameter were determined from 10 randomly selected corms.
The dry stigma and corm water productivity were calculated using the following formula (1):

$$\text{Dry stigma/corm WP (g/kg m}^{-3}) = \frac{\text{Dry stigma/corm yield (g/kg ha}^{-1})}{\text{Irrigation water applied (m}^3\text{ ha}^{-1})} \quad (1)$$

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.2 (SAS, 2008), and the difference between treatment means was separated using the least significant difference test (LSD) at a 5% probability level.

Table 4. Mean comparison for the saffron flower indices as affected by nutrition programs and maternal corm weight.

Treatments	Flower number (no. m^{-2})		Fresh flower yield (g m^{-2})		Dry flower yield (g m^{-2})		Fresh stigma yield (g m^{-2})		Dry stigma yield (g m^{-2})	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
Nutrition program (NP)	**	**	**	**	**	**	**	**	**	**
NP0 (control)	27.6^{d}	39.9^{d}	10.06^{d}	11.94^{d}	1.94^{c}	2.45^{c}	0.93^{c}	1.46^{d}	0.17^{c}	0.29^{d}
NP1	33.1^{b}	53.1^{c}	13.12^{b}	15.25^{c}	2.38^{b}	3.01^{c}	1.22^{b}	1.88^{c}	0.22^{b}	0.36^{c}
NP2	30.3^{c}	74.6^{b}	11.58^{c}	24.45^{b}	2.18^{b}	4.75^{b}	1.05^{c}	2.97^{b}	0.20^{bc}	0.59^{b}
NP3	43.3^{a}	85.6^{a}	16.46^{a}	28.21^{a}	2.78^{a}	5.48^{a}	1.62^{a}	3.35^{a}	0.27^{a}	0.67^{a}
Corm weight (CW)	**	**	**	**	**	**	**	**	**	**
CW1	30.4^{c}	45.4^{c}	11.56^{c}	15.06^{c}	1.92^{c}	3.03^{c}	1.06^{c}	1.95^{c}	0.20^{b}	0.38^{c}
CW2	33.6^{b}	64.5^{b}	12.84^{b}	20.28^{b}	2.30^{b}	3.89^{b}	1.21^{b}	2.41^{b}	0.22^{ab}	0.48^{b}
CW3	36.8^{a}	79.9^{a}	14.02^{a}	24.57^{a}	2.72^{a}	4.85^{a}	1.35^{a}	2.90^{a}	0.24^{a}	0.58^{a}
NP×CW	NS	*	NS	*	*	*	NS	*	NS	*
NP0CW1		25.7^{h}		8.48^{g}	1.70^{g}	2.04^{f}		1.10^{i}		0.22^{g}
NP0CW2		43.7^{g}		11.43^{f}	1.90^{efg}	2.11^{f}		1.35^{h}		0.28^{f}
NP0CW3		50.3^{efg}		15.91^{e}	2.20^{d}	3.20^{e}		1.94^{f}		0.38^{e}
NP1CW1		42.3^{g}		10.42^{f}	2.08^{def}	2.24^{f}		1.56^{gh}		0.27^{f}
NP1CW2		49.0^{fg}		15.92^{e}	2.34^{cd}	3.19^{e}		1.71^{fg}		0.34^{e}
NP2CW3		68.0^{c}		19.41^{d}	2.74^{b}	3.61^{e}		2.38^{e}		0.48^{d}
NP2CW1		54.3^{ef}		18.96^{d}	1.78^{fg}	3.41^{e}		2.32^{e}		0.46^{d}
NP2CW2		77.0^{ef}		26.19^{b}	2.22^{d}	4.90^{cd}		3.19^{c}		0.64^{b}
NP2CW3		92.3^{c}		28.21^{b}	2.54^{bc}	4.95^{ab}		3.42^{b}		0.68^{b}
NP3CW1		59.3^{d}		22.35^{c}	2.14^{de}	4.43^{d}		2.81^{d}		0.57^{c}
NP3CW2		88.3^{b}		27.56^{b}	2.80^{b}	5.37^{bc}		3.38^{c}		0.66^{b}
NP3CW3		109.0^{a}		34.73^{a}	3.40^{a}	6.65^{a}		3.86^{a}		0.78^{a}
C.V (%)	10.63	9.21	9.96	7.22	9.71	8.72	13.04	6.77	11.27	6.13

* and **: Significant at $p<0.05$ and $p<0.01$, respectively. NS: Non-significant according to the LSD test.
Means within each column with same letters are not significantly different by LSD test ($p<0.05$).
NP0 to NP3 indicate nutrition programs. CW1 to CW3 indicate maternal corm weights.

RESULTS AND DISCUSSION

Flowering indices

All the nutrition treatments increased the flower number (FN) compared to the control treatment. In the first year, the highest value (43.3 flowers m^{-2}) was obtained from the NP3 treatment, and the NP1 treatment was ranked next with 33.1 flowers m^{-2}, that increased the FN by 56.9 and 19.9%, respectively, compared to the control. In the second year, the NP3 treatment, achieved 85.6 flower m^{-2}, caused the highest increase than control (114.5%), and the NP2 treatment, with a value of 74.6 flowers m^{-2} (86.0% increase) was ranked in second place (Table 4). It seems that the NP3 treatment with a suitable combination of organic, biological and chemical fertilizers has a better balance of nutrients. For this reason, it has provided better growth conditions for the saffron plant that, ultimately, led to more flower production. Probably, the increase in the FN as a result of the application of the NP2 treatment is more due to the application of slaughterhouse waste in this treatment because this organic fertilizer, not only increases the organic matter in the soil but, is also able to release various hormones and amino acids that can increase photosynthesis and assimilates production by the plant (Besharati & Saffari, 2022). Changing the trend from the chemical fertilizers to organic and biological fertilizers in saffron cultivation is vital. Several studies have shown favorable responses of saffron to the use of organic and biological fertilizers alone or in combination with chemical fertilizers (Ahmadi & Nazari Alam, 2015; Ekta et al., 2017; Ebrahimi et al., 2021; Esmaeilian et al., 2022b).

Planting heavier maternal corms increased the emergence of saffron flowers so that in the first year, the CW2 (8-12 g) and CW3 (12-16 g) treatments increased the FN by 10.4 and 21.05%, respectively, and 42.0 and 75.9% in the second year, respectively, compared with the small corms (4-8 g). The interaction effect of the experimental factors on the FN in the second year was significant so that the highest value (109.0 flowers m^{-2}) was obtained from the NP3CW3 treatment and followed by the NP3CW2 treatment with 88.3 flowers m^{-2}. Table 4 also shows that the nutrition treatments had more effect on the flowering of small corms than on the large ones, which was more evident in the case of the smallest corms (4-8 g). In a situation where one of the main obstacles and limitations in the crop production in the arid and semi-arid regions is the organic matter content as well as the poor nutrients in the soil (Reynolds, 2017), the integrated use of chemical, organic and biological fertilizers along with the cultivation of appropriate maternal corms can significantly increase the probability of success in saffron cultivation.

The fresh flower yield (FFY) significantly responded to the experimental treatments. The NP3 treatment with the FFY of 8.2 and 28.2 g m^{-2} obtained the highest value in the first and second year, respectively. While the lowest values (5.0 and 11.9 g m^{-2}, respectively) belonged to the control (Table 4). The combined use of nitrogen chemical fertilizer with poultry manure in this treatment, in addition to lowering the ratio of C/N, has prevented the lack of nitrogen required by the plant during the early growth period and through the acceleration in mineralization and release of minerals from organic fertilizer has provided the nutritional requirements of the plant (Salehi et al., 2014). Increasing the maternal corms weight led to an increase in the FFY, gradually. This increase in the second (medium-weighted corms) and third (big corms) levels compared to the first level (small corms), was 11.1 and 21.3%, respectively, and in the second year was 34.7 and 63.1%, respectively (Table 4). The saffron corm characteristics can determine the growth status of this plant in the following years. It has been found that the corm properties have a high effect on the emergence of flowers and the flower yield of saffron (Esmaeilian et al., 2022a). A comparison of the mean values of the interaction effect treatments showed that the NP3CW3 treatment achieved the highest FFY (34.7 g m^{-2}) and the NP2CW3 treatment, with a value of 28.2 g m^{-2} ranked in the second place

(Table 4). As mentioned above, the growth and yield of saffron are highly dependent on the growth situation of replacement corms in the previous year. Therefore, planting corms with appropriate size and weight and optimum reservoir status can improve the regrowth and flower yield of saffron in the following year (Renau-Morata et al., 2012; Koocheki & Seyyedi, 2015).

The dry flower yield (DFY) was significantly influenced by the experimental treatments in all the experiment years. The data in Table 4 clearly show that the nutrition program treatments had different effects on this trait. The highest DFY obtained in the first year (1.39 g m^{-2}) belonged to the NP3 treatment, and the NP1 (1.19 g m^{-2}) and NP2 (1.09 g m^{-2}) treatments were ranked in second and third place, respectively. The NP3 treatment in the second year also had the highest effect on the improvement of this trait by obtaining a DFY of 5.48 g m^{-2}. The NP2 and NP1 treatments were placed in the following places with values of 4.75 and 3.01 g m^{-2}, respectively. Optimum formation of saffron replacement corms can improve the growth characteristics and production of saffron flowers in the following year, due to the proper agronomic management operations (de Juan et al., 2009). The results of this research show that the integrated nutrient management by means of the effective and homogeneous combination of different types of fertilizers leads to their adequate and balanced use in terms of the quantity and quality, that results in the nutrient uptake at the right time and during the plant growth stages. This ultimately, can lead to better growth and higher yields (Selim, 2020). Planting corms with higher weight improved the DFY. By increasing the maternal corm weight from the first level to the second and third level, the values of this trait increased by 20.8 and 41.7% in the first year and 29.0 and 60.1% in the second year, respectively (Table 2). The mean comparison of the interaction treatments (Table 4) showed that in both years of the experiment, the plants that were treated with the fourth nutrition program and had the highest weight of the maternal corm (NP3CW3 treatment) achieved the highest DFY (1.70 and 6.65 g m^{-2} in the first and second year, respectively). A more effect of the fertilizer treatments on the small than large corms was also observed regarding DFY (Table 4). Koocheki and Seyyedi (2015) stated that although the amount of nutrient reserve of maternal corm has a determining role in the growth and production of saffron flowers, the soil nutrient content, especially from the second year of growth, when the reserves of maternal corms decreased and the growth of replacement corms increased, is more critical.

The variation in the fresh stigmas yield (FSY) as a result of the implementation of different nutrition programs and plantation of corm with different weight groups was also significant. In the first year, the NP3 treatment achieved the highest value (1.62 g m^{-2}), and the NP1 treatment, with a value of 1.22 g m^{-2}, was in second place. In the second year, the NP3 treatment also obtained the highest value (3.35 g m^{-2}), while the NP2 treatment, with a value of 2.97 g m^{-2}, was in second place (Table 4). Numerous researchers have emphasized that the growth and yield of saffron are greatly influenced by the plant environment and nutritional condition of the corms (Lage & Cantrell, 2009; Siracusa et al., 2011; Ghorbani et al., 2019). The use of humic acid fertilizer in the integrated nutrition systems can be considered an essential factor in achieving these results. It has been reported that humic acid, as a growth stimulating fertilizer, directly or indirectly increases the nutrients uptake and improves plant physiological processes (Nourihoseini et al., 2016). By increasing the maternal corm weight from the first to the second and third levels, the FSY increased by 14.1 and 27.4% in the first year and by 23.6 and 48.7% in the second year, respectively (Table 4). The mean comparison of interaction effect treatment in the second year also showed that the NP3CW3 treatment had the highest value (3.86 g m^{-2}), and the second place (3.42 g m^{-2}) was related to the NP2CW3 treatment (Table 4).

The response of dry stigma yield (DSY) to the experimental treatments was also almost similar to the FSY, although its increase was higher than the FSY. Applying the second to fourth nutrition programs in the first year caused 29.4, 17.6, and 58.8%, and in the second year, 24.1, 103.4, and 130.0% increase, respectively, compared to the control (Table 4). This increase in the case of corm weight treatments from the second and third levels compared to the first level was 7.3 and 16.7% in the first year and 26.3 and 52.7% in the second year, respectively (Table 4). Our results also indicated that applying the NP3CW3 treatment obtained the highest DSY (0.78 g m^{-2}), and the NP2CW3 treatment placed in the next rank (0.68 g m^{-2}). Temperini et al. (2009) stated that corm weight and nutrition management are two determining factors for saffron yield. Khorramdel et al. (2015) concluded that maternal corm characteristics have more importance than other agronomic factors affecting saffron production. The results indicate that the selection of appropriate maternal corm and implementation of integrated nutrition that provides sufficient and balanced macro and micro elements required by the saffron plant are key factors in saffron production.

Corm indices

Replacement corm number (RCN) in both years of the experiment showed a significant response to the nutrition program treatments. In the first year, the NP3 treatment showed the highest value (163.4 corm m^{-2}), although it didn't show a significant difference from the NP1 treatment. In the second year, different results were obtained, so the highest value (319.6 corm m^{-2}) belonged to the NP2 treatment, and the NP1 treatment ranked second with 314.8 corm m^{-2} (Table 5). Although the saffron plant is considered an adaptable and low-input plant in terms of the need for fertilization and supply of nutrients (Gresta et al., 2008), the results of numerous researches show a reasonable response of this crop to the use of chemical and biological fertilizers (Koocheki & Seyyedi, 2015; Ghanbari et al., 2019; Esmaeilian et al., 2022a,b). As shown in Table 5, the variation in the weight of maternal corms hadn't any significant effect on the RCN in the first year while, in the second year, the CW2 and CW3 treatments without statistically significant differences with values of 313.6 and 322.7 corm m^{-2}, respectively obtained higher values than control (278.8 corm m^{-2}). It seems that planting corms with more weight due to more nutrition reserves and producing an extensive root system that can absorb more water and nutrients resulted in the production of more replacement corms by more buds (Renau-Morata et al., 2012; Koocheki et al., 2014).

Replacement corm diameter (RCD) significantly responded to the nutrition program treatments. As Table 5 shows, all the nutrition treatments increased the RCN than the control; however, the highest value in the first and second year (3.74 and 8.54 cm, respectively) was obtained from NP3 treatment, which showed a 70 and 78% increase, respectively compared to control. Planting heavier corms increased the RCN, so a 56.5 and 39.5% increase in RCN in the first and second year of the experiment, respectively, was achieved due to planting 12-16 g maternal corms (Table 5). Among the interaction treatments, the highest values in two years of the experiment (5.09 and 5.15 cm, respectively) were obtained due to planting 12-16 g maternal corms under the third nutrition program (Table 5).

Replacement corm weight (RCW) was also significantly influenced by the experimental treatments. The highest value in the first year (5.42 g) was obtained due to the implementation of the fourth nutrition program. However, it didn't show a significant difference with the third nutrition program (4.97 g). Similar results were obtained in the second year, and the NP3 and NP2 treatments without any significant difference had the highest effect on the increase in the RCW by obtaining values of 6.86 and 6.65 g, respectively (Table 5). The difference in the RCW was very evident regarding the levels of maternal corm weight so that the increase in corm weight from the first to the second and third levels led to 28.2 and 53.7% increases in

the first year and 24.7 and 48.7% in the second year, respectively (Table 5). Amirnia et al. (2014), by comparing the effects of maternal corm weight on saffron characteristics, concluded that corms with a weight of 10 grams and above are the most suitable corms to obtain more replacement corms with a higher weight, while corms with a weight of 6 grams and below are not recommended for cultivation. The results of our experiment also indicated that planting the heaviest maternal corms and applying the fourth nutrition program in both years of the experiment had the most significant effect (6.73 and 8.82 g, respectively) in improving this trait. The second rank in both years of the experiment (5.89 and 7.78 g, respectively) belonged to the third nutrition program and the third level of maternal corm weight (Table 5).

The corm yield (CY) was also significantly affected by the experimental treatments. The NP3 treatment achieved the highest CY in both years of the experiment however; its effect was more noticeable in the second year so that the CY as a result of the implementation of the fourth nutrition program was 15.5% in the first year and 26.3% in the second year (Table 5). Rasouli et al. (2014) also compared different types of nutrient systems on the characteristics of saffron and, concluded that the integrated nutrition system consisting of organic, biological and chemical fertilizers had the highest effect on the corm yield. Planting the maternal corms with higher weight resulted in higher CY values, so the CW2 and CW3 treatments showed 8.6 and 15.2% increase in the first year, respectively, and 7.2 and 13.2% increase in the second year, respectively, compared to the CW1 treatment (Table 5).

Table 5. Mean comparison for the saffron corm indices as affected by nutrition programs and maternal corm weight.

Treatments	Corm number (no. m^{-2})		Corm diameter (cm)		Corm weight (g)		Corm yield (g m^{-2})	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
Nutrition program (NP)	*	*	**	**	**	**	**	**
NP0 (control)	143.4^{b}	278.1^{b}	2.20^{c}	2.79^{d}	3.37^{b}	4.11^{c}	533.8^{c}	1239.3^{d}
NP1	160.4^{a}	314.8^{ab}	2.77^{b}	2.92^{c}	3.97^{b}	5.37^{b}	580.1^{bc}	1310.5^{c}
NP2	157.0^{ab}	319.6^{a}	3.08^{b}	3.21^{b}	4.97^{a}	6.65^{a}	599.5^{b}	1441.5^{b}
NP3	163.4^{a}	312.1^{ab}	3.74^{a}	4.27^{a}	5.42^{a}	6.86^{a}	639.7^{a}	1565.4^{a}
Corm weight (CW)	NS	**	**	**	**	**	**	**
CW1		282.2^{b}	2.30^{c}	2.67^{c}	3.48^{c}	4.62^{c}	549.6^{c}	1298.5^{c}
CW2		313.6^{a}	2.95^{b}	3.20^{b}	4.46^{b}	5.75^{b}	597.2^{b}	1392.5^{b}
CW3		322.7^{a}	3.60^{a}	3.72^{a}	5.35^{a}	6.87^{a}	632.9^{a}	1476.5^{a}
NP×CW	NS	NS	*	**	*	*	*	*
NP0CW1			1.63^{e}	1.61^{h}	2.78^{h}	3.37^{f}	509.3^{f}	1156.2^{h}
NP0CW2			2.34^{de}	2.53^{g}	3.57^{fgh}	4.23^{ef}	554.3^{de}	1256.4^{g}
NP0CW3			2.64^{cd}	2.89^{ef}	3.76^{efgh}	4.72^{e}	597.6^{de}	1305.2^{f}
NP1CW1			2.38^{d}	2.65^{fg}	2.91^{gh}	4.63^{e}	533.9^{ef}	1219.1^{g}
NP1CW2			2.85^{cd}	2.99^{ef}	3.96^{ef}	5.34^{de}	599.8^{cd}	1307.5^{f}
NP2CW3			3.11^{b}	3.13^{de}	5.04^{bcd}	6.14^{cd}	606.5^{cd}	1405.0^{d}
NP2CW1			2.65^{cd}	3.71^{c}	4.35^{def}	5.24^{de}	568.8^{de}	1373.0^{e}
NP2CW2			3.05^{b}	3.13^{de}	4.68^{cde}	6.95^{bc}	611.3^{bc}	1466.6^{cd}
NP2CW3			3.57^{b}	3.71^{c}	5.89^{ab}	7.78^{b}	618.5^{bc}	1484.9^{b}
NP3CW1			2.56^{cd}	3.48^{cd}	3.89^{efg}	5.26^{de}	586.4^{cd}	1445.7^{cd}
NP3CW2			3.58^{b}	4.16^{b}	5.65^{bc}	6.51^{c}	623.5^{b}	1539.6^{b}
NP3CW3			5.09^{a}	5.15^{a}	6.73^{a}	8.82^{a}	709.3^{a}	1710.9^{a}
C.V (%)			5.67	6.75	3.42	2.71	10.35	8.16

* and **: Significant at $p<0.05$ and $p<0.01$, respectively. NS: Non-significant according to the LSD test.
Means within each column with same letters are not significantly different by LSD test ($p<0.05$).
NP0 to NP3 indicate nutrition programs. CW1 to CW3 indicate maternal corm weights.

Stigma and corm water productivity

Considering water shortage and poor soils in dry areas such as the experiment area where saffron occupies a major proportion of the land under cultivation of crops, one of the effective approaches for water conservation is the implementation of a suitable nutrition program for saffron growing. Increasing the saffron water use efficiency can play a significant role in the sustainable production of saffron (Koocheki & Seyyedi, 2020). The dry stigma water productivity (DSWP) significantly responded to the experimental treatments. Among the nutrition program treatments, the NP3 treatment by achieving the values of 0.885 and 1.942 g m^{-3} for the first and second year of the experiment, respectively, had the highest effect on the improvement of this index. The NP1 treatment in the first year (0.721 g m^{-3}) and the NP2 treatment in the second year (1.720 g m^{-3}) were in second place in terms of the effect on increasing DSWP (Table 6). Numerous researches revealed the significant effects of integrated nutrient management by organic, inorganic, and biological fertilizers on the WP of crops (Singh et al., 2019; Faloye et al., 2019; Midya et al., 2021). The DSWP increased as a result of planting maternal corms with more weight. The CW3 treatment, by obtaining the values of 0.754 and 1.680 g m^{-3} for the first and second year of the experiment, respectively, showed the highest DSWP. The CW2 treatment with 0.718 and 1.390 g m^{-3}, respectively, was in the next place (Table 6). Koocheki and Seyyedi (2020) found that farms with larger corms had more water use efficiency compared to smaller corms. They also stated that farms that received manure had higher water use efficiency compared to farms that were fertilized with chemical fertilizers.

The interaction effect of the experimental factors on the DSWP in the second year of the experiment was significant. The NP3CW3 treatment and, after it, the NP2CW3 treatment (2.260 and 1.971 g m^{-3}, respectively) caused the highest increase in the DSWP, while the lowest value (0.640 g m^{-3}) was observed in the NP0CW1 treatment (Table 6). The results of this research show that the integrated nutrition of saffron through the improvement of soil properties and the nutrient availability, mobility in soil and dynamics (Midya et al., 2021) and in overall improvement of the plant's growth environment has provided the conditions that the physiological processes of the plant done in a better manner that finally improved the vegetative and reproductive growth of the plant.

Investigating the response of corm water productivity (CWP) to different nutrition programs and different corm weight groups showed a significant variation of this index in response to the experimental treatments. As shown in Table 6, there was a significant difference among the nutrition treatments in terms of the effect on the CWP, so the highest value in the first and second years (2.10 and 4.45 kg m^{-3}, respectively) belonged to the NP3 treatment. The NP2 treatment, with values of 1.97 and 4.08 kg m^{-3}, respectively, and the NP1 treatment, with values of 1.90 and 3.77 kg m^{-3}, respectively, were ranked in the second and third place. The lowest value (1.81 and 3.65 kg m^{-3}, respectively) belonged to the control. Because of the limitation in arable lands and water resources, especially in arid and semi-arid regions, development of the agricultural sector will be possible only by increasing the resource use efficiency with minimal harmful effects on the agroecosystem through the use of modern and adaptable technologies (Amirnia et al., 2014). Soil fertility management by improving soil physicochemical and biological properties and enhancing quantitative and qualitative yields of crops plays an essential role in improving crop water productivity (Rockström et al., 2010).

Planting corms with more weight resulted in higher CWP, so the CW3 treatment had the highest value (2.07 and 4.18 kg m^{-3} in the first and second years of the experiment, respectively). The CW2 treatment ranked next with values of 1.96 and 3.99 kg m^{-3}, respectively, while the lowest values (1.80 and 3.67 kg m^{-3}, respectively) belonged to the

CW1 treatment (Table 6). The mean comparison of interaction effects showed that the NP3CW3 treatment, with values of 2.33 and 4.86 kg m^{-3} in the first and second year of the experiment, respectively, caused the highest increase in the CWP. The NP3CW2 and NP2CW3 treatments with values of 2.04 and 2.02 kg m^{-3} in the first year and 4.37 and 4.21 kg m^{-3} in the second year, respectively, ranked in the next place. The lowest values (1.67 and 3.25 kg m^{-3} in the first and second year, respectively) belonged to the NP0CW1 treatment (Table 6).

Table 6. Mean comparison for the dry stigma and corm water productivity as affected by nutrition programs and maternal corm weight.

Treatments	Dry stigma WP (g m^{-3})		Corm WP (kg m^{-3})	
	2016-17	2017-18	2016-17	2017-18
Nutrition program (NP)	**	**	**	**
NP0 (control)	0.557^{c}	0.850^{d}	1.81^{d}	3.65^{d}
NP1	0.721^{b}	1.053^{c}	1.90^{c}	3.77^{c}
NP2	0.656^{bc}	1.720^{b}	1.97^{b}	4.08^{b}
NP3	0.885^{a}	1.942^{a}	2.10^{a}	4.45^{a}
Corm weight (CW)	**	**	**	**
CW1	0.669^{c}	1.101^{c}	1.80^{c}	3.67^{c}
CW2	0.718^{b}	1.391^{b}	1.96^{b}	3.99^{b}
CW3	0.754^{a}	1.681^{a}	2.07^{a}	4.18^{a}
NP×CW	NS	**	*	**
NP0CW1		0.640^{h}	1.67^{c}	3.25^{b}
NP0CW2		0.810^{gh}	1.81^{bc}	3.54^{b}
NP0CW3		1.101^{ef}	1.95^{b}	3.69^{ab}
NP1CW1		0.780^{gh}	1.75^{bc}	3.43^{b}
NP1CW2		980^{gf}	1.97^{b}	3.89^{ab}
NP2CW3		1.391^{de}	1.99^{ab}	3.97^{ab}
NP2CW1		1.333^{de}	1.86^{b}	3.88^{ab}
NP2CW2		1.855^{bc}	2.00^{ab}	4.15^{ab}
NP2CW3		1.971^{b}	2.02^{ab}	4.21^{ab}
NP3CW1		1.652^{c}	1.92^{b}	4.09^{ab}
NP3CW2		1.913^{b}	2.04^{ab}	4.37^{ab}
NP3CW3		2.261^{a}	2.33^{a}	4.86^{a}
C.V (%)	11.14	6.50	10.05	7.94

* and **: Significant at $p<0.05$ and $p<0.01$, respectively. NS: Non-significant according to the LSD test. Means within each column with same letters are not significantly different by LSD test ($p<0.05$). NP0 to NP3 indicate nutrition programs. CW1 to CW3 indicate maternal corm weights.

CONCLUSION

The results of our research indicate a highly significant response of saffron to all types of nutrition systems. Based on the obtained results, the best nutrition program to improve the saffron flower indices (flower number, fresh and dry flower yield, fresh and dry stigma yield) and corm indices except for corm number (corm diameter, corm weight, and corm yield) was the NP3 treatment, in which the integrated nutrition system included various chemical and organic fertilizers in different forms was applied. Furthermore, the NP2 treatment (mineral fertilizers containing macro- and micronutrients and humic acid) was associated with the second-highest values of most of the mentioned saffron traits. The NP3 treatment led to the highest dry stigma and corm water productivity, significantly followed by the NP2 treatment. Additionally, the research results indicate the highly significant effects of maternal corm weight on the flower and corm properties of saffron. Planting corms with a higher weight

group resulted in the improvement of all studied traits of saffron so the highest values were obtained as a result of planting corms with a weight group of 12-16 g, while the lowest values were obtained due to planting corms with a weight group of 4-8 g. Mean comparisons of the nutrition system and maternal corm weight interactions revealed that the best treatment for improving the saffron studied traits was the NP3CW3 treatment.

Conflict of interest

The authors have no conflict of interest to report.

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Changes in color, vitamin C, carotenoids and tocopherols during ripening and senescence of tomato fruit

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ABSTRACT

Purpose: Changes in color, vitamin C, β-carotene, lycopene, α- and δ-tocopherol were followed during ripening and senescence of mature-green tomatoes (*Lycopersicon esculentum* Mill cv. Rhapsody) maintained at 22 °C and 85% RH for up to 5 weeks. **Research method:** Tomatoes were harvested at the mature-green stage and valuated for color and the content of selected bioactive compounds (β-carotene, lycopene, α- and δ- tocopherols and vitamin C) during simulated retail market conditions (22 °C and 85% RH for 5 weeks). **Findings:** The tested tomato cultivar had a long postharvest life under the tested conditions, as the fruit maintained in edible conditions during the whole storage period. Vitamin C, lycopene, α- and δ-tocopherol presented their highest values after about 14 to 18 days after harvest, and β-carotene maintained a maximum content (0.84 mg/100 g) 8 days after harvest. The maximum content of vitamin C, lycopene, α- and δ-tocopherol were 0.036, 30, 0.27 and 0.0045 mg 100 g^{-1} of fresh tissue, respectively. Our results indicate that 'Rhapsody' tomatoes harvested at the mature-green stage have shown important levels of vitamin E (tocopherols), C, and carotenoids (lycopene and β-carotene) after 14 to 17 days from harvest. **Research limitations:** There were no limitations. **Originality/Value:** This study evaluated the changes in the content of bioactive compounds in long shelf life tomatoes, of great importance for human health, for up to 5 weeks to determine the ideal moment for their consumption.

INTRODUCTION

Tomato is an essential vegetable in the diet of people around the world and is the main vegetable produced worldwide; in 2022, up to 186 million tons were produced (FAOSTAT, 2022). Mexico is among the 10 biggest world producers of tomatoes (FAOSTAT, 2022), and is the main exporter to United States and Canada markets (SAGARPA, 2017). Tomato has become one of the main sources of nutritional and bioactive compounds for human health, fundamentally antioxidant vitamins, such as C, E and B12, with content of 4–22 mg/ 100 g fw, 0.9-3.8 mg/100 g fw, and 4–60 µg/100 g fw, respectively (Bianchi et al., 2023; Raiola et al., 2015; Figueira et al., 2017; Vats et al., 2022). Additionally, tomato fruit has high content of carotenoids, mainly lycopene (2.2–41.8 mg/100 g fw), α- and β-carotenes (0.1–0.5 mg/100 g fw), and lutein (0.1-19.7 mg/100 g fw) (Flores et al., 2017; Loayza et al., 2021a,b; Vats et al., 2022; Bianchi et al., 2023). The intake of tomato fruit and its lycopene has been related with a lower risk of cancer mortality and prevention of cardiovascular diseases (Mazidi et al., 2020a,b). The consumption of bioactive components found in tomato fruit was reported to increase the natural antioxidants, such as plasma total antioxidant capacity, erythrocyte superoxide dismutase and glutathione peroxidase, and reduce the oxidative stress, serum malondialdehyde, and the inflammatory markers such as TNF-α (Ghavipour et al., 2015; Widjaja et al., 2022).

Tomato is a climacteric fruit, and therefore it is usually harvested unripen, including in the green-mature stage, and continues its ripening after harvest (Quinet et al., 2019; Islam et al., 2023). Tomato fruit maturation and ripening involve a series of biochemical, physiological and structural changes, triggered by the production and action of ethylene (Tilahun et al., 2017). These changes can influence the content of nutrients and functional phytochemicals (Yahia et al., 2001; Tilahun et al., 2017). Some of the fruit maturation and ripening changes are used as maturation and ripening indices as well as harvesting indices, and as quality standards and attributes. These include color (due to pigments) changes, structural (softening) changes, changes in the contents of sugars, acids, and volatile compounds (Quinet et al., 2019). The main indicator of fruit ripening processes is associated with the degradation of chlorophylls and the synthesis of carotenoids with the consequent dedifferentiation of chloroplasts to chromoplasts (Tilahun et al., 2019), and therefore the maturation and ripening stages of tomatoes are commonly classified according to the color change of the fruit, mainly the intensity of the red color caused primarily by the carotenoid lycopene (USDA, 2005). However, there has been an increased interest in objective and integral measurements for a better selection of cultivars, maturation and ripening stages, harvesting indices, quality standards and attributes, and therefore for effective pre- and post-harvest practices to optimize the content of bioactive components and to extend the storage life of the fruit.

Tomatoes are highly perishable after harvest affected by several important factors that affect fruit quality, such as temperature, relative humidity, atmosphere, and other treatments (Kefas et al., 2024). Fruit of traditional tomato cultivars have a postharvest life of about 15-20 days at ambient temperature, but some cultivars have an extended postharvest life, and these are becoming more popular in the international market (Bal, 2021). Changes in bioactive compounds, of great importance for human health, in the long shelf life cultivars have received little attention. Therefore, the objective of this work was to follow the changes of some of the important bioactive compounds, during the ripening and senescence of 'Rhapsody' tomato fruit after harvest, over a long period extending from the mature-green stage to over ripening.

MATERIALS AND METHODS

Tomato samples

Forty tomatoes (*Lycopersicon esculentum* Mill cv. Rhapsody) free of blemishes, with uniform color and size, with an average weight of 210 g, was harvested at the mature-green stage, from a commercial hydroponic greenhouse in Querétaro, Mexico. After harvest, the tomatoes were placed to ripen naturally at 22 °C and 85 % RH. Fruit evaluation was done over a period of 35 days, and consisted of a sample of four fruits each time.

Color measurement

Fruit external color was determined on two readings from each fruit in longitudinal adjustment (one near of peduncle and the other in the apex), using a CM-2002 Minolta colorimeter (model CM-2002, Minolta Co. Ltd., Osaka, Japan). The colorimeter was calibrated with a white pattern and zeroed during each evaluation. The variables a*, b*, L*, C*, and h° were recorded.

Extraction and HPLC analysis of vitamin C

Five grams of fresh fruit pulp were homogenized with 10 mL of 0.1 M citric acid solution containing 0.05% EDTA and 5% methanol, and pH was adjusted to 2.35-2.40. The mixture was centrifuged at 11, 960 x *g* for 10 min at 2 °C, and 2.4 mL of the supernatant was filtered through a number 3 Whatman paper, and at least one hour before the analysis 1 mL of 1,2-benzenediamine in water (83.2 mg/100 mL) was added to it. Of this mixture, 40 µL were injected automatically into an HP 1100 series high pressure liquid chromatography (HPLC) system (Agilent Technologies Co., Palo alto, CA, USA) equipped with an inline degasser, a thermostatic auto-sampler, 100 µL loop and a diode array detector (DAD). The mobile phase was water/methanol (95:5 % v/v) containing 5 mM of hexadecyltrimethyl-ammonium bromide and 50 mM of KH_2PO_4, and it was pumped at 1.5 mL min^{-1} through a Waters µBondapak C_{18} analytical column (3.9 x 300 mm, 10 µm) kept at 25 °C. The ascorbic acid (AA) and the isoascorbic acid (IAA) were monitored at λ= 261 nm, and the dehydroascorbic acid (DHAA) at λ= 348 nm. The identification and quantification of AA, IAA, and DHAA were achieved by comparing the retention time values and the integrated peak areas with those of known amounts of the standards of these compounds purchased from Sigma (Sigma-Aldrich, St. Louis, MO). The peaks obtained were processed using the HP Chem Station program, and reported as total vitamin C.

Extraction and HPLC analysis of tocopherols

Ten grams of fresh tomato pulp were homogenized with 20 mL of ethanol for 2 min and centrifuged at 5000 x *g* for 5 min at 2 °C. The pellet was eliminated and 3.5 mL of petroleum ether were added to the extract, shaken, and 5mL of water were added. The mixture was centrifuged again, under the same conditions, and the upper layer was then separated and evaporated in a rotavapor (Yahia et al., 2007). The obtained mixture was dissolved in 1.5 mL of methanol and filtered through a polyethylene membrane of 0.45 µm of pore prior to the injection of 50 µL to the HPLC system. The HPLC system described above was used, but using a fluorescence detector. The mobile phase was methanol/water (95:5 % v/v) and it was pumped at 1mL/min through a Waters Symmetry C_{18} analytical column (4.6 x 150mm, 3.5 mm) that was kept at 25°C. The tocopherols were monitored at λ_{ex}= 294 nm and λ_{em}= 326 nm. The identification and quantification of α- and σ-tocopherols were achieved by comparing the retention time values and the integrated peak areas with those of the samples

obtained from solutions of known concentrations of standard tocopherols purchased from Sigma-Aldrich (Saint Luis, MO, USA).

Extraction and HPLC analysis of carotenoids

Six grams of fresh tomato pulp were dehydrated with $NaSO_4$, and 30 mL of hexane-acetone-toluene-ethanol solution (33.3:23.3:23.3:20 v/v) were added. The saponification of samples was done by adding 1 mL of 40% KOH in methanol. This mixture was placed in a water bath at 56 °C and shaken for 20 min, after which it was cooled, and 15 mL of hexane were added, sample was shaken again, and 15 mL of aqueous 10% $NaSO_4$ were added. The samples were then kept in the darkness for 15 min, and after filtration of the extracts through polyethylene membranes of 0.45 µm pore, 25 µL were taken from the upper layer and injected into the HPLC system. The determinations were made at λ= 471 nm using a diode array detector. A YMC Carotenoid C_{30} (4.6x150 mm, 3 µm) analytical column was used. A gradient system of a mobile phase was employed, which begun with 4% water, 81% methanol and 15% *tert*-butyl methyl ether, and finalized after 75 minutes to 4%, 18% and 78%, respectively (Yahia et al., 2007). As it is the case in the other HPLC analyses, lycopene and β-carotene were identified and quantified using commercial standards obtained from Sigma-Aldrich (Saint Luis, MO, USA).

Statistical analysis

The experiment was repeated 3 times, using a completely randomized design, and the collected data were analyzed by comparing the means using the Tukey-Kramer test and linear regressions, employing the JMP software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Color development and carotenoids

Significant color changes were observed during the first 8 to 11 days after harvest, followed by very slight variations (Fig. 1). The development of the tomato pigmentation consisted in the disappearance of the green color, and the acquisition of red tonalities (increases in *a** value). These changes in color are also reflected in hue (h^o) value. The quotient of the parameters *a** (redness) and *b** (yellowness) (*a*/b**) was also useful in describing these changes, whose biochemical explanation is based on the degradation of chlorophyll (green pigment) and a rapid biosynthesis and accumulation of carotenoids (Yahia et al., 2007), principally lycopene, responsible for the red pigmentation characteristic of tomato fruit (Tilahun et al., 2017). The a* and *a*/b** values showed good correlation with lycopene content (Fig. 2), according to equations 1 and 2, whose correlation coefficients (r^2) were 0.97 and 0.94, respectively, where lycopene values were in mg/100 g of fresh tissue.

$$\text{Ln (lycopene)} = -0.87767667 + 0.07675095\ (a^*) + 0.00691\ (a^*)^2 \quad (1)$$

$$\text{Ln (Lycopene)} = -0.73015483 + 2.19329353\ (a^*/b^*) \quad (2)$$

In contrast, Loayza et al. (2021a) did not find significant correlations between the color changes and lycopene content in 'BHN-602' tomatoes, while other studies have reported associations between color changes in tomatoes with carotenoids such as β-carotene (Flores et al., 2017).

The luminosity (L*) value decreased almost 21% after 36 days of storage, and the major decrease was observed during the first 12 days. The chroma (C*) values slightly increased

(14%) during ripening. Soto-Zamora et al. (2005) also found similar reductions (20%) in tomatoes stored at 20 °C. Changes in color during ripening have been reported in shorter postharvest periods in other cultivars. Mazón-Abarca et al. (2022) found higher reductions in the hue value after 9 days of storage in fruit of the cultivar TA234 in comparison with our study, where major changes were found after 12 days of storage of 'Rhapsody' tomatoes.

Lycopene content increased continuously after 21 days of storage to up to 30 mg/100 g fw and then slightly decreased until day 36 to 23 mg/ 100 g fw (Fig. 2). These lycopene levels are within the range reported for tomatoes of other cultivars (2.2–54.9 mg/100 g fw) (Flores et al., 2017; Loayza et al., 2021a; Vats et al., 2022; Bianchi et al., 2023). Once the tomatoes have developed their red color (after 8 to 11 days, see Fig. 1), the maximum β-carotene content (0.84 mg 100 g^{-1}) has been reached (Fig. 2), which surpassed the content (0.51 ± 0.1 mg/ 100 g fw) reported in 21 red tomato cultivars (Flores et al., 2017). In our study, the β-carotene content after 35 days was similar with that at day 0 (0.41 mg 100 g^{-1}). It has been suggested that lycopene content increases during tomato ripening due to an increase in the activity of enzymes such as 6-methyl-5-hepten-2-one, promoted by ethylene action (Pu et al., 2020). Few studies have followed the changes in the content of carotenoids during the ripening and senescence periods of tomatoes (only 10-20 d postharvest) (Tilahun et al., 2019; Pu et al., 2020). Yahia et al. (2007, 2005) reported major variations related to storage temperatures, where chilling temperatures (4°C) delayed the carotenoid accumulation in tomato fruit.

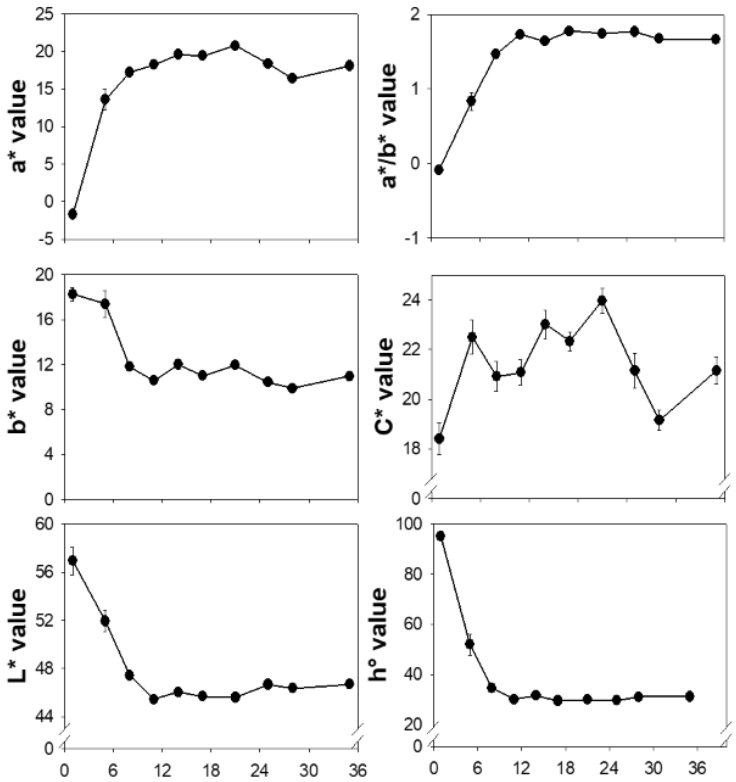

Fig. 1. Changes of color parameters during the ripening and senescence of 'Rhapsody' tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

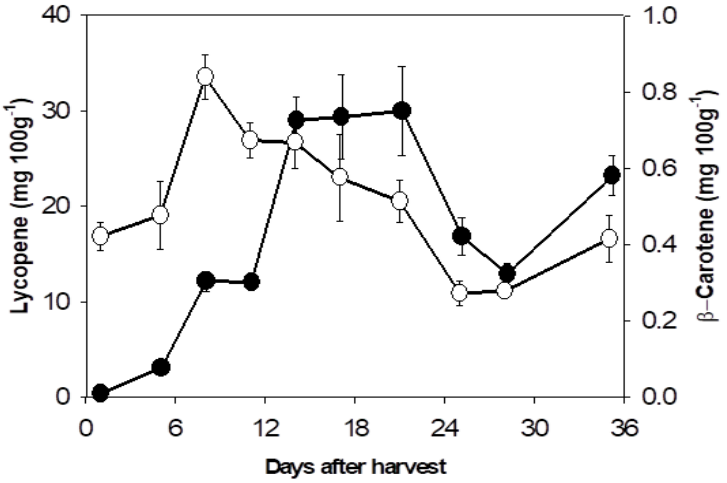

Fig. 2. Changes in lycopene (—●—) and β-Carotene (—○—) content during the ripening and senescence of 'Rhapsody' tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

Changes in tocopherols and vitamin C

The maximum levels of δ- and α-tocopherols were reached after 14 and 17 days after harvest, respectively, and then started to decrease (Fig. 3). This tendency, especially the peak of these two vitamers of vitamin E agrees with the development of the red color of the fruit. Chlorophyll biodegradation produces phytol, which is a limiting factor in the synthesis of tocopherols by the 2-methyl-6-phytylquinol methyltransferase during tomato ripening (Vats et al., 2022). The limited amount of chlorophyll and its degradation during ripening, and thus the limited production of phytol groups might be the reason behind the reduction of the tocopherols after reaching their peak (Soto-Zamora et al., 2005). The reduction was slower for α-tocopherol (Fig. 3), probably because other tocopherols are good substrates for methyltransferase (Guo et al., 2022), which is responsible for the formation of this compound. In contrast, Figueira et al. (2017) found higher losses of α-tocopherol, up to 62.8% less α-tocopherol in ripe fruit than in full mature green 'Gordal' tomatoes, and slight increase in the content of δ- and γ-tocopherols. Yahia et al. (2007) reported a reduction in the content of α-tocopherol after 14 days and then increased until day 30 of storage at 20 °C, whereas in fruit stored at 4 °C, the content slightly increased during storage. The obtained tocopherol chromatograms (Fig. 4C) imply that analyzed tomatoes may contain at least another isomer of tocopherol (β or γ or both together), which is in agreement with results reported by Abushita et al. (2000) and Figueira et al. (2017). The chromatograms (Fig. 4) show two compounds (X and XX), eluted between α- and δ-tocopherol (α- as three-methylated and δ- as the mono-methylated vitamers, respectively), which exhibited fluorescence response at λ_{ex}= 294 nm and λ_{em}= 326 nm, a chemical property that characterizes tocopherols. The peaks X, XX**,** or both of them, could represent a mixture of bimethylated tocopherols as well as tocotrienols. The content of tocopherols in tomato fruit has been reported from 0.17 up to 3.83 mg/100 g fw, being α-tocopherol the most abundant (Raiola et al., 2015; Figueira et al., 2017).

The content of vitamin C (Fig. 5) was within the reported range for tomato fruit (4–23 mg/ 100 g fresh fruit) (Tilahun et al., 2017; Bianchi et al., 2023). It decreased slightly during the first 11 days after harvest and then increased during 11 and 17 days, and gradually decreased again, however, there were no significant differences between the initial and final amounts of this vitamin. Yahia et al. (2007 and 2005) reported similar contents of ascorbic acid, but an increase of isoascorbic acid during tomato ripening after 30 and 35 days of

storage at 10 and 20 °C. To the contrary, Tilahun et al. (2017) reported a slight decrease (17.2–20.3%) in the content of ascorbic acid from tomatoes at breaker (initiation of green-red) stage and red fruits of cv. 'TY Megaton' and 'Yureca' at similar storage conditions (20 ± 2 °C). The increase of vitamin C during fruit ripening has been related to the production of the intermediary GDP-1-galactose promoted by the GDP-mannose 3', 5'-epimerase family (Vats et al., 2022). However, the ripening metabolism varied in different cultivars.

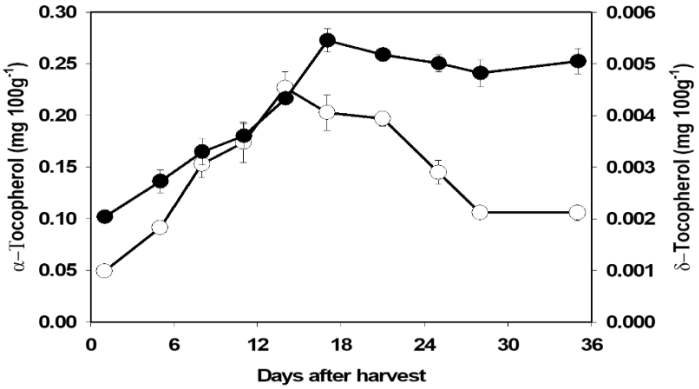

Fig. 3. Changes in α-tocopherol (—●—) and δ-tocopherol (—○—) content during the ripening and senescence of 'Rhapsody' tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

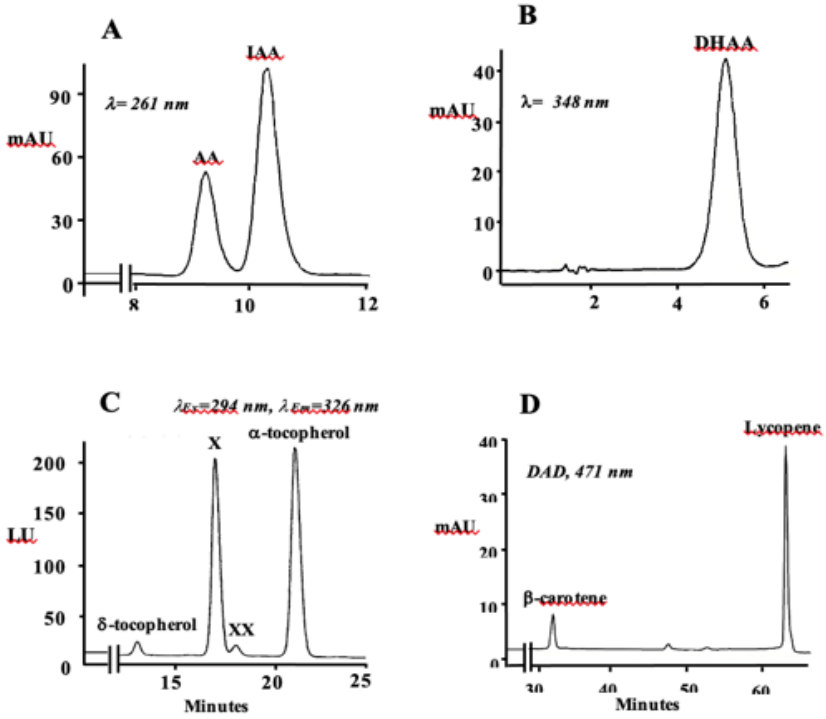

Fig. 4. Typical chromatograms of ascorbic acid (AA). A: isoascorbic acid (IAA), B: dehydro ascorbic acid (DHAA), C: δ-tocopherol, α-tocopherol, and D: β-carotene and lycopene. X and XX are thought to be γ- and β-tocopherol, respectively. The response of the photodiodes array detector (DAD) and the fluorescence detector (FLD) were in milli absorbance units (mAU) and luminescence units (LU), respectively.

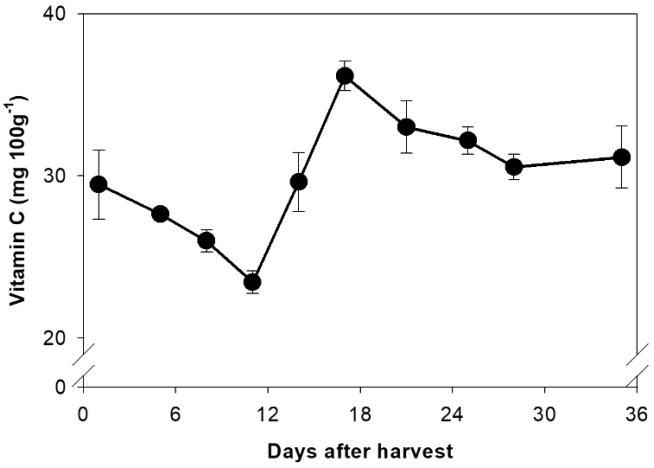

Fig. 5. Changes in vitamin C content during ripening and senescence of 'Rhapsody' tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

CONCLUSION

Our results indicated that 'Rhapsody' tomatoes harvested at the mature-green stage and ripened naturally had adequate levels of vitamin E (tocopherols), vitamin C, lycopene, and β-carotene for 14 to 17 days. The storage at 20 °C was suitable for the promotion of carotenoids accumulation in tomato fruit. The content of these bioactive antioxidant compounds was maintained during the ripening stage, and therefore the consumption of the fruit or its use for processing during this period would not cause major losses in these health-promoting phytochemicals. However, extended periods, beyond 11-17 days of ripening when the fruit are harvested at the mature-green stage, could result in significant losses in important bioactive components, and therefore decreased health benefits.

Conflict of interest

The authors declare that there is no conflict of interest.

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A comprehensive study of qualitative and biochemical characteristics of dried seedless barberry fruits from different regions of South Khorasan Province, Iran

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ABSTRACT

Purpose: There is no report regarding the physicochemical properties of dried barberry fruits from main production regions of the South Khorasan Province, Iran. **Research Method**: Therefore, we investigated the nutritional quality and bioactive compounds of dried, seedless barberry fruits from different regions of South Khorasan Province, Iran. Dried barberries were evaluated after being purchased and collected. **Findings:** The highest total soluble solids (TSS) and the highest taste index (TSS/TA) were obtained for the barberries from the Birjand region, which indicates that the barberries from this region have better and sweeter tastes than those from other regions. However, the barberry fruits of the Qaen region had the greatest amount of titratable acidity (TA) and the lowest amount of TSS, which indicates that the fruits of this region are more sour than those of other regions. The examination of color indices (*L**, *a**, *b**, *Hue* and *chroma*) showed that the lowest values of *a** (redness) and chroma were related to the dried barberry of the Darmian region. Additionally, the highest total phenol content and anthocyanin content were detected in fruits from the Birjand region. However, barberry fruits from the Darmian region had the lowest phenol content and the lowest levels of anthocyanin and vitamin C. Positive strong correlations revealed between anthocyanin and TSS (r = 0.82), anthocyanin and phenol (r =0.95), anthocyanin and vitamin C (r =0.77), and anthocyanin and chroma (r =0.81). **Research limitations:** No limitations were found. **Originality/Value:** In general, it can be concluded that the dry, seedless barberry fruits from the Birjand region had higher quality and nutritional value than those from other regions. Nevertheless, the barberries of the Zirkoh and Qaen regions also had acceptable quality and nutritional value. However, it seems necessary to control and review the storage and drying conditions of barberry fruits in the Darmian region.

INTRODUCTION

Seedless barberry (*Berberis integerrima* cv. Zereshk bidaneh), is a small fruit and contain essential nutrients, minerals, and health-promoting phytochemicals, including the treatment of liver and vascular problems and the prevention of many diseases (Sarraf et al., 2019; Khayyat, 2022). One of the most important functional compounds in the barberry plant is berberine, which is present in various parts of the plant. Studies have shown that berberine in barberries reduces cholesterol and blood glucose (Sarraf, et al., 2019). It can help prevent Alzheimer's disease and neoplastic diseases. In addition, barberry fruits have antimicrobial and antifungal effects (Rahimi Kakolaki et al., 2024), and are rich source of natural antioxidant substances (Sun et al., 2021; Sarraf et al., 2019).

Investigations have indicated that preharvest factors (Zeraatgar et al., 2019) and harvest time significantly influence on the fruit quality at postharvest stage (Amiri, et al., 2022; Tatari et al., 2022; Tatari, 2023; Moradinezhad et al., 2008; Shikwambana et al., 2023). In the research conducted by Alavi and Mazloumzadeh (2012), different methods of collecting barberries (branch-cutting, cluster picking and impact force), harvesting times (mid-September-late October to mid-November) and drying methods (shade drying, sun drying and industrial drying) were investigated to determine the effects of these treatments on achieving optimal production conditions and quality. They found that the highest quality of barberry fruit was obtained when they were harvested at late October, harvested in clusters and dried in the shade. In another study conducted by Moghaddam et al. (2013), the effects of harvest date (September 9, September 20, November 1, and November 13), harvest time (07:00, 10:00, 13:00, 16:00, and 19 00:00), picking method (branching and berry picking) and drying method (sun drying and shade drying) on the quantity and quality of seedless barberry fruits were investigated. They found that the best harvesting time is November 13, which is the cool hour of the day (07:00), at which the berries are picked and dried in the sun, which increases the quality and preserves the nutritional value of the dried berries.

Approximately 80% of barberries are water. This product is therefore highly perishable, which is why the most common type of barberry used is dried to reduce moisture content and microbial infections (Moradinezhad & Ranjbar et al., 2023), and to prolong its storage life and is available for consumers in all seasons. Barberry fruits are harvested by three methods: branch-cutting, cluster-picking and impact force. The barberry is also dried in three ways: shade-drying, sun-drying and industrial-drying. Traditionally, in the shade-drying approach, harvested fruits are distributed or scattered on wooden or metal scaffolds.

South Khorasan is the main region of seedless barberry production in Iran and in the world (Goodarzi et al., 2018). Although geographical conditions, diverse weather conditions and structural characteristics causes physicochemical changes in the dried barberry fruit in different regions of the province, but literature shows that previous postharvest studies on barberry mainly focused on the effect of harvest method, harvest date and time. In addition, there is no comprehensive study to compare produced and dried barberry fruits from main production regions of the South Khorasan province, Iran. Therefore, this experiment was conducted with the aim of investigating and comparing the physical and biochemical characteristics of dried barberries prepared from different regions of the province as the main production area of seedless barberry in the world.

MATERIALS AND METHODS

Plant material preparation

To conduct this experiment, first, the main areas of barberry planting in South Khorasan Province were determined. Four regions, namely, the Birjand, Zirkoh, Darmian and Qaen were selected. The barberry is dried by the farmers as the following method. To dry fresh seedless barberry, fruit branches were stored traditionally under shade-house during long-term storage from November 2021 to March 2022. Then, from March 10 to 15, 2022, dried barberry fruits were purchased and collected from each region. Four kilos of dried barberry were prepared from each region (one kilo per replicate), for a total of 16 kilos of barberry. Samples were then transferred to the Postharvest Physiology Laboratory, University of Birjand, South Khorasan Province, Birjand, Iran. The method of fruit picking and storage conditions of used shade-house in all studied regions are presented in Table 1. Additionally, analyses of the water, soil, and geographical coordinates of the studied regions (Birjand, Zirkoh, Darmian, and Qaen) in the Khorasan province, and the corresponding data are presented in Table 2. Barberry samples from Birjand, Zirkoh, Darmian, and Qaen regions were collected from the cities of Arian Shahr, Zohan, Qahestan and Mohammad Abad Alam, respectively.

Physicochemical assessments

Total soluble solids (TSS), titratable acidity (TA) and TSS/TA of dried barberry fruits

To prepare the extract from dried barberry fruit, first, 10 grams of dried fruit were weighed and turned into powder through a mortar. Then, 10 ml of water was added, and the mixture was placed in a shaker (Orbital shaker made by IKA Company, Germany; KS260 digital) for 25 minutes at 50 rpm. The solution was passed through filter paper. This prepared extract was used to determine the biochemical characteristics of dried barberry fruit (Niazmand et al., 2021).

Table 1. The type of fruit picking, height of branches mass and storage conditions in the four studied regions of South Khorasan province, Iran.

Regions	Picking fruit	Height of fruit branches mass (cm)	Storage conditions
Birjand	With branches	30	One-row metal drying rack, with ventilation
Zirkoh	With branches	40	Double-row wooden drying rack, with ventilation
Darmian	With branches	10	On the cement floor, no ventilation
Qaen	With branches	40	One-row metal drying rack, with ventilation

Table 2. Characteristics of soil, water, and coordinate of the four studied regions.

Regions	Soil			Water		Coordinates		
	pH	EC (ds/m)	SP (mg)	pH	EC (ds/m)	Latitude	Longitude	Altitude(m)
Birjand	7.98	4.63	37.40	7.84	2.85	59° 17′	33° 19′	1698
Zirkoh	8.39	1.56	48.22	8.24	1.37	59° 47′	33° 24′	1710
Darmian	8.20	2.85	59.31	8.13	2.02	59° 43′	33° 9′	1966
Qaen	7.82	16.51	33.00	7.09	13.15	59° 11′	33° 54′	1471

To determine the TSS, a few drops of the prepared extract were placed on the prism of an optical refractometer (Model RHB-611-made in China), and the desired number was read in the light (Moradinezhad et al., 2013). The TSS data are presented as °Brix. To measure TA, 10 ml of fruit extract was diluted with 100 ml of distilled water and 0.1% NaOH at pH 8.23 (Moradinezhad et al., 2019). The results are expressed as percentages (Mabunda et al., 2023).

Color indices (L*, a*, b*, Hue° and Chroma) of dried barberry fruits

The color indices of the samples were measured based on *a**, *b** and *L** components by a HUNTER LAB colorimeter (Model TES-135A, Taiwan TES Company).

*L** indicates a dark or light color, ranging from L* = 0 (dark) to L* = 100 (white). The indicators *a**+ (red) and −*a** (green), +*b** (yellow), and −*b** (blue) are shown. Chroma describes the luminosity, intensity, and degree of color purity. Hue represents the degree of color being displayed (McGuire, 1992).

Total phenol (TPC), total anthocyanin and vitamin C contents of the dried barberry fruits

The TPC was measured according to Samadi et al. (2020) method. 300 µL of the extract was added to 1.2 mL of 7.5% sodium carbonate and 1.5 mL of 10% Folin-Ciocalteu solution. The reaction mixtures were placed in the dark for 30 min and then measured at 765 nm via a spectrophotometer (Model UV/Vis 2100, 200-1000 nm; UNICO, USA) and compared to a gallic acid calibration curve to estimate the mg of gallic acid/g extract. For the blank, all processes were the same and 300 µL of methanol was added instead of plant extract. The data are presented as mg.100 g^{-1} DW.

To evaluate the total anthocyanin content, we utilized the pH differential approach of the AOAC, as explained by (Brito et al., 2014; Abbasi Bastami et al., 2022). Potassium chloride buffer with a pH of 1.0 was employed for the evaluation of the absorbance at 510 nm, and sodium acetate buffer (pH 4.5) was utilized for the evaluation of the absorbance at 700 nm. The pigment level (mg cyanidin 3-glucoside equivalents (CGE) per 100 g) was calculated according to the following equations (1 and 2) and expressed as mg.100 g^{-1} DW.

$$A=(A_{510}-A_{700})_{pH\ 1}-(A_{510}-A_{700})_{pH\ 4.5} \quad (1)$$

$$\text{Total anthocyanin content}= (A\times MW\times DF\times 100)/(\varepsilon\times L) \quad (2)$$

MW= the molecular weight (449.2 g/mol), DF= dilution factor, Ɛ= molar extinction coefficient for cyanidin 3-O b-D-glucoside (26.900 L/mol cm), and L= cuvette path length (cm).

The vitamin C concentration was determined by the titration method described by Rangana (1979). For this purpose, 10 ml of extract was diluted with 3% metaphosphoric acid to a volume of 100 ml and filtered. Then, 10 ml of the prepared solution was titrated using 2,6-dichlorophenol indophenol until a blue color appeared. The following formula (3) was used to calculate the observed vitamin C content.

$$\text{Ascorbic acid (mg. 100 g}^{-1}\text{ DW)} = \frac{F\times T\times 100}{D\times S}\times 100 \quad (3)$$

T= Solution (Dye) taken for the burette (mm), F= Dye factor, S= Fruit juice (g) used in dilution, and D=Diluted sample used for titration (mm).

Statistical analysis

Analyses were performed by JMP statistical software, Discovery Pro V. 13.2.1, and Excel Ver. 2019 software. Additionally, comparisons of the means were performed based on the least significant difference (LSD) test at the 5% probability level. The statistical plan used for this experiment was a completely randomized design with four replications, and the data were analyzed. To assess the correlation between evaluated traits the Pearson's correlation coefficient was used.

RESULTS AND DISCUSSION

The results of the analysis of variance of the data showed that the contents of TSS, TA and TSS/TA in the different regions significantly differed (Table 3). A comparison of the averages revealed that the greatest amount of TSS was obtained from dry barberries in the Birjand region (6.96±0.27 °Brix), followed by those in the Zirkoh region (5.33±0.26 °Brix). Additionally, the lowest amount of TSS was obtained from the samples prepared from the Qena region (3.76±0.24 °Brix). By examining the TA values of dry barberries, it was found that the highest and lowest TA values were obtained from the Qaen region (3.60±0.11%) and Darmian region (2.82±0.15%), respectively .The results obtained from the comparison of TSS/TA in different regions indicated that the highest value of this trait was related to the samples from the Birjand region (2.14±0.29), followed by those from the Zirkoh region (1.76±0.27). Additionally, there was no statistically significant difference in the amount of TSS/TA between the Birjand region, Zirkoh region and Darmian region (1.48±0.24).

Table 3. Comparison of the effect of different regions on total soluble solids (TSS), titratable acidity (TA) and taste index (TSS/TA) of dried barberry fruit.

Regions	TSS (°Brix)	TA (%)	TSS/TA
Birjand	6.96^{a}±0.27	3.24^{b}±0.11	2.14^{a}±0.29
Zirkoh	5.33^{ab}±0.26	3.01^{c}±0.17	1.76^{a}±0.27
Darmian	4.20^{b}±0.21	2.82^{d}±0.15	1.48^{ab}±0.24
Qaen	3.76^{b}±0.24	3.60^{a}±0.11	1.05^{b}±0.20
LSD	1.80	0.18	0.45
The significance level	*	**	*

Means ± SEs followed by different letters in the same column for the same evaluated parameter are significantly different (P ≤ 0.05) according to the LSD test. * and ** indicate significance levels at 1% and 5%, respectively.

Table 4. Comparison of the effect of different regions on of color indices (*L**, *a**, *b**, *Hue°* and *Chroma*) of dried barberry fruit.

Regions	*L*	*a*	*b*	*Hue°*	*Chroma*
Birjand	34.43^{a}±3.28	20.72^{a}±2.02	0.55^{a}±0.09	1.54^{a}±0.31	20.72^{a}±2.24
Zirkoh	37.52^{a}±4.03	20.30^{a}±1.23	0.60^{a}±0.11	1.71^{a}±0.33	20.30^{a}±2.21
Darmian	37.60^{a}±3.68	16.34^{b}±1.28	0.57^{a}±0.10	2.01^{a}±0.35	16.35^{b}±1.87
Qaen	36.34^{a}±1.34	20.86^{a}±2.87	0.71^{a}±0.11	1.47^{a}±0.18	20.90^{a}±2.91
LSD	5.39	4.26	0.31	0.94	4.24
The significance level	ns	**	ns	ns	**

Means ± SEs followed by different letters in the same column for the same evaluated parameter are significantly different (P ≤ 0.05) according to the LSD test. *,** and ns indicate significance at 1%, 5% and non-significance levels, respectively.

The level of total soluble solids (TSS) accumulation in fruit can change under the influence of various conditions, including soil and climate, fruit yield and degree of ripeness. The dominant effect of weather factors on fruit TSS accumulation compared to other factors has been proven (Serdyuk et al., 2020). In a study, it was shown that in southern Ukraine, sweet cherry fruit contained 12.1-19.9% soluble solids, while in the central part of the country, the same variety contained only 11.3-12.8% soluble solids (Bublyk et al., 2014; Ivanova et al., 2019). Several scientists have related changes in the biochemical composition of fruits to different ripening conditions and cultivation areas (Basanta et al., 2014; Long et al., 2018). Caprio and Quamme (2006) showed that one of the most important and influential climatic factors in the accumulation of total soluble solids in fruits are the difference between day and night temperatures. In other words, the greater the temperature differences between night and day, the greater the amount of TSS accumulation in the fruit. In the present research, the highest amount of TSS accumulation was found in the fruits of the Birjand region. This increase in TSS may be justified by the presence of warm days and cool nights in this region. In addition, water and soil salinity are likely other mechanisms for changing fruit quality in different regions. Studies have shown that when crops are irrigated with saline water, the macromolecular substances in the cells (such as sucrose) tend to be hydrolyzed to soluble sugars to regulate osmosis and increase the protoplasm's protective ability (Li et al., 2022; Munns and Tester, 2008), which increases solids. Li et al. (2022) showed that with increasing irrigation water salinity (salinity level, 1 and 2 g/liter), the amount of glucose in tomato plants increased with increasing irrigation water salinity, which shows that an appropriate increase in salt concentration in irrigation water is favorable for the formation of fruit glucose. However, when the salinity reached 3 grams per liter, the amount of fruit sugars decreased especially glucose and fructose, which was probably due to ion toxicity at this level of salinity. They stated that the maximum acceptable amount of water salinity in terms of tomato quality characteristics is 2 mg/L. According to Table 1, in this study, the water and soil salinities in the Birjand region were greater than those in the Zirkoh region and Darmian region, which is likely because this level of salinity is tolerable for barberry plants and has increased to increase the osmotic regulation of dissolved solids. And improves the quality of the fruit. For this reason, the taste index (TSS/TA) of fruits from the Birjand region was greater than that of fruits from other regions. However, the salinity levels of the soil and water in the Qaen region are high, which likely causes a decrease in water absorption in the roots and a decrease in soluble solids, which ultimately reduces the taste quality of barberry fruits. As shown in Table 3, the fruits from the Qaen region had the lowest taste indices. The results of the present study agreed with the results of other researchers (Ivanova et al., 2021; Li et al., 2022; Yang et al., 2019) in this regard. Additionally, in the present study, the TA of barberry fruit was greater in the Qena region than in other regions. Studies have shown that with increasing water salinity, the amount of TA in fruits increases (Zhang et al., 2017; Van Meulebroek et al., 2015).

The results presented in Table 4 show that a^* and color chroma of the barberry fruits significantly differed among the different regions. The lowest a^* value for dry barberries was obtained from the Darmian region. Since the values of b^* (fruit yellowness) in barberry fruits ranged from 0.55 to 0.71, the values of a^* and chroma were similar. The color of the products is an important quality factor because the consumer evaluates the products by observing the color in the first stage. In addition, the color of fruits reveals several nutritional parameters. It has been proven that anthocyanin compounds are responsible for the red color of barberry fruits. Therefore, it can be concluded that the redder the barberry fruit is, the more anthocyanin it contains. The color of the products is an important quality factor because the consumer evaluates the color of the products in the first step. In addition, the color of fruits

indicates several nutritional parameters. Anthocyanin compounds have been proven to be responsible for the red color of barberry fruits (Sarraf et al., 2019; Moradinezhad et al., 2018). Therefore, it can be concluded that the redder the barberry fruit is, the more anthocyanin it contains (Kamiab et al., 2023). The bright red color of the fresh barberry gradually turned dark red with dehydration. Additionally, by destroying and changing the pigment compounds of barberries, especially anthocyanins, barberries turn brown or dark (Ardestani et al., 2013). The effects of enzymes, oxidation, light and heat may change pigments into other components and reduce the appearance quality of barberries. Valipoor Motlagh et al. (2013) showed that with increasing oxidation and storage temperature of dry barberry fruit, the red color of the fruit decreased. Sharifi and Hassani (2012) showed that as the storage temperature of barberries increases, red pigments are degraded. In other words, increasing the storage temperature changes the color of barberry fruits from red to brown. In the present study, the barberries in the Darmian region had the lowest amount of redness, which was probably due to the high temperature and lack of ventilation during product storage. The numerical ranges obtained for the values of *L** (34.43-37.60), *a** (16.34-20.86), *b** (0.55-0.71), *Hue*° (1.47-20.1) and *Chroma* (16.35-20.90) for dry cranberry fruit were in accordance with the ranges reported by other researchers (Serdaroğlu et al., 2023; Nadali et al., 2022; Alavi & Mazloumzadeh, 2012).

The results of the phenol evaluation of dried barberry fruit in four regions showed that the highest total phenol content (TPC) was obtained from the fruits of the Birjand region (840.3 $mg.g^{-1}$ DW), and the lowest TPC was from the fruits of the Darmian region (134.61 $mg.g^{-1}$ DW) (Fig. 1). Phenolic compounds such as flavonoids, phenolic acids and stilbenes are the main compounds responsible for the high antioxidant activity of fruits, which can help reduce the oxidative damage of free radicals to the human body and strengthen the immune system (Lin et al., 2016). These compounds are not necessary for plant growth. However, to protect plants against various stresses, they are synthesized as secondary metabolites in plants (Vilvert et al., 2024). The synthesis of phenolic compounds, in addition to playing a defensive role in plants, acts as a warning signal for the synthesis of other antioxidants in plants. These compounds are synthesized through the shikimate and phenylpropanoid pathways (Yeshi et al., 2022; Lin et al., 2016). The basic function of phenolic compounds is their antioxidant activity, which leads to the prevention and reduction of oxidative damage by free radicals to vital cellular components such as lipids, proteins and nucleic acids. Phenolic compounds act as hydrogen or electron donors to free radicals, removing and reducing the amount of free radicals (Kalinowska et al., 2014; Vuolo et al., 2019). In the present research, it was shown that the content of total phenol in the fruits of the Darmian region was lower than that in other regions. In this area, fruits may be stored under inappropriate environmental conditions. As mentioned, free radicals are produced in the face of various stresses, and compounds such as phenols are synthesized in plants to address these conditions. Most likely, in the process of reducing free radicals, phenol is consumed, and its accumulation in the plant decreases. In a study on dried barberry, the authors reported that the release of phenolic compounds bound to the cell matrix due to rapid drying and limited exposure to oxygen near the product during processing could maintain total antioxidant activity (Nadali et al., 2023). Nateghi and Kavian (2024) investigated the effect of different drying conditions on the total phenol content of seedless barberry juice. They showed that the application of low temperature (evaporator) and high temperature (microwave) along with increasing pressure leads to the destruction of phenols and reduces the total phenol content. Most likely, the reduction in environmental stresses, especially temperature, during the storage period has a direct effect on the content of total phenol in barberry fruits.

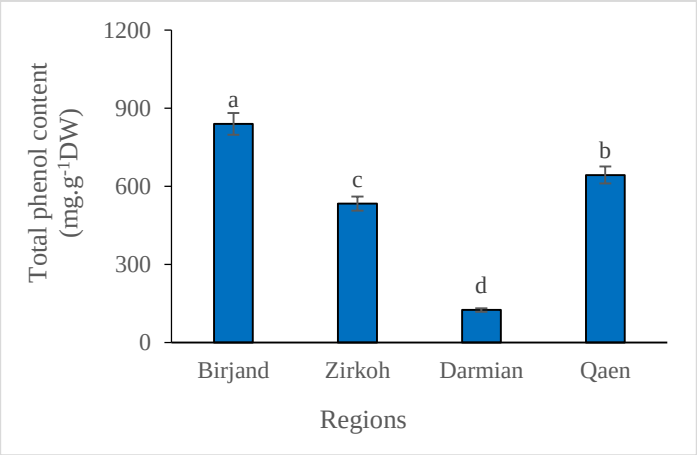

Fig. 1. Comparison of total phenol content of dried barberry fruit from different regions. of South Khorasan province, Iran. Error bars represent the error deviation. Symbols with the same letter are not significantly different between them, at P ≤ 0.05 (LSD test)

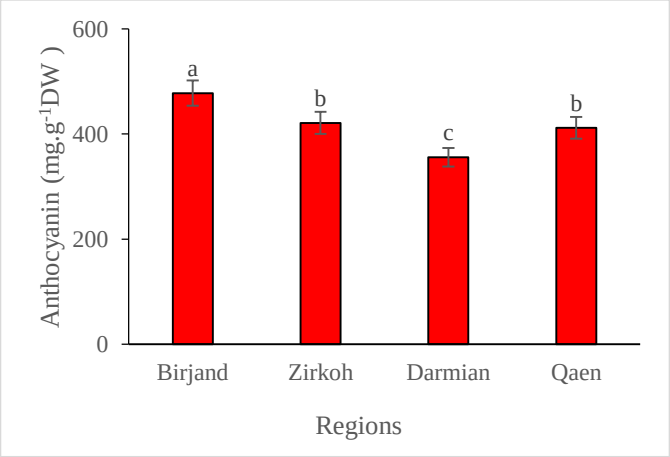

Fig. 2. Comparison of anthocyanin content of dried barberry fruit from different regions of South Khorasan province, Iran. Error bars represent the error deviation. Symbols with the same letter are not significantly different between them, at P ≤ 0.05 (LSD test).

According to the results presented in Figure 2, the anthocyanin contents of the barberries prepared from different regions were significantly different from each other. The highest content of anthocyanin was obtained from barberry fruits in the Birjand region (477.92 mg.m^{-1} DW), and the lowest amount was related to samples prepared from the Darmian region (355.81 mg.m^{-1} DW). Anthocyanins are vital indicators of the nutritional and commercial value of fruits. Anthocyanin biosynthesis is affected by various environmental factors (Zhao et al., 2023). Plants have developed an efficient system for anthocyanin production as a protective mechanism against environmental stressors (Bendokas et al., 2020). Two very important factors in the synthesis of anthocyanins are light and temperature. High temperature inhibits the synthesis of anthocyanins, and low temperatures cause the accumulation of these compounds (Gouot et al. 2019). In addition to its role in disrupting anthocyanin biosynthesis, high temperature causes anthocyanin degradation in fruit due to increased peroxidase activity (Movahed et al., 2016). The main anthocyanins in seedless barberries are delphinidin-3-glucoside (D-3-G) and pelargonidin-3-glucoside (P-3-G) (Berenji Ardestani et al., 2016). Laleh et al. (2006) evaluated the effect of temperature on the amount of anthocyanin in four varieties of barberry (*B. integerima*, *B. vulgaris*, *B. khorasanica* and *Orthobotrys*) at

temperatures of 5, 15, 25 and 35°C. The authors reported that the anthocyanin content decreased significantly with increasing storage temperature. In this regard, in the present study, it was shown that the barberry fruits prepared from the Darmian region had the lowest anthocyanin content, while the fruits prepared from the Birjand region had the greatest amount of anthocyanin. These results were probably due to the good ventilation and favorable storage conditions in the Birjand region, which decreased the storage temperature and caused the accumulation of anthocyanin in the seedless barberry fruit. As mentioned in the section on fruit color results in this experiment, anthocyanins are responsible for the red color of barberry fruits. In the present research, the results obtained from the fruit color indices and anthocyanin content was consistent with each other.

An examination of vitamin C in barberry fruit showed that the lowest level of vitamin C was related to fruits prepared from the Darmian region. Additionally, vitamin C levels in the Birjand region, Zirkoh region and Qaen region were not significantly different and were greater than those in the Darmian region. The most important vitamin in fruits and vegetables for human nutrition is vitamin C (Santos & Silva, 2008). Fruits and vegetables meet more than 90% of the body's need for vitamin C (Rasanu et al., 2005). Vitamin C is defined as the general term for all compounds that exhibit the biological activity of L-ascorbic acid (AA) (Lee & Kader, 2000). The vitamin C content in fruits and vegetables can be affected by various factors, such as preharvest weather conditions, maturity, harvesting methods and postharvest storage methods (Mditshwa et al., 2017). The greater the light intensity during the growing season is, the greater the vitamin C content in the plant tissue (Mditshwa et al., 2017). Temperature management after harvesting is the most important factor for preserving vitamin C in fruits and vegetables (Magwaza et al., 2017, Kumar et al., 2024). With increasing temperature and duration of storage, more vitamin C products are exposed to degradation. Vitamin C is a plant compound that is sensitive to high temperatures, and very low temperatures are also effective at increasing the loss of vitamin C in fruits (Wang et al., 2017). It should be noted that a significant amount of vitamin C in barberries is lost during the drying process. However, by keeping the product at the right temperature, the loss of vitamin C during the drying period can be reduced. In the postharvest phase, the amount of O_2 and CO_2 around the product increases with the breathing of the product, this leads to the oxidation and severe reduction of vitamin C (Mditshwa et al., 2017; Kader et al., 2000). Therefore, researchers have concluded that to store products for a long time, optimal ventilation and appropriate temperature are necessary to maintain the nutritional value of the products. In one study, the effect of different drying conditions on the level of vitamin C in kiwi fruits was investigated (Kaya et al., 2010). The test is performed using air temperatures of 35, 45, 55 and 65 °C, average velocities of 0.3, 0.6 and 0.9 m/s and relative humidity values of 40%, 55%, 70% and 85%. Their results showed that increasing the temperature of the drying air causes more loss of vitamin C in dried fruits, while increasing the relative humidity of the drying air decreases the degradation of vitamin C. The results obtained from the present research showed that the majority of vitamin C losses were related to barberries prepared from the Darmian region. Most likely, these results were obtained due to unfavorable storage conditions (including temperature, humidity and ventilation) in the Darmian region. Similar results were reported by other researchers (Talebzadeh et al., 2022; Santos and Silva, 2008; Mengyun et al., 2018).

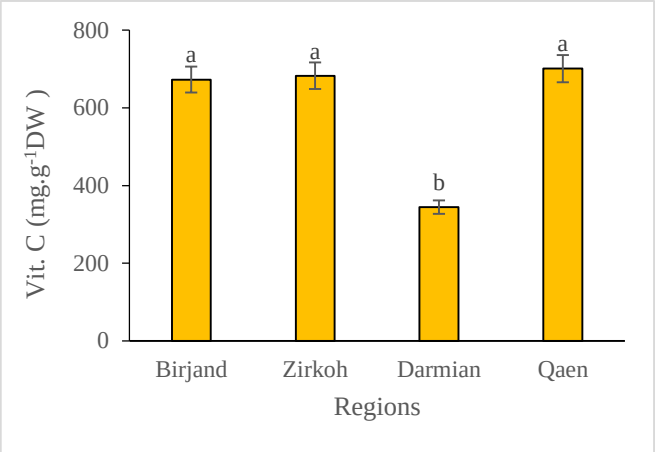

Fig. 3. Comparison of vitamin C of dried barberry fruit from different regions of South Khorasan province, Iran. Error bars represent the error deviation. Symbols with the same letter are not significantly different between them, at $P \leq 0.05$ (LSD test).

Correlation coefficients of evaluated traits

Significant strong correlations found among investigated variables. The results showed positive significant correlations between anthocyanin and TSS (r = 0.82), anthocyanin and phenol (r =0.95), anthocyanin and vitamin C (r =0.77), anthocyanin and an index (r =0.81), and anthocyanin and chroma (r =0.81). Also negative significant correlations between L index and anthocyanin (r = -0.85), and L index and phenol (r = -0.82) were observed.

Previous studies on barberry fruits also have been reported significant correlations. Total antioxidant activity correlates significantly with total flavonoids, soluble solid content, pH, and brightness (L value) in barberry fruit genotypes from Sivasli, Usak, Turkey (Okatan & Çolak, 2019). Further, barberry fruits from CKNP region of Pakistan show correlations between pH, TSS, acidity, sugars, proteins, antioxidants, and phytochemicals like carotenoids, flavonoids, phenolics, and anthocyanins (Awan et al., 2014). However, barberry yield negatively correlated with fruit length, titratable acidity, anthocyanin, DPPH, and phenol (Tavakoli-Kaghaz et al., 2023).

CONCLUSION

The results of this experiment show that fruits from different regions have different tastes and food qualities. The highest TSS and the highest taste index (TSS/TA) were obtained for the barberries from the Birjand region, which indicates that the barberries from this region have better and sweeter tastes than those from other regions. The examination of color indices (*L**, *a**, *b**, Hue and Chroma) showed that the lowest color quality was related to dry barberry in the Darmian region. Additionally, the highest amount of total phenol and anthocyanin was obtained from the fruits of the Birjand region. However, the lowest amount of phenol and the lowest amount of anthocyanin and vitamin C were detected in the Darmian region, and in general, it can be concluded that the dry, seedless barberry fruits of the Birjand region have greater quality and nutritional value than those of other regions. It seems that the drying and storage conditions of barberry fruit have a direct relationship with the nutritional value of this product. Therefore, according to the results obtained and the comparisons made between different regions of barberry cultivation, in the Darmian region, the type of fruit picking and the conditions of drying and keeping the fruit need more supervision and control. However, in

the Birjand region, Zirkoh region and Qaen region, the conditions for drying and storing dry barberries are optimal.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Evaluation the phenotypic diversity of some grapevine cultivars and genotypes based on morphological, phenological, biochemical and fruit characteristics (Case study: Khuzestan province, Iran)

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ABSTRACT

Purpose: This study aimed to gain knowledge about the genetic reserves of native Iranian grapevine (*Vitis vinifera* L.) cultivars and genotypes in tropical regions and to identify the best grapevine cultivars and genotypes existing in vineyards of Khuzestan province. **Research Method:** This study evaluated the phenotypic diversity of 60 grapevine cultivars and genotypes existing in tropical, subtropical region of Khuzestan province in Iran. **Findings:** The result showed that the most descriptive statistics in the most important quantitative traits are related to fresh weight of bunch, bunch length, bunch width, the number of berries per bunch, berry length, berry width, protein content, total soluble solids and titratanbe acidity. The native Iranian grape cultivars and genotypes of Khuzestan province included 'Bangi', 'Soltani' and 'Yershi' as the earliest, Iranian cultivars including 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' as mid-ripening and foreign cultivars including 'Flame Seedless' and 'Perlette' as late ripening respectively. The results of factor analysis showed that the highest coefficients of eigenvectors in 7 main components are related to the most important traits including fresh weight of bunch, fresh weight of berries, berry diameter, berry length, the number of berries per bunch, protein content, total soluble solids, titratable acidity and the content of chlorophyll which accounted for 84.28% of the total variance variation. To group cultivars and genotypes based on investigated traits from cluster analysis by Ward's method was used. Cultivars and genotypes were grouped in 9 main clusters in 5 Euclidean distances. **Research limitations:** No limitations were encountered. **Originality/Value:** In this research, the significant diversity of grapevine cultivars and genotypes existing in vineyards of Khuzestan province showed the superiority of native cultivars and genotypes such as 'Soltani', 'Bangi (Ghermez)' and 'Yershi' in some traits compared to other foreign cultivars.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) has been known for a long time and has been used in various ways throughout the centuries. *Vinifera* cultivars have the best product quality and are cultivated and consumed in most temperate regions (Salimov et al., 2017). Grapes, as a prominent horticultural product, can be cultivated in different parts of the world and are among the most profitable fruit products worldwide. They are mainly consumed fresh or used to produce raisins and processed products (Kupe et al., 2021).

There are numerous theories about the evolution of the European grapevine (*Vitis vinifera* L.) (Jahnke et al., 2021). According to De-Candolle (1985), grapevines originated outside the Caucasus and Middle East regions of Asia. According to this classification, the European grapevine belongs to the Central Asian center of diversity, along with pistachios and almonds. The genus *Vitis* is believed to have more than 100 species. This genus is distributed in 10 regions of the world, all located in the northern hemisphere, including five regions in North America, where 29 species have been described; four regions in Asia, with at least 11 species; and only *Vitis vinifera* in a wide range including the Mediterranean and sub-Mediterranean and Caucasian floristic regions, extending to the Pontic, Caspian and Central Asian regions (Sargolzaei et al., 2021).

Grapevines are rare fruits, cultivated from tropical to temperate regions due to their great diversity. The common grapevine is widely distributed in temperate and subtropical regions of the world. It is estimated that 10,000 known grapevine cultivars are distributed in the world's wine-growing regions, and about 13 cultivars dominate global production, covering more than one-third of the world's vineyard area (FAO, 2020; Lacombe et al., 2013; Imazio et al., 2013).

Breeding programs to produce new cultivars for predicted future environmental conditions may be one of the most promising solutions to stabilize production, although this strategy is part of long-term solutions. Choosing the right cultivar reduces the inputs required for crop management and increases the sustainability of production (Santos et al., 2020).

Due to the interest of grape growers in developing new vineyards, it is critical for them to accurately identify and ensure the accuracy of the grapevine cultivar. Mistakes in grapevine cultivar identification can result in significant financial costs, not only for the growers, but also for the grape industry (Mena et al., 2014). For many years, the traditional identification of grapevine cultivars has been done by visual inspection of grapevines, known as amplography. The results of using amplography to identify grapevine cultivars are not accurate, and there may be changes in the definition of the descriptor due to environmental conditions and genetic variations (Razi et al., 2021). For example, the same grapevine cultivar in different environments shows variation in the size, shape, and color of berries and bunch. Amplography descriptions vary slightly depending on the health status of the grapevine cultivars and the interpretation of the observer (Antolin et al., 2020).

To determine grapevine traits, several multivariate statistical analyses such as principal components analysis and cluster analysis are used for quantitative and qualitative analysis. These techniques describe the morphological characteristics of horticultural crops (Zahedi et al., 2023). The berry shape index is used to differentiate grapevine cultivars, but fruit shape is a three-dimensional trait and should be defined using pleiotropic explanatory variables instead of a simple single index (Maeda et al., 2018).

Local grapevine cultivars are mainly grown in old vineyards located in old settlements and vineyards. These cultivars differ in terms of morphological traits and berry and grape size. They also differ in terms of phenology, harvest time, productivity and quality indicators. Local grapevine cultivars are essential for maintaining crop diversity and can also be important for the nutritional and economic security of many people. For smallholder farmers and farming

communities in rural and marginal areas, the diversity of local grapevines can be a guarantee against low yields and provide specific raw materials for the preparation of traditional local foods. In each country where grapevines are grown, many local species contribute to the global diversity of grapevines (Antolin et al., 2020).

Diverse grapevine cultivars are important sources of locally adapted genes for breeding new grapevine cultivars. Significant clonal diversity can be observed in a grapevine cultivar. Therefore, the definition and identification of cultivars are of considerable scientific and practical importance in modern viticulture and amplography (Sargolzaei et al., 2021).

The important point is the lack of studies to identify local grapevine cultivars and genotypes in tropical and subtropical regions of Iran, especially in vineyards of Khuzestan province, the center of agriculture and horticulture. Khuzestan has an area of 64055 km^2 and a population of 4.7 million. It is located in the southwestern region of Iran and has a hot, arid and semi-arid climate; and with a suitable climate for growing grapevines, has a cultivated area of about 2164 ha (both fertile and non-fertile) with an average yield per unit area of 12662 $kg.ha^{-1}$ and a total production of 17245 tons (FAO, 2020).

This study aimed to gain knowledge about the genetic reserves of native Iranian cultivars and genotypes in tropical regions and to identify the best grapevine cultivars and genotypes existing in vineyards of Khuzestan province. Considering the different and unique climatic conditions of Khuzestan province, by studying the phenological and morphological traits and evaluating the diversity of cultivars and genotypes, it is possible to identify the important traits affecting grapevine yield and use them in breeding programs to identify important traits affecting the differentiation of cultivars and genotypes.

MATERIALS AND METHODS

The environmental and climatic characteristics of region

The Khuzestan province with an area of nearly 64000 km^2 in the southwestern region of Iran has an altitude lower than the level of the open seas to about 3400 m above the level of the open seas and between the longitude of 47º and 41′ to 50º and 39′ and the latitude of 29º and 58′ to 33º and 4′ (Fig. 1). The Khuzestan province has three regions in terms of elevation and altitude: mountains, foothills, and plains, of which about 25% is mountainous, about 20% is foothills and about 60% is plains (Ministry of Agricultural Jahad of Iran, 2021; Agricultural Jahad Organization of Khuzestan Province, 2021).

The Khuzestan province has different weather conditions from hot and dry to semi-arid, which during the years 1990 to 2020 (with 30-year average) has an average annual rainfall of 284.3 mm, an average annual relative humidity of 3.3 42%, the average minimum and maximum daily temperature ranges from 13.9°C to 33.9°C and the average minimum and maximum annual temperature ranges from -4.8°C to 53.7°C (Meteorological Organization of Khuzestan province, 2021).

Fig. 1. Location of Khuzestan Province in the southwest of Iran (Safieddin-Ardebili & Khademalrasoul, 2022).

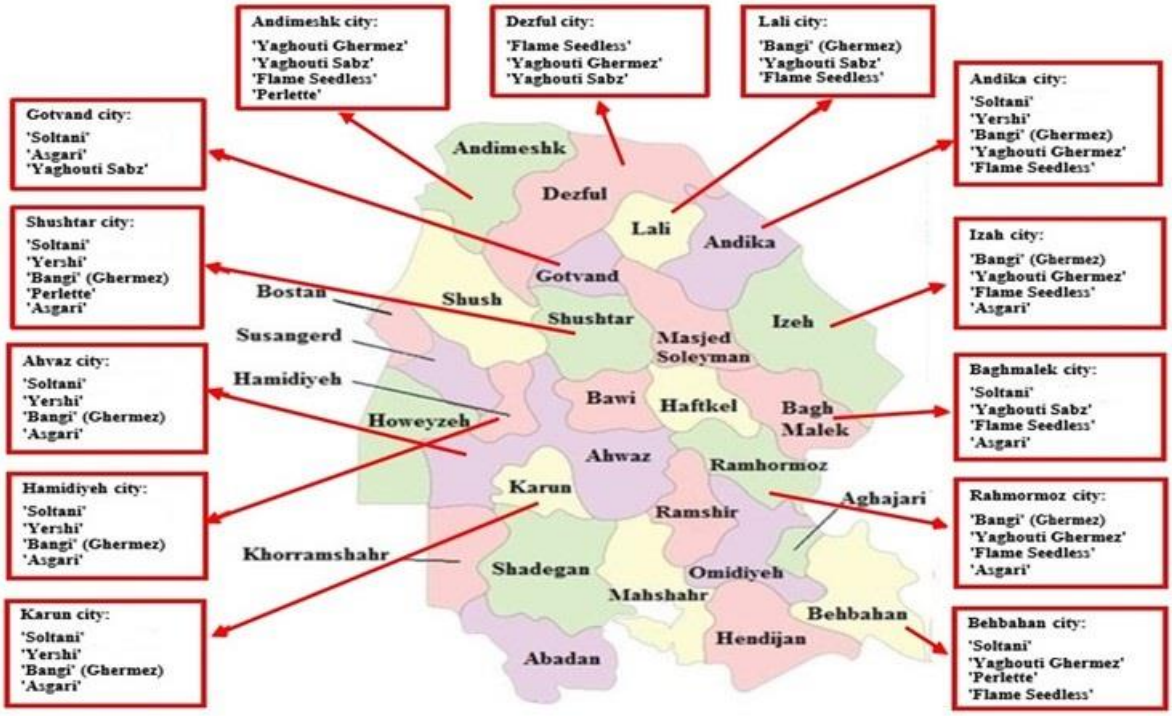

Fig. 2. Geographical location of cultivars or genotypes sampled in vineyards by each selected city in Khuzestan province of Iran (13 selected regions).

Plant Materials

In order to evaluate the phenotypic diversity based on the morphological and phenological characteristics of some cultivars and genotypes of grapevine (*Vitis vinifera* L.) existing in the vineyards of Khuzestan province, firstly based on the geographical location including the type of region based on the plain, foothills and mountains and also the climatic and ecological conditions of the place, including the height above sea level, temperature, humidity, rainfall and other indicators, the location of the vineyards were identified based on the priorities mentioned above. After determining the location of the vineyards and by identifying the existing cultivars and genotypes, the types of cultivars and genotypes were determined based on whether they are native or foreign, and they were identified for testing and sampling. The phenotypic diversity of 60 grape varieties, including cultivars, genotypes and clones of native, local and foreign varieties, including 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Sabz Dorosht', 'Mocheh', 'Roghani', 'Nameless' 'Asgari', 'Yaghouti Ghermez', 'Yaghouti Sabz', 'Flame Seedless' and 'Perlette' and etc. In the vineyards of Khuzestan province, which were evaluated based on 105 morphological, phenological, fruit and biochemical traits in 3 years from 2019 to 2022 (Table 1 and Fig. 2).

Table 1. The names of grape cultivars and genotypes of vineyards in Khuzestan province to study phenotypic diversity.

No.	Cultivar/Genotype	Origin	Place of sampling		No.	Cultivar/Genotype	Origin	Place of sampling	
			Region name	Altitude above sea level (m)				Region name	Altitude above sea level (m)
1	'Soltani'	Iran	Ahvaz	16	31	'Yaghouti Sabz'	Iran	Andimeshk	146
2	'Soltani'	Iran	Karun	12	32	'Yaghouti Sabz'	Iran	Dezful	143
3	'Soltani'	Iran	Hamidiyeh	15	33	'Yaghouti Sabz'	Iran	Lali	493
4	'Soltani'	Iran	Shushtar	65	34	'Yaghouti Sabz'	Iran	Gotvand	599
5	'Soltani'	Iran	Andika	339	35	'Yaghouti Sabz'	Iran	Baghmalek	917
6	'Soltani'	Iran	Baghmalek	917	36	'Yaghouti Sabz'	Iran	Ramhormoz	179
7	'Bangi' (Ghermez)	Iran	Ahvaz	16	37	'Flame Seedless'	USA	Andimeshk	146
8	'Bangi' (Ghermez)	Iran	Karun	12	38	'Flame Seedless'	USA	Dezful	143
9	'Bangi' (Ghermez)	Iran	Hamidiyeh	15	39	'Flame Seedless'	USA	Baghmalek	917
10	'Bangi' (Ghermez)	Iran	Shushtar	65	40	'Flame Seedless'	USA	Behbahan	325
11	'Bangi' (Ghermez)	Iran	Andika	339	41	'Flame Seedless'	USA	Izeh	835
12	'Bangi' (Ghermez)	Iran	Ramhormoz	179	42	'Flame Seedless'	USA	Andika	339
13	'Yershi'	Iran	Ahvaz	16	43	'Perlette'	USA	Andimeshk	146
14	'Yershi'	Iran	Karun	12	44	'Perlette'	USA	Dezful	143
15	'Yershi'	Iran	Hamidiyeh	15	45	'Perlette'	USA	Shushtar	65
16	'Yershi'	Iran	Shushtar	65	46	'Perlette'	USA	Ramhormoz	179
17	'Yershi'	Iran	Andika	339	47	'Perlette'	USA	Behbahan	325
18	'Yershi'	Iran	Behbahan	325	48	'Perlette'	USA	Lali	493
19	'Asgari'	Iran	Ahvaz	16	49	'Roghani'	Iran	Ahvaz	16
20	'Asgari'	Iran	Karun	12	50	'Roghani'	Iran	Karun	12
21	'Asgari'	Iran	Hamidiyeh	15	51	'Roghani'	Iran	Hamidiyeh	15
22	'Asgari'	Iran	Shushtar	65	52	'Mocheh'	Iran	Ahvaz	16
23	'Asgari'	Iran	Ramhormoz	179	53	'Mocheh'	Iran	Karun	12
24	'Asgari'	Iran	Gotvand	599	54	'Mocheh'	Iran	Hamidiyeh	15
25	'Yaghouti Ghermez'	Iran	Izeh	835	55	'Nameless'	Iran	Ahvaz	16
26	'Yaghouti Ghermez'	Iran	Andika	339	56	'Nameless'	Iran	Karun	12
27	'Yaghouti Ghermez'	Iran	Andimeshk	146	57	'Nameless'	Iran	Hamidiyeh	15
28	'Yaghouti Ghermez'	Iran	Lali	493	58	'Sabz Dorosht'	Iran	Dezful	143
29	'Yaghouti Ghermez'	Iran	Behbahan	325	59	'Sabz Dorosht'	Iran	Dezful	143
30	'Yaghouti Ghermez'	Iran	Dezful	143	60	'Sabz Dorosht'	Iran	Dezful	143

Measurement of traits

To determine the phenological traits, coding was performed based on OIV (2020), IPGRI (2008) and UPOV (2008) grapevine descriptors (Table 2). Quantitative and qualitative differentiating and diversifying traits were identified in the cultivar and genotype study, and the traits were evaluated and compared among cultivars and genotypes.

Some of the important qualitative traits are berry color, the depth of the cut of leaf blade, the number of leaf lobes, leaf width folding, color of upper and lower leaf surface, and shape of young shoot terminal. The quantitative traits are divided into two categories; the first category includes some traits that can be measured in the vineyard, such as growth size of shoot, the length and width of leaf, plant height, the number of bunches per plant, the number of bunch shoulders per bunch, the number of berries per bunch shoulders (Rasouli et al., 2013; Khadivi-Khub et al., 2014). The second category includes some traits such as leaf area, specific leaf area (Koundouras et al., 2008), fresh and dry weight of bunch, fresh and dry weight of shoot, fresh and dry weight of leaf, titratanbe acidity (Amerine & Ough, 1980) and sugar content (Khochert, 1987) that can be measured in the laboratory. The quantitative measuring of total soluble solids was done using a MASTER-3M manual analog optical refractometer, ATAGO CO, made in Japan (Khochert, 1987). The protein content was determined according to the method of Bradford (1976). Using bovine serum albumin as the standard. To measure the amount of cis and trans-resveratrol according to the method of Cvejic et al. (2010), Sykam HPLC device made in Germany equipped with Sykam S3210 UV/V is detector was used. Based on the inhibition time and using a spectrophotometer, the amount of cis and Trans resveratrol in the sample of different parts of the plant was measured at the wavelength of 280 and 306 nm and expressed as micromoles pergram of fresh weight.

Table 2. Some evaluated characteristics and their measurement in the investigated grape samples based on the grape descriptor of OIV (2020), IPGRI (2008) and UPOV (2008).

No.	Traits	Unit	Measurement method
1	Flowering time	score	1= too early, 2= very early, 3= early, 4= early to medium, 5= medium, 6= medium late, 7= late, 8= very late, 9= too late
2	Leafing time	score	1= early, 3= medium, 5= late
3	Bush growth	score	3= weak, 5= moderate, 7= strong
4	Shape of the tip of young shoot	score	1= closed, 3= slightly open, 5= half open, 7= wide open, 9= fully open
5	Strength of shoot	score	1= 0-60 cm, 3= 60-90 cm, 5= more than 90 cm
6	Leaf width	score	1= less than 100 square centimeters, 3= 100 to 125 square centimeters, 5= 125 to 150 square centimeters, 7= 150 to 175 square centimeters, 9= more than 175 square centimeters
7	The number of leaf lobes	score	1= no lobes, 2= three lobes, 3= five lobes, 4= seven lobes, 5= six of seven lobes
8	Color of the upper surface of leaf	score	1= very light green, 3= light green, 5= medium green, 7= dark green, 9= very dark green
9	The cutting depth of the leaf	score	1= less than 4 mm, 3= 4 to 8 mm, 5= 8 to 12 mm, 7= 12 to 16 mm, 9= more than 16 mm
10	Bunch size	score	3= small, 5= medium, 7= large, 9= very large
11	Bunch density per plant	score	3= open, 5= medium, 7= tight, 9= very tight
12	Bunch shoulder density per bunch	score	3= open, 5= medium, 7= compact
13	Ripening time of fruit	score	41= very early, 3= early, 5= medium, 7= late, 9= very late
14	Berry density per bunch	score	3= low, 5= medium, 7= high
15	Peduncle separation	score	1= difficult, 2= fairly easy, 3= very easy
16	Anthocyanin color of pedicel	score	0= no color, 1= very weak, 3= weak, 5= medium, 7= strong, 9= very strong

Table 2 (*Continued*). Some evaluated characteristics and their measurement in the investigated grape samples based on the grape descriptor of OIV (2020), IPGRI and UPOV (2008).

No.	Traits	Unit	Measurement method
17	Anthocyanin color of berry mesocarp	score	1= none or very little, 3= little, 5= moderate, 7= high, 9= very high
18	Thickness of berry skin	score	3= thin, 5= medium, 7= thick
19	Juiciness of berry	score	1= little water, 2= slightly watery, 3= very watery
20	Berry color	score	1= green-yellow, 2= light red, 3= dark red, 4= gray, 5= purple, 6= navy blue
21	Berry firmness	score	1= soft, 2= slightly hard, 3= hard
22	Berry shape	score	1= rectangular, 2= oval, 3= broad oval, 4= round, 5= flat, 6= ovoid, 7= open ovoid, 8= ovoid, 9= conical
23	Berry size	score	1= very small, 3= small, 5= medium, 7= large, 9= very large
24	Seed presence	score	1= none, 2= incomplete growth, 3= complete growth
25	Growth size of Shoot	cm	Digital Caliper
26	Tendril length	cm	Digital Caliper
27	Length of internode	mm	Digital Caliper
28	Fresh weight of shoot	g	Digital Scale
29	Dry weight of shoot	g	Digital Scale
30	Fresh weight of leaf	g	Digital Scale
31	Dry weight of leaf	g	Digital Scale
32	Leaf Area	cm^2	Digital Caliper
33	The cutting depth of leaf	mm	Digital Caliper
34	The ratio of leaf dry weight to leaf area	$mg.cm^{-2}$	Calculate ratio
35	Specific leaf area (SLA)	$cm^2.mg^{-1}$	Calculate Leaf Area/Occupied Area Ratio
36	The rachis length	mm	Digital Caliper
37	Bunch length	mm	Digital Caliper
38	Bunch width	mm	Digital Caliper
39	The ratio of length to width of the bunch	ratio	Digital Caliper
40	The peduncle length	mm	Digital Caliper
41	The length of bunch shoulder	mm	Digital Caliper
42	The width of bunch shoulder	mm	Digital Caliper
43	The ratio of length to width of bunch shoulder	ratio	Calculate ratio
44	The ratio of rachis length of bunch to rachis length of bunch shoulder	ratio	Calculate ratio
45	Berry length	mm	Digital Caliper
46	Berry width	mm	Digital Caliper
47	The ratio of length to width of berry	ratio	Calculate ratio
48	Berry Diameter	mm	Digital Caliper
49	The number of berries per bunch	number	Count
50	The number of berries per bunch shoulder	number	Count
51	The number of bunch shoulder per bunch	number	Count
52	Fresh weight of the bunch	g	Digital Scale
53	Fresh weight of bunch shoulder	g	Digital Scale
54	Fresh weight of berries	g	Digital Scale
55	Fresh weight of berries per bunch	g	Digital Scale
56	Fresh weight of berries per bunch shoulder	g	Digital Scale
57	Total pedicels of fresh weight of berries per bunch	g	Digital Scale
58	Dry weight of bunch	g	Digital Scale
59	Dry weight of bunch shoulder	g	Digital Scale
60	Dry weight of berry	mg	Digital Scale
61	Dry weight of berries per bunch	g	Digital Scale
62	Dry weight of bunch shoulders per bunch	g	Digital Scale

Table 2 (*Continued*). Some evaluated characteristics and their measurement in the investigated grape samples based on the grape descriptor of OIV (2020), IPGRI and UPOV (2008).

No.	Traits	Unit	Measurement method
63	Fresh weight of rachis	mg	Digital Scale
64	Fresh weight of peduncle	mg	Digital Scale
65	The pedicel of fresh weight of berries	mg	Digital Scale
66	Fresh weight of rachis of the total bunches	g	Digital Scale
67	The ratio of fresh weight of rachis of bunch shoulder to fresh weight of rachis bunch	ratio	Calculate ratio
68	Dry weight of rachis	g	Digital Caliper
69	Dry weight of peduncle	g	Digital Scale
70	Dry weight pedicel of berry	mg	Digital Scale
71	The total pedicels of dry weight of berries per bunch	g	Digital Scale
72	The ratio of dry weight of rachis of bunch shoulder to dry weight of rachis bunch	g	Digital Scale
73	The peduncle length of the bunch	ratio	Calculate ratio
74	The pedicel length of bunch	mm	Digital Caliper
75	Fresh weight of peduncle	mm	Digital Caliper
76	The pedicel length of berry	mm	Digital Caliper
77	Seed weight	mg	Digital Scale
78	Seed diameter	mm	Digital Caliper
79	Seed length	mm	Digital Caliper
80	Titratanbe acidity	mEq	$^{(1*)}$A= S.N.F.E/C× 100
81	Total soluble solids (TSS)	$mg.g^{-1}$ F.W.	Khochert (1987)
82	Protein content	$mg.g^{-1}$ F.W.	Bradford method (1976)
83	Content of trans-resveratrol in tendril	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
84	Content of trans-resveratrol in petiole	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
85	Content of trans-resveratrol in leaf	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
86	Content of trans-resveratrol in pedicel	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
87	Content of trans-resveratrol in the skin of unripe berries	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
88	Content of trans-resveratrol in mesocarp of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
89	Content of trans-resveratrol in skin of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
90	Content of trans-resveratrol in mesocarp of ripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
91	Content of trans-resveratrol in seeds of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
92	Content of trans-resveratrol in seeds of ripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
93	Content of cis-resveratrol in the tendril	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
94	Content of cis-resveratrol in petiole	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
95	Content of the cis-resveratrol content in leaf	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
96	Content of cis-resveratrol in pedicel	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
97	Content of cis-resveratrol in the skin of unripe berries	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
98	Content of cis-resveratrol in mesocarp of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
99	Content of cis-resveratrol in the skin of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
100	Content of cis-resveratrol in the mesocarp of ripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
101	Content of cis-resveratrol in seeds of unripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
102	Content of cis-resveratrol in seeds of ripe berry	$mg.L^{-1}$	$^{(2*)}E_{1cm}^{1\%}=(A\times100/M\times100)\times(100/100-H)$
103	Total chlorophyll content	$mg.g^{-1}$ F.W.	Lichtenthaler & Buschmann method (2001)
104	The content of chlorophyll a	$mg.g^{-1}$ F.W.	Lichtenthaler & Buschmann method (2001)
105	The content of chlorophyll b	$mg.g^{-1}$ F.W.	Lichtenthaler & Buschmann method (2001)

$^{(1*)}$ A= The amount of fruit acid (gr/100ml), S= the amount of NaOH consumed, N = NaOH normalization, F = invoice naoh, C = The amount of fruit extract, E = Ekiwalan acid included.
$^{(2*)}$ A= absorption rate, H = moisture content, M = sample mass in grams. measured by Spectrophotometer.

Statistical analysis

Frequency of traits, descriptive statistics, the simple correlation between the traits and cluster analysis were performed in SPSS (Version 21.0). The correlation between traits was calculated using Pearson's correlation analysis. Through the factor rotation technique and the maximum variance method (Varimax Method), the separation of factors was carried out, and factor coefficients of 0.5 and above were considered significant in each main and independent factor. Cluster analysis and grouping of cultivars and genotypes were performed using Ward's or the minimum variance method, based on the square of the Euclidean distance and calculating the distances after standardizing the data (Rasouli et al., 2013; Zahedi et al., 2023).

RESULTS

Descriptive statistics of traits

In examining quantitative and qualitative traits measured in the studied cultivars and genotypes of grapevines in vineyards of Khuzestan province, the parameters of minimum, maximum, mean, standard deviation and coefficient of variation (CV%) are presented in Table 3. The results showed that in evaluated quantitative traits such as growth size of shoot (73.80-104.71 cm), the length of internode (68.65-99.56 mm), leaf area (53.13-153.76 cm^2) and specific leaf area (135.45-344.28 $cm^2.g^{-1}$), the rachis length (112.17-313.28 mm), the bunch length (95.98-279.68 mm), the bunch width (61.69-157.03 mm), the peduncle length (37.59-146.09 mm), the length of bunch shoulder (28.84-113.52 mm), the width of bunch shoulder (22.82-64.06 mm), berry length (11.03-30.89 mm), the number of berries per bunch (73.90-688.83 berries), fresh weight of bunch (165.172-1062.57 g), fresh weight of bunch shoulder (15.08-91.74 g), dry weight of bunch (17.76-217.73 g), dry weight of berry (44.36-397.38 mg) had the largest range of difference between minimum and maximum.

The measurement of the diversity of the most important vegetative characteristics between grape cultivars and genotypes in the vineyards of Khuzestan province showed that the native cultivar or genotype 'Soltani'in the vineyards of Khuzestan province in terms of shoot growth size with 100.5 cm and specific leaf area with 339.45 $cm^2.g^{-1}$ and the native cultivar or genotype had the highest value, and the leaf area with 151.94 cm in the native cultivar or genotype 'Sabz Dorosht' had the highest amount.

In the vineyards of Khuzestan province, the highest bunch length with 311.78 mm, bunch width with 154.65 mm, the length of bunch shoulder with 105.77 mm, the width of bunch shoulder with 58.86 mm, the number of berries per bunch with 633.55 berries, fresh weight of bunch with 958.77 g, it is related to the native cultivar or genotype of 'Soltani'.

The highest fresh weight of bunch shoulder with 88.33 g was in the native cultivar or genotype 'Sabz Dorosht' in the vineyards of Khuzestan province. The native cultivar or genotype 'Yershi' in the vineyards of Khuzestan province had the largest berry length with 9.89 mm. 'Yaghouti Ghermez' cultivar had the highest dry weight of bunch with 212.73 g and dry weight of berries with 390.45 mg.

The native cultivar or genotype 'Mocheh' had the highest shoot growth with 65.40 cm, the 'Flame Seedless' cultivar with 45.30 cm^2 had the highest leaf area and the 'Perlette' cultivar with 339.45 $cm^2.mg^{-1}$ had the highest specific leaf area. The smallest bunch length with 102.66 mm, bunch width with 50.5 mm, length of bunch shoulder with 31.47 mm, width of bunch shoulder with 17.94 mm, berry length with 8.88 mm, the number of berries per bunch with 65.10 berries, fresh weight with of bunch 197.44 cm, fresh weight of bunch shoulder 11.20 g, dry weight of bunch with 12.69 g and the dry weight of berry with 374.39 mg was related to the native cultivar or genotype 'Mocheh' in the Khuzestan province conditions.

The highest percentage of variation coefficient (CV%) in examining qualitative morphological and phenological traits such as flowering time (21.29%), leafing time (58.05%), leaf width (71.88%), bunch size (39.96%), bunch density per plant (28.36%), berry density per bunch (29.41%), berry size (31.87%), the presence of seeds (50.42%), berry shape (39.13%), juiciness of berry (44.09%), berry color (49.67%) and anthocyanin color of the berry mesocarp (85.04%) which has a high phenotypic diversity among the cultivars and genotypes of grapes available in the vineyards of Khuzestan province.

Also, the bunch length (22.46%), bunch width (28.18%), berry length (26.75%), berry width (27.15%), the number of berries per bunch (78.44%), fresh weight of bunch (50.51%), fresh weight of bunch shoulder (46.74%), dry weight of bunch (60.07%), fresh weight of berry (50.52%), protein content (37.20%) had the highest percentage of coefficient of variation (CV.) in genetic diversity.

The highest coefficient of variation (CV.) in content of trans-resveratrol in different plant organs including tendril (60.38%), petiole (55.97%), ripe berry skin (60.13%) and also the content of cis-resveratrol in different plant organs including tendril (63.21%), petiole (39.69%), leaf (52.65 %), pedicel (65.19%) unripe berry skin (60.80%) had the highest percentage of coefficient of variation.

According to Table 1, out of the 60 clones of the studied, only the native 'Yershi' cultivar and genotype in the vineyards of Khuzestan province (including 6 clones) had complete seeds and 54 clones had no seeds, and the reason for the high coefficient of variation of trans and cis-resveratrol content in unripe and ripe berry seeds is that only the 'Yershi' cultivar or genotype had developed seeds. While in the studied clone, other native cultivars or genotypes and foreign cultivars did not have seeds.

Frequency distribution of traits

The frequency percentages of some of the most important qualitative traits examined in the cultivars and genotypes studied in parts A to J is presented in Figure 3. Various traits related to morphological characteristics, including flowering time, leafing time, leaf width width, the cutting depth of leaf, bunch size, berry shape, berry color, berry size, berry density per bunch, and late or early fruit showed a relatively high diversity among the studied cultivars and genotypes.

Native grape cultivars and genotypes in Khuzestan province such as 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Roghani' and 'Mocheh', due to suitable climatic and ecological conditions in terms of sufficient water, light, temperature, humidity, etc., have higher growth rate and efficiency. They have earlier flowering (March) compared to other Iranian grape cultivars 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' (March-April) and foreign grape cultivars 'Flame Seedless' and 'Perlette' (April) available in vineyards of Khuzestan province. Other studied Iranian grape cultivars, such as 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' had earlier flowering compared to foreign cultivars such as 'Flame Seedless' and 'Perlette' (Fig. 3a).

Table 3. Descriptive statistics of quantitative traits (phenological, morphological and pomological traits) in grape cultivars and genotypes studied in Khuzestan province.

No.	Traits	Unit	Range	Min.	Max.	Average	Std.	CV.%
1	Flowering time	score	2	3	5	3.70	0.79	21.29
2	Leafing time	score	4	1	5	2.60	1.51	58.05
3	Bush shape	score	3	1	4	2.25	1.10	48.84
4	Shape of the tip of the young shoot	score	4	5	9	6.20	1.34	21.58
5	Strength of the shoot	score	2	5	7	6.3	0.96	15.27
6	Leaf width	score	6	1	7	2.73	1.96	71.88
7	The number of leaf lobes	score	2	5	7	5.10	0.44	8.62
8	Color of the upper surface of the leaf	score	2	3	5	4.30	0.96	22.37
9	The cutting depth of the leaf width	score	6	1	7	4.70	1.72	36.60
10	Bunch size	score	6	3	9	5.50	2.20	39.96
11	Bunch density per plant	score	4	5	9	6.40	1.82	28.36
12	Bunch shoulder density per bunch	score	4	3	7	5.60	1.29	23.06
13	Fruit ripening time	score	4	3	7	4.80	1.41	29.41
14	Berry density per bunch	score	4	3	7	4.80	1.41	29.41
15	Peduncle separation	score	4	3	7	4.40	1.58	35.80
16	Anthocyanin color of pedicel	score	6	3	9	4.90	1.62	33.12
17	Anthocyanin color of berry mesocarp	score	6	1	7	3.00	2.55	85.04
18	Thickness of berry skin	score	4	3	7	5.40	1.51	27.95
19	Juiciness of berry	score	2	1	3	1.90	0.84	44.09
20	Berry color	score	2	1	3	1.30	0.65	49.67
21	Berry firmness	score	1	2	3	2.40	0.49	20.58
22	Berry shape	score	4	2	6	3.90	1.85	39.13
23	Berry size	score	6	3	9	5.80	0.61	31.87
24	Seed presence	score	2	1	3	1.20	0.51	50.42

Also, more benefit and better efficiency from the climatic and ecological conditions in Khuzestan province due to the native Iranian grape cultivars and genotypes, including Soltani', 'Bangi' (Ghermez), 'Yershi', 'Roghani' and 'Mocheh' in earlier leafing (February) compared to other Iranian grape cultivars including 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' (February-March) and foreign cultivars 'Flame Seedless' and 'Perlette' (March-April) are available in the vineyards of Khuzestan province (Fig. 3b).

The climatic and ecological conditions in Khuzestan province, in terms of the presence of sufficient water, light, temperature, humidity, etc., will cause favorable vegetative growth of the plant, especially in the leaves, which will cause a favorable and rapid increase in the leaf surface. In native Iranian grape cultivars and genotypes of grapes in vineyards of Khuzestan province, 'Soltani', 'Bangi' (Ghermez) and 'Yershi' had more leaf area than foreign cultivars ('Flame Seedless' and 'Perlette'), but compared to other Iranian grape cultivars ('Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari') studied, the leaf area was slightly more and the difference didn't show much. The native Iranian grape cultivars and genotypes of 'Roghani' and 'Mocheh' grapes available in vineyards of Khuzestan province had the lowest amount of leaf area compared to other cultivars (Fig. 3c).

The results showed that the native Iranian grape cultivars or genotypes of Khuzestan province 'Soltani', 'Bangi' (Ghermez) and 'Yershi' had more and deeper incisions in the leaves compared to the other Iranian and foreign cultivars. The reason for these differences in the leaves of native plants is probably some kind of adaptation to the hot weather conditions of the region (Fig. 3d).

The native cultivars and genotypes of grapes in the vineyards of Khuzestan province, including 'Soltani' and 'Yershi' and Iranian grape cultivars 'Yaghouti Ghermez' and 'Yaghouti Sabz' were large and almost the same size in terms of grape size. The foreign cultivars 'Flame Seedless' and 'Perlette' had a smaller bunch size. The native Iranian grape cultivars and genotypes of 'Roghani' and 'Mocheh' had the smallest bunch size among the investigated grape cultivars and genotypes (Fig. 3e).

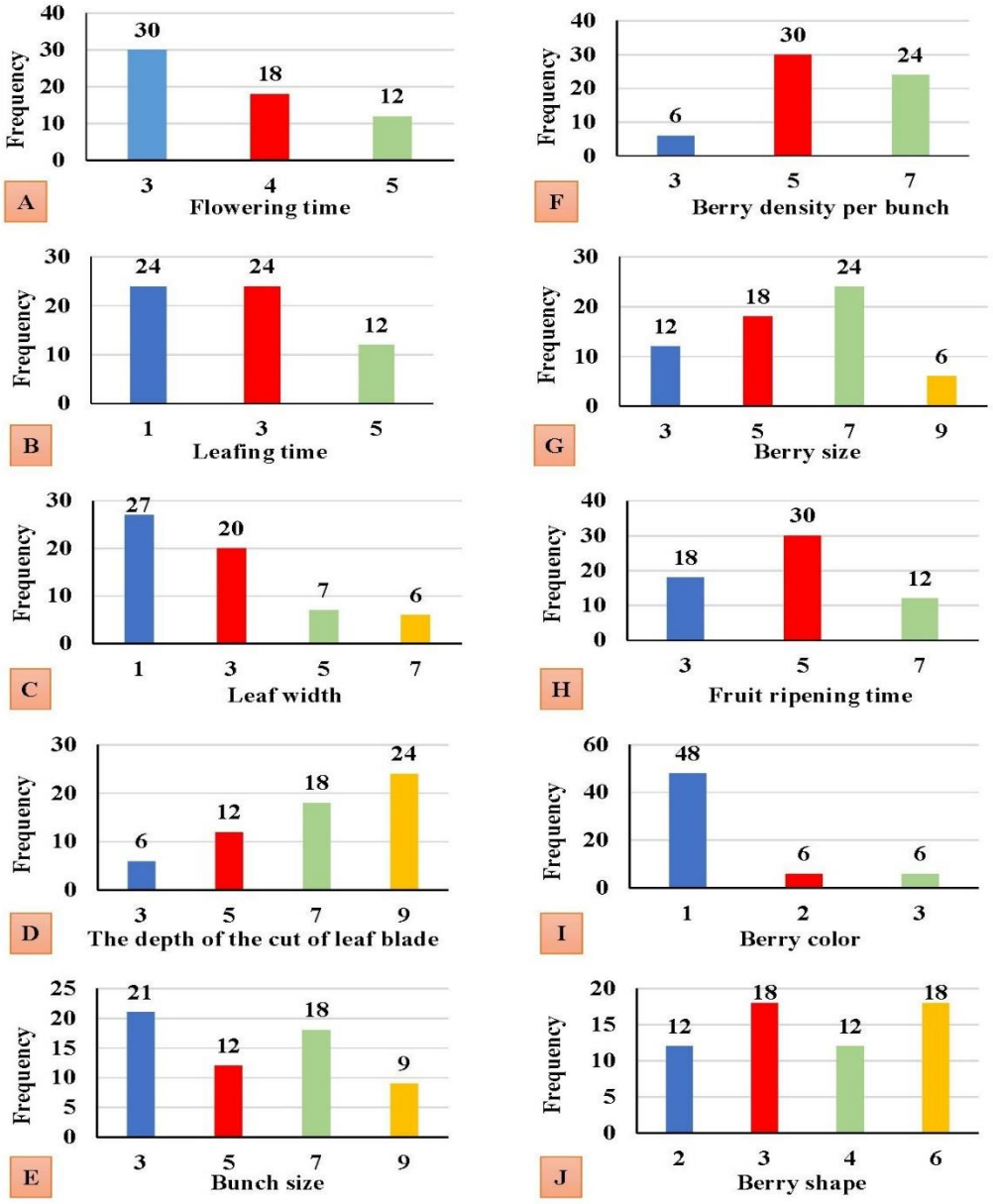

Fig. 3. The frequency of some important studied traits in different cultivars and genotypes of grapes existing in the vineyards of Khuzestan province as follows. **A)** The frequency of flowering time in the studied cultivars and genotypes of grapevines. 1=Too early, 2= Very early, 3= Early, 4= Early to moderate, 5= Moderate, 6= Moderate to late, 7= Late, 8= Very late, 9= Too late. **B)** Frequency of leafing time in the studied cultivars and genotypes of grapevines. 1=early, 3= intermediate, 5= late. **C)** The frequency of leaf width in the studied cultivars and genotypes of grapevines. 1= less than 100 cm^2, 3= 100 to 125 cm^2, 5= 125 to 150 cm^2, 7= 150 to 175 cm^2, 9= more than cm^2. **D)** The frequency of the depth of wide leaf incisions in the studied cultivars and genotypes of grapevines. 1= less than 4 mm, 3= 4 to 8 mm, 5= 8 to 12 mm, 7= 12 to 16 mm, 9= more than 16 mm. **E)** The frequency of bunch size in the studied cultivars and genotypes of grapevines. 3= small, 5= medium, 7= large, 9= very large. **F)** The frequency of berry density in the bunch in studied cultivars and genotypes of grapevine s. 3= open, 5= medium, 7= compact. **G)** The frequency of seed size in the studied cultivars and genotypes of grapevines. 1= very small, 3= small, 5= medium, 7= large, 9= very large. **H)** The frequency of fruit ripening time in the studied cultivars and genotypes of grapevines. 1= very early, 3= early, 5= medium, 7= late, 9= very late. **I)** The frequency of berry color in the studied cultivars and genotypes of grapevines. 1= green-yellow, 2= light red, 3= dark red, 4= gray, 5= purple, 6= navy blue. **J)** The frequency of berry shape in the studied cultivars and genotypes of grapevines. 1= rectangular, 2= oval, 3= broad oval, 4= round, 5= flat, 6= ovoid, 7= open ovoid, 8= ovoid, 9= conical.

The native Iranian grape cultivars and genotypes in vineyards of Khuzestan province, including 'Soltani' and 'Yershi' and Iranian grape cultivars ('Yaghouti Ghermez' and 'Yaghouti Sabz'), had the highest berry density per bunch compared to foreign cultivars ('Flame Seedless' and 'Perlette'). Native and local Iranian grape cultivars and genotypes of 'Roghani' and 'Mocheh' had the lowest berry density per bunch among the studied grape cultivars and genotypes (Fig. 3f).

Also, native grape cultivars and genotypes of Khuzestan province such as 'Soltani' and 'Yershi' grapes had larger berry size than other Iranian grape cultivars ('Yaghouti Ghermez','Yaghouti Sabz' and 'Asgari') and non- Iranian grape cultivars ('Flame Seedless' and 'Perlette'). The smallest berry size was obtained by native Iranian grape cultivars and genotypes 'Roghani' and 'Mocheh' (Fig. 3g).

The native Iranian grape cultivars and genotypes including 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Roghani' and 'Mocheh' were earlier (May) than other cultivars. The grape cultivars 'Yaghouti Ghermez', 'Yaghouti Sabz', and 'Asgari' (May-June) were medium, and the foreign cultivars 'Flame Seedless' and 'Perlette' (June) were late (Fig. 3h).

By studying the cultivars and genotypes of grapes available in the vineyards of Khuzestan province, it was observed that the color of the berries in the native 'Soltani' cultivar or genotype is from bright yellow to greenish yellow, the native 'Bangi' (Ghermez) cultivar or genotype has a light red to medium red color, the native 'Yershi' cultivar or genotype has a yellow to green and bright red color, the native 'Sabz Dorosht' cultivar or genotype has a green color, the native 'Roghani' cultivar or genotype has a shiny green color, the native 'Mocheh' cultivar or genotype has a green color, 'Nameless' cultivar or genotype has a yellow to green color, the 'Yaghouti Sabz' cultivar has a green color, the 'Yaghouti Ghermez' cultivar has a light red to medium red color, the 'Asgari' cultivar has a yellow-green color, the 'Flame Seedless' cultivar has a light red to medium red color, and the 'Perlette' cultivar has a variable green color (Fig. 3i).

The berry shape varied from round to oval in Iranian grape cultivars and genotype and round to elongated oval in foreign cultivars (Fig. 3j).

Simple correlation of traits

There was a significant correlation between variables related to vegetative growth and fruit characteristics. The results showed that the growth size of shoot had a positive and significant relationship with the dry weight of shoot (r=0.99). The relationship between tendril length and dry weight of leaf (r=0.97) was positive and significant. Internode length had a significant positive relationship with dry weight of shoot (r=0.99). The length of bunch shoulder showed a positive and significant relationship with bunch length (r=0.87). Berry length had a positive and significant relationship with berry width (r=0.95), fresh weight of berry (r=0.84), dry weight of berry (r=0.71), dry weight of bunch shoulder (r=0.71). Also, the fresh weight of bunch shoulder had a significant and negative relationship with rachis length (r= -0.26). The length of bunch shoulder has a negative and significant correlation with dry weight of berry (r= -0.49). The specific leaf area has a negative and significant relationship with the characteristics of bunch length (r= -0.31) and bunch width (r= -0.49).

Principal Component Analysis

Component analysis prior to cluster analysis is useful to determine the relative importance of the role of variables. In general, component analysis is performed to determine the role of each trait in the diversity among the genotypes under study. The first component contains the most variance, followed by the second component, and the last component contains the least variance. The main purpose of this analysis is to obtain eigenvalues in the hope that the variances of many components are so small that they can be ignored. The best results from this

analysis are obtained when the primary variables have a high correlation; otherwise, this analysis is useless. Under favorable conditions (high correlation), the principal components can serve as criteria to show different aspects of the data. It is also important to know that it is possible to reduce the number of primary variables in this analysis (Soltani, 2002).

The results showed that the 7 principal components accounted for 22.77, 17.27, 12.77, 11.39, 9.14, 5.99, and 4.95% of the variance changes, respectively, and a total of up to 84.28% of the total variance of the variables (Table 4). The relative variance of each factor indicates its importance, expressed as a percentage of the total variance of the characteristics studied. The results show the placement of some important traits in different factors, with their positive and negative factor coefficients.

In the first factor (PC_1), fruit vegetative and biochemical traits explained 22.77% of the variance as the most important traits for grouping genotypes in cluster analysis (Table 5).

Important vegetative characteristics in the first factor (PC_1) such as shoot growth size, length of internode, fresh weight of the shoot, dry weight of shoot, the ratio of the length to the width of the berry, and traits as content of trans-resveratrol in leaf, content of trans-resveratrol in the skin of unripe berries, total chlorophyll content, the content of chlorophyll a and the content of chlorophyll b the most important biochemical of fruit have the highest coefficients were eigenvectors (Table 5).

In the second factor (PC_2), the length of bunch shoulder, the number of berries per bunch, Dry weight of berry, trans-resveratrol content in tendril and petiole trans-resveratrol content were salient and important characteristics and had the highest coefficients. This group explained 17.27% of the variance (Table 5).

In the third factor (PC_3), seed weight, seed diameter and seed length had the highest eigenvector coefficients and explained 12.77% of the variance (Table 5).

In the fourth factor (PC_4), the peduncle length of the bunch, the pedicel length of the bunch and the pedicel length of the berry were the primary characteristics with the highest coefficients and explained 11.39% of the variance (Table 5).

In the fifth factor (PC_5), the total dry weight of peduncle had the highest coefficients and explained 9.14% of the variance (Table 5).

In the sixth factor (PC_6), the ratio of leaf dry weight to leaf area**,** specific leaf area and protein content had the highest coefficients and justified 5.99 % of the variance of the variables (Table 5).

In the seventh factor (PC_7), the ratio of the length to the width of the bunch has the highest coefficients with 4.95 % of the variance (Table 5).

Table 4. The amount of eigenvalues, percentage of variance and cumulative variance of the decomposition into factors in the first 7 factors.

Factor	Eigen values	Eigen values to Percent Variance	Cumulative percent variance
1	18.21	22.77	22.77
2	13.81	17.27	40.04
3	10.22	12.77	52.81
4	9.12	11.39	64.20
5	7.32	9.14	73.34
6	4.79	5.99	79.33
7	3.96	4.95	84.28

Table 5. Coefficients related to 1 to 7 principal components of grape cultivars and genotypes studied in Khuzestan province.

No.	Traits	Main Component						
		1	2	3	4	5	6	7
1	Growth size of shoot	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175
2	Tendril length	0.538	0.225	0.215	0.367	0.454	-0.081	-0.067
3	Length of internode	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175
4	Fresh weight of shoot	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175
5	Dry weight of shoot	0.822	-0.343	-0.056	-0.147	-0.249	-0.035	-0.176
6	Fresh weight of leaf	0.537	0.223	0.214	0.366	0.457	-0.082	-0.068
7	Dry weight of leaf	0.532	0.138	0.129	0.245	0.577	-0.059	-0.106
8	Leaf Area	0.271	0.387	-0.167	0.434	0.465	0.397	-0.314
9	The cutting depth of the leaf	0.137	-0.299	0.269	-0.298	-0.104	-0.619	0.407
10	The ratio of leaf dry weight to leaf area	-0.062	0.396	-0.283	0.290	0.024	0.580	-0.361
11	Specific Leaf Area (SLA)	0.631	0.342	-0.089	0.141	0.335	-0.212	0.441
12	The rachis length	0.592	0.406	0.173	-0.196	0.127	-0.236	0.466
13	Bunch length	0.467	0.021	0.420	-0.487	0.164	-0.143	-0.088
14	Bunch width	0.042	0.350	-0.372	0.390	-0.022	-0.055	0.547
15	The ratio of length to width of the bunch	0.466	0.510	-0.296	0.541	-0.090	0.195	0.136
16	The peduncle length	0.374	0.662	-0.133	0.187	-0.361	0.315	0.097
17	The length of bunch shoulder	0.507	0.460	-0.420	0.349	0.013	0.143	0.009
18	The width of bunch shoulder	0.025	0.364	0.336	0.099	-0.494	0.242	0.250
19	The ratio of length to width of bunch shoulder	-0.127	-0.308	0.146	-0.483	0.520	-0.362	0.183
20	The ratio of rachis length of bunch to rachis length of bunch shoulder	0.421	-0.631	-0.487	-0.028	0.173	0.104	-0.017
21	Berry length	0.498	-0.613	-0.366	0.011	-0.009	0.036	-0.062
22	Berry width	-0.439	-0.066	-0.302	-0.048	0.447	0.162	0.037
23	The ratio of length to width of berry	0.760	-0.250	0.117	0.228	-0.039	0.333	0.222
24	Berry Diameter	0.212	0.865	0.227	-0.343	-0.106	-0.013	-0.048
25	The number of berries per bunch	0.529	0.449	0.280	0.076	0.043	-0.341	0.096
26	The number of berries per bunch shoulder	0.652	0.477	0.248	0.118	-0.052	-0.251	0.107
27	The number of bunch shoulder per bunch	0.507	0.602	0.159	-0.490	0.097	0.007	-0.006
28	Fresh weight of the bunch	0.746	-0.310	-0.089	0.245	0.093	-0.261	-0.024
29	Fresh weight of bunch shoulder	0.446	-0.621	-0.243	0.198	-0.061	-0.012	-0.202
30	Fresh weight of berries	0.510	0.655	0.212	-0.406	0.027	-0.012	-0.034
31	Fresh weight of berries per bunch	0.749	-0.231	0.000	0.349	0.013	-0.282	-0.025
32	Fresh weight of berries per bunch shoulder	0.026	0.227	0.147	-0.296	0.112	-0.098	0.467
33	Total pedicels of fresh weight of berries per bunch	0.549	0.225	-0.061	-0.543	0.141	0.185	0.026
34	Dry weight of bunch	0.536	-0.478	-0.395	-0.316	0.276	0.063	0.141
35	Dry weight of bunch shoulder	0.201	-0.837	-0.329	-0.053	0.060	0.004	-0.185
36	Dry weight of berry	0.585	-0.003	-0.121	-0.632	0.270	0.277	0.021
37	Dry weight of berries per bunch	0.697	-0.132	-0.072	-0.013	0.031	-0.243	0.179
38	Dry weight of bunch shoulders per bunch	0.439	0.244	-0.207	-0.288	0.187	0.379	0.037
39	Fresh weight of rachis per bunch	-0.406	0.189	-0.510	-0.412	0.223	0.362	0.298
40	Fresh weight of peduncle	-0.108	-0.305	-0.189	-0.341	0.345	-0.083	0.408
41	The pedicel of fresh weight of berries	0.323	0.738	-0.203	-0.182	0.001	0.077	0.352

Cluster Analysis

Cluster analysis is a method for grouping different populations. In this method, different cultivars or genotypes are measured based on P (variable) and items that are very similar to each other are placed in the same group. The advantage of this analysis is to find those elements that have the greatest genetic distance from each other for use in breeding programs. The other advantages of this method are finding the true groups and reducing the data (Soltani, 2002; Zahedi et al., 2023).

In this study, cluster analysis by Ward's method was used to group the genotypes based on the studied traits and at 5 Euclidean distances the cultivars and genotypes were grouped into 9 main clusters, (Fig. 4). The most obvious distinguishing characteristics of the groups included phenological traits such as leafing time, flowering time, early maturity and vegetative vigor, and morphological characteristics such as all traits related to bunches and berries such as bunch and berry size, berry shape, color and weight.

Table 5 (*Continued*). Coefficients related to 1-7 principal components of grape cultivars and genotypes studied in Khuzestan province.

No.	Traits	Main Component						
		1	2	3	4	5	6	7
42	Fresh weight of rachis of the total bunches	-0.518	0.245	-0.383	-0.333	0.026	0.068	0.428
43	The ratio of fresh weight of rachis of bunch shoulder to fresh weight of rachis bunch	0.368	-0.083	-0.400	-0.586	0.502	0.174	-0.014
44	Dry weight of rachis	0.170	-0.354	-0.465	-0.522	0.542	0.167	0.021
45	Dry weight of peduncle	0.411	-0.286	0.147	0.441	-0.476	0.002	0.040
46	Dry weight pedicel of berry	0.432	0.546	0.426	-0.026	-0.242	0.099	0.158
47	The total pedicels of dry weight of berries per bunch	0.615	0.086	-0.347	-0.277	0.550	0.016	0.178
48	The ratio of dry weight of rachis of bunch shoulder to dry weight of rachis bunch	-0.581	-0.526	-0.287	-0.082	0.315	-0.202	0.059
49	The peduncle length of the bunch	0.208	0.017	-0.457	0.734	0.410	-0.051	0.057
50	The pedicel length of bunch	0.282	0.118	-0.349	0.805	0.280	-0.085	0.101
51	The pedicel length of berry	-0.178	0.059	-0.331	0.773	0.373	-0.232	-0.051
52	Seed weight	0.140	-0.077	0.841	0.105	0.436	0.212	-0.011
53	Seed diameter	0.140	-0.077	0.841	0.105	0.436	0.212	-0.011
54	Seed length	0.139	-0.076	0.842	0.105	0.436	0.212	-0.011
55	Titratanbe acidity	-0.149	0.185	-0.104	0.158	0.282	-0.203	-0.148
56	Total soluble solids (TSS)	0.408	-0.144	0.364	0.341	-0.553	0.149	0.314
57	Protein content	0.069	-0.309	0.055	0.488	-0.194	0.504	0.425
58	Content of trans-resveratrol in tendril	0.353	0.847	-0.069	-0.122	-0.140	-0.120	-0.261
59	Content of trans-resveratrol in petiole	0.080	-0.809	0.367	-0.035	-0.036	-0.284	0.195
60	Content of trans-resveratrol in leaf	0.786	-0.077	-0.249	-0.278	-0.022	0.333	0.205
61	Content of trans-resveratrol in pedicel	-0.037	-0.719	0.365	0.167	-0.299	0.065	0.446
62	Content of trans-resveratrol in the skin of unripe berries	0.761	-0.107	-0.297	-0.205	-0.047	0.316	0.321
63	Content of trans-resveratrol in mesocarp of unripe berry	-0.171	0.031	-0.083	0.130	-0.307	0.794	0.270
64	Content of trans-resveratrol in skin of unripe berry	-0.045	-0.648	0.527	0.005	-0.265	-0.335	0.219
65	Content of trans-resveratrol in mesocarp of ripe berry	0.493	-0.479	-0.323	-0.370	0.414	0.098	0.045
66	Content of trans-resveratrol in seeds of unripe berry	0.141	-0.077	0.841	0.105	0.436	0.212	-0.011
67	Content of trans-resveratrol in seeds of ripe berry	0.141	-0.077	0.841	0.105	0.436	0.212	-0.011
68	Content of cis-resveratrol in the tendril	0.387	0.784	-0.199	0.197	0.028	-0.213	-0.232
69	Content of cis-resveratrol in petiole	0.301	0.516	-0.127	0.261	-0.033	-0.470	0.389
70	Content of the cis-resveratrol content in leaf	0.271	0.693	0.130	-0.457	-0.347	-0.050	-0.201
71	Content of cis-resveratrol in pedicel	0.275	0.792	-0.063	-0.187	-0.097	-0.414	-0.054
72	Content of cis-resveratrol in the skin of unripe berries	0.442	-0.423	0.221	0.196	-0.464	0.351	0.321
73	Content of cis-resveratrol in mesocarp of unripe berry	0.469	-0.159	-0.295	0.739	0.164	-0.091	0.120
74	Content of cis-resveratrol in the skin of unripe berry	0.331	-0.101	-0.348	0.747	0.356	-0.155	0.005
75	Content of cis-resveratrol in the mesocarp of ripe berry	0.685	-0.092	0.176	-0.039	-0.256	0.023	-0.423
76	Content of cis-resveratrol in seeds of unripe berry	0.138	-0.076	0.843	0.105	0.437	0.212	-0.011
77	Content of cis-resveratrol in seeds of ripe berry	0.138	-0.076	0.843	0.105	0.437	0.212	-0.011
78	Total chlorophyll content	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175
79	The content of chlorophyll a	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175
80	The content of chlorophyll b	0.846	-0.336	-0.044	-0.131	-0.265	-0.029	-0.175

Group 1: This group included 6 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the native Iranian grape cultivars and genotypes of 'Mocheh' (regions of Ahvaz, Hamidiyeh, Karun) and 'Roghani' (regions of Ahvaz, Hamidiyeh, Karun). The native Iranian grape cultivars and genotypes of Khuzestan province with similar geography and tropical climate were included in this group. The cultivars and genotypes of this group had relatively early setting, earlier ripening; small bunches with low density of berries per bunch, small berries, almost firm with berries of spherical shape and green color which is shiny in

'Roghani' genotype. In this group, cultivars and genotypes had lower sugar and higher titratable acidity and were completely seedless or had incomplete seeds, which is the result of stenospermocarpy. The average of some important characteristics in the native Iranian grape cultivar or genotype of 'Mocheh' is the leaf area with 77 cm^2, specific leaf area with 215 $cm^2.g^{-1}$, bunch length with 100 mm, berry length with 11 mm, number of berries per bunch with 78 berries, bunch weight with 220 g and berry weight with 1.3 g. Also, the average of some important characteristics in native Iranian grape cultivar or genotype of 'Roghani' are leaf area with 91 cm^2, specific leaf area with 214 $cm^2.g^{-1}$, bunch length with 126 mm, berry length with 15 mm, number of berries per bunch with 106 berries and berry weight with 2.15 g.

Group 2: This group included 6 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the foreign cultivar of 'Perlette' (regions of Lali, Ramhormoz, Behbahan, Gotvand, Izeh and Andimeshk) which has medium to late setting, mid-season ripening, medium to large bunches with medium density of berries in the bunch. This group had relatively elongated berry shape and medium firmness. The grapes were juicy, completely seedless or with incomplete seeds. Due to the fact that this variety was foreign and its geographical distance and peculiarities, it was placed in a separate group and the most distant group compared to the Sultani variety. In the foreign cultivar of 'Perlette', the average of some important characteristics are examples of leaf area with 109 cm^2, specific leaf area with 275 $cm^2.g^{-1}$, bunch length with 89 mm, berry length with 14 mm and berry weight with 2.1 g.

Group 3: This group included 6 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the Iranian grape cultivar 'Asgari' (regions of Ahvaz, Shushtar, Baghmalek, Izeh, Gotvand and Ramhormoz). This group had medium to late bearing cultivars, mid-season ripening, medium to large bunches, medium berry density in the bunch, relatively elongated berries and medium firmness. They were juicy, completely seedless or with incomplete seeds. The average of some important characteristics in the non-native Iranian grape cultivar 'Asgari' are leaf area with 55 cm^2, specific leaf area with 165 $cm^2.g^{-1}$, bunch length with 67 mm, berry length with 14 mm and berry weight with 2.35 g.

Group 4: This group included 6 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the foreign cultivar of 'Flame Seedless' (regions of Andimeshk, Dezful, Andika, Behbahan, Lali and Baghmalek) which, due to the climate of the region, had later flowering and ripening than other native and local cultivars of the region. They had medium sized vines with favorable growth and varying bunch sizes from small to large and with open to compact vine density. They also had yellow to medium red berries with oval to egg-shaped oval berries. In the foreign cultivar of 'Flame Seedless', the average of some important characteristics are leaf area with 143 cm^2, specific leaf area with 259 $cm^2.g^{-1}$, bunch length with 78 mm, berry length with 18 mm and berry weight with 2.2 g.

Group 5: This group included 12 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the Iranian grape cultivars 'Yaghouti Ghermez' (regions of Andika, Dezful, Shushtar, Izeh, Behbahan and Andimeshk) and 'Yaghouti Sabz' (regions of Gotvand, Ramhormoz, Andimeshk, Dezful, Baghmalek and Lali). Both cultivars had medium to high vegetative vigor, early to medium fruit ripening, medium flowering time, early to mid-season ripening and a relatively full crop with large and dense bunches, spherical and red to purple and green berry color. Both varieties were used for two purposes (fresh consumption and raisin production) and had a medium to high sugar content. The leaf area with 111 cm^2, specific leaf area with 205 $cm^2.g^{-1}$, bunch length with 60 mm, berry length with 13 mm and berry weight with 2.8 g as the most important characteristics of non-native Iranian grape cultivar is 'Yaghouti Ghermez'. Also, the average of some important characteristics in the native Iranian grape cultivar or genotype of 'Yaghouti Sabz' is the leaf area with 92 cm^2, specific leaf

area with 178 $cm^2.g^{-1}$, bunch length with 84 mm, berry length with 12 mm and berry weight with 2 g.

Group 6: This group included 6 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the native grape cultivar and genotype of 'Yershi' (regions of Karun, Hamidiyeh, Ahvaz, Shushtar, Andika and Ramhormoz) as the native cultivar or genotype of Khuzestan which, according to the climate of the province, had earlier leafing, flowering and ripening than other non-native and local cultivars. They had medium sized bushes with favorable growth and large and compact bunches. They also have yellow to medium red berries that are oval to ovoid. The average of some important characteristics in the native and local Iranian grape cultivar or genotype of 'Yershi' are leaf area with 111 cm^2, specific leaf area with 205 $cm^2.g^{-1}$, bunch length with 60 mm, berry length with 13 mm and berry weight with 2.8 g.

Group 7: This group included 3 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the native Iranian grape cultivar or genotype of 'Sabz Dorosht' (regions of Dezful 1, Dezful 2 and Dezful 3). Its berries were large, seedless, green and had a larger bunch with higher density and weight compared to other cultivars. In native and local Iranian grape cultivar or genotype 'Sabz Dorosht', the average of some important characteristics are leaf area with 111 cm^2, specific leaf area with 235 $cm^2.g^{-1}$, bunch length with 63 mm, berry length with 24 mm and berry weight with 4.9 g.

Group 8: This group included 3 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the native Iranian grape cultivar or genotype of 'Soltani', (regions of Andika, Shushtar and Karun). leaf area with 93 cm^2, specific leaf area with 184 $cm^2.g^{-1}$, bunch length with 71 mm, berry length with 15 mm and berry weight with 3.3 g are the main characteristics of the native and local cultivar or genotype of Iranian 'Soltani' grapes in the eighth group.

Group 9: This group included 12 cultivars and genotypes out of 60 investigated grapes cultivars and genotypes such as the native Iranian grape cultivars and genotypes of Khuzestan province 'Bangi' (Ghermez), (regions of Andika, Lali, Hamidiyeh, Ramhormoz, Shushtar and Karun) and 'Soltani', (regions of Behbahan, Gotvand and Hamidiyeh) and 'Nameless', (regions of Ahvaz, Hamidiyeh and Karun). The average of some important characteristics in the native and local Iranian grape cultivar or genotype of 'Bangi' (Ghermez) are leaf area with 82 cm^2, specific leaf area with 219 $cm^2.g^{-1}$, bunch length with 65 mm, berry length with 20 mm and berry weight with 6.3 g. Also, the average of some important characteristics in the native Iranian grape cultivar or genotype of 'Soltani' are leaf area with 77 cm^2, specific leaf area with 194 $cm^2.g^{-1}$, bunch length with 63 mm, berry length with 16 mm and berry weight with 3.5 g. In the native Iranian grape cultivar or genotype of 'Nameless', the average of some important characteristics are leaf area with 66 cm^2, specific leaf area with 189 $cm^2\ g^{-1}$, bunch length with 67 mm, berry length with 18 mm and berry weight with 4.58 g.

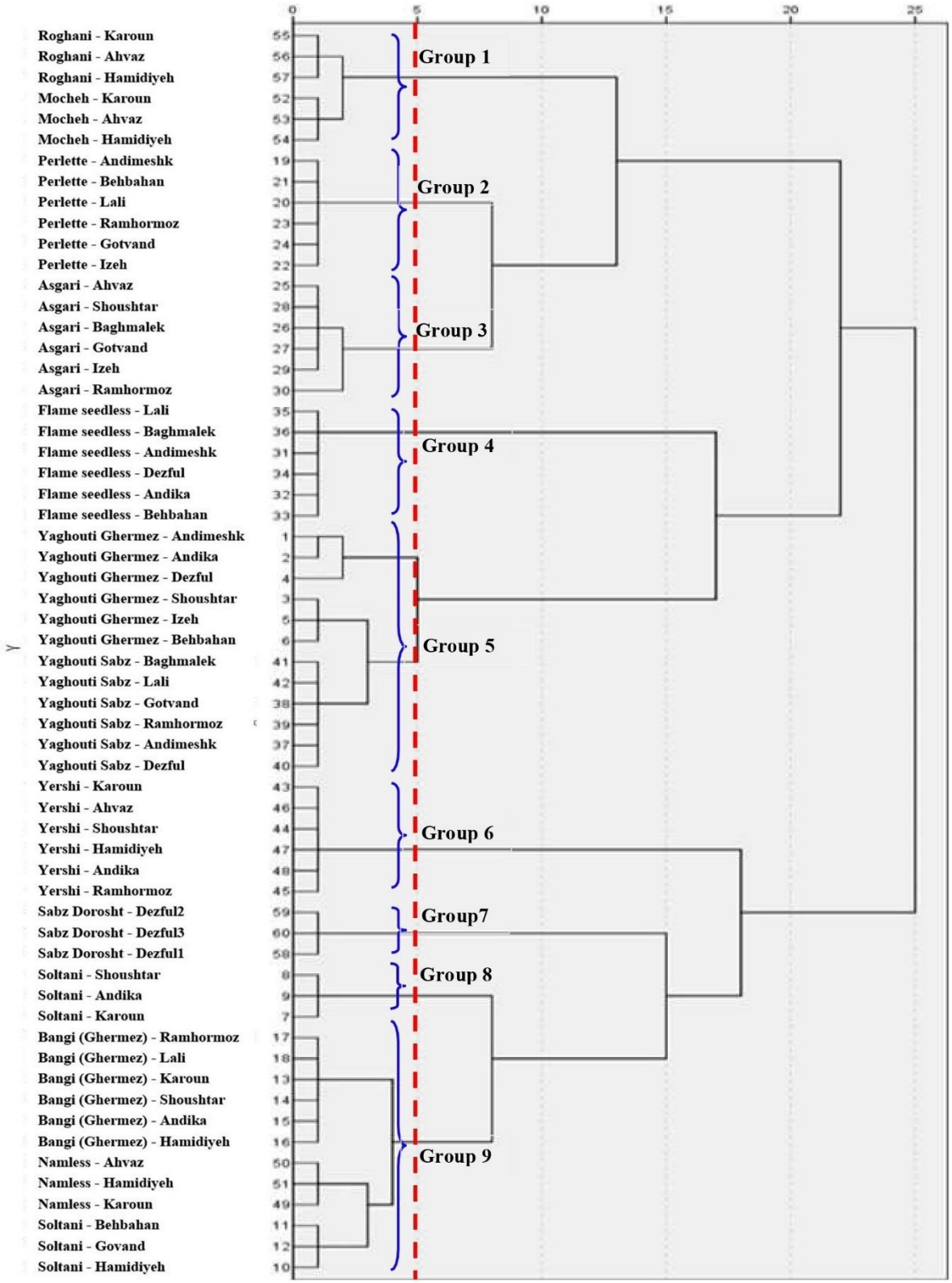

Fig. 4. Dendrogram showing relationship between 60 cultivars and genotypes of grapes, available in the vineyards of Khuzestan province located in the southwest of Iran, and at 5 Euclidean distances based on studied traits using cluster analysis by Ward's method.

The groups 8 and 9, included the native Iranian grape cultivar and genotype of Khuzestan province with similar geography and tropical climatic conditions. According to the climate of the region, these cultivars and genotypes have earlier leafing, flowering and ripening than the cultivars of other regions, and they have medium bushes with favorable growth and diverse bunch sizes from small to large and with open to compact density. They also have yellow to medium-red berry color and oval to egg-shaped oval berries and are fully seeded or have incomplete seeds. Cultivars and genotypes in these two groups were similar to each other in most of the traits compared to other cultivars and genotypes.

According to the separation of factors, determination of the effect of each trait on the diversity of phenological, morphological and pomological traits and identification of the most important traits affecting the diversity and difference of cultivars and genotypes in vineyards of Khuzestan province, it was concluded that the first factor belongs to vegetative traits of shoot, the second factor to berry traits, the third factor to seed traits, the fourth factor to rachis, peduncle, pedicel and the fifth factor to leaf traits. The most important traits include internode length, branch length, branch weight, number of berries per bunch, dry weight of berries, seed weight, seed diameter, seed length, content of trans-resveratrol in seeds of unripe grapevine, content of trans-resveratrol in seeds of ripe berry, content of cis-resveratrol in seeds of unripe berry, content of cis-resveratrol in seeds of ripe berry, pedicel length, leaf area, leaf specific area, leaf dry weight, and total soluble solids.

These results indicate that the cultivars and genotypes studied in terms of best quality traits had visible differences in growth and reproduction, and these superior traits create diversity. The variations observed are due to the different occurrence of the traits in the cultivars and genotypes (Fig. 5 and Fig. 6).

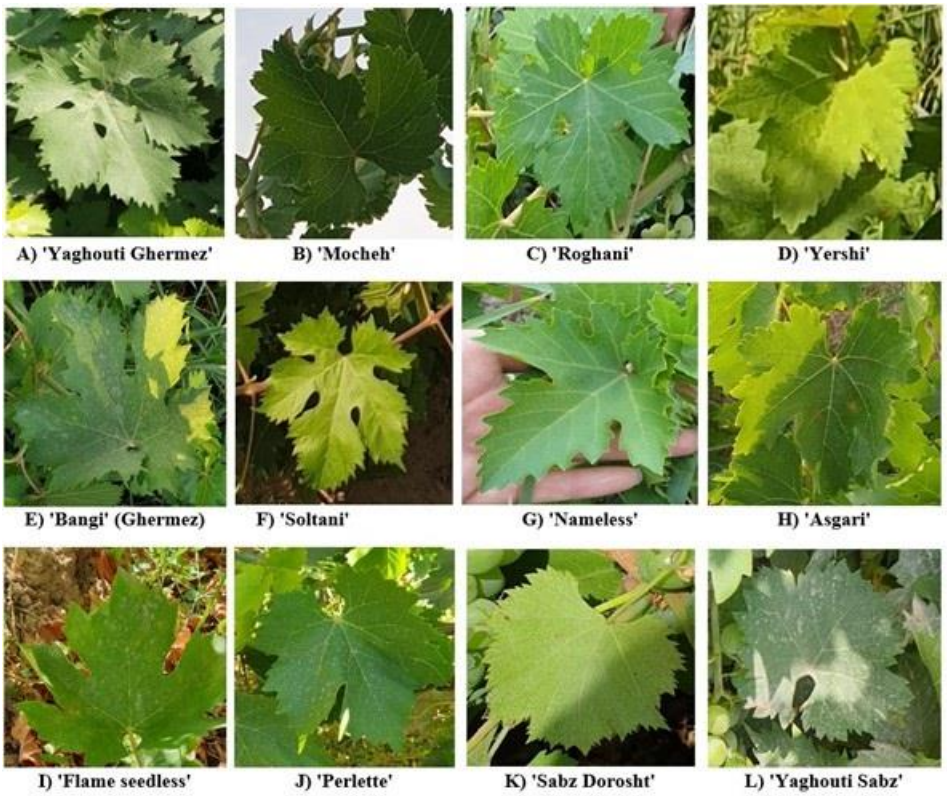

Fig. 5. Variations observed in leaf characteristics (included size, color, shape) of grape cultivars and genotypes grown in vineyards of Khuzestan province. A) 'Yaghouti Ghermez', B) 'Mocheh', C) 'Roghani', D) 'Yershi' , E) 'Bangi' (Ghermez), F) 'Soltani', G) 'Nameless', H) 'Asgari', I) 'Flame Seedless', J) 'Perlette', K) 'Sabz Dorosht' and L) 'Yaghouti Sabz'.

The difference in vine biomass, berry size, berry density and weight, presence or absence of seeds in the berry, seed size, leaf width size and leaf area, total soluble solids, and resveratrol content in different parts of the plant are among the most important traits that differ among cultivars; and the native and local Iranian grape genotypes such as 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Mocheh', 'Roghani', 'Sabz Dorosht' and grape cultivars such as 'Yaghouti Ghermez', 'Yaghouti Sabz', 'Asgari' and foreign cultivars such as 'Flame Seedless' and 'Perlette' in vineyards of Khuzestan province.

By identifying the most prominent characters to separate and group the cultivars and genotypes in the vineyards of Khuzestan province, it was determined that among the native Iranian grape cultivars and genotypes, 'Mocheh' and 'Roghani' from the first group had the most distant genetic relationship with 'Soltani' and 'Bangi' (Ghermez) from the ninth group. The native Iranian grape cultivars and genotypes of Khuzestan province 'Mocheh' and 'Roghani' had more relationship with other Iranian grape cultivars such as 'Asgari', 'Yaghouti Ghermez' and 'Yaghouti Sabz' as well as foreign cultivars of 'Flame Seedless' and 'Perlette'. In addition, other local and endemic Iranian grape cultivars and genotypes such as 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Sabz Dorosht' and 'Nameless' showed more genetic relationship with each other (Fig. 5 and Fig. 6).

Fig. 6. Variations observed in bunch characteristics (included size, color, shape) of grape cultivars and genotypes grown in vineyards of Khuzestan province. A) 'Yaghouti Ghermez', B) 'Mocheh', C) 'Roghani', D) 'Yershi' , E) 'Bangi' (Ghermez), F) 'Soltani', G) 'Nameless', H) 'Asgari', I) 'Flame Seedless', J) 'Perlette', K) 'Sabz Dorosht' and L) 'Yaghouti Sabz'.

DISCUSSION

Determination of genetic diversity in plant material is very important and is the first and fundamental step to identify, conserve and maintain genetic resources, which is the basic foundation for genetic research and breeding programs. In order to improve and produce new cultivars, it is necessary to have the power of accurate selection among plants, which depends on the identification of cultivars and the diversity in them. Studying the latent genetic diversity in the plant population, selecting the traits that are effective in production, and introducing superior cultivars will help. Also, the study of phenotypic and genotypic diversity is very important to identify similar genotypes in order to evaluate, use and conserve genetic resources.

The findings of this research showed that most of the traits among the cultivars and genotypes of the studied grapes, especially in the local grape cultivars of the tested region, were significantly different from each other due to their diverse morphological and phenological characteristics, which the research results of Salimov et al. (2017) and Razi et al. (2021) and Habib et al. (2020, 2021) were in agreement.

Jahnke et al. (2021) reported that genotypic differences cause variation in leafing time, flowering and fruiting time between different grape cultivars and genotypes. Based on the codes determined in the grape descriptor and in accordance with the results of Rasouli et al. (2013), Khadivi-Khub et al. (2014) and Salimov et al. (2017), in our research, flowering time with code 3-7 (early flowering - late flowering), leafing time with Code 3-5 (early leafing-late leafing) and fruit ripening time with code 3-7 (early ripening-late) were variable in grape cultivars and genotypes of Khuzestan province.

In this research, the shape of berries in the studied cultivars and genotypes was oval, wide oval and round, and the reason for the difference is mostly related to the type of variety and its distribution, which is according to the findings of Rasouli et al. (2013), Khadivi -Khub et al. (2014), Razi et al. (2021) have been more consistent regarding the variety of berry shape in similar growing conditions. According to him, different cultivars of grapes differ from each other in terms of length and width of berries, and seedless cultivars have small to medium bunch. In terms of the color of the skin and mesocarp in the berries, juiciness, berry weight and the presence of seeds, they differ from each other, which were similar to the opinion of Salimov et al. (2017) and Janke et al. (2021).

According to Dilli et al. (2014), vegetative and reproductive morphological traits such as leaf area, plant growth size, bunch weight and berry weight are highly correlated with changes in genetic traits. The results of the present research show a positive and significant correlation between the bunch and berry traits (r=0.71) with the findings of Rasouli et al. (2013). It matched. Cargnin (2018) in the study of ′Cabernet Sauvignon′ cultivars showed that fruit yield (weight) has a high and significant correlation with cluster weight (r=0.98) and berry weight (r=0.98). Also, in similar results, Cargenin (2018) in the study of ′Chardonnay′ cultivars showed that fruit yield (weight) had a positive and significant phenotypic correlation with bunch weight (r=0.91) and number of berries per bunch (r=0.88). According to Cargnin (2018), the higher the number of bunch per plant, the lower the weight of the bunch and berry weight, and as a result, the fruit yield is lower. But the selection based on traits with positive and significant correlation showed the potential of high fruit yield in the plant. These results confirm the findings obtained by different researchers who have studied the correlation between variables in grape production (Akram et al., 2021; Khalil et al., 2017; Vujović et al., 2017) and correlations that can be they observed significant correlations between grape variables (for example, between yield components, bunch weight and bunch size; berry weight and berry size and physicochemical characteristics).

The results of this research showed that bunch weight and berry weight have an inverse relationship with the number of bunch per plant and the number of berries per bunch. Increasing berries weight and bunch weight had a positive effect on increasing crop yield. The decrease in crop weight was due to the connection between the sink and the source due to the increase in the number of berries and the limitation in photosynthetic production. As the number of berries increased, the amount of assimilate produced was divided between them and led to a decrease in berry weight. Reducing the number of bunches per plant and the number of berries per bunch has increased the weight of the product. The decrease in vine yield weight was due to the increase in leaf weight and shoot weight. The distribution of photosynthetic substances in the plant during the growing season considering that the leaves are the main factor of photosynthesis. For the growth of leaves, the priority is to use photosynthetic materials with leaves, and when we reach the critical level of leaves, the priority is to use photosynthetic materials with shoot and roots, respectively. But in the stage of reproductive growth, with the growth of berries, the movement of materials to this part is prioritized and the growth of leaves and roots is stopped to a large extent. Knowing which sinks or sources limit the performance of a genotype can determine genotype improvement strategies using selection and breeding.

The results obtained from this research are consistent with the results of other researchers and show that increasing yield components such as number of berries per bunch, berry weight and number of berries per bunch leads to an increase in fruit yield. The results of the researchers show that the phenological and morphological traits with the range of low to high changes are significant, which indicates the heritability and genetic progress of the traits in different cultivars (Silva et al., 2009). Significant positive correlations between economic traits such as bunch length, bunch weight, number of berries per bunch, and berry width with fruit yield indicate that selection for these characteristics leads to an increase in grape yield (Dolkar et al., 2017; Gupta et al., 2015).

In this research, 7 principal components explained 28.84% of the total variance of the measured variables, which is in agreement with the results of Rasouli et al. (2013) in the study of 32 cultivars and genotypes of grapes with 10 factors in total 22.74% and the results of good Khadivi-khub et al. (2014) in the study of genetic diversity in 22 different grape cultivars explained 76.96% of the traits variance with 5 factors. In these studies, the most important traits included bunch weight, berry weight, bunch length and width, berry length and width, number of bunch per plant and number of kernels per berries, which were similar to the results obtained from our experiment.

Leão and Oliveira (2023) reported that the first and second principal components explained 59.2% of the variation. In PC1 (42.76%), the variable was related to the number of clusters, cluster weight, seed length and seed diameter, and in PC2 (16.4%) it was related to vine yield. which was consistent with our results.

The results of PCA analysis of this research were consistent with the findings of Ibacache et al. (2016) and Silva et al. (2017) reported that the variation in the number of clusters in Flame Seedless, Red Globe and Thompson cultivars on different bases is very high. Also, Leão et al. (2010) identified yield per vine, number of bunch per plant, bunch length, bunch weight, berry weight and size, titratable acidity and total soluble solids as prominent variables, which is consistent with the results of our experiment.

The results of cluster analysis by Ward's method at 5 Euclidean distances in this research showed that the native Iranian grape cultivars and genotypes available in the vineyards of Khuzestan province, including 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Sabz Dorosht' and 'Nameless', are more closely related to each other due to their distribution in the vineyards of the province, and Iranian grape cultivars including 'Asgari', 'Yaghouti Ghermez' and 'Yaghouti Sabz' and the non-Iranian grape cultivars including 'Flame Seedless' and 'Perlette' had a much

greater genetic distance from each other, which is consistent with the reports of Gholami et al. (2018) regarding the better efficiency of grouping based on the cultivars and genotypes studied, as well as the results of Basafa et al. (2008), Al-Saady et al. (2018) and Morales-Castilla et al. (2020), who found that genetic diversity and geographic diversity corresponded. Also, the native grape cultivars and genotypes of 'Mocheh' and 'Roghani' were genetically closely related to 'Perlette' and 'Asgari' cultivars and had a small genetic distance with 'Flame Seedless', 'Yaghouti Ghermez' and 'Yaghouti Sabz' cultivars. However, compared to other native and local Iranian grape cultivars and genotypes, they had a much larger genetic distance and showed higher genetic diversity. This high genetic difference between native and local grape cultivars and genotypes in vineyards of Khuzestan province shows that their primary habitats were probably far from each other and they were later transferred to secondary origin. In the grouping of grape cultivars and genotypes in vineyards of Khuzestan province, phenological characteristics such as leafing time, flowering time, earliness, growth strength and morphological characteristics such as all traits related to bunches and berries such as bunch shape, bunch and berry size, berry color and berry weight were the most obvious discriminating characteristics of grape cultivars and genotypes in different groups.

Furthermore, the results of cluster analysis in this research with the findings of Haj-Amiri (2011), Rasouli et al. (2013), Alizadeh (2013), Zainalu (2013), Nejadian (2015), Rasouli and Kalvandi, (2022), and Mirfatah et al. (2024) in the varieties available in Iran (from Kermanshah, Hamedan, West Azarbaijan, Qazvin and Isfahan provinces) in terms of different traits related to vegetative parts, bunch size, bunch weight, berry density per bunch, berry color, seeded or seedless, time of fruit ripening, as well as genetic affinity they had reported, were consistent.

The native grape cultivars and genotypes from Khuzestan province had higher plant growth, growth size of shoot, leaf area, bunch weight and berry weight compared to Iranian cultivars of 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' and foreign cultivars of 'Flame Seedless' and 'Perlette'.

The native grape cultivar of 'Bangi' (Ghermez) has a relatively high anthocyanin content in the mesocarp and skin of berry, as well as the Iranian cultivar of 'Yaghouti Ghermez' and foreign cultivars of 'Flame Seedless' and the native cultivar of 'Yershi' also has some anthocyanin in the mesocarp and skin of berry.

In terms of seed size and shape, the native cultivars and genotypes from Khuzestan province had bigger seeds and larger dimensions and relatively more seed hardness and seed skin thickness compared to other Iranian cultivars of 'Yaghouti Ghermez', 'Yaghouti Sabz' , 'Asgari' and the non-Iranian cultivars of 'Flame Seedless' and 'Perlette'.

The difference in vine growth vigour , berry size, berry density per bunch and berry weight, the presence of seeds per berry, seed size and leaf area, total soluble solids (TSS), and the content of resveratrol in different organs of the plant are among the most important different characteristics between and the native and local grape cultivars and genotypes from Khuzestan province such as 'Soltani', Bangi' (Ghermez), 'Yershi', 'Roghani', 'Mocheh', 'Sabz Dorosht' and 'Nameless' with other Iranian cultivars such as 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' and foreign cultivars such as 'Flame Seedless' and 'Perlette'were available in vineyards of Khuzestan province.

CONCLUSION

The purpose of this research, considering the specific climatic conditions of Khuzestan province of Iran, which has a tropical climate with a very short winter, without frost and with a temperature above zero degrees, knowledge of grape genetic reserves and identification of local cultivars and genotypes in the vineyards of Khuzestan province using phenological, morphological and pomological traits. In the climatic conditions of Khuzestan province, grape cultivars and genotypes, compared to other grape growing regions in Iran, come out of dormancy earlier and leaf buds are activated earlier. After that, the flowers appear faster. In this region, the grapes ripen and are harvested earlier and are not exposed to the late spring cold. Of course, this time coincides with the beginning of the peak of heat in the Khuzestan region, but the native cultivars do not face high environmental temperatures due to their early maturity. The native varieties and genotypes of grapes available in the region include 'Soltani', 'Bangi' (Ghermez), 'Yershi', 'Sabz Dorosht' and 'Nameless' in terms of final fruit yield, bunch weight, berry weight, berry number per bunch and bunch length, they assigned the highest amount. These cultivars and genotypes were superior compared to other investigated cultivars including 'Yaghouti Ghermez', 'Yaghouti Sabz' and 'Asgari' and even foreign cultivars 'Flame Seedless' and 'Perlette'. The native grape cultivars and genotypes 'Roghani' and 'Mocheh' were ranked lower than the others in this respect. Finally, it can be reported that the local cultivar or genotype of 'Soltani' grapes has the most diversity in the traits related to fruit yield, including bunch length, bunch width, bunch shoulder length, bunch shoulder width, number of berries in the bunch, fresh has had the weight of the bunch. Also, the 'Yaghouti Ghermez' cultivar had the highest dry weight of the bunch and the dry weight of the berries, and the local variety or genotype 'Yershi' had the longest bunch length.

Conflict of interest

All authors declare that they have no competing financial or personal relationships that could have influenced the work reported in this paper.

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Evaluating the efficacy of *Moringa oleifera* leaf extracts prepared using different solvents on growth, yield and quality of tomatoes and peppers

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ABSTRACT

Purpose: The study aimed to explore different extraction methods, extraction solvents, as well as solvent/water mixtures that could potentially yield(s) the best growth-enhancing, yield- and quality-promoting effects of *Moringa oleifera* leaf extracts (MLEs), when applied foliarly to tomatoes and peppers. **Research Method:** This study was laid out following a complete randomized design with three replications. Foliar application of MLEs tested included: control, aqueous (hot water, MLE HW), aqueous (cold water, MLE CW), ethanolic (MLE ETH) and methanolic (MLE METH) extracts. These treatments were repeatedly sprayed onto the leaves of selected plants, from two weeks after transplanting in weekly intervals until fruit set. **Findings:** Foliar application of all MLEs significantly enhanced growth of both pepper and tomato plants compared with the control. MLE HW application positively affected yield parameters, followed by MLE ETH and MLE METH. All MLEs significantly enhanced the colour coordinate a* and TSS, excluding MLE CW. Carotenoids in red peppers were significantly higher, following all MLE treatments, excluding the MLE CW, while in red tomatoes MLEs enhanced lycopene and β-carotene content. The concentration of Vitamin C was also significantly enhanced by MLE application to peppers, while in tomatoes, only MLE METH and MLE ETH positively altered the fruit Vit C concentration. These results generally prove that MLE application could potentially be used to improve crop production and their nutritive value. **Research limitations:** There were no limitations identified. **Originality/Value:** The results obtained in this study highlight the potential MLEs, particularly hot water MLE, to enhance growth, yield and nutritional quality of pepper and tomato, without compromising human health and environmental sustainability.

INTRODUCTION

Consumption of vegetable crops is a very important part of a well-balanced diet, since their intake provides nutrients, fibre, fat and carbohydrates to meet human dietary needs, thereby sustaining a healthy body (Hounsome et al., 2008). Amongst fruit vegetable crops, peppers (*Capsicum annum*) and tomatoes (*Solanum lycopersicum*) are recognised as high value crops with high nutritive value (Friedman, 2013; Salehi et al., 2019; Diouf et al., 2023). These vegetable crops are good sources of health-promoting constituents, such as vitamins, proteins, minerals and various antioxidants. In peppers, the antioxidants capsaicin, capsorubin and capsanthin are present, while in tomato lycopene and β-carotene are important phytonutrients (Topuz and Ozdemir, 2007; Dorais et al., 2008; Bae et al., 2014; Salehi et al., 2019).

To achieve high yields in horticultural commodities, the use of moringa (*Moringa oleifera*) plant extracts, particularly of leaf extracts, is an innovative, promising approach that has been tested by various authors using different agricultural crops, including cereals (Phiri, 2010), legumes (Phiri et al., 2010) tomato (Culver et al., 2012) and potato (Mbuyisa et al., 2023). Moringa leaves have high nutritional value, their extract (MLE) has been found to contain high concentrations of vitamins, minerals and other phytochemicals; the extract is also a good source of growth-regulating hormones, such as cytokinin zeatin, which makes MLE a natural plant growth regulator (Hassanein et al., 2018; Siddhuraju et al., 2003; Sreelatha et al., 2011; Goordeen & Mohammed, 2021).

Extraction from and separation of bioactive components found in plant material varies with the solvent, the extraction conditions and the extraction duration (Hayat et al., 2009). The extraction process also affects the active ingredients present in the extract substantially (Mustafa et al., 2011). In addition, in order to become a technology that is adopted by farmers, the method of extraction should be simple, financially feasible and applicable to both small-scale farmers (based in rural areas with little access to resources) and commercial, large-scale farmers (Pothitirat et al., 2010). Furthermore, it is also crucial to ensure that the selected method of extraction is environmentally friendly and thereby part of the green economy.

Despite MLE being an environmentally sustainable and low-cost approach to modify the efficiency of photosynthesis and assimilate partitioning of crop plants, various authors select chemical-based methods, when extracting moringa leaves. Such an extraction might, however, pose health risks and compromise environmental sustainability of plant production. Keeping in mind the reported positive impacts of MLE on plant growth and development, this study was conducted to compare the efficacy of organic and inorganic solvents, as well as various solvent/ water mixtures, to determine, which extraction method (s) yield (s) the best growth-enhancing effects of MLE, when applied foliarly to tomatoes and peppers.

MATERIALS AND METHODS

Plant material and growing conditions

Two experiments were performed separately in a glasshouse at the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'32.9"S 30°24'18.8"E). Plants of cherry tomato (*Solanum lycopersicum*), cv. 'Gardener's Delight', and pepper (*Capsicum annum*), cv. 'Revelation', were established from seed. Seedlings were transplanted, when the second true leaf was fully expanded, into 5 L plastic pots filled with Organic for Africa® (Greytown, South Africa) potting mix. Physical and chemical characteristics of the growing medium used in the study were analysed and amended before transplanting. The environmental conditions in the glasshouse were maintained at 25 ± 2°C, 65% relative humidity during the day and 15 ±

2°C, 72% relative humidity during the night with a light/dark cycle of 13 h/11 h. Plants were pruned and trained when necessary and irrigated with drip irrigation system supplying only water to the plants.

Preparation and analysis of moringa leaf extracts

Moringa leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa). The nutrient composition of the powder (Table 1) was analysed prior to the study. Several extractions were performed using different solvents. Moringa powder was extracted repeatedly until extracts were colourless.

Aqueous moringa leaf extracts (MLE HW, MLE CW)

For preparation of aqueous moringa extracts, the dry MLP was boiled, with continuous stirring, in distilled water (5 g MLP + 450 ml distilled water) for 30 min. For cold water extraction, 35 g MLP was soaked in 1 L distilled water for 48 h with continuous stirring. Each extract was then filtered through muslin cloth to remove any larger particles, before filtration through Whatman No. 1 filter paper and centrifugation at 20 000 g for 15 min. The supernatants were stored at 5°C.

Table 1. Chemical composition of dry *Moringa oleifera* leaf powder (average of five analyses).

Property	Value	Unit
N	4.49	%
P	0.36	%
K	1.62	%
C	44.28	%
Ca	2.71	%
Mg	0.51	%
Na	993.7	mg/kg
Zn	24	mg/kg
Cu	8.5	mg/kg
Mn	57	mg/kg
Fe	842	mg/kg
Al	795	mg/kg
B	18	mg/kg

Table 2. Phytochemical profile of different *Moringa oleifera* leaf extracts.

Components	Treatments				
	MLE CW	MLE HW	MLE ETH	MLE MET	Units
N	0.14	0.05	0.07	0.08	%
P	76.40	4.93	0.00	0.40	mg/L
K	436.47	53.07	0.13	5.87	mg/L
Ca	324.40	53.47	9.20	1.73	mg/L
Mg	105.20	15.33	0.40	0.13	mg/L
Na	46.53	41.60	0.80	0.53	mg/L
Mn	1.00	0.00	0.00	0.00	mg/L
Zn	0.00	0.00	0.00	0.00	mg/L
Fe	0.00	0.00	0.00	0.00	mg/L
Al	0.00	0.00	3.67	1.00	mg/L
Ascorbic acid	22.92	121.02	63.16	16.51	mg AAE/g DM
Total phenolics	204.48	216.60	103.46	174.41	mg GAE/g DM
Total flavonoids	215.33	155.64	487.89	472.67	mg QE/g DM
DPPH	14.18	125.16	66.83	5.18	mg AAE/g DM
FRAP	175.35	394.92	239.40	256.32	mg AAE/g DM

Maceration of dry MLP in methanol (MLE METH) and ethanol (MLE ETH)

Preparation of MLE was carried out according to (Vongsak et al., 2013), with slight modifications. Moringa leaf powder (35 g) was macerated in 1 L 80% methanol (MLE METH) or 80% ethanol (MLE ETH) (1:35, w/v) and left to stand for 48 h at room temperature with occasional shaking. The mixture was then filtered through Whatman No. 1 filter paper to obtain precipitate-free extracts. Extractions were carried out on the day of MLE application.

Quantitative analysis of phytochemicals and antioxidant capacity in moringa leaf extracts

Following the preparation of moringa leaf extracts using various solvents, the mineral analysis was performed using inductively coupled plasma mass spectrometry (ICPMS), while the phytochemical analysis for the quantitative detection of total phenolics, flavonoids and vitamin C, as well as total antioxidant activities (using DPPH and FRAP methods) was performed following the protocols described by Wang et al. (2003); Boonkasem et al. (2015); Rocchetti et al. (2019) and the results are presented in Table 2.

Experimental design and foliar application

A completely randomised design (CRD) with three replications was followed in this experiment. Fifteen healthy, similar-sized plants of both the pepper and the tomato cultivars were selected per treatment, with five plants per replicate. There were five applications (treatments); namely, hot water at 100°C used to extract moringa leaf powder (MLE HW), control (water), cold water (10°C) used to extract moringa leaf powder (MLE CW), ethanol (room temperature) used to extract moringa leaf powder (MLE ETH) and methanol (room temperature) used to extract moringa leaf powder (MLE METH). Applying only water (excluding moringa) did not have any effects on tested parameters, as preliminary results and a previous study determined (Ngcobo et al., 2021). The above-mentioned treatments were sprayed directly onto the leaves of selected pepper and tomato plants to run-off using a hand-held pressure sprayer. The first foliar application of MLE was carried out two weeks after transplanting and treatments were repeated weekly until fruit set as described in our previous study (Ngcobo et al., 2021).

Determination of dependent variables

Measurement of vegetative growth parameters

Data on growth parameters (number of leaves, number of branches, plant height) were recorded immediately after application of the initial moringa treatments, in 14 day-intervals, until maturity. Plant height was measured using a tape measure from the base of the stem to the tip of the terminal bud.

Measurement of yield parameters

A once-over harvest of red ripe fruit was performed. Fruit at the red ripe stage were harvested from each plant of the various replicates. Yield was recorded as the number of fruit/plant and the total fruit mass per plant (g).

Measurement of fruit quality parameters

External quality parameters (colour and size) of five fruit per plant from similar positions (75 fruit per treatment) were evaluated at harvest. The same fruit were utilized for destructive measurements.

Fruit colour

A chroma meter CR-400 (Minolta Co. Ltd., Osaka, Japan) was used to evaluate the surface colour of red-mature fruit. The colour parameters *L**, *a**, and *b** were determined, with (*L**) representing lightness (black (0) to white (100)), *a** characterising the green to red colour ranging from green (-) to red (+)), and *b** representing the yellow to blue colour axis (ranging from blue (-) to yellow (+)).

Fruit size

Fruit size (diameter) of all red-mature fruit in each treatment was measured using 150 mm digital callipers.

Carotenoid analysis

The concentration of carotenoids in the tomato fruit pericarp was determined spectrophotometrically according to (Nagata et al., 1992), using exact absorbance readings of the fruit pericarp material extracted in acetone-hexane (2:3). Individual fruit (1 g) were macerated in a 100 mL acetone: hexane (2:3) solution, centrifuged in a table-top centrifuge and the supernatant collected to read its absorbance at 663, 645, 505, 453 nm using a spectrophotometer (IRMECO GmbH, Germany, Model U2020). The following equations were used to calculate lycopene and β-carotene concentrations of the sample solution:

$$\text{Lycopene (mg/g FM)} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453} \quad (1)$$

$$\beta\text{-carotene (mg / g FM)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453} \quad (2)$$

In pepper fruit, however, the concentrations of carotenoids were determined according to Hornero-Méndez and Mínguez-Mosquera (2001); briefly, 0.5 g fresh pericarp sample was extracted with 75 mL acetone for 1 h, the extract filtered through Whatman No. 1 filter paper to obtain precipitate-free extracts, transferred to a volumetric flask and made up to 100 mL, before its absorbance was measured spectrophotometrically at 472 and 508 nm. The following equations were used to calculate the red (C^R) and the yellow (C^Y) carotenoid fractions:

$$(CR) = (A508 \times 2144.0 - A472 \times 403.3) / 270.9\ (\mu g\ red/mL) \quad (3)$$

$$(CY) = (A472 \times 1724.3 - A508 \times 2450.1) / 270.9\ (\mu g\ yellow\ carotenoids/mL) \quad (4)$$

Total soluble solids

A digital refractometer (RFM340+ refractometer, Bellingham and Stanley Ltd, Basingstoke, Hants, UK) was used to determine the percentage of TSS in the fruit juice.

Ascorbic acid

Ascorbic acid was quantitatively determined according to a slightly modified method of (Boonkasem et al., 2015). Briefly, freeze-dried pericarp portions (0.5 g DM) were extracted with 20 mL 3% (w/v) metaphosphoric acid, followed by shaking the sample at 300 rpm for 30 min. The extracts were subsequently centrifuged for 10 min at 2000 g; thereafter, 1 mL sample extract was added to 3 mL 0.2 mM 2, 6-dichlorophenolindophenol (DCPIP) and the solution was measured immediately at 515 nm after mixing for 15 s. The results were expressed in mg ascorbic acid per 100 g dry mass (mg/ 100 g DM).

Statistical analysis

Results obtained were subjected to one-way analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18th edition, VSN International, UK). Duncan's Multiple Range Tests was used to compare means. Treatments were accepted as significantly different at $P < 0.05$.

RESULTS

Growth and yield parameters

The effects of moringa leaf extracts (MLE) on vegetative growth parameters of tomato and pepper plants are presented in Figure 1. The plant height, number of leaves and number of ranches in pepper plants (Fig. 1a, c, and e) were significantly increased by the application of MLE HW, MLE ETH, MLE METH and MLE CW, while the control (water only) had a lesser and non-significant effect. The response to the MLE application was similar in tomatoes and peppers, as all MLE treatments increased the number of branches and leaves on tomato and pepper plants; similarly, plant height was positively affected (Fig. 1b, d, and f). More notably, MLE HW tended to outperform the organic solvent extracts; however, the difference was not significant. Overall, foliar application of MLE, more so of MLE HW, improved growth of pepper and tomato under greenhouse conditions (Fig. 1).

The effects of various MLEs on pepper and tomato yield parameters is presented in Figure 2 and 3, briefly, a significant increase in fruit diameter, fruit mass and the number of fruits per plant was observed following the foliar application of MLE in both pepper and tomato (Fig. 2 and 3). Treatment with MLE HW affected yield parameters more positively than other treatments (Fig. 2 a-d and Fig. 3a and b), while the MLE ETH and MLE METH treatments were not as effective, but still outperformed the control. On the other hand, even though MLE CW produced yield parameter values lower than other MLEs, it had, however, the potential to improve yield (Fig. 2a, c and d, and Fig. 3a).

Physical and chemical constituents

The application of MLE HW, MLE ETH and MLE METH significantly enhanced fruit colour (a^*, Fig. 4c and d) of red pepper and tomato; this was achieved mostly by treatment with MLE HW. These three extracts had also a significant effect on the lightness (L^*) of fruit, increasing the $L^*$of red pepper and tomato (Fig. 4a and b). The control and MLE CW, however, did not produce notable effects on colour of treated fruit. Similar to colour, TSS in peppers improved following MLE treatment, except for the cold-water treatment (MLE CW, Fig. 5a), whereas in tomatoes all MLEs affected fruit TSS positively (Fig. 5b).

The MLE treatment affected the tomato and pepper fruit carotenoids differently (Table 3). All the MLEs except the MLE CW had a significant effect on the red fraction of peppers while having no significant effect on the yellow fraction. Similar to peppers, all the treatments did not have a significant effect on lycopene and β-carotene, the major carotenoids in tomatoes; however, the MLE HW and MLE ETH had the potential to increase these carotenoids (Table 3). On the other side, the ascorbic acid concentration was significantly enhanced by all the MLEs except the aqueous extracts in tomatoes (Table 3).

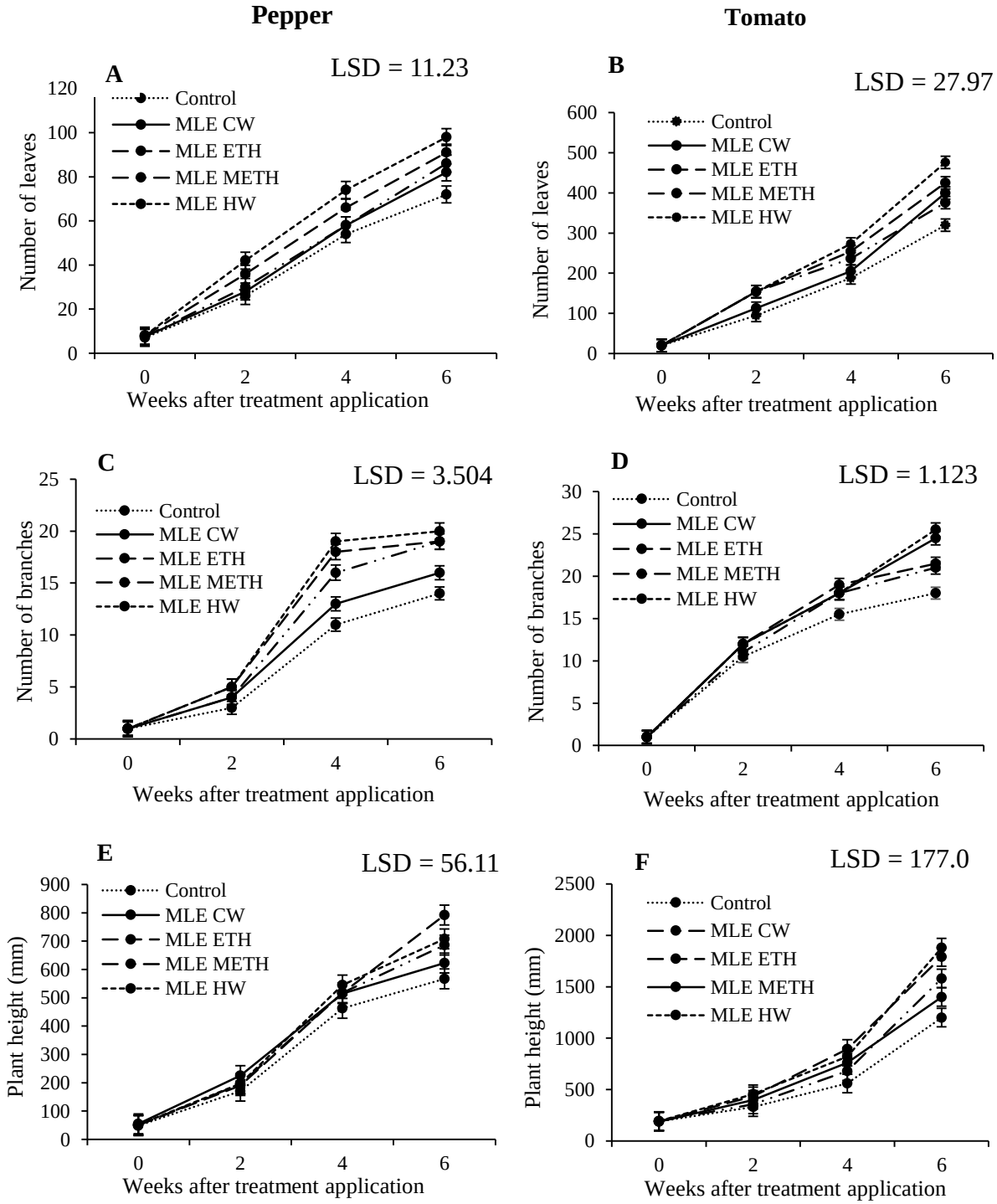

Fig. 1. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on vegetative growth parameters of red pepper and tomato. MLE CW = Moringa leaf powder extracted with cold water; MLE HW = Moringa leaf powder extracted with hot water; MLE ETH = Moringa leaf powder extracted with ethanol; MLE METH = Moringa leaf powder extracted with methanol. *(number of leaves and branches are measured per plant). A = pepper number of leaves/plant, B = tomato number of leaves/plant, C = pepper number of branches/plant, D = tomato number of branches/plant, E = pepper plant height, and F = tomato plant height.

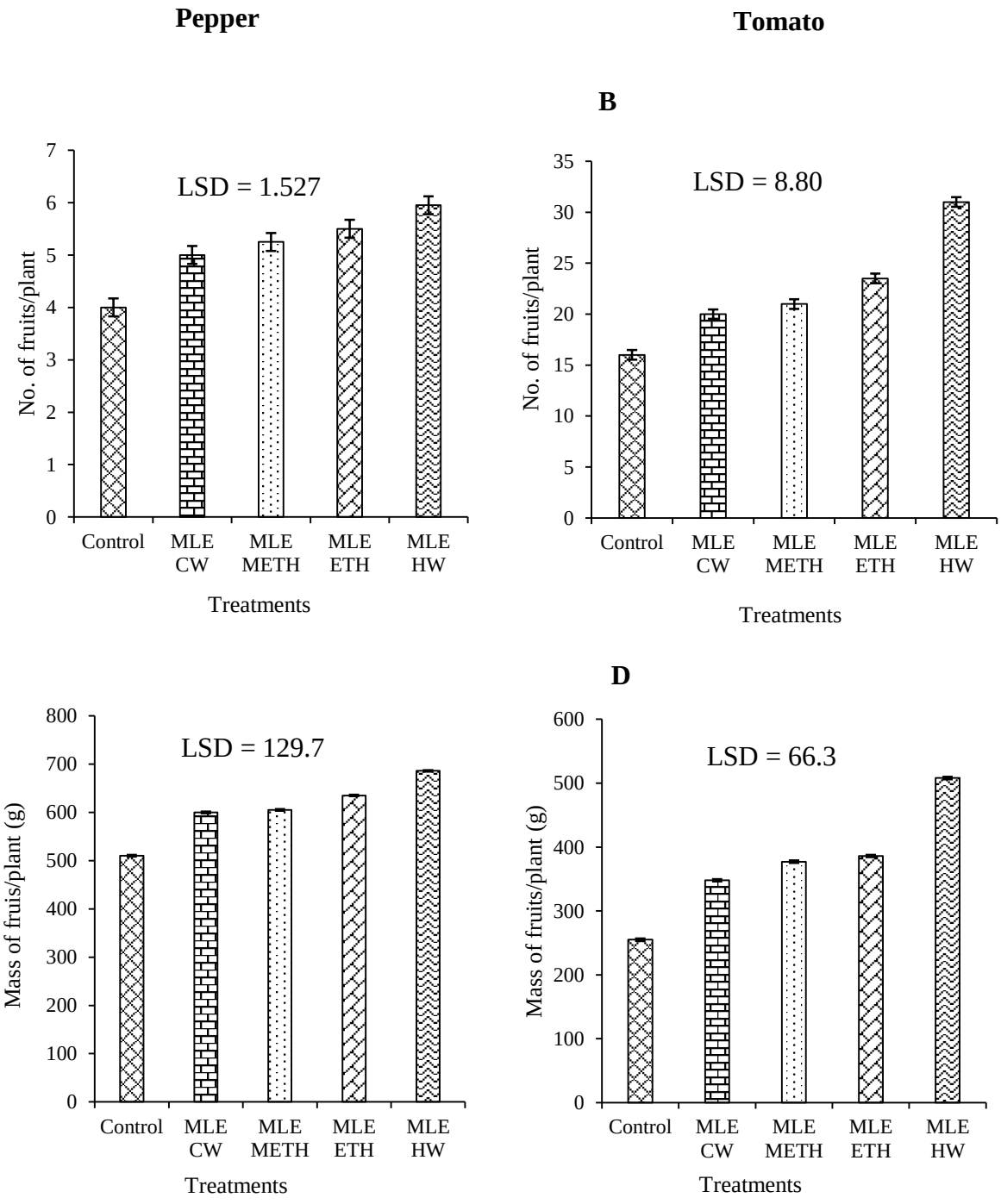

Fig. 2. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on yield parameters of red pepper and tomato. MLE CW = Moringa leaf powder extracted with cold water; MLE HW = Moringa leaf powder extracted with hot water; MLE ETH = Moringa leaf powder extracted with ethanol; MLE METH = Moringa leaf powder extracted with methanol. A = Number of pepper fruits/plant, B = Number of tomato fruits/plant, C = Mass of pepper fruits/plant, and D = Mass of tomato fruits/plant.

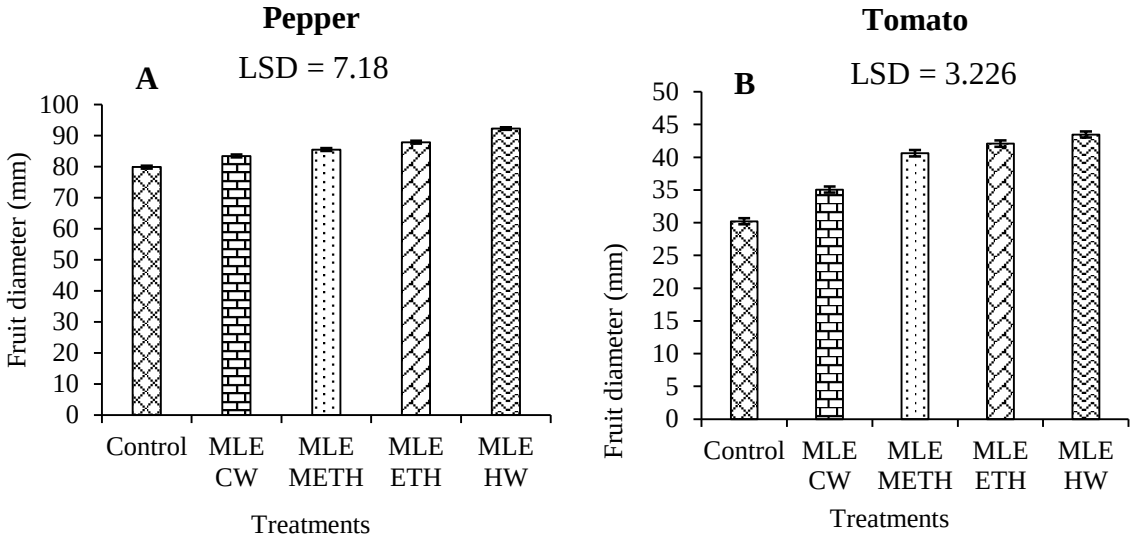

Fig. 3. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on fruit diameter of red pepper and tomato. MLE CW = Moringa leaf powder extracted with cold water; MLE HW = Moringa leaf powder extracted with hot water; MLE ETH = Moringa leaf powder extracted with ethanol; MLE METH = Moringa leaf powder extracted with methanol. A = Pepper fruit diameter, B = Tomato fruit diameter.

Table 3. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on carotenoid and ascorbic acid concentrations determined in red pepper and tomato fruit.

Treatment	Red pepper		Red tomato	
	Carotenoids (µg. g^{-1} DM)		Carotenoids (mg. g^{-1} FM)	
	Yellow fraction (C^Y)	Red fraction (C^R)	Lycopene	β-carotene
Control	2.434a	4.231a	43.32a	5.956a
MLE CW	2.787a	6.587a	51.68a	6.555a
MLE HW	3.481a	10.914b	52.63a	8.274a
MLE ETH	3.051a	7.812ab	51.25a	10.634a
MLE METH	3.246a	10.876b	42.35a	9.313a
LSD	0.993	3.983	12.84	4.894
CV (%)	21.5	35.8	21.5	19.4
Treatment	Red pepper		Red tomato	
	Ascorbic acid (Vitamin C) (mg.$100g^{-1}$ DM)			
Control	400.3a		253.4a	
MLE CW	484.1b		262.7a	
MLE HW	487.4b		282.4abc	
MLE ETH	481b		308.6bc	
MLE METH	483.3b		319.6c	
LSD	12.89		46.99	
CV (%)	2.2		16.6	

MLE CW = Moringa leaf powder extracted with cold water; MLE HW = Moringa leaf powder extracted with hot water; MLE ETH = Moringa leaf powder extracted with ethanol; MLE METH = Moringa leaf powder extracted with methanol; C^R = Red carotenoid fraction; C^Y = Yellow carotenoid fraction. *Values followed by different lower-case letters in each column are statistically different at $P \leq 0.05$.

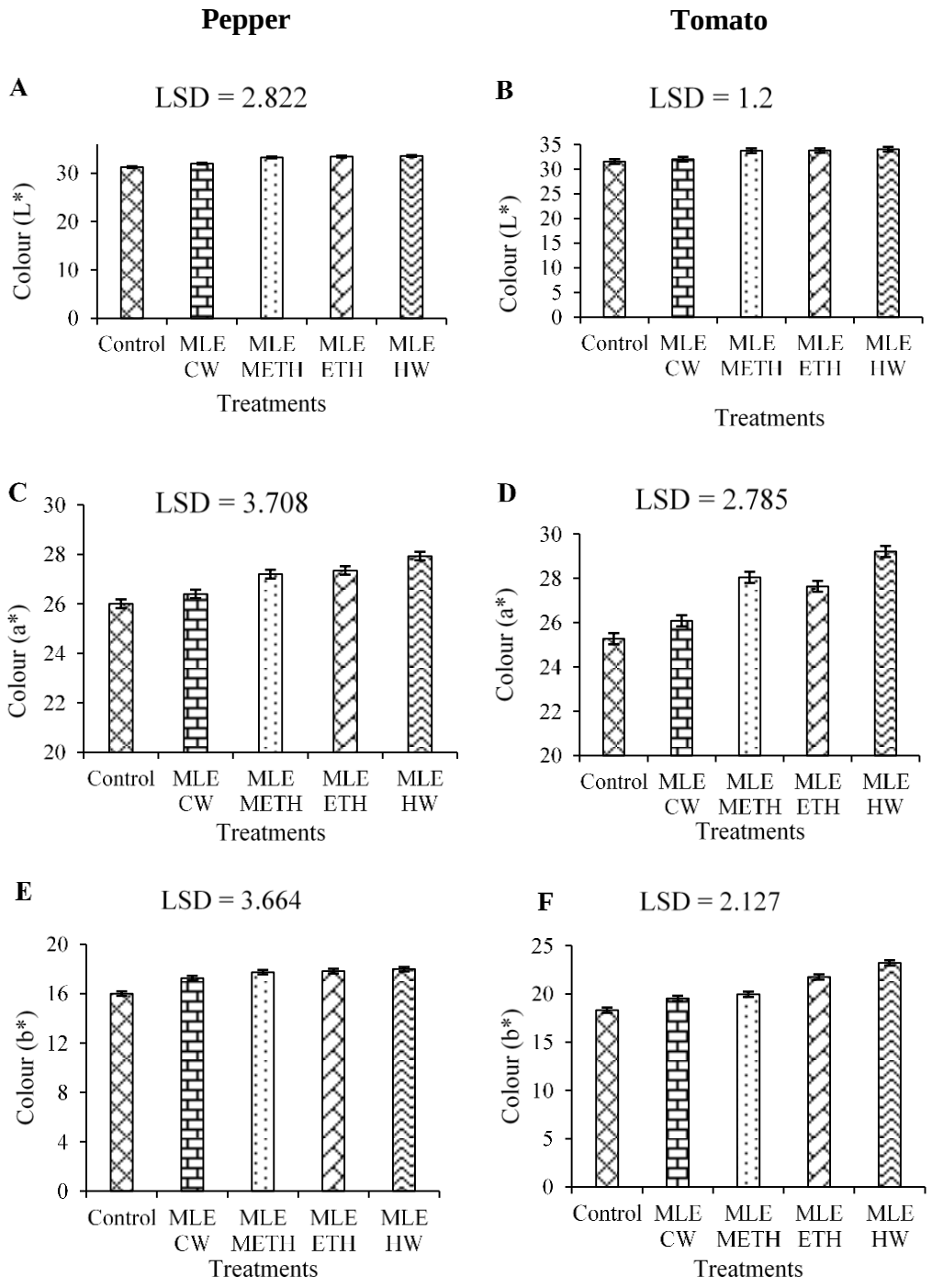

Fig. 4. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on colour coordinates (*L**, *a** and *b**) of red pepper and tomato. MLE CW = Moringa leaf powder extracted with cold water; MLE METH = Moringa leaf powder extracted with methanol; MLE ETH = Moringa leaf powder extracted with ethanol; MLE HW = Moringa leaf powder extracted with hot water. . A = pepper colour parameter (L*, lightness), B = tomato colour parameter (L*, lightness), C = pepper colour parameter (a*, green-red axis), D = tomato colour parameter (a*, green-red axis), E = pepper colour parameter (b*, yellow-blue axis), and F = tomato colour parameter (b*, yellow-blue axis).

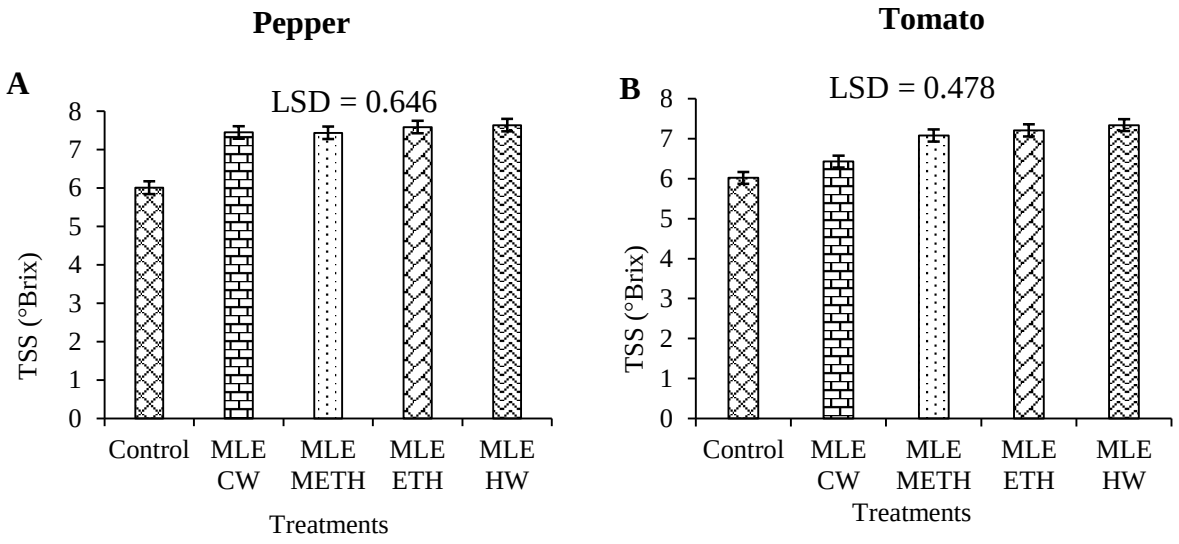

Fig. 5. Effect of foliar application of moringa leaf extract (MLE) prepared using different solvents on total soluble solids (TSS) of red pepper and tomato. MLE CW = Moringa leaf powder extracted with cold water; MLE METH = Moringa leaf powder extracted with methanol; MLE ETH = Moringa leaf powder extracted with ethanol; MLE HW = Moringa leaf powder extracted with hot water. A = Pepper TSS, B = Tomato TSS.

DISCUSSION

The use of biostimulants, as growth and yield enhancers, has recently gained enormous attention. This is due to the ability of such biostimulants to increase plant vigour and yield parameters by modifying plant physiological reactions and increasing plant nutrient acquisition (Zulfiqar et al., 2020). The present study evaluated, hence, the effect of foliar application of MLEs, extracted using different solvents, on pepper and tomato plant growth and development.

The effect of moringa leaf extracts on vegetative growth and yield characteristics

In the present study, foliar application with moringa leaf extracts enhanced the vegetative growth attributes of tomato and pepper plants (Fig. 1). It is not surprising that methanolic and ethanolic extracts enhanced the vegetative growth of the vegetable crops tested in this study since the effect of these extracts has been tested extensively; however, the performance of the aqueous extracts especially the 'MLE HW' tended to outperform the organic solvent extracts; but the difference was not significant. The effectiveness of MLE in enhancing plant height, number of leaves and number of branches in pepper and tomato seedlings (Fig. 1) could be due to the high nutrient concentration in the extracts, as MLEs have been reported to be particularly high in N, K, Ca, Mg, Fe, Zn, Mn, P, these essential nutrients play a pivotal role on growth and development of plants (Table 2; Abd El-Mageed et al., 2017; Gopalakrishnan et al., 2016). In addition to minerals, MLE also contains high concentration of certain plant growth-promoting substance, such as cytokinin zeatin (Rehman et al., 2017), as well as in antioxidant phytonutrients, such as vitamin C, flavonoids and phenolics (Table 2) (Foidl et al., 2001; Siddhuraju et al., 2003; Sreelatha et al., 2011). As a rich source of these compounds, MLE is an ideal natural growth enhancer (Jacob et al., 2011; Makkar et al., 2007); moreover, cytokinin zeatin is a plant hormone, present in high concentrations in moringa leaves

(Yasmeen, 2011). Zeatin plays a crucial role in cell division, resulting in cell multiplication, and general cell enlargement or elongation; leading to growth promotion of greenhouse-grown pepper and tomato plants (Fig. 1a, b, c, d, f) (Anwar et al., 2007; Siddhuraju et al., 2003). In addition, the high concentration of both total phenolics and ascorbic acid (Table 2) from the MLE HW, particularly, compared to other MLEs could be the reason this treatment performed well. The rapid increase in vegetative growth following MLE application is not only accredited to the presence of cytokinins. Besides zeatin, there is also dihydrozeatin, isopentyladenine and high content of crude protein in MLE (Busani et al., 2011). These authors studied the crude protein concentration, and the growth-promoting hormones present in moringa leaves and found that this protein/hormone combination is responsible for growth acceleration. Numerous studies on the response of several crops, such as pear (*Pyrus communis*) (El–Hamied et al., 2015), 'Kinnow' mandarin (*Citrus nobilis x Citrus deliciosa*) (Nasir et al., 2020), sunflower (*Helianthus annuus*) (Iqbal et al., 2020); tomato (Ngcobo et al., 2021) to MLE have been reported; however, there is no study that reported the comparison of hot and cold aqueous moringa leaf powder extracts, or ethanolic and methanolic moringa leaf powder extracts on fruit vegetable crops. It is clear from the analysis presented in table 2 that inorganic solvents (hot and cold water) are also suitable for extracting phytochemicals due unique properties they possess as they are non-carbon-based solvents in nature (Matshediso et al., 2015). Choosing a suitable moringa leaf powder extraction solvent is particularly important, as MLEs can be used in organic food production and the use of water, to extract the moringa leaf powder, is an environmentally friendly method, while organic solvents pose several health problems.

In terms of yield, treatment with MLE HW, again, affected yield parameters more positively than other treatments (Fig. 2a-d and Fig. 3a and b), while the chemical-based treatments were not as effective, but still outperformed the control. It was unsurprising that the application of only cold water (control) to the leaves of tomato and pepper did, in accordance with our recent study (Ngcobo et al., 2021), not produce any effect on yield parameters. The study by Foidl et al. (2001) supports our study, as these authors reported that MLE applied in low concentration showed prominent effects on yield. Similarly, (Cheema et al., 2013) demonstrated that the application of watery moringa extract to wheat (*Triticum aestivum*) improved crop yield considerably. Iqbal et al. (2020) conducted a comparative study using cold, aqueous extract of moringa leaves as well as roots to improve sunflower growth and yield, and reported that moringa extracts, obtained by extraction with cold water, significantly improved growth and yield of sunflower. The high content of vitamin C, total carotenoids and phenolics (Table 2) in aqueous extracts particularly MLE HW could be the possible reason this extract performed well by modifying plant physiological reactions and increasing plant nutrient acquisition. High antioxidant activity in both water extracts potentially resulted in a high photosynthetic rate because antioxidants prevent chlorophyll degradation, protect against oxidative damage, and improve nutrient uptake by the roots (Moyo et al., 2011). There seems, however, no study that investigated the comparison of extraction solvent on the efficacy of fruit yield and quality.

Moringa leaves are a rich source of natural growth-enhancing substances and contain amino acids, minerals, ascorbate and other active ingredients, which are considered "growth supporters" that are likely to enhance yield (Mahmood et al., 2010). These compounds were possibly present on the MLEs, more so on MLEs derived from water solvents. Foliar application of moringa hot water extract, and that of MLEs produced with inorganic solvents, increased the resistance of pepper and tomato plants to pests and diseases and also increased growth parameters (Fig. 1). The number and size of roots was also enhanced by moringa application, seemingly aligned with plants creating more (Fig. 2) and superior fruit (Fig. 3)

resulting in increased yield. In addition, an increase in yield may be due to the stimulating effect of MLE, particularly when MLE is produced via a hot water extract, on the vigour of plants and photosynthate accumulation, stimulating pepper and tomato plants to produce larger and more fruit (Abdel-Rahman et al., 2020).

The effect of moringa leaf extracts on physical and chemical constituents of pepper and tomato

Recently, consumers' attention to the intake of fruit and vegetables with a high phytochemical concentration has increased significantly. Consumers are aware of the health benefits associated with the consumption of nutrient-rich fruit and vegetables, including the prevention of chronic, degenerative disorders. In the present study, the effect of MLE on colour, sugars and carotenoids of two solanaceous crops was evaluated at harvest (Fig. 4, 5; Table 3). Studies of this nature focus mostly on growth and yield attributes, while studies evaluating quality parameters, particularly postharvest, are scarce. This study revealed that MLE HW, MLE ETH and MLE METH significantly enhanced fruit colour (a^*, Fig. 4c and d) of red pepper and tomato; again, treatment with MLE HW outperformed other treatments. These three extracts had also a significant effect on the lightness (L^*) of fruit (Fig. 4a and b). These findings are important for producers and consumers alike, as colour is one of the main characteristics of foodstuffs noted by consumers, relating directly to consumer purchase decisions and fruit market value. Similar to colour, TSS in peppers improved following MLE treatment, except for the MLE CW (Fig. 5a), while in tomatoes all MLEs affected fruit TSS positively (Fig. 5b). The increase in pepper sugars may be due to the high content of zeatin in MLE, a cytokinin involved in the translocation of photo-assimilates towards the fruit (Yasmeen, 2011). On the other hand, carotenoids in red peppers, probably the major pepper pigments capsanthin and capsorubin, were significantly enhanced by MLE treatments, excluding the cold water MLE treatment and the control (Table 3), while MLEs seemingly enhanced lycopene and β-carotene on red tomatoes (Table 3), the activity of antioxidants in the extracts could potentially enhance the concentration of phytochemicals in the treated fruits. These results are in line with those of (Basra et al., 2016), who reported that sugars and carotenoids, particularly lycopene and β-carotene in cherry tomato, were enhanced by foliar application of MLE. Manuscripts assessing postharvest quality of fruit and vegetables after applying preharvest moringa treatment are scarce. Foliar MLE application significantly enhanced vitamin C in peppers, fruit known to be an excellent source of vitamin C (Guil-Guerrero et al., 2006), while the organic-solvent extracts significantly enhanced this parameter compared with the control in tomato fruit; the aqueous extracts, however, only had a potential to do so (Table 3).

The increased carotenoid concentrations in the pericarp and, therefore, colour in pepper and tomato fruit may be attributed to the presence of various secondary metabolites in MLE, such as β-carotene, ascorbate, phenolics and other antioxidants (Table 2; Foidl et al., 2001), as well as zeatin (Yasmeen, 2011). Together with the minerals (Ca, K) found in MLE, zeatin, through its effects on root growth (Atzmon et al., 1993), is likely to have promoted nutrient uptake and photosynthesis, ultimately resulting in an increase in fruit antioxidant concentration or maybe MLE caused a slight stress to plants that contributed to an increase in fruit antioxidant concentration. Carotenoids act as radical scavengers, preventing oxidative damage caused by free radicals (Rao et al., 2007). The carotenoid concentration of fruit, including tomatoes and peppers, determines fruit colour and ultimately also affects the nutritional status of the fruit (Cömert et al., 2020).

The effects of MLE differed with extraction solvent. The use of water as an extraction solvent is slowly gaining popularity due to its high extraction efficiency (Castro-Puyana et al.,

2017). Water is obviously the most readily available and environmentally friendly extraction solvent. While the polarity of water does not allow it to dissolve hydrophobic substances which are the most active compounds present in medicinal plants, such as moringa, it has been demonstrated that increasing water temperature improves the extraction efficiency of organic compounds (Castro-Puyana et al., 2017). The ability of water to efficiently extract growth-stimulating compounds presented in Table 1 is linked to the fact that the dielectric constant (polarity) of water can be reduced significantly with increasing temperature (Castro-Puyana et al., 2017; Ong et al., 2006). The polarity of water at 25°C is around 80, indicating that it is an extremely polar solvent (Ong et al., 2006); however, at temperatures higher than 100°C water's polarity is reduced to approximately 27, falling between the polarity of methanol (33) and ethanol (24) at 25°C (Ong et al., 2006). Hot water might, therefore, have been able to extract certain compounds from moringa powder that were not extractable by other solvents. It must, however, be noted that while increasing the water temperature to the boiling point resulted in higher extraction efficiency, the high temperature could also have degraded other compounds (Hawthorne et al., 1994; Miller et al., 1998; van Bavel et al., 1999). Apart from our study, the higher extraction efficiency of hot water was aslo witnessed when antioxidant compounds were extracted from rosemary plants and catechin and epicatechin from tea leaves and grape seeds; these antioxidant compounds could be successfully extracted by hot water at the boiling point, as reviewed by (Ong et al., 2006). A study by (Matshediso et al., 2015) further revealed that extraction of moringa leaf powder with hot water results in a high yield of phytochemicals and this study supports our results.

CONCLUSION

Various solvents used in this study to prepare MLE differ in extraction efficacy. Certain MLEs, particularly hot water MLE (MLE HW) and ethanolic MLE (MLE ETH), were more effective in enhancing vegetative growth parameters, yield and fruit colour of both pepper and tomato plants or fruit. Treatment with MLE HW and MLE METH enhanced the carotenoid concentration in pepper; therefore, MLE application holds the potential to enhance the carotenoid and vitamin C of fruit, thereby improving the nutritive value of these commodities. Results presented in this research are of high significance to both commercial and small-scale pepper, as well as tomato growers, as hot water extracts which is an environmentally friendly, financially feasible and sustainable approach, outperformed other treatments. The positive effect of MLE HW on tomato and pepper, therefore, requires further investigation.

Conflict of interest
The authors declare that they have no conflict of interest

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Data availability statement
The data that support the findings of this study are available from the corresponding author, [BLN], upon reasonable request

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Ripening and postharvest quality of guavas treated with plant regulators

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ABSTRACT

Purpose: Guava is a tropical and subtropical fruit recognized for its nutritional quality. However, o it is a climacteric fruit, that is, with high respiratory activity and ethylene production during ripening, it becomes extremely perishable under environmental conditions, requiring conservation technologies that allow its commercialization without compromising post-harvest quality during storage. Therefore, the objective of this study was to evaluate the effect of plant regulators (gibberellic acid – GA_3 and ethephon) on ripening and quality preservation during storage at room temperature. **Research method**: Physiologically mature guavas (stage 3) were harvested in a commercial orchard, selected and sanitized in a chlorinated solution, and immersed in the following solutions: distilled water (control), GA_3, and ethephon (150 $mg.L^{-1}$) for a period of 10 minutes and subsequently stored under room temperature conditions (28 ± 2 ºC) for 12 days with physical-chemical quality assessments carried out every three days. **Findings**: Treatment with GA_3 provided lower values of mass loss, soluble solids, titratable acidity, and total sugars, in addition to higher values of firmness and vitamin C of the fruits analyzed, while the opposite effect was observed in guavas treated with ethephon. In general, the postharvest application of GA_3 delays ripening, making it possible to extend marketing for up to 9 days, on the other hand, ethephon anticipates ripening, making them fully ripe after 6 days of storage. **Research limitations**: There were no limitations to carrying out this research. **Originality/Value**: This research's results support staggering the ripening of guava over time, allowing the fruit's commercialization period to be extended.

INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical and subtropical fruit belonging to the Myrtaceae family and recognized for its high nutritional content, such as minerals (Ca, Mg, K, P), vitamins (A, thiamine, riboflavin), bioactive compounds (phenolic, flavonoids, ascorbic acid), pectin and fibers, among others (Shao et al., 2023). However, all this nutritional potential is limited by the postharvest physiology of the fruit, which is classified a climacteric fruit, and has high perishability, and limits its commercialization potential.

Knowledge of fruit physiology is important, as it provides technical support for extending storage time without altering their physical, sensory, and nutritional characteristics (Karagiannis, 2024). In the case of guava, loss of quality is generally associated with immediate softening and color change from green to yellow during postharvest storage (Anjum et al., 2020; Supa et al., 2024). Therefore, postharvest technologies such as the application of plant regulators must be adopted to increase storage potential and preserve quality.

Studies have shown that the application of plant regulators postharvest can reduce physiological disorders, improve quality, increase shelf life, and standardize ripening (Mostert et al., 2024). Gibberellins, such as gibberellic acid (GA_3), are essential plant hormones in regulating the growth and development of fruits (Kamiab et al., 2023). Recent studies have demonstrated that gibberellins are important in delaying the ripening and senescence of horticultural crops, improving internal and external quality, and resistance to stress and disease (Zhang et al., 2023). In turn, ethylene is an important regulator of various aspects of development and physiological processes: from seed dormancy to germination, fruit ripening, defense against biotic and abiotic stresses. Regarding its role in ripening, ethylene accelerates the deterioration of vegetable products resulting in a shorter shelf life (Asrey et al., 2023), but depending on management, its application can anticipate maturation allowing marketing in a short period of time.

Considering that the exogenous application of GA_3 and ethylene can delay or anticipate ripening, allowing the expansion of the supply in the commercialization of guava at different times, this study aimed to evaluate the effect of the application of these plant regulators in the standardization of ripening and maintenance of quality during storage under ambient conditions (28 ºC).

MATERIALS AND METHODS

Plant material

Physiologically ripe guavas 'Paluma' were harvested at maturity stage III with light green epicarp color (Azzolini et al., 2004) in a commercial orchard located in the municipality of Marituba, PA, Brazil at 1° 21′ 19″ South, 48° 20 ′36″ West. When harvesting, fruits free from physiological defects or affected by pests and diseases were taken into consideration. These were packed in thermal boxes and transported to the Postharvest Laboratory of the Isacta Institute, Belém, PA, Brazil.

Application of treatments and storage

In the laboratory, the fruits were washed in running water, sanitized in a chlorinated solution (150 $mg.L^{-1}$), and air-dried at room temperature (28 ºC). These were then divided into batches (75 fruits) and immersed in the following solutions: control (distilled water), GA_3 (150 $mg.L^{-1}$) Sigma Aldrich®, and ethephon (150 $mg.L^{-1}$) Ethrel® for 10 minutes, with subsequent drying under a screen nylon to drain excess liquid. The concentration of the regulators was

defined after preliminary tests. After drying, the fruits (5 units) were placed in polyethylene styrofoam trays, and stored under ambient temperature conditions (28 ± 2 ºC) simulating points of sale for 12 days.

Evaluation of physical-chemical quality

Evaluations were carried out in triplicate at intervals of three days under the following aspects:

The shelf life of the fruits was measured in days from the initiation of the experiment up to 25% rotting.

Fresh mass loss determined by weighing the fruits on an analytical balance (Mars, model AS 2000, São Paulo, Brazil) at the beginning of the experiment (initial weight) and again at each evaluation period (final weight). The results were expressed as a percentage (percentage) based on the following equation (1):

PMF (percentage) = Initial mass - Final mass / Initial mass × 100 (1)

Pulp firmness determined with a digital penetrometer (53200 TR Turoni, Italy), with an 8 mm tip, taking 2 readings on opposite sides in the region with the largest diameter of the fruits. The fruit peel was previously removed with the help of a manual peeler. The data were expressed in Newtons (N), considering the average of the two readings per fruit.

The color of the peel and pulp was determined using a colorimeter (Minolta, CR-400, Osaka, Japan), positioned in the central region of the opposite sides of the fruit to obtain the variables L*, a* and b*, which were used to calculate luminosity (L*), chromaticity (C*) and hue angle (hº), respectively (McGuire, 1992).

Soluble solid content (SSC) of the liquid obtained by pressing 10 g of pulp was determined using a digital refractometer (Alpha, Atago Co., Ltd, Japan) and the results expressed as °Brix. Titratable acidity (TA) was determined by titrating 10 g of pulp with 0.1 N NaOH, using 0.1% phenolphthalein as an indicator. The results were expressed as equivalent grams of citric acid in 100 g of pulp. The pH of the samples was measured using a pHmeter (Thermo Scientific, Orion 3 Star, USA) directly on the fruit pulp. The vitamin C (AsA) content was determined by titrating a 10 mL aliquot of the extract with 2,6-dichlorophenolindophenol. The results were expressed as mg 100 g^{-1} (AOAC, 2020).

Total sugars determined according to the methodology described by Yemn and Willis (1954). The extract was obtained by diluting 1.0 g of the pulp in 100 mL of distilled water. The samples were prepared in an ice bath, adding 150 µL of the extract, 850 µL of distilled water and 2.0 mL of 0.2% anthrone solution to a tube, followed by shaking and resting in a water bath at 100 °C for 8 minutes. The reading was carried out on a spectrophotometer at 620 nm, using glucose as a reference to obtain the standard curve and the results were expressed in g.100g-1 pulp.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design (CRD), with a factorial scheme (3×5), with PGR treatments (control, GA_3, and ethephon), and 5 evaluation times (0, 3, 6, 9, and 12 days), with three replications and five fruits composing the experimental plot. The results were analyzed using analysis of variance and presented with mean and standard deviation after Tukey's test at a 5% level of significance, with the aid of the statistical program SAS® version 9.1.

RESULTS

The shelf life of guavas was significantly affected by plant regulators. In general, the longest shelf life was observed in fruits treated with GA_3 (12 days), control (9 days), and the shortest in fruits exposed to ethephon (3 days) (Fig. 1).

There were significant effects of treatments and days of storage ($p<0.05$) on the loss of mass and firmness of 'Paluma' guavas treated with plant regulators and stored under ambient conditions (28 ± 2 ºC) for 12 days (Table 1).

There was an increase in mass loss, regardless of the treatments applied throughout the storage period. Generally, guavas treated with GA_3 showed the lowest mass losses up to the 12th day of storage (8.96%). On the other hand, fruits treated with ethephon exhibited the greatest losses with an average percentage exceeding 10% after 6 days of storage (Table 1).

Firmness was significantly affected by treatments and storage time ($P<0.05$). It was observed that fruits treated with GA_3 presented the highest firmness values and that this regulator kept the fruits firmer until the 12th day (26.62 N). On the other hand, the lowest firmness was observed in guavas treated with ethephon, which on the 3th day of storage already stood out from the different treatments with lower values (53.89 N), showing total softening on the 6th day of storage (17.65 N) (Table 1).

There was a significant effect of regulators ($P<0.05$) on the color of the peel and pulp during storage (Table 2).

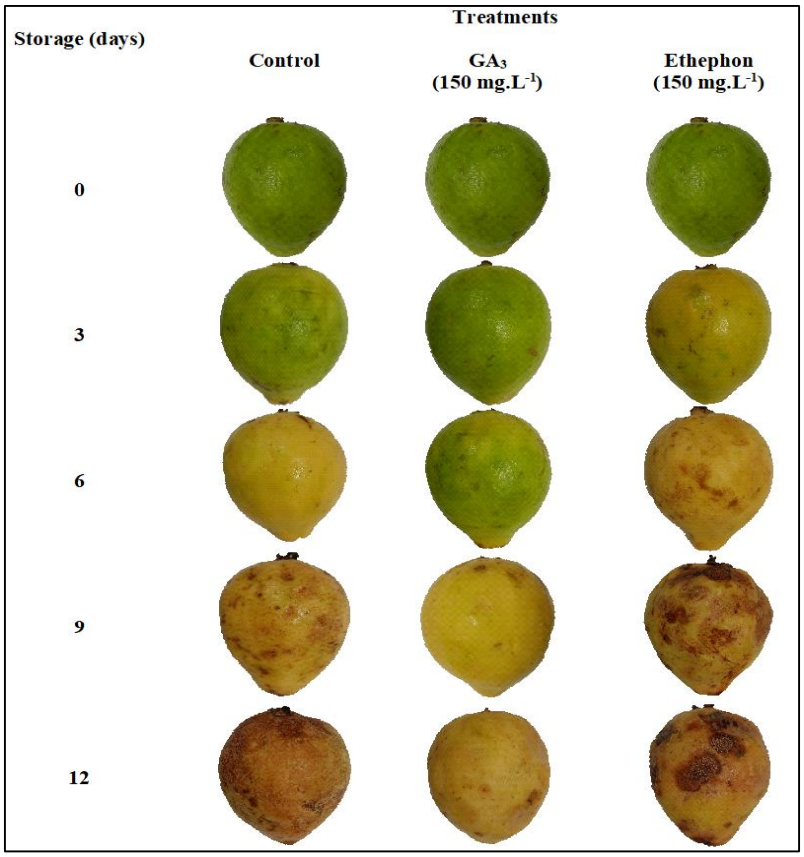

Fig. 1. Shelf life and visual quality of guavas treated with GA_3 and ethephon during storage at ambient conditions (28 ± 2 ºC) for 12 days.

Table 1. Evolution of mass loss (%) and reduction in firmness (N) in guavas treated with GA_3 and ethephon during storage under ambient conditions (28 ± 2 ºC) for 12 days.

Storage (days)	Treatments		
	Control	GA_3 (150 mg.L^{-1})	Ethephon (150 mg.L^{-1})
	Mass loss (%)		
0	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.00 ± 0.00 Aa
3	2.89 ± 0.36 bB	1.69 ± 0.06 bA	3.86 ± 0.31 bC
6	5.54 ± 0.29 cB	2.74 ± 0.19 cA	10.05 ± 0.33 cC
9	9.31 ± 0.43 dB	4.07 ± 0.33 dA	*
12	*	8.96 ± 0.41 eA	*
CV (%)	4.11		
	Firmness (N)		
0	86.11 ± 2.10 aA	86.11 ± 2.10 aA	86.11 ± 2.10 aA
3	80.19 ± 2.05 bA	84.05 ± 2.83 aA	53.89 ± 1.28 bB
6	52.18 ± 1.94 cB	63.89 ± 2.11 bA	17.65 ± 1.92 cC
9	21.07 ± 3.65 dB	41.77 ± 1.98 cA	*
12	*	26.62 ± 3.71 dA	*
CV (%)	3.91		

Means followed by the same letter in the row (treatments) and in the column (storage days) do not differ from each other using the Tukey test ($p<0.05$). *not evaluated.

Regarding luminosity (L*), a reduction of more than 65% was observed in the values of the peel and pulp of fruits treated with ethephon and control on the 6th and 9th days of storage, respectively, while the treatment with GA_3 maintained the L* with higher values in the peel (16.86 L*) and pulp (21.05 L*) for up to 12 days (Table 1).

In the peel, chromaticity (C*) reduced by 40.63% in fruits treated with ethephon after 6 days of storage, while in the pulp there was an increase of 38.82% for the same period (Table 2). In turn, fruits treated with GA_3 exhibited higher and lower C* values in the peel (29.77 C*) and pulp (54.04 C*) up to the 9th day of storage in relation to the control (16.74 and 65.32), respectively ($p<0.05$).

Likewise, the hue angle (hº) reduced in the peel and increased in the pulp at different fruit storage periods. In the peel of guavas treated with ethephon, a sharp reduction of around 78.47% was observed between day 0 and day 6 of storage, while in the pulp an increase (35.49%) was observed for the same period (Table 2). In contrast, fruits treated with GA_3 exhibited a slower reduction in peel hº, approximately 48.67% lower than control fruits over 9 days. In the pulp, a smaller increase (8.45%) was also observed in relation to control fruits for the same period (Table 2).

The effects of regulators on physicochemical quality (soluble solids, titratable acidity, pH, vitamin C, and total sugars) are presented in Table 3.

Regarding soluble solids (SS), there was an increase as storage progressed, regardless of the treatments applied (Table 3). In general, SS levels increased until the 6th (10.41 ºBrix), 9th (10.67 ºBrix), and 12th day (10.52 ºBrix) in fruits treated with ethephon, control, and GA_3, respectively ($p<0.05$).

Table 2. Coloration of peel and pulp in guavas treated with GA_3 and ethephon during storage at ambient conditions (28 ± 2 ºC) for 12 days.

Storage (days)	Treatments (peel)		
	Control	GA_3 (150 mg.L^{-1})	Ethephon (150 mg.L^{-1})
	Luminosity (L*)		
0	74.19 ± 2.05 aA	74.19 ± 2.05 Aa	74.19 ± 2.35 aA
3	63.36 ± 1.97 bB	71.26 ± 1.71 bA	43.86 ± 1.86 bC
6	44.31 ± 3.06 cB	62.50 ± 2.47 cA	25.05 ± 2.38 cC
9	23.39 ± 2.53 dB	44.07 ± 2.76 dA	*
12	*	16.86 ± 3.41 eA	*
CV (%)	4111		
	Chroma (C*)		
0	50.13 ± 1.74 aA	50.13 ± 1.74 aA	50.13 ± 1.74 aA
3	40.11 ± 2.35 bA	46.15 ± 2.31 aA	29.76 ± 2.56 bB
6	31.18 ± 1.44 cB	40.89 ± 2.63 bA	11.04 ± 1.53 cC
9	16.74 ± 2.14 dB	29.77 ± 1.18 cA	*
12	*	13.68 ± 3.02 dA	*
CV (%)	2.83		
	Angle hue (hº)		
0	82.01 ± 2.36 aA	82.01 ± 2.36 aA	82.01 ± 2.36 aA
3	80.11 ± 2.05 bA	84.05 ± 2.83 aA	43.89 ± 1.28 bB
6	52.18 ± 1.94 cB	64.89 ± 2.11 bA	17.65 ± 1.92 cC
9	21.14 ± 3.65 dB	41.77 ± 1.98 cA	*
12	*	23.67 ± 3.71 dA	*
CV (%)	3.05		
Storage (days)	Treatments (pulp)		
	Control	GA_3 (150 mg.L^{-1})	Ethephon (150 mg.L^{-1})
	Luminosity (L*)		
0	58.34 ± 1.81 aA	58.34 ± 1.81 aA	58.34 ± 1.81 aA
3	47.04 ± 2.49 bA	51.04 ± 3.93 bA	35.11 ± 1.21 bC
6	28.76 ± 2.18 cB	42.33 ± 2.06 cA	19.58 ± 2.54 cC
9	16.44 ± 1.71 dB	34.07 ± 1.80 dA	*
12	*	21.05 ± 2.13 eA	*
CV (%)	3.47		
	Chroma (C*)		
0	33.06 ± 1.74 aA	33.06 ± 1.74 aA	33.06 ± 1.74 aA
3	39.61 ± 2.73 bA	37.67 ± 2.54 aA	49.76 ± 2.56 bB
6	51.10 ± 1.41 cB	47.12 ± 2.91 bA	64.04 ± 1.53 cC
9	65.32 ± 2.14 dB	54.04 ± 1.83 cA	*
12	*	65.11 ± 2.19 dA	*
CV (%)	2.86		
	Angle hue (hº)		
0	46.22 ± 2.06 aA	46.22 ± 2.06 aA	46.22 ± 2.06 aA
3	52.91 ± 1.33 bA	50.93 ± 1.47 aA	59.41 ± 2.54 bB
6	66.18 ± 1.61 cB	59.61 ± 1.18 bA	71.65 ± 1.46 cC
9	72.14 ± 2.20 dB	66.04 ± 2.50 cA	*
12	*	73.17 ± 1.03 dA	*
CV (%)	3.72		

Means followed by the same letter in the row (treatments) and in the column (storage days) do not differ from each other using the Tukey test ($p<0.05$). *not evaluated.

Table 3. Soluble solids, titratable acidity, pH, vitamin C, and total sugars in guavas treated with GA_3 and ethephon during storage at ambient conditions (28 ± 2 ºC) for 12 days.

Storage (days)	Treatments		
	Control	GA_3 (150 mg.L^{-1})	Ethephon (150 mg.L^{-1})
	Soluble solids (ºBrix)		
0	8.61 ± 0.26 aA	8.61 ± 0.26 aA	8.61 ± 0.26 aA
3	9.17 ± 0.31 bB	8.87 ± 0.34 aA	9.50 ± 0.35 bC
6	9.95 ± 0.35 bB	9.28 ± 0.21 aA	10.41 ± 0.23 cC
9	10.67 ± 0.26 cB	9.94 ± 0.30 bA	*
12	*	10.52 ± 0.17 cA	*
CV (%)	1.92		
	Titratable acidity (g.100g^{-1} citric acid)		
0	0.61 ± 0.03 aA	0.61 ± 0.03 aA	0.61 ± 0.03 aA
3	0.52 ± 0.05 aA	0.56 ± 0.04 aA	0.37 ± 0.02 bB
6	0.34 ± 0.04 cB	0.48 ± 0.03 bA	0.23 ± 0.03 cC
9	0.27 ± 0.03 dB	0.35 ± 0.04 cA	*
12	*	0.26 ± 0.0 dA	*
CV (%)	0.67		
	pH		
0	4.41 ± 0.12 aA	4.41 ± 0.12 aA	4.41 ± 0.12 aA
3	4.81 ± 0.17 bA	4.65 ± 0.23 bA	4.93 ± 0.18 bB
6	5.08 ± 0.21 cB	4.74 ± 0.21 bA	5.31 ± 0.12 cC
9	5.26 ± 0.14 dB	5.06 ± 0.14 cA	*
12	*	5.29 ± 0.11 dA	*
CV (%)	1.03		
	Vitamin C (g.100g-1)		
0	20.31 ± 0.45 aA	20.31 ± 0.45 aA	20.31 ± 0.45 aA
3	26.56 ± 0.42 bB	24.89 ± 0.30 bA	16.54 ± 0.19 bC
6	19.18 ± 0.21cB	31.03 ± 0.28 cA	11.85 ± 0.22 cC
9	11.06 ± 0.61 dB	21.56 ± 0.26 dA	*
12	*	12.15 ± 0.31 eA	*
CV (%)	3.48		
	Total sugar (g.100g^{-1})		
0	5.83 ± 0.21 aA	5.83 ± 0.21 aA	5.83 ± 0.21 aA
3	6.31 ± 0.17 bA	6.14 ± 0.16 aA	8.46 ± 0.11 bB
6	9.16 ± 0.14 cB	7.40 ± 0.20 bA	9.44 ± 0.22 cC
9	9.75 ± 0.25 dB	9.21 ± 0.13 cA	*
12	*	9.81 ± 0.21 dA	*
CV (%)	2.75		

Means followed by the same letter in the row (treatments) and in the column (storage days) do not differ from each other using the Tukey test ($p<0.05$). *not evaluated.

Regardless of the treatments applied, a reduction in titratable acidity (TA) was observed (Table 3). However, guavas treated with GA_3 presented the highest TA levels, which remained with minimal variations until the 6th day of storage, with values around 0.26 g.100g^{-1} of citric acid at the end of 12 days. In turn, the acidity in fruits treated with ethephon reduced by around 39.43% over 3 days and an average content of 0.23 g.100g^{-1} of citric acid after 6 days (Table 3).

pH values increased during storage periods (Table 3), with a significant interaction between storage periods and treatments ($P<0.05$). In general, pH increased as a result of the reduction in acidity, in fruits treated with ethephon and control, for example, there was an

increase of around 16.0% until the 6th and 9th day, respectively (Table 3). For the same period, the pH of fruits treated with GA_3 increased by only 13.51% and reached a percentage of 16.63% at the end of 12 days (Table 3).

The vitamin C content varied during storage, with a significant interaction between storage periods and treatments ($P<0.05$) (Table 3). In fruits treated with ethephon, there was a reduction of around 41.65% with average values going from 20.35 $mg.100g^{-1}$ on day 0 to 11.85 $mg.100g^{-1}$ at 6 days. On the other hand, the control and GA_3 treated fruits showed an increase of 23.53 and 34.56% between the 3th and 6th day, respectively. After this period, a reduction in average levels was noted, with control fruits exhibiting a lower value (11.06 $mg.100g^{-1}$) compared to those treated with GA_3 (12.15 $mg.100g^{-1}$) over 9 and 12 days, respectively (Table 3).

DISCUSSION

Shelf life represents the commercial quality of the fruit during the storage period. In this sense, the longer shelf life of fruits treated with GA_3 is due to the inhibition of ethylene synthesis caused by this regulator, which delayed ripening and extended the marketing period. In turn, exposure to ethephon anticipated the ripening of guavas, allowing them to be sold in a shorter time (Fig. 1). These strategies are important as they provide subsidies for producers and retailers to obtain better prices when selling their fruits. Bananas treated with GA3 (300 $mg.L^{-1}$), for example, had approximately 5 days more shelf life when compared to control fruits during storage at 28 ºC (Ghimire et al., 2021).

The maximum tolerated mass loss to prevent the appearance of wilting and wrinkling on the surface ranges between 5 and 10% for most fresh vegetables (Iakimova et al., 2024). In this study, fruits showed losses above this range after 6 days of storage, especially in those treated with ethephon (Table 1). This fact can be attributed to the effect of ethylene which, after being released into the tissues, causes an increase in respiratory activity favoring water loss due to greater transpiration.

On the other hand, the lower loss of mass in guavas treated with GA_3 can be attributed to the inhibition of ethylene synthesis and consequent delay in the ripening process, caused by the reduction in metabolic activity in the fruits due to the action of GA_3, which acts as an antagonist to the effects ethylene degradative agents (Mukherjee et al., 2022). Similar results were obtained in bananas where the immersion treatment at 300 $mg.L^{-1}$ of GA_3 resulted in less mass loss (3.52%) in relation to the control (9.21%) after 15 days under conditions of room temperature.

The firmness of the pulp is determined by the strength of cohesion between the pectins and as ripening progresses, pectinolytic enzymes act, which transforms insoluble pectin into soluble one, promoting the softening of the fruits (Sanches & Repolho, 2022). In this study, the greatest reduction in firmness in fruits treated with ethephon (Table 1) is due to the accelerated ripening caused by this regulator, which promotes the solubilization of the pectins that make up the cell wall, leading to loss of firmness.

For Mukherjee et al. (2022), GA_3 can partially inhibit the action of ethylene, thereby delaying metabolic processes related to ripening, especially fruit softening. This justified the greater firmness in guavas treated with this regulator throughout the storage period (Table 1). Similarly, Bagnazari et al. (2018) also observed that the firmness of peppers treated with 50 $mg.L^{-1}$ was 21.11% greater than control fruits after 20 days of storage at 10 ºC. In okra, treatment with GA_3 (100 $mg.L^{-1}$) delayed softening, corroborated by the inhibition of genes that regulate cell wall degradation during refrigerated storage (10 ºC) over 12 days (Li et al., 2023).

Regarding the color of the skin and pulp, the reduction in L* with storage time is an indication that the fruits were losing their natural shine with the ripening process, in this case, the lower L* values observed in fruits treated with ethephon are consequences of rapid maturation driven by the effects of ethylene. These results agree with Zhang and Zhou (2023) who relate the loss of brightness and darkening of tissues with ripening in lemon fruits exposed to ethephon (1000 $mg.L^{-1}$) and stored at 20 ºC for 9 days. In turn, the higher L* values up to 9 (control) and 12 days (GA_3) suggest greater preservation of natural brightness and consequent delay in maturation (Table 2).

Chroma (C*) is considered the quantitative attribute of coloring and is used to determine the degree of difference in a shade, the higher the chroma values, the greater the intensity of the color. In this study, the reduction in C* values in the peel, regardless of the treatment applied, indicates that the color intensity decreased in different periods depending on ripening (Table 2). Likewise, the increase in C* in the pulp shows that the color intensity/saturation increased with ripening, changing from pink to red as storage progressed, being more pronounced in fruits treated with ethephon (6 days) and lower in those treated with GA_3 (12 days) (Table 2).

The hue angle (h*) is considered a qualitative attribute of color, according to which colors are traditionally defined as reddish, greenish, etc. In this study, the decrease in hº in the fruit peel with storage time indicates a change from green to yellow, suggesting that treatment with ethephon accelerated this change. In contrast, treatment with GA_3 retained the green color for a longer period (Table 2). In the pulp, the increase in hº values demonstrates that the color became redder in different storage periods due to the metabolic role of regulators in anticipating (ethephon) or delaying (GA_3) ripening.

SS is an important parameter, as in addition to being an indicator of the harvest point, it is the main criterion used in establishing postharvest quality standards for fruit in market regulations (Kyriacou & Rouphael, 2018). In this study, the increase in SS levels during storage is due to the advancement of fruit ripening, associated with the loss of water resulting from respiratory activity or transpiration, as well as the degradation of the cell wall, increasing the concentration of soluble solids levels (Zhao et al., 2024). This would justify the lower and higher SS contents in guavas treated with GA_3 and ethephon, respectively (Table 3), demonstrating the antagonistic capacity of the regulators on ripening.

The presence of organic acids gives the acidity of a fruit and is one of the criteria used to classify the fruit by flavor (Chen et al., 2021). Normally, the content of organic acids tends to decrease during the maturation process due to the oxidation of acids in the tricarboxylic acid cycle as a result of respiration. Thus, the variation in acidity can be an indication of the fruit's ripening stage, as acidity decreases as maturation progresses (Sanches et al., 2021). In this sense, the smaller reduction in TA levels in fruits treated with GA_3 may be an indication that there was a reduction in the metabolism of the fruits, showing few transformations due to the delay in ripening. Regarding the treatment with ethefon, on average, the fruits became less acidic than the other treatments on the 6th day, probably due to the use of organic acids in respiratory metabolism driven by rapid ripening (Table 3).

The increase in pH during storage is related to the reduction in organic acids (TA) that are consumed by the respiratory metabolism of fruits, promoting ripening and senescence (Sanches et al., 2021). Thus, the higher pH values in fruits treated with ethephon (6 days) and control (9 days) are indicative of a greater stage of maturation/senescence of these fruits, in turn, the smaller increases in pH values in fruits treated with GA_3 over 12 days suggests a delay in these physiological events (Table 3). Similar results were obtained in mangos where treatment with 400 $mg.L^{-1}$ of GA_3 delayed ripening in relation to the control, corroborated by

lower SS accumulation, higher concentration of organic acids (TA) and lower pH increase during 12 days of storage at room temperature (30 ºC) (Chhetri & Ghimire, 2023).

According to Macedo et al. (2023), vitamin C content can be used as an index of food quality, because it varies in the product according to cultivation, storage, and processing conditions. In the case of fruits treated with ethephon, the reduction in average values results from the acceleration of ripening/senescence, as ascorbic acid is used in oxidative reactions, which are activated by the stresses suffered in cell membranes during storage. In this sense, the increase and preservation of vitamin C levels in control fruits and, especially in those treated with GA_3, may be a reflection of less oxidative damage, mainly from respiration, which caused the delay in ripening (Table 3). Likewise, the application by immersion of 'rabbiteye' blueberries in GA_3 solution (500 $mg.L^{-1}$) was able to delay the degradation of ascorbic acid, maintaining the postharvest quality of the fruits in relation to untreated ones (Zang et al., 2016).

Regarding total sugar levels (Table 3), there was an increase in average values at different storage times between treatments ($P<0.05$). In guavas treated with ethephon a pronounced increase was observed between day 0 and day 3 (32.63%) with a maximum peak at 6 days of storage (9.44 $g.100g^{-1}$). In control fruits and those treated with GA_3, this most significant increase was obtained on the 6th and 9th days, with an average percentage of 36% and a maximum peak on the 9th and 12th days (9.75 and 9.81 $g.100g^{-1}$), respectively (Table 3).

According to Wee et al. (2023) during the fruit ripening process, one of the main changes in their characteristics is the accumulation of sugars, reaching their maximum at the end of ripening. In this study, treatment with GA_3 provided the lowest total sugar values up to 12 days of storage, this fact is possibly related to the delay in fruit maturation due to the reduction in metabolic rate and consequent decrease in sugar conversion. On the other hand, the opposite occurred for fruits treated with ethephon up to the 6th day of storage, with increased fruit metabolism corroborated by greater sugar synthesis.

CONCLUSIONS

The postharvest treatment of guavas with GA_3 delayed ripening, making it possible to extend commercialization for up to 12 days under room temperature conditions (28 ± 2 ºC), due to the preservation of firmness, less loss of mass, less accumulation of SS, the content of vitamin C and total sugars.

Ethephon anticipated ripening, allowing the fruits to be commercialized in up to 6 days, without compromising the physical-chemical quality during the storage period.

Conflict of interest

The authors have no conflict of interest to report.

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