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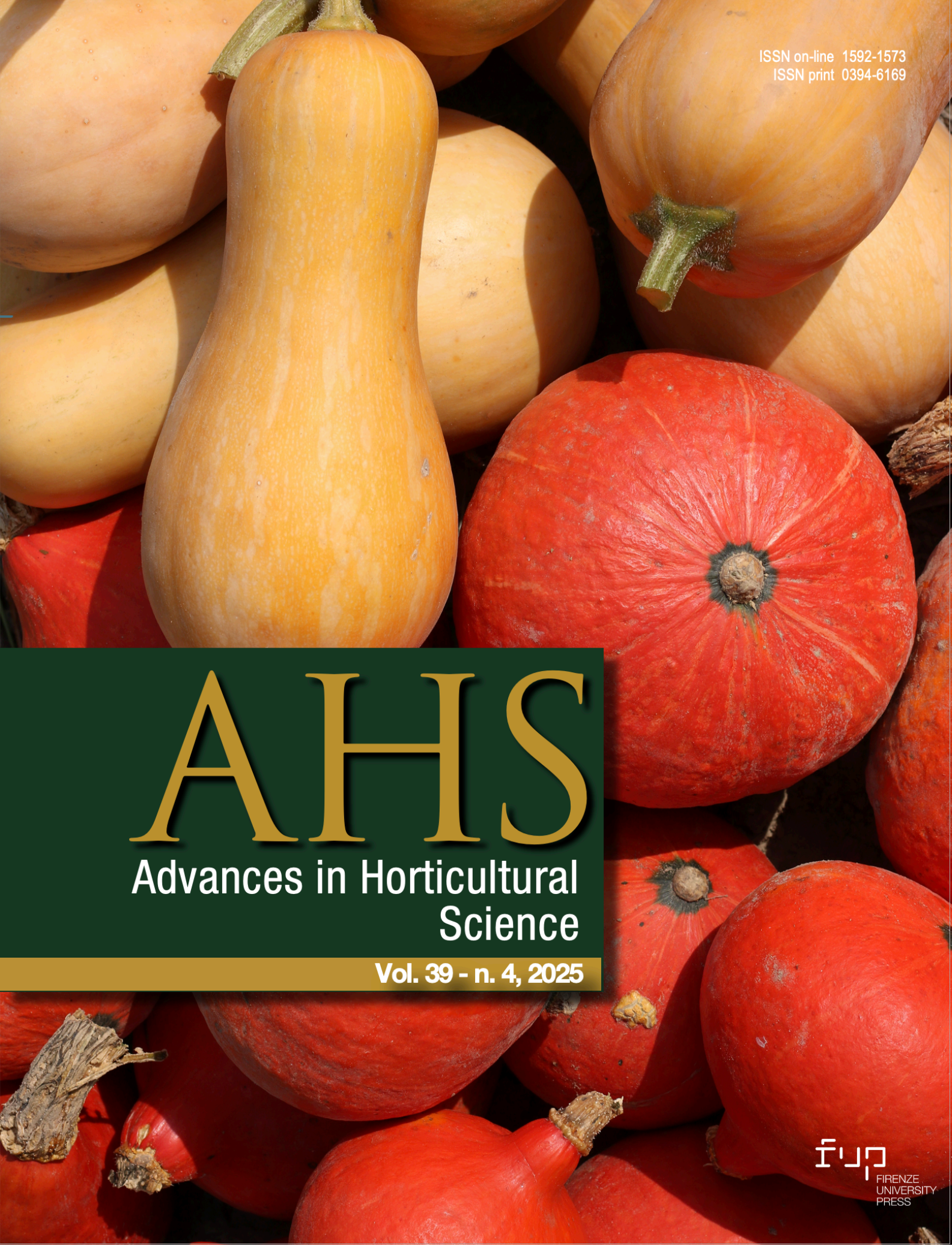

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Effects of high temperature on the fruit development and sugar concentration in everbearing strawberry cultivars

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Key words: Flower bud, *Fragaria × ananassa*, fruit set, sucrose.

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Abstract: In everbearing strawberry cultivars, understanding the effects of high temperature is crucial. Long-day everbearing cultivars are promoted for summer and autumn production whereas this is off-season for June-bearing varieties. This study investigated the effects of high temperature on the development and fruit quality of the everbearing cultivars Natsuakari and Dekoruju in a phytotron-simulating summer condition. Both cultivars showed a smaller number of flowering inflorescences at 30°C/25°C (day/night) compared to 20°C/15°C, indicating that flower bud development is sensitive to high temperatures. Fruit set rates were also negatively affected by the higher temperature. Fruit sugar composition of 'Natsuakari' fruits showed an increased sucrose concentration during the developmental stage from 75% colored fruit to 100% colored fruit. In the ripe fruit of 'Natsuakari,' the sugar content was measured higher due to greater amount of sucrose accumulation, whereas a remarkable decrease in the sucrose concentration was observed at 30°C/25°C temperature compared to 20°C/15°C. These results suggested that summer high temperatures negatively impacted flowering, fruit set, and sucrose content in everbearing cultivars, ultimately leading to reduced fruit production and quality. Additionally, the temperature effects varied between the two cultivars strawberry.

1. Introduction

Strawberries currently grown in Japan and many other countries are mainly June-bearing cultivars, whose flower buds differentiate under low temperatures and short days (Yano *et al.*, 2021; Janssens *et al.*, 2024). In the cultivation of June-bearing strawberries in Japan, fruits are produced from November to June of the following year by artificially controlling flowering and dormancy and performing forcing culture or semiforcing culture. The summer and fall seasons, from July to October, are the off-season of these cultivars, and strawberries are imported to meet the market demand. However, since imported fruits have problems with freshness and taste, there is a demand for domestically produced fruits

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(Nishiyama *et al*., 2020).

Methods for producing strawberries in summer and fall using June-bearing cultivars include retarding culture and short-day treatment. Retarding culture, which involves delaying plant development through long-term refrigeration which has several disadvantages, such as high refrigeration costs, plant weakening, and the increased risk of gray mold during refrigeration. As a result, this cultivation method is not widely practiced (Kobayashi and Yamamoto, 1994; Yamazaki, 2012). Similarly, June-bearing strawberry production through short-day treatment is also limited due to the high facility and labor requirements, as well as the instability of flowering, which can be affected by weather conditions (Hamano *et al*., 2012).

In addition to June-bearing cultivars, there are everbearing cultivars, which are quantitatively long-day plants. Everbearing cultivars can be differentiated from flower buds under high temperature and long-day conditions in summer (Durner *et al*., 1984; Smeets, 1980). Nishiyama *et al.* (1998, 1999) reported that these cultivars have critical day lengths for differentiating flower buds under summer conditions. Under high-temperature conditions simulating the summer season, the critical photoperiods of four everbearing cultivars were examined, and they varied among cultivars, ranging from 12 to 15 hours (Nishiyama *et al*., 2009). Due to their quantitatively long-day nature, everbearing strawberry cultivars are seen as a potential solution to the challenges of off-season fruit production faced by June-bearing cultivars.

In fruit vegetables, including strawberries, high temperatures in summer and fall often cause problems with fruit set, growth, and quality (Ito *et al*., 2022). Regarding the effects of high temperatures on strawberries, there are some findings in June-bearing cultivars. Regarding fruit set and fruit growth, it is reported that high temperatures during the flowering period reduce fruit achenes (Mori, 1998) and decrease fruit weight in fall and winter (Miura *et al*., 1994). In the former study, the effect of high temperature was examined by applying five temperature regimes ranging from a high of 32°C/27°C (day/night) to a low of 16°C/11°C (day/night), and in the latter study, the effect was investigated under constant temperatures of 19°C (high) and 15°C (low), respectively. Additionally, fruit sugar concentration tends to decrease under comparatively high temperature conditions, depending on the cultivar (Kumakura and Shishido, 1994; Ogiwara *et al*., 1999; Sato and Kitajima, 2007). High night temperatures have also been found to negatively affect fruit sugar content, as demonstrated by comparisons between night temperatures of 10°C and 20°C (Matsuzoe *et al*., 2006).

Compared to June-bearing cultivars, the high temperature problem is even more severe in everbearing cultivars because their production is expected from summer to fall. However, there is little knowledge regarding the effect of high temperatures in everbearing cultivars. In addition, in previous reports using June-bearing cultivars, most of the experiments assumed the production period of June–bearing cultivars (Ono *et al*., 2023), and the experiments that assumed under high temperatures in summer like the present study, are lacking. In Japan, since the rise in temperature due to global warming is exceeding the world average, and the increase in annual average maximum temperature is higher in agricultural land as well as in urban areas (Murakami *et al*., 2011), it would be meaningful to investigate the impact of high summer temperature on fruit production. Furthermore, few reports have comprehensively investigated flowering, fruit growth, sugar concentration, and even sugar composition. Therefore, in this study, we controlled the temperature in a phytotron and investigated the effects of high summer temperature on various factors related to fruit production using two everbearing strawberry cultivars.

2. Materials and Methods

Plant materials

We used everbearing strawberries (*Fragaria × ananassa* Duch.) 'Natsuakari' and 'Dekoruju.' The cultivation method was the same followed by Nishiyama *et al*. (1998), in which runner seedlings were potted up, grown in an unheated greenhouse, and then moved to a phytotron and subjected to temperature treatments. The temperature treatments started in early November, with day (6:00 to 18:00)/night (18:00 to 6:00) temperatures set at 20°C/15°C or 30°C/25°C. Long-day treatment with a day length of 24 h was given to promote flowering, that is, sunlight from 9:00 to 17:00 and incandescent light bulb radiation (3.23 $W \cdot m^{-2}$) from 17:00 to 9:00. Because the critical photoperiod is influenced by cultivar and temperature (Smeets, 1980; Nishiyama

et al., 2009), a 24-h day length was set as the maximum possible photoperiod to ensure stable flowering. Hand pollination was performed to ensure fruit set.

Measurement of flowers and fruits

The inflorescences were removed before starting the temperature treatment. The number of inflorescences with an open first flower (referred to as flowering inflorescence) was counted weekly during the temperature treatment. The number of flowers and fruits was also counted to calculate the fruit set rate. Fruit length and diameter of the first fruit in each inflorescence were measured using a vernier caliper every 2 to 3 days until ripening (100% coloration). Fruit length was measured as the maximum distance between the apical and peduncle ends of each fruit. The fruit diameter was measured as the maximum transverse diameter.

Determination of the fruit sugar concentration

Glucose, fructose, and sucrose concentrations were analyzed in white (W), 50%, 75%, and 100% colored fruit according to Sagor *et al*. (2015), Corvino *et al*. (2023), and Jia *et al*. (2011). Briefly, sugars were extracted with ethanol at 80°C, and maltose solution was added as an internal standard. After filtration using a Sep-pack C18 cartridge (Waters) and chloroform extraction, the sugars were measured using a sugar analyzer SU-300 (Toa DKK Co., Ltd., Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan). Twelve plants were used in the temperature treatment at 20°C/15°C of 'Natsuakari' and at 20°C/15°C and 30°C/25°C of 'Dekoruju.' Ten plants were used in the temperature treatment at 30°C/25°C of 'Natsuakari' because two plants died during the experiment. The percentage data for the fruit set was subjected to arcsine transformation. Sugar concentration and fruit size changes were measured in three biological replicates.

3. Results

Effect of high temperature on flowering and fruit development

Figure 1 shows the changes in the number of flowering inflorescences during the temperature treatment, which changed similarly for both cultivars. During weeks 1-3, the number of flowering inflorescences was higher at 20°C/15°C than at 30°C/25°C in 'Natsuakari.' Similarly, during weeks 4-6, the number of flowering inflorescences was higher at 20°C/15°C than at 30°C/25°C in both cultivars. In weeks 7-9, the number of flowering inflorescences was similarly low under both temperature treatments. Temperature significantly affected the number of flowering inflorescences throughout the treatment period, but no cultivar differences or interactions were observed (Table 1). However, temperature difference and their interaction with cultivars was observed in case of fruit set rate although there were no cultivar differences (Table 1). The fruit set rate of 'Natsuakari' was reduced to one-third at 30°C/25°C compared to 20°C /15°C, whereas the reduction in 'Dekoruju' was moderate. The results suggest that 'Dekoruju' is more tolerable to high temperature in terms of fruit set rate.

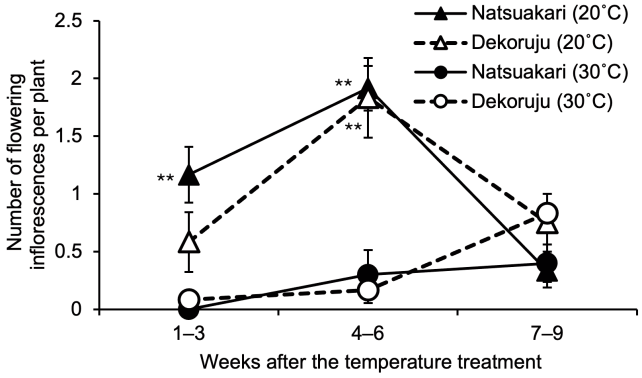

Fig. 1 - Changes in the number of flowering inflorescences per plant of 'Natsuakari' and 'Dekoruju' during the temperature treatment at 20°C/15°C (20°C on the graph) and 30°C/25°C (30°C on the graph). The number of flowering inflorescences is the sum of inflorescences in each 3-weeks shown on the horizontal axis. Values indicate means with SEs (n=10 for 'Natsuakari' at 30°C/25°C, n=12 for others). ** indicates significant differences at the 1% level between temperatures for each cultivar by t-test.

Fruit development during temperature treatment is shown in figure 2. The changes in fruit length were similar regardless of cultivars and temperatures, whereas fruit diameters tended to be larger at 30°C/25°C for both cultivars. Since the fruit length and diameter were measured until ripening, the timing of ripening can be seen on the graph. The result indicated that the fruit ripened earlier at 30°C/25°C than at 20°C/15°C. Higher temperatures

Table 1 - Effect of temperature on the number of flowering inflorescences and fruit set rate

Cultivar	Temperature day/night (°C)	Number of inflorescences [z]	Fruit set rate (%)
Natsuakari	20/15	3.42	91.4
	30/25	0.70	30.0
Dekoruju	20/15	3.17	71.4
	30/25	1.08	62.1
ANOVA [y]	Cultivar	NS [x]	NS
	Temperature	**	*
	Interaction	NS	*

[z] Number of flowering inflorescences per plant.
[y] Analysis of the variance.
[x] NS, **, and * indicate not significant and significant differences at P < 0.01 and P<0.05, respectively (two-way ANOVA).

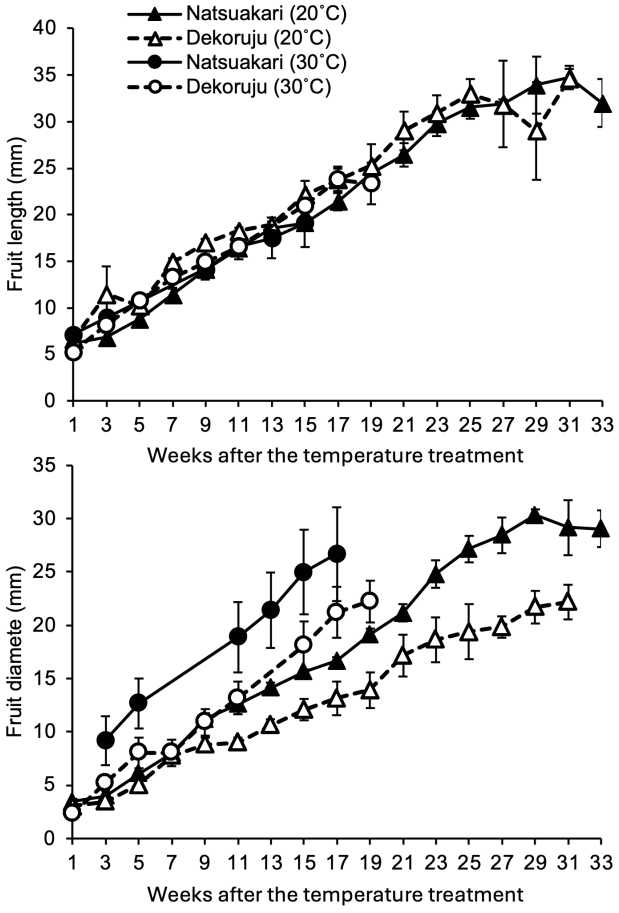

Fig. 2 - Changes in the length and diameter of 'Natsuakari' and 'Dekoruju' fruits during the temperature treatment at 20°C/15°C (represented as 20°C on the graph) and 30°C/25°C (represented as 30°C on the graph). The first fruit from each inflorescence was measured from flowering to ripening (100% coloration). Values represent means with SEs (n = 3). For 'Natsuakari,' there was considerable variation in fruit diameter at high temperatures and no significant difference between the temperature treatments. However, for 'Dekoruju,' a significant difference in fruit diameter was observed between the temperature treatments during the later stages of fruit development (P<0.05, t-test).

are considered to accelerate strawberry ripening primarily by enhancing the activity of enzymes involved in key ripening processes.

Fruit sugar accumulation and the effects of temperature on sugar concentration

To clarify the cultivar's characteristics of fruit sugar accumulation, sugar concentrations were measured at different ripening stages of 'Natsuakari' and 'Dekoruju' fruits at 20°C/15°C. Changes in glucose and fructose concentrations were similar in both cultivars with concentrations remaining at similar levels from white fruit to 75% colored fruit, then increasing from 75% to 100% colored fruit (Fig. 3). On the other hand, changes in sucrose concentrations differed between cultivars; that is, sucrose concentrations did not increase in 'Dekoruju,' whereas they increased in 'Natsuakari' from 75% to 100% colored fruit. The effects of temperature on the sugar concentrations in ripe fruits are shown in Table 2. There were no significant differences in the glucose and fructose concentrations in the cultivars at different temperatures, whereas significant differences in the sucrose concentrations were found between the cultivars at different temperatures.

4. Discussion and Conclusions

High temperature treatment at 30°C/25°C reduced the number of flowering inflorescences in both everbearing strawberry cultivars, indicating that such high temperatures affect fruit yield. The difference in the number of flowering inflorescences was greater during weeks 4-6 after the start of the treatment. However, the flower buds that opened

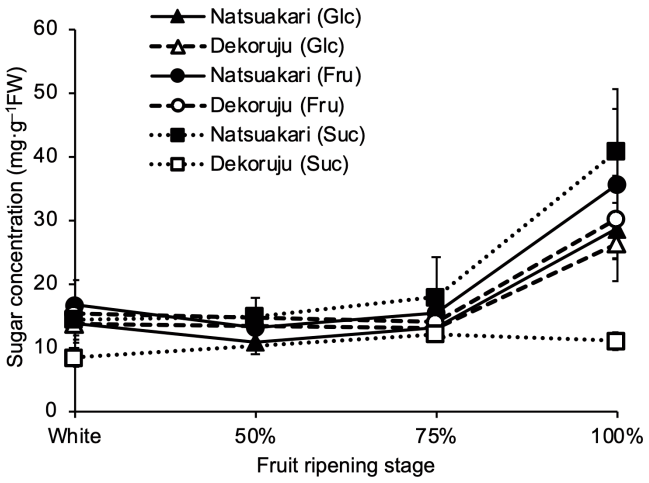

Fig. 3 - Changes in sugar concentration during the fruit ripening stages of 'Natsuakari' and 'Dekoruju.' The plants were grown at 20°C/15°C, and glucose (Glc), fructose (Fru), and sucrose (Suc) concentrations in white, 50%, 75%, and 100% colored fruit were measured. Values indicate means with SEs (n = 3). For 'Dekoruju,' data for 50% colored fruit is missing. Small error bars are hidden by the marker and cannot be seen.

fairly long period after flower bud differentiation. Until now, the effects of high temperatures have mainly been considered to be poor fruit set due to decreased fertility (Pipattanawong *et al*., 2009; Ledesma and Sugiyama, 2005). Therefore, a significant finding of this study is that flower bud development for several weeks after differentiation is inhibited, leading to a reduction in the number of flowering inflorescences under high temperatures. In the near future, it will be crucial to identify the stages of flower bud development that are sensitive to high temperatures and to develop efficient temperature control techniques based on the findings.

Seven to nine weeks after the start of the temperature treatment, the number of flower inflorescences at 20°C/15°C decreased to the same level as that at 30°C/25°C. In everbearing cultivars, in the case of strong promotion of flowering, most buds differentiate into flower buds instead of vegetative buds, leading to the loss of meristems and growth inhibition (Yoshida, 2009; Nishiyama *et al*., 2020).

Table 2 - Effect of temperature on the sugar concentration in ripe fruit

Cultivar	Temperature day/night (°C)	Sugar concentration (mg g^{-1} FW)		
		Glucose [z]	Fructose	Sucrose
Natsuakari	20/15	28.7	35.6	40.9
	30/25	23.8	28.1	22.9
Dekoruju	20/15	26.4	30.2	11.0
	30/25	12.8	15.1	5.6
ANOVA [y]	Cultivar	NS [x]	NS	**
	Temperature	NS	NS	*
	Interaction	NS	NS	NS

[z] Glucose, fructose, and sucrose concentration were determined.
[y] Analysis of the variance.
[x] NS, **, and * indicate not significant and significant differences at $P < 0.01$ and $P < 0.05$, respectively (two-way ANOVA).

during this period likely already differentiated, based on the time required for flower bud differentiation to flowering (Morishita, 2014). These results suggest that the development of flower buds after differentiation is inhibited by high temperatures such as 30°C/25°C, and this high temperature sensitivity is common to both cultivars. Furthermore, the number of flowering inflorescences was lower at 30°C/25°C during weeks 1-3 and weeks 4-6, in at least one of the two cultivars indicating that the development of flower buds is sensitive to high temperature during a

Besides, excessive fruit set in strawberries is known to weaken plant growth. Our results suggest that under optimal temperature and long-day conditions, it is necessary to regulate the number of flowering inflorescences. To solve this problem, light quality control and intermittent lighting techniques are promising (Nishiyama *et al*., 2020; Yamazaki *et al*., 2009).

Changes in fruit length and diameter from flowering to ripening are shown in figure 2. The change in fruit length was similar between 20°C/15°C

and 30°C /25°C until ripening. At 30°C/25°C, fruit diameter was larger due to the occurrence of malformed fruit. Fruit ripened faster at 30°C/25°C, with a similar growth rate based on fruit length between the two temperature treatments, which may have resulted in a smaller fruit size at ripening. Therefore, early ripening under high temperatures is likely to influence yield, together with reductions in flower number and fruit set rate. Early ripening is also reported in June-bearing cultivars, where fruit harvested in April when temperatures are relatively high, ripens in fewer days (Ogiwara *et al*., 1999). Additionally, fruit ripens faster at higher temperatures within an average daily temperature range of 15°C to 25°C (Kumakura and Shishido, 1994). These previous reports and our results suggest that early ripening under high temperatures is common to everbearing and June-bearing cultivars. However, in this study, we investigated the effects of high temperatures simulating summer conditions, indicating that summer temperatures have a significant impact on everbearing strawberries. Regarding fruit growth curves, a previous report on June-bearing cultivars (Ogiwara *et al*., 1999), showed that fruits harvested in April rose faster than those harvested in January, which differs from our results. Therefore, the response of growth curves to temperature may differ depending on the temperature combinations or cultivars being compared.

Strawberries set fruit and develop fruit receptacles through the fertilization of many pistils on the flower receptacle. As a result, even if some pistils remain unfertilized, the fruit does not necessarily drop. A decrease in fertility at daytime temperatures of 30°C to 32°C has been reported in June-bearing cultivars (Pipattanawong *et al*., 2009; Ledesma and Sugiyama, 2005). In this study, the fruit set rate decreased under high temperatures, whereas there were cultivar-specific differences in fruit set inhibition caused by high temperatures. Additionally, the morphology of floral organs, such as filament length and sepal size, appeared to be affected in the 30°C 25°C area. Therefore, the effects of high summer temperatures should be examined from multiple perspectives, including fertility and morphology.

Strawberries are classified into cultivar groups based on their sugar composition, including the fructose/glucose accumulating type, the sucrose accumulating type, and the intermediate type (Ogiwara *et al*., 1998). Although the cultivars used in this study were not included in the same report, they were considered to be intermediate types. However, the sucrose content at 20°C/15°C was higher at approximately 40% in 'Natsuakari' and low at around 15% in 'Dekoruju' showing characteristics more aligned with the sucrose accumulating type and the fructose/glucose accumulating type, respectively. The sucrose concentration in 'Natsuakari' fruit increased from 75% to 100% ripeness, whereas no enhancement was observed in 'Dekoruju,' suggesting that sucrose accumulation in the late stages of fruit development is a characteristic of the sucrose accumulating type. In tomatoes and pears, invertase and sucrose synthase play key role in determining the sucrose ratio (Kanayama, 2017), and recent genome editing of the invertase inhibitor-related factor has been recently reported (Kawaguchi *et al*., 2021). Therefore, it is expected that the development of this research will lead to the elucidation of the determining mechanism of fruit sugar composition and the improvement of fruit quality through its control.

In this study, the sum of the three sugar concentrations decreased by approximately 30% in 'Natsuakari' and by approximately 50% in 'Dekoruju' at 30°C/25°C compared to 20°C/15°C. One likely reason for this decrease is the reduction in sucrose concentration, which significantly differed between the two temperature treatments. A previous report on June–bearing cultivars suggested that low fruit sugar concentrations in April, from winter to spring, may be related to high fruit load or early fruit coloration (Ogiwara *et al*., 1999). In this study, the number of fruits was smaller at 30°C/25°C, and ripening was determined by fruit color; thus, early ripening likely contributed to the lower total sugar and sucrose concentrations. In particular, 'Natsuakari' may have been more strongly affected by early ripening, as sucrose accumulates during the later stage of fruit development, as described in this study. In tomatoes, sucrose synthase has been reported to play a role in reducing fruit sucrose concentration under high temperature conditions (Rosales *et al*., 2007). Therefore, understanding the relationship between sugar composition and sucrose-related enzymes in everbearing strawberries is essential to elucidate the mechanism behind quality decline during summer.

We conducted a temperature treatment assuming high temperatures in summer, which are important

in the production of everbearing strawberries, using a phytotron and revealed its effects on the number of flowering inflorescences, sucrose concentration, and fruit set. The differences between cultivars in these traits under different temperatures described in this study can be useful for breeding and cultivation. There are divergent phenotypes for the flowering response to temperature, as described by the comparison of many everbearing cultivars in warm and cold regions (Hikawa-Endo *et al*., 2019). Although the sugar composition was not investigated by Hikawa-Endo *et al*. (2019), it is expected that there will be diversity in the high temperature sensitivity and sugar accumulation. The controlled environment experimental system in this study will contribute to the selection of high temperature resistance genes and biomarkers from such a variety of everbearing cultivars to maintain fruit production and quality in everbearing strawberries grown under high temperature conditions.

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Efficacy of different bio-control agents for managing *Colletotrichum* leaf blight of large cardamom in Taplejung, Nepal

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Key words: AUDPC, biocontrol agents, blight, *Colletotrichum*, large cardamom, leaf blight.

Abstract: Large cardamom (*Amomum subulatum*) is a high-value spice crop primarily grown in the mid-hills of Nepal, providing significant economic benefits to smallholder farmers. However, the crop faces substantial threats from *Colletotrichum* blight, which has led to severe yield losses, prompting the need for effective disease management strategies. This study was conducted in Phungling-06, Taplejung district, within the cardamom cultivation zone, to evaluate the efficacy of various biocontrol agents in managing *Colletotrichum* blight. Using a randomized complete block design (RCBD) with five treatments, the study applied three foliar sprays of biocontrol agents (*Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens, Pseudomonas fluorescens* + *Bacillus subtilis*, and a control). Disease severity and AUDPC scores were measured over a period of four weeks. The results demonstrated that *Trichoderma harzianum* significantly reduced disease severity and AUDPC, followed by *Trichoderma viride*. Additionally, *Pseudomonas fluorescens* and its combination with *Bacillus subtilis* showed promising effects compared to the control. These findings suggest that biocontrol agents can provide an eco-friendly and sustainable alternative to chemical fungicides in large cardamom production. Future research should focus on optimizing the application techniques and exploring the long-term benefits of these biocontrol strategies in integrated pest management systems for large cardamom cultivation.

1. Introduction

Large cardamom (*Amomum subulatum* roxb.), locally known as "Alaichi" in Nepal, is a high-value perennial herbaceous spice crop belonging to the Zingiberaceae family. It is extensively cultivated across the mid-hills of eastern Nepal and serves as a vital source of income for thousands of smallholder farmers (Shrestha *et al*., 2018; Subedi *et al*.,

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All relevant data are within the paper and its Supporting Information files.

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2022). The plant typically reaches 1.5 to 3.0 meters in height, producing pseudostem (tillers) from underground, tuberous rhizomes (Pathak, 2008; Singh and Pothula, 2013). Its leaves are long and lanceolate, ranging from 30-60 cm in length and 5-15 cm in width. The flowering spike bears 30-40 flowers per inflorescence, with blooming occurring in spring; the flowers are characterized by white petals with bluish stripes and yellow margins, each lasting about three days, while the overall flowering period extends for approximately a month (Vijayan *et al.*, 2013; Belbase *et al*., 2018; Shrestha *et al*., 2018). The fruit is a small, three-chambered capsule that encloses 20-30 fragrant brown seeds, which are commonly utilized for both culinary and medicinal purposes (Joshi *et al.*, 2013).

Large cardamom thrives in subtropical, humid climates at altitudes of 900-2,000 meters above sea level, typically under the partial shade of nitrogen-fixing trees such as *Alnus nepalensis* (Paudel *et al*., 2018; Kharel *et al.*, 2025). It prefers acidic soils (pH 5.5-6.5), high rainfall (2,000-3,500 mm), and temperatures ranging from 10°C to 30°C. Traditional cultivation practices involve rhizome propagation, organic mulching, and manual harvesting, making it a labor-intensive but culturally significant crop (Sharma *et al*., 2021). Dried capsules are consumed whole or ground, while essential oils extracted from seeds find applications in food, confectionery, perfumery, and cosmetic industries (Bhandari and Bhandari, 2018; Shrestha *et al*., 2018). Medicinally, large cardamoms have been used in ayurvedic and traditional healing systems to treat respiratory disorders, digestive issues, throat infections, and even envenomations from snakes and scorpions (Pathak, 2008; Belbase *et al.*, 2018; Basnet *et al*., 2021). Chemically, its capsules contain approximately 8% essential oil, with bioactive compounds such as cineol (up to 70%), terpineol (45%), myrcene (27%), limonene (2-14%), menthone (6%), borneol, sabinene, α- and β-pinene, and α-terpinyl acetate (Vijayan *et al*., 2013; Shrestha *et al*., 2018; Gurung *et al*., 2021). Despite its economic and ethnobotanical importance, large cardamom cultivation faces significant threats from *Colletotrichum* blight, primarily caused by *Colletotrichum* spp., with outbreaks frequently reported in Taplejung, Ilam, and other eastern districts of Nepal (Joshi *et al*., 2013; Paudel *et al*., 2018). The pathogen proliferates in the moist, shaded microclimate typical of cardamom plantations, particularly under sprinkler irrigation systems that favor fungal growth (Feksa *et al*., 2019; Kharel *et al.*, 2025). Overreliance on chemical fungicides has been largely ineffective in the long term, often leading to fungicide resistance and environmental degradation (Gautam *et al.*, 2020; Zubair *et al*., 2022; Tiwari *et al*., 2023). These chemicals can harm beneficial soil microbiota, contaminate soil and water, and pose health risks to both farmers and consumers (Subedi *et al*., 2022).

Epidemiologically, *Colletotrichum* blight is most severe during the pre-monsoon period, with symptoms including water-soaked lesions and necrotic spots on leaves, leading to premature leaf drop and reduced photosynthetic capacity (Pun, 2019). Infected old tillers serve as the primary inoculum for subsequent seasons, facilitating the spread of the disease. The disease has been associated with significant yield reductions, with reports indicating up to 46.8% reduction in dry yield in infected plants compared to healthy ones (Shrestha *et al.*, 2018). Given these challenges, biological control agents have emerged as sustainable alternatives. Beneficial microorganisms such as *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus subtilis* suppress pathogens through mechanisms including competition, antibiosis, mycoparasitism, and induced systemic resistance (Gautam *et al.*, 2016; Shrestha *et al.*, 2018). For instance, *Trichoderma viride* has demonstrated significant efficacy in reducing *Colletotrichum* blight under field conditions in Nepal, with studies indicating its effectiveness when combined with fungicides like azoxystrobin (Subedi *et al.*, 2022). Their application not only reduces pathogen load but also enhances soil health and stimulates plant immunity (Subedi *et al.*, 2022; Kharel *et al.*, 2025). Integrated disease management (IDM) approaches that incorporate these agents promote eco-friendly, economically viable crop protection strategies for resource-constrained farmers in Nepal's hill regions (Tiwari *et al*., 2023). Therefore, evaluating the efficacy of biocontrol agents in managing *Colletotrichum* blight is crucial for sustainable large cardamom production.

The present study aims to assess the effectiveness of different eco-friendly biocontrol agents under field conditions, providing viable alternatives to chemical fungicides while supporting environmental sustainability and farmer health.

2. Materials and Methods

The field research was carried out in phungling-6, located in the Taplejung district of eastern Nepal, within the temperate climatic zone. The experiment took place in a mature large cardamom plantation (variety: Ramsai), where the plants were approximately 15 to 20 years old. The experimental site was situated at an altitude of 1690 meters above sea level. In terms of geographical coordinates, the site lay at 27°2'1"51 n latitude and 87°4'0"45 e longitude. During the study period, the highest recorded temperatures ranged from 26.06°C to 31.26°C, while the lowest temperatures fluctuated between 12.78°C and 16.77°C. Relative humidity showed a peak value of 67.31% and declined to a minimum of 32.16% which were collected from https://power.larc.nasa.gov/. No rainfall was observed at the initial and final stages of the trial, with a maximum recorded rainfall of 6.82 mm in the middle phase. Figure 1 illustrates the geographical location of the research site, and figure 2 presents the meteorological data recorded during the experiment.

Experimental design

The experimental layout was structured using a randomized complete block design (RCBD), incorporating five different treatments replicated four times. This resulted in a total of 20 experimental plots, each measuring 4 meters by 3 meters. All treatments were implemented in accordance with recommended agronomic practices and standard operational guidelines (Fig. 3).

The treatment details are summarized in Table 1.

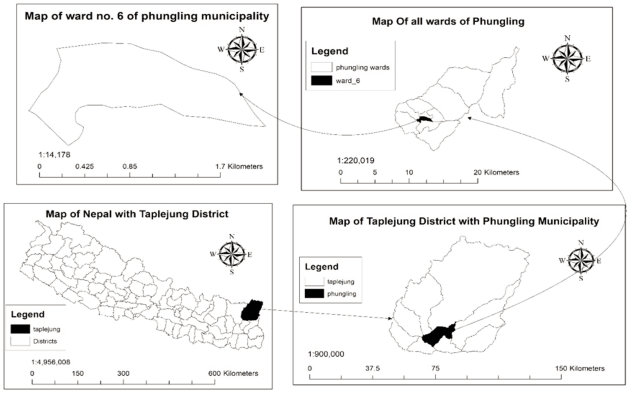

Fig. 1 - Mapping of a study area.

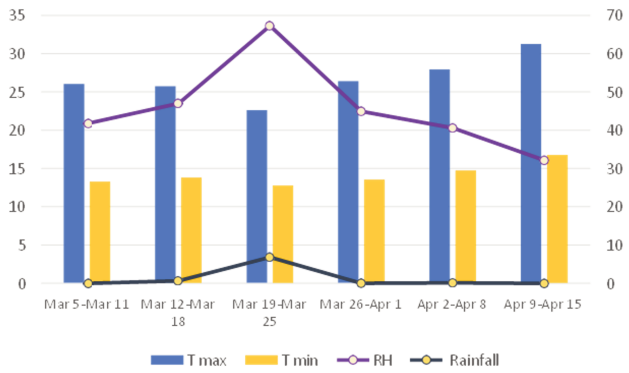

Fig. 2 - Climatic conditions of the research area (temperature, rainfall, and relative humidity).

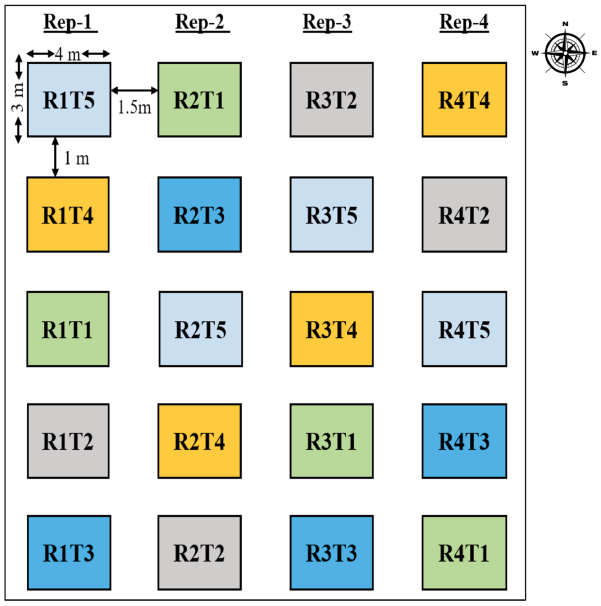

Fig. 3 - Layout of experimental site.

Table 1 - Treatment details with company name, trade name, and doses

Treatments	Company	Trade name	Bio-control agents	Dose	Cfu per l
t1	multiplex	Nisarga	*Trichoderma viride*	10 gm/l water	2×10^9 cfu/l
t2	multiplex	Safe root	*Trichoderma harzianum*	10 gm/l water	2×10^9 cfu/l
t3	multiplex	Sparsha	*Pseudomonas fluorescens*	5 gm/l water	1×10^9 cfu/l
t4	multiplex	Bio-jodi	*Pseudomonas fluorescens* + *Bacillus subtilis*	5 gm/l water	1×10^9 cfu/l
t5	-	-	Control	-	-

Allocation and application of biopesticides

Treatment allocation within each experimental unit across all replications was carried out using the lottery method to ensure randomness. The bioagents were applied in three separate sprayings throughout the trial period. The initial application was conducted when disease severity reached approximately 5% in the field, followed by two subsequent sprays at seven-day intervals. A high-volume knapsack sprayer was utilized for the application, delivering the required solution at a rate of 4 liters per plot.

Observation and data collection

Disease severity assessment. From each experimental plot, ten leaves were randomly chosen from plants in four different directions to ensure representative sampling. Disease severity was visually monitored and recorded every three days. The evaluation was conducted using a 0-9 scale based on the amended 10% ordinal scale (Table 2, Fig. 4).

Disease scoring. The disease severity was assessed using a modified 10% ordinal rating scale, as outlined by Chiang *et al.* (2017).

Table 2 - Amended 10% ordinal scale

Score	Description	Midpoint
0	0% (no infection)	0
1	0+ - 1%	0.5
2	1+ - 4%	2.5
3	4+ - 10%	7
4	10+ - 20%	15
5	20+ - 30%	25
6	30+ - 40%	35
7	40+ - 50%	45
8	50+ - 70%	60
9	70+ - 100% disease	85

Disease severity index. The disease severity index (DSI) was calculated based on the midpoint of the severity range of each class rather than based on the severity score of each class to avoid overestimation (Chiang *et al*., 2017).

$$\text{DSI}\ (\%) = \sum \frac{\text{Frequency of each class x Midpoint}}{\text{Total no. of observations x Maximum midpoint of scale}}\ \text{x}\ 100$$

AUDPC calculation

The area under the disease progress curve (AUDPC) was computed following the formula proposed by Simko and Piepho (2012),

$$\text{AUDPC} = \sum_{i=1}^{\text{n-1}} \frac{y_{\text{i}}+y_{\text{i+1}}}{2}\ \text{x}\ (t_{\text{i+1}} - t_{\text{i}})$$

where,

y_i = Disease severity (%) recorded at each i^{th} observation,

t_i = Time (in days, hours) at the i^{th} observation.

n = Total no. of observations.

Statistical analysis

The experimental data were initially entered into MS-excel (2019) for subsequent analysis. To test for variance homogeneity, a square root transformation (SQRT) was applied to the original data, following the method recommended by Gomez and Gomez (1984). Analysis of variance (ANOVA) was performed using r-studio statistical software (version 4.3.1). Significant differences among the variables were assessed through Duncan's multiple range test (DMRT) at a 5% significance level ($p \leq 0.05$). Figures and tables were generated using MS excel.

Fig. 4 - Symptoms of *Colletotrichum* leaf blight in the field.

3. Results

Effect of different biocontrol agents against leaf blight severity

The disease severity index (DSI) observed at 7, 14, 21, and 28 days after sowing (DAS) demonstrated progressive disease development and significant treatment effects from 14 das onward, clearly highlighting the efficacy of different biocontrol agents in suppressing *Colletotrichum* leaf blight in large cardamom. Initially, at 7 DAS, no significant differences were observed among treatments; however, from 14 DAS onwards, *Trichoderma harzianum* (3.75±0.30) and *T. viride* (3.74±0.14) recorded significantly lower DSI values compared to *Pseudomonas fluorescens* + *Bacillus subtilis* (4.45±0.25), *P. fluorescens* (4.93±0.45), and the control (6.07±0.23). This trend persisted through 21 and 28 das, with *T. harzianum* and *T. viride* consistently maintaining the lowest DSI values (4.80±0.55 and 4.88±0.22 at 28 das, respectively), while the control exhibited the highest disease severity (10.70±0.30). Mean DSI values across all observation periods confirmed the superior performance of *T. harzianum* (3.80±0.30) and *T. viride* (4.02±0.15) compared to moderate suppression by bacterial treatments and high susceptibility in the control. These results are visually reinforced by the boxplot (Table 3, Fig. 5), where *Trichoderma* spp. treatments show the lowest median DSI and narrow variability, indicating strong and consistent disease suppression, while the control shows the highest median and widest range, reflecting greater disease progression. Overall, the temporal disease data and graphical evidence confirm that *Trichoderma harzianum* is the most effective biocontrol agent against leaf blight, closely followed by *T. viride*, whereas bacterial treatments provide moderate control and the untreated control is the most susceptible. These findings underscore the robustness and sustainability of Trichoderma spp. as efficient biocontrol strategies for managing *Colletotrichum* leaf blight in large cardamom.

Effect of different biocontrol agents against leaf blight on area under disease progress curve (AUDPC)

The results for total AUDPC (area under disease progress curve) showed significant differences

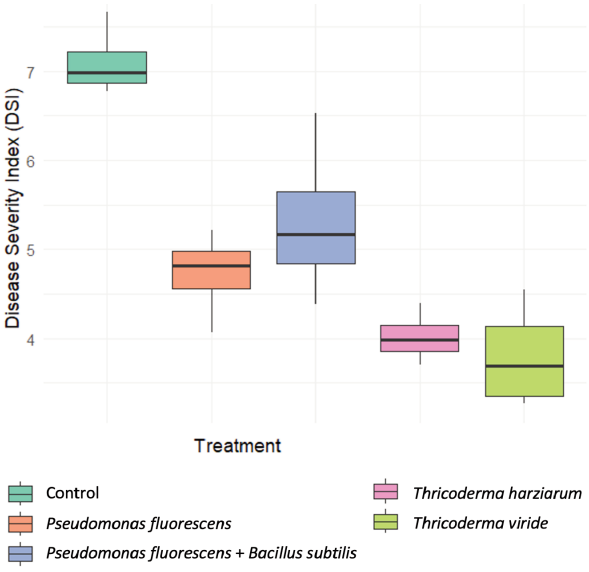

Fig. 5 - Effect of different biocontrol agents against leaf blight severity.

Table 3 - Effect of different biocontrol agents against leaf blight severity

Treatments	DSI (7 das)	DSI (14 das)	DSI (21 das)	DSI (28 das)	Mean disease severity
Trichoderma harzianum	2.79a±0.22	3.75c±0.30	3.90c±0.23	4.80d±0.55	3.80d±0.30 (2.07)
Trichoderma viride	3.22a±0.12	3.74c±0.14	4.23bc±0.22	4.88d±0.22	4.02cd±0.15 (2.13)
Pseudomonas fluorescens + Bacillus subtilis	3.33a±0.21	4.45bc±0.25	4.92bc±0.32	6.21c±0.40 (2.6)	4.73bc±0.24 (2.30)
Pseudomonas fluorescens	3.11a±0.21 (1.9)	4.93b±0.45	5.70b±0.81 (2.5)	7.50b±0.60	5.31b±0.45 (2.41)
Control	3.37a±0.12	6.07a±0.23	8.30a±0.40	10.70a±0.30	7.04a±0.22 (2.75)
mean	3.16	4.59	5.39	6.82	4.98
cv (%)	11.47	12.63	17.61	11.02	11.12
f-test	NS	**	**	**	**

Cv= Coefficient of variation; NS= non-significant ** = significant at 1% level of significance; (±) indicates standard error of the mean; in a column, means followed by a common letter(s) are not significantly different at the 5% level by duncan multiple range test (DMRT). figures outside the parenthesis are original values; figures inside the parenthesis are square root transformed values. DSI= disease severity index. DAS= Days after spraying.

among treatments, indicating varying levels of disease suppression effectiveness (Table 4, Fig. 6). The lowest AUDPC value was recorded in *Trichoderma harzianum* (79.80), followed closely by *T. viride* (84.11), with both treatments statistically similar and significantly more effective in reducing disease progression compared to other treatments. The combined application of *Peudomonas fluorescens* and *Bacillus subtilis* (98.95) showed moderate effectiveness, significantly lower than the control but higher than both *Trichoderma* treatments. *P. fluorescens* alone had a higher AUDPC value (111.54), which was significantly different from *T. harzianum* and *T. viride* but still performed better than the control. The highest AUDPC was observed in the untreated control (148.73), indicating the greatest disease severity over time. These findings confirm that *T. harzianum* and *T. viride* were the most effective bio-agents in suppressing disease development, with significantly lower AUDPC values compared to all other treatments.

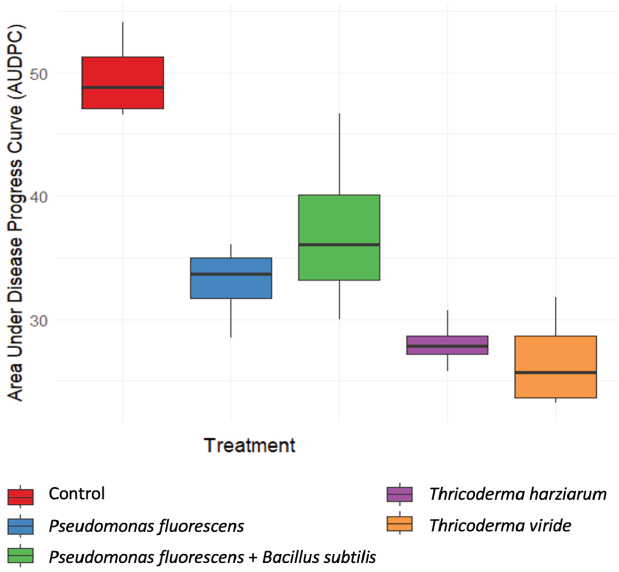

Fig. 6 - Effect of different biocontrol agents against leaf blight on AUDPC

Table 4 - Effect of different biocontrol agents against leaf blight on AUDPC

Treatments	Total AUDPC
Trichoderma harzianum	79.80±5.94 c
Trichoderma viride	84.11±3.07 c
Pseudomonas fluorescens + Bacillus subtilis	98.95±4.94 bc
Pseudomonas fluorescens	111.54±10.66 b
Control	148.73±5.12 a
Mean	104.62
cv (%)	12.19
f-test	**

Cv= Coefficient of variation; AUDPC = Area under disease progress curve; ** = significant at 1% level of significance; (±) indicates standard error of the mean; in a column, means followed by a common letter(s) are not significantly different at the 5% level by duncan multiple range test (DMRT).

Effect of different bio-agents on vegetative and yield parameters

The study revealed that the application of different bio-agents had significant effects on all observed parameters, with notable differences among treatments (Table 5, Figs. 7 and 8). For plant height, the tallest plants were observed in *Trichoderma harzianum* (55.52 cm), which was

Table 5 - Effect of different bio-agents on vegetative and yield parameters

Treatments	Plant height	Leaf length	Leaf breadth	NLPP	Yield (kg/ha)
Trichoderma harzianum	55.52 a	28.32 a	5.82 a	5.47 a	496.25 a
Trichoderma viride	53.0 ab	26.83 ab	5.21 ab	5.75 a	496.0 a
Pseudomonas fluorescens + Bacillus subtilis	51.81 ab	26.44 ab	5.53 a	5.10 ab	483.25 b
Pseudomonas fluorescens	50.15 b	24.92 b	5.07 ab	5.00 ab	476.0 b
Control	44.79 c	20.99 c	4.47 b	4.45 b	454.25 c
Mean	51.05	25.50	5.22	5.15	481.15
cv (%)	5.34	6.57	10.20	9.45	6.49
f-test	*	*	*	*	**

Cv= Coefficient of variation; NLPP= Number of leaves per plant; **= Significant at 1% level of significance; *= Significant at 5% level of significance; in a column, means followed by a common letter(s) are not significantly different at the 5% level by Duncan Multiple Range Test (DMRT).

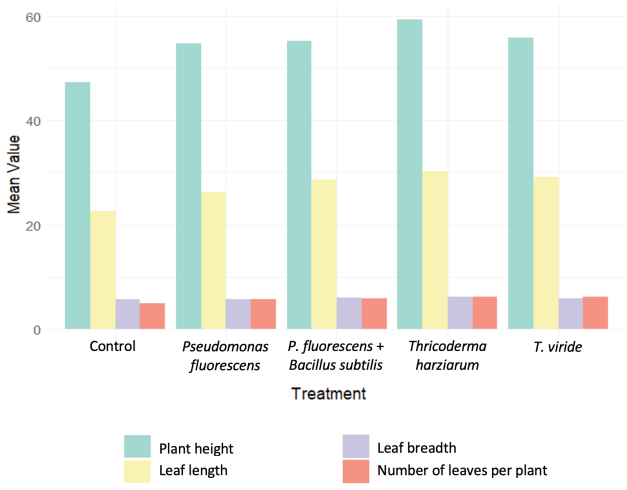

Fig. 7 - Effect of different bio-agents on vegetative parameters.

significantly higher than *Pseudomonas fluorescens* (50.15 cm) and the control (44.79 cm). Meanwhile, *T. viride* (53.0 cm) and *P. fluorescens* + *Bacillus subtilis* (51.81 cm) were statistically similar and intermediate between the highest and lowest values. This trend is visually evident in figure 7, where *T. harzianum* shows the tallest bar for plant height, confirming its superior vegetative growth. In terms of leaf length, *T. harzianum* again recorded the highest value (28.32 cm), followed by *T. viride* (26.83 cm) and *P. fluorescens* + *B. subtilis* (26.44 cm), which were statistically similar, while the control had the shortest leaves (20.99 cm). For leaf breadth, the widest leaves were found in *T. harzianum* (5.82 cm), significantly broader than the control (4.47 cm), with other treatments showing intermediate values without significant differences. Regarding number of leaves per plant (NLPP), *T. viride* (5.75) and *T. harzianum* (5.47) had the highest and statistically similar values, both significantly higher than the control (4.45), while *P. fluorescens* and *P. fluorescens* + *B. subtilis* produced moderate and comparable results. These vegetative improvements are clearly illustrated in figure 7, where *T. harzianum* and *T. viride* consistently outperform other treatments across all parameters. As for yield, both *T. harzianum* (496.25 kg/ha) and *T. viride* (496.0 kg/ha) produced the highest and statistically similar yields, significantly outperforming the control (454.25 kg/ha).

P. fluorescens + *B. subtilis* (483.25 kg/ha) and *P. fluorescens* (476.0 kg/ha) gave moderate yields that did not differ significantly from each other. The yield trend is visually supported by figure 8, which shows the highest bars for *T. harzianum* and *T. viride*, confirming their positive influence on productivity. overall, the findings suggest that *T. harzianum* and *T. viride* were the most effective bio-agents for enhancing both vegetative growth and yield. *P. fluorescens* and its combination with *B. subtilis* also provided moderate benefits. A key insight from this study is that *T. harzianum*, in addition to promoting vegetative traits, also achieved the highest yield, highlighting its potential as a dual-benefit bio-agent in crop production.

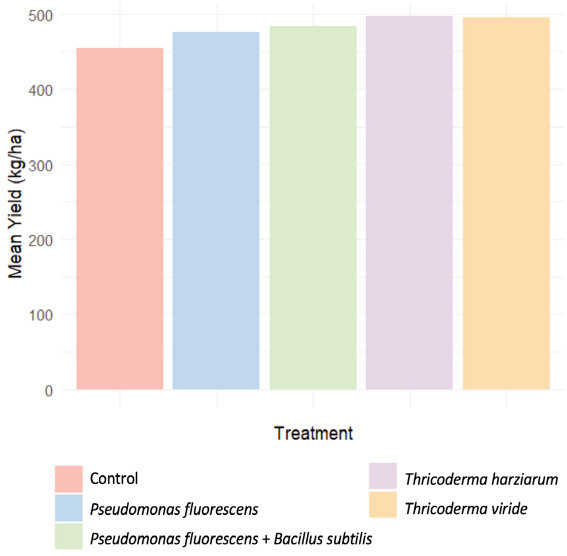

Fig. 8 - Effect of different bio-agents on yield of large cardamom.

Relationship between microbial concentration (cfu/l) and disease parameters in large cardamom

Application of *Trichoderma* spp. at a higher microbial concentration (2×10^9 cfu/l) resulted in a significant reduction in disease parameters compared to treatments with *Pseudomonas fluorescens* and *Pseudomonas* + *Bacillus* at 1×10^9 cfu/l. As shown in figure 9, *Trichoderma viride* exhibited the lowest disease severity index (DSI) and area under disease progress curve (AUDPC), indicating strong suppression of disease development in large cardamom. The reduction in both DSI and AUDPC at higher CFU levels demonstrates that increased microbial concentration enhances biocontrol efficacy. These findings suggest that *Trichoderma* spp. is more effective at controlling disease progression when applied at higher viable counts.

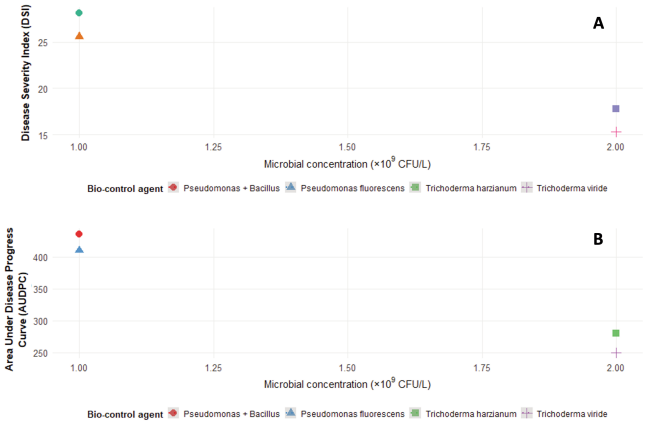

Fig. 9 - Relationship between microbial concentration (CFU/l) and disease parameters in large cardamom. A) Effect of microbial concentration on disease severity (DSI); b) Effect of microbial concentration on disease progress (AUDPC).

Effect of microbial concentration on yield of large cardamom

In addition to disease suppression, the impact of microbial concentration on yield was assessed to further validate dose efficacy. As shown in figure 10, *Trichoderma* spp. applied at 2×10^9 CFU/l resulted in the highest yield response (up to 450 kg/ha), compared to the 1×10^9 CFU/l treatments of *Pseudomonas fluorescens* and *Pseudomonas + bacillus*, which produced lower yields (approximately 360-390 kg/ha). These findings demonstrate a strong positive correlation between microbial concentration and yield performance. The improved plant health and productivity observed under higher *Trichoderma* CFU levels suggest a dual benefit - enhanced biocontrol and growth promotion - affirming that the increased dose rate is microbiologically justified and not merely an arbitrary increase in formulation quantity.

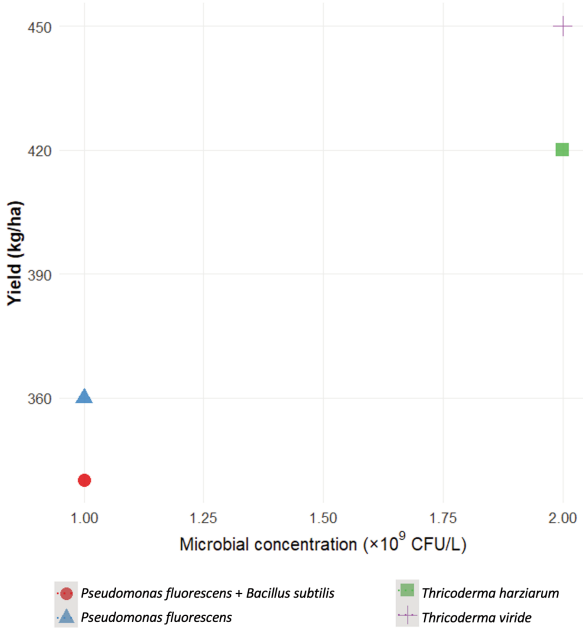

Fig. 10 - Effect of microbial concentration on yield of large cardamom.

Multivariate analysis of growth, yield, and disease parameters

The multivariate analysis provides a comprehensive and integrated understanding of the impact of different biocontrol agents on the growth, yield, and disease dynamics of large cardamom affected by *Colletotrichum* leaf blight in Taplejung, Nepal. The heatmap (Fig. 11) clearly illustrates significant variations in vegetative and yield parameters across treatments, with plants treated with *Trichoderma viride* and the combined application of *Pseudomonas fluorescens* + *Bacillus subtilis* exhibiting the highest values for yield, plant height, and leaf traits compared to the control, highlighting their strong positive influence on plant performance.

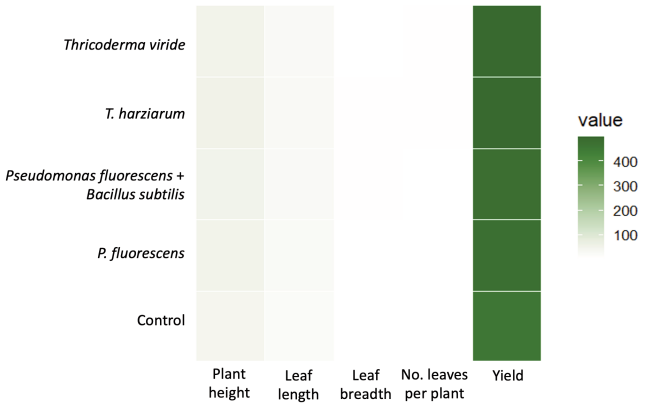

Fig. 11 - Heatmap of vegetative and yield parameters against different bioagent treatments

This observation is further reinforced by the PCA biplot (Fig. 12), where clear separation of treatment groups along the principal component axis (dim1 = 74.7%, dim2 = 10.3%) reflects the substantial contribution of biocontrol treatments in improving vegetative and yield characteristics. The overlapping yet distinct ellipses in the PCA indicate consistent but varied effects of different bioagents, emphasizing

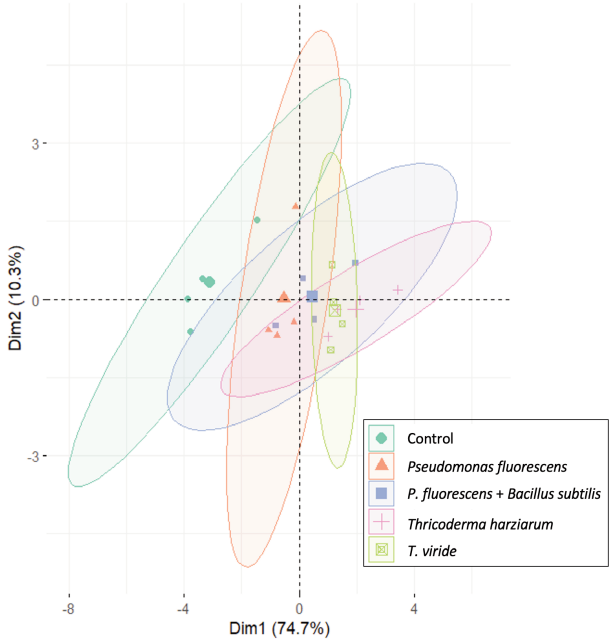

Fig. 12 - Principal component analysis of vegetative and yield traits.

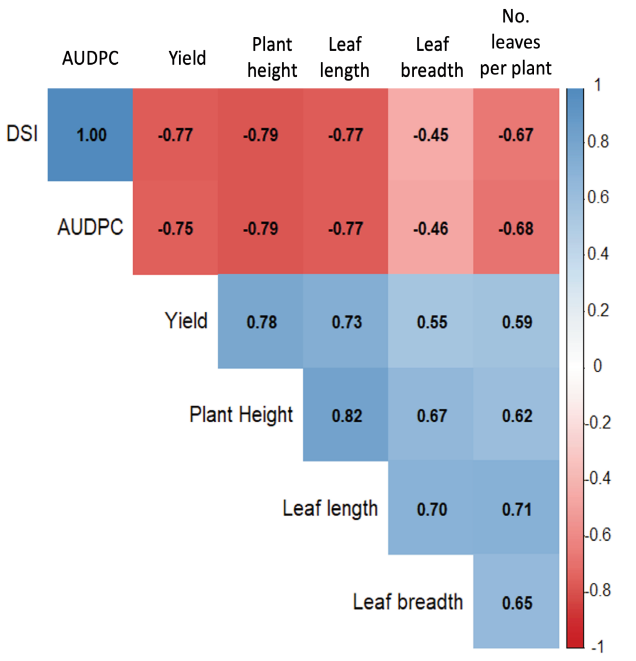

Fig. 13 - Correlation matrix of growth, yield, and disease parameters.

their differential roles in enhancing plant vigor and productivity. The correlation matrix (Fig. 13) strengthens these findings, showing strong positive associations among yield, plant height, and leaf traits, indicating that better vegetative growth directly contributes to increased yield. Conversely, disease parameters such as disease severity index (dsi) and area under disease progress curve (AUDPC) show strong negative correlations with vegetative and yield variables, underscoring the detrimental impact of disease pressure on crop performance. Complementing these results, the canonical correlation biplot (Fig. 14) and comprehensive radar chart (Fig. 15) demonstrate a clear and strong positive relationship between vegetative-yield traits and disease resistance parameters under different treatments. Control treatments clustered at lower canonical variable values, indicating poor growth and weak resistance, whereas *Trichoderma viride* and *Trichoderma harzianum* treatments exhibited higher values, reflecting superior growth performance and enhanced resistance to leaf blight. The radar chart further highlights that these treatments cover the largest polygonal areas across plant height, leaf length, leaf breadth, number of leaves, yield, audpc, and dsi, confirming their broad-spectrum effectiveness. *Pseudomonas fluorescens* and its combination with bacillus subtilis showed intermediate performance, while the control

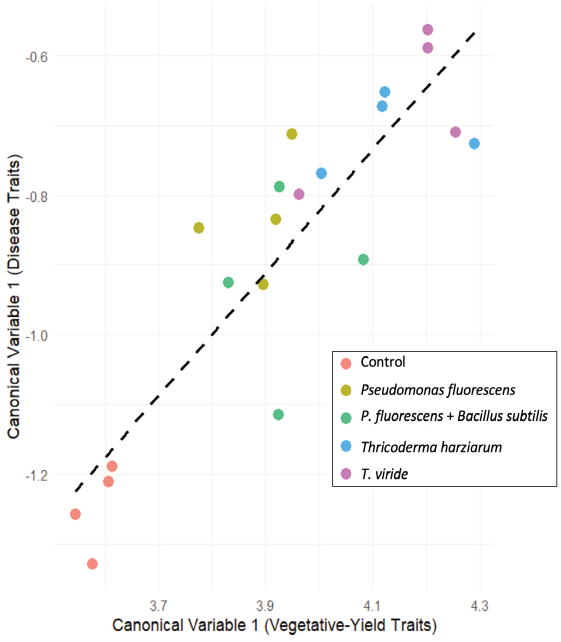

Fig. 14 - Canonical correlation biplot.

consistently displayed the lowest values across all traits. Collectively, these multivariate analyses provide strong evidence that biocontrol agents, particularly *Trichoderma viride* and *Pseudomonas fluorescens* + *Bacillus subtilis*, not only suppress *Colletotrichum* leaf blight but also significantly

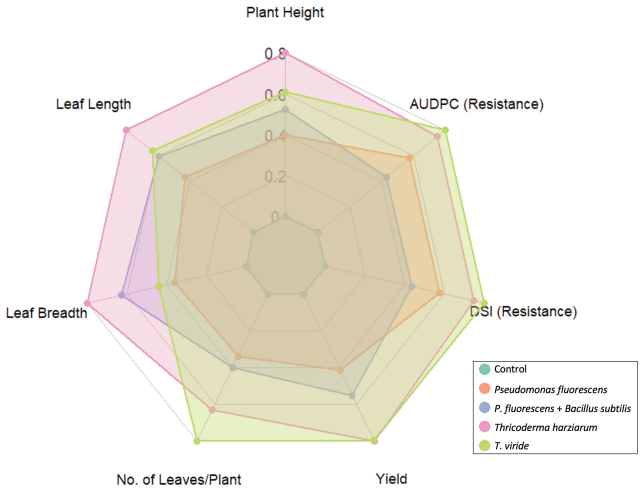

Fig. 15 - Comprehensive radar chart of growth, yield, and disease resistance parameters.

promote vegetative vigor, enhance yield, and improve disease resistance. This integrated approach underscores their potential as effective, sustainable, and eco-friendly alternatives to chemical control measures, offering a promising strategy for improving large cardamom production in the Himalayan agroecosystem.

4. Discussion and Conclusions

The present study underscores the superior efficacy of *Trichoderma harzianum* and *T. viride* in mitigating leaf blight severity caused by *Colletotrichum gloeosporioides* in large cardamom. treatments with *Trichoderma* consistently exhibited lower disease severity index (DSI) values throughout all observation periods, with *T. viride* achieving the lowest DSI of 18.5%, compared to 32.4% in *Pseudomonas fluorescens* treatments and 65.7% in the untreated control. Similarly, area under disease progress curve (AUDPC) metrics were significantly reduced in *Trichoderma*-treated plots, with an average reduction of 52% relative to bacterial treatments and 78% compared to the control. These quantitative comparisons clearly demonstrate the enhanced disease suppression capacity of *Trichoderma* spp., marking a key novelty of the current research. These findings align with previous studies highlighting the potent antagonistic capabilities of *Trichoderma* species against phytopathogens. Asalkar *et al*. (2019) reported *in vitro* bio-efficacy of various *Trichoderma* spp. against *C. gloeosporioides*, while Saju *et al*. (2013) observed effective control of leaf blight in large cardamom under field conditions using *Trichoderma*. The multifaceted mechanisms underlying *Trichoderma's* biocontrol efficacy include mycoparasitism, competition for nutrients and space, and production of antifungal metabolites (Shrestha *et al*., 2018; Dhanya *et al*., 2021). These traits enable *Trichoderma* spp. to suppress pathogen proliferation effectively while also enhancing the host plant's resistance. Beyond disease suppression, treatments with *T. harzianum* and *T. viride* significantly improved vegetative growth and yield, suggesting additional plant growth-promoting effects. The increase in plant height, number of tillers, and capsule yield in *Trichoderma*-treated plots may be attributed to the production of growth-promoting substances and enhanced nutrient uptake (Krishna *et al*., 2021). In contrast, *Pseudomonas fluorescens*, alone or in combination with *Bacillus subtilis*, provided moderate disease control (DSI 32-36%), which was notably less effective than trichoderma treatments. This discrepancy could stem from differences in colonization efficiency, metabolite production, or interactions with the host plant and pathogen, underscoring *Trichoderma's* superior field performance. Despite the comparatively lower efficacy of bacterial bio-agents, their use remains a valuable component of integrated disease management (IDM), particularly when applied in combination with other measures (Krishna *et al*., 2021). The untreated control consistently exhibited the highest DSI and lowest yield, highlighting the severe impact of *C. gloeosporioides* on large cardamom productivity and emphasizing the necessity of effective management strategies. The present study contributes novel insights by providing quantitative evidence of *Trichoderma's* superior performance over *Pseudomonas* in field conditions, reinforcing its potential as a primary biocontrol agent for large cardamom leaf blight. The dual role of *Trichoderma* in suppressing disease and promoting plant growth positions it as a sustainable and eco-friendly alternative to chemical fungicides (Dhanya *et al*., 2019; Dhanya *et al*., 2021). Previous research supports these findings; Belbase *et al*. (2018) emphasized the importance of IDM approaches incorporating biocontrol agents, while Subedi *et al.* (2022) and Biju *et al*. (2018) highlighted the field efficacy of *Trichoderma* and other bio-agents against

Colletotrichum blight. In conclusion, the application of *T. harzianum* and *T. viride* demonstrates significant promise in managing leaf blight in large cardamom, offering a sustainable alternative to conventional chemical controls. Integrating these biocontrol agents into disease management strategies can lead to improved plant health, higher yields, and reduced environmental impact, thereby supporting the goals of sustainable agriculture in the hill regions of Nepal.

The study demonstrated that among seven treatments evaluated against leaf blight of large cardamom caused by *Colletotrichum gloeosporioides*, *Trichoderma harzianum* was the most effective, recording the lowest disease severity index (DSI) and area under disease progress curve (AUDPC), along with the highest yield. *Trichoderma viride* also performed significantly better than other treatments, indicating its strong biocontrol potential. Both agents not only suppressed the pathogen effectively but also promoted plant vigor, making them sustainable alternatives to chemical fungicides. For practical use, farmers are advised to apply *Trichoderma* formulations at 15-day intervals starting one month after planting and continuing during the early monsoon period when disease incidence is high. Regular soil and foliar applications can enhance colonization and provide season-long protection. Integrating *Trichoderma* with organic mulching, field sanitation, and proper shade management can further strengthen disease resistance. However, the present study was conducted for a single season and at one location, which may limit the broader applicability of the results. Therefore, multi-season and multi-location trials are recommended to validate the findings. Overall, *T. harzianum* and *T. viride* hold strong potential for inclusion in integrated disease management (IDM) programs to ensure sustainable and eco-friendly large cardamom production.

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Genotype × environment effects on yield and fruit quality in cherry tomato (*Solanum lycopersicum* var. *cerasiforme*)

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Key words: Antioxidant pigments, hydroponic cultivation, lycopene, soil-based cultivation, total soluble solids (TSS), vitamin C.

Abstract: This study compared six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines - 'Yellow pear', 'Red pear', 'Yellow lamp', 'Red olive', 'Big red orbicular', and 'Small yellow orbicular' - grown under hydroponic and soil-based greenhouse conditions to identify superior genotypes for yield and fruit quality. The experiment was conducted during the 2023 winter-spring season in Borazjan, Iran, using a factorial experiment arranged in a completely randomized design with four replicates. Hydroponic plants (perlite:cocopeat, 40:60) received Hoagland solution, while soil-grown plants were supplied with a mixture of organic and inorganic fertilizers. Yield components (fruit weight, fruit volume, fruit set, and yield), growth (plant height), and biochemical attributes (vitamin C, total soluble solids, citric acid, carotenoids, lycopene, chlorophyll a, and chlorophyll b) were measured. Significant effects (p<0.01) of genotype, cultivation system, and their interaction were observed for all traits. 'Big red orbicular' recorded the highest fruit weight, fruit volume, yield, chlorophyll b, and carotenoid contents, while 'Red pear' showed the greatest vitamin C and chlorophyll a, and 'Small yellow orbicular' had the highest total soluble solids and citric acid. Hydroponic cultivation enhanced yield, vitamin C, and soluble solids, whereas soil cultivation favored carotenoid and lycopene accumulation. Overall, 'Big red orbicular' under hydroponic culture emerged as the most promising genotype for high-yield greenhouse production, while soil-grown tomatoes may be preferable for maximizing pigment content. These results provide practical guidance for genotype selection and cultivation system optimization in cherry tomato production.

1. Introduction

Cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) is a high-value horticultural crop appreciated for its unique flavor, attractive appearance,

and health-promoting properties. Its fruits are rich in vitamins, minerals, and bioactive compounds, particularly carotenoids (e.g., lycopene and β-carotene), vitamin C, phenolic compounds, and flavonoids (Martí *et al*., 2016; Ilahy *et al*., 2019). The high nutritional value and antioxidant potential of cherry tomatoes have been associated with a reduced risk of chronic diseases, including cardiovascular disorders and certain cancers (Mozos *et al*., 2018; Petrović *et al.,* 2022). Global demand for cherry tomatoes has been rising in both fresh and processed markets due to their diverse shapes, sizes, and colors, each associated with distinctive phytochemical profiles (Casals *et al*., 2018; Londoño-Giraldo *et al*., 2020).

The quality and yield of tomato fruit are influenced by both genetic and environmental factors, as well as their interactions. Genotypic differences affect yield components, fruit set, pigment composition, and biochemical traits (Khan *et al*., 2017; Renna *et al*., 2019). Environmental factors, including temperature, light intensity, and nutrient availability, further modulate plant physiology and fruit quality attributes (Pék *et al.*, 2014). Optimizing genotype × environment (G×E) interactions is therefore crucial for achieving stable production of high-quality fruit (Londoño-Giraldo *et al*., 2020; Petrović *et al*., 2022).

Hydroponic cultivation systems are increasingly popular for tomato production in controlled environments, offering advantages such as precise nutrient management, improved water-use efficiency, reduced soil-borne diseases, and higher yield potential (Gruda, 2009; Singh *et al*., 2020; Verdoliva *et al*., 2021). Balanced nutrient solutions such as Hoagland's provide optimal macro- and micronutrient supply, promoting growth and fruit development (Woldemariam *et al.*, 2018; Tavallali *et al.,* 2018). Studies have shown that hydroponic tomatoes often exhibit higher vitamin C and total soluble solids (TSS) due to efficient nutrient uptake and environmental control (Verdoliva *et al.,* 2021; Fernandes *et al.,* 2021).

In contrast, soil-based cultivation remains the dominant system globally and offers its own advantages, such as the complex rhizosphere microbiome that can stimulate secondary metabolite biosynthesis (Dorais *et al*., 2008; Fernandes *et al.,* 2021). Soil-grown tomatoes often accumulate more lycopene and carotenoids, likely due to mild abiotic stresses that enhance antioxidant pathways (Rasheed *et al*., 2018). However, soil cultivation is more prone to variability in nutrient availability, pathogen pressure, and environmental fluctuations, which can negatively affect yield and fruit quality (Olle *et al*., 2012; Hernandez-Perez *et al*., 2020).

Evaluating genotypes under contrasting cultivation systems is an effective approach to identify lines that combine high yield potential with desirable fruit quality traits (Khan *et al*., 2017; Renna *et al*., 2019). While several studies have compared commercial and experimental tomato cultivars under hydroponic and soil systems (Maboko *et al*., 2009; Kumar *et al*., 2022; Mohamed *et al*., 2023), comprehensive evaluations that simultaneously address yield components, growth traits, and biochemical attributes across multiple cherry tomato lines remain limited. Understanding the trade-offs between yield and quality traits such as vitamin C, TSS, citric acid, and pigment content is essential for tailoring breeding and production strategies (Pestoric *et al.,* 2021).

Given the increasing demand for nutrient-rich cherry tomatoes, detailed genotype evaluation under contrasting cultivation systems is required to provide growers and breeders with actionable recommendations. Therefore, the objective of this study was to compare six cherry tomato lines with distinct yield and biochemical profiles under hydroponic and soil-based greenhouse conditions, in order to identify superior performers and provide insights into the influence of cultivation system on yield components and fruit quality.

2. Materials and Methods

Plant material and growth conditions

The experiment was conducted during the 2023 winter-spring season at Borazjan (29°16' N, 51°13' E; 68 m asl), Bushehr Province, Iran, under greenhouse conditions. Six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines - 'Yellow pear', 'Red pear', 'Yellow lamp', 'Red olive', 'Big red orbicular', and 'Small yellow orbicular' - were evaluated.

Two cultivation systems were compared: a hydroponic (soilless) system and a soil-based greenhouse system. In the hydroponic system, plants were grown in a perlite:cocopeat substrate (40:60, v/v). Nutrients were supplied using a modified Hoagland solution prepared with analytical-grade

salts (Merck, Germany) (Table 1). The final macronutrient concentrations in the solution were 210 mg L^{-1} N, 31 mg L^{-1} P, 235 mg L^{-1} K, 200 mg L^{-1} Ca, 48 mg L^{-1} Mg, and 64 mg L^{-1} S. Micronutrient concentrations were 5 mg L^{-1} Fe, 0.5 mg L^{-1} B, 0.05 mg L^{-1} Mn, 0.05 mg L^{-1} Zn, 0.02 mg L^{-1} Cu, and 0.01 mg L^{-1} Mo. The pH of the nutrient solution was maintained at 5.8-6.0 and electrical conductivity (EC) at 2.0-2.2 dS m^{-1}.

Table 1 - Composition of the modified Hoagland nutrient solution used for hydroponic cultivation

Hoagland components	Final concentration of elements (mg/l)
Macroelements	
Nitrogen (N)	210
Phosphorus (P)	31
Potassium (K)	235
Calcium (Ca)	200
Magnesium (Mg)	48
Sulfur (S)	64
Microelements	
Iron (Fe)	5
Boron (B)	0.5
Manganese (Mn)	0.05
Zinc (Zn)	0.05
Copper (Cu)	0.02
Molybdenum (Mo)	0.01

In the soil-based system, plants were grown in a sandy-loam soil amended with well-rotted cattle manure and compost before transplanting. The organic fertilizer consisted of farmyard manure containing approximately 1.2% total N, 0.6% P_2O_5, and 1.3% K_2O, applied at a rate equivalent to 20 t ha^{-1}. Mineral fertilizers-calcium nitrate [$Ca(NO_3)_2$], magnesium sulfate ($MgSO_4 \cdot 7H_2O$), mono-potassium phosphate (KH_2PO_4), and a biostimulant product (Bioradicante, Valagro, Italy) were applied at 1-2 g L^{-1} according to crop stage. Fertilizers in the soil system were supplied in three split applications: at transplanting, at the onset of flowering, and at early fruit set.

Before transplanting, the physical and chemical properties of the soil were analyzed. The soil had a sandy-loam texture with pH 7.4, EC 1.5 dS m^{-1}, 1.2% organic matter, 0.08% total N, 18 mg kg^{-1} available P, and 160 mg kg^{-1} exchangeable K. These baseline characteristics are essential for comparing nutrient dynamics between soil and hydroponic systems.

Environmental conditions in the greenhouse were monitored throughout the growing period. Daytime air temperature ranged from 20 to 28°C and nighttime temperature from 15 to 20°C. Relative humidity was maintained between 60% and 70%, and ventilation was provided when necessary to avoid excessive heat accumulation. Plants in both systems were spaced 40 cm within rows and 70 cm between rows. Each plant was trained to a single stem, pruned weekly, and vertically supported with plastic twine. Pollination was assisted manually by vibrating inflorescences during anthesis to ensure uniform fruit set.

Irrigation and fertigation management differed between cultivation systems and were adjusted dynamically according to plant growth stage. In the hydroponic system, fertigation was supplied via drip irrigation multiple times per day. Young transplants initially received approximately 0.3-0.5 L $plant^{-1}$ day^{-1}, delivered in one to two irrigation events (7-10 min each). As plants developed a larger canopy and entered the reproductive stage, both irrigation volume and frequency increased. During peak fruiting, fertigation was applied three to four times per day, each lasting 10-15 min, delivering a total of approximately 2-3 L $plant^{-1}$ day^{-1}. Drainage was maintained at 10-20% to prevent salt accumulation and to stabilize EC within the optimal range.

In the soil-based system, irrigation was applied by drip lines approximately every two days, with volume adjusted to maintain soil water content close to field capacity. Fertilizers were delivered with the irrigation water at the predefined phenological stages described above, rather than continuously as in the hydroponic system.

The nutrient solution was delivered via drip irrigation, with pH maintained at 5.8-6.0 and electrical conductivity (EC) at 2.0-2.2 dS m^{-1}. In the soil culture, plants were grown in sandy-loam soil amended with compost and well-rotted manure. Fertilization included applications of calcium nitrate, magnesium sulfate, mono-potassium phosphate, and Bioradicante (Valagro, Italy) at 1-2 g L^{-1}, adjusted according to plant growth stage. Greenhouse temperature ranged from 20 to 28°C during the day and 15 to 20°C at night, with a relative humidity of 60-70%. Supplemental ventilation was provided when necessary.

Experimental design and treatments

The experiment was arranged as a two-factor factorial based on a completely randomized design (CRD), with four replicates per treatment and four plants per replicate. The two experimental factors were (1) cultivation system (hydroponic and soil-based) and (2) genotype (six cherry tomato lines: 'Yellow pear', 'Red pear', 'Yellow lamp', 'Red olive', 'Big red orbicular', and 'Small yellow orbicular').

Each replicate consisted of a separate cultivation unit containing four individually managed plants, considered as experimental units for data collection. Standard crop management practices - including pruning, training, and pest control - were applied uniformly across all treatments to minimize non-treatment variability.

Data were subjected to two-way analysis of variance (ANOVA) to evaluate the main effects of genotype and cultivation system, as well as their interaction (G × C). When significant differences were detected, treatment means were compared using Duncan's multiple range test at the 5% probability level.

Measurements and analytical methods

Yield components and plant height. Fruit weight (g) was measured using an electronic balance (AND EK-3000i, Japan) from a random sample of ten marketable fruits per plant. Fruit volume (cm^3) was determined by the water displacement method. Plant height (cm) was measured from the base of the stem to the shoot apex using a graduated measuring tape at the final harvest stage.

Fruit set (%) was calculated as the ratio of the number of fruits to the total number of flowers per truss × 100. Fruit yield (g $plant^{-1}$) was recorded as the total fresh weight of all marketable fruits harvested from each plant. For each variable, data were collected from four replicates (each comprising four plants) and averaged prior to statistical analysis.

Biochemical analyses. All biochemical determinations were performed on fresh fruit samples collected at the red-ripe stage from each replicate.

Vitamin C (ascorbic acid, mg 100 g^{-1} FW) was quantified by titration with standardized potassium iodate (KIO_3) in the presence of starch as an indicator, following the method described by Pearson (1976).

Total soluble solids (TSS, °Brix) were measured with a digital refractometer (Milwaukee MA871, USA) using filtered fresh fruit juice at 25°C.

Titratable acidity, expressed as citric acid (mg 100 g^{-1} FW), was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator (OECD, 1998).

Photosynthetic pigments - chlorophyll a, chlorophyll b, and total carotenoids (mg 100 g^{-1} FW) - were extracted with 80% (v/v) acetone and quantified spectrophotometrically (UV-Vis spectrophotometer, Jenway 7315, UK) at wavelengths of 663, 645, and 470 nm, respectively, according to Arnon (1949).

Lycopene (mg 100 g^{-1} FW) was extracted with a mixture of acetone:hexane (4:6, v/v) and quantified spectrophotometrically at 453, 505, 645, and 663 nm, with concentrations calculated following Ravelo-Pérez *et al.* (2008). All biochemical measurements were performed in triplicate for each replicate sample to ensure analytical precision.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with cultivation system (hydroponic vs. soil) and genotype (six cherry tomato lines) as fixed factors. The interaction between cultivation system and genotype (G × C) was included in the model. Before ANOVA, data were examined for normality and homogeneity of variances.

The experiment followed a completely randomized design (CRD) with four replicates per treatment, and the mean of four plants per replicate was used for analysis. When the ANOVA indicated significant effects, mean separation was performed using Duncan's multiple range test at $p \leq 0.05$.

All statistical analyses were carried out in SAS software (version 9.1; SAS Institute, Cary, NC, USA). Data are presented as mean ± standard deviation (SD).

3. Results and Discussion

The analysis of variance (Tables 2 and 3) showed that genotype, cultivation system, and their interaction (G × C) had highly significant effects ($p < 0.01$) on both production-related traits (plant height, fruit set, fruit weight, fruit volume, and yield per plant) and biochemical/quality traits (vitamin C, total soluble solids, citric acid, carotenoids, lycopene, and chlorophyll pigments). This demonstrates that (i) the

Table 2 - Analysis of variance (ANOVA) for plant height, fruit set, fruit weight, fruit volume, and fruit yield of six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines grown under two cultivation systems (hydroponic and soil-based)

Source of variation	df	Fruit weight	Fruit volume	Plant height	Fruit set	Yield
Genotype (G)	5	108.65 **	10685.52 **	3705.45 **	179.15 **	270057.27 **
Cultivation system (C)	1	53.34 **	5271.02 **	11041.33 **	6533.33 **	17706196.02
G × C	5	2.06 **	336.02 **	91.98 **	7.13 **	85449.27 **
Error		0.13	14.33	19.12	1.57	3087.20
CV (%)		2.68	2.70	2.19	1.56	2.65

Data were analyzed using a two-way ANOVA (completely randomized design with four replicates). ** = significant at p≤0.01.

Table 3 - Analysis of variance (ANOVA) for biochemical and pigment-related traits of six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines grown under hydroponic and soil-based greenhouse systems

Source of variation	df	Vitamin C	TSS	Citric acid	Carotenoids	Lycopene	Chlorophyll a	Chlorophyll b
Genotype (G)	5	13.79 **	2.78 **	10.19 **	3.65 **	79.04 **	0.06 **	0.04 **
Cultivation system (C)	1	457.50 **	120.33 **	60.26 **	86.30 **	262.17 **	0.21 **	0.09 **
G × C	5	14.06 **	2.00 **	0.88 **	3.07 **	8.38 **	0.02 **	0.08 **
Error		0.15	0.08	0.07	0.06	0.17	0.05	0.04
CV (%)		2.98	2.80	3.72	5.49	6.35	33.45	25.91

Data were analyzed using a two-way ANOVA (completely randomized design with four replicates). ** = significant at p≤0.01.

six cherry tomato lines are physiologically distinct, (ii) the growing system (hydroponic vs. soil) exerts a major influence on both yield and fruit quality, and (iii) most importantly, genotype performance is environment-dependent. The significant G × C interaction means that genotype ranking changed between hydroponic and soil cultivation; in other words, no single line was universally superior across all traits and both systems. For this reason, the following sections focus on mean comparisons (Tables 4 and 5) to interpret how specific genotypes responded under each cultivation system.

Fruit set and yield performance

Significant differences were observed among genotypes, cultivation systems, and their interaction

Table 4 - Mean values of plant height, fruit set, fruit weight, fruit volume, and fruit yield per plant for six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines grown under hydroponic and soil-based greenhouse cultivation

Genotype	Cultivation system	Fruit set (%)	Yield (g plant^{-1})	Fruit weight (g)	Fruit volume (cm^3)	Plant height (cm)
Yellow pear	Hydroponic	88.00±0.82 c	2405.00±21.21 c	15.77±0.26 b	169.00±1.15 b	243.75±1.89 a
Yellow pear	Soil	66.25±0.96 f	1186.25±51.21 g	13.25±0.29 d	139.50±2.89 d	210.25±2.06 b
Red pear	Hydroponic	96.00±0.82 b	2863.75±24.96 a	15.00±0.00 c	160.00±0.00 c	184.50±3.70 d
Red pear	Soil	70.75±1.26 e	1255.00±71.41 g	12.75±0.29 d	135.00±3.46 d	163.75±2.87 f
Yellow lamp	Hydroponic	98.75±1.50 a	2590.00±50.50 b	12.75±0.29 d	136±4.62 d	212.50±2.08 b
Yellow lamp	Soil	73.75±2.22 e	1475.00±66.71 f	11.00±0.00 f	117.50±0.58 f	189.50±7.00 cd
Red olive	Hydroponic	85.50±1.00 d	2701.25±129.25 b	11.12±0.25 f	109.25±1.50 g	211.00±2.00 b
Red olive	Soil	62.25±1.26 g	1536.25±41.10 f	8.25±0.29 h	87.50±2.89 i	173.75±6.24 e
Big red orbicular	Hydroponic	87.50±1.00 cd	2883.25±34.04 a	20.00±0.00 a	200.00±0.00 a	196.00±7.16 c
Big red orbicular	Soil	67.00±0.82 f	1706.25±25.62 e	19.75±0.96 a	203.00±10.00 a	163.50±4.12 ef
Small yellow orbicular	Hydroponic	95.00±0.82 b	2780.00±14.72 b	12.00±0.00 e	130.00±0.00 e	238.75±3.50 a
Small yellow orbicular	Soil	70.75 ±1.71 e	1776.25±28.69 d	9.00±0.41 g	96.00±4.24 h	203.75±4.79 c

Different letters within a column indicate significant differences at p ≤ 0.01 (Duncan's multiple range test).

Table 5 - Mean values of biochemical and pigment-related traits of six cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) lines under hydroponic and soil-based greenhouse cultivation

Genotype	Cultivation system	Vitamin C (mg 100 g^{-1} FW)	TSS (°Brix)	Citric acid (mg 100 g^{-1} FW)	Carotenoids (mg 100 g^{-1} FW)	Lycopene (mg 100 g^{-1} FW)	Chlorophyll a (mg 100 g^{-1} FW)	Chlorophyll b (mg 100 g^{-1} FW)
Yellow pear	Hydroponic	14.90±0.34 d	11.82±0.09 b	5.42±0.14 g	2.32±0.24 h	1.30±0.08 h	0.03±0.01 c	0.08±0.01 b
Yellow pear	Soil	11.82±0.09 b	7.45±0.33 h	6.96±0.13 e	4.62±0.47 de	3.51±0.43 f	0.23±0.16 b	0.30±0.17 a
Red pear	Hydroponic	18.82±0.17 a	11.10±0.08 c	5.44±0.19 g	3.16±0.10 g	5.03±0.59 e	0.31±0.05 b	0.03±0.00 c
Red pear	Soil	9.29±0.38 h	9.72±0.50 f	7.15±0.23 d	6.52±0.17 b	10.77±0.39 c	0.45±0.03 a	0.37±0.03 a
Yellow lamp	Hydroponic	16.45±0.19 c	11.65±0.10 b	6.36±0.12 f	4.45±0.19 e	2.56±0.16 g	0.09±0.02 b	0.18±0.01 a
Yellow lamp	Soil	11.24±0.51 g	8.47±0.33 g	8.45±0.17 b	5.99±0.27 c	5.17±0.51 e	0.30±0.15 ab	0.33±0.13 a
Red olive	Hydroponic	18.32±0.24 b	11.85±0.06 b	6.04±0.33 f	3.08±0.21 g	6.92±0.62 de	0.12±0.02 b	0.37±0.02 a
Red olive	Soil	9.10±0.27 h	8.80±0.29 g	8.05±0.19 c	5.79±0.29 c	13.75±0.19 a	0.28±0.05 b	0.36±0.03 a
Big red orbicular	Hydroponic	13.62±0.21 f	10.60±0.18 e	4.12±0.57 h	2.91±0.03 g	6.02±0.48 e	0.19±0.03 b	0.41±0.02 a
Big red orbicular	Soil	8.25±0.64 h	7.27±0.27 h	7.34±0.26 d	7.65±0.31 a	12.76±0.35 b	0.13±0.04 b	0.16±0.01 a
Small yellow orbicular	Hydroponic	14.12±0.15 e	12.30±0.08 a	7.42±0.28 d	3.58±0.28 f	3.68±0.49 f	0.13±0.01 b	0.16±0.01 a
Small yellow orbicular	Soil	9.21±0.51 h	8.60±0.48 g	10.30±0.09 a	5.03±0.15 d	7.60±0.33 d	0.27±0.03 b	0.25±0.03 a

Different letters within a column indicate significant differences at $p \leq 0.01$ (Duncan's multiple range test).

(G × C) for all yield-related parameters (Table 4). Overall, plants grown under hydroponic conditions exhibited markedly higher fruit set, fruit weight, fruit volume, and yield per plant compared with those grown in soil. The improved performance in hydroponics can be attributed to the more uniform nutrient availability, stable water supply, and optimal root-zone aeration, which collectively enhance flower retention and assimilate transport to developing fruits (Singh *et al.*, 2020; Verdoliva *et al.*, 2021). In contrast, soil-grown plants likely experienced mild fluctuations in soil moisture and nutrient concentration, which may have reduced flower fertility and increased fruit drop, leading to lower yields.

Among the evaluated lines, 'Big red orbicular' showed the highest yield potential under hydroponic cultivation, producing an average yield of 2883 g $plant^{-1}$ and fruit weight of 32.5 g. This genotype combined vigorous vegetative growth with a high fruit set ratio (86%), suggesting strong sink strength and efficient translocation of carbohydrates from leaves to fruits. Such performance is often linked to a greater photosynthetic capacity and a balanced source-sink relationship that supports continuous fruit filling (Khan *et al*., 2017). 'Big red orbicular' therefore appears physiologically adapted to high nutrient and water availability, conditions that typify hydroponic systems.

Conversely, under soil conditions, the same genotype maintained good but not superior yield, while 'Red olive' and 'Small yellow orbicular' performed relatively better in terms of fruit number, suggesting that these lines tolerate moderate substrate stress more efficiently. Similar environment-specific genotype responses have been reported by Renna *et al.* (2019) and Ilahy *et al.* (2019), who observed that genotype ranking in cherry tomato changes substantially between soilless and soil cultivation due to differences in nutrient-use efficiency and reproductive plasticity.

The strong G × C interaction observed for yield parameters demonstrates that yield performance is highly environment-dependent. In hydroponics, the yield advantage of 'Big red orbicular' and 'Red pear' was driven primarily by larger fruit size and more efficient resource use, while in soil, the yield of other lines was constrained by nutrient diffusion and water fluctuations. This confirms that hydroponic systems magnify the expression of genotypic potential by minimizing environmental limitations, whereas soil-based systems expose differences in stress tolerance among genotypes.

The average yields obtained in this study (ranging from 1780 to 2880 g $plant^{-1}$) are within or slightly higher than those reported by Fernandes *et al*. (2021) for cherry tomatoes grown in comparable greenhouse hydroponic systems (1500-2600 g $plant^{-1}$), indicating that the nutrient and irrigation management used here was appropriate.

In summary, the superior performance of 'Big red orbicular' under hydroponic conditions reflects its high physiological efficiency and adaptability to non-stress environments, whereas genotypes such as 'Red olive' and 'Small yellow orbicular' maintain more stable performance in soil. These contrasting responses highlight the importance of evaluating genotype × environment interactions for selecting suitable cherry tomato lines for either yield-oriented hydroponic production or resource-limited soil cultivation.

Fruit weight and fruit volume

Fruit weight and fruit volume were strongly affected by genotype, cultivation system, and their interaction (Table 4). In general, fruits produced in the hydroponic system were heavier and larger than those from the soil system, confirming that a controlled root environment and continuous nutrient availability favor cell expansion and fruit filling. The line 'Big red orbicular' consistently produced the largest fruits in both systems, with individual fruit weight above 19 g and fruit volume close to or above 200 cm^3. In contrast, 'Red olive' under soil culture showed the smallest fruits (<9 g and <90 cm^3), reflecting a strategy biased toward higher fruit number rather than individual fruit size.

The superiority of 'Big red orbicular' in fruit size under hydroponic cultivation suggests that this genotype has a strong sink capacity at the fruit level. Large-fruited lines typically exhibit prolonged cell expansion phases, thicker pericarp tissues, and more efficient assimilate unloading into the fruit, all of which depend on uninterrupted potassium and calcium supply (Khan *et al.*, 2017; Hernandez-Perez *et al.*, 2020). This is consistent with the fertigation regime in the present study, where hydroponic plants received balanced K and Ca throughout fruit development. Adequate K and Ca are known to support turgor-driven enlargement of parenchyma cells and reinforce cell wall structure, leading to increased firmness and fruit mass.

By contrast, the much smaller fruits observed in soil-grown 'Red olive' and 'Small yellow orbicular' indicate a different reproductive strategy: these lines produced many small fruits rather than fewer large fruits. This pattern is agronomically relevant because it implies that "high yield" can arise from different physiological routes - either high mean fruit weight (as in 'Big red orbicular') or high fruit set and fruit number (as in 'Red olive'). Similar genotype-dependent trade-offs between fruit number and fruit size have been described in cherry tomato under contrasting cultivation systems (Renna *et al*., 2019; Kumar *et al*., 2022).

The cultivation system also played a direct role. Hydroponic plants had access to a stable nutrient and water supply, which reduces transient water deficits around the fruit and prevents temporary restrictions in phloem unloading. This favors continuous cell expansion and results in larger fruit volume. In soil, even mild fluctuations in water availability or root-zone salinity can transiently limit expansion during the critical sizing phase, producing smaller fruits despite acceptable fruit set. Such stress-driven limitation of fruit enlargement is well documented in tomato exposed to variable irrigation regimes (Pék *et al*., 2014; Hernandez-Perez *et al*., 2020).

Taken together, these results indicate that fruit size in cherry tomato is not solely an inherent varietal characteristic; it is an emergent property of genotype × environment. 'Big red orbicular' expresses its large-fruit phenotype most strongly under hydroponic conditions, where mineral nutrition and water are non-limiting, whereas smaller-fruited lines such as 'Red olive' maintain their characteristic fruit size even under more variable soil conditions. From a production standpoint, this means growers targeting premium markets that demand larger cherry-type fruits can exploit hydroponic cultivation of large-fruited genotypes, while cultivars that naturally produce numerous smaller fruits may be more appropriate in soil-based systems where maximizing fruit number per plant is economically relevant.

Plant height

Height was significantly affected by genotype, cultivation system, and their interaction (Table 4). In general, plants grown in the hydroponic system were taller than those grown in soil, with 'Yellow pear' and 'Small yellow orbicular' reaching the greatest heights (>235 cm) under hydroponic conditions. In contrast, the shortest plants were observed in soil-grown 'Red pear' and 'Big red orbicular' (<170 cm). These differences indicate that stem elongation in cherry tomato is plastic and strongly influenced by the growing environment.

The consistently greater plant height in hydroponics can be explained by the continuous nutrient and water supply and the highly aerated

root zone of the perlite-cocopeat substrate. Under these conditions, nitrogen and potassium availability is not temporarily restricted, and osmotic stress in the root zone is minimized. This supports vigorous vegetative growth, faster internode elongation, and sustained apical dominance throughout the cropping cycle. Similar stimulation of shoot vigor under soilless culture systems has been reported for tomato, where improved root-zone oxygenation and balanced fertigation promote canopy expansion and leaf area development (Gruda, 2009; Verdoliva *et al*., 2021).

By contrast, shorter plants in the soil-based system likely reflect intermittent constraints on water and nutrient availability, especially during periods of high transpiration demand. Even mild fluctuations in soil moisture can transiently limit cell expansion in elongating internodes, resulting in more compact canopies. This is agronomically relevant: in soil culture, part of the reduction in plant height may not necessarily be a weakness, but a stress-mediated growth regulation that reduces vegetative vigor at the expense of canopy volume. Such growth restriction can also shift assimilate allocation toward reproductive sinks under certain genotypes.

The observed genotypic differences in plant height also point to contrasting growth habits and vigor potential among lines. For example, 'Yellow pear' and 'Small yellow orbicular' expressed a strongly indeterminate growth pattern under hydroponic conditions, maintaining rapid stem extension and requiring regular pruning and training. In contrast, 'Red pear' exhibited a more compact growth habit, particularly in soil, which implies inherently lower apical dominance or a stronger allocation of assimilates to fruit rather than continued stem elongation. Comparable genotype-specific variation in canopy architecture and internode length has been described in tomato germplasm panels and has been linked to differences in hormonal regulation of shoot growth and source-sink balance (Khan *et al.,* 2017; Kumar *et al*., 2022).

From a production standpoint, these results underline two practical points. First, hydroponic systems favor highly vigorous canopies that demand more frequent pruning, training, and support, especially for tall, indeterminate lines such as 'Yellow pear'. Second, more compact genotypes such as 'Red pear' may be easier to manage in soil-based greenhouses with lower input intensity, where controlling excessive vegetative growth is desirable. Together, this confirms that canopy architecture in cherry tomato is not fixed but emerges from a genotype × environment interaction, and should be considered when matching cultivars to production systems.

Vitamin C and total soluble solids (TSS)

Vitamin C and total soluble solids (TSS) were both strongly influenced by genotype, cultivation system, and their interaction (Table 5). In general, hydroponic cultivation increased vitamin C concentration compared with soil culture, with 'Red pear' and 'Red olive' showing the highest ascorbic acid contents under hydroponics (up to ~18-19 mg 100 g^{-1} FW). These values are comparable to, or slightly above, those previously reported for cherry tomato grown under controlled soilless systems (typically 14-16 mg 100 g^{-1} FW) (Fernandes *et al.*, 2021), indicating that the fertigation management used in this study supported efficient accumulation and retention of ascorbic acid in the fruit.

The enhancement of vitamin C in hydroponically grown fruits can be explained physiologically. Continuous and balanced nutrient delivery, especially potassium, supports redox homeostasis and the biosynthesis of ascorbic acid. Potassium is known to regulate key enzymatic steps in ascorbate metabolism and to stabilize antioxidant pools in tomato fruit tissues (Woldemariam *et al.*, 2018; Hernandez-Perez *et al.*, 2020). In addition, the relatively stable root-zone environment in hydroponics limits oxidative damage during fruit development, so vitamin C is not degraded as rapidly. In contrast, mild fluctuations in water and nutrient availability in the soil system may induce oxidative bursts that consume ascorbic acid rather than allowing it to accumulate at high levels.

TSS (°Brix), which reflects soluble sugars and other soluble solids, also showed a clear genotype-dependent response to cultivation system. The highest TSS values were recorded in 'Small yellow orbicular' under hydroponic conditions (up to ~12 °Brix), indicating strong sugar loading into the fruit. This level is commercially relevant because TSS above ~10 °Brix is typically associated with intense sweetness and enhanced flavor perception in cherry-type tomatoes, which is desirable in fresh markets. The consistent increase in TSS under hydroponics is consistent with previous reports that precise water control and high nutrient-use efficiency promote carbohydrate accumulation and reduce dilution effects in the fruit (Tavallali *et al*., 2018; Verdoliva *et*

al., 2021). In practical terms, this means that hydroponic growing conditions in this study favored not only yield but also sensory quality (sweetness).

Interestingly, genotype effects were not uniform across systems. While 'Red pear' and 'Red olive' clearly excelled in vitamin C under hydroponics, 'Small yellow orbicular' maintained high TSS regardless of cultivation system, suggesting that sugar accumulation in this line is strongly under genetic control and less environmentally plastic. This is valuable for breeding and cultivar recommendation: such genotypes can deliver high perceived sweetness even outside of fully optimized hydroponic conditions.

Overall, these results indicate that hydroponic production systems can be used strategically to increase nutritional (vitamin C) and sensory (TSS) quality in selected genotypes, and that specific lines such as 'Red pear' (vitamin C enrichment) and 'Small yellow orbicular' (high °Brix) can be targeted for fresh-market niches that demand high antioxidant value or high sweetness. This supports the concept that optimizing cherry tomato quality is not only a question of cultivar choice, but of matching the correct cultivar to the correct production environment.

Citric acid, carotenoids, and lycopene

Citric acid, carotenoids, and lycopene contents were significantly affected by genotype, cultivation system, and their interaction (Table 5). Soil-grown plants generally accumulated higher levels of carotenoids and lycopene, whereas hydroponic cultivation favored greater vitamin C and chlorophyll retention (as discussed above). The higher carotenoid and lycopene concentrations in soil-grown fruits indicate that mild environmental stress - such as moderate fluctuations in soil moisture, root-zone salinity, or nutrient availability - triggered the activation of antioxidant pathways, stimulating the biosynthesis of secondary metabolites that protect against oxidative stress (Dorais *et al.*, 2008; Ilahy *et al.,* 2019).

In this study, lycopene concentrations reached up to 8.2 mg 100 g^{-1} FW in soil-grown 'Red olive' and 'Big red orbicular', values slightly higher than those reported for field-grown cherry tomato under moderate stress conditions (6-8 mg 100 g^{-1} FW) (Renna *et al.*, 2019). This suggests that the soil-based greenhouse environment imposed a mild oxidative challenge that enhanced carotenoid biosynthetic activity. Carotenoid accumulation is known to depend on phytoene synthase and other enzymes of the lycopene pathway, which are upregulated under limited nitrogen or mild water deficit. These stress-related signals increase the expression of genes such as *PSY1* and *PDS*, resulting in enhanced pigment accumulation as a photoprotective mechanism (Ilahy *et al.*, 2019; Liu *et al.,* 2022).

Conversely, hydroponic-grown fruits displayed lower lycopene and carotenoid concentrations but retained higher chlorophyll levels during ripening. This pattern reflects reduced oxidative stress and faster fruit development under optimal nutrient and water supply. In hydroponics, high N availability and low environmental stress favor chlorophyll stability and rapid fruit filling, shortening the period during which carotenoid biosynthesis peaks. Although this leads to slightly paler fruits, it also results in higher yields and better nutrient use efficiency (Olle *et al.,* 2012; Verdoliva *et al.*, 2021).

Citric acid concentration also showed clear genotype-specific and environment-dependent variation. Hydroponically grown fruits of 'Small yellow orbicular' and 'Red pear' contained lower citric acid levels compared to their soil-grown counterparts, reflecting the dilution effect associated with faster fruit growth. Meanwhile, soil-grown fruits of the same lines exhibited higher acidity, likely because slower fruit expansion allowed greater organic acid accumulation relative to sugars. This inverse relationship between fruit growth rate and acid concentration has been reported previously in cherry and cocktail tomato (Pék *et al.*, 2014; Hernandez-Perez *et al*., 2020).

Taken together, these results demonstrate that hydroponic cultivation promotes primary metabolism -faster growth, higher yield, greater vitamin C - while soil conditions favor secondary metabolism - enhanced carotenoid and organic acid synthesis through mild stress signaling. The contrasting behavior of genotypes such as 'Big red orbicular' (yield- and size-oriented in hydroponics) and 'Red olive' (quality- and pigment-oriented in soil) exemplifies this genotype × environment interaction. From a breeding and production perspective, this implies that hydroponic systems should be used for high-yield production of visually uniform, less pigmented fruits, while soil-based systems remain valuable for niche markets targeting high color

intensity, antioxidant content, and stronger flavor.

Chlorophyll a and chlorophyll b

Chlorophyll a and chlorophyll b concentrations were significantly affected by genotype, cultivation system, and their interaction (Table 5). In all genotypes, hydroponic cultivation resulted in higher chlorophyll a and b contents compared with soil-based culture. The highest total chlorophyll concentration was observed in 'Big red orbicular' and 'Red pear' grown hydroponically, whereas soil-grown plants of the same lines showed a noticeable decline in both pigments. These results suggest that photosynthetic pigment accumulation is closely linked to plant nutritional status and environmental stability.

The consistently higher chlorophyll content under hydroponics can be explained by the continuous nutrient and water supply and by the absence of transient root-zone stress. Adequate nitrogen availability is particularly critical because N is a major component of chlorophyll molecules and of the enzymes involved in their synthesis, such as glutamate dehydrogenase and δ-aminolevulinic acid synthase. Stable potassium and magnesium nutrition also contributes to chlorophyll stability and thylakoid membrane integrity (Singh *et al.,* 2020; Verdoliva *et al.*, 2021). Therefore, under hydroponic conditions, efficient nutrient uptake and absence of salinity or water stress maintained active chlorophyll biosynthesis and delayed pigment degradation during the reproductive phase.

In contrast, plants cultivated in soil experienced lower chlorophyll levels, which may be attributed to temporary nutrient limitation or mild oxidative stress during the growth cycle. Even moderate fluctuations in soil moisture or electrical conductivity can increase chlorophyllase activity and reactive oxygen species (ROS) production, both of which promote chlorophyll breakdown (lahy *et al.*, 2019; Hernandez-Perez *et al*., 2020). The observed reduction in chlorophyll concentration in soil-grown plants thus reflects a shift from active photosynthesis to stress adaptation, where part of the nitrogen pool is remobilized toward antioxidant compound synthesis, including carotenoids and ascorbic acid. This trade-off between chlorophyll stability and secondary metabolite accumulation is consistent with the trends observed in the present study.

Genotypic differences were also evident. 'Big red orbicular' maintained the highest chlorophyll levels under both systems, confirming its strong vegetative vigor and photosynthetic potential, while 'Small yellow orbicular' and 'Red olive' showed lower pigment concentrations, consistent with their more compact canopy and higher investment in fruit biochemical traits. Such genotype-dependent variation in chlorophyll metabolism has been attributed to differences in leaf structure, nitrogen use efficiency, and the hormonal control of senescence (Khan *et al.,* 2017; Renna *et al.*, 2019).

From a physiological perspective, the balance between chlorophyll and carotenoids reflects how each genotype allocates resources between growth and stress defense. Hydroponic systems favor primary metabolism, sustaining chlorophyll synthesis and photosynthetic productivity, whereas soil conditions promote secondary metabolism, enhancing antioxidant pigment accumulation. The strong G×E interaction found for chlorophyll a and b further supports the conclusion that leaf pigment content is not fixed but dynamically regulated by both genotype and cultivation environment.

4. Conclusions

This study demonstrated that both genotype and cultivation system strongly influence growth, yield, and fruit biochemical traits of cherry tomato, with significant genotype × environment (G×E) interactions detected for all measured parameters. Hydroponic cultivation promoted vegetative vigor, fruit weight, and overall yield through improved nutrient and water availability, whereas soil-based cultivation enhanced secondary metabolism, resulting in greater carotenoid, lycopene, and organic acid accumulation.

The contrasting responses among genotypes highlight that no single line performs best across all traits or environments. 'Big red orbicular' expressed outstanding performance in hydroponics, combining high yield and uniform fruit size with balanced vitamin C and pigment content. By contrast, 'Red olive' and 'Small yellow orbicular' showed superior quality attributes - higher total soluble solids, citric acid, and lycopene - particularly under soil cultivation, indicating a stronger sensory profile (sweetness, acidity, color intensity) and antioxidant enrichment.

From a physiological perspective, hydroponic systems favor primary metabolism and rapid fruit development, while soil systems impose mild and structured stress that stimulates antioxidant and pigment biosynthesis. These findings confirm that cherry tomato fruit quality is not a fixed varietal property, but an environmentally modulated trait that can be directed through appropriate genotype × system matching.

In practical terms, cultivar selection in cherry tomato production should be guided by the target market segment rather than by yield alone:

• For high-yield fresh-market production requiring uniform fruit size and reliable volume (e.g. supermarket-oriented supply), vigorous genotypes such as 'Big red orbicular' and 'Red pear' are most suitable for hydroponic or other high-input greenhouse systems with stable fertigation.

• For premium fresh-market niches that emphasize intense flavor, sweetness, color, and nutraceutical value (e.g. specialty salad mixes, direct farm-to-consumer sales, high-antioxidant labeling), genotypes such as 'Red olive' and 'Small yellow orbicular' are preferable, especially under soil-based cultivation that enhances pigment and acid accumulation.

These results provide a physiological and agronomic basis for matching cherry tomato genotypes to specific production environments and market targets, and they offer actionable guidance for breeding programs aiming to balance yield, flavor, and nutritional quality under contrasting cultivation systems.

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AI statement

The authors used ChatGPT solely for improving the English language and clarity of the manuscript. All scientific content, data interpretation, and conclusions were entirely produced by the authors, who take full responsibility for the final version.

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Optimal storage duration for dormancy break and early growth in shallot bulbs

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Abstract: Shallots (*Allium ascalonicum* L.) are a critical horticultural crop in Indonesia; however, their production remains unstable owing to the inconsistent availability of quality planting materials. This study aimed to determine the optimal storage duration to break dormancy and enhance the early growth of Super Philips shallot bulbs under ambient conditions. A randomised complete block design was used to assess five storage durations (2, 4, 6, 8, and 10 weeks) by evaluating the sprouting percentage, rooting percentage, time to emergence, and early vegetative traits, including root volume, root length, plant height, and number of leaves. The results showed that bulbs stored for eight weeks exhibited the highest sprouting (98%) and rooting (99%) rates, along with the shortest mean emergence times and superior vegetative growth. Statistical analyses confirmed strong correlations and predictive regressions linking storage duration and physiological performance. These findings indicate that an eight-week storage period provides optimal physiological conditions for dormancy release, improves seedling vigour, and supports synchronised root and shoot development. This study offers actionable insights for seed management practices and validates empirical storage traditions, with implications for more stable and productive cultivation systems.

1. Introduction

Shallot (*Allium ascalonicum* L.) is one of Indonesia's most vital horticultural commodities and holds significant national economic value due to its indispensable role in daily cuisine. Used ubiquitously across

households and the food industry, its irreplaceable culinary function underscores its strategic agricultural status. Beyond its role as a kitchen staple, shallots offer substantial nutritional and medicinal properties. They contain essential macronutrients and micronutrients, such as carbohydrates, proteins, and minerals including calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), and zinc (Zn). Additionally, shallots are rich in bioactive compounds like phenols, flavonoids, and antioxidants, which possess powerful free radical-scavenging activities (Adeyemo *et al*., 2023). The growing population and the expanding food processing sector in Indonesia further drive the consistent increase in demand for shallots.

Despite the growing need, shallot production remains volatile. A persistent imbalance between supply and demand contributes to market instability and price inflation. For example, in April 2024, shallots contributed the highest food inflation rate of 30.75% month-to-month due to reduced availability across regions (Liman, 2024). Per capita consumption of shallots rose from 2.802 kg/year in 2019 to 2.861 kg/year in 2023 (Central Bureau of Statistics of Indonesia, 2024). However, national shallot production has shown fluctuations: 2,004,590 tons in 2021, decreasing to 1,982,360 tons in 2022, and slightly increasing to 1,985,233 tons in 2023 (Central Bureau of Statistics of Indonesia, 2024). This instability is attributed to multiple agronomic and logistical constraints, notably the inconsistent availability of high-quality planting materials.

One major constraint in shallot cultivation is the dependency on bulbs as the primary planting material. These bulbs serve as the foundation for initial plant growth and significantly affect crop productivity. However, their use is complicated by the natural dormancy phase post-harvest, which hinders immediate replanting (Arif *et al*., 2022). Consequently, bulbs require storage to allow dormancy to break before planting. This storage period, while necessary, introduces a new challenge: quality deterioration. Improper storage conditions can compromise bulb viability and vigor, resulting in suboptimal germination and plant development (Syam'un *et al.*, 2017). Typically, shallot bulbs are stored for 3 to 4 months post-harvest to overcome dormancy (Nurfaida *et al*., 2024). Yet, extended or poorly managed storage can lead to reduced sprouting rates, delayed emergence, weight loss, and lower dry matter accumulation (Ansar *et al*., 2022).

The general solution employed by farmers involves storing the bulbs under ambient conditions until dormancy subsides. However, this common practice lacks standardization and often depends on empirical tradition rather than scientific guidance. Variables such as bulb variety, pre- and post-harvest conditions, and storage environment (temperature, humidity, and airflow) heavily influence the dormancy period and the outcome of sprouting and rooting. Deviations in these parameters can result in mechanical, physiological, or microbial damage. Typical manifestations include moisture loss, abnormal sprouting and rooting, softening, and fungal decay (Maemunah *et al.*, 2015; Ayu *et al.*, 2023). These issues pose serious risks to planting efficiency and yield.

Scientific studies have attempted to identify optimal storage conditions and durations to manage bulb dormancy. Tarigan *et al.* (2019) reported that two months of storage influenced the prevalence of diseases caused by pathogens such as *Peronospora destructor*, *Alternaria porri*, and *Fusarium* spp. Kusmali *et al.* (2020) found that shallot bulbs stored for 16 weeks exhibited significant quality decline, with 32% rot and a weight loss of 38.7%. Meanwhile, Sarjani *et al.* (2018) observed that dormancy in shallots ends after approximately 12 weeks at 5°C, with sprouting viability and vigor exceeding 90% and a relatively low damage rate (9.8%). Such findings highlight that both the duration and condition of storage are crucial to maintaining bulb quality and performance.

Despite these insights, many farmers in regions like Enrekang Regency in South Sulawesi continue to rely on traditional practices, commonly storing shallot bulbs for approximately two months by hanging them at room temperature. Although this method is widely used, it lacks scientific validation and fails to account for environmental variability and physiological responses of different varieties. Therefore, refining the storage duration to match the physiological needs of the crop under local conditions could improve dormancy management, seed quality, and ultimately productivity.

Recent investigations into physiological changes during dormancy offer deeper insights into dormancy break mechanisms. Sarjani *et al.* (2018) emphasized that hormonal shifts-specifically increases in gibberellins, auxins, and cytokinins are instrumental in initiating sprouting, counteracting inhibitory effects of abscisic acid. Similarly, Sohany *et al.* (2016)

demonstrated that storing bulbs for 60 days at 13°C resulted in 68% sprouting. Pasigai *et al.* (2016) stated that two months of storage at room temperature is generally adequate to break dormancy in many shallot varieties. These studies underscore the interplay between environmental stimuli and internal biochemical changes in bulbs during storage, suggesting the possibility of identifying a critical storage period that maximizes seedling vigor while minimizing losses.

While existing research provides foundational knowledge on dormancy and sprouting behavior in shallots, comprehensive studies tailored to specific varieties and regional contexts remain limited. In particular, there is a lack of detailed assessments of the Super Philips variety a cultivar commonly used by farmers in Enrekang. The current literature has yet to fully quantify how different storage durations under practical room conditions affect physiological responses such as sprouting rate, rooting success, and early vegetative growth in this variety. Addressing this gap is essential for evidence-based agronomic recommendations that align with local practices and ecological conditions.

Therefore, this study aims to determine the optimal storage duration of Super Philips shallot bulbs to effectively break dormancy and enhance early seedling performance under ambient storage conditions. By evaluating key parameters such as sprouting percentage, rooting time, root and shoot development, and correlating them with storage periods, this research offers a practical solution grounded in physiological evidence. The novelty of this work lies in its focus on a locally important cultivar, its use of ambient storage mirroring field conditions, and its systematic assessment of multiple physiological parameters. The study provides actionable recommendations that can improve seed management, reduce crop failure, and contribute to more stable shallot production.

2. Materials and Methods

Study location and duration

This study was conducted at the Greenhouse of the Agrotechnology Department, Faculty of Science and Technology, Muhammadiyah University of Enrekang, located in South Sulawesi, Indonesia. The experiment took place between December 2024 and March 2025, encompassing the entire period from post-harvest bulb storage to the early growth stages of the shallot seedlings. The environmental conditions during the study closely reflected typical ambient storage and cultivation conditions in the region, thus ensuring the relevance and applicability of the results to local agricultural practices.

Plant material and bulb harvesting

The plant material used in this research was the Super Philips variety of shallot (*Allium ascalonicum L.*), a cultivar widely cultivated by farmers in the Enrekang region due to its favorable agronomic traits and market demand. The bulbs were harvested on 10 December 2024 from a shallot field located in Bulo Village, Bungin Subdistrict, Enrekang Regency. Bulbs selected for the study were uniform in size, free from visible defects, and representative of healthy post-harvest quality to reduce variability due to initial physiological differences.

Storage conditions and duration treatments

Immediately following harvest, shallot bulbs were subjected to five different storage durations under ambient room temperature conditions, which ranged between 20 and 30°C, with an average relative humidity of approximately 60%. The bulbs were stored in mesh containers to allow for sufficient air circulation and to prevent moisture accumulation, which could otherwise promote microbial growth. During the storage period, regular inspections were conducted to identify and discard any bulbs showing signs of rotting, fungal infection, or physical damage. The storage duration treatments were set at 2, 4, 6, 8, and 10 weeks, labeled respectively as P1, P2, P3, P4, and P5. These durations were chosen to reflect commonly observed storage periods in traditional farming practices while enabling an analytical comparison of physiological outcomes.

Experimental design

The experiment was arranged in a one-factor Randomized Complete Block Design (RCBD) to control for potential variability in greenhouse microclimate and ensure the statistical robustness of treatment comparisons. The single experimental factor was storage duration, with five treatment levels as described above. Each treatment was replicated four times, resulting in 20 experimental units. Within each unit, bulbs were planted and monitored under standardized agronomic practices to isolate the effect of storage duration.

Cultivation practices

After completing their respective storage durations, the shallot bulbs were transplanted into greenhouse beds. The planting dates corresponded to each storage treatment: bulbs in P1 were planted on 24 December 2024, P2 on 7 January 2025, P3 on 21 January 2025, P4 on 4 February 2025, and P5 on 18 February 2025. Soil preparation included tilling, weed removal, and application of organic fertilizers. Additionally, poultry manure was incorporated as an organic amendment to enhance soil fertility and structure. Standard cultivation procedures such as irrigation, manual weeding, pest and disease monitoring, and thinning were performed uniformly to maintain plant health and minimize the influence of external variables on seedling performance.

Observed parameters

To assess the impact of storage duration on dormancy break and early seedling growth, various physiological and morphological parameters were observed. These included sprouting metrics such as the percentage of bulb sprouting (%), the time taken to reach 10% sprouting (d), and the mean sprouting time (d). Rooting performance was evaluated by measuring the percentage of bulbs that developed roots (%), the time to reach 10% rooting (d), and the mean rooting time (d). Furthermore, vegetative growth parameters including root volume (mm^3), root length (cm), plant height (cm), and number of leaves per plant were recorded. Data collection commenced immediately after planting and continued through the early stages of vegetative development to capture reliable indicators of bulb vigor and physiological response to storage duration.

Statistical analysis

The data obtained were processed and analyzed using R Studio statistical software. A one-way analysis of variance (ANOVA) was employed to test the effects of different storage durations on each observed parameter. The level of significance was set at $\alpha = 0.05$. Where significant differences were detected, post-hoc analysis was conducted using Duncan's Multiple Range Test (DMRT) to compare treatment means. Regression analysis was also conducted to evaluate the linear relationships between storage duration and each physiological response. Coefficients of determination (R^2) were reported to indicate the strength of association, and the regression equations were used to model predictive responses. In addition, correlation analysis was performed to determine the relationships among all measured parameters, particularly focusing on the interactions between sprouting, rooting, and vegetative traits. The analysis was carried out in the latest version of R Studio using the cor() function with the Pearson method, and the absolute correlation coefficients (|r|) were obtained by applying the abs() function to the resulting correlation matrix to evaluate the strength of association irrespective of direction. The strength of correlations was interpreted based on |r| values, where <0.3 indicated a weak, 0.3-0.7 a moderate, and >0.7 a strong relationship. To visualize the overall interrelationships among parameters, a correlation web was constructed using the qgraph and corrplot packages in R Studio, with edge thickness representing the magnitude of |r| and color gradients (blue to pink) indicating the direction of the correlations.

3. Results

Effect of storage duration on bulb sprouting

The results of this study demonstrate that storage duration significantly influenced the sprouting capacity of Super Philips shallot bulbs (Fig. 1). Bulbs stored for eight weeks exhibited the highest sprouting percentage at 98%, which was statistically different ($p<0.05$) from all other treatments. In

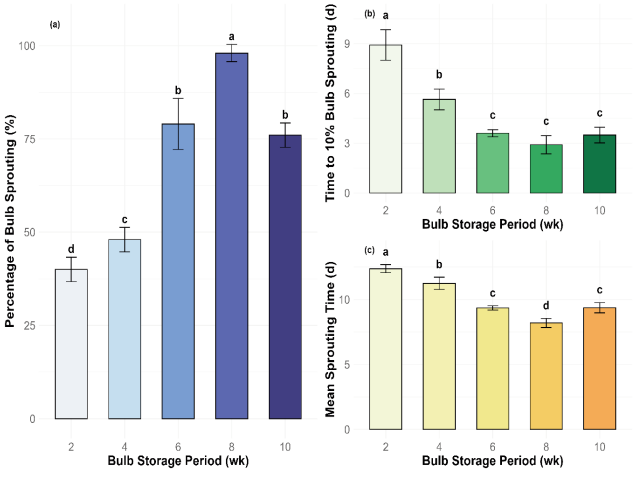

Fig. 1 - Sprouting traits. Note: Week (wk). Bars represent mean values (± standard error, n = 3). Different lowercase letters above bars indicate significant differences among storage durations according to Duncan's Multiple Range Test ($p<0.05$).

contrast, the lowest sprouting percentage was recorded in the two-week storage treatment (40%), followed by four weeks (48%), six weeks (79%), and ten weeks (76%). These findings indicate that an eight-week storage period is optimal for promoting dormancy break and achieving maximum sprouting efficiency.

The time required to reach 10% sprouting (T10S) also varied significantly across treatments. Bulbs stored for eight weeks reached this threshold in just 2.91 days, which was statistically different ($p < 0.05$) from all other treatments. Although storage durations of six and ten weeks (3.1 and 3.3 days, respectively) were not statistically different from the eight-week treatment, the shortest duration was consistently achieved with eight-week storage. This result supports the hypothesis that dormancy break is most effectively initiated around this period. Furthermore, the mean bulb sprouting time (MBS) reinforced this trend. Bulbs stored for eight weeks recorded the shortest MBS at 6.7 days, which was significantly shorter than the values for two weeks (10.8 days), four weeks (9.4 days), six weeks (7.7 days), and ten weeks (7.8 days). This pattern confirms that eight weeks of storage not only maximized the percentage of sprouting but also accelerated the initiation and uniformity of sprout emergence.

Effect of storage duration on bulb rooting

Storage duration also had a significant impact on the rooting response of shallot bulbs (Fig. 2). The highest percentage of bulb rooting (99%) was observed in the eight-week storage treatment, significantly outperforming all other treatments. Rooting percentages for the remaining durations were as follows: two weeks (48%), four weeks (77%), six weeks (92%), and ten weeks (92%). Like the sprouting results, these data suggest that an eight-week storage duration supports optimal physiological readiness for root initiation.

In terms of the time to reach 10% rooting (T10R), bulbs stored for eight weeks demonstrated the fastest performance at 1.54 days. This was significantly different from the two-week (5.20 days), four-week (2.99 days), and ten-week (2.78 days) treatments. Although the six-week treatment (1.98 days) did not differ significantly from the eight-week treatment, the consistent superiority of the eight-week period in both sprouting and rooting metrics confirms its central role in dormancy termination.

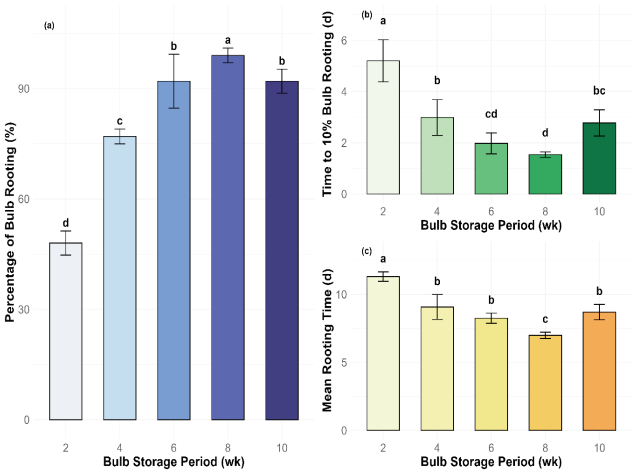

Fig. 2- Rooting traits. Note: Week (wk). Bars represent mean values (± standard error, n = 3). Different lowercase letters above bars indicate significant differences among storage durations according to Duncan's Multiple Range Test ($p<0.05$).

The mean bulb rooting time (MBR) further emphasized these differences. The shortest MBR was found in the eight-week treatment at 6.9 days, followed by six weeks (8.24 days), ten weeks (8.69 days), four weeks (9.07 days), and two weeks (11.30 days). These results highlight the advantage of storing bulbs for eight weeks to achieve faster and more synchronized rooting responses, thereby improving early seedling vigor.

Relationship between sprouting and rooting dynamics

Interestingly, the study revealed that rooting does not necessarily precede or guarantee sprouting. Across all treatments, the percentage of rooted bulbs consistently exceeded the percentage of sprouted bulbs (Fig. 3). However, the smallest discrepancy between these two indicators was recorded in the eight-week storage treatment, suggesting improved synchronization of root and shoot development at this stage of storage.

This asynchronous behavior implies that root emergence may serve as an earlier physiological marker of dormancy break, with sprouting following shortly thereafter. These findings align with the observations of Nurfaida *et al.* (2024), who noted that while rooting may begin before visible sprouting, the concurrent occurrence of both phenomena marks the end of the dormancy phase.

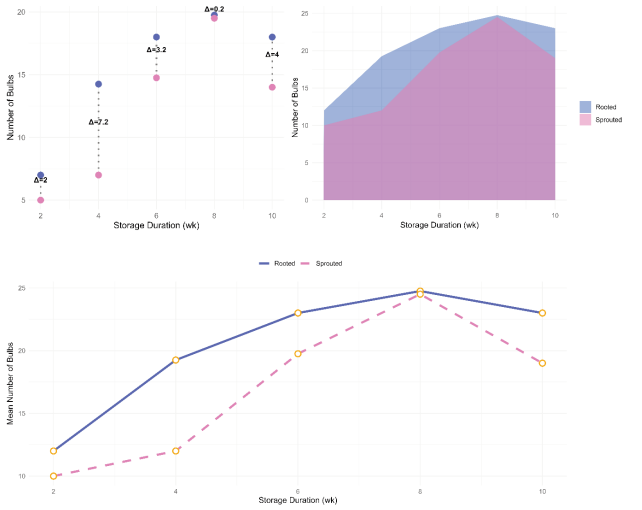

Fig. 3 - Sprouting traits. Note: Week (wk). The symbol Δ (delta) represents the difference between the number of bulbs that produced roots and those that initiated shoot sprouting.

Vegetative growth performance

Storage duration significantly affected all observed vegetative growth parameters, including plant height, number of leaves, root volume, and root length (Fig. 4). The most robust vegetative growth was observed in the eight-week treatment. Plants derived from bulbs stored for eight weeks attained an average height of 19.12 cm, significantly greater than those from two weeks (8.18 cm), four weeks (12.18 cm), six weeks (14.80 cm), and ten weeks (16.10 cm) treatments.

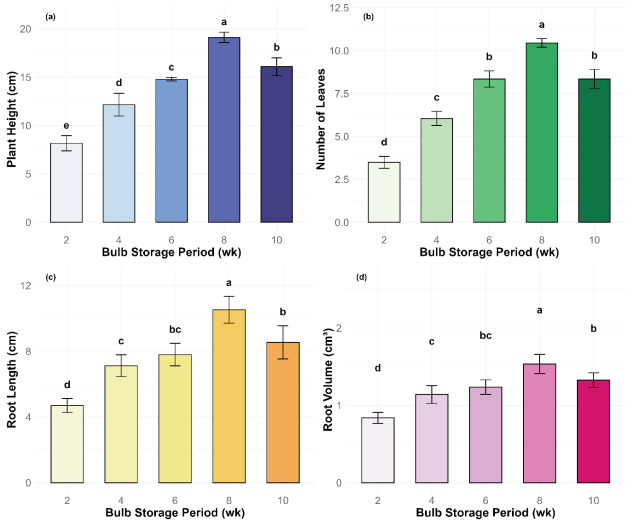

Fig. 4 - Morphology traits. Note: Week (wk). Bars represent mean values (± standard error, n = 3). Different lowercase letters above bars indicate significant differences among storage durations according to Duncan's Multiple Range Test (p<0.05).

Similarly, the number of leaves was highest in the eight-week treatment (10.45 leaves per plant), statistically surpassing the other treatments, which recorded the following averages: two weeks (3.5), four weeks (6.05), six weeks (8.35), and ten weeks (8.35). These results underscore the direct influence of optimal storage duration on above-ground vegetative development.

Below-ground traits exhibited the same trend. The highest root volume was recorded in the eight-week treatment at 1.54 mm^3, while the lowest was seen in the two-week treatment (0.84 mm^3). Intermediate values were recorded for four weeks (1.14 mm^3), six weeks (1.24 mm^3), and ten weeks (1.33 mm^3). Likewise, the longest root length (10.53 cm) was observed in the eight-week treatment, compared to 4.70 cm (two weeks), 7.13 cm (four weeks), 7.80 cm (six weeks), and 8.55 cm (ten weeks). These findings indicate that an eight-week storage period enhances the early vigor and morphological development of shallot seedlings, confirming its importance in postharvest management practices.

Correlation analysis of growth parameters

Pearson correlation analysis revealed strong and statistically significant relationships among most of the measured parameters (Fig. 5). The percentage of bulb sprouting (PBS) showed a strong negative correlation with mean sprouting time (MST, r = -0.96), time to 10% sprouting (T10S, r = -0.86), and mean rooting time (MRT, r = -0.83). This suggests that bulbs with a higher sprouting percentage tended to sprout and root earlier and more uniformly. PBS also had strong positive correlations with vegetative growth parameters, including root volume (RV, r = 0.89), root length (RL, r = 0.90), number of leaves (NOL, r = 0.97), and plant height (PH, r = 0.94), indicating a clear linkage between sprouting success and early vigor.

The time to 10% sprouting (T10S) was negatively correlated with most vegetative parameters, including RV (r = -0.85), RL (r = -0.85), NOL (r = -0.93), and PH (r = -0.91). Similar trends were observed for MST and MRT, both showing strong negative associations with early growth metrics such as NOL (r = -0.97 and -0.91, respectively) and PH (r = -0.96 and -0.91, respectively), indicating that slower sprouting and rooting were consistently associated with reduced growth performance. The percentage of bulbs rooted (PBR) also exhibited positive

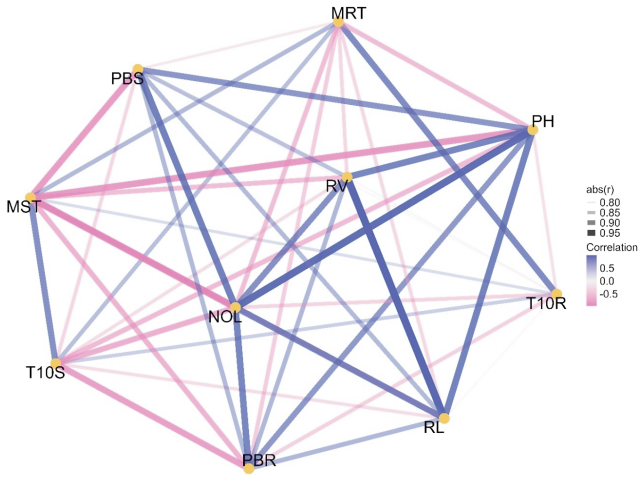

Fig. 5 - Correlation web. Note: percentage of bulb sprouting (PBS), time to 10% sprouting (T10S), mean sprouting time (MST), percentage of bulb rooting (PBR), time to 10% rooting (T10R), mean rooting time (MRT), root volume (RV), root length (RL), plant height (PH), and number of leaves (NOL).

correlations with RV (r = 0.90), RL (r = 0.90), NOL (r = 0.96), and PH (r = 0.94), reinforcing the importance of rapid and uniform root development in promoting aboveground growth. Notably, NOL and PH were highly correlated (r = 0.98).

Regression analysis

Regression models further illustrated the relationship between storage duration and key physiological parameters (Fig. 6). The regression equation for the percentage of bulb sprouting was y = 11.2 + 6.1x, with a coefficient of determination (R^2) of 0.64, indicating that 64% of the variation in sprouting percentage could be explained by storage duration. For the time to reach 10% sprouting, the regression equation was y = 7.11 − 0.486x (R^2 = 0.59), and for mean sprouting time, y = 11.2 − 0.443 x (R^2 = 0.62). These negative slopes suggest that longer storage durations generally result in faster sprouting responses.

Similarly, for the percentage of bulb rooting, the regression equation was y = 28.6 + 5.5x with R^2 = 0.70, while for time to 10% rooting, y = 3.86 − 0.227x (R^2 = 0.38), and mean rooting time, y = 9.07 − 0.32x (R^2 = 0.37). These values reinforce the previous findings, showing a strong linear influence of storage duration on both rooting and sprouting dynamics. The regression for root volume was y = 0.805 + 0.0683x (R^2 = 0.62), while root length followed y = 4.41 + 0.555x (R^2 = 0.61). Number of leaves was best

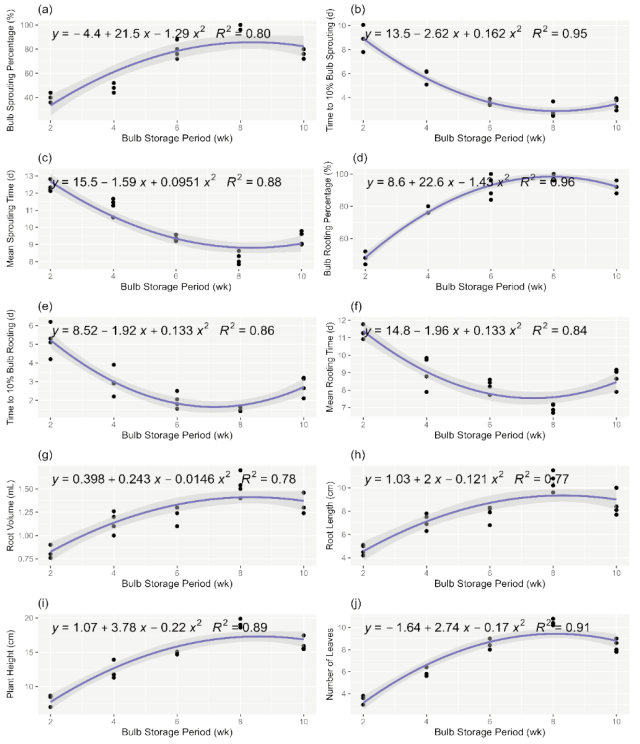

Fig. 6 - Regression analysis. Note: Week (wk). Black dots represent observed values. The blue line indicates the fitted quadratic regression model for each variable, while the grey shaded band represents the 95% confidence interval of the fitted line, illustrating the uncertainty around the model-estimated mean response.

described by the equation y = 3.11 + 0.705x (R^2 = 0.69), and plant height followed y = 7.24 + 1.14x (R^2 = 0.73), indicating a strong predictive relationship with storage duration. These models confirm that an eight-week storage duration corresponds to the inflection point for optimal growth. In summary, the results of this study consistently show that storing shallot bulbs for eight weeks prior to planting produces the best outcomes across all major physiological indicators. This storage period not only accelerates and synchronizes the processes of sprouting and rooting but also enhances early vegetative growth. The convergence of results from statistical tests, correlation matrices, and regression models provides robust evidence supporting eight weeks as the ideal duration for pre-planting bulb storage in Super Philips shallots.

4. Discussion and Conclusions

The results of this study demonstrate that storage

duration significantly affects the dormancy status, sprouting behaviour, and early vegetative growth of Super Philips shallot bulbs. Among the tested durations, an eight-week storage period consistently produced the best outcomes in terms of sprouting and rooting percentages, as well as subsequent shoot and root development. These findings align with earlier research that identifies storage time as a critical factor influencing physiological readiness and viability of shallot bulbs (Pasigai *et al.*, 2016; Sarjani *et al.*, 2018). The enhanced sprouting and rooting performance observed at eight weeks can be attributed to the natural progression of dormancy release. Dormancy in shallot bulbs is governed by a complex interplay of hormonal and environmental factors. As the storage period progresses, inhibitory compounds such as abscisic acid (ABA) gradually decline, while promotive hormones like gibberellins (GA), cytokinins, and auxins increase. This pattern is supported by evidence in onion bulbs showing that ABA concentration consistently declines during storage across cultivars and storage conditions (Chope *et al*., 2007 a; Chope and Terry, 2008), with the greatest reduction reported during the first 80 days of storage (Chope *et al.*, 2006). A pronounced decrease has also been observed between harvest and early storage, likely due to curing effects (Chope *et al*., 2007 b). Sarjani *et al.* (2018) reported that these hormonal shifts are critical for triggering metabolic activities required for sprouting. In the present study, the peak physiological response at eight weeks suggests that this duration coincides with a hormonal balance conducive to rapid shoot and root emergence. In addition to hormonal regulation, dormancy termination and sprouting onset are also marked by shifts in carbohydrate metabolism, including decreases in sucrose levels accompanied by increased respiration rate and enzyme activity (Benkeblia *et al.*, 2005; Abrameto *et al*., 2010).

Moreover, the acceleration of sprouting and rooting processes observed in the eight-week treatment can also be linked to favourable environmental storage conditions. The storage environment, characterized by room temperatures ranging from 20 to 30°C and relative humidity of approximately 60%, likely supported biochemical transitions necessary for dormancy break. Sohany *et al*. (2016) observed that shallot bulbs stored for 60 days at 13°C exhibited a 68% sprouting rate, supporting the hypothesis that controlled storage environments can enhance physiological performance. The higher percentages of both sprouting and rooting observed in the eight-week treatment confirm that this duration effectively breaks dormancy and enhances bulb viability. Root growth generally occurs before shoot emergence in dormant bulbs, as root initiation requires different hormonal activation than shoot development (Dubrovsky *et al*., 2008). This sequence may reflect the transition of the bulb from a sink organ to a source organ, which is necessary to sustain cell division at the meristematic tissue of the basal plate where rooting is initiated (Chope *et al.*, 2012 a; Chope *et al*., 2012 b). However, for optimal field performance, synchronized development of roots and shoots is crucial. The smaller discrepancy between rooting and sprouting percentages at eight weeks indicates improved physiological synchronization, which enhances transplant success and early growth (Nurfaida *et al*., 2024).

In contrast, the reduced performance in the two- and four-week storage treatments likely results from incomplete dormancy release. At these stages, inhibitory hormones may still be present in significant concentrations, delaying the onset of sprouting and reducing rooting activity. Ansar *et al.* (2022) emphasized that insufficient storage durations negatively affect vigour and lead to non-uniform seedling emergence. Similarly, the relatively moderate improvements seen at six and ten weeks suggest that eight weeks represents an optimal physiological threshold, beyond which bulb quality begins to decline. The decrease in physiological performance observed in the ten-week treatment may be attributed to the onset of senescence and cumulative exposure to storage-related stressors. Over time, stored bulbs are prone to water loss, nutrient depletion, and susceptibility to mechanical damage or microbial attack (Maemunah *et al.*, 2015; Ayu *et al*., 2023). Kusmali *et al.* (2020) found that bulbs stored for 16 weeks exhibited a 32% damage rate and 38.7% weight loss, indicating a decline in quality due to prolonged storage. The diminishing returns in sprouting, rooting, and vegetative traits observed in the ten-week treatment in this study support this conclusion.

In terms of vegetative performance, plants from eight-week stored bulbs exhibited superior shoot height, leaf number, root length, and root volume. These indicators are commonly associated with higher seedling vigour and better adaptation to field

conditions. Lestari *et al.* (2018) noted that high-vigour bulbs tend to produce uniform and robust seedlings, while low-vigour bulbs generate weaker and less consistent growth. The clear advantage of the eight-week treatment across all vegetative parameters highlights the importance of aligning storage practices with physiological readiness to maximize seedling quality. Regression and correlation analyses further validated these findings. The strong linear relationship between storage duration and sprouting/rooting percentages, as well as plant height and leaf number, indicates that physiological responses are predictably linked to storage time. For example, the coefficient of determination (R^2) for plant height was 0.73, suggesting that 73% of the variation in shoot growth could be explained by storage duration. This statistical robustness provides practical implications for farmers and seed producers aiming to optimize bulb performance.

The synchronization between root and shoot development observed at eight weeks is particularly noteworthy. Root development is essential for water and nutrient uptake, while shoot emergence facilitates photosynthesis and energy production. Enhanced coordination of these two processes ensures better establishment after transplanting and contributes to overall crop productivity (Leskovar and Othman, 2021). Synchronized sprouting ensures more uniform plant development, minimizing competition for light, water, and nutrients among individuals, which can ultimately contribute to improved plant health and higher yields (Kimmelshue *et al.*, 2022; McDonald *et al.*, 2024). The concurrent increase in root length and volume with plant height and leaf number suggests that dormancy break not only initiates sprouting but also prepares the entire plant system for active growth. Another critical insight from this study is the differential timing of physiological events. While rooting may occur earlier than sprouting in many treatments, true dormancy break is marked by the emergence of both roots and shoots. This observation aligns with the findings of Nurfaida *et al.* (2024), who emphasized that dormancy termination should be assessed through a combination of rooting and sprouting metrics. The practical implication is that seed quality assessments should include both parameters rather than rely solely on sprouting percentages.

The findings also validate the traditional practice in Enrekang Regency, where farmers typically store shallot bulbs for approximately two months before planting. Although largely empirical, this practice closely corresponds with the optimal storage duration identified in the present study. By quantifying the physiological advantages associated with eight-week storage, this research provides an evidence-based framework to support and refine local knowledge, thereby strengthening the link between scientific evidence and farmer-based management practices. Supporting this concept, evidence from other bulbous ornamentals such as lilies and tulips indicates that dormancy release and subsequent growth are also promoted by an optimum storage period (e.g., 6-8 weeks under cold storage), emphasizing that dormancy termination in bulb crops often occurs within a defined physiological window rather than increasing indefinitely with longer storage (Yang *et al.*, 2015 a; Yang *et al.*, 2015 b). However, despite the strong evidence supporting the benefits of eight-week storage, the influence of cultivar specificity and environmental variability should be carefully considered. Different shallot cultivars may exhibit distinct dormancy behaviours and physiological thresholds. Moreover, fluctuations in storage temperature, relative humidity, and light exposure may modify hormonal regulation and influence dormancy progression. Therefore, site-specific validation and cultivar-based comparisons are required to confirm the broader applicability of these findings across diverse production environments.

Finally, although this study focused on early physiological responses, future research should explore the long-term effects of storage duration on crop yield, bulb size, and postharvest quality. Integrating physiological assessments with yield-based outcomes will provide a comprehensive understanding of how pre-planting storage influences the entire growth cycle and commercial viability of shallot crops.

This study demonstrates that storage duration plays a critical role in the dormancy release and early vegetative performance of Super Philips shallot bulbs. Among the five evaluated durations, eight weeks of ambient storage consistently resulted in the highest sprouting and rooting percentages, fastest emergence times, and the most vigorous seedling development in terms of root volume, root length, plant height, and number of leaves. These findings suggest that an eight-week period aligns with optimal physiological

readiness, supported by hormonal transitions favorable for growth. The observed synchronization between sprouting and rooting, as well as the strong correlations with vegetative traits, indicate a compounded benefit for crop establishment. This study contributes empirical validation to traditional practices, reinforces the importance of pre-planting storage management, and enhances the broader understanding of dormancy physiology in shallots. Future research should explore the long-term impacts of storage duration on yield and postharvest quality, as well as varietal responses across diverse agroecological zones.

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Efficacy of humic acid and seaweed extracts on the growth, yield and biochemical properties of chilli

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Key words: Antioxidant, chilli, humic acid, seaweed, TSS, vitamin C.

Abstract: Chilli is a significant horticultural crop in Bangladesh, contributing to spice production and the rural economy. This investigation aimed to evaluate the effect of humic acid and seaweed extracts as bio-stimulants on plant growth, yield, and biochemical properties of chilli (var. Hira 1701 Supreme). There were four treatments in this research: T_0 (control), T_1 (recommended fertilizer + humic acid), T_2 (recommended fertilizer + seaweed extract), and T_3 (recommended fertilizer + both seaweed extract and humic acid). The results indicated that T_2 possessed the highest values in the most studied parameters, including plant height (60.13 cm), number of leaves (98.75), number of branches (13.25), number of flowers (37.63), fruit length (7.92 cm), seeds per fruit (79.75), yield per plot (1156.5 g), yield per hectare (7.23 ton), plant fresh weight (187.50 g^{-1} Plant), plant dry weight (41.00 g^{-1} Plant), vitamin C (177.80 mg 100 g^{-1} FW), total phenolic content (164.87 mg 100 g^{-1} FW), total flavonoid content (35.95 mg 100 g^{-1} FW), crude protein content (8.63%), and total soluble solids content (5.80 °Brix). While T_1 and T_3 also performed better than the control (T_0), T_2 consistently outperformed all other treatments. Correlation analysis revealed strong positive associations among yield, plant physiology, and key biochemical traits, indicating their interdependence. Moreover, PCA-based biplot analysis clearly separated the treatments and confirmed that T_2 showed a strong correlation with enhanced agronomic performance and superior nutritional quality. These findings show that seaweed extract foliar spray with the recommended fertilizer is superior to humic acid alone for enhancing chilli growth, productivity, and nutritional quality.

1. Introduction

The chilli pepper (*Capsicum annuum* L.) is an herbaceous perennial spice crop belonging to the Solanaceae family, commonly grown in tropical regions, and is believed to have originated in Central America (Idrees *et al*., 2020; Adom *et al*., 2024). Chilli is a significant vegetable and spice crop consumed worldwide, both domestically and commercially. It

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is an important cash crop and spice in many nations worldwide. To give food a strong, hot flavor, chillies are frequently used in a variety of dishes (Al-Imran *et al*., 2025; Kim *et al.,* 2025). Chilli is widely used in the production of curry paste, curry powder, and various pickles, as well as in the preparation of soups and salads. In the human diet, it is regarded as a significant source of β-carotene, total soluble solids, phenolic compounds, flavonoid compounds, vitamin A, vitamin C, and other phytochemicals that are essential antioxidants (Lahbib *et al*., 2021; Azlan *et al*., 2022). Chilli's pungency is caused by the alkaloid «capsaicin,» which also has a strong physiological effect and is utilized in a variety of pharmaceutical preparations, including ointments for chest congestion, colds, and sore throats (Rezazadeh *et al*., 2023). Chilli's pungency is caused by the presence of capsaicin, a crystalline, acrid, volatile alkaloid found in the placenta and pericarp of the fruit. Capsaicin has numerous preventive and therapeutic benefits in allopathic and ayurvedic medicine (Aimol *et al*., 2023). Modern agriculture is seeking new technologies to reduce the use of chemical inputs without compromising crop production or farm income worldwide. In agriculture and horticulture, seaweed and humic acid are used as nutritional supplements, bio-stimulants, or bio-fertilizers to promote plant growth and yield (Pavani *et al.,* 2022). Seaweed extracts are eco-friendly alternatives to chemical fertilizers, as they are nontoxic and do not pollute the soil or the environment. Rich with growth-promoting substances such as auxins, cytokinins, kinetin, zeatin, and gibberellins, seaweeds positively influence germination, crop establishment, and yield while also increasing tolerance to biotic and abiotic stresses (Mughunth *et al*., 2024; Sobuj *et al*., 2024).

Chilli, a widely consumed and economically significant crop, is an ideal subject for examining the impact of bio-stimulants such as seaweed and humic acid. A previous study indicated that the use of SLFs increases germination, growth, and yield of chilli plants (Vijayakumar *et al*., 2019). Auxins and cytokinins, two naturally occurring plant growth regulators (PGRs) found in seaweed extracts, regulate plant growth and structural development. Seaweed contains very low levels of PGRs, measured in parts per million. However, buds and cytokinins promote plant growth, while the indole compounds in the seaweed extract aid root development. It revitalizes leaves and promotes photosynthesis when applied to foliage. Consequently, a higher yield was obtained when the seaweed extract was used in the formulation. Additionally, humic acids encourage plants to produce antioxidants, which help reduce free radicals caused by stressors such as heat, UV light, and dehydration. Because they are potent oxidants, these radicals damage the DNA, proteins, and lipids in plant cells. Enzymes and metabolites known as antioxidants hunt down free radical molecules and shield plants from harm. They consist of water-soluble substances, such as vitamin C, and lipid-soluble substances, such as beta-carotene and vitamin E (Mukherjee and Patel, 2020; Nanda *et al*., 2022). Positive ions are drawn to humic acid, which chelates with micronutrients and releases them gradually when plants need them. By acting as a chelating agent, it stops micronutrients in the soil from precipitating, fixing, leaching, and oxidizing. Furthermore, humic compounds exhibit auxin-like activity, triggering hormonal responses that increase nutrient absorption, cell permeability, and catalytic activity, thereby enhancing dry matter production. Humic acids have great potential for foliar application in sustainable agricultural production as nutrient transporters (Eshwar *et al*., 2017). This study explores environmentally friendly alternatives, specifically seaweed extract and humic acid, to enhance chilli crop morphology and yield, and improve biochemical attributes. Chilli was selected for its susceptibility to various agronomic challenges. The effects of these bio-stimulants on growth, yield, and biochemical parameters were evaluated via a scientific experimental design. The primary objective of this study was to generate evidence-based recommendations for sustainable chilli cultivation. Ultimately, this study aims to advance agricultural knowledge of the effective use of bio-stimulants in crop production.

2. Materials and Methods

Experiment material and growing condition

The experiment was conducted from April to August 2023 at the IUBAT Agricultural Research Station, Rajendrapur, Gazipur, Bangladesh, located in AEZ 28 (Greater Dhaka). The location of Rajendrapur, Gazipur is 23.8734°N, 90.438° E, and it is approximately 9-10 meters above sea level (Quddus, 2009). The soil, a sandy loam, was prepared following the BARC Fertilizer Recommendation Guide (BARC,

2012), with cow dung (5 t/ha), TSP (330 kg/ha), gypsum (110 kg/ha), borax (1.5 kg/ha), zinc sulfate (3.91 kg/ha), and half of urea (210 kg/ha) and MoP (200 kg/ha) applied during final land preparation, while the remaining urea and MoP were added 30 days post transplantation. Humic acid (Shakti Humic Acid; Ispahani Agro Limited) was used as a powder at 1.5 g L^{-1}, as per the manufacturer's recommendations and those of Alizadeh *et al*. (2022). The *Sargassum wightii* specimens were harvested from the coast of Bangladesh, cleaned and dried in either sunlight or shade, and then pulverized. Seaweed extract (2 g/L) was prepared by grinding 2 g of seaweed powder in 1 L of distilled water for 24 h at room temperature, then filtering the extract and storing it in airtight, opaque containers at <25°C, as described by Al-Hasany *et al.* (2019).

A locally bred high-yielding chilli (*Capsicum annuum* L.) hybrid, 'Hira 1701 Supreme' (Fig. 1), popularly grown in Bangladesh, was used as a planting material. Medium-long, glossy red, very hot fruit are borne on this productive variety known for its versatility and tolerance to common field pressures. Thirty-day-old seedlings were transplanted at 60 cm × 40 cm spacing in a RCBD with four replications, comprising 16 plots (1.6 m × 1.0 m each). The treatments consisted of T_0 (RDF only), T_1 (RDF + humic acid 1.5 g/L), T_2 (RDF + seaweed extract 2 g/L) and T_3 (RDF + humic acid 1.5 g/L + seaweed extract 2g/L). Humic acid and seaweed extract were first sprayed 15 days after transplanting, then once a week during evening hours for a total of 4 applications. Irrigation and field management, as well as other standard agronomic and intercultural practices, were uniformly followed. Growth and yield characters of plants were noted at regular intervals. Fruit was harvested several times at commercial maturity (visually mature green stage) for yield, and pooled representative samples from each plot were transported to the laboratory for biochemical analysis.

Assessment of bio-chemical properties

Assessment of vitamin c (ascorbic acid). Vitamin C from fresh chilli was measured according to a modified method of Salkic *et al.* (2009). A 1 g sample was mixed with 0.056 M sodium oxalate for 2 minutes in a 10 mL solution, then allowed to stand for 5 minutes to obtain a suitable result before filtration. The filtered extract (0.5 mL) was diluted to 5 mL with sodium oxalate. The absorbance was measured using a UV-Vis spectrophotometer (UV-1900i, Shimadzu, Japan) against a sodium oxalate reference. The final vitamin C concentration was determined using a standard curve.

Assessment of total phenolic content (TPC). TPC photochemical was determined in some fresh chilli using the modified method of Al Kafi *et al*. (2025). Six milliliters of 80% ethanol were mixed with 1.0 g of fresh chilli material, and the supernatant obtained after centrifugation at 10,000 RPM and 4°C for 25 minutes was kept for further analysis. For the determination of phenolic content of the extract, the extract (1.0) was combined with Folin-Ciocalteu reagent (0.75), 7.5% sodium carbonate solution (0.25), and distilled water (1.0). The solution turned blue after 90 min in a water bath at 30°C. Absorbance data at 765 nm were collected using a

Fig. 1 - (A) Experimental field, (B) Chili plant with fruits, and (C) Effects of different treatments on chili, where T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

spectrophotometer (UV-1900i, Shimadzu, Japan). Finally, the total phenolic content was determined using a gallic acid standard curve.

Assessment of total flavonoid content (TFC). The modified Wolfe *et al*. (2003) method was used to determine the phytochemical total flavonoid content of fresh chilli. After thoroughly mixing the sample (1.0 g) with 6 mL of chilled 80% ethanol, it was centrifuged for 25 min at 10,000 RPM and 4°C. The following reagents were combined with 0.4 mL of the extract for analysis: 2 mL of distilled water, 0.12 mL of 5% sodium nitrite (which was incubated for 5 minutes), 0.24 mL of 10% aluminum nitrate, 0.8 mL of 1 mol/L sodium hydroxide, and finally 0.44 mL of distilled water. A UV-Visible spectrophotometer (Model UV-1900i, Shimadzu, Japan) was used to measure absorbance at 420 nm. TFC, which is measured in milligrams of rutin equivalents per gram of fresh weight (mg RE/g FW), was computed using a rutin standard curve.

Assessment of β Carotene. After preparing a 4:6 acetone-hexane solution, 10 mL of the solution was mixed with 1.0 g of fresh chilli, and the mixture was centrifuged. Following the procedure outlined by Nagata and Yamashita (1992) and Sharmin *et al.* (2024), the optical density (OD) of the clear supernatant was measured at 663 nm, 645 nm, 505 nm, and 453 nm using a UV-Visible spectrophotometer (Model UV-1900i, Shimadzu, Japan). The amount β-carotene was calculated using the following formula:

$$\beta\text{carotene (mg/100 g)} = 0.216 \times OD_{663} + 0.452 \times OD_{453} - 1.22 \times OD_{645} - 0.304 \times OD_{505}.$$

Here, OD represents the optical density readings at the respective wavelengths.

Assessment of total antioxidant. The antioxidant ability of the methanol extract of the plant was determined using a DPPH radical scavenging assay based on the procedures of Brand-Williams *et al.* (1995) and Susanti *et al.* (2007). 4 mg of DPPH were dissolved in 100 mL of 95% methanol to create a 0.004% solution, which was then left in the dark. The chile extract was made at a concentration of 1 mg/mL by dissolving 5 mg of crude extract in 5 mL of methanol, whereas ascorbic acid was utilized as a control at 0.1 mg/mL. Three milliliters of DPPH solution were combined with one milliliter of the powder or sample, and the mixture was left in the dark for 30 minutes. Antioxidant activity (%) was measured only at 100 µg/mL. Absorbance was measured at 517 nm to determine the radical scavenging activity, and the calculation was done using the following equation:

$$\text{Percent inhibition} = [(\text{ADPPH} - \text{A sample})/\text{ADPPH}] \times 100$$

Where, ADPPH indicated Absorbance of Control DPPH and A sample indicated Absorbance of sample

Assessment of crude protein (%). The Kjeldahl method for protein measurement in crude form was used to determine nitrogen content, following a slightly modified version of the method described by Mortuza *et al.* (2009). In a Kjeldahl flask, 0.5 g of a chilli sample was digested at 200°C with 10 mL of concentrated H_2SO_4, 9 g of K_2SO_4, and 1 g of $CuSO_4$ until a clear green solution was seen. After cooling, the liquid was steam-distilled, and 150 milliliters of distilled water were added. The distilled ammonia was trapped in a mixed indicator containing 4% boric acid. Ammonia was liberated by adding a 40% NaOH solution, and total nitrogen was determined by titrating the distillate with 0.2N HCl. Nitrogen content (N_2%) was calculated, and crude protein (CP) was determined using the formula:

$$CP\ (\%) = N_2\ (\%) \times 6.25$$

Assessment of total soluble solids (TSS). Total soluble solids (TSS) were determined using a digital refractometer, which expressed as °Brix (°B). First of all, chilli pulp was blended and filtered through filter paper. Finally, a drop of the filtered juice was given on the refractometer to check the reading and determine the result (Kafi *et al*., 2025).

Data collection and statistical analysis

Data on morphological, yield traits and biochemical properties including plant height, branches, leaves, flowering, fruit characteristics, root traits, yield, Vitamin C, TPC, TFC, β Carotene, Antioxidants, TSS etc. were collected from cultivated chilli among the treatments. Statistical analysis was performed using STATISTIX-10, R software and treatment means were compared using the LSD test at a 5% significance level.

The following formula was used to compute the increased yield percentage compared to the control:

$$\text{Increased yield (\%)} = (\text{Yield with treatment} - \text{Yield of control})/\text{Yield of control} \times 100.$$

3. Results

Morphological characteristics

The results demonstrated that chilli plant height was significantly influenced by humic acid and seaweed levels. After 90 days of transplanting, treatment T_2 (60.13 cm) showed the highest plant height, while T_0 (48.38 cm) had the lowest. Similar trends were observed at 45, 60, and 70 days of measurement. The results indicated that the different treatments significantly influenced the number of chilli leaves. The highest leaf count at 90 days after planting was observed in T_2 (98.75), while the lowest was recorded in the control treatment, T_0 (77.19). Similar trends were observed at 45, 60, and 70 days. The highest number of chilli branches was recorded at 70 days after planting in T_2 (13.25), while the control treatment T_0 showed the lowest number of branches (8.19). Similar trends were observed in the branch count at 90 days. The highest number of flowers was observed at 70 and 90 days after transplanting (DAT) in T_2, with counts of 37.63 and 32, respectively, in chilli. In contrast, the lowest number of flowers was recorded in T_0, with values of 20.88 and 8.69, respectively (Table 1).

Yield and yield attributes

The highest fruit length was observed in T_2 (7.92 cm), while the lowest fruit length was recorded in T_1 (5.69 cm) among the four treatments in chilli. Similarly, T_2 showed a larger fruit diameter (7.48 cm) than the control. These results also suggest that humic acid and seaweed did not significantly affect fruit diameter. The highest chilli seed count was observed in treatment T_2 (79.75), while the lowest was recorded in the control treatment T_0 (64.44). No significant differences in seed weight were observed, with values around 10.79 g across treatments, suggesting that humic acid and seaweed applications did not have a notable effect on seed weight. The results indicated that the highest chilli yield was achieved in treatment T_2 (1156.5 g per plot and 7.23 tons per hectare), while the lowest yield was recorded in the control treatment (T_0) at 801.75 g per plot and 5.01 tons per hectare (Table 2). The yield in T_2 showed the most significant increase over the control, with a 44.24% improvement compared to all other treatments. Both humic acid and seaweed treatments had a substantial effect on yield and yield-related attributes, with the seaweed treatment (T_2) performing best.

Table 1 - Morphological characteristics of Chili under different humic acid and seaweed treatments

Treatment	Plant height (cm)				Number of leaves			Number of branches		Number of flowers	
	45 days	60 days	70 days	90 days	45 days	60 days	70 days	90 days	70 days	90 days	70 days	90 days
T_0	26.56±2.20 b	28.25±2.25 b	31.44±3.66 b	48.38±3.84 c	48.94±5.13 ab	33.56±3.88 b	49.94±6.11 a	77.19±3.30 c	8.19±1.34 b	9.13±0.67 a	20.88±1.47 c	8.69±2.78 a
T_1	30.44±3.63 a	47±6.43 a	41.75±3.51 a	58.5±3.24 a	71.50±7.36 ab	52.38±2.87 a	84.88±7.03 a	96.19±4.67 ab	10.63±1.65 ab	11.56±0.84 a	29.75±2.92 b	26.06±4.45 a
T_2	28.44±2.72 ab	46.50±8.32 a	44.69±2.80 a	60.13±3.21 a	104.75±8.91 a	38.48±6.07 ab	84.06±4.19 a	98.75±7.89 a	13.25±1.21 a	10.38±0.80 a	37.63±2.03 a	32±3.34 a
T_3	29.56±2.20 ab	39.69±2.33 ab	40.88±3.14 a	54.19±3.12 b	33.13±6.92 b	46.94±6.63 ab	75.19±6.18 a	80.31±3.70 bc	12.75±0.83 a	9.31±0.06 a	22.93±2.05 c	29.81±5.79 a
CV	7.69	22.90	8.83	4.01	54.20	27.21	38.34	12.92	20.88	15.14	18.74	69.18
LSD	3.54	14.78	5.60	3.55	56.38	18.64	45.02	18.25	3.74	2.44	2.57	26.72
Level of significance	NS	NS	**	***	NS	NS	NS	*	*	NS	***	NS

*** = Significant at a 0.1% probability level; ** = Significant at a 1% probability level; * = Significant at a 5% probability level; NS = Not significant;
LSD = Least significant difference; CV = Coefficient of variation; Letters a, b, and c represent statistically significant differences among treatments and same letters did not show any difference; Data were represented as mean ± standard error for four replications; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

Table 2 - Yield and yield attributes of chili under different humic acid and seaweed treatments

Treatment	Fruit length (cm)	Fruit diameter (cm)	Seed per fruit	Seed weight per 1000 seed (g)	Yield per plot (g)	Yield ton per hectare	Increased yield over control (%)
T0	5.99±0.31 ab	5.56±0.47 b	64.44±3.57 c	10.64±1.15 a	801.75±30.06 d	5.01±0.19 d	0
T1	5.69±0.45 b	7.40±0.12 a	73.66±1.62 bc	10.52±0.58 a	939.50±19.77 c	5.87±0.12 c	17.8
T2	7.92±0.72 a	7.48±0.36 a	79.75±5.47 ab	10.75±1.15 a	1156.50±66.40 a	7.23±0.41 a	44.24
T3	6.62±1.22 ab	7.34±0.11 a	88.63±4.31 a	10.79±1.00 a	892.50±56.04 b	5.58±0.35 b	11.31
CV	19.74	8.11	11.61	16.89	1.67	1.67	
LSD	2.07	0.90	14.19	3.60	31.61	0.86	
Level of significance	NS	**	*	NS	***	***	

*** = Significant at a 0.1% probability level; ** = Significant at a 1% probability level; * = Significant at a 5% probability level; NS = Not significant; LSD = Least significant difference; CV = Coefficient of variation; Letters a, b, c, and d represent statistically significant differences among treatments and same letters did not show any difference; Data were represented as mean ± standard error for four replications; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

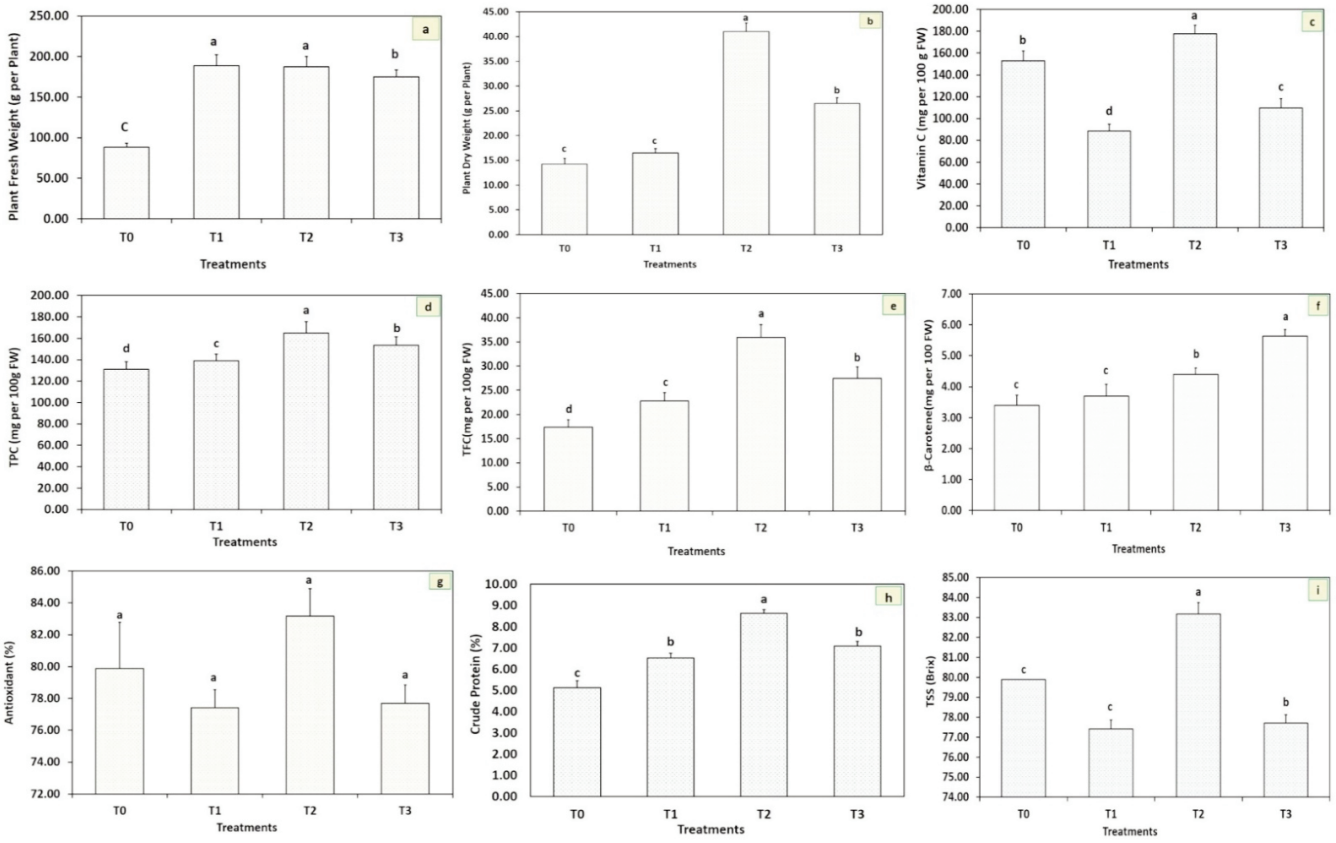

Fig. 2 - Plant physiological and biochemical properties of chili under different humic acid and seaweed treatments. Here, (a)=Plant Fresh Weight (g plant-1); (b)=Plant Dry Weight (g plant-1); (c)=Vitamin C (mg 100 g^{-1} FW); (d)=TPC (mg 100 g^{-1} FW); (e)=TFC (mg 100 g^{-1} FW); (f)=β Carotene (mg 100 g^{-1} FW); (g)=Antioxidant (%); (h)=Crude Protein (%); (i)=TSS; Letters a, b, c, and d represent statistically significant differences among treatments and same letters did not show any difference; The figure also indicates the standard error bar; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

Plant physiological and biochemical properties of chilli

The highest and lowest weights were recorded for both fresh and dry chilli plants (Fig. 2a and Fig. 2b). The relatively maximum fresh weight was observed in T_2 (187.50 g), while the lowest fresh weight was

found in T_0 (88.25 g). In terms of dry weight, the highest value was recorded in T_2 (41 g), while the lowest was in T_0 (14.25 g). The results revealed significant variation in ascorbic acid (vitamin C) content among treatments in chilli fruits. Treatment T_2 exhibited the highest vitamin C content (177.80 mg 100 g^{-1} FW). In comparison, the lowest level was observed in T_1 (88.39 mg 100 g^{-1} FW) among all treatments (Fig. 2c). The application of bio-stimulants significantly influenced the total phenolic and flavonoid content in chilli fruits (Fig. 2d). The highest TPC was recorded in the seaweed-treated plants (T_2) with 164.87 mg 100 g^{-1} FW. The lowest TPC value, 131.07 mg 100 g^{-1} FW, was observed in the control (T_0). Bio-stimulant treatments enhanced the total flavonoid content of chilli. T_2 showed the highest flavonoid concentration, 35.95 mg 100 g^{-1} FW. At the same time, the lowest TFC was recorded in the control, 17.37 mg 100 g^{-1} FW (Fig. 2e). In chilli, the combined treatment (T_3) resulted in the highest accumulation of β-carotene, 5.63 mg 100 g^{-1} FW. In contrast, the control T_0 exhibited the lowest value, 3.40 mg 100 g^{-1} FW (Fig. 2f). These findings suggest that seaweed and humic acid, particularly when applied individually or in combination, effectively enhance the phytochemical composition of chilli fruits. The results indicated that all four treatments did not influence the total antioxidant percentage in chilli fruits. No significant differences were observed in Total antioxidant percentage, which ranged from 77.41% to 83.16%, and T_2 showed the best result. However, it was not statistically higher than the other treatments (Fig. 2g). The application of biostimulants significantly increased crude protein content in chilli fruits. The highest crude protein percentage was observed in plants treated with seaweed extract (T_2), recording 8.63%. Humic acid alone (T_1) resulted in a moderate protein content of 6.53%. In comparison, the lowest value was recorded in the control treatment (T_0) at 5.13% (Fig. 2h). The results demonstrated a significant difference in the total soluble solids (TSS) content across the treatments in chilli fruits. Treatment T_2 recorded the highest TSS value (5.8 °Brix), while the lowest was observed in the control treatment (T_0) at 4 °Brix (Fig. 2i).

Comparative evaluation of different traits among the treatments

A Pearson correlation heatmap was generated to elucidate the associations among the studied morphological, yield-contributing, and biochemical traits (Fig. 3). The results revealed several significant positive correlations, underscoring strong interdependence among growth, productivity, and nutritional attributes. Out of the 171 trait pairs analyzed, 18 traits exhibited statistically significant positive correlations, indicating a focused set of interrelated parameters with potential breeding value. Yield per plot (YP) exhibited a highly significant positive correlation with increased yield over control (IYC; r = 1, ***p < 0.001), plant fresh weight (PFW; r = 1, **p < 0.01), number of flowers at 70 days (NF.70D; r = 0.97, *p < 0.05), plant dry weight (PDW; r = 0.93, *p < 0.05), and fruit length (FL; r = 0.98, *p < 0.05). These findings suggest that biomass accumulation and floral characteristics are key determinants of yield performance. Strong inter-correlations were also observed among biochemical traits. Crude protein (CP) exhibited highly significant positive correlations with total phenolic content (TPC; r =

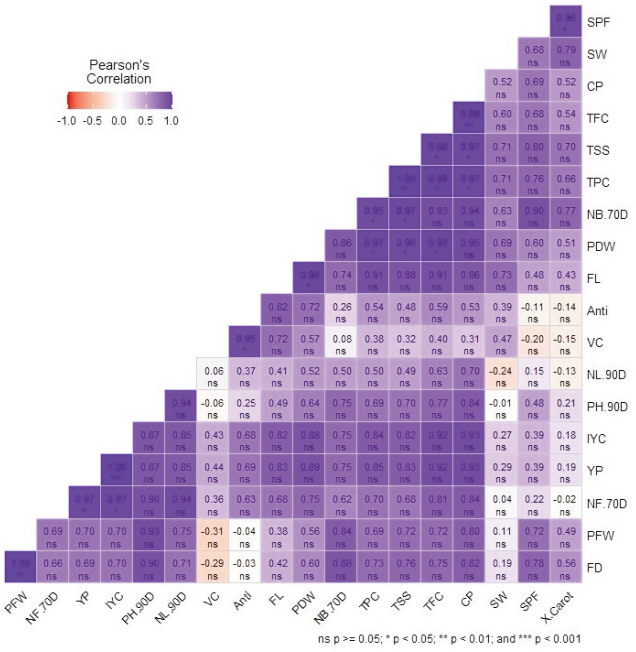

Fig. 3 - Correlation heatmap of yield and biochemical traits of chili under different humic acid and seaweed treatments. Here, PH 90D-Plant height at 90 days; NL 90D-Number of leaves at 90 days ; NB 70D-Number of branches at 70 days; NF 70D-Number of flowers at 70 days; FL- Fruit length; FD-Fruit diameter; SPF- Seed per fruit; SW-Seed weight per 1000 seed; YP- Yield per plot; mIYC-Increased yield over control; PFW-Plants fresh weight ; PDW- Plants dry weight ; VC- Vitamin C; TPC-Total phenolic content; TFC- Total flavonoids content; XCarot-βCarotene; Anti-Total antioxidants; CP-Crude protein; TSS-Total soluble solids.

0.99, *p < 0.05), total flavonoid content (TFC; r = 0.99, **p < 0.01), and total soluble solids (TSS; r = 0.98, *p < 0.05), indicating their potential co-regulation and contribution to fruit quality. Furthermore, antioxidant activity (Anti) showed a strong positive correlation with vitamin C (VC; r = 0.95, *p < 0.05), suggesting their synergistic role in enhancing nutraceutical value. In contrast, certain traits exhibited weak or negative associations. Notably, some negative non-significant correlations were found. Overall, the correlation analysis highlights meaningful trait interrelationships, emphasizing the importance of simultaneously considering both agronomic and biochemical characteristics in breeding strategies aimed at developing high-yielding, nutritionally enhanced cultivars.

Principal Component Analysis (PCA) explained 84.18% of the total variability, with PC_1 accounting for 66.89% and PC_2 for 17.29% (Fig. 4). The biplot clearly separated the treatments, with T_2 and T_3 showing strong positive associations with key agronomic and nutritional traits. Yield per plot (YP), increased yield over control (IYC), plant dry weight (PDW), total phenolic content (TPC), total flavonoid content (TFC), and crude protein (CP) were the dominant contributors along PC_1. Traits such as antioxidant activity (Anti) and vitamin C (VC) were mainly associated with PC_2. T_0 and T_1 were negatively associated with most traits, indicating lower performance. Traits such as β-carotene (βCarot), seed per fruit (SPF), and plant fresh weight (PFW) contributed moderately. Close grouping of YP, IYC, PDW, and TFC vectors indicated strong positive inter correlations. The biplot effectively illustrated the interactions between traits and treatments, with T_2 identified as the most effective treatment for enhancing yield and improving biochemical quality.

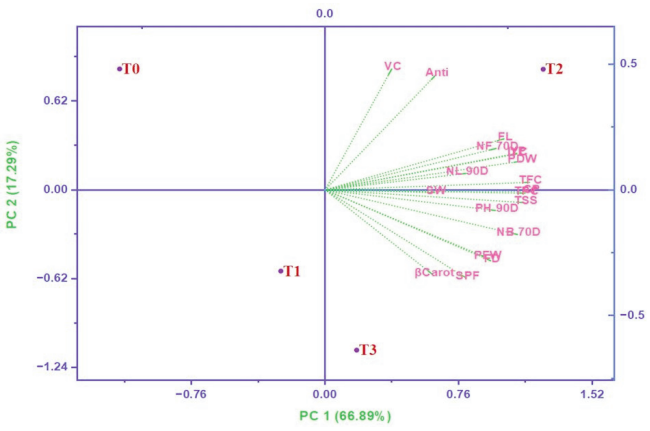

Fig. 4 - Biplot of yield and biochemical traits of chili under different humic acid and seaweed treatments. Here, PH 90D-Plant height at 90 days; NL 90D-Number of leaves at 90 days ; NB 70D-Number of branches at 70 days; NF 70D-Number of flowers at 70 days; FL- Fruit length; FD-Fruit diameter; SPF- Seed per fruit; SW-Seed weight per 1000 seed; YP- Yield per plot; IYC-Increased yield over control; PFW-Plants fresh weight ; PDW-Plants dry weight ; VC-Vitamin C; TPC-Total phenolic content; TFC- Total flavonoids content; βCarot-βCarotene; Anti- Total antioxidants; CP-Crude protein; TSS-Total soluble solids; T0 (RDF only), T1 (RDF + Humic acid 1.5 g/L), T2 (RDF + Seaweed extract 2 g/L), and T3 (RDF + Humic acid 1.5 g/L + Seaweed extracts 2 g/L).

4. Discussion and Conclusions

Bio-stimulants such as seaweed extract and humic acid are increasingly recognized as sustainable inputs that enhance plant growth, yield, and biochemical composition by modulating key physiological and metabolic processes. In this study, seaweed extract promoted chilli vegetative growth and fruit quality more effectively than humic acid under regular fertilizer application. This finding is in agreement with Alaway and Hasan (2023) and Shahen *et al.* (2019), who reported pronounced improvements in vegetative development in Capsicum following seaweed treatments. Similar observations were made by Jan *et al*. (2020) regarding branching enhancement after seaweed application, while humic acid has also been shown to stimulate branch formation, indicating that both bio-stimulants can improve plant architecture, albeit to different extents and depending on crop-specific responses. The positive impact of seaweed extract on reproductive performance is also consistent with previous studies. Jayasinghe *et al.* (2016) reported increased flower production in seaweed-treated chilli plants, supporting the reproductive improvement observed here. The enhanced fruit characteristics reported in this study align with the findings of Segmen and Ozdamar (2023) and Dutta *et al.* (2019), who documented improvements in fruit size and seed parameters following seaweed application. From a yield and quality perspective, our results align with Ashour *et al*. (2021) and Ali *et al.* (2023), who demonstrated that seaweed-based treatments significantly improved yield components and the biochemical composition of *Capsicum annuum*. Similarly, Mohamed *et al.* (2021) observed

substantial increases in pepper biomass following seaweed extract application, reinforcing its positive effect on vegetative accumulation.

The application of seaweed to chilli plants increased the chilli's biochemical properties, including vitamin C levels, as reported by Segmen and Ozdamar (2023). Increasing vitamin C content and other biochemical properties, as reported here, have been linked to enhanced nutrient availability and uptake, photosynthetic efficiency, and stress tolerance in response to various biostimulants (Khan *et al*., 2022; Mohamed and Hassan, 2025). The total phenolic content (TPC) was also significantly enhanced by both seaweed and humic acid in our study, in agreement with Musolo *et al.* (2020) and Jalali *et al*. (2022). Humic acid-treated peppers showed 36.4% and 31.8% increases in TPC relative to the controls, consistent with this study's findings and those reported by Zamljen *et al.* (2025). Similarly, Ashour *et al.* (2021) reported that the most excellent TFC value was recorded from seaweed-treated plants and associated this response with the presence of phytohormones, polyphenols, and flavonoids in the biostimulant. Mohamed *et al.* (2021) and Zohaib *et al.* (2023) demonstrated that combined seaweed and humic acid applications increased the β-carotene content in chilli fruits, suggesting a synergistic effect on pigment biosynthesis and overall nutritional potential. The differences between studies on the impact of biostimulants have been widely described and linked to differences in environmental conditions, crop species, and their physiological status, as stressed by both Roleda and Hurd (2019). These response context-dependencies need to be taken into account when extrapolating our findings to larger agro-ecological scales. In terms of protein, Yilmaz *et al.* (2018) and Veliz *et al.* (2023) demonstrated that seaweed and humic acid improve nitrogen assimilation and metabolic processes, resulting in higher crude protein accumulation in the biomass. Comparini *et al.* (2021) also reported significant responses in hot chilli peppers, including variable morphological traits, field yield, and protein content, following biostimulant application. Similarly, applying biostimulants or seaweed to chilli plants increases TSS content (Mohamed *et al*., 2021). All these combined lines of evidence strongly support the higher efficiency of seaweed extract compared with humic acid in improving chilli growth, yield, and biochemical quality. Seaweed extract, when added to regular fertilizer schedules, appears to be a good, sustainable option for enhancing chilli production and nutritional quality.

This study explored the application of seaweed and humic acid as biostimulants on growth, yield attributes, and biochemical constituents in chilli. The idea was to investigate alternative, eco-friendly soil amendments to increase chilli yield and improve fruit nutritional quality. Among the treatments, the highest response in plant growth, yield, and biochemical contents was observed with seaweed extract spray (T_2-RDF + Seaweed extract 2 g/L), followed by the suggested level of fertilizers. The treatment T_2 had the highest yield parameters, as well as elevated nutritional value of chilli fruits in terms of vitamin C, phenolic compounds, flavonoids, β-carotene, and crude protein. The correlation and PCA analyses also supported the conclusion that T_2 was strongly associated with superior agronomic traits and biochemical characters, suggesting it as an efficient bio-stimulant for chilli production. In conclusion, this study's results show that seaweed extract could be a promising, environmentally friendly input for sustainable chilli cultivation systems. Given their ability to increase crop yields without causing additional environmental stress, seaweed-based biostimulants could be a pragmatic solution for sustainable agriculture. In the future, more studies are required to refine application rates, evaluate performance in multi-season field trials, and assess the cross-crop relevance of seaweed-based bio-stimulants. Such a development can contribute to broader agricultural advancement, enabling the sector to create sustainable, high-quality crop production systems that are profitable and beneficial to the environment.

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In-situ assessment of the traditional Lebanese walnut germplasm using morphological characteristics and ISSR markers

OPEN ACCESS

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Abstract: The characterization of walnut cultivars is an important step towards conservation and exploitation of the Lebanese walnut diversity. In this study, we aimed to evaluate the genetic and phenotypic diversity within traditional Lebanese walnut cultivars grown in different regions of Lebanon. A total of 35 accessions were submitted to an ampelographic assessment; 26 of them were genetically studied using Inter Simple Sequence Repeat (ISSR) markers. A set of 27 morphological characters, including 13 qualitative traits and 14 quantitative traits related to the tree, leaf, nut, and kernel, was examined. Seven specific characters of the walnut were revealed to be the most discriminating. These characters included: leaflet shape, shell texture, shell color, nut shape, nut weight, kernel weight, and kernel color. Based on these traits, the clustering analysis identified three distinct groups that are clearly differentiated by the leaflet shape and margin. Molecular assessment was conducted using nine ISSR primers already used for walnut trees. These primers generated 72 bands, of which 67 were polymorphic. The average of the observed alleles was equal to eight in each locus. ISSR clustering based on Jaccard distance did not reveal similarity between accessions with the molecular studies. The highest genetic distance was observed between genotypes T34 and T2, differing by 45 bands (62.5%), while the lowest was between genotypes T2 and T6, differing by only four bands (5.5%). Accordingly, the results revealed a high diversity among Lebanese walnut accessions that should be further investigated to determine the major walnut genotypes and to evaluate the effect of agroclimatic conditions on the morphotypes.

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1. Introduction

Walnuts belong to the genus *Juglans* of the family *Juglandaceae* of which the most commonly grown species is the Persian walnut (*Juglans regia*). Walnuts are native to Central Asia, Eastern Europe and North America. Walnut trees usually require higher altitudes for growing well (1200 -2000 m above sea levels). They are adapted to sunny climate in summers and moderate winters. However, the trees grow well in cool and dry conditions, with optimum rainfall exceeding 800 mm. Walnuts, due to their high nutritional value, are prioritized by the Food and Agriculture Organization (FAO) (Hassan *et al.,* 2013). They are consumed in numerous ways and are also pressed for oil (Gao *et al*., 2024). Nuts are highly valued in the pharmaceutical and food industries for their high content of proteins, carbohydrates, lipids, and micronutrients. Additionally, walnuts contain bioactive compounds such as sterols, dietary fiber, and polyphenols (Sharma *et al*., 2022), which possess antimicrobial, antifungal, anti-inflammatory, antiviral, and anticancer properties. Walnuts provide value for the ecosystem in terms of offering a habitat and nourishment for many animals. The wood of some species of walnut is highly prized for its color, hardness, and grain, being used for furniture and other purposes.

The geographical nature of Lebanon is the ideal habitat for the growth of walnut trees. It is considered one of the traditional and economically important crops, and its cultivation is widespread in most Lebanese regions, especially in the north of Lebanon and the Bekaa region, near the rivers (Janta, Labweh, Ajar), in irrigated areas, and in home gardens. According to the agricultural census (MOA, 2012), the area of nut trees was approximately 1282.5 ha, with a majority of walnut trees, covering an approximate area of 1205 ha.

In Lebanon, walnut genotypes are highly diverse, often derived from nuts or cuttings, resulting in numerous landraces that are adapted to different conditions. There are many local traditional accessions but no named cultivars. Walnut accessions are distinguished and denominated according to the shape of the fruit as Dmagh shape (Brain shape), Kalb shape (Heart shape) or the hardness of the nuts (Ferk, Kasi). These accessions may harbor important traits for future breeding programs and for enhancing the genetic database of international cultivars. Over the last few decades, the traditional walnut germplasm has been threatened by various anthropogenic pressures, particularly the progressive replacement of local cultivars by more advantageous, improved varieties imported from abroad. Moreover, walnut germplasm is threatened by urbanization, climate change, outbreaks of new diseases and pests, and de-vegetation due to timber harvesting. Little information is available about the genetic diversity of Lebanese walnuts. Therefore, the preservation of traditional cultivars, which are at risk of disappearance, is a critical necessity in order to maintain the local genetic diversity of walnuts. Accordingly, characterizing the autochthonous cultivars is an important step towards conserving and exploiting the diversity of the Lebanese walnut.

Several techniques have been developed that can be used to estimate the genetic diversity of walnuts, including morphological characteristics (Atefi, 1997; Einollahi and Khadivi, 2024) and various molecular markers, such as ISSR (Potter *et al.*, 2002; Abbasi Holasou *et al*., 2023), RFLP (Fjellstrom and Parfitt, 1995; Bernard *et al*., 2018), SSR (Wang *et al.,* 2008; Wani *et al.*, 2024) and RAPD (Fatahi *et al.*, 2010). The objectives of this study were to determine the level and distribution of genetic diversity of the Lebanese traditional walnut accessions using morphological traits and ISSR markers.

2. Materials and Methods

Field survey

Field survey was performed during vegetation (Early Spring 2024) and production periods (September 2024) with the aim of collecting traditional cultivars growing in family gardens and commercial plantations. Samples of 35 walnut accessions were collected from 12 cultivated stands that are located in major walnut producing villages, which spread over four main agricultural areas (the North plain, Bekaa plain, Mount Lebanon and the South). These sites are subjected to varying climatic conditions (precipitation and temperature) and different agricultural practices. They are situated at an altitude between 262 and 1566 m, a latitude ranging from N33.27762° to N34.46924° and a longitude between E35.34504° and E36.2242° (Table 1, Fig. 1). Trees were selected based on their age (minimum of 10 years) and their sanitary state. Samples consisted of 20 mature fruits and leaves were collected from one tree per accession. The

Table 1 - Geographic and climatic information of 12 walnut sites surveyed in Lebanon

Sites	Zone	Latitude (°/N)*	Longitude (°/E) [z]	Altitude (m) [z]	Precipitation (mm)	Average annual mean surface air temperature (°C)	Number of accessions
Nabi sheeth	Beqaa	33.52478	36.06654	1187	570-750	13.6	5
Janta	Beqaa	33.51527	36.0416	1106	580-750	13.2	4
Nahle	Beqaa	34.03535	36.17998	1434	500-700	13	5
Labweh	Beqaa	34.12528	36.20107	872	350-450	15.5	2
Anjar	Beqaa	33.44001	35.56798	882	550-680	15.28	5
kfardabch	Beqaa	33.94350	36.03667	1180	540-640	13.10	2
Tal Al-amara	Beqaa	33.86388	35.98658	910	540-650	15	1
sohmor	Beqaa	33.53194	35.69833	850	600-800	15	2
Maaroub	south	33.27762	35.34504	262	650-750	20	1
Fnaydeq	North	34.46924	36.2242	1566	750-1000	14	5
Alay	Mount Lebanon	33.80485	35.60151	911	1000-1200	15.69	2
Bisour	Mount Lebanon	33.75450	35.57685	350	1000-1200	15.69	1

[z] Data listed by the Global Positioning System (GPS).

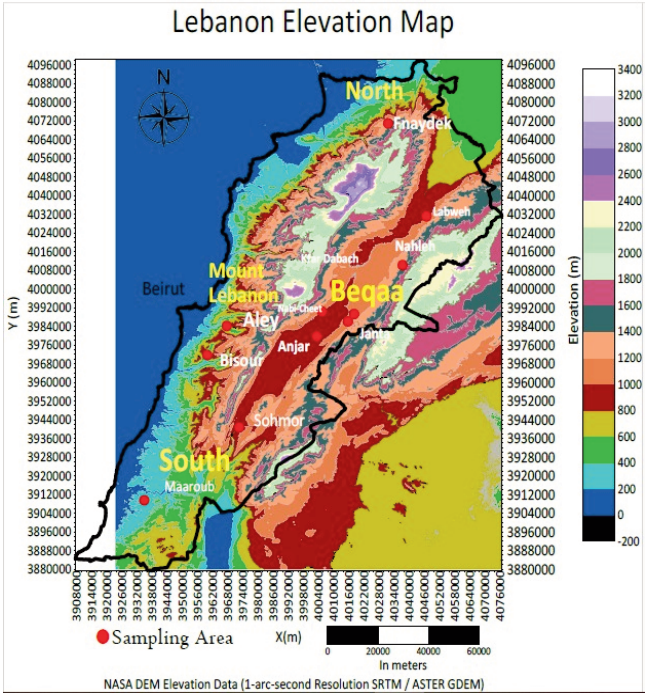

Fig. 1 - Geographic distribution of the studied walnut accessions as visualized with DIVA-GIS program.

sampled young leaves were stored at -20°C pending DNA extraction.

Morphological descriptors

Twenty-seven morphological traits related to the tree, leaf, nut and kernel extracted from the list of walnut descriptors previously developed by International Plant Genetic Resources Institute (IPGRI, 1994) were used in this study, of which thirteen qualitative (Growth habit, leaflet shape, leaflet margin, leaflet color, rachis color, nut shape, shell texture, shell color, shell strength, nut length of tip, nut shape of base, nut shape of apex, kernel color) and fourteen quantitative (Leaf length, leaf petiole, leaf width, number of leaflets, leaflet length, leaflet width, leaflet length/leaflet width, leaflet petiole, nut width, nut length, nut length/ nut width, nut weight, kernel weight, kernel percentage).

Molecular characterization

DNA for each walnut accession was extracted from 300 mg of young leaves using cetyl-trimethyl ammonium bromide (CTAB) procedure described by Doyle and Doyle (1987). DNA quantity and quality were determined spectrophotometrically at 260 nm and 280 nm (using a NanoDrop) and by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualised under UV light.

Nine ISSR primers (UBC807, UBC810, UBC811, UBC814, UBC818, UBC819, UBC821, UBC826, UBC865) previously developed for the assessment of the walnut germplasm (Christopoulos *et al.*, 2010; Aiqing *et al.*, 2014; Shamasbi *et al.*, 2018; Çilesiz, 2025) were used in this study based on their good results for amplification and high power of discrimination on walnut.

Microsatellite amplifications were performed in a total volume of 20 µl with 2 µl of PCR buffer (10mM Tris-HCl, 50 mM KCl, 0.1% Triton x 100 and 0.02% of gelatin), 2.5 mM MgCl2, 0.2 mM of each dNTP, 2.5 µM primer, 1,5 U of Taq DNA polymerase and 50 ng of walnut genomic DNA. PCR was carried out using a Bio-rad thermocycler. PCR reactions were run with a temperature pattern of initial DNA denaturation at 95°C for 4 minutes, followed by 35 cycles of DNA denaturation at 94°C for 45 seconds, primer annealing at 45-47-51.3°C according to primer for 45 seconds, the extension stage with 72°C for 2 minutes and a final extension step of 7 min at 72°C. Five µl of the PCR products were separated on a 2% agarose gel electrophoresis prepared in TBE 1X stained with ethidium bromide to check the PCR amplification and determine the size of the amplified fragments.

Data analysis

Qualitative characteristics have been described and scored according to IPGRI standards (IPGRI, 1994). For quantitative traits, the mean ± standard deviation was calculated. Means were compared using analysis of variance (Anova) followed by the Least Significant Difference (LSD) tests, performed with SAS software (SAS, 1995).

Trait evaluation was performed by using the principal component analysis (PCA). The relationships between walnut leaves and fruits based on their quantitative and qualitative traits were analyzed using Hierarchical Cluster Analysis with Euclidean distance in the PAST software (Hammer *et al*., 2001).

To assess the information given by ISSR markers, amplified bands were scored as 1 for presence or 0 for absence. The following parameters were calculated as follow: number of total alleles per locus, the percentage of polymorphic band (PPB), Polymorphism Information Content for the dominant markers (PIC = 2 x f x (1-f), where f is the frequency of present allele) and power of discrimination (PD= 1 -Σ gi^2, where gi is the frequency of the i_{th} genotype). Genetic distances were calculated according to Jaccard. Trees clustering the data with the unweighted pair-group method (UPGMA) with SAHN-clustering and tree programs of PAST software (Kriege *et al.,* 2014).

3. Results and Discussion

Morphological characterization analysis

In this study, a set of 27 descriptors was examined for 35 walnut accessions collected from various agroclimatic areas in Lebanon, encompassing 13 qualitative and 14 quantitative traits related to the tree, leaf, nut, and kernel. Residual normality was assessed for 14 quantitative traits using the Shapiro-Wilk test. Six traits (Leaf length, leaf width, leaf petiole, leaflet length, leaflet width and kernel weight) were normally distributed (P>0.05) and were analyzed with Anova test followed by LSD. The walnut samples of these traits showed significant differences among walnut trees (p<0.05) and pairwise comparisons using LSD indicated which walnut trees differed significantly.

The remaining eight traits violated the normality assumption (p<0.05) were showed statistically significant differences between walnut tress by using nonparametric procedures (Mann-Whitney U test, data not shown).

Morphological characteristics of the Trees and leaves

The growth habit of the tree showed diversity among the studied accessions, varying between erect, semi-erect, and spreading. Differences were clearly observed in the leaflet shape (Fig. 2), which ranged from narrow elliptic (Two accessions) to elliptic (23 accessions) and broad elliptic (10 accessions), predominantly exhibiting entire margins. The leaflet and rachis color of nearly 71% and 66% of the accessions were green and green to yellow, respectively. The two accessions, T27 (Tal Al-Amara) and T44 (Bisour), exhibit unique traits, such as narrow-elliptic leaflets and serrate margins, which

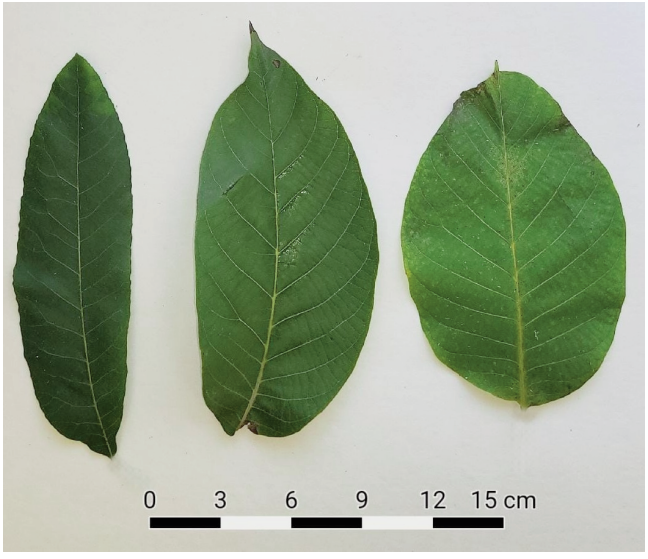

Fig. 2 - Leaflet shapes, from left to right: Narrow elliptic, Elliptic, and Broad elliptic.

emphasise morphological diversity (Table 2).

The accessions showed significant differences in the leaf length ranging from 21.81 ± 3.88 cm (T44, Bisour) to 44.60 ± 4.29 cm (T16, Nahle) and leaf width between 18.69 ± 3.66 cm (T44, Bisour) and 40.88 ± 3.75 cm (T43, Alay) (Table 3). Petiole length average of the leaf was between 4.20 ± 0.71 cm (T44, Bisour) and 10.75 ± 1.55 cm (T43, Alay). The leaflet number of the leaf was between 3 (T11, Janta) to 15 (T27, Tal Amara). The average leaflet length ranged between 9.32 ± 1.38 cm (T44, Bisour) and 20.59 ± 1.47 cm (T43, Alay) and width between 2.70 ± 0.31 cm (T44, Bisour) and 8.99 ± 0.66 cm (T43, Alay). The petiole length of the leaflet of nearly 77% of the accessions was 0.1 cm. The ratio leaflet length to leaflet width in the majority of the samples exceeded a ratio of two, reflecting the narrow elliptic shape of the leaflets. Accordingly, Bisour accession (T44) had the lowest leaf length, leaf width, petiole length, leaflet length, leaflet width, while Alay accession (T43) had the highest leaf width, petiole length, leaflet length, and leaflet width.

Table 2 - Qualitative descriptors of the Leaf and tree recorded for the 35 walnut accessions

Sample	Site	Growth habit	Leaflet shape	Leaflet margin	Leaflet color	Rachis color
T1	Nabi sheeth	Spreading	Broad elliptic	Entire	Green	Yellow
T2		Semi-erect	Broad elliptic	Entire	Green	Green to Yellow
T6		Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T7		Spreading	Broad elliptic	Entire	Green	Green to Yellow
T8		Spreading	Broad elliptic	Entire	Dark Green	Green to Yellow
T9	Janta	Spreading	Broad elliptic	Entire	Green	Green to Yellow
T10		Spreading	Broad elliptic	Entire	Green	Green to Yellow
T11		Semi-erect	Broad elliptic	Entire	Dark Green	Green to Yellow
T12		Spreading	Elliptic	Entire	Green	Green to Yellow
T13	Nahle	Spreading	Elliptic	Entire	Green	Green to Yellow
T14		Semi-erect	Broad Elliptic	Entire	Green	Green
T15		Semi-erect	Elliptic	Entire	Green	Green
T16		Erect	Broad elliptic	Entire	Dark Green	Green
T17		Spreading	Elliptic	Entire	Green	Green to Yellow
T18	Labweh	Spreading	Elliptic	Entire	Green	Green
T19		Spreading	Elliptic	Entire	Green	Green
T20	Anjar	Spreading	Elliptic	Entire	Green	Green to Yellow
T21		Spreading	Elliptic	Entire	Green	Green to Yellow
T22		Erect	Elliptic	Entire	Green	Green to Yellow
T23		Spreading	Elliptic	Entire	Green	Green to Yellow
T24		Spreading	Elliptic	Entire	Green	Green to Yellow
T25	kfardabch	Spreading	Elliptic	Entire	Green	Green to Yellow
T26		Spreading	Elliptic	Entire	Green	Green to Yellow
T27	Tal Al-amara	Semi-erect	Narrow elliptic	Serrate	Dark Green	Green
T28	Maaroub	Semi-erect	Elliptic	Entire	Green	Green to Yellow
T31	sohmor	Spreading	Elliptic	Entire	Green	Green to Yellow
T32		Spreading	Elliptic	Entire	Green	Green
T34	Fnaydeq	Spreading	Elliptic	Entire	Green	Green to Yellow
T35		Semi-erect	Elliptic	Entire	Green	Green to Yellow
T36		Spreading	Broad Elliptic	Entire	Dark Green	Green
T37		Spreading	Elliptic	Entire	Dark Green	Green
T39		Semi-erect	Elliptic	Entire	Green	Green
T41	Alay	Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T43		Spreading	Elliptic	Entire	Dark Green	Green to Yellow
T44	Bisour	Erect	Narrow elliptic	Serrate	Dark Green	Green

Table 3 - Quantitative characteristics of walnut leaves recorded for the 35 walnut accessions

Sample	Site	Leaf length (cm)	Leaf width (cm)	Leaf petiole (cm)	Leaflet length (cm)	Leaflet width (cm)	Leaflet length/Leaflet width	Leaflet petiole (cm)	Number of leaflet
T1	Nabi sheeth	30.90 ± 4.13 cdefg [(z)]	28.10 ± 4.19 efghijkl	8.50 ± 1.66 cdefgh	14.15 ± 2.10 efghij	8.13 ± 1.11 bcd	1.11 ± 0.13	0.1	3
T2		32.00 ± 4.00 cdefg	29.50 ± 0.50 bcdefghijk	7.75 ± 0.87 fghij	14.88 ± 1.81 defg	7.00 ± 0.46 fghi	2.12 ± 0.15	0.1	7
T6		34.75 ± 7.09 cd	30.00 ± 3.56 bcdefghij	9.50 ± 1.47abcde	15.55 ± 1.64 cdef	6.29 ± 0.49 hijk	2.47 ± 0.15	0.1	7
T7		29.95 ± 6.06 cdefgh	28.30 ± 3.00 defghijkl	6.59 ± 1.17 ij	13.90 ± 2.29 fghijk	6.87 ± 0.87 fghi	2.04 ± 0.33	0.1	7
T8		26.70 ± 6.02 fghijk	30.10 ± 5.03 bcdefghij	7.90 ± 0.22 efghi	14.15 ± 3.03 efghij	7.02 ± 1.42 fghi	2.01 ± 0.11	0.1	5
T9	Janta	27.50 ± 3.83 efghijk	25.50 ± 3.35 ghijkl	6.08 ± 0.80 j	10.90 ± 2.04 op	5.70 ± 1.18 mn	1.93 ± 0.21	0.1	5
T10		34.17 ± 7.08 cd	31.67 ± 5.39 bcdefgh	8.50 ± 0.87 cdefgh	14.88 ± 2.91 def	7.56 ± 1.43 def	1.98 ± 0.23	0.1	7
T11		23.33 ± 1.89 ijk	23.33 ± 2.52 jklm	7.67 ± 2.52 fghij	10.63 ± 2.40 op	5.75 ± 1.25 klm	1.87 ± 0.30	0.1	3
T12		26.46 ± 4.69 fghijk	24.48 ± 4.29 ijklm	7.90 ± 0.82 efghi	11.72 ± 2.48 lmno	4.78 ± 0.72 n	2.44 ± 0.25	0.1	5
T13	Nahle	29.90 ± 4.16 cdefgh	32.10 ± 5.41 bcdefg	7.70 ± 0.97 fghij	16.10 ± 2.51 cbd	7.65 ± 0.41 cdef	2.10 ± 0.28	0.1	5
T14		41.00 ± 3.67 ab	35.40 ± 3.83 abc	8.90 ± 1.43 bcdefg	17.60 ± 1.97 b	8.43 ± 0.93 abc	2.09 ± 0.11	0.1	8
T15		35.80 ± 3.49 bc	34.70 ± 2.17 abcde	9.60 ± 1.08 abcd	15.50 ± 1.80 def	7.26 ± 0.71 efg	2.16 ± 0.38	0.1	7
T16		44.60 ± 4.29 a	35.00 ± 2.03 abcd	9.50 ± 2.06 abcde	17.25 ± 1.18 bc	8.80 ± 0.65 ab	1.96 ± 0.13	0.1	10
T17		32.40 ± 3.27 cdef	35.90 ± 3.09 ab	9.90 ± 1.14 abc	17.60 ± 1.47 b	8.02 ± 1.24 bcde	2.22 ± 0.25	0.1	5
T18	Labweh	26.20 ± 2.08 ghijk	25.60 ± 1.82 fghijkl	7.60 ± 0.96 fghij	13.05 ± 1.14 hijklm	5.53 ± 0.29 klmn	2.36 ± 0.17	0.1	7
T19		28.00 ± 3.67 efghij	26.10 ± 1.78 fghijkl	9.68 ± 1.69 abcd	13.19 ± 0.99 ghijkl	6.27 ± 0.59 ijk	2.11 ± 0.18	0.1	6
T20	Anjar	35.40 ± 1.95 bcd	28.30 ± 3.82 defghijkl	8.96 ± 2.77 bcdefg	15.75 ± 1.92 cde	6.66 ± 0.62 ghij	3.28 ± 0.03	0.3	7
T21		35.17 ± 4.67 bcd	30.33 ± 3.44 bcdefghij	9.17 ± 0.68 abcdef	15.36 ± 1.07 def	6.88 ± 0.87 fghi	2.25 ± 0.17	0.3	7
T22		30.33 ± 3.82 cdefg	32.50 ± 1.91 bcdef	9.50 ± 0.58 abcde	14.81 ± 1.30 defg	6.06 ± 0.88 jkl	2.47 ± 0.23	0.3	7
T23		34.17 ± 4.07 cd	29.67 ± 3.06 bcdefghij	7.33 ± 0.58 ghij	14.60 ± 1.45 defghi	6.70 ± 0.67 ghij	2.19 ± 0.24	0.3	8
T24		30.38 ± 2.72 cdefg	27.50 ± 3.87 fghijkl	7.00 ± 2.71 hij	13.25 ± 2.15 ghijkl	6.03 ± 0.57 jklm	2.19 ± 0.29	0.3	7
T25	kfardabch	32.83 ± 5.48 cde	29.50 ± 6.50 bcdefghijk	8.00 ± 1.00 defghi	12.91 ± 3.13 ijklm	5.40 ± 0.93 lmn	2.37 ± 0.20	0.3	7
T26		31.13 ± 4.98 cdefg	31.33 ± 4.04 bcdefghi	8.33 ± 0.58 cdefgh	15.69 ± 1.81 cde	7.11 ± 0.87 fg	2.23 ± 0.32	0.3	6
T27	Tal Al-amara	34.52 ± 4.84 cd	26.16 ± 4.39 fghijkl	7.10 ± 1.69 hij	10.76 ± 1.10 op	3.65 ± 0.50 o	2.97 ± 0.25	0.1	15
T28	Maaroub	31.59 ± 6.87 cdefg	27.22 ± 7.19 fghijkl	8.91 ± 1.42 bcdefg	12.63 ± 1.45 jklmn	5.74 ± 0.41 klm	2.20 ± 0.23	0.1	7
T31	sohmor	23.80 ± 3.59 ijk	21.90 ± 5.59 lm	8.30 ± 0.45 cdefgh	10.98 ± 2.55 op	5.25 ± 0.80 mn	2.07 ± 0.19	0.1	5
T32		26.80 ± 1.52 efghijk	24.24 ± 2.13 jklm	8.26 ± 1.30 defghi	12.20 ± 0.92 klmno	5.33 ± 0.27 lmn	2.29 ± 0.13	0.1	6
T34	Fnaydeq	22.13 ± 4.84 jk	22.00 ± 4.30 lm	8.17 ± 1.26 defghi	10.73 ± 2.10 op	5.51 ± 1.25 klmn	1.97 ± 0.18	0.1	6
T35		22.90 ± 4.71 jk	24.40 ± 3.23 ijklm	7.08 ± 0.73 hij	11.90 ± 1.61 lmno	5.93 ± 0.78 jklm	2.02 ± 0.20	0.1	6
T36		29.36 ± 5.52 defghi	25.10 ± 6.02 hijklm	10.50 ± 0.79 ab	12.64 ± 2.14 jklmn	7.06 ± 1.29 fgh	1.80 ± 0.21	0.1	6
T37		26.33 ± 7.68 ghijk	22.68 ± 8.66 klm	7.80 ± 1.15 fghij	11.98 ± 1.68 lmno	5.94 ± 0.53 jklm	2.02 ± 0.20	0.1	6
T39		24.00 ± 1.26 hijk	22.67 ± 2.64 klm	8.25 ± 1.17 defghi	11.46 ± 1.20 mno	5.42 ± 0.90 lmn	2.13 ± 0.07	0.1	7
T41	Alay	27.67 ± 2.79 efghijk	28.75 ± 4.58 cdefghijkl	8.85 ± 0.90 bcdefg	14.65 ± 2.35 defgh	5.97 ± 0.49 jklm	3.16 ± 0.11	0.1	5
T43		42.23 ± 5.47 a	40.88 ± 3.75 a	10.75 ± 1.55 a	20.59 ± 1.47 a	8.99 ± 0.66 a	3.02 ± 0.11	0.1	5
T44	Bisour	21.81 ± 3.88 k	18.69 ± 3.66 m	4.20 ± 0.71 k	9.32 ± 1.38 p	2.70 ± 0.31 p	1.92 ± 0.04	0.3	11
LSD		60.565	4.578.488	16.962	17.163	0.78			

[(z)] For each trait, means followed by different letters are significantly different at P≤0.05 (Least Significant Difference (LSD) test).

Morphological characteristics of the nuts and kernels

For the 35 accessions, the nut and kernel traits showed significant diversity. The shell texture varies from smooth (Nine accessions) to rough (Four accessions), with medium texture being the most common (22 accessions) (Fig. 3). The shell color ranges from very light to dark shades. Regarding the tip of the nut, 91% of the accessions had a medium tip, and the rest varied between a long and absent tip, as observed in specific accessions, such as T17 and T27 (Table 4). Significant differences were observed in the nut shape, walnuts exhibit a wide range of forms, including round (Eight accessions), ovate (Three accessions), elliptic (Two accessions), Broad Elliptic (Seven accessions), elongate (One accession), short trapezoid (Seven accessions), long

Fig. 3 - Shell texture, from Left to right: Rough, Medium, and Smooth.

trapezoid (Seven accessions) (Fig. 4). The majority of the walnut accessions had a round apex and base. For instance, the Tal-Amara sample features an ovate nut with a round apex and base. Shell strength is generally intermediate, with some accessions exhibiting strong or weak shells. Kernel coloration ranges from light to amber hues.

Nuts had generally presented an average length ranging from 2.50±0.00 cm (T27, Tal Amara) to 5.22± 0.26 cm (T1, Nabi sheeth) and width from 1.18±0.10 cm (T39, Fnaydeq) to 4.15±0.15 cm (T1, Nabi sheeth),

Table 4 - Qualitative traits of nut and kernel recorded for the 35 walnut accessions

Sample	Site	Shell texture	Shell color	Nut tip	Nut shape	Apex shape	Base shape	Shell strength	Kernel color
T1	Nabi sheeth	Rough	Light	Medium	Long trapezoid	Emarginate	Round	Intermediate	Amber
T2		Medium	Medium	Medium	Short trapezoid	Obtuse	Round	Intermediate	Light amber
T6		Medium	Medium	Medium	Broad Elliptic	Round	Round	Strong	Light amber
T7		Medium	Light	Medium	Broad Elliptic	Round	Round	Intermediate	Light
T8		Medium	Very light	Medium	Long trapezoid	Truncate	Truncate	Strong	Light
T9	Janta	Smooth	Light	Medium	Broad Elliptic	Round	Cuneate	Strong	Light
T10		Rough	Medium	Medium	Short trapezoid	Truncate	Truncate	Intermediate	Light amber
T11		Smooth	Medium	Medium	Round	Round	Round	Intermediate	Amber
T12		Medium	Medium	Medium	Short trapezoid	Truncate	Truncate	Intermediate	Amber
T13	Nahle	Medium	Medium	Medium	Round	Obtuse	Truncate	Intermediate	Light amber
T14		Medium	Light	Medium	Broad Elliptic	Obtuse	Round	Intermediate	Light amber
T15		Medium	Medium	Medium	Long trapezoid	Truncate	Truncate	Intermediate	Light
T16		Medium	Medium	Medium	Round	Round	Truncate	Weak	Light amber
T17		Smooth	Light	Absent	Round	Round	Truncate	Intermediate	Amber
T18	Labweh	Smooth	Light	Medium	Round to ovate	Obtuse	Cuneate	Weak	Light
T19		Medium	Dark	Medium	Broad Elliptic	Round	Round	Weak	Light amber
T20	Anjar	Medium	Dark	Medium	Short trapezoid	Truncate	Round	Intermediate	Amber
T21		Medium	Light	Medium	Short trapezoid	Obtuse	Truncate	Intermediate	Amber
T22		Smooth	Medium	Medium	Round	Round	Truncate	Intermediate	Light amber
T23		Smooth	Light	Medium	Round to ovate	Obtuse	Truncate	Intermediate	Light
T24		Rough	Dark	Medium	Short trapezoid	Truncate	Round	Intermediate	Amber
T25	kfardabch	Medium	Light	Medium	Long trapezoid	Round	Round	Intermediate	Amber
T26		Medium	Dark	Medium	Round	Round	Truncate	Intermediate	Light amber
T27	Tal Al-amara	Smooth	Very light	Absent	Ovate	Round	Round	Paper	Light
T28	Maaroub	Smooth	Very light	Medium	Broad Elliptic	Truncate	Cuneate	Intermediate	Light amber
T31	sohmor	Medium	Dark	Medium	Short trapezoids	Emarginate	Round	Intermediate	Amber
T32		Medium	Dark	Medium	Round	Truncate	Truncate	Intermediate	Light
T34	Fnaydeq	Medium	Medium	Medium	Broad Elliptic	Round	Round	Intermediate	Light amber
T35		Medium	Medium	Medium	Long trapezoids	Round	Truncate	Intermediate	Light amber
T36		Medium	Medium	Medium	Long trapezoid	Truncate	Round	Intermediate	Light amber
T37		Medium	Dark	Medium	Round	Truncate	Truncate	Intermediate	Light amber
T39		Medium	Dark	Medium	Long trapezoid	Obtuse	Round	Intermediate	Amber
T41	Alay	Rough	Dark	Long	Elliptic	Obtuse	Cuneate	Weak	Light amber
T43		Medium	Medium	Medium	Elliptic	Round	Round	Weak	Amber
T44	Bisour	Smooth	Dark	Medium	Elongated	Round	Cuneate	Paper	Light

Fig. 4 - Nut shapes. From left to right, Top: Short trapezoid, Ovate, Long trapezoid; Bottom: Elliptic, Broad elliptic, Round.

resulting in length/width ratios typically around 1.2 (Table 5). The accessions showed significant differences in the nut and kernel weight ranging from 2.51 ± 0.02 g (T27, Tal Amara) to 19.81 ± 3.92 g (T7, Nabi sheeth) and from 0.70±0.02 (T27, Tal Amara) to 8.06 ± 0.33 g (T8, Nabi sheeth) respectively, contributing to kernel percentages from 27% (T36, Fnaydeq) to 60% (T8, Nabi sheeth). Accordingly, Tal Amara accession (T27) was distinguished by the lowest nut length, nut weigth, kernel weight while Nabi sheeth accession (T1) showed the highest nut length and nut width. Results from other Research showed that the fruit weight in Iran varied between 8.58 and 19.8 g, and the kernel percentage varied from 17.57 to 62.6% (Sarikhani Khorami *et al.,* 2014). Additionally, research on 58 walnut genotypes in the Himachal Region, India, revealed that fruit weight ranged from 6.4 to 20.55 g, and kernel percentage varied from 12 to 62.5% (Sharma and Sharma, 2001).

Validation of the variables

To validate the descriptors used in this study, a principal component analysis was performed on 27 morphological characters for the 35 different walnut accessions. This analysis will enable us to identify the most discriminating characters.

The principal components revealed that the first three components contributed 68% of the total variation (Table 6). The nut shape dominates the first component, which provides 48% of the total variation. The second component, which accounts for 13% of the total variation, includes leaflet shape, nut, and kernel weight. The third component, representing 7% of the total variation, comprises the shell texture, nut, and kernel color.

The biplot analysis was performed using PC1 and PC2 (Fig. 5), which together accounted for 61% of the variance (Table 6). The green arrows (Fig. 5) indicate the direction and magnitude of each trait's contribution to the first and second principal components. Among the 27 descriptors used in this study, the biplot analysis allowed to extract four traits (Q: Nut Shape, B: Leaflet shape, W: Nut weight and Y: Kernel weight) contributed strongly to the separation of the accessions. Most of the variation among the accessions was explained by nut shape (Q, component 1), resulting a broad horizontal distribution of the accessions. Highly correlated morphological components pointed in roughly the same direction. Accordingly, nut weight (W) was positively correlated with kernel weight (Y). Positive correlations were also observed among leaf traits such as Leaf length (F), Leaf width (H), leaflet length (J) and leaflet width (K).

PCA biplot segregated walnut accessions based on distinct morphological traits. For example, seven accessions (T1, T2, T7, T8, T9, T10 and T36) with broad elliptic leaflet shape and medium to high nut and kernel weights clustered together. The three accessions T11, T16 and T17 characterized by a round nut shape and intermediate to high leaflet width formed a separate group. Additionally, fourteen accessions (T6, T12, T14, T15, T19, T20, T21, T24, T25, T28, T31, T34, T35 and T39) with elliptic leaflet shape were grouped together.

The results confirmed the efficiency of the ampelographic descriptors in distinguishing among walnut varieties, consistent with previous studies on walnut trees (Karimi *et al*., 2014), *Ensete ventricosum* (Haile *et al.*, 2023), grapevine (Chehade *et al*., 2022; Khater *et al.*, 2025).

Morphological clustering of the accessions

The dendrogram constructed based on the seven discriminating descriptors validated by PCA allowed for clustering the 35 evaluated accessions into three main groups at an Euclidean distance of -9 (Fig. 6).

The first group clustered 11 accessions: Labweh (T27, T18), Janta (T11), Sohmor (T32), kafardabch (T26), Fnaydeq (T37), Anjar (T22, T23), Nahle (T17, T16, T13). The majority of the accessions in this group share an elliptic leaflet shape, a round nut shape, medium shell color, and low to medium nut

Table 5 - Quantitative traits of nut and kernel recorded for the 35 walnut accessions

Sample	Site	Nut length (cm)	Nut width (cm)	Nut length/ Nut width	Nut weight (g)	Kernel weight (g)	kernel %
T1	Nabi sheeth	5.22 ± 0.17	4.15 ± 0.15	1.26 ± 0.08	18.78 ± 4.42	5.46 ± 1.49 bcde [z]	30.0 ± 7.0
T2		3.99 ± 0.15	3.27 ± 0.12	1.22 ± 0.02	12.78 ± 0.90	6.31 ± 0.57 ab	50.0 ± 6.0
T6		3.79 ± 0.46	3.17 ± 0.17	1.19 ± 0.11	12.54 ± 1.11	5.19 ± 0.48 bcdefg	42.0 ± 4.0
T7		4.32 ± 0.15	3.39 ± 0.29	1.28 ± 0.13	19.81 ± 3.92	6.67 ± 1.35 ab	33.0 ± 7.0
T8		4.16 ± 0.13	3.44 ± 0.24	1.22 ± 0.12	14.56 ± 5.18	8.06 ± 0.33 a	60.0 ± 16.0
T9	Janta	3.93 ± 0.10	3.38 ± 0.06	1.16 ± 0.02	12.84 ± 0.95	5.14 ± 0.64 bcdefg	40.0 ± 2.0
T10		4.06 ± 0.19	3.24 ± 0.05	1.25 ± 0.05	12.57 ± 0.79	4.11 ± 0.57 fghijk	33.0 ± 4.0
T11		3.18 ± 0.08	3.08 ± 0.08	1.03 ± 0.00	9.70 ± 0.77	4.02 ± 0.40 ghijkl	41.0 ± 1.0
T12		3.70 ± 0.26	3.58 ± 0.26	1.03 ± 0.01	13.96 ± 2.05	4.87 ± 1.52 cdefgh	34.0 ± 8.0
T13	Nahle	3.74 ± 0.11	3.39 ± 0.20	1.11 ± 0.05	13.00 ± 0.98	4.47 ± 0.44 efghij	34.0 ± 2.0
T14		3.94 ± 0.23	3.25 ± 0.17	1.21 ± 0.05	12.41 ± 2.33	4.53 ± 1.18 efghij	36.0 ± 3.0
T15		4.20 ± 0.28	3.45 ± 0.11	1.24 ± 0.14	15.63 ± 2.53	6.25 ± 0.70 abc	38.0 ± 5.0
T16		3.62 ± 0.28	3.00 ± 0.14	1.21 ± 0.09	9.49 ± 0.52	4.33 ± 0.72 efghijk	45.0 ± 5.0
T17		3.65 ± 0.07	3.50 ± 0.06	1.04 ± 0.01	12.16 ± 1.56	5.05 ± 0.33 bcdefg	39.0 ± 2.0
T18	Labweh	3.49 ± 0.11	2.84 ± 0.13	1.23 ± 0.08	9.55 ± 0.68	4.97 ± 0.45 cdefgh	52.0 ± 2.0
T19		3.64 ± 0.11	2.77 ± 0.10	1.31 ± 0.05	9.14 ± 0.27	3.45 ± 0.34 jkl	38.0 ± 4.0
T20	Anjar	3.35 ± 0.1	3.28 ± 0.03	1.02 ± 0.02	11.56 ± 2.49	5.37 ± 0.26 bcde	49.0 ± 19.0
T21		3.54 ± 0.23	3.11 ± 0.13	1.14 ± 0.10	9.14 ± 1.58	3.94 ± 0.90 hijkl	43.0 ± 3.0
T22		3.52 ± 0.23	3.29 ± 0.11	1.07 ± 0.07	12.56 ± 1.07	5.52 ± 0.65 bcde	44.0 ± 2.0
T23		3.46 ± 0.18	3.31 ± 0.12	1.05 ± 0.06	10.37 ± 1.24	4.58 ± 1.01 efghij	44.0 ± 5.0
T24		3.95 ± 0.11	3.23 ± 0.19	1.22 ± 0.04	11.39 ± 1.90	5.93 ± 0.85 bcd	52.0 ± 2.0
T25	kfardabch	4.43 ± 0.20	3.37 ± 0.20	1.32 ± 0.08	12.79 ± 1.34	5.97 ± 0.73 bc	47.0 ± 2.0
T26		3.71 ± 0.21	3.20 ± 0.14	1.16 ± 0.05	12.87 ± 1.75	5.00 ± 0.74 cdefgh	39.0 ± 1.0
T27	Tal Al-amara	2.50 ± 0.00	1.20 ± 0.00	2.08 ± 0.00	2.51 ± 0.02	0.70 ± 0.02 m	28.0 ± 0.0
T28	Maaroub	3.84 ± 0.13 f	3.00 ± 0.12	1.28 ± 0.06	12.23 ± 1.32	4.72 ± 0.93 defghi	38.0 ± 4.0
T31	sohmor	3.34 ± 0.17	3.21 ± 0.14	1.03 ± 0.08	12.19 ± 3.73	5.54 ± 0.45 bcde	40.0 ± 2.0
T32		3.38 ± 0.18	3.16 ± 0.19	1.07 ± 0.07	11.40 ± 1.11	4.62 ± 1.01 efghij	40.0 ± 6.0
T34	Fnaydeq	3.24 ± 0.09	2.82 ± 0.15	1.15 ± 0.04	8.63 ± 1.63	2.97 ± 1.15 l	33.0 ± 8.0
T35		3.92 ± 0.18	3.16 ± 0.09	1.24 ± 0.06	12.17 ± 1.92	4.40 ± 1.08 efghij	36.0 ± 7.0
T36		4.08 ± 0.25	3.23 ± 0.15	1.26 ± 0.03	11.93 ± 2.72	3.07 ± 0.30 l	27.0 ± 6.0
T37		3.28 ± 0.21	3.33 ± 0.17	0.99 ± 0.05	11.23 ± 1.67	3.95 ± 1.93 hijkl	34.0 ± 13.0
T39		4.14 ± 0.33	1.18 ± 0.10	2.14 ± 0.19	14.90 ± 2.77	5.92 ± 1.42 bc	40.0 ± 8.0
T41	Alay	4.56 ± 0.39	1.44 ± 0.12	2.44 ± 0.25	9.94 ± 1.22	3.63 ± 1.03 ijkl	36.0 ± 5.0
T43		3.96 ± 0.43	1.31 ± 0.12	2.30 ± 0.15	9.54 ± 1.50	4.10 ± 1.18 fghijkl	42.0 ± 7.0
T44	Bisour	3.74 ± 0.30	1.95 ± 0.20	2.01 ± 0.40	6.47 ± 0.38	3.17 ± 0.19 kl	49.0 ± 3.0
LSD						12.224	

[z] For each trait, means followed by different letters are significantly different at P≤ 0.05 (Least Significant Difference (LSD) test).

and kernel weight.

The second group contained eight accessions: Janta (T10, T9), Nabi-Sheeth (T1, T2, T7, T8), Fnaydeq (T36) and Nahle (T14), which share, on one hand, broad elliptic leaflet shapes. On the other hand, the majority of these accessions had medium to high nut and kernel weight.

The third and largest group consisted of 16 accessions: Alay (T41, T43), Kfardabch (T25), Bisour (T44), Maaroub (T28), Nabi-Sheeth (T6), Labweh (T19), Nahle (T15), Fnaydeq (T34, T35, T39), Janta (T12), Anjar (T21, T24, T20), Sohmor (T31). The majority of these accessions are characterised by an elliptic leaflet shape, an amber shell color, and low to medium nut and kernel weights.

Three cases of similarity were observed within the evaluated accessions: the first case was revealed between T18 (Labweh) and T23 (Anjar), the second case between T26 (Kafardabch) and T37 (Fnaydeq) and the third case was detected between T20 (Anjar)

Table 6 - Principal components analysis of the 27 morphological characters evaluated for the 35 walnut accessions

Variables	Factor 1	Factor 2	Factor 3
Growth Habit	-0.2605	0.1511	-0.4106
Leaflet Shape	-0.1451	**0.9298 ***	0.1581
Leaflet Margin	0.1059	-0.4854	0.5637
Leaflet Color	0.28	0.0429	0.2204
Rachis Color	-0.07275	0.3707	-0.1822
Leaf Length	0.1052	0.2692	-0.004267
Leaf Petiole	0.1797	-0.0005028	-0.3164
Leaf Width	0.07981	0.2107	0.01889
Number of Leaflet	0.1045	-0.4429	0.4273
Leaflet Length	0.1623	0.2281	-0.3419
Leaflet Width	0.1665	0.4709	-0.3174
leaflet L/ Leaflet W	0.006946	-0.5072	0.07997
Leaflet Petiole	0.007297	-0.3471	-0.09709
Shell Texture	-0.4299	0.2027	**-0.62417**
Shell Color	0.03561	-0.3428	**-0.6081**
Nut tip	-0.2038	0.06415	-0.2275
Nut Shape	**-0.9978**	-0.05072	0.02065
apex shape	-0.243	0.2149	-0.3307
base shape	0.2937	0.1901	-0.4649
shell strength	-0.2361	0.5061	-0.2951
Nut Length	-0.2389	0.3161	-0.1177
Nut width	-0.184	0.4765	-0.5116
Nut L/Nut W	-0.02781	-0.4988	0.1539
Nut Weight	-0.1082	**0.6299**	-0.2755
Kernel Weight	-0.1296	**0.6**	-0.1125
Kernel Percentage	-0.02273	-0.195	0.1534
Kernel Color	-0.1722	0.01275	**-0.6292**
Percentage of total variation	**48**	**13**	**7**

*= The characters in bold are discriminant.

and T31 (Sohmor).

Analysis of ISSR

The molecular analysis of walnut accessions revealed a high degree of variability. The ISSR markers exhibited distinct polymorphism among the various Juglans accessions. A total of 67 polymorphic bands were detected across 26 accessions out of 35 studied (nine accessions not amplified) through the use of 9 ISSR primers: UBC 807, UBC 810, UBC 814, UBC 811, UBC 819, UBC 865, UBC 818, UBC 821, and UBC 826 (Table 7). Similarly, 67 polymorphic bands were reported in Kashmir valley by Shah *et al.* (2019) in studying the genetic diversity 96 walnut genotypes using 19 ISSR markers, while 82 polymorphic bands were obtained in Iran by Sharifi *et al.* (2021) in studying 82 walnut accessions using 10 ISSR markers; 123 polymorphic bands were obtained by Kabiri *et al.* (2019) for 66 Moroccan walnuts using 11 ISSRs markers.

The size of the amplified products ranged from 195 to 1300 bp (Table 7). The number of polymorphic bands varied between five (UBC810) and nine (UBC818 and UBC821), with an average of 7.4 per primer. Five primers had one monomorphic band (UBC 810, UBC814, UBC819, UBC826 and UBC 865). The percentage of polymorphism varied between 83% for the ISSR primer UBC 810 and 100%

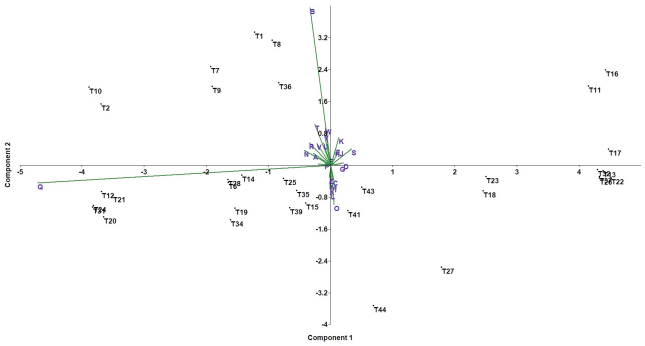

Fig. 5 - PCA biplot for 27 traits among 35 walnut accessions. The contribution of each trait to principal components 1 and 2 is shown in green arrows. A: growth habit; B: leaflet shape; C: leaflet margin; D: leaflet color; E: rachis color; F: leaf length; G: Leaf petiole; H: leaf width; I: number of leaflets; J: leaflet length; K: Leaflet width; L: leaflet length/leaflet width; M: leaflet petiole; N: Shell texture; O: Shell color; P: nut tip; Q: Nut shape; R: apex shape; S: base shape; T: shell strength; U: nut length, V: nut width; W: Nut weight; X: nut length/nut width; Y: Kernel weight; Z: kernel percentage; kernel color.

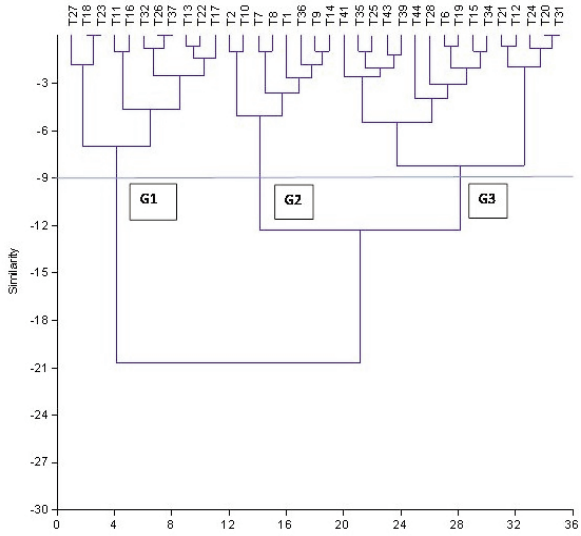

Fig. 6 - Dendogram of the 35 Lebanese walnut accessions, constructed with the most seven discriminant morphological traits, using Euclidean distance and UPGMA clustering.

Table 7 - Primer sequences, annealing temperature, band sizes, monomorphic band, polymorphic band, percentage of polymorphism, polymorphic information content and discriminating power of the nine ISSR markers used in this study

Primers	Sequence 5' to 3'	Annealing temperature (°C)	Band sizes (bp)	Total number of bands	Mono-morphic band	Poly-morphic band	% Poly-morphism	Polymorhic Information Content (PIC)	Dicrimination power
UBC 807	A(GA)7GGT	45	225-800	7	0	7	100%	0.29	0.9
UBC 810	(GA)8T	45	225-800	6	1	5	83%	0.27	0.78
UBC 811	CAC(CA)6AT	47	250-1100	8	0	8	100%	0.29	0.79
UBC 814	(CT)8A	45	275-1200	8	1	7	88%	0.35	0.87
UBC 818	(CA)8G	45	250-700	9	0	9	100%	0.31	0.914
UBC 819	(GT)8A	47	250-900	8	1	7	88%	0.26	0.86
UBC 821	(GT)8T	51.3	260-1300	9	0	9	100%	0.41	0.94
UBC 826	(AC)8C	51.3	195-650	8	1	7	88%	0.24	0.8
UBC 865	(ATG)5TCC	47	275-950	9	1	8	89%	0.37	0.9
Mean	-	-	-	8	-	7.4	92.8	0.31	0.86

for UBC 807, UBC 811, UBC 818 and UBC 821, with an average 92.8%. This finding is higher than that shown by Malvolti *et al.* (2010) for Italian walnut (73.8%) and Christopoulos *et al.* (2010) for Greek walnut (82.8%). Nevertheless, it is similar with the result reported by Aiqing *et al.* (2014) for Chinese walnut (92.31%).

The Polymorphism Information Content (PIC) of the 9 primers varied from 0.24 to 0.41 with an average of 0.31. The results indicate that UBC 821 with GT dinucleotide repeat which has the highest polymorphism. The minimum polymorphism content was obtained from the UBC826 with AC dinucleotide repeat. The average PIC obtained in our present results was higher than those previously found in ISSR studies in walnut by Potter (2002), Christopoulous (2010), Mahmoodi *et al.* (2012), Ji *et al.* (2014) and Shah *et al.* (2019).

The calculated discrimination power (PD) ranged from 0.78 for the ISSR primer UBC 810 to 0.94 for the ISSR primers UBC 821, with an average of 0.86, indicating a high diversity of the loci and confirming the efficiency of these primers in studying the polymorphism of the Lebanese walnut accessions. This value is similar to the work of Kabiri *et al.* (2019) for 66 Moroccan walnuts using ISSRs markers.

Molecular classification of the accessions

The allelic data were utilized to generate a dendrogram through the Jaccard distance and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method, revealing the genetic relationships among walnut accessions (Fig. 7). The dendrogram did not reveal similarity between accessions as observed in the molecular studies. The highest genetic distance was observed between genotypes T34 (sohmor) and T2 (Nabi sheeth), differing by 45 bands (62.5%), while the lowest was between two genotypes from the same location (Nabi sheeth) T2 and T6, differing by only 4 bands (5.5%). In addition, this dendrogram enabled the distinction of four groups at a Jaccard similarity distance of -9.

Group 1 included seven accessions with an elliptic leaflet shape and an entire margin. These accessions shared a common amplification of 225 bp and 275 bp

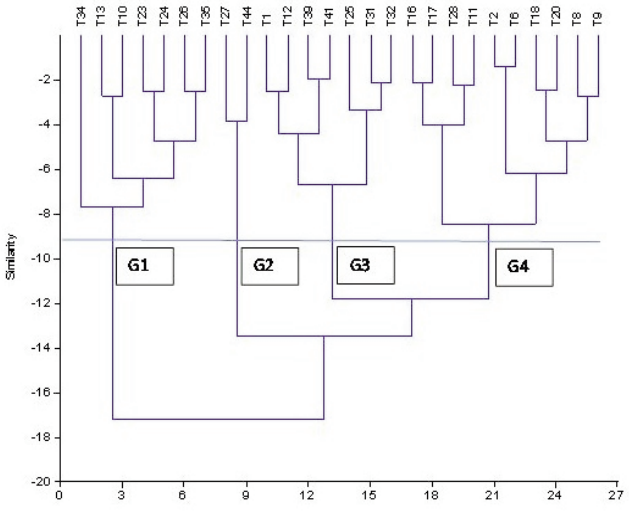

Fig. 7 - Dendogram of the 26 Lebanese walnut accessions, constructed from 9 ISSR, using Jaccard distance and UPGMA clustering.

using the UBC 807 primer, 300 bp and 400 bp using the UBC 818 primer, and 275 bp using the UBC 814 primer.

Group 2 consisted of two accessions (T27 and T44), which had a narrow, elliptic leaflet shape with a serrate margin and a dark green color. The two accessions had 19 common bands, including: five bands of UBC 821 (400 bp, 480 bp, 600 bp, 700 bp, and 900 bp), three bands of each: UBC 807 (300 bp, 400 bp, and 500 bp) and UBC 818 (275 bp, 350 bp, and 500 bp), two bands of each: UBC 819 (450 bp, and 500 bp) and UBC 826 (400 bp, and 650 bp), one band of each: UBC 810 (300 bp), UBC 814 (375 bp), UBC 811 (680 bp) and UBC 865 (750 bp). Indeed, this group exhibits a specific molecular pattern characterized by the presence of a unique band at 250 bp for UBC 811 and the absence of two bands that are present in other groups (275 bp at UBC 807 and 300 bp at UBC 818). This result confirms that these two accessions are not related to *Juglans regia* L, but they are related to *Carya illinoinensis* (Pecan) L.

Group 3 included seven accessions characterized by an elliptic leaflet shape with entire margin and medium nut tip which share 23 bands, comprising: five bands of UBC 818 (275 bp, 300 bp, 350 bp, 550 bp, and 700 bp), four bands of UBC 807 (225 bp, 275 bp, 400 bp, and 500 bp), three bands of each: UBC 810 (300 bp, 350 bp, and 480 bp), UBC 811 (425 bp, 680 bp, and 750 bp), UBC 819 (500 bp, 600 bp, and 800 bp), two bands of each: UBC 865 (700 bp, and 750 bp), UBC 826 (400 bp, and 550 bp) and one band of UBC 814 (700 bp)

Group 4 included ten accessions characterized by entire leaflet margin with twenty one common bands comprising: four bands of UBC 807 (275 bp, 400 bp, 500 bp and 800 bp), three bands of each: UBC 865 (700 bp, 750 bp and 950 bp), UBC 814 (275 bp, 375 bp and 700 bp), UBC 811 (425 bp, 680 bp and 750 bp), UBC 818 (275 bp, 300 bp and 700 bp), two bands of UBC 826 (225 bp and 550 bp), one band of each: UBC 810 (480 bp), UBC 819 (500 bp), UBC 821 (700 bp). Indeed, this group exhibits a specific molecular pattern characterized by the presence of a unique band at 800 bp for UBC 807.

Accordingly, the results of the genetic structure by using ISSR markers of the walnut trees were not correlated with the geographical regions of the sites. All the groups comprised accessions from different geographical regions.

The results of this study revealed a large morphological diversity and high genetic variation among the Lebanese walnut accessions. The dendrogram based on ISSR markers did not correspond to the one generated from morphological traits. These traits can be influenced by environmental factors, whereas molecular data are largely unaffected by such factors. Therefore, results derived from molecular data are generally considered more precise and reliable for assessing genetic relationships. Similar results were obtained in Iran by Sharifi *et al.* (2021) in studying walnut genetic diversity investigation using phonological and morphological characteristics and ISSR markers.

4. Conclusions

This study involves a morphological and molecular characterization of walnut accessions grown in various areas of Lebanon, with the objective of assessing the genetic diversity among walnut cultivars. Numerous discriminant traits were initially responsible for the diversity detected within the accessions, such as leaflet shape, shell texture, shell color, nut Shape, nut weight, kernel weight, and kernel color. These traits can be used by researchers and nursery owners to select the best single accession of walnut cultivars and meet the needs of growers for particular desired traits. Such traits include kernel weight and kernel percentages are a desirable character for commercial acceptance of a variety. In this context, T8 accession had the highest kernel weight and kernel percentages, making it suitable for propagation by nursery owners to meet farmers' demand.

No clear structure of the accessions with their geographical growing areas was observed in this study. Such results have been reported in different crops by several studies, e.g. on chest-nut (Marinoni *et al.*, 2013), almond (Chalak *et al.*, 2007) and olives (Chehade *et al.*, 2015). This variability could be attributed to the free exchange of planting material between different Lebanese villages. The combination of ampelographic descriptions and molecular markers generated effective differentiation of potential clones within the walnut cultivars studied. No significant correlation was found between the results of morphological and molecular characterization. This correlation could be further improved, first by extending the number of accessions, second by involving more descriptors allowing for the characterization of more traits and

secondly, at the molecular level, by using a larger set of molecular markers such (SSR, SNP).

Finally, the walnut accessions listed in our study should be gathered into specific collections located in different environmental conditions, in order to evaluate their agronomic potential (bloom, maturity dates, yield, nutritive composition and disease resistance). Based on the morphological, molecular characterization and agronomic evaluation of walnut accessions, conservation strategies should be implemented. The selected material should constitute a potential wealth of genetic diversity, which can be used for the improvement of walnut trees in Lebanon and elsewhere.

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Review paper

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Beyond salt stress: Unlocking the potential of sugar beet in saline environments

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Abstract: Soil salinity is a growing constraint on crop production, especially in arid and semi-arid regions of the world where freshwater is scarce and irrigation water often has poor quality. Sugar beet (*Beta vulgaris* L.) is an important crop with relatively high salt tolerance that is increasingly valued for its potential to grow on marginal lands. This review combines current knowledge and recent advances in improving sugar beet's tolerance to salinity stress through agronomic practices, as well as physiological and environmentally friendly methods to manage salinity. Key topics include how sugar beet responds to salinity at the morphological and physiological levels, tolerance mechanisms such as osmotic adjustment and antioxidant activity, effects of salinity on yield and sugar quality, and various salinity mitigation strategies. These strategies involve the application of organic amendments (biochar, compost, humic substances), improved nutrient management (potassium, phosphorus, silicon, and micronutrients), biostimulants and plant hormones applied to the foliage (salicylic acid, melatonin, GABA), microbial inoculants (PGPR and AMF), and seed priming techniques. The review also discusses regulated deficit irrigation and the development of salt-tolerant cultivars. The importance of sustainable, low-impact approaches to enhance soil health, boost plant tolerance to stress, and improve water efficiency will be emphasized. Ultimately, this review identifies gaps in our understanding of sustainable interventions and offers guidance for future research to expand sugar beet cultivation in saline environments.

1. Introduction

In semi-arid and arid regions, precipitation is often infrequent and

irregular, access to freshwater is limited, and high temperature causes high rates of evapotranspiration (Ribeiro *et al*., 2024). Under such conditions, farmers rely on irrigation to prevent crops from experiencing drought stress and to maintain productivity (Gadelha *et al*., 2021). However, due to the scarcity of freshwater, low-quality or brackish water is often used for irrigation (El-Kady *et al*., 2021). Over time, this practice tends to increase soil salinity and degradation, ultimately reducing both the yield and economic value of the cultivated crops (Ahmed *et al*., 2007).

Soil salinity and sodicity pose a serious threat to food security by reducing crop productivity through mechanisms such as physiological drought, nutrient imbalances, and oxidative stress. Moreover, high concentrations of sodium (Na^+) and chloride (Cl^-) ions contribute to soil dispersion and can exert toxic effects on plant growth (Hafez *et al*., 2021; Othman *et al*., 2023). Therefore, adopting appropriate agricultural practices in arid regions is essential to enhance water use efficiency under drought and saline conditions (Pereira Filho *et al*., 2019). One promising strategy to address both freshwater scarcity and soil salinity, both of which are acute in semi-arid regions, is cultivating salt-tolerant crops such as sugar beet (*Beta vulgaris* L.) (Flowers, 2004). This crop offers additional benefits, including reducing dependency on imported sugar and providing valuable by-products for animal feed. It also has potential in renewable energy production through bioethanol and biomethane (Gumienna *et al*., 2016).

Sugar beet is a major industrial sugar crop, ranking second globally after sugarcane in production (Stevanato *et al*., 2001). The cultivation of sugar beet is expanding into marginal environments, particularly in arid and saline regions, due to its relatively high tolerance to soil salinity, with successful growth reported in soils with an electrical conductivity (EC) of up to 7.0 dSm^{-1} (Lv *et al*., 2019). Sugar beet exhibits strong agronomic potential for arid and semi-arid regions due to its relatively low water and fertilizer requirements (Mekdad *et al*., 2021). For instance, studies have shown that the sugar beet crop consumes 30-40% less water than sugarcane (Carr and Knox, 2011), making it a more sustainable alternative in water-scarce environments. Its high water-use efficiency, coupled with relatively high salinity tolerance, underscores its suitability as a strategic industrial crop for marginal lands, especially those characterized by salt-affected soils and limited freshwater resources.

Nonetheless, elevated salinity in both soil and irrigation water can negatively impact root yield and sugar quality (Munns, 2002). As a result, sugar beet cultivation in semi-arid and arid regions faces dual challenges of low-quality irrigation water and degraded soils (Alharbi *et al*., 2022). Under such conditions, plant growth and metabolism are significantly disrupted, with the extent of damage depending on the type of stress, its duration, the developmental stage, and prevailing environmental conditions (Silva *et al*., 2022). For example, sugar beet is particularly sensitive to salinity during early growth stages, resulting in a reduced germination rate, seedling growth, and plant density, which ultimately lead to lower root and sugar yields (Ghoulam and Fares, 2001). However, during later growth stages, sugar beet can withstand high salinity levels without considerable yield loss. Cultural practices play a critical role in enhancing WUE and mitigating salt stress, particularly through the use of soil-tolerant varieties, adoption of appropriate irrigation techniques, and improvement of soil physical and chemical properties via organic acids and biostimulant treatments (Pereira Filho *et al*., 2019; El-Kady *et al*., 2021).

Countries relying on dryland farming can benefit from introducing sugar beet as a strategic, non-traditional crop. It would diversify agricultural systems, reduce dependence on water-intensive or imported crops, and improve resilience to climate variability by utilizing marginal saline soils and low-quality irrigation water. Additionally, sugar beet cultivation holds significant economic potential by creating employment opportunities on farms and in associated sugar processing facilities. It can also help reduce reliance on imported refined sugar, thereby decreasing national import expenditure. Moreover, utilizing sugar beet by-products, such as beet pulp for animal feed and molasses, adds circular economic value and promotes resource efficiency within the agro-industrial sector.

This review aims to assess the introduction of sugar beet as a salt-tolerant alternative crop in agricultural systems of semi-arid and arid regions, with the goal of enhancing resilience and sustainability. Current knowledge regarding agronomic, physiological, and environmental factors, as well as associated challenges, will be synthesized from comparable regions to identify key traits and

agricultural practices needed for successful sugar beet cultivation under conditions such as salt-affected soils and low-quality irrigation water. By highlighting knowledge gaps, this review will provide information to support decision-making by researchers and policymakers on the integration of sugar beet into crop portfolios, aiming to improve crop diversification and sustainable agricultural practices under saline and arid conditions.

2. Botanical characteristics and salinity adaptation

Sugar beet (*Beta vulgaris* L.), a tuberous root crop belonging to the family Amaranthaceae, is native to the temperate regions of Europe and North Africa (Ribeiro *et al*., 2024). Within the cultivated beets, four primary groups are taxonomically identified: leaf beet, table beet, fodder beet, and sugar beet (Goldman and Janick, 2021). Sugar beet is primarily cultivated for sugar production, human consumption, and animal feed, and is globally recognized as the second most important sugar crop after sugarcane (*Saccharum officinarum* L.). Sugar derived from sugar beet accounts for approximately 30% of total global sugar production (Yolcu *et al*., 2021).

Compared to sugarcane, sugar beet is distinguished by a shorter growing season, lower water requirements, and a higher sugar content. For example, it has been reported that producing one kilogram of sugar from sugar beet requires approximately 1.4 m^3 of water, which is less than half the volume needed to produce the same amount of sugar from sugarcane (Brar *et al*., 2015). Sugar beet can be cultivated under a wide range of climatic conditions and is recognized for its tolerance to drought and salinity. It is believed to have evolved from sea beet (*Beta maritima* L.), a wild ancestor adapted to saline soils along the coasts of Western Europe and the Mediterranean regions (Rozema *et al*., 2015). Consequently, numerous sugar beet cultivars are regarded as salt-tolerant due to the inheritance of various physiological and morphological traits that enable them to withstand drought and salinity in both soil and irrigation water (Lv *et al*., 2019; Wang *et al*., 2024). For instance, it has been reported that sugar beet can tolerate high concentrations of sodium chloride (NaCl), up to 500 mM, for extended periods without loss of viability (Yang *et al*., 2012).

3. Effect of salinity on sugar beet

In general, soil salinity negatively impacts plant growth and development by altering various morphological and physiological characteristics, as well as disrupting biochemical processes at the cellular level (Abd El-Mageed *et al*., 2019). Salinity induces osmotic and oxidative stress, resulting in damage to cellular membranes and organelles (Ashrafi *et al*., 2018). Additionally, salinity is known to impair the synthesis of proteins and metabolic enzymes, thereby reducing the rate of photosynthesis. When salinity is combined with drought, as commonly occurs in arid and semi-arid regions, the resulting impact on plant growth can be particularly detrimental.

Sugar beet is classified as a salt-tolerant glycophytic species, exhibiting optimal growth under low concentrations of sodium (Na^+), which can partially substitute for potassium (K^+) in specific non-specific metabolic processes (Wakeel *et al*., 2011). The optimal growth of sugar beet has been reported to occur under 10% seawater salinity; however, exposure to 25% seawater salinity resulted in a significant reduction in fresh biomass compared to plants grown under 10% salinity (Daoud *et al*., 2008). Under low-salinity conditions, sugar beet exhibits enhanced growth performance, characterized by efficient water and nutrient uptake, stimulated root development, and improved physiological responses to salt stress (Liu *et al*., 2008; Wang *et al*., 2024). These traits indicate that sugar beets can be successfully cultivated under mild saline, alkaline, and drought conditions; however, it is essential to note that salinity levels exceeding specific thresholds can lead to growth inhibition due to salt-induced damage at the morphological, cellular, and molecular levels (Wang *et al*., 2024).

At the morphological level, sugar beet plants exposed to saline stress exhibit reduced seed germination and seedling growth, a decrease in leaf number and surface area, as well as leaf deformation and discoloration. Additionally, overall root development is adversely affected, particularly in terms of root length and lateral root formation (Wang *et al*., 2017). At the cellular level, the detrimental effects of salinity on sugar beet plants encompass osmotic, oxidative, and ionic (toxic) stress (Mulet *et al*., 2020). Osmotic stress arises from elevated external salinity, which restricts water

uptake and can lead to dehydration by drawing water out of the cells. Consequently, osmotic stress is primarily manifested by cellular dehydration, disintegration of membrane structures, and disruption of metabolic processes (Rasouli *et al*., 2020).

On the other hand, oxidative stress results from the excessive accumulation of reactive oxygen species (ROS), which are harmful to various cellular components, including proteins and nucleic acids such as DNA (Zhao *et al*., 2020). Moreover, toxic stress results from elevated concentrations of Na^+ and Cl^- ions, leading to nutrient imbalances that hinder the uptake of essential minerals such as K^+, Ca^{2+}, and Mn^{2+}, and disrupt cellular osmoregulation (Yolcu *et al*., 2021). However, increasing the salinity of irrigation water corresponds with an increased concentration of soluble Ca, Mg, and Na, whereas K exhibits decreased solubility (El-Kady *et al*., 2021). At the molecular level, sugar beet responses to salinity include modifications to gene expression, protein synthesis, stress response mechanisms, and other metabolic pathways (Yu *et al*., 2016). For instance, ABA signaling is impacted under salt stress through the expression of specific transcription factors (Wang *et al*., 2024). Additionally, the functions of specific stress proteins in sugar beet (such as 14-3-3 proteins) are also modified, for example, through induced protein phosphorylation, helping to mitigate the effect of salinity by enhancing ROS detoxification and cell wall synthesis (Sheikh *et al*., 2024).

Salinity also tends to disrupt photosynthesis in sugar beet by inducing stomatal closure, limiting CO_2 uptake, and impairing photosynthesis efficiency (Skorupa *et al*., 2019). Furthermore, ionic imbalances, particularly K^+ deficiency and Na^+ accumulation, damage photopigments, chlorophyll content, and certain enzymes, resulting in lower photosynthetic rates (Yolcu *et al*., 2021). These effects highlight the complex responses of sugar beet to salt stress and collectively contribute to its adaptive tolerance to salinity. Figure 1 provides an overview of the multilevel impact of salinity on sugar beet, as well as the adaptive mechanisms under mild salt stress.

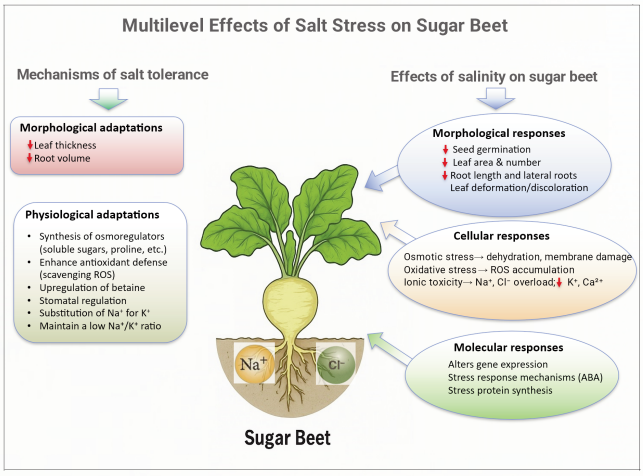

Fig. 1 - Multilevel effects of salinity stress on sugar beet growth and physiology. The diagram illustrates the morphological, cellular, molecular, and physiological disruptions caused by salt stress. Under mild salinity conditions, sugar beet exhibits adaptive traits, including enhanced root growth, efficient water and nutrient uptake, partial substitution of Na^+ for K^+ in metabolic processes, and activation of antioxidant defense systems. These responses contribute to the species' relatively high tolerance and potential for cultivation in saline environments.

4. Mechanisms of salt tolerance in sugar beet

Sugar beet displays a range of morphological and physiological adaptations to salinity stress, including reduced root volume, increased leaf thickness, and regulation of stomatal opening to minimize transpiration losses (Van Zelm *et al*., 2020). Physiological adaptations include the synthesis of osmoregulatory compounds - such as proline, soluble sugars, and organic acids - to maintain cellular osmotic balance, as well as the enhancement of antioxidant defense systems to mitigate oxidative stress by scavenging ROS and preventing cellular damage (Zhang *et al*., 2021). An experiment investigating the effects of varying seawater concentrations on sugar beet growth demonstrated that osmotic adjustment, achieved through the accumulation of intracellular solutes, constitutes a primary mechanism underlying sugar beet's tolerance to salinity (Daoud *et al*., 2008).

In addition to the aforementioned mechanisms, salt tolerance in sugar beet also involves the upregulation of betaine synthesis and accumulation, which contributes to the protection of photosynthetic enzyme activity and alleviation of osmotic stress by regulating intracellular water potential (Russell *et al*., 1998). Furthermore, under potassium-deficient conditions resulting from nutrient imbalance, sugar beet has been shown to partially substitute Na^+ for K^+ in key physiological

processes, such as stomatal regulation, enzyme activation, osmoregulation, and long-distance transport, thereby supporting continued growth under salt stress (Faust and Schubert, 2017). The relatively higher salt tolerance observed in specific sugar beet cultivars has been attributed to their capacity to maintain a low Na^+/K^+ ratio and to accumulate greater concentrations of compatible solutes (Wu *et al*., 2019). Sugar beet also possesses the ability to compartmentalize and sequester salt ions (Na^+ and Cl^-) in petioles and older leaves, thereby mitigating their toxic effects on metabolically active, functional leaves (Wang *et al*., 2012).

5. Salt stress mitigation strategies for sugar beet cultivation

Soil salinity is a major abiotic stress that constrains crop cultivation by adversely affecting plant growth and yield. Its multifaceted impacts include impaired water and nutrient uptake, toxicity from excessive sodium and chloride ions, and increased oxidative damage resulting from elevated levels of ROS (Abu-Ellail and Sasy, 2021; Mosaad *et al*., 2022; El-Atrony *et al*., 2025 a).

The potential for expanding sugar beet cultivation into saline-affected regions is supported by its relatively high tolerance to salt stress, as previously demonstrated in the preceding sections. However, when salinity levels exceed a critical threshold, even relatively high salt-tolerant glycophytic crops, such as sugar beet, exhibit substantial reductions in growth, root biomass, and sugar yield. Consequently, the development and implementation of effective salt stress mitigation strategies are essential for sustaining successful sugar beet cultivation in saline soils, particularly in arid and semi-arid regions. The following sections will present a range of ameliorative strategies, derived from recent research, examining their impact on sugar beet growth and development under saline conditions, as well as their effectiveness in mitigating the detrimental effects of salinity. Figure 2 presents an overview of the primary strategies employed to mitigate salt stress in sugar beets.

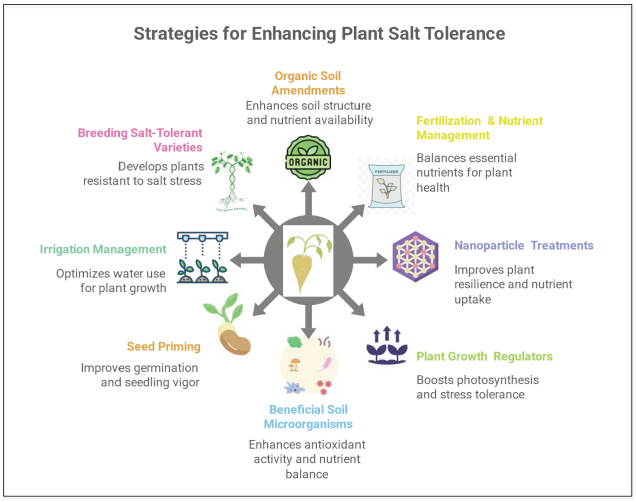

Fig. 2 - Overview of mitigation strategies for improving salt stress tolerance in sugar beet. The visual summarizes eight major approaches: (1) application of organic amendments such as biochar, compost, and humic substances; (2) nutrient management including potassium, micronutrients, silicon, and phosphorus; (3) foliar and seed treatment with nanoparticles; (4) use of plant growth regulators and biostimulants; (5) inoculation with beneficial soil microorganisms such as PGPR and AMF; (6) seed priming techniques; (7) optimized irrigation management through deficit irrigation; and (8) breeding of salt-tolerant varieties. These strategies enhance physiological, biochemical, and agronomic traits to improve sugar beet performance under saline conditions.

6. Organic amendments

As shown in Table 1, soil amendments with various types of organic materials have demonstrated significant potential to enhance the growth and yield of sugar beet under cultivated saline conditions. The following sections provide a detailed discussion of the impact of organic amendments in mitigating salt stress in sugar beet.

Biochar

Soil amendments using organic materials - particularly compost, biochar, and humic substances - have recently garnered considerable attention as eco-friendly strategies for sustaining soil quality through the improvement of physicochemical properties (Tomczyk *et al*., 2020; Su *et al*., 2022; Abdou *et al*., 2023; Abdou *et al*., 2024). These amendments increase soil carbon content, which enhances nutrient exchange capacity, stimulates microbial activity, promotes balanced nutrient uptake, and supports overall plant growth (Ghorbani *et al*., 2022). Consequently, the application of organic matter improves key soil functional properties,

Table 1 - Organic amendments for salt stress mitigation

Organic amendment	Salinity level (EC dSm^{-1})	Rate/(Additives)	Mechanism of action/benefits	Key findings/impact on Sugar Beet under salinity	References
Biochar	10.3	10-20 (t/ha)	Improved water productivity as well as soil physical and chemical properties	20 t/ha biochar gave the highest root and sugar yields	Abdou *et al.*, 2024
Biochar	10.94	0/10/20 (t ha^{-1})	20 t ha^{-1} reduced bulk density (-2.9%) and soil EC (-12.5%). Enhanced water availability and CEC	Improved growth, yield, quality and physiological attributes	El-Samnoudi *et al.*, 2021
Compost + Glauconite	4.3	Compost (0-150% of recommendation)/ + (Glauconite at 0-190 kg/ha)	Reduces soil EC[1] and ESP[2]; increases soil nutrients	Increase sugar beet root yield, sugar yield and quality	Shabana *et al.*, 2024
Molasses (M) + Humic acid (HA)	7.9-8.8	HA (12 kg/ha), M (60 kg/ ha) + foliar application of Ha and M/ + (Lithovit/Boron)	Increased growth parameters (leaf area index, dry weight, root weight, length, diameter)	Combined soil and foliar application of Ha, M, and Lithovit/Boron produced the highest root and sugar yield	Sorour *et al.*, 2021
Humic acid (HA)	45-4.6	Humic acid (0-24 kg/ha) + foliar salicylic acid, fulvic acid, hydroxyproline	Enhancing growth characteristics and helping mitigate soil salinity	HA achieved the highest growth at 24 kg/ha to the soil, combined with foliar spraying of Fulvic acid and Hydroxyproline	Nassar *et al.*, 2023
Humic substances	9.7-11.3	Soil humic/fulvic acids + Bacillus biofertilizer	Increased proline level and nutrient uptake; reductions in soil EC and ESP	Higher root yield and quality	El-Atrony *et al.*, 2025 a
Novel compost (NC)	6.4-7.1	bagasse + animal blood. NC with 70:30 ratio (bagasse:blood) at 10–20 t/ha)	Improved soil properties and water productivity; reduced the uptake of Cd	The highest yields (root yield 97.2 t/ha) were observed under full irrigation with 20 t/ha NC	Abd El-Mageed *et al.*, 2019
Compost tea (CT)	11.1-12.1	Foliar compost extract + soil PSB[3]	Enhance physiological functions; boost antioxidants and osmolytes; enhance nutrient (P) availability	PSB and CT have beneficial effects on growth and quality of sugar beet growing in salt-affected soil.	Osman *et al.*, 2022
Moringa leaf extract (MLE)	0-120 mM NaCl[4]	Seed priming (soaking) in MLE at 100-300 ml/L + Algae and yeast extracts	Improves seedling parameters (root and shoot lengths, seedling length, and seedling vigor index)	300 mL/L moringa extract yielded the highest seedling growth under salinity	Kandil *et al.*, 2023
Compost (manure)	6.0-6.2	Soil compost (0.8-2.4 ton/ha)	Influence various growth, yield, and quality parameters (e.g., increasing root diameter, fresh weight, sucrose, and decreasing alpha-amino N)	2.4 ton/ha compost increased root diameter, fresh weight and sugar yield	Abu-Ellail and Sasy, 2021
Humic substances + Nitrogen fertilization	8.63	Soil humic/fulvic acid (30 kg/ha) + N (71–213 kg/ha)	Increases nutrient uptake and sugar synthesis; reduces Na content	Humic substances (30 kg/ha) and 213 kg N/ha + gave the highest root and sugar yields	Mosaad *et al.*, 2022

EC= Electrical conductivity; ESP= Exchangeable sodium percentage; PSB= Phosphate-solubilizing bacteria.

particularly those linked to enhancing crop resilience to abiotic stresses such as salinity (Abd El-Mageed *et al*., 2019; El-Samnoudi *et al*., 2021; Osman *et al*., 2022).

Among organic amendments, biochar has attracted particular interest due to its capacity to enrich soil with a high content of stable carbon, which, unlike other organic materials such as compost, remains persistent in the soil over the long term (Haider *et al*., 2020). Biochar is produced through thermal decomposition of biomass, typically under limited or no oxygen conditions, via processes such as pyrolysis or hydrothermal carbonization (Saravanan and Kumar, 2022). These processes break down biopolymers, yielding structurally stable carbon-rich materials. In addition to increasing soil carbon content, biochar enhances soil water retention, reduces bulk density, improves nutrient retention by increasing cation exchange capacity (CEC), and stimulates microbial activity (Alkharabsheh *et al*., 2021; Singh *et al*., 2022). Soil amendment with biochar has been proposed as a promising strategy to enhance sugar beet resilience to abiotic stresses, particularly salinity and drought. For instance, the application of biochar has been shown to mitigate the adverse effects of salinity and

drought on sugar beet by enhancing physiological and biochemical processes under stress conditions (Abdou *et al*., 2024).

In sugar beet field studies, biochar has been applied at 10-20 t ha^{-1} across two successive seasons under saline conditions (ECe ≈ 10-11 dS m^{-1}) and deficit irrigation (≈ 60-80% ETc), with the 20 t ha^{-1} rate improving soil physical status (e.g., lower bulk density and ECe; higher field capacity and available water), growth, and water productivity (El-Samnoudi *et al*., 2021; Abdou *et al*., 2024). Complementary long-term evidence from highly saline-alkali paddy soils shows that a one-off biochar application sustained annual improvements in soil physical and chemical properties and increased rice yield over six years (Jin *et al*., 2024). The benefits were most significant at 3.0% (w/w) and were enhanced when combined with nitrogen fertilizer. In contrast to the consistent yield responses, data on sugar composition and juice-quality parameters (e.g., sucrose %, purity, α-amino N, K/Na) were insufficient across the present field investigations, underscoring the need for targeted studies to quantify biochar's effects on sugar quality under salinity. Further details are provided in Table 1.

Compost

The application of various types of compost as soil amendments has also been investigated for its effectiveness in improving sugar beet cultivation under saline conditions (Abu-Ellail and Sasy, 2021; El-Atrony *et al*., 2025 a). For instance, the application of a novel compost composed of sugarcane bagasse and animal blood in a 70:30 (w/w) ratio at a rate of 20 t/ha effectively mitigated the adverse effects of salinity and drought on sugar beet cultivation, resulting in approximately a 50% increase in root yield compared to the control treatment (Abd El-Mageed *et al*., 2019). In that study, soil EC was 6.89 dS m^{-1}; applying 20 t/ha increased white sugar yield and juice purity by 65.5% and 3.23%, respectively, and reduced α-amino N and sugar loss to molasses by 11% and 12.20% relative to the control. The application of tea compost in combination with phosphate-solubilizing bacteria has also been reported to enhance sugar beet performance under salinity and drought stress by improving osmotic balance, photosynthetic efficiency, and root development (Osman *et al*., 2022). In these salt-affected trials, soil and irrigation-water ECs were 11.14 and 1.37 dS m^{-1}, respectively; improvements in sugar quality were associated with decreased impurity constituents (α-amino N, Na, K) and higher juice purity under water stress. Similarly, a mixture of compost and glauconite (a mineral rich in potassium) significantly improved soil physical properties such as bulk density and porosity, increased the availability of macro- and micronutrients, and enhanced both sugar yield and quality in sugar beet grown under saline and sodic conditions (Shabana *et al*., 2024). At that site (EC = 3.41 dS m^{-1}), the combination reduced EC, improved organic matter and soil physical status, increased N, K, Fe, Mn, Zn, and Cu, and enhanced sugar quality with minimal sugar loss to molasses (~2.43%) alongside reductions in K, Na, and α-amino N. Further details are provided in Table 1.

Humic substances

The application of humic substances has also demonstrated promising potential in enhancing sugar beet resilience to salinity stress. Several field studies have reported that the application of humic acid, fulvic acid, and related organic materials significantly enhances the growth, yield, and physiological performance of sugar beet under saline conditions. For instance, Sorour *et al*. (2021) reported that the combined application of humic acid and molasses, applied through both soil and foliar treatments, significantly improved the growth parameters of sugar beet cultivated under saline conditions (soil EC ≈ 8.8 dS m^{-1}; irrigation-water EC ≈ 2.01 dS m^{-1}) (Sorour *et al*., 2021). Similarly, under saline-sodic conditions (soil EC ~11.3 dS/m and exchangeable sodium percentage (ESP) >15%), the application of a mixture of organic substances - including humic acid, fulvic acid, and potassium humate - in combination with a Bacillus-based biofertilizer, enhanced sugar beet productivity and nutrient uptake, while also mitigating the impact of soil salinity through significant reductions in both EC and ESP (El-Atrony *et al*., 2025 a). Furthermore, another study demonstrated that the application of humic and fulvic acids, in conjunction with high nitrogen fertilization, resulted in a more than 15% increase in both root yield and sugar extraction percentage in sugar beets cultivated under saline conditions (soil EC ≈ 8.63 dS m^{-1}; irrigation-water EC ≈ 3.2 dS m^{-1}) (Mosaad *et al*., 2022).

Soil-applied humic substances act through the rhizosphere and soil matrix, improving aggregation and water storage, enhancing nutrient availability/CEC, and moderating salinity (e.g.,

reductions in EC/ESP), which together support higher growth and extracted sugar under saline conditions (Mosaad *et al*., 2022; Abdel-Salam *et al*., 2025; El-Atrony *et al.*, 2025 a). In contrast, foliar applications of humic/fulvic compounds primarily exert physiological effects, raising chlorophyll and canopy vigor, improving membrane stability and photosynthetic efficiency, and can contribute to improved juice-quality indices under stress, particularly when integrated with soil applications (Sorour *et al*., 2021). In practice, combined soil and foliar applications can outperform single-route applications under salinity by simultaneously improving soil-plant conditions and canopy physiology (Sorour *et al.*, 2021; Mosaad *et al*., 2022; El-Atrony *et al*., 2025 a). These studies provide evidence that soil organic amendments, including compost and humic substances, represent effective strategies for mitigating salinity-induced stress in sugar beet cultivation. Further details are provided in Table 1.

7. Fertilization and nutrient management

Worldwide, arable lands are increasingly subjected to salinization due to soil and irrigation water salinity. One ameliorative measure to help crops withstand the adverse effects of salinity is optimizing fertilization practices. Recent studies have investigated various fertilization management strategies to mitigate the impact of salinity on the growth and productivity of sugar beet (Table 2).

Potassium

Exploring the role of potassium and micronutrient fertilization in alleviating the adverse effects of salinity on sugar beet has received considerable attention. Under salinity conditions, potassium is essential for maintaining osmotic balance and supporting membrane function. It has been found that foliar application of potassium silicate (K_2SiO_3, 20 mmol L^{-1}) significantly alleviates salt stress (EC ≈ 7 dS m^{-1}) by enhancing water productivity, chlorophyll content, and root yield under combined drought and salinity conditions (Shaaban *et al*., 2025). In another study, it has been reported that a combined treatment of potassium at a rate of 180 kg/ha and foliar application of Zn at a rate of 300 ppm resulted in a substantial improvement in root and sugar yield, up to 23% and 38%, respectively (EC ≈ 8.6 dS m^{-1}) (Mekdad *et al*., 2021). Physiological traits such as membrane stability, antioxidant activity, and relative water content of salt-stressed sugar beet were improved by high potassium application (144 kg/ha), alongside a 42% reduction in sodium and a 35% increase in root yield (EC ≈ 3.5-9.3 dS m^{-1}) (El-Mageed *et al*., 2022).

Moreover, synergistic effects between potassium fertilization and foliar application of salicylic acid were also observed in sugar beet. For example, it has been found that potassium fertilization at a rate of 200 kg/ha, combined with foliar application of salicylic acid, improved sugar content by 20% (Nemeat Alla, 2023). Meanwhile, a combination of 115 kg K_2SO_4/ha with salicylic acid significantly increased root yield and sugar quality under salinity conditions (EC ≈ 6.9 dS m^{-1}). These integrated fertilization strategies can be used practically to enhance sugar beet tolerance to salinity through improving physiological and biochemical responses. Further details are provided in Table 2.

Micronutrients

Micronutrients such as iron, manganese, zinc, and selenium play a crucial role in osmoprotection and the regulation of stress enzymes. Foliar application of Fe (150 ppm), Zn (100 ppm), and Mn (50 ppm) increased root and sugar yield, as well as quality index under saline conditions (EC ≈ 9.3-9.5 dS m^{-1}) (Abd El-Mageed *et al*., 2021). In this experiment, higher sugar and root yields of salt-stressed sugar beet were attributed to improved nutrient uptake, leaf hydration, and photosynthetic efficiency. Moreover, seed priming of sugar beet with selenium (Na_2SeO_3) at concentrations of 20 µM and 30 µM enhanced germination and seedling vigor, increased photosynthesis, and increased the activity of antioxidant enzymes under 300 mM Na^+ salinity (Liu *et al*., 2025). These results were primarily attributed to the modification of the rhizosphere microbial community. In the absence of imposed salinity, under an alkaline soil environment, foliar application of zinc (100 mg L^{-1}) and molybdenum (40 mg L^{-1}) increased sugar percentage, sugar purity, and growth parameters by improving balanced nutrient uptake and translocation (Zewail *et al*., 2020). Further details are provided in Table 2.

Silicon

Silicon applications have demonstrated beneficial effects on sugar beet performance by improving ion

Table 2 - Nutrient management for salt stress mitigation

Nutrient/Strategy	Salinity level (dSm^{-1})	Application method/rate	Mechanism of Action/Benefits	Key Findings/Impact on Sugar Beet under Salinity	References
Foliar micronutrient mix (Fe, Zn, Mn)	9.3-9.5	Foliar spray 0-150 ppm $FeSO_4^+$ 0-100 $ZnSO_4^+$ 0-50 $MnSO_4$	Supplies essential micronutrients to support chlorophyll formation, enzyme activity, and osmotic balance; raises K/Na ratio	A 150-300 ppm mix significantly boosted growth, water status, and yield; 300 ppm increased root yield by 42% and sugar yield by 93% compared to the control	Abd El-Mageed *et al.*, 2021
Soil K fertilizer	3.5-9.3	Soil K at 0, 48, 96, 144 kg K ha^{-1}	Improves osmotic/ionic balance; enhances antioxidant capacity and photosynthetic performance	K = 144 kg ha^{-1} maximized gross and white sugar; Na ↓ 42%, root yield ↑ 35.9%	El-Mageed *et al.*, 2022
Foliar silicon (various forms)	---	Foliar spray of potassium silicate (PS), calcium silicate (CS), sodium metasilicate (SM), orthosilicic acid (OSA)	Enhance photosynthesis and stress signaling; affect sugar technological quality; reduce sodium content in roots	PS and OSA sprays increased sugar yield; OSA most reduced root Na and i ncreased sugar content; spray form/ timing influenced sugar and K content	Siuda *et al.*, 2023
Soil K fertilizer + Salicylic acid (SA)	7.6	Soil K_2SO_4 at 0, 100, 150, 200 kg/ha + two foliar SA sprays (1000 mg/L each)	Enhances root length and diameter, shoot and root yield, sucrose content, juice purity, sugar yield, and uptake of N, P, and K	200 kg K/ha + SA gave the highest root/sugar yields, sucrose% and purity	Merwad, 2016
Soil K + Foliar Zn	8.6	Soil K at 120 or 180 kg/ha + foliar Zn at 0, 150, 300 ppm	Correct K and Zn deficiencies in saline soil; improve sugar beet growth, yield, quality, and K-use efficiency	K-180 + Zn-300 produced ~23% higher root yield and 38% higher pure sugar vs control; reduced impurities (Na/α-amino N)	Mekdad *et al.*, 2021
K-fertilizer + SA	6.9	Soil K_2SO_4 10 or 20 kg/ha and foliar K + SA foliar spray at 100,150, or 200 ppm	K increases growth parameters yield components; SA improves growth, yield, and sugar quality	48 kg K + 2 foliar K + 200 ppm SA gave the highest root diameter, yields, and quality	Nemeat Alla, 2023
Phosphorus	12	Soil P_2O_5 at 100, 120, 140 kg/ha	P enhances sugar beet's tolerance to salinity and improves both yield and sugar content	120 kg P_2O_5/ha was optimal, improving root and sugar yields under saline irrigation; higher P increased sugar content at moderate salinity	Bouras *et al.*, 2021
Soil K × P interaction	5.0-9.0	K_2SO_4 at 0, 75, 150 kg K_2O ha^{-1} × DAP at 0, 60, 120 kg P_2O_5 ha^{-1}	K lowers leaf Na and Na:K ratio; P improves P nutrition	Fresh beet yield ↑ 15-84% across K×P vs control; shoot yield gains; strong leaf K/yield positive relationship	Hussain *et al.*, 2014
Foliar Zn, B, Mo	--	Foliar Zn (50 and100 mg/L), B (50 and 100 mg/L), Mo (20 and 40 mg/L)	Balancing nutrient uptake and translocation. Increased growth parameters (root diameter, length, fresh/dry weight), and improved nutrient contents (N, P, K, C	Zn 100 mg/L and Mo 40 mg/L gave the highest root yield and sugar%; all micronutrient sprays increased growth and leaf nutrient (NPK, Ca, Mg) content	Zewail *et al.*, 2020
Selenium (Se) seed priming (Na_2SeO_3)	300 mM Na^+	Seed priming at 20 and 30 µM Na_2SeO_3; salinity during germination and pot stages	Enhances antioxidant enzymes, photosynthetic pigments, and ion balance; modulates rhizosphere microbiome	Se-priming improved germination and seedling vigor, ↑ soluble sugars/ proteins, ↓ MDA, and optimized microbial community	Liu *et al.*, 2025
Foliar potassium silicate (K_2SiO_3)	7	Foliar K_2SiO_3 at 0, 10, 20 mmol/L under three deficit irrigation regimes	Improves physiological and biochemical traits, photosynthetic efficiency, osmolyte accumulation, antioxidant activity, and nutrient uptake	K_2SiO_3 (20 mmol/L) resulted in the highest root yield (88.97 t/ha) and sugar yield (14.43 t/ha)	Shaaban *et al.*, 2025
Soil K-humate + foliar biostimu-lants (SA, fulvic acid (FA), hydroxy-proline (HP)	4.7	Soil K-humate at 0, 12, 24 K-muhate at 0 -24 kg/ha + foliar (SA 100 mg/L, FA 1.2 kg/ha, HP 1000 mg/L)	Enhanced growth traits, higher yields, increased sucrose, and reduced sodium content in the juice	24 kg/ha K-humate + foliar FA+HP gave highest growth, root and sugar yields and lowest juice Na under salinity	Nassar *et al.*, 2023

balance and physiological functioning. Under combined salinity and drought, foliar potassium silicate at 20 mM significantly enhanced physiological responses, increasing sugar yield and water-use efficiency (EC ≈ 7 dS m^{-1}) (Shaaban *et al.*, 2025). Complementarily, in a study without imposed

salinity, foliar potassium silicate and orthosilicic acid (at 49 g ha^{-1} and 3 g ha^{-1}, respectively) enhanced the quality of sugar beet yields by reducing Na and α-amino nitrogen levels while increasing sugar content (Siuda *et al*., 2023). Further details are provided in Table 2.

Phosphorus

Phosphorus application has also been found to counter salinity-induced yield reduction in sugar beet. The application of phosphorus at a rate of 120 P2O5/ha enhanced the salt stress resilience of sugar beet, as it significantly improved sugar yield under salinity levels of up to 12 dS/m (Bouras *et al.*, 2021). Similar results were also observed for a combined application of potassium and phosphorus in saline and sodic soils (EC = 5.0-9.0), where both nutrients improved ionic balance and the yield of sugar beets, particularly by enhancing the Na: K ratio (Hussain *et al*., 2014). Further details are provided in Table 2.

8. Nanoparticle treatments

Recently, the application of nanoparticles (NPs) in agriculture has emerged as a promising and sustainable strategy to help crops mitigate the adverse effects of abiotic stress, improve nutrient balance, and improve growth parameters. NPs are characterized by nanoscale size, enhanced penetration, and high reactivity with plant cell components (Singh *et al*., 2024). Therefore, NPs can offer practical and novel approaches to alleviate the adverse effects of salt stress on sugar beet mainly by enhancing antioxidant activities, nutrient uptake, and helping plants to maintain ionic balance. For instance, sugar beet grown in saline soil (EC=6.8) and irrigated with saline water (EC = 5.7) and cultivated in saline soils exhibited improved growth and yield parameters, along with reduced salt-induced oxidative stress, when treated with a foliar application of silica nanoparticles (SiO_2-NPs; 12.5 mg L^{-1}) in combination with rhizobacteria (Alharbi *et al*., 2022).

In another experiment, seed priming and foliar application of nanoparticles composed of magnesium oxide (MgO-NPs at 50 mg L^{-1}) and silicon oxide (SiO_2-NPs at 50 mg L^{-1}) improved the growth and yield of sugar beet irrigated with wastewater (EC = 1.61) by increasing chlorophyll content and sucrose accumulation in leaves, as well as enhancing the activity of antioxidant enzymes (Ali *et al*., 2025). Moreover, foliar application and seed priming with titanium dioxide nanoparticles (TiO_2-NPs) at a rate of 100 ppm priming and 200 ppm spray combined with silver nanoparticles ($AgNO_3$-NPs) at a rate of 30 ppm priming +75ppm spray in sugar beet cultivated under saline soil conditions (EC = 4.2) significantly enhanced sugar content and extraction efficiency, while concurrently reducing sugar impurities compared to untreated controls (Gomaa *et al*., 2022). Further details are provided in Table 3.

9. Plant endogenous metabolites

Among hormones, indole-3-acetic acid (IAA) plays a role in enhancing plant resilience to abiotic stress. Under salinity (EC ≈ 7 dS m^{-1}), exogenous IAA - particularly 300 mg L^{-1} combined with 340 kg N ha^{-1} - enhanced root growth and nutrient uptake (higher K^+/Na^+, Ca^{2+}/Na^+), increasing root (97.6 t ha^{-1}) and pure sugar (14.50 t ha^{-1}) yields (Shaaban *et al*., 2025). In another experiment, the use of growth-promoting rhizobacteria has been shown to enhance the performance of sugar beet under salinity conditions (Alharbi *et al*., 2022); in salt-affected soil (EC 6.8) with saline irrigation (EC 5.7), seed inoculation with PGPR (*Pseudomonas koreensis*, *Bacillus coagulans*) at 150 mL of 1 × 10^8 CFU mL^{-1}, enhanced antioxidant defenses (SOD up to ~1.9-fold, CAT ~1.4-fold, POX ~2.5-fold), reduced H_2O_2, lipid peroxidation, and Na^+, increased K^+, and improved RWC, RMSI, stomatal conductance, chlorophyll, and ultimately root and sugar yields across two seasons. In general, rhizobacteria such as *Bacillus* and *Pseudomonas* enhance plant tolerance by the biosynthesis of PGRs such as IAA, gibberellins, and cytokinins, in addition to the production of exopolysaccharides that can chelate sodium ions in the soil and reduce their influx (Yang *et al*., 2016).

Also the use of plant hormone-like substances, such as salicylic acid (SA), have been widely utilized in sustainable agriculture to mitigate the effects of adverse abiotic factors (Rašovský *et al*., 2022). For instance, under saline condition (EC 7.5), the salt tolerance of sugar beet was improved by foliar application of SA (at the rate of 200 ppm), which enhanced the activities of antioxidant enzymes, resulting in higher root biomass and sugar yield (El-Gamal *et al*., 2021). The beneficial effect of SA in alleviating abiotic stress has been attributed to the

Table 3 - Nanoparticles, microbes, seed priming, and hormones

Mitigation strategy	Salinity level (dSm^{-1})	Application Method/Rate	Mechanism of action/benefits	Key findings/ Impact on Sugar Beet under salinity	References
γ-Aminobutyric Acid (GABA) treatment	300 mM NaCl	Exogenous GABA at 1.5 mM L	Enhance antioxidant enzyme activity, gas exchange and fluorescence parameters; bilize photosynthesis and maintain normal growth	Effectively alleviated salt stress damage; improve dry matter accumulation in salt-stress	Yu *et al.*, 2024
SiO_2 or MgO nanoparticles	EC soil 1.6. EC water 3.5	Seed priming and foliar 0, 50, 100, 200 mg L^{-1}	↑ CAT, POX, PPO, APX; mitigates oxidative stress	Low dose (50 mg L^{-1}) improved root traits; SiO_2 best for antioxidant enzymes; MgO improved chlorophyll and sucrose accumulation	Ali *et al.*, 2025
Salicylic acid (SA)	4.5-7.15	foliar aplication 0, 1000, 2000 ppm	Enhanced root length and LAI	2000 ppm SA significantly increased top fresh mass and root biomass; SA enhanced sugar yield, sucrose% and purity%	El Gamal *et al*., 2021
Seed coating	NaCl with osmotic pressure from (-0.4) to (-1.2 dS/m)	Coating with combinations of N, P, K, 6 micronutrients + GA_3 + humic acid)	Improved germination and seedling growth with no significant effect on antioxidant enzymes	Treatments improved seedling establishment, and enhanced root and shoot growth	Neamatollahi *et al.*, 2024
Seed priming (hydropriming/ $ZnSO_4$)	NaCl 0, 2, 5, 12 S m^{-1}	ZnSO4 (0.5%) suspension for about 12 hours at 15 °C	↑ pigments, antioxidant enzymes, proline; better germination indices	Seed priming increased values of germination attributes	Shokouhian and Omidi, 2021
Halotolerant endophytic bacteria	NaCl 0, 50, 150, 300 mM	Inoculation under NaCl with *Pseudomonas stutzeri Kushneria marisflavi*	↓ H_2O_2 and proline; ↑ chlorophyll; growth promotion	Both strains improved growth; *K. marisflavi* generally more effective	Szymańska *et al.*, 2020
Halotolerant PGPR from halophytes	NaCl 50-125 mM	Inoculation (*Micrococcus yunnanensis*, *Planococcus rifietoensis*, *Variovorax paradoxus*)	ACD-producing PGPR; ↓ stress ethylene; ↑ photosynthesis	Enhanced germination, biomass, photosynthetic capacity under saline stress	Zhou *et al.*, 2017
Humic extracts and *Bacillus megaterium*	Saline-sodic soil: EC 9.7-11.3; ESP >15	Combinations of k-humate, humic acid, fulvic acid, P, and *B. megaterium*	↓ EC & ESP; ↑ SOM; improved structure (↓ BD) and P availability	Significant shoot/tuber yield increases	El-Atrony *et al.*, 2025 b
PGPR[1] + Si nanoparticles (Si-NP)	EC soil 6.9, EC water 5.8	Inoculation with PGPR (*Pseudomonas koreensis*, *Bacillus coagulans*) + foliar SiO_2 NP	decrease oxidative stress indicators (hydrogen peroxide, lipid peroxidation) and sodium ions	Improve growth characteristics, physiological processes, root yield, and sugar yield	Alharbi *et al.*, 2022
Nanoparticles of Titanium Dioxide (TiO_2NPs) and Silver Nitrate ($AgNO_3$NPs) + Gibberellic acid	4.1	TiO_2NPs (100 ppm priming + 200 ppm spray) and $AgNO_3$NPs (30 ppm priming + 75 ppm foliar)	Decrease in potassium, α-amino nitrogen, and sodium in the sugar beet root, influencing nutrient balance and quality	increased sugar yield, total soluble solids, and sugar content	Gomaa *et al.*, 2022
Indole-3-acetic acid (IAA) and Nitrogen (N)	7.0	Foliar IAA (0, 150, 300 mg/L) + soil N (240, 290, 340 kg/ha)	Enhance root diameter, leaf fresh weight, and leaf area index; Improves ionic homeostasis (increasing leaf K^+/Na^+ and Ca^{2+}/Na^+ ratios)	The combination of IAA300 × N340 was the most effective in enhancing root yield and sugar yield	Shaaban *et al.*, 2025
Melatonin (MT)	300 mM Na^+	Foliar melatonin (0, 30, 60, 90 µM)	Increases antioxidant enzyme activities (SOD, POD, CAT); reduces ROS accumulation; enhances photosynthesis in seedlings	The application of 60 µM melatonin was identified as a feasible way to alleviate salt stress in sugar beet	Liu *et al.*, 2022
Tryptophan priming	320-800 ppm	Seed soaking in tryptophan (0, 2.5, 5.0 mM)	Tryptophan has a promotive effect on increasing sugar beet yield under water salinity	Pre-soaking in tryptophan (2.5 mM) was the most effective treatment, leading to an increase in all tested growth, yield, and root quality parameters	Hozayn *et al.*, 2020
Allantoin	100-300 mM Na+	Exogenous allantoin (0.01, 0.1, 1 mM)	Reduce accumulation of ROS; increase activities of antioxidant enzymes; improve ion homeostasis by decreasing the Na^+/K^+	Exogenous allantoin effectively mitigated salt-adverse effects in a dose-dependent manner, with 0.1 mM being most effective	Liu *et al.*, 2020
PGPR + Proline + salicylic acid	7.6	Bacterial Inoculation + proline at 40 g/ha + salicylic acid at 80 g/ha	Combine application enhance root length, root diameter, sugar yield, sucrose% and purity%	Yield, its components (root diameter, sugar yield), and juice quality parameters, enhanced by the bacterial inoculation and inducing material combinations	Mehasen, 2022

PGPR= Plant growth-promoting rhizobacteria.

maintenance of membrane integrity, enhanced photosynthetic efficiency, and the regulation of antioxidant enzymes (Miao *et al*., 2020). Additionally, under soil salinity (EC ≈ 4.7 dS m^{-1}), soil application of potassium humate (24 kg ha^{-1}) combined with foliar application of salicylic acid 100 mg L^{-1}, fulvic acid 1.2 kg ha^{-1}, hydroxyproline 1000 mg L^{-1}increased root yield and sucrose/sugar yield and reduced juice Na; the K-humate 24 kg ha^{-1} + FA + HP combination performed best (Nassar *et al*., 2023).

Exogenous application and seed priming with other plant metabolites have also shown significant effects in alleviating salt tolerance in sugar beet. For example, the application of melatonin (at 60 µM), an hormone-like substance, significantly enhanced the activities of antioxidant enzymes, reduced ROS concentration, and improved photosynthetic efficiency and biomass of sugar beet under salt stress conditions (300 mM Na^+) (Liu *et al*., 2022). Similarly, the treatment with γ-aminobutyric acid (GABA), a plant signaling compound, at 1.5 mM has been shown to alleviate salt stress (300 mM Na^+) damage in sugar beet by stabilizing membrane integrity, enhancing photosynthetic efficiency, and increasing dry matter accumulation (Yu *et al*., 2024). Also the treatment of sugar beet seedlings subjected to salt stress (300 mM Na^+) with another signaling compound, allantoin at 0.1 mM, showed a reduction in salt-induced oxidative damage, a reduced Na/K^+ ratio (enhanced homeostasis), and an increased accumulation of osmoprotectants, such as betaine and soluble sugars (Liu *et al*., 2020). Moreover, sugar beet seed pre-soaking in tryptophan (aminoacid precursor) at 2.5 mM under different salinity levels (320 to 8000 ppm), improved chlorophyll pigments, root purity, and overall growth (Hozayn *et al*., 2020). The above findings affirm the potential of plant endogenous metabolites in enhancing sugar beet resilience to salt stress by regulating various physiological and biochemical processes. Further details are provided in Table 3.

10. Beneficial soil microorganisms and plant growth stimulators (PGS)

Bioaugmentation with soil microorganisms such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) has shown promising results for sustaining the growth and yield of sugar beet cultivated under salinity stress. For instance, seed and root inoculation with halotolerant endophytic bacteria such as *Pseudomonas stutzeri* and *Kushneria marisflavi* resulted in improved sugar beet growth under saline conditions (up to 300 mM Na^+), with *K. marisflavi* showing higher efficiency (Szymańska *et al*., 2020). Growth improvement and salt stress mitigation of these bacterial strains were attributed to increased chlorophyll content and reduced oxidative stress. Similarly, the seed application of two PGPR (*Pseudomonas koreensis* and *Bacillus coagulans*) alone or in combination with foliar application of silica nanoparticles (12.5 mg L^{-1}) significantly improved antioxidant activity, decreased ion imbalances (lower K+/Na+ ratio), and enhanced the yield of sugar beet under saline water irrigation and soil salinity (EC 5.7- 6.8) (Alharbi *et al*., 2022). The above results highlight the potential role of PGPR, particularly halotolerant strains, in improving sugar beet productivity under saline conditions.

Synergistic benefits were also achieved by combining microbial inoculation with other PGS, such as humic acid, proline, and salicylic acid, under salinity stress. It has been reported that bacterial inoculation, combined with foliar application of salicylic acid (~80 g ha^{-1}) and proline (~40 g ha^{-1}), significantly improved root growth, sugar content, and sugar purity under saline soil conditions (EC 8.12 dSm^{-1}) (Mehasen, 2022). In another study, inoculation with microorganisms isolated from halophytes, such as *Micrococcus yunnanensis*, *Planococcus rifietoensis*, and *Variovorax paradoxus*, reduced salt-induced stress (up to 150 mM Na^+) by improving biomass and photosynthesis under salinity (Zhou *et al*., 2017). Yield of salt-stressed sugar beet and soil characteristics such as EC (9.7-11.3 dSm^{-1}) and ESP (15.8%) were enhanced as a result of co-application of *Bacillus megaterium* and humic substances such as fulvic acid and humic acid (El-Atrony *et al*., 2025 b). Inoculation with AMF was also found to be beneficial in mitigating salt stress by enhancing antioxidant activity and stress hormone signaling in sugar beet (Cui *et al*., 2025). These results highlight the crucial role of soil microorganisms in mitigating salt stress and enhancing sugar beet growth through multifaceted mechanisms, including physiological and biochemical effects, as well as improvements in soil quality. Further details are provided in Table 3.

11. Seed priming

Seed priming can be used as an effective and practical approach to mitigate salt stress in sugar beet. Priming with sodium selenite (Na_2SeO_3) at 20-30 µM significantly improved germination and seedling growth parameters in sugar beet under salinity conditions (300 mM Na^+) (Liu *et al*., 2025). Similarly, osmopriming sugar beet seeds with $ZnSO_4$ (0.5%) increased the germination percentage and seedling vigor under salinity levels of up to 12 dS/m (Shokouhian and Omidi, 2021). Seed coating with various combinations of micro- and macronutrients, humic acid, and gibberellic acid resulted in improved germination and seedling growth of sugar beet under drought and saline conditions (NaCl solutions with osmotic pressure up to - 1.2 dS/m) (Neamatollahi *et al*., 2024). Similarly, tryptophan priming (2.5 mM) of sugar beet seeds enhanced germination and seedling growth under salinity conditions (up to 8000 ppm), highlighting the effect of amino acid priming in mitigating abiotic stress in sugar beet (Hozayn *et al*., 2020).

Seed priming can enhance physiological and biochemical processes that contribute to salt tolerance in sugar beet. For example, priming with Na_2SeO_3 helped maintain ion homeostasis, increased chlorophyll content, and enhanced antioxidant activity under salt stress (Liu *et al*., 2025). Tryptophan priming (Hozayn *et al*., 2020) also improved chlorophyll a, chlorophyll a/b ratio, and carotenoids, mitigating the impact of salinity on the root quality of sugar beet. Melatonin priming at 60 µM enhanced the salt tolerance of sugar beet by improving antioxidant defenses, reducing reactive oxygen species, and increasing osmolyte accumulation, such as proline and betaine, thereby maintaining higher photosynthetic efficiency in salt-stressed sugar beet (300 m Na^+) (Liu *et al*., 2022). Recent advances in the use of nanoparticles, beneficial microbes, seed priming, and plant growth regulators are presented in Table 3.

12. Irrigation management

Under salinity conditions, irrigation management optimization can be employed as an effective strategy to sustain sugar beet productivity while preserving precious water resources, particularly in salt-affected semi-arid and arid environments. Regulated deficit irrigation (DI) is an approach to improve water use efficiency (WUE) by reducing the amount of irrigation water below the maximum potential requirements at specific developmental stages. Reportedly, regulated DI (50% FC) combined with optimized N fertilization (150 kg ha^{-1}) positively affected the yield, WUE, and photosynthesis of sugar beet, albeit reducing leaf area index (Zhou *et al*., 2022). Similarly, applying DI to sugar beet resulted in improved root dry matter, WUE, and photosynthesis efficiency at a moderate DI level (50% of FC) (Li *et al*., 2019 a). In the formal experiment, it has been demonstrated that rehydration following moderate DI treatment enhanced the allocation of photosynthate to the taproot. In another study, timed water stress (controlled water deficit) suppressed excessive vegetative growth in sugar beet and led to higher yield and improved WUE (Fabeiro *et al*., 2003). Therefore, moderate DI can be used to improve the yield and reduce water consumption in sugar beet production. Li *et al*. (2019 b) have found that moderate DI increased sugar yield by 27% compared to the control treatment (70% of FC), while severe DI (30% of FC) at the phase of storage root development resulted in a 45% improvement in sugar yield. Moreover, both DI levels (30% and 50%) enhanced antioxidant defense, expressed by higher peroxidase activity and proline content after rehydration.

However, the outcomes of deficit irrigation are not always consistent and often highlight a critical trade-off between maximizing absolute yield and optimizing water productivity. The contradiction in results across different studies can be attributed to several factors, including the severity and timing of the water stress, prevailing environmental conditions, the specific sugar beet cultivar, and other interacting agronomic practices. For instance, while the aforementioned studies show yield improvements under specific DI regimes, others demonstrate that maximum yield is still achieved under full irrigation, especially in arid climates. This is illustrated in a study by Yetik and Candoğan (2022), who reported that an irrigation regime replenishing 100% of the soil water depletion resulted in the highest root and sugar yields. In contrast, a 33% DI treatment, while yielding less overall, achieved the highest water productivity. Therefore, moderate DI can be a powerful tool. However, its application must be calibrated to local conditions and specific production goals, whether that be achieving

maximum biomass or maximizing the efficiency of water use.

Fewer studies have explicitly tested irrigation strategies under salt stress. In clay saline soil (ECe = 10.1 dS m^{-1}) with irrigation waters of 0.5, 1.8, and 3.8 dS m^{-1}, shortening the irrigation interval to 2 weeks with fresh water produced the highest root yield and sugar %, while saline water reduced yield and quality; acceptable outcomes were still attainable at short intervals (2-3 weeks) even with saline water (Eid and Ibrahim, 2010). In salt-affected soil (ECe = 10.94 dS m^{-1}), combining DI (100/80/60% ETc) with biochar (0/10/20 t ha^{-1}) improved soil moisture retention; while water productivity (WP) peaked at 18.18 kg m^{-3} under 80% ETc (El-Samnoudi *et al.*, 2021). Under furrow irrigation, cut-off at 80% of furrow length produced the highest root and sugar yields and the highest WP; improvements in salinity metrics (ECe, SAR, ESP) were greatest with 100% irrigation and least with 70% cut-off (Zoghdan *et al.*, 2019). With saline irrigation water (ECiw = 6.2 vs 0.8 dS m^{-1}), high salinity reduced root mass, length, and yield but increased soluble soilds ; similar responses were also observed for irrigation suppression (Costa *et al.*, 2025). This increase is consistent with the stress-induced accumulation of soluble solids (sugars) and earlier root maturation under water deficit (Mahmoud *et al.*, 2018); however, responses can vary with irrigation depth/regime (Ribeiro *et al.*, 2024). Finally, in salt-affected soil (EC 2.9 dSm-1) managed by 80/100/120% pan-evaporation schedules and organic inputs (compost fractions; K-humate 12/24 kg ha^{-1}), the 80% pan combined with N fertilization and 24 kg K-humate ha^{-1} improved CEC, soil organic matter, infiltration, hydraulic conductivity, water productivity, and yield (Amer *et al.*, 2020).

13. Breeding of salt-tolerant varieties

As demonstrated in the formal sections, cultural practices and agronomic treatments can help alleviate the adverse effects of salinity stress on root yield and sugar yield in sugar beet. However, long-term and sustainable mitigation of salt stress can be achieved by developing salt-resilient sugar beet varieties. In this regard, comparisons of salt-tolerant and salt-sensitive cultivars at the proteomic level can be utilized, particularly for the upregulation of stress proteins, the activity of ROS detoxification, Na^+ extrusion, and the accumulation of osmoprotectants (such as betaine) (Wang *et al*., 2024). Identifying potential salt-stress markers based on physiological and biochemical traits helps screen sugar beet lines to develop new salt-tolerant cultivars.

Sugar beet is considered a salt-tolerant crop; however, high salinity levels negatively affect the growth, development, and yield of sugar beet, particularly at the seedling stage. In an experiment to determine the selection criterion for salt tolerance among three sugar beet cultivars, the proline content, soluble sugar concentration, Na^+/K^+ ratio, and Na^+/Ca^{2+} ratio were analyzed and used as criteria to screen for salt stress resistance among the seedlings of the cultivars (Wu *et al*., 2013). Physiological and proteomic profiles of sugar beet cultivars can also be used to analyze salt tolerance. For instance, proteomic results indicate that salt-sensitive and salt-tolerant cultivars exhibit distinct responses to salinity stress, with the tolerant cultivar showing enhanced antioxidant activity, higher proline content, and lower sodium accumulation (Wang *et al*., 2019). In another experiment, 11 morphological and physiological traits were used in a cluster analysis to evaluate the salt stress tolerance levels of sugar beet genotypes, and the results were successfully used to identify cultivars as tolerant, moderately tolerant, and sensitive to salt stress (Abbasi, 2020). The identification of reliable and rapid measurable traits is vital for effective salt-stress screening, facilitating selection in breeding programs. In this regard, it has been indicated that chlorophyll content and electrolyte leakage could be used as indicators for selecting salt-tolerant sugar beet cultivars (Kulan *et al*., 2021).

Polyploidy was also shown to influence the response to salt stress in sugar beet, where diploid genotypes exhibited superior germination, seedling growth, and chlorophyll retention under salt stress compared to triploid and tetraploid genotypes (Aycan *et al*., 2023). It is also worth mentioning that recent advancements in sugar beet biotechnology have facilitated the development of in vitro regeneration, somatic hybridization, and genetic transformation techniques, such as Agrobacterium-mediated transfer (Mukherjee and Gantait, 2023). These powerful tools can be employed to develop transgenic lines with enhanced tolerance to salt stress (Subrahmanyeswari and Gantait, 2022). In this regard, advanced techniques have been used to rapidly introduce salt tolerance and other stress-

resilient traits into sugar beet, providing a faster and more precise alternative to conventional breeding methods (Pattanayak *et al*., 2023).

14. Research gap and future perspectives

A substantial body of evidence supports the use of mitigation strategies to enhance salinity tolerance in sugar beet, yet several key research gaps remain. It is crucial to first acknowledge a critical limitation across many existing studies: they are often conducted under a wide range of soil conditions (not only varying in salinity) and diverse climates. This variability restricts the direct applicability and generalization of their findings, making it challenging to recommend universal solutions for all saline environments.

Regarding fertilization management, most studies have focused on the effects of individual macro- or micronutrients, with a primary emphasis on potassium, phosphorus, zinc, and molybdenum. Few studies have addressed the synergistic effects of combined nutrient application. There is potential in using strategies that incorporate multiple beneficial elements/nutrients to enhance the physiological responses of sugar beets to salinity by applying a more balanced nutrient regimen. Such integrated management might be more effective in improving nutrient uptake efficiency, ion homeostasis, and osmotic adjustment. Likewise, hormone-like substances, such as salicylic acid, and biostimulants could complement nutrient strategies by enhancing stress adaptation mechanisms in sugar beet. However, comprehensive evaluations of such combined approaches remain scarce, and future research should prioritize the design of experiments that not only evaluate individual strategies but also systematically test combinations of organic amendments, nutrient management, biostimulants, and microbial inoculants. This will be essential to understand their complex interactions and optimize plant responses. Other non-essential but beneficial elements, such as silicon, have also been demonstrated to play a crucial role as an ameliorative agent under saline and drought conditions. To convey the results of these studies as practical solutions, further investigations are needed to examine their optimal form, timing, and dose of application.

Another area that remains understudied is how different sugar beet genotypes respond to specific fertilization strategies under salinity and drought stress**.** Sugar beet genotypes with various levels of salt tolerance may respond differently to fertilization management, particularly when the salinity threshold level is exceeded.

The application of organic amendments, such as compost, biochar, and humic substances, has also shown promising results. However, long-term evaluations of organic amendments remain limited, particularly in terms of their effects on the soil's chemical, physical, and biological properties, which require more attention in future studies. We also note that the optimal type, timing, and rates of application remain poorly defined and require optimization for varying salinity levels in soil and irrigation water. Further investigation is needed to clarify the physiological and biochemical mechanisms, such as enhanced ion uptake, antioxidant activity, and microbe-plant interactions, that contribute to the beneficial effects of organic amendments. In parallel with organic amendments, several emerging tools have also been explored, such as nanoparticles, plant hormones and hormone-like substances, and microbial inoculants, which hold promise in mitigating salt stress in sugar beets. Yet, their combined effects and underlying mechanisms are not well understood. Biostimulants, such as tryptophan seed soaking or the application of hormone-like substances like melatonin, have recently garnered special attention in the literature for their potential to improve photosynthesis and strengthen antioxidant defenses under saline conditions. Further research is needed to evaluate their effectiveness and determine how they may be integrated into comprehensive salinity mitigation strategies for sugar beet.

The combined application of microbial inoculants, such as plant-growth-promoting rhizobacteria (PGPR), nanoparticles, and plant hormones, including auxins, remains largely uninvestigated, primarily due to the limited data available on their effectiveness in mitigating salt stress. While the use of microbial inoculants holds promise, significant knowledge gaps persist. For example, inoculating sugar beets with halotolerant endophytes has been shown to enhance growth under salinity conditions, yet the mechanisms underlying PGPR-mediated stress alleviation and the roles of other symbionts like arbuscular mycorrhizal fungi are still not well understood. Addressing these gaps will require multi-factorial studies to identify

potential synergistic or additive effects among microbial inoculants, biostimulants, plant hormones, and beneficial elements/nutrients. Detailed physiological and molecular research will be essential to clarify the tolerance pathways activated by such combinations.

Ultimately, to move beyond isolated findings and develop robust, field-ready solutions, future research programs must be designed to explore the complex interactions between Genotype × Environment × Management (G×E×M). Such studies would provide crucial guidance on which cultivars are best suited to specific saline conditions or respond most effectively to particular agronomic practices. Conducting field-based trials across different sugar beet cultivars, combining microbial inoculation with optimized fertilization and biostimulant treatments, will be crucial for translating experimental findings into practical and scalable solutions for mitigating salinity.

This G×E×M approach will not only test strategies but also help build predictive models that can guide farmers in selecting the optimal combination of cultivar and management practice for their unique environmental challenges.

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